

COMPUTATIONAL APPROACHES TO *D. RERIO* RETINAL ORGANOGENESIS

by

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Abstract

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Increasing integration of sophisticated statistical and computational techniques into the analysis of cell and molecular biological data has created many opportunities for explaining organogenic phenomena by numerical analysis. This thesis first examines the suitability of the most developed of these explanations for the development of the *D. rerio* eye, the stochastic mitotic mode explanation (SMME) for retinal progenitor cell (RPC) function, and demonstrates that it is neither the best available explanation, nor theoretically defensible. It is shown that a deterministic alternative model is an improved explanation for the data, and that the SMME cannot explain postembryonic RPC function. These studies support earlier hypotheses of a linear progression of competency phases over the hypothesized “stochastic” nature of RPC lineage commitment.

A Bayesian model comparison framework, relying centrally on the Galilean Monte Carlo nested sampling algorithm for estimation of model evidence and posterior parameter distributions, is subsequently elaborated as an improved method for testing models in organogenesis. The utility of this approach is proved by comparing models of *D. rerio* RPC populations in the postembryonic Circumferential Marginal Zone (CMZ). The inferential framework is used to show that the activity of CMZ RPCs in the postembryonic period is best characterised by two distinct phases of proliferative activity with similar timing across morphological axes, despite asymmetrical population dynamics. Changes to RPC lineage commitment across the two phases suggest an explanation for the changing photoreceptor mosaic. These studies establish that CMZ RPCs are a dynamic population, whose postembryonic function is not adequately described as a recapitulation of an embryonic program.

Finally, the *D. rerio* mutant *rys* is shown to arise from a lesion in the npat locus, and the apparently paradoxically enlarged CMZ RPC population observed in these animals is demonstrated to arise from a failure of these cells to specify and exit the proliferative niche, and not from a proliferative defect. Nested sampling is used to prove that unique populations of nucleosome positions in *rys* mutants and sibling arise from separate causal processes. A mechanism for these observations involving simultaneous changes to the subunit composition of the nucleosome pool and loss of translational control is advanced.

This work is dedicated to the memory of my brother Gareth Akerman.

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Introductory Notes

Thesis Guide

This document is split into three parts. Part I contains results and discussion pertaining to empirical modelling studies that will be of interest to committee members and developmental biologists. Readers wishing to confine themselves to results chapters should examine [Chapter 2](#), [Chapter 4](#), and [Chapter 5](#). Part II contains technical reports pertaining to novel software that was written in order to perform the analyses presented in Part I. Part III contains supplementary materials arising from Parts I and II. These include detailed descriptions of the methods used in Part I, less-technical explanations of relevant statistical and model theory, the source code of all software and analyses, and the bibliography. The source code has been omitted from the print document.

Readers of the .pdf document will find some text highlighted in plum throughout the thesis. Section indices highlighted in this way link to the specified section. Technical terms have also been highlighted when pertinent material is available in Part III to explain them for the reader who may be unfamiliar. Because empirical results in Part I are separated from relevant methods and code in Part III, links to the methods and code used to generate the figure's data and analysis are given in figure captions.

Terminology and Style

Throughout [Chapter 1](#), [Chapter 2](#), and [Chapter 3](#), I have used the appellation "Harris" when referring to the output of William Harris' research group. This is a large body of research spanning several decades, and involves many co-authors. My use of "Harris" here is a convenience, as Harris is the only common author across the period in question, and presumably the agent carrying these ideas forward from project to project. It is not intended to slight or minimize the contributions of any of the other members of Harris' group (many of whom are now senior scientists in their own right). I have used "Raff" in an identical sense in referring to the Raff group's work in [Chapter 3](#).

I have preferred the terms "specification", "determination", and their derivatives, to refer to cells assuming a particular lineage fate. "Differentiation" is well-understood, but ambiguous, and often understood to relate to the mitotic event itself, which I generally do not intend. "A cell has specified" means it has assumed a stable macromolecular identity or "fate". This term is intended to correspond exactly with the appearance of stable markers of cell type.

I have occasionally used two useful terms from explanation theory, when discussing models, for concision. These were unfamiliar to me from my biological training and so are defined here for others: explanandum - the fact to be explained explanans - the explanation itself

Unless otherwise noted, formatting of quoted material is preserved, so that italic emphases appear in the original.

Part I

Modelling Studies

Précis of Modelling Studies

The primary concern of this thesis is developing and assessing computational models of retinal progenitor cell (RPC) phenomena. While many different explanations for various aspects of eye development exist, these have usually been supported by assessing the frequentist significance of observed effects (i.e. t-testing), not by building formally testable models of the phenomena themselves. Of the explanations which could be subject to model comparison, virtually all of these are derivatives of the Simple Stochastic Model (SSM), dating to the earliest work, performed by Till and McCulloch [TMS64].

The explosion of molecular biological information has massively increased the number of parameters that might be included in RPC models. The question of how RPC activity might be successfully predicted and controlled *in vivo* may revolve around finding adequate models that explain retinogenetic processes. The development and assessment of such models is, therefore, of fundamental basic and applied interest. This is particularly so, in light of increasing interest in entraining RPCs for the purpose of retinal regenerative medicine. Our group is particularly interested in the possibility that RPCs located in the peripheral retinal annulus of the circumferential marginal zone (CMZ), which are present in zebrafish and mammals alike, could be harnessed for retinal repair.

It is surprising that, despite the use of SSMs, the literature on neural stem cells contains virtually no systematic statistical approaches to the construction, optimization, or comparison of models. This thesis is an attempt to address this problem. It proceeds in three basic strokes. Firstly, Chapters 1 and 2 evaluate the existing explanations of RPC behaviour, and find that those with formal model components are deficient, and cannot explain the activities of RPCs in the CMZ. Secondly, Chapters 3 and 4 propose a general CMZ modelling framework under which the Bayesian evidence for different model-hypotheses might be assessed, and, by testing a variety of population-level hypotheses, provide guidance and develop the methods required to test cell-based hypotheses. Lastly, Chapters 5 and 6 introduce the zebrafish CMZ mutant *rys*, and apply some of the methods developed in the second stroke to explain aspects of the aberrant morphology and behaviour of *rys* RPCs.

Chapter 1 begins by summarizing the range of explanations that have been offered for RPC activities, and introduces the primary set of formal models that have recently been used to favour an explanation centered around a supposed stochastic mitotic mode of RPCs. Chapter 2 elucidates the structure and development of the stochastic mitotic mode explanation (SMME) models, finding fundamental logical errors in their interpretation of "stochasticity". Moreover, by pursuing a basic information theoretical model selection approach, it is demonstrated that the SMME is not even the best available explanation relying on this flawed notion of stochasticity.

Chapter 3 discusses the implications of Chapter 2 for models of RPCs generally, and in the CMZ particularly; it also recommends a better model selection approach from Bayesian theory, nested sampling. Chapter 4 surveys the activity of RPCs in the post-embryonic zebrafish CMZ, developing Bayesian methods for doing so. These methods include the use of nested sampling to solve the long-standing problem of estimating cell cycle parameters of subpopulations of cells from cumulative thymidine labelling measurements of the entire super-population. It concludes with recommendations for future modelling approaches to the CMZ, structured by the foregoing Bayesian analysis.

Chapter 5 identifies the causative mutation in *rys* and shows that the zebrafish CMZ mutant *rys* CMZ RPCs have disorganized chromatin, likely blocking differentiation but not proliferation. Nested sampling is used to demonstrate that the nuclear phenotype is likely the consequence of a shift in nucleosome histone composition in *rys* progenitors. Chapter 6 discusses some of the theoretical issues

raised by the *rys* analysis, and their implications for modelling retinogenesis more broadly, especially in integrating nuclear dynamics into models of RPCs.

Chapter 1

Canonical retinal progenitor cell phenomena and their explanations

1.1 The Harris Stochastic Mitotic Mode Explanation (SMME)

The work presented in [Chapter 2](#) examines the best-developed theory of *D. rerio* retinal progenitor cell (RPC) function. The theory in question is the work of preëminent retinal biologist William Harris' research group, referred to hereafter Stochastic Mitotic Mode Explanation (SMME). This theory purports to explain the function of zebrafish RPCs in terms of stochastic effects on mitotic mode, which specifies the mitotic state and fate propensity of offspring.

The SMME for RPC function is of fundamental theoretical and practical interest. It is a concerted effort by the Harris group to explain how a complex tissue like a retina can arise from a field of similar proliferating cells. In 2009, Harris coauthored a detailed review chapter, documenting the bewildering array of macromolecules and cellular processes thought to be involved in RPC function [\[AH09\]](#). This enumeration contains many caveats and notes that the effects of particular macromolecules routinely differ between developmental stages, cell types, organisms, and so forth. At this time, no clear, detailed, comprehensive models of RPC function had been advanced, and the review is typified by statements like "It is difficult to reconcile all the studies on the initiation and spread of neurogenesis in a single model."¹ It is therefore remarkable that, over the next nine years, the Harris group would go on to promulgate a simple stochastic model of zebrafish RPC function invoking only two named macromolecules.

Harris thus seems to be making a bold attempt to cut the Gordian knot of conflicting explanatory threads and advance a simple, comprehensible, "mind-sized" model of RPC function. Harris' explanation for zebrafish RPC function is a microcosm of the broader promise of "Systems Biology" to make sense of the contemporary welter of conflicting datasets; by using sophisticated mathematical methods drawn from information and complexity theories, the apparent confusion will be clarified and underlying molecular mechanisms will be revealed. Given Harris' track record and preëminence in the field, we have good reason to take seriously the possibility that he has succeeded. Examining whether this is the case is our first priority. To appreciate the scope of his theoretical maneuver, and possible alternatives

¹This was in no sense a problem with Harris' understanding. A similarly high-level review coauthored by Pam Raymond [\[AR08\]](#) concluded, with regard to models of photoreceptor fate specification: "The data reviewed in the preceding sections indicate that a 'one-size-fits-all' model is not possible..."

to it, we begin by summarising the state of the art at the time of his 2009 review (as well as relevant subsequent additions).

1.2 Explanations for RPC function in 2009 and the drive to unification

Molecular biologists have sought explanations for the same remarkable features of retinal progenitor cells for decades. Animal retinas, having well-understood functions and stereotyped structures, promise tractable targets for typical molecular biological explanations. With well defined cell types present in tightly regulated proportions and topological organisations, both theoretically-inclined molecular biologists and clinically-inclined regenerative medical practitioners have found the retina to be a useful model tissue². The regular, easily detected order in eyes seemed to suggest a similar level of order and regularity in the macromolecular processes which underlay the formation of the tissue. Older explanations commonly suggested that RPCs are more-or-less identical and go through rigidly stereotypical macromolecular processes. It is, however, the persistently observed departures from this conception that have occupied much of our attention.

The simplest explanation for the regularity of retinal development is that any given RPC is executing the same strict “developmental program” as its neighbours. If every progenitor produces a similar number of cells, and the progeny are specified in similar proportions, well-understood principles of cellular adhesion could give rise to the characteristic laminar organisation observed in animal retinas. However, by the 1980s, vertebrate lineage tracing experiments revealed a surprising degree of inter-lineage variability in many neural progenitor systems, not least of which was the retina. In their seminal 1987 paper, David Turner and Connie Cepko, using retroviral lineage labelling techniques, demonstrated that individual RPC lineages in rats had diverse proliferative and fate specification outcomes [TC87]; Harris’ group confirmed this result in *Xenopus* the subsequent year [HBEH88], suggesting this variability was a common feature of vertebrate RPC function.

Indeed, at this point, we find fairly clear accounts of what retinal biologists took their theoretical options to be in explaining this variability. As Harris’ 1988 report states:

Changes in cell character associated with cell type diversification may be controlled in an autonomous way, reflecting either a temporal program inside the cell (Temple and Raff, 1986), the asymmetrical segregation of cytoplasmic determinants (Strome and Wood, 1983; Sulston and Horvitz, 1977), stochastic events inside the cell (Suda et al., 1984), or some combination of these processes. Alternatively, cell type may be controlled in a nonautonomous way, as in cases in which the extracellular environment (Doupe et al., 1985) or cellular interactions (Ready et al., 1976) elicit or limit cell fate. With its multiplicity of cell types, the vertebrate nervous system would seem to require the ultimate sophistication in its means of cellular determination. [HBEH88]

It is striking, then, that Harris’ review of the literature two decades later describes the situation similarly:

Once differentiation is initiated, regulatory mechanisms within the retina ensure that progenitors retain the capacity to undergo more divisions, in parallel with churning out differentiated

²Indeed, if central neuroregenerative medicine is to become a clinical possibility, it seems likely that the theoretical and practical issues are most likely to be resolved in eyes before other areas of the CNS.

cells, and that progenitors cease dividing at variable times. There is still debate about the extent of early programming that allows progenitors to step through a series of stereotypical divisions and the extent of regulation from within the whole retina. The production of differentiated cells alters the retinal environment with time...

Moreover, cells from the same clone do not all differentiate at the same time, suggesting three possibilities: a stochastic mechanism for the decision to differentiate, exposure of the two daughters to different environments, or asymmetric inheritance of determinants. [AH09]

In the same paper, he states that the “simple structure and accessibility of the retina make it a useful model to study cell division and differentiation, and as a result most aspects of this have been studied, from lineage tracing of progenitors, to the morphological aspects of division, to the molecular mechanisms involved.” Thus, by Harris’ own account, some twenty years of additional research into almost every variety of macromolecular explanation for a huge range of RPC-related phenomena had not provided any means to narrow down the possibilities he had already laid out in 1988. We still have Raff’s temporal program (“early programming … step[ping] through a series of stereotypical divisions”), asymmetric segregation of cytoplasmic inheritants during mitotic events, “stochastic” events internal to the cells, and possible “environmental” extracellular determinants. While the number of particular macromolecules functionally implicated in proliferative, specificative, and organisational RPC phenomena had greatly expanded, this had not provided any means to differentiate between these theoretical options. This is only one example of a general phenomenon, in which enumerationist research programs, directed at producing more and more facts about macromolecular involvement in cellular phenomena, have failed to generate additional theoretical understanding [Kan06]. Harris’ SMME therefore represents an example of a “Systems” biological explanation, in which biologists apply the analytical and interpretative methods of the physical and mathematical sciences in an effort to resolve the problems posed by biological complexity [Mor09].

Before proceeding to the SMME itself, let us briefly summarise the diversity of phenomena implicated in RPC function by 2009, as well as the panoply of mechanisms offered as explanations. In doing so, it will become clear what has been elided in the SMME, and what may need to be restored in any alternative modelling approach.

1.3 Canonical vertebrate RPC phenomena: the RPC “morphic-genetic alphabet”

The bulk of our knowledge of RPC behaviour stems from histological observation employing a limited number of techniques. Simple observations of mitotic figures in a variety of animals had, by the 1950s, revealed the surprising diversity of RPC proliferative phenomena across vertebrate clades. However, it was the advent of lineage tracing techniques, particularly those marking single clonal lineages in whole retinae, and the extensive use of these techniques in the 1980s-90s, that formed most of the basic body of observations that any macromolecular explanation is now called upon to account for.

Since the majority of vertebrate retinas of biomedical interest are mammalian, and these retinae are fully formed in an early developmental period, RPC behaviour has been best-studied in an embryonic and early developmental context. Here, vertebrate RPCs are derived from the eye field population of the early neural plate and later neural tube. This population is separated into left and right eye primordia, which in turn pouch outwards toward the ectoderm, and, in conjunction with the lens placode (itself induced

from the ectoderm), form the optic vesicle. The primitive eye is formed when this vesicle completes a complex morphological folding process, resulting in the cup-shaped structure of the retina [Cav18]. During this process, the cells of the neural retina are differentiated from the overlying retinal pigmented epithelium (RPE). Sometime after the formation of the retinal cup, RPCs begin to exit the cell cycle and are specified as retinal neurons. Studies of this early period revealed numerous difficult-to-explain features of RPC behaviour. Following Larsen’s observation that tissue form is attributable to only six behaviours [Lar92], which she describes as the “morphogenetic alphabet”, I have categorised RPC phenomena as relating to proliferation, fate specification, migration, growth, death, and extracellular matrix formation. Since the vast majority of reported phenomena fall under the first two categories, proliferation and specification, these are given separate sections below, while those belonging to the last four are described collectively.

1.3.1 Proliferative phenomena

Clonal lineage tracing experiments reliably find that vertebrate RPCs give rise to highly variable numbers of offspring over the collective “lifetime” of the lineage. The most dramatic of these findings demonstrate that rat RPC lineage sizes vary across two orders of magnitude *in vivo*, from 1 to over 200 [TSC90]. While at least some of this variability must be related to differential integration of lineage markers into “older” (giving fewer offspring) and “newer” (giving more) RPCs, vertebrate RPC lineages nevertheless differ widely in their fecundity. The physical organisation of these clones is complex; as detailed below, RPC progeny may appear in any of the 3 retinal layers in a wide variety of specified fate combinations, and may engage in short-range migrations to appropriate positions for their specified fates. Most clonal lineages are “extinguished”; that is, after some time, all of its members have become postmitotic. However, it has long been noted that not all cells produced by RPCs are strictly postmitotic neurons; specified Müller glia retain the ability to reenter the cell cycle in response to stimuli (normally, retinal damage) [DC00, FR03], and peripheral CMZ RPCs remain proliferative in those vertebrates whose eyes grow beyond early development (notably in frogs and fish, while the chick retina has a CMZ of more limited output [FR00]). Therefore, some clonal lineages may be organised into clumps associated with Müller responses, while others in frogs and fish may continue to be “plated out” in a more-or-less linear manner at the retinal periphery for as long as the lineage “lives” [CHW11]. This ongoing RPC contribution to peripheral neurogenesis has long been recognised, so that by 1954 we find the following statement introducing a study of unusual mitoses in the retina of a deepsea fish:

It is conventional³ to hold that the growth of the vertebrate retina is only possible due to the presence, in this tissue, of a peripheral germinal zone. In this region, young elements actively multiply, and, by subsequent differentiation, give rise to the diverse nervous and sensory constituents of the retina. [VL54]

[author’s translation from the French]

Despite this, the proliferating RPCs in this “peripheral germinal zone” (also known as the “ciliary marginal zone”, or CMZ, for its proximity to the retinal ciliary body) have not received the same level of attention as those associated with the central retina. As a consequence, these RPCs are generally treated as though they are a type of “frozen” progenitor population, recapitulating spatially, along

³Unfortunately, I am unable to locate the source of this convention, likely due to the poor preservation of many of these older reports. That this required no citation in 1954 suggests the original observations of CMZ proliferation may be in the early 20th century.

the peripheral-central axis, the process which RPCs in the central retina undergo in a time-dependent fashion[HP98].

The length of the RPC cell cycle is of considerable interest, since the evolution of this parameter in time, in conjunction with the RPC population size (the number of cells specified in the eye field), determines the eventual size of differentiated retinal neural population, and therefore the retina. RPC cell cycle length is usually inferred from clonal lineage size, although it has also been assayed directly in cumulative thymidine analogue labelling experiments [AC96]. Vertebrate RPCs undergo a period of relative quiescence, in which the cell cycle lengthens, before the neural retina begins to be specified (in zebrafish, this period is 16-24 hpf). The cell cycle shortens as RPCs begin to exit the cell cycle [HH91, LHO⁺00]. After the central retina is specified, the RPC cell cycle again lengthens, and is presumed to continue to slow until RPCs have completed specifying⁴.

Finally, the orientation of the RPC division plane in mitosis is also implicated in retinal organisation. The orientation of divisions is associated with both proliferation and fate specification of RPC progeny. For instance, interfering with spindle orientation in the developing rat retina, such that more RPC divisions occur parallel to the neuroepithelial plane (rather than along the apico-basal axis) results in more proliferative and fewer postmitotic, specified progeny[ZCC⁺05]. That said, it seems that whatever effects are attributed to mitotic orientation are likely species-specific, as zebrafish RPCs display a different pattern of axis orientation, dividing mainly in the epithelial plane[DPCH03].

1.3.2 Fate specification phenomena

Offspring of vertebrate RPC lineages may enter any of the three cellular layers of the retina. Moreover, single lineages can include any possible combination of cell fates, so that RPCs cannot be readily divided into “types” on the basis of lineage fate outcomes [HBEH88, TSC90, WF88]. While some progenitors have propensities to generate similar cell types, these relations seem species-specific, and may not define separate progenitor pools [AR08]. In general, then, RPCs are taken to be totipotent with respect to the neural retina- all of the cell types⁵ of the differentiated retina are derived from similar RPC lineages. Little about this picture has changed since its initial development, using a variety of lineage tracers (including retroviruses, thymidine analogues, and injectable dyes) and histochemical markers to supplement morphological identification of specified neurons. In particular, sophisticated modern live imaging experiments in zebrafish (many pioneered by Harris), have broadly confirmed the findings of the 80s and 90s in mammalian fixed specimens, explants, and live animals [BRD⁺15].

Of particular note are the observations of the Raff group [WR90, CBR03], who demonstrated that dissociated rat RPCs, cultured at clonal density, took on morphological and histochemical features associated with different specified neural types in similar numbers and proportions, and on a similar schedule, to same-aged RPCs cultured in retinal explants. These results dramatically suggested that both the proliferative and fate specification behaviour of RPCs depends less on intercellular contact, and the complex signalling environment of the developing retina, than on factors intrinsic to the RPCs themselves. These studies contain the essential germ of Harris’ eventual commitment to SSM explanations, purporting as they do to “test the relative importance of cell-intrinsic mechanisms and extracellular

⁴This presumption is demonstrably incorrect in the *D. rerio* eye, see Section 4.4 for a more plausible model of cell cycle dynamics in this organism.

⁵The “cell type” concept is unusually well-defined in the retina, as there are an abundance of distinct morphological and molecular features which differentiate numerous subtypes of the seven general types of retinal neuron.

signals in cell fate choice”, and providing convincing evidence for the preponderant importance of the purported cell-intrinsic mechanisms.

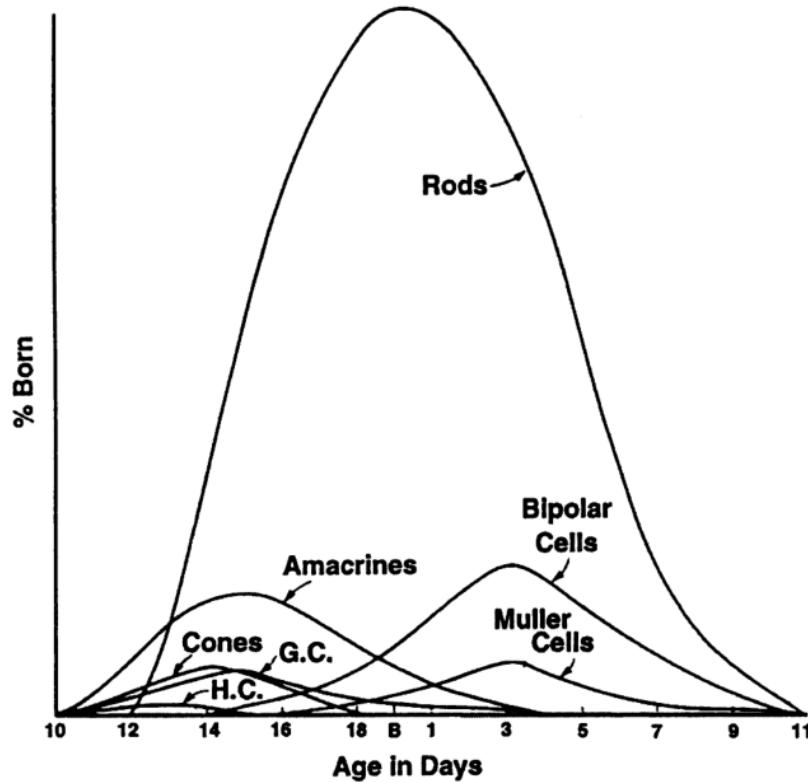


Figure 1.1: **Histogenetic birth order of retinal neurons**

Adapted from [You85] by [CAY⁺96]. G.C., Ganglion Cells; H.C., Horizontal Cells. Data from embryonic and perinatal mouse eyes.

In spite of the apparent ability of RPCs to produce offspring specified to any of the possible neural cell fates, at any time during their lineage history⁶, vertebrate retinal development displays a temporal ordering, such that in any particular location, retinal ganglion cells (RGCs) tend to be produced first, followed by the other cell types in what has been described as an overlapping “histogenetic order”, pictured in Figure 1.1. As noted above, RPCs also exit the cell cycle in a spatiotemporally defined order (from central to peripheral over time), and specification follows the same pattern⁷. This naturally gave rise to questions about the origin of the “overlap” observed in the sequential production of various cell types; conceptually, this overlap could be produced by identical RPCs executing identical rigid specification programs if they begin to execute it along the spatiotemporal gradient noted above, as originally suggested by Cepko et al. [CAY⁺96]. However, it is impossible to reconcile this notion with more recent results from Harris’ *in vivo* zebrafish lineage tracing studies [DPCH03, HZA⁺12, BRD⁺15],

⁶Even in papers arguing for a strict, linear sequence of speciative outcomes in all RPC lineages, the actual data show RPCs occasionally giving rise to “late-born” photoreceptors subsequent to their first division[WR09], giving lie to the notion that the process is particularly strict.

⁷In many studies, cell cycle exit is conflated with specification such that evidence of the former is taken as evidence of the latter. There are, however, reasons to believe cell cycle exit and specification are not the same process, discussed below.

which confirm mammalian cell culture work in demonstrating that vertebrate RPC lineages do not execute similar specifitative programs. More recent articulations of Cepko’s concept of linear specification programs incorporate variable sub-programs to account for this [Cep14].

1.3.3 Other morphogenetic phenomena

The outcomes of RPC proliferation and lineage commitment are often taken as sufficient explanation for the formation of the neural retina. Indeed, after the eye cup has been formed (prior to the specification of any neurons), RPCs are, in effect, already “in place”. Therefore, there are few documented RPC phenomena outside of the “proliferation” and “specification” categories of the morphogenetic alphabet [Lar92], which enumerates the cellular processes contributing to tissue form and structure. RPCs do not seem inclined to migrate long distances, they seem not to generate much in the way of extracellular material⁸, and, in non-pathological conditions, they are rarely seen to die. Still, there are a number of phenomena which appear to be important for proper retinal organisation that fall into these other “alphabet” categories.

The most notable of these is interkinetic nuclear migration (INM), in which RPC nuclei move back and forth between the apical and basal surfaces of the retina. Common in many neural tissues, INM affects both proliferation and fate specification, but is dissociable from them- both cell cycle and specification proceed (albeit in a precocious manner) when INM is disrupted[MZLF02]. The apical retinal surface provides a microenvironment which appears to be required for RPC mitosis to occur⁹, and INM seems to consist of a directed, probably actomyosin-mediated movement to the apical surface, followed by an undirected “random walk” away from the apical surface and hence toward the basal retina [NYLH09]. This “random walk” may arise from displacements due to the directed INM of neighbouring progenitors [AHW²⁰]. More committed RPCs also appear to actively migrate to positions appropriate for the specified cell type [CAR¹⁵, IKRN16], although it is unclear if this short-range migration is caused by INM undergone by more actively proliferating progenitors. It seems likely that these short-range migrations of more specified cells are especially significant in the early retina, before the neural plexiform layers (consisting of axons and other neural processes) have begun to divide the cellular layers into bounded compartments.

Notable lacunae in the study of the RPC “morphogenetic alphabet” include the regulation of cell size and growth, and potential roles for cell death, both of which have received little attention. While cell size and growth are tightly linked to proliferative behaviour in yeast [YDH¹¹], any related effects in RPCs have not been elucidated. This may be a significant oversight, given the requirement for RPCs to continuously grow during their proliferative lifespan. Cell death does not seem to play the same “pruning” role for RPCs that it often does in other neural tissues, and observed rates of cell death in normal RPC populations are very low, so few studies have been conducted.

⁸The formation of a laminin-rich basement membrane seems to be necessary for optic vesicle formation [ICW13], but it is not clear that RPCs produce this. ECM function in eye formation remains under-studied.

⁹Nonapical divisions do occur, notably in specified, but proliferative cells [GWC⁰⁷]. The extent to which RPCs depend on the apical surface may depend on how “RPC” is defined. In general, RPCs are no longer considered as such when they acquire characters associated with differentiated neurons (cell type markers, morphological traits, etc.), although it is clear that the acquisition of these characters does not necessarily imply that the cell is postmitotic. Any complete explanation of how RPCs give rise to the structure of the eye must also consider these nonapical divisions.

1.4 Macromolecular mechanistic explanations for RPC phenomena

Having surveyed the cellular phenomena pertaining to RPC function in retinal development, we proceed to a selection of the many macromolecular mechanistic explanations (MEx) [Fag12] that have been offered to explain them. Unsurprisingly, given the field’s focus on the early events of eye development, these MEx are mainly targeted at this period. Thus, the majority explain tissue-level phenomena like the initial “wave” of cell cycle exit and specification, without necessarily seeking a global explanation for RPC behaviour irrespective of context, so it is unknown how many of these might pertain to ongoing peripheral neurogenesis or other, adult neurogenic phenomena like those exhibited by Müller glia. That said, we now turn to examine some of the best-developed of these explanations.

1.4.1 Transcription factor networks

Perhaps the most notable MEx offered to explain RPC specification and development is the eye field transcription factor network, or EFTFN. The roots of this explanation are found in the Pax6 “master gene” explanation popularised in the 1990s [Geh96]. This explanation revolved around the apparently universal involvement of Pax6 gene products in eye formation in model organisms, and the promiscuous inter-species effects of Pax6 (with mouse RNA able to induce ectopic formation of eye structures in *Drosophila* imaginal discs, for instance [HCG95]), so that it appeared to be a highly conserved genetic “switch” for eye development.

Importantly, this explanation purported to resolve what Darwin regarded as a serious problem for his theory, the apparent implausibility of the gradual evolution of eyes (and other “organs of extreme perfection”) from some primitive ancestral structure [Dar88, p.143-4]. In particular, Pax6 suggested to some theorists an alternative to the surprising hypothesis of Mayr and Salvini-Plawen, that differences in eye structure and function across clades indicate the independent appearance of eyes in more than 40 clades [vM77]. Pax6 thus provided a molecular pointer to a potential common ancestor for all animal eyes [EHML09].

Subsequent investigations revealed that vertebrate Pax6 is a conserved member of a complex network of cross-activating and inhibiting transcription factors, including Pax6, Rx1, Six3, Six6, Lhx2, ET, and Tll [Zub03]. Members of this network tend to promote proliferation and suppress markers of differentiated neurons, and their loss commonly results in the failure to form the eye field at all [AH09]. The expansion of this explanation to include other TFs in a network revealed significant differences between species [Wag07]- while the role of Pax6 is conserved between *Drosophila* and vertebrates, the roles of other members of the EFTFN are not. Moreover, the universality of Pax6 was only apparent, and not real, as there are bilaterian eyes whose development is Pax6 independent (including in *Platynereis*, *Branchiostoma*, and planarians) [Koz08]. This highlighted the great difficulty in connecting morphological characters such as those observed by Mayr with a genetic basis- it is simply not clear that Pax6 conservation points to a common ancestor for all eyes, or even all photoreceptive neurons¹⁰. Moreover, expansion of the moncausal “master gene” explanation, to include a network of TFs with broad gene regulatory effects, highlighted the problems of complexity in offering MEx for RPC function. The components of this network interact in complex, context-dependent ways. While the EFTFN as a whole is

¹⁰Indeed, the relevant “unit” of homology for evolutionary explanations for eyes remains contested, with some arguing for the cell itself over any particular set of gene sequences [EHML09]

taken to promote RPC proliferation and to delay specification¹¹, its components have also been held responsible for the specification of particular classes of differentiated neurons, and remain expressed in those postmitotic cells. Notably, Pax6 is implicated in the expression of bHLH TFs required to specify multiple classes of retinal neuron [MAA⁺01], and is known to directly activate Ath5, necessary for RGC specification [WSP⁺09]. The EFTFN has thus been offered as an explanation for the maintenance of the multipotent, proliferative RPC state, but how this network is disassembled, and its components repurposed to promote specification, remains obscure.

The EFTFN is not the only transcription factor network offered as a MEx for RPC function. Another well-developed explanation involves Chx10 (aka vsx2), a transcription factor important for normal proliferation of RPCs, its loss causing microphthalmia in the mouse [BNL⁺96]. Chx10 was subsequently found to repress Mitf, involved in RPE specification, and hence to promote neural retinal fates over pigmented epithelial ones [Hor04]; in the absence of Chx10 the early eye cup does not stratify properly between apical pigmented cells and the neural retina. Much like the multifunctional EFTFN components, Chx10/vsx2 has also been implicated in the specification of particular neural fates, notably bipolar neurons [BNL⁺96] and the regulation of Vsx1 (a parologue of Chx10), Foxn4, and Ath5, associated with specification of subpopulations of bipolar cells, horizontal and amacrine cells, and RGCs and PRs, respectively [CYV⁺08, VJM⁺09].

Clear hypotheses advocating for particular relationships between different TF-centric explanations are rarely stated. It is tempting simply to arrange them in some kind of “developmental order”, perhaps with the Chx10-Mitf network “downstream” of the EFTFN. That this would be facile is evident from the changing roles of these transcription factors, depending on developmental and cellular context. To date, no unifying framework has been applied. Obvious candidates include the “developmental gene regulatory network” concept [LD09], a type of cybernetic explanation which assembles genes into feedback networks. Given the popularity of this type of explanation, it is worth noting that no one has yet had any success in offering one for RPC function.

These transcription factor network MEx frequently incorporate extracellular signals (often as an explanation for the appearance or “set-up” of the TF network), and it is generally recognised that these signals have a profound influence on these networks, and on RPC behaviour generally. We therefore turn to explanations invoking these signalling mechanisms.

1.4.2 Intercellular signalling networks

Virtually every developmentally significant class of signal has been implicated in RPC function, so that Harris, by 2009, simply glosses over a majority of these pathways by briefly summarising them in tabular form and not otherwise mentioning them [AH09]. These include BMP, CNTF, FGF, Glucagon, Hedgehog, IGF, Notch, TGF α , TGF β , VEGF, wnt, and a host of neurotransmitters. This diversity of signalling pathways has proved to be a formidable problem for integrated explanations, since almost all of these pathways converge on the same two cellular outcomes in RPCs, that is, proliferation and specification. Thus, most signalling MEx for RPC function elide the majority of other signals which are known, or thought, to affect the same processes. That said, let us explore a few of the more detailed signalling explanations.

¹¹This is sometimes referred to as “promoting RPC fate”, since RPCs are taken to be those cells which proliferate but do not yet display markers of specification. Since it is, by now, widely recognised that cells that appear to be well-specified may remain in cell cycle [GWC⁺07, ESY⁺17] this terminology should probably be jettisoned.

In developmental terms, the first phenomenon requiring explanation is the appearance of the eye field to begin with- what is it that accounts for the differentiation of RPCs from the rest of the anterior neural plate and tube? Wnt signalling MEx have been offered to explain the appearance of the *Xenopus* eye field. Fz3 signalling seems to promote expression of eye field transcription factors (see below)[RDT⁺01], while an unspecified non-canonical interaction between Wnt11 and Fz5 inhibits canonical β -catenin signalling through Wnt8b/Fz8a, which would otherwise promote prospective anterior forebrain fates [CCY⁺05]. Inhibition of FGFR2 signalling, and activation of ephrinB1 signalling have also been implicated in early *Xenopus* eye field cell movements [MMDM04]. Subsequent experiments determined that the xenopus ADP signalling through the P2Y1 receptor directly activates the EFTFN [MBE⁺07], described above. More recent experiments in zebrafish suggest that precocious acquisition of neuroepithelial apicobasal polarity, probably driven by interactions with a Laminin1 basement membrane, distinguishes the early eye field [ICW13].

Subsequent to the appearance of eye field RPCs and their rearrangement into the optic cup, the apparent central-to-peripheral “wave” of RPC exit from cell cycle and specification of early RGCs [HE99], has had detailed MEx advanced to explain it. In both zebrafish and chick retina, FGF3 and FGF8, originating from the optic stalk, initiate this early cell cycle exit and specification [MDBN⁺05], while inhibiting FGF signalling prevents this from occurring, and ectopic expression of FGF can cause it to occur inappropriately. The progression of this “wave” of cell cycle exit and specification has been separately explained, by Sonic Hedgehog (Shh) signalling from the newly specified RPCs inducing cell cycle exit and specification in adjacent cells [Neu00]. This process is dependent on, and downstream of, the above-mentioned FGF induction [MDBN⁺05]. The role of Hh signalling has been challenged on the basis that Hh inhibition in subsequent experiments did not display the same effect size [SF03], and that its effects on Ath5 expression, required for RGC specification, are ambiguous [AH09]. More recent MEx advanced by Harris have suggested that Hh signals may decrease the length of the cell cycle, resulting in increased proliferation and earlier cell cycle exit and specification [LAA⁺06, ALHP07].

A well-developed “local” signalling MEx (mainly advanced by Harris) invokes the classic Notch/Delta lateral inhibition model, with small fluctuations in Notch/Delta activity giving rise to a positive feedback response that differentiates neighbouring cells. Cells which have high Delta expression tend to be specified as retinal neurons, while those with high Notch tend to remain proliferative, either as RPCs or Müller glia [DRH95, DCRH97]. Such a mechanism could regulate the activity of both early, central RPCs, as well as peripheral RPCs, and may contribute to inter-RPC variability. These differences between RPCs located in different parts of the developing retina have been of significant interest, and it is worth briefly examining patterning MEx that may also explain spatial differentiation between RPCs.

1.4.3 Patterning mechanisms

Among the most interesting features of RPCs is that they reliably give rise to specified neurons, in particular RGCs, that seem to “know where they are” in the retina, enabling them to wire their axons in correct retinotopic order in the superior colliculus (SC) or optic tectum (OT). The most robust MEx explaining this refer to gradients of EphA and EphB receptors expressed in RGCs, and their respective ephrin ligands expressed in the SC or OT. In the retinal RGC population, an increasing nasotemporal gradient of EphA is paired with an increasing dorsoventral gradient of EphB. A corresponding increasing rostrocaudal gradient of ephrin-A is paired with an increasing lateromedial gradient of ephrin B in the SC/OT. This allows for a two-axis encoding of an RGCs’ position in the retina [TK06]. As the RGCs’

axon pathfinding depends on repulsive effects mediated by Eph receptors, this code is sufficient to allow correct wiring of even single RGCs [GNB08]. The action of Gdf6a seems to establish this code in RPCs themselves, prior to specification [FEF⁺09].

Indeed, there are numerous similar observations of expression gradients that create spatial differences between RPCs themselves. Most relevant to the proliferation dynamics highlighted in this chapter is the observation that, in *Xenopus* eyes, a decreasing dorsoventral gradient of type III deiodinase renders the cells of the dorsal CMZ refractory to thyroid hormone (as the deiodinase inactivates TH) [MHR⁺99]. The effect of this is to set up a differential response to TH in post-metamorphic RPCs, so that the ventral population selectively expands in response to TH [BJ79].

These patterning mechanisms are of particular interest here, in large part because they clearly establish that the RPC population is heterogenous, both with respect to proliferative and specificative behaviours, and perhaps others as well. This is of critical importance for any modelling effort, as virtually all mathematical models used by stem cell biologists (and those used to justify Harris' SMME) assume, at least initially, homogenous populations of stem or progenitor cells. Since RPCs do not meet this condition, special care is needed to use these models.

1.4.4 Chromatin dynamics

In recent years, the great importance of chromatin conformation in RPC proliferation and specification has become more clear. Indeed, chromatin dynamics are now widely invoked in explaining stem and progenitor cell behaviour, and suggested as a target for cell reprogramming [Kon06, TR14]. In RPCs, detailed accounts of three-dimensional chromatin dynamics have yet to appear. However, a number of studies point to the importance of chromatin state in informing the overall cellular state. In particular, histone deacetylation seems to be important for RPC specification, as the loss of histone deacetylase 1 (HDAC1) in zebrafish results in overproliferation and decreased specification, and correlated increases in Wnt and Notch activity [Yam05]. In mouse retinal explants, decreased proliferation and specification result from pharmacological inhibition of HDAC [CC07]. Additionally, the chromatin remodelling complex SWI/SNF has repeatedly been implicated in RPC function. Notably, one particular component of this complex seems to be particularly associated with vertebrate RPCs (BAF60c, an accessory subunit) [LHKR08]. A switch to other subunits seems to be necessary for specification [LWR⁺07]. Details regarding the subunits involved in specification and their downstream effects are complex and context dependent, much like the signalling pathways mentioned above.

1.5 A unified theory of RPC function? “Blurring” to order

From the foregoing discussion, we can see Harris' theoretical conundrum. Macromolecular explanations for RPC behaviours, like those throughout the molecular biological tradition, have generally been built outwards from particular transcription factors, receptors, etc. The result is an archipelago of MEx, at best connected by tenuous speculation, and in most cases, without any known means to form an integrated model. Furthermore, the degree of complexity and context-dependence evident from the literature might seem to preclude such a model. As we have seen, Harris found that the evidence did not allow for clear discrimination between logically distinct types of mechanisms for producing the observed variability in RPC lineage outcomes.

In this situation, Harris effectively had two theoretical options. The first is simply to “crank the handle”- to generate more and more facts describing the difference particular molecules make to RPC outcomes in dozens of relevant contexts, piling up exceptions and idiosyncrasies, in the hope that doing so will eventually bridge the explanatory “islands” of the MEx archipelago. This has been referred as the enumerationist approach by Kaneko, who ably explains why it is doomed to failure [Kan06, pp.31-32]. The second option, the one actually chosen by Harris, is more theoretically sophisticated. As Nicholas Rescher has noted regarding in-principle limits to scientific knowledge, the phenomenal universe has infinite descriptive complexity- one can always add more detail to a description of some phenomenon, and no such description is ever complete [Res00, p.22-9]. Moreover, “even as the introduction of greater detail can dissolve order, so the neglect of detail can generate it.” [Res00, p.62] As Rescher goes on to comment:

[W]e realize that in making the shift to greater detail we may well lose information that was, in its own way, adequate enough ... information at the grosser level may well be lost when we shift to the more sophisticated level of greater fine-grained detail. The ‘advance’ achieved in the wake of ‘superior’ knowledge can be - and often is - purchased only at a substantial cognitive loss.

...

It is tempting on first thought to accept the idea that we secure more - and indeed more useful and more reliable - information by examining matters in greater precision and detail. And this is often so. But the reality is that this is not necessarily the case. It is entirely possible that the sort of information we need or want is available at our ‘natural’ level of operation but comes to be dissolved in the wake of greater sophistication. [Res00, p.65-6]

Rescher’s greater point is that “blurring” detail, at levels below the phenomenal one under consideration (for RPCs, generally, the cell or lineage), may be necessary to produce an ordered explanation that is useful for some objective. Given the number of overlapping and contradictory MEx for RPC behaviour, we have the situation Rescher is describing- more sophistication, and more detail, has dissolved order, not revealed it¹². The inability to assemble an unified explanation for RPC function has left us without fundamental understanding of how highly ordered neural tissues like eyes are generated from composites of units with highly variable, temporally and spatially ordered outcomes like RPC lineages. As this is a common feature of vertebrate neurogenesis more generally, this leaves us without the ability to produce complete models of neurodevelopmental processes in many species. Moreover, in the absence of a clear framework for comparing the explanatory power of the diverse array of MEx so far advanced, practical contributions of clinical relevance have been scanty and tentative, with RPC transplantation, and more recently, gene therapies taking little note of complex MEx for RPC function[CAI⁺04, GS07, YQW⁺18].

In a situation of this kind, this type of “blurring” is required, and it seems that is what Harris is attempting in advancing his SMME. Harris is asking, in some sense, whether most of the mechanisms offered as explanations for RPC behaviour are extraneous to an adequate understanding of how RPCs work. By cutting down to the simplest possible explanation, Harris hopes to bring into view order that was previously obscured by detail. There is, of course, a significant danger here: how does one decide what is “blurred out” and what remains? We can easily understand how a practitioner’s biases could

¹²At least part of this problem is likely related to the fact that the majority of biomedical findings cannot be replicated [Ioa05]. The finding, mentioned above, that Shh effect sizes on RPC function were not as large as initially reported when subsequently investigated, is typical and symptomatic of this replication problem. “Blurring” may therefore be necessary not only because of fundamental epistemic limits, but also because it is often difficult to distinguish bona fide results and explanations from spurious ones.

lead to a sort of relativism, where the “blurring” makes apparent a spurious order that conforms to these biases rather than to reality as such. With this in mind, let us survey the general thrust of Harris’ SMME, before proceeding to examine it in detail, in Chapter 2.

1.6 Explanatory Strategy and Intent of the SMME

As we have seen in Section 1.2, Harris’ long-held understanding of the explanatory options for RPC function divides them into four broad categories:

1. A linear algorithmic “program” of proliferation and specification
2. Asymmetric segregation of specificative determinants
3. “Stochastic processes” internal to the cells
4. Influences of extracellular factors

Harris’ sophisticated discussions of RPC MEx rarely treat these categories as exclusive, and concede that good explanations for RPC behaviour may involve phenomena from more than one of them. Indeed, the SMME necessarily contains elements that Harris concedes are “linear” and “deterministic” [HZA⁺12]. Still, his overall strategy for the SMME is, first, to substantiate the predominant influence of one of these categories of phenomena (that is, category 3, internal stochastic processes or effects), and subsequently to specify an actual macromolecular system that could plausibly be such an “internal stochastic process”. These two theoretical maneuvers, while tightly linked, serve different purposes within Harris’ overall explanatory framework, which must be examined separately.

The SMME for zebrafish RPC function has been developed across three separate papers [HZA⁺12, BRD⁺15, WAR⁺16]. Each builds on the earlier publications, collectively purporting to explain the behaviour of RPCs wherever, and whenever, they may be found in the zebrafish eye. The underlying model is originally derived from an earlier paper pertaining to rat RPCs [GZC⁺11]. He et al. [HZA⁺12] and Wan et al. [WAR⁺16] use essentially the same model and make up the substance of the first of these two maneuvers. Boije et al. [BRD⁺15] substantially modifies this model, specifying the activity of two known transcription factors (Ath5 and Ptf1a) as the model’s biological referents. This paper constitutes the second theoretical thrust.

The first maneuver intends to support the contention that zebrafish RPCs are equipotent progenitors with variable lineage outcomes that depend on independent “stochastic” processes within each of these cells. This is to be provided by demonstrating that a Simple Stochastic Model (SSM) of an RPC, numerically simulated many times by Monte Carlo methods to represent a population of RPCs, produces similar outcomes to populations of RPCs *in vivo*. This explanatory strategy is common in the stem cell literature, the original example having been published in 1964 by Till, McCulloch, and Siminovitch[TMS64]. The SMME therefore represents an example of a traditional scientific logic- an explanatory pattern deployed by stem cell biologists in diverse contexts, and widely accepted because of its ongoing use in the literature, although with varying interpretations.

The success of this first maneuver thus depends on two outcomes. Firstly, the output of the SSM should accurately reflect the observed proliferative and specificative outcomes of zebrafish RPC lineages, giving weight to Harris’ claim that it ”provides a complete quantitative description of the generation of

a CNS structure in a vertebrate *in vivo*” [HZA⁺12]¹³ Secondly, the internal structure of the SSM should provide good reason to believe that one of the stochastic options is a better explanation for RPC lineage outcomes than those identified by the other three categories of theoretical options enumerated above.

The second theoretical maneuver is the specification of particular biological referents for entities in the model. This offers an opportunity to move beyond a purely conceptual argument about the kind of process that might produce variable RPC lineage outcomes, and to begin the work of explaining how the behaviour of a particular macromolecular system constitutes such a process, so that empirically verifiable hypotheses may be generated. The success of this maneuver depends on the biological plausibility of the identification between model structure and the biological function of transcription factors, *Ath5* and *Ptf1a*, that the model names. A good SSM-based explanation would point the way for further research by identifying *how* so-called “stochastic processes” in RPCs might function, and make some predictions about this. With that said, let us turn to the SMME and determine how well these maneuvers have succeeded.

¹³This claim is somewhat extravagant; the SSM, by definition, includes no spatial information, so it is unclear how one could be a “complete description” of any spatially organised structure. Still, it can be complete with regard to cell population numbers.

Chapter 2

“Stochastic mitotic mode” models do not explain zebrafish retinal progenitor lineage outcomes

The text of this chapter has been adapted from a manuscript originally prepared for submission to PLOS One and is formatted accordingly; the methods and supplementary materials are available in ??

2.1 Introduction

Mechanistic explanations (MEx) derive their utility from the resemblance of the conceptual mechanism’s output to empirically observed outcomes. As maps to biological territories, biological MEx are habitually identified with the living systems they represent. Most well-developed MEx take the form of a model, whose internal structure is taken to reflect the underlying causal structure of a biological phenomenon. The nature of the causal relationship between a mechanistic model and the phenomenon it purports to explain remains a topic of active discussion in the philosophy of biology [Fag15]. Biologists, nonetheless, usually accept that a model which explains empirical observations well (usually measured by statistical or information theoretic methods), and reliably predicts the results of interventions, bears a meaningful structural resemblance to the actual causal process giving rise to the modelled phenomenon.

Biological phenomena are notable for exhibiting both complex order and unpredictable variability. A significant challenge for MEx in multicellular systems is to explain how complex, highly ordered tissues, like those produced by neural progenitors, can arise from unpredictably variable cellular outcomes. Stem cell biologists have often used to Simple Stochastic Models (SSMs) in order to explain the observed unpredictable variability in clonal outcomes of putative stem cells [Fag13]. Because SSMs are susceptible to Monte Carlo numerical analysis as Galton-Watson branching processes, they have been convenient explanatory devices, appearing in the literature for more than half a century [TMS64]. By specifying the probability distributions of symmetric proliferative (PP), symmetric postmitotic (DD), and asymmetric proliferative/postmitotic (PD) mitotic modes, SSMs allow cell lineage outcomes to be simulated.

SSMs are “stochastic” insofar as they incorporate parametric random variables. As Jaynes has noted, “[b]elief ... that the property of being ‘stochastic’ rather than ‘deterministic’ is a real physical property of

a process, that exists independently of human information, is [an] example of the mind projection fallacy: attributing one’s own ignorance to Nature instead.” [JBE03] Despite this, macromolecular processes are often described as “stochastic” in the stem cell literature. Generally speaking, the behaviour of an SSM’s random variable is taken to represent sequences of outcomes that are produced by multiple, causally independent events. Recently, the influence of “transcriptional noise” on progenitor specification has been identified as a candidate macromolecular process that may produce unpredictable variability in cellular fate specification. Therefore, one explanatory strategy for stem and progenitor cell function compares SSM model output to observed lineage outcomes, in order to argue that “noisy”, causally independent events give rise to the proliferative and specific outcomes of progenitor lineages.

In this report, we evaluate the best-developed of these explanations, proffered by William Harris’ retinal biology group. We have dubbed this the “Stochastic Mitotic Mode Explanation” (SMME) for zebrafish retinal progenitor cell (RPC) function. The SMME is noteworthy because it claims: (1) to “provid[e] a complete quantitative description of the generation of a CNS structure in a vertebrate *in vivo*” [HZA⁺12]; (2) to have established the functional equivalency of embryonic RPCs and their descendants in the postembryonic circumferential marginal zone (CMZ) [WAR⁺16]; and (3) to have established the involvement of causally independent transcription factor signals in the production of unpredictably variable RPC lineage outcomes [BRD⁺15]. Moreover, the SMME is taken to supply evidence for the predominance of stochastic effects over other explanations for RPC lineage outcomes, such as the classical explanation of a temporal succession of competency states [TR86]. These would be significant achievements with important consequences for both our fundamental understanding of CNS tissue morphogenesis and for retinal regenerative medicine. However, these models were not subjected to model optimization or selection procedures, as advocated by model selection theorists [BA02], and widely adopted in ecology and evolutionary biology [JO04].

In order to evaluate of the two SSMs which form the SMME’s MEx for RPC function, we have re-expressed the models as cellular agent simulations conducted using the open source C++-based CHASTE cell simulation framework [MAB⁺13]. We explored the structure of the SSMs, dubbed the He and Boije SSM respectively, compared to their explanatory forebear, dubbed the Gomes SSM [GZC⁺11]. This analysis suggested the progression of temporal “phases” in the models was largely responsible for the SMME model fits to data. In order to investigate this possibility, we built an alternative model with a deterministic mitotic mode and compared its output to the He SSM. We found that the deterministic alternative model was a better explanation for the observations, demonstrating that SMME fails when compared to alternatives. We therefore suggest that an explanatory approach based on the use of SSMs is incapable of distinguishing between theoretical alternatives for the causal structure of RPC lineage behaviours. Furthermore, by comparing the output of the models with novel postembryonic measurements of proliferative activity, we find that the SMME explanation cannot account for quantitative majority of retinal growth in the zebrafish, driven by the CMZ. Finally, we discuss the place of SSMs, the concept of “mitotic mode”, and the role of “noise” in explaining RPC behaviour, and suggest ways to avoid the modelling pitfalls exemplified by the SMME.

2.2 Results and Discussion

The SMME for zebrafish RPC function has been advanced using two SSMs. One first appears in He et al., and again, unmodified, in Wan et al. [HZA⁺12, WAR⁺16]; it explains lineage population statistics and

time-dependent rates of the three generically construed mitotic modes (that is, PP, PD, DD). We have called this the He SSM. The second appears in Boije et al. [BRD⁺15]; its intent is both to introduce the role of specified macromolecules into the mitotic mode process, and to explain neuronal fate specification in terms of the process. We have called this the Boije SSM. The He model is directly descended an SSM advanced to investigate causally independent fate specification in late embryonic rat RPCs, formulated in Gomes et al. [GZC⁺11]. The Boije SSM differs substantially from the He and Gomes models, but inherits its general structure from the He SSM.

The metascientific analysis of the development of biological explanations remains undertheorized. Perhaps most refined tool for global evaluation of biological theories (Schaffner's "Extended Theories"), treats biological explanations as hierarchically organised logical structures, after the fashion of Imre Lakatos, and proposes the use of Bayesian logic to distinguish between them [Sch93]. However, biologists rarely offer explanations in this form; we rather prefer mechanisms, expressed in diagrammatic form or as mathematical model-objects.

In this report, we accept Fagan's view that MEx for the behaviour of stem and progenitor cells consist of assemblages ("mechanisms") of explanatory components which are understood to be causally organised by virtue of their intermeshing properties [Fag15]. While Fagan treats SSMs separately from macromolecular MEx, and we find that the Gomes SSM was not deployed in this role, the He and Boije SSMs were used as explanations for the behaviour of RPCs. As Feyerabend famously observed, scientists operate as epistemological anarchists; the development of our explanatory logic is not bound by a set of rules, but rather arises organically from our scientific objectives, extrascientific context, and so on [Fey93].

We have therefore chosen to examine the structure of the Gomes, He, and Boije SSMs arranged in chronological order, to highlight how the explanatory logic of zebrafish SMME SSMs differs from their immediate ancestor, and from other uses of SSMs in stem cell biology. We have diagrammatically presented these SSMs as MEx, consisting of components describing the proliferative and fate specification behaviour of cellular agents. The proliferative and specificative components of the MEx are causally organised by their Faganian intermeshing property, mitotic mode. We have used abbreviations to denote important classes of model components and inputs, informed by the emphasis of Feyerabend on the persuasive role of metaphysical ingredients and auxiliary scientific material; these are as follows:

- MI - Model ingredient, making reference to some conceptual or metaphysical construct
- AS - Auxiliary scientific content
- RV - Random variable
- PM - Parameter measurement, model parameter set by measurements, independently of model output considerations
- PF - Parameter fit, model parameter set without reference to measurement, in order to produce model output agreement with observations

We draw the reader's attention to the expansion of the "mitotic mode" intermeshing property in later SSMs; this explanatory component comes to dominate the later models, which makes its interpretation critical to the success of the mechanistic explanation in meaningfully representing a real biological process.

Numerous explanations for variability in RPC lineage outcomes have been considered. Harris has argued that these belong to three cell-autonomous categories of process, in addition to extracellular influences: (1) a linear temporal progression of competency states, (2) asymmetric segregation of determinants during mitoses, and (3) intracellular “stochastic events” [HBEH88, AH09]. All of the SSMs are used to argue for the predominant influence of the third type of process in RPC lineage outcomes, essentially by demonstrating that the output of the SSM resembles observations.

2.2.1 Gomes SSM: Ancestral Model of the SMME SSMs

The Gomes SSM is presented in Fig 2.1. The model’s structure is straightforward; there are three independent random variables, drawing from empirically-derived probability distributions for the time each cell takes to divide, the mitotic mode of the division, and the specified neural fate of any postmitotic progeny. The random mitotic mode variable functions as the “intermeshing property” linking cycle behaviour to fate specification. In this scenario, the processes governing proliferation, leading to cell cycle exit, and governing cell fate commitment are causally independent of each other and of their history. The objective of the Gomes et al. study is to compare the lineage outcomes of dozens of individual E20 rat RPCs, in clonal-density dissociated culture, with the model output, developing earlier work in this system [CBR03]. Although the model incorporates conventional proper time (clock-time), none of the RVs reference it to determine their values. The abstract “cells” represented by this model do not have any timer or any source of information about their relative lineage position. Fate specification is construed in terms of conventional histochemical markers of stable cell fates; only the neural types generated late in the retinal histogenetic order are represented, as these are the only neurons specified by E20 rat RPCs.

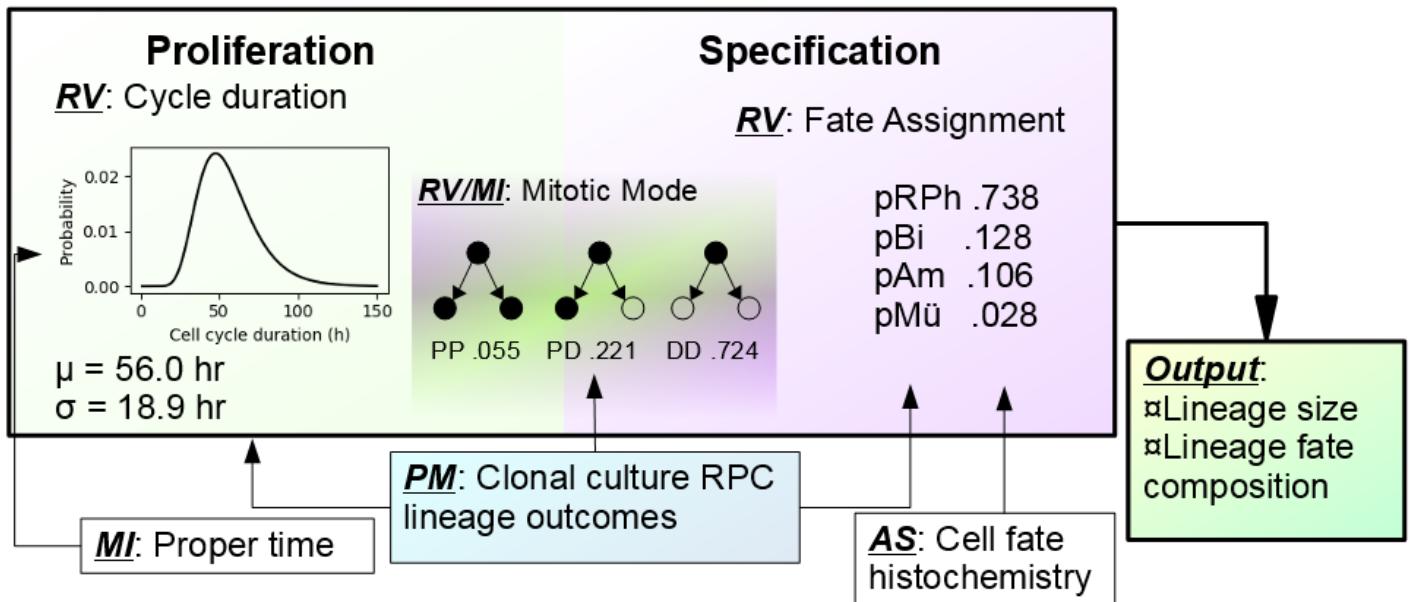


Figure 2.1: Structure of Gomes SSM

Structure of the Gomes SSM [GZC⁺11]. pRPh, probability of rod photoreceptor specification. pBi, probability of bipolar cell specification. pAm, probability of amacrine cell specification. pMü, probability of Müller glia specification.

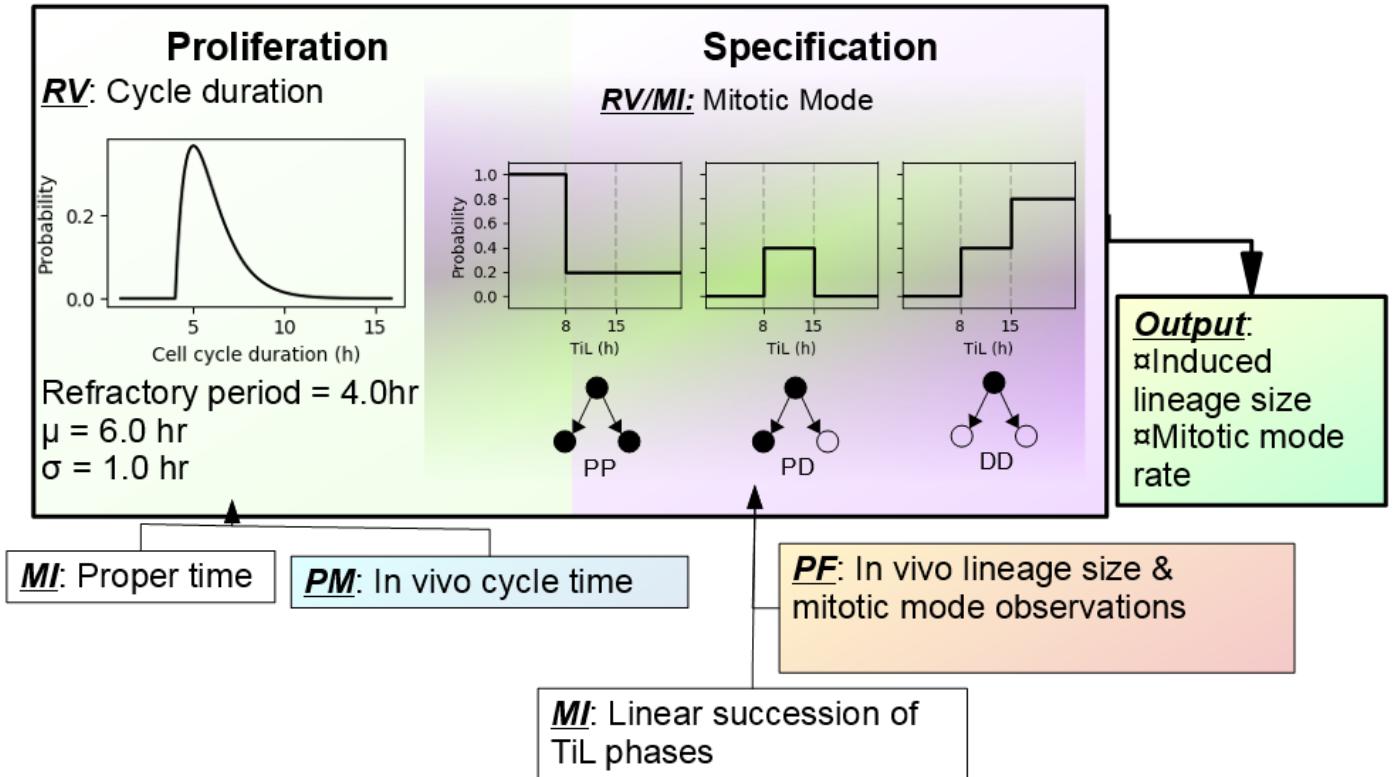
This SSM is explicitly built as a null hypothesis, or a model of background noise. It represents an extreme case in which all of the specificative and proliferative behaviours of an RPC are totally independent of all other RPCs and events in its clonal lineage. The stated purpose of this model is “to calibrate the data”, serving “as a benchmark” [GZC⁺11], a purely hypothetical apparatus to produce sequences of causally unrelated lineage outcomes. Substantial deviations of the observed data from the fully independent events of the SSM may then be interpreted as causal dependencies between RPC outcome and their relative lineage relationships, as might be observed in a developmental “program” or algorithmic process. Finding that, with some exceptions, observed proliferative and specificative outcomes generally fall within the plausible range of Gomes SSM output, Gomes et al. conclude that RPC lineage outcomes in late embryonic rat RPCs seem to be dominated by causally independent events, suggesting that causally isolated “noisy” macromolecular processes may give rise to the unpredictable variability in the fate outcomes of these cells.

2.2.2 He SSM: Explaining variability in zebrafish neural retina lineage size

The He SSM, shown in Fig 2.2, is deployed in a explanatory role rhetorically identical to the Gomes SSM. The extent to which model output “captures.. aspects of the data” is taken to obviate the need for explicit “causative hypothes[e]s”. On this account, only residual error between model output and observations may be ascribed to non-stochastic processes like “histogene[tic ordering] of cell types or a signature of early fate specification”. That is, if the model can be fit to observations, this is taken to exclude the presence of any cell-autonomous temporal program, asymmetric segregation of fate determinants, extracellular influences, and the like.

There are fewer direct empirical inputs to the He model’s parameters than the Gomes SSM, limited to the duration of the cell cycle. The close synchrony of zebrafish RPC divisions is modelled by assigning sister RPCs the same cycle length, shifted by a normal distribution with a variance of one hour, in contrast to Gomes RPCs, which are treated as fully independent. The lineage outcomes the He SSM is called on to explain differ significantly from those referred to by the Gomes SSM. Most notably, the Gomes SSM does not account for the early appearance of RGCs, which are not produced by the late E20 progenitors examined in that study. The zebrafish RPC lineages studied by He et al. produce all of the retinal neural types, including RGCs, which are typically produced by PD-type divisions. The He SSM does not model particular cell fates, supposing that mitotic mode is “decoupled” from fate specification. The mitotic mode RV linking cell cycle to fate specification in the Gomes SSM has thus subsumed the specification outcomes of RPC lineages entirely, and the model is concerned only to explain the sizes of lineages (marked by an inducible genetic marker at various times), and the observed progression of mitotic modes in these early RPCs.

The He model is, therefore, called on to explain the temporal structure of the proliferative behaviour of early zebrafish RPCs that dissociated late rat RPCs do not exhibit. A Gomes-type SSM, in which the RVs determining RPC behaviour are independent of any measure of time, cannot account for this temporal progression. In order to address this, He et al., assume a linear progression of three phases which cells in each lineage pass through, the timing of these phases being determined relative to the first division of the RPC lineage, called here “Time in Lineage” or TiL. The parameters of the mitotic mode RV are determined by these TiL phases. The temporal structure of the phases and their effect on the mitotic mode RV are selected to produce a model fit. While He et al. acknowledge that the model therefore represents a “combination of stochastic and programmatic decisions taken by a population of equipotent

Figure 2.2: **Structure of He SSM**

Structure of the He SSM [HZA⁺12]. TiL, Time in Lineage.

RPCs,” no test is performed to determine the relative contribution of the model’s stochastic vs. linear programmatic elements. Instead, the purportedly stochastic nature of mitotic mode determination is emphasized throughout the report.

2.2.3 Boije SSM: Explaining variability in zebrafish RPC fate outcomes

The second SMME model advanced to explain zebrafish RPC behaviour, the Boije SSM, is displayed in Fig 2.3. This model is primarily concerned with the lineage fate outcomes that the He SSM does not treat, while abandoning the explicit proper time of the He and Gomes SSMs in favour of abstract generation-counting. The mitotic mode model-ingredient now subsumes all RPC behaviours. Boije et al. make a laudable effort to specify the particular macromolecules ostensibly involved in determining mitotic mode, nominating the transcription factors (TFs) Atoh7, known to be involved in RGC specification, and Ptfla, known to be involved in the specification of amacrine and horizontal cells, as primary candidates. The contribution of vsx2 is taken to determine the balance between PP and DD divisions late in the lineage, with the latter resulting in the specification of bipolar or photoreceptor cells. The binary presence or absence of these signals is determined by independent RVs structured by the phase structure present in the He SSM, translated into generational time from proper time.

By this point, the SMME has become a very different type of explanation from its Gomes SSM

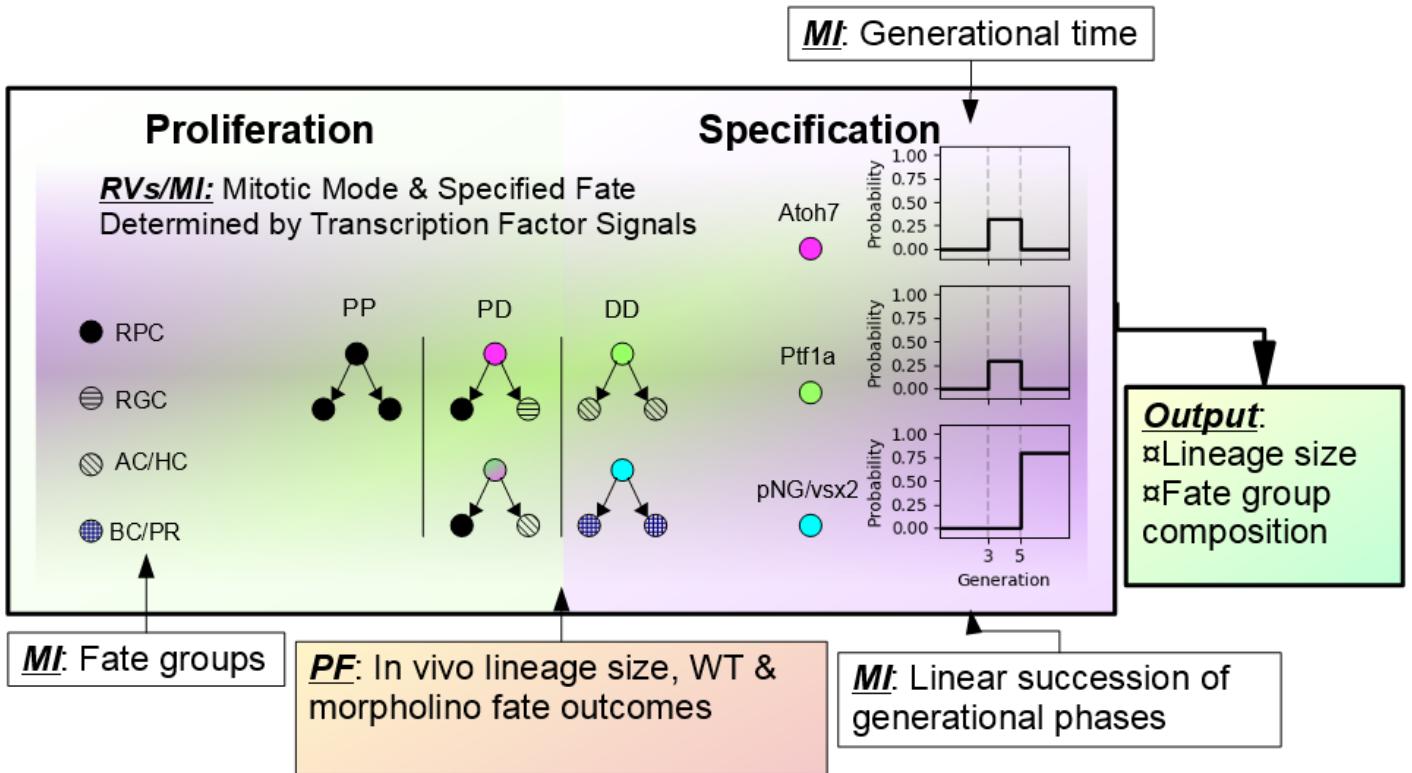


Figure 2.3: Structure of Boije SSM

Structure of the Boije SSM [BRD⁺15]. RPC, retinal progenitor cell. RGC, retinal ganglion cell. AC, amacrine cell. HC, horizontal cell. BC, bipolar cell. PR, photoreceptor. pNG- parameter representing contributions of Vsx1 and Vsx2 to specification of BC & PR fates in absence of Atoh7 or Ptf1a signals.

forebear. We are no longer dealing with RVs that model causally and temporally independent processes for different aspects of RPC behaviour. There is, rather, one temporally dependent process, the determination of mitotic mode, which is explained by unpredictable subsets of each lineage generation expressing particular TF signals. Where the Gomes SSM takes its parameters directly from empirical measurements of lineage outcomes, asking whether it is sufficient to assume that these are independently determined, the Boije SSM's parameters are derived solely from model fit considerations. In spite of these considerable differences, the explanatory role of the SSM is effectively the same: the model's fit to observations is taken as evidence of the predominant influence of stochastic processes determining mitotic mode on RPC behaviour. While Boije et al. acknowledge that whether some process is called "deterministic" or "stochastic" is "a matter of the level of description" [BRD⁺15] (i.e. is a property of the model-description and not of the physical process), the explanatory role of stochasticity for RPC lineage outcomes is emphasized throughout.

2.2.4 Model selection demonstrates the SMME is not the best available explanation for RPC lineage outcomes

From a modeller’s perspective, it is notable that neither of the reports which use the He SSM [HZA⁺12, WAR⁺16], nor that using the Boije SSM [BRD⁺15] report in any detail their fitting procedures, nor do any of the above report any statistical measures of goodness-of-fit. Additionally, no models representing the alternative “theoretical options” available to explain variability in RPC lineage outcomes are compared to those advanced as evidence for stochastic processes. Given that the He SSM and Boije SSM depart from the Gomes SSM by the addition of an unexplained temporal structure to the mitotic mode model ingredient, it is striking that the overall argument remains similar to Gomes et al.’s, despite the persuasive force of the latter deriving from the lack of such structures. While He et al. and Boije et al. acknowledge that their models involve both stochastic and linear programmatic elements, their relative influence on model output is not measured, and macromolecular explanation is only applied to the stochastic elements. Moreover, the emphasis on this mitotic mode model construct increases with each successive model, to the extent that in the Boije model there are no other elements that are used to explain RPC behaviours. All cellular behaviours are, in effect, progressively collapsed into the stochastic mitotic mode concept. Finally, the He and Boije SSMs contain more parameters, which are determined by fewer empirical measurements than the Gomes SSM. It is therefore important to test whether the most important ingredient in these models is the stochastic mitotic mode, against the possibility the unexplained temporal structure is the truly explanatory element.

Fortunately, we may employ model optimisation and selection techniques to adjudicate this. The most straightforward way to do so is to reexamine the He SSM alongside a competing alternative model. The data to which the He SSM has been applied is amenable to a scheme in which the models are fit to a “training” dataset, followed by a “test” dataset. In this case, the training data are the induced lineage size and mitotic mode rate data from He et al. The test dataset comprises the Atoh7 morpholino observations from He et al., and the CMZ lineage size data from Wan et al. This allows us to test how well the He SSM, and an alternative, hold up under novel experimental conditions, without relying on a trivial ordering of goodness-of-fit to training data. Since the Boije SSM draws straightforwardly on the He SSM for its temporal phase-parameterisation and stochastic mitotic mode model ingredient, this analysis of the He SSM also bears directly on the validity of the Boije SSM.

As an alternative to the SMME He SSM, we constructed a model that has a deterministic mitotic mode with variable phase lengths. Rather than variability arising from a stochastic-process mitotic mode changing across phases of fixed length, we simply supposed that mitotic mode is deterministic in each phase (guaranteed PP mitoses in the first phase, PD in the second, and DD in the third), but the phase lengths are variable between lineages and shift slightly between sister cells. That is, we represented this linear progression of deterministic mitotic mode phases using the same type of statistical construct the He SSM applies to model cell cycle length, to avoid introducing any novel or contentious elements into the model comparison. More specifically, each lineage has a first PP phase length drawn from a shifted gamma distribution, followed by a second phase length drawn from a standard gamma distribution. Upon mitosis, these phase lengths are shifted in sister cells by a normally distributed time period, exactly like cell cycle lengths in the He SSM.

We take this to be a reasonable representation of the classic suggestion that RPCs step through linear succession of competency phases, given the conceptual tools that the He SSM uses to model mitotic phenomena. If we suppose that this temporal program is governed by RPC lineages passing through a

stereotypical series of chromatin configurations which allow for PP, then PD, then DD mitoses in turn, along with the associated competence to produce the particular cell fates associated with PD and DD mitoses, it seems entirely plausible to suggest that lineages differ in the lengths of time they occupy each state. This is particularly true if we concede that an SSM, foregoing any spatial modelling whatsoever, must necessarily abstract extracellular and spatial influences on these processes. Moreover, since these chromatin configurations must be broken down and rebuilt with each mitosis, the re-use of the “sister shift” model ingredient from the He SSM’s cell cycle RV is congenial, representing the same sort of cell-to-cell variability that results in the small differences between sister cells in cycle timing.

While the code used to implement the SSMs mentioned above has not been published, the relevant reports provide enough detail to reconstruct these models in full, which we did using the CHASTE cell-based simulation framework, in order to provide transparent and reproducible implementations of the SSMs. Because the values selected for the He SSMs’ parameters seem to have been selected as a series of rough estimates, with only the value for the probability of PD-type mitoses in the second model phase being varied to produce the fit, we suspected that the fit would not be at or near the local minimum for a loss function. That is, a model fit produced in this manner is likely to be located in a region of the parameter space that is highly sensitive to small perturbations, and therefore may depend strongly on implementation-specific idiosyncrasies. He et al. report that they experienced difficulty in obtaining a good fit to their 32 hour induction data, with changes in the phase two PD probability producing large differences in fit quality. Unsurprisingly, when we rebuilt the He SSM with the original fit parameterisation, we substantially reproduced the original fit, except for the 32 hour data, where the model output diverges substantially from that reported in He et al. (see [S1 Fig](#)). Since this is clearly not the best fit available for the He SSM, and we wish to directly compare the best fits (i.e. the parameterisations at minima of some loss function) for the He SSM and our putative alternative model, we used the simultaneous perturbation stochastic approximation (SPSA) algorithm [[Spa98](#)] to optimise both models against test data. SPSA is particularly convenient for complex, multi-phase models like the He SSM, because no knowledge of the relationship between the model’s parameters and the loss function is required. We used Akiake’s information criterion (AIC) as the loss function to be minimised, in order to provide a rigorous comparison between the two differently-parameterised models (the deterministic alternative has two fewer parameters than the He SSM).

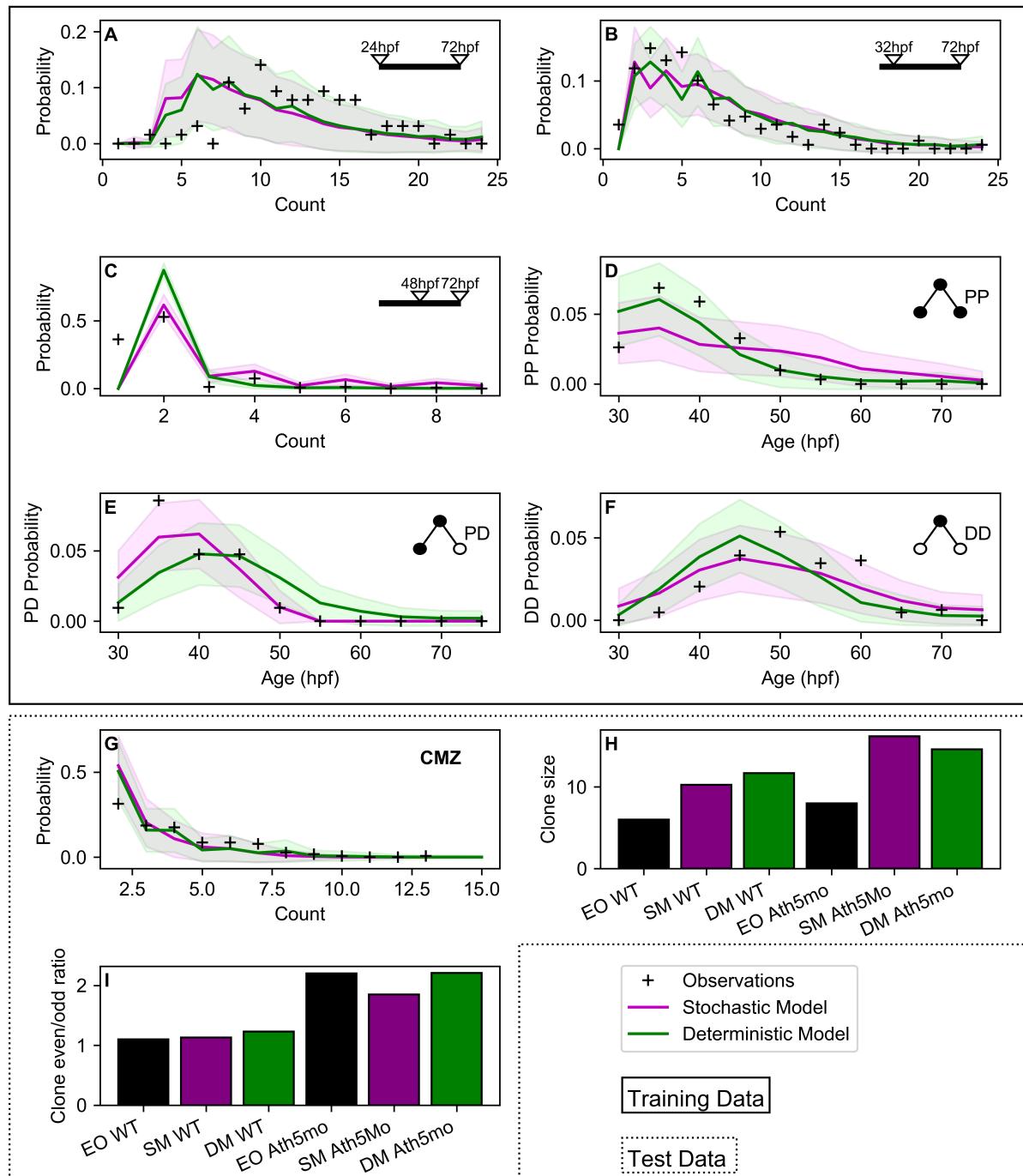


Figure 2.4: Model comparison: the SPSA-optimised He SSM and a deterministic alternative

Empirical observations (black crosses and bars) and SPSA-optimised model output (magenta, stochastic mitotic mode model; green, deterministic mitotic mode model). Model output in panels A-G is displayed as mean \pm 95% CI. Panels A-F: Training dataset, to which models were fit. Panels A-C: Probability of observing lineages of a particular size ("count") after inducing single RPC lineage founders at (A) 24, (B) 32, and (C) 48 hours with an indelible genetic marker, tallying their size at 72hpf. Panels D-F: Probability density of mitotic events of modes (D) PP, (E) PD, and (F) DD over the period of retinal development observed by He et al. Panel G: Probability of observing lineages of a particular size ("count") originating from the CMZ; RPCs are taken to be resident in the CMZ for 17 hours before being forced to differentiate, lineage founders are assumed to be evenly distributed in age across this 17 hour time period. Panel H: Average clonal lineage size of wild type and Ath5 morpholino-treated RPCs. Ath5mo treatment is taken to convert 80% of PD divisions, which occur in the second phase of the He model, to PP divisions, resulting in larger lineages. Panel I: Ratio of even to odd sized clones in wild type and Ath5 morpholino-treated RPCs. Methods in Section 11.1.7. Code in Section 16.2.14.

After fitting to the training dataset, we calculated AIC for the He SSM (hereafter SM, for stochastic model) and our deterministic mitotic mode alternative (hereafter DM), for both training and test datasets. The combined results are displayed in Fig 2.4, with the output of the two models being presented separately in S2 Fig and S3 Fig. Remarkably, the DM closely recapitulates the output of the SM for both datasets. Moreover, while the more highly-parameterised SM permits a better fit to the training data, the DM proves to be a better fit to the test dataset, as summarised in Table 2.1. This can be understood as a case of model overfitting: a higher-parameter model fits some training dataset better than a simpler model, but fails upon challenge with a new dataset. Given this model selection scheme, it is plain that we should choose the DM over the SM as a superior explanatory model, both on the basis of explanatory power and of Occam’s Razor.

Table 2.1: **AIC values for models assessed against training and test datasets**

Model	Training AIC	Test AIC
Stochastic (He fit)	-93.26	255.64
Stochastic (SPSA fit)	-93.80	92.24
Deterministic (SPSA fit)	-69.33	86.60

AIC: Akaike’s information criterion. Lower values reflect better model explanations of the noted dataset. Lowest values are noted in bold. Methods in Section 11.1.7. Code in Section 16.2.14.

We therefore conclude that the SMME is not the best available explanation for variability in zebrafish RPC lineage outcomes. It is clear from this analysis that the assumed, but unexplained, linear succession of mitotic mode phases is what provides the overall structure of the model output. Variability in lineage outcomes may be supplied by entirely different model ingredients without any loss of explanatory power on new datasets (indeed, with some improvement, in our case). To conclude that these models support a stochastic process governing mitotic mode, ruling out other types of explanation, is unjustified. It is likely that any number of different types of SSMs, representing other sorts of processes (such as asymmetric segregation of fate determinants, or differential spatial exposure to extracellular signals), can produce identical model output. Given this, we conclude that SSM models of this type are inadequate for the task of locating the source of variability in RPC lineage outcomes.

2.2.5 SMME SSMs cannot explain the post-embryonic phase of CMZ-driven zebrafish retinal formation

The zebrafish retina, like other fish retinas, and unlike the mammalian retina, continues to grow long after the early developmental period, indeed, well past the organism’s sexual maturity. This may be unsurprising, given that zebrafish increase in length almost ten-fold over the first year of life [PEM⁺09], necessitating a continuously growing retina during this period. In fact, the quantitative majority of zebrafish retinal growth occurs post-metamorphosis, outside of the early developmental period. This growth occurs due to the persistence of a population of proliferative RPCs present in an annulus at the periphery of the retina, called the ciliary or circumferential marginal zone (CMZ), which plates out the retina in annular cohorts. A typical “tree-ring” analysis from our studies, marking the DNA of cohorts of cells contributed to the retina at particular times with indelible thymidine analogues, is shown in Fig 2.5. These experiments show the structure of the adult zebrafish retina is quantitatively dominated by contributions from the CMZ in the period between one and three months of age. Why peripheral

RPCs in zebrafish remain proliferative, while those in mammals are quiescent [Tro00], and whether and how their behaviour might differ from embryonic RPCs, remain unresolved. Answers to these questions may have significant fundamental and therapeutic implications, especially given e.g. the possibility of harnessing endogenous, quiescent, peripheral RPCs in humans for regenerative retinal medicine.

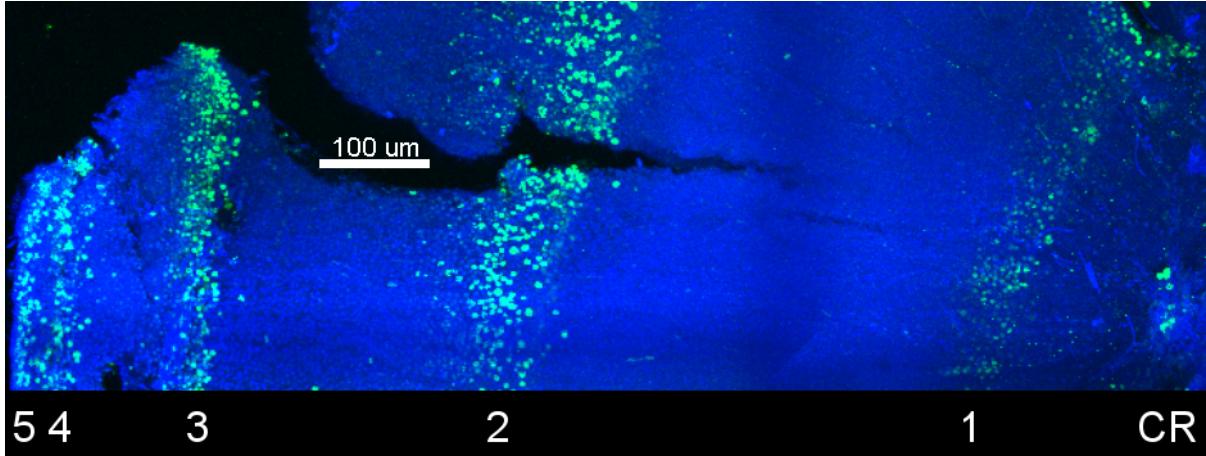


Figure 2.5: Most zebrafish retinal neurons are contributed by the CMZ between one and three months of age

Maximum intensity projection derived from confocal micrographs of a zebrafish whole retina dissected at 5 months post fertilisation (mpf). The animal was treated with BrdU at 1, 2, 3, 4, and 5 mpf for 24hr. Anti-BrdU staining of the whole retina reveals the extent to which the CMZ (which is responsible for retinal growth after approximately 72hpf) contributes new neurons in tree-ring fashion, extending out from the center of the retina (CR), which is formed before 72hpf. Monthly cohorts are labelled appropriately. Scale bar, 100 μ m. Methods in Section 11.1.2.3.

The He SSM was deployed in Wan et al. [WAR⁺16], with the claim that the He SSM explains the behaviour of CMZ RPCs. Wan et al. argue that a slowly mitosing population of bona fide stem cells, at the utmost retinal periphery, divides asymmetrically to populate the CMZ with He-SSM-governed RPCs. In other words, the usual suggestion that CMZ RPCs undergo a somewhat different process than embryonic RPCs, perhaps recapitulating across the peripheral-central axis some progression of states or lineage phases that embryonic RPCs pass through in time [HP98], is repudiated in favour of one model which describes the behaviour of all RPCs throughout the life of the organism, with the addition of a small population of stem cells to keep the CMZ stocked with RPCs. If so, this might suggest that the problem of activating quiescent stem cells in the retina is simply that- one need only sort out how to throw the proliferative switch in these cells, since the proliferative and fate specification behaviours of the resultant RPCs will reliably be the same as those observed in development.

While we determined that the SMME is not the best available explanation for RPC lineage outcomes, we still felt that the SSMs associated with this explanation might be used to elucidate this point. In particular, if it is the case that the He SSM provides good estimates of lineage size and proliferative dynamics in the early zebrafish retina, it should be possible, using this model, and the estimates of putative stem cell proliferative behaviour provided by Wan et al., to simulate the population dynamics of the CMZ, at least through the first few weeks of the organism's life.

In pursuing this point, we noted a peculiar feature of the He SSM not documented by any of the

SMME reports: the cell cycle model overstates the *per-lineage* rate of mitoses by as much as a factor of 3. That is, the mitotic mode rate data presented in He et al., recapitulated here in Fig 2.4, panels D, E, and F, and used to optimise both the SM and DM, are probability density functions that are not standardised on a per-lineage basis. These data simply indicate the distribution of mitotic events of a particular type. When we take all of the mitotic events documented by He et al. and calculate the probability of any such event occurring per lineage, per hour, we obtain the values presented in Fig 2.6.

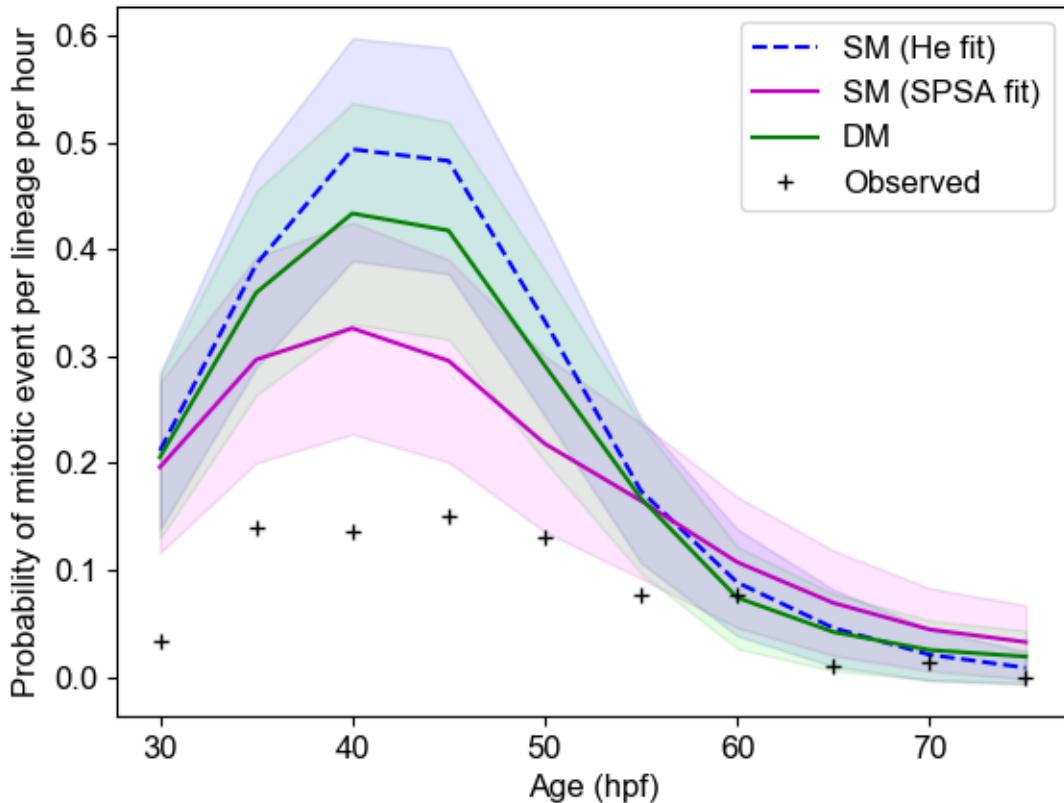


Figure 2.6: **Per-lineage probabilities of mitoses, He et al. observations compared to model output**

Probability of observing a mitotic event over the period studied by He et al., per hour, per lineage. Model output is presented as mean \pm 95% CI. Methods in Section 11.1.7. Code in Section 16.2.16.

Similarly, when we performed cumulative thymidine analogue labelling of the 3dpf CMZ, (using the assumptions of Nowakowski et al. [NLM89], which treat the proliferating population as a homogenous, and dividing asymmetrically; these assumptions are flawed but adequate for a rough estimate) we obtain an average cell cycle length of approximately 15 hours, more than twice as long as the He SSM's mean cycle length. These data are displayed in 11.2. Therefore, the He SSM (in both its original and refit parameterisation, as well as the deterministic alternative, since they all rely on the same proliferative model elements) substantially overstates the proliferative potential of both embryonic and early CMZ RPCs. Since we observed a massive build-up of proliferating RPCs between two and four weeks post-

fertilisation, we thought this might actually suit this later context better.

To estimate of total annular CMZ population in zebrafish retinas over time, we counted proliferating RPCs present in central coronal sections of zebrafish retinas throughout the first year of life, treating these as samples of the annulus, and calculated the total number of cells that would be present given the diameter of the spherical lens measured at these times. Our simulated CMZ populations were constructed at 3dpf by drawing an initial population of RPCs governed by the He SSM (using the original fit parameters, which further exaggerate the proliferative potential of these lineages) from the observed distribution. We added immortal stem cells amounting to one tenth of this total. This is likely an overestimate, given that these putative stem cells are thought to be those in the very peripheral ring of cells around the lens, of which typically two to four may be observed in our central sections with an average of over one hundred proliferating RPCs. Moreover, these simulated stem cells were given a mean cycle time of 30 hours, proliferating about twice as quickly as Wan et al. suggest. Finally, to reflect the fact that these stem cells contribute to the retina in linear cohorts [CAH⁺14], and more of them are therefore required as the retina grows, the stem cells were permitted to divide symmetrically when necessary to maintain the same density of stem cells around the annulus of the lens. Two hundred such CMZ populations were simulated across one year of retinal growth.

The results of these simulations are displayed in Fig 2.7, overlaid over CMZ population estimates derived from observations. Given the generous parameters of the population model, consistently overestimating the proliferative potential of embryonic and early CMZ RPCs, it is surprising that the He SSM proves completely unable to keep up with the growth of the CMZ in the first two months of life. Indeed, the unrealistically active stem cell population the simulated CMZs are provided with is unable to prevent a near-term collapse in RPC numbers, only catching up after the months later as the number of stem cells increases with the growth of the lens. Thus, even given permissive model parameters, the He SSM is not able to recapitulate the quantitatively most important period of retinal growth in the zebrafish.

This analysis suggests that observations of RPCs in embryogenesis and early larval development are unlikely to provide a good quantitative model of the development of the zebrafish retina, even abstracting away spatial and extracellular factors as SSMs necessarily do. Our data point to a second, quantitatively more important phase of retinal development, between approximately one and four months of age, in which RPCs are far more proliferatively active than in early development. It is likely that models that closely associate proliferative behaviour with fate specification, like those of the SMME, will be unable to explain this period. Recent evidence suggests that mitotic and fate specification behaviours in RPCs may be substantially uncoupled [ESY⁺17]. This would permit the CMZ population to scale appropriately with the growing retina. Alternatively, it is possible that RPCs have heterogenous proliferative behaviour, and that this heterogeneity is mainly apparent later in development.

2.3 Conclusion

Simple stochastic models are familiar tools for stem cell biologists. Introduced by Till, McCulloch, and Siminovitch in 1964 [TMS64], they proved their utility in describing variability in clonal lineage outcomes of putative stem cells, originating from macromolecular processes beyond the scope of cellular models (and beyond the reach of the molecular techniques of the time). More prosaically, their simplicity afforded computational tractability in an era when processing time was relatively scarce, allowing early access

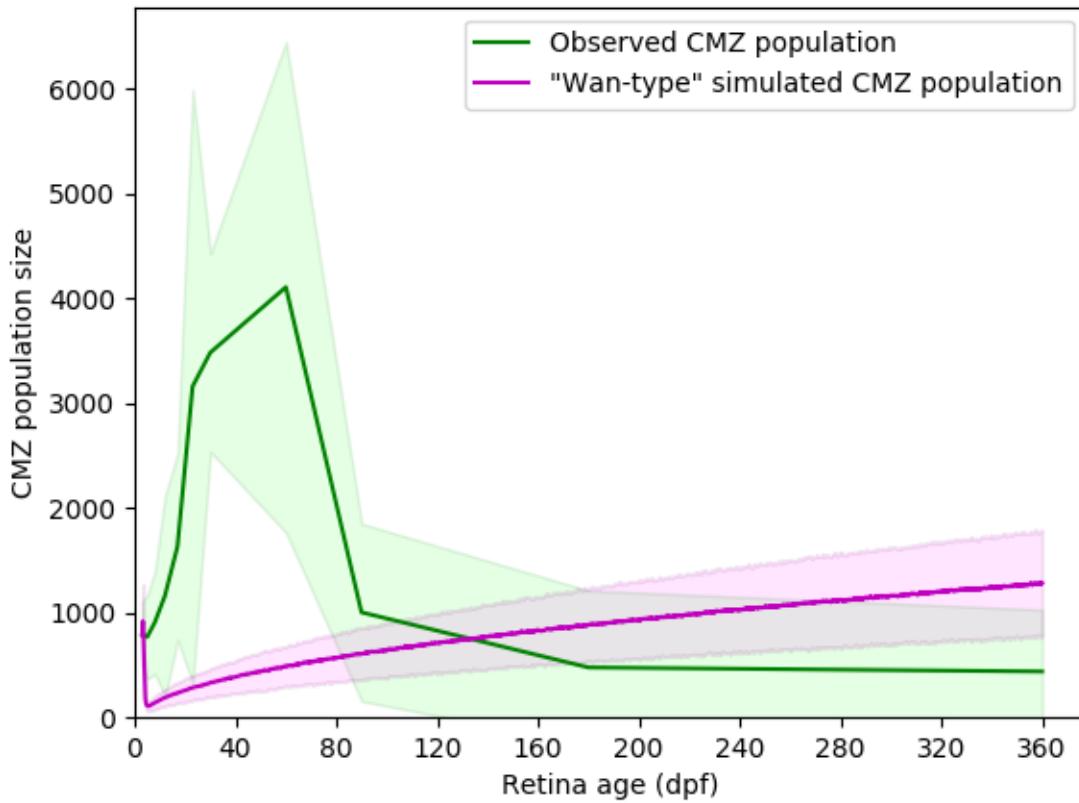


Figure 2.7: CMZ population of proliferating RPCs: estimates from observations and simulated Wan-type CMZs

Annular CMZ population was estimated from empirical observations of central coronal cryosections stained with anti-PCNA, a marker of proliferating cells, standardising by the diameter of the spherical lens observed in these animals. "Wan-type" CMZs were initialised with a number of RPCs drawn from the observed 3dpf population distribution, and given an additional 1/10th of this number of immortal, asymmetrically dividing stem cells, as described in the text, and simulated for the first year of life. All data is presented as mean \pm 95% CI. Methods in [Section 11.1.2.1](#), [Section 11.1.7](#). Code in [Section 16.2.17](#).

to Monte Carlo simulation techniques. That said, their abstract nature emphasizes an aspatial, lineage-centric view of tissue development, which cannot account for the generation of structurally complex tissues like retinas beyond cell numbers, and, perhaps, fate composition. As a result of this relatively loose relationship to the complex morphogenetic environment, there are few constraints on the model configurations that may produce similar outputs. This can result in modellers being led astray by their apparently good fits to observations, which is why the fit of one highly parameterised model cannot be taken, alone, as evidence for some theory; it is very rare that there is not a model representing an alternative theory that cannot be made to produce a reasonable model fit. Indeed, as we demonstrate here, the use of standard model selection techniques may make plain that a completely contradictory theory is a better explanation for the data.

To some extent, this can be ameliorated by careful attention to the particular macromolecular or cellular referents that particular model constructs represent. The “mitotic mode” construct present in all SSMs is a particularly ambiguous and problematic one in this regard. “Mitotic mode” is not, itself, a property of a mitotic event, but is rather a retrospective classification of the event after an experimenter observes whether progeny resulting from the event continue to proliferate. Its original appearance in SSMs was simply to allow calculation of clonal population sizes; it was never intended to represent a particular type of process or “decision” made by cells at the time of mitosis to continue proliferating or not. Any number of pre- or post-mitotic signals and processes may result in a particular mitosis being classified as PP, PD, or DD, without anything about the mitotic event itself determining this. The use of such a retrospective classification, rather than the identification of some physical property of the mitotic event (such as the asymmetric inheritance of fate determinants), is straightforwardly a concession that the actual macromolecular determinants of cellular fate are outside the scope of the model.

The SMME represents an attempt to connect this abstract, retrospective model construct to observations of noisy gene transcription [Rv08]. Motivated by the observation that momentary mRNA transcript expression in RPCs is highly variable from cell-to-cell [TSC08], the suggestion is that this transcriptional noise may be responsible for the observed variability in RPC lineage outcomes. Since this noise may be “tuned” to a degree by e.g. promoter sequences [RO04], it could plausibly be under selective pressure. There are two significant problems with identifying the SMME’s stochastic mitotic mode with this type of process. The first we have demonstrated by constructing an alternative model with deterministic mitotic mode but variable phase lengths: the source of variability may be located elsewhere without compromising the explanatory power of the model. It is therefore impossible to determine what sort of process might give rise to variability in RPC lineage outcomes by using SSMs in this fashion. The second, more fundamental problem is that a causal explanation of the presence of the signal, for which noise is a property, is elided entirely in favour of emphasis on “stochasticity”. This is most apparent in the Boije SSM. In this model, Atoh7 and Ptfla TFs are available to provide their noisy signal in the 4th and 5th lineage generations, and at no other time. We do not dispute that a noisy signal may contribute to variability in that signals’ effects; rather, we suggest that it is the temporal structure of such a signal (assuming this structure can be empirically demonstrated, rather than assumed) that calls for causal explanation. The SMME reports explicitly disclaim the necessity for “causative hypotheses” in the case that a stochastic model provides a good fit to observations. As Jaynes remarked in his classic text on probability theory, “[stochasticity] is always presented in verbiage that implies one is describing an objectively true property of a real physical process. To one who believes such a thing literally, there could be no motivation to investigate the causes more deeply ... and so the real processes at work might never be discovered.” [JBE03]

In identifying the model construct with the physical processes determining RPC outcomes, the SMME obscures what seems to us to be the primary lesson to be drawn from the Harris groups’ beautiful *in vivo* studies of zebrafish RPCs. That is, compared to late rat RPCs in dissociated clonal culture, RPCs in intact zebrafish retinas produce far more orderly outcomes. To the extent that these outcomes are variable, the source of variability remains unidentified. We suggest that resort to “stochasticity” as an explanatory element should not be made in the absence of model comparisons that rule out alternatives with well-defined causal structures, lest we fall into the trap Jaynes warned us about.

Chapter 3

Toward a computational CMZ model comparison framework

3.1 SMME Postmortem: a wrong turn at Gomes

To return to the question posed in [Section 1.6](#): has the SMME succeeded in finding order by blurring out the chaotic welter of mechanistic explanations for RPC function in retinogenesis? [Chapter 2](#) argues the structure of the SMME models support explanations based on the original idea of a linear, deterministic series of stages through which RPCs progress, originally put forward by Cepko et al. [[CAY⁺96](#)], rather than the stochastic processes which are their rhetorical emphasis. The important feature of the SMME models is where they depart from the original Gomes SSM: the assumption of the linear structure of temporal phases. The succession of stages is the model ingredient that produces the structure resembling the data, not the various random variables associated with mitotic mode, which we have proven by demonstrating that a deterministic progression of mitotic mode models the observations better than its stochastic counterpart. By introducing variability into the lengths of the linear temporal program already assumed by the SMME models, we can remove variability from the mitotic mode and produce a superior model with fewer parameters. Only by the process of counterinduction [[Fey93](#)], the comparison of models with different propositional structures about reality, does this become clear. The introduction of the linear phase structure is required to model the early contribution of RGCs in zebrafish. If this is left without some biological rationale, retinogenesis remains unexplained. The SMME thus does not achieve its aim of being a complete description of lineage outcomes in retinogenesis, and this is confirmed by our observation that the Wan model [[WAR⁺16](#)] has little explanatory power outside the first few days of life, which makes up a small portion of the total retinal contribution to the zebrafish retina.

We must, therefore, assess that Harris' first theoretical maneuver, explaining the data with a model whose structure supports a stochastic resolution of mitotic mode "decisions", fails in the face of an alternative which locates stochasticity elsewhere. The underlying data used to inform these models speak better to an entirely different selection from the array of theoretical options outlined in [Section 1.2](#), the linear progression of competencies. Because the SSM model form does not usually have spatial dimensions, we did not test models involving variability in extracellular signals, but it is a good bet that such a model could be made to fit about as well as either of the ones tested in the previous chapter. In effect, if we are to follow Harris' inferential logic, variability in RPC outcomes can be explained by

“stochastic variability” in any model parameter which affects fate outcomes. Because of the seriousness of this problem for biological inferences, an explanation of the Bayesian epistemological view of probability has been provided in ???. Moreover, a thorough argument that “stochasticity” cannot be a property of real existents is provided in Section 15.1. It suffices here to conclude that the SMME models do not achieve the aim of supporting “stochastic processes” as explanations over the alternatives.

The second theoretical maneuver was to nominate a particular macromolecular system as the physical locus of the stochastic process, in this case represented by *Atoh7* and *Ptfla* as labels for abstract Bernoulli distributed processes. It remains obscure in what sense the model variables relate to their namesake transcription factors (transcription of the TF itself? activation of other genes?), and no measurements of these factors inform parameter selection. Plainly, these factors are involved in relevant RPC behaviours, but it is unclear why they have been nominated as causally upstream of the “mitotic mode” selection. Since the Boije model inherits the assumption of a linear progression of stages, it is very likely that a model with similar explanatory power could be built using the same strategy outlined above, locating random variability outside mitotic mode, although this is outside the scope of the present work.

On the basis of the above, we can conclude that the sole formally testable model of RPC function in the zebrafish retina is not adequate for our purposes. But how did we get here? As noted in Section 1.3.2, the notion that the most significant behaviours associated with RPCs are produced by mechanisms intrinsic to the cells derives much of its empirical support from the Raff group’s work. This story began with the observation that co-culturing E15 rat RPCs in dissociated pellet cultures with P1 cells did not accelerate the appearance of the first rods derived from the E15 progenitors (which occurred at a similar time as *in vivo*), suggesting a partially intrinsic commitment “schedule” for these cells¹ [WR90]. The scope of these observations were dramatically expanded by Raff’s subsequent work, intended to address the relative significance of intrinsic versus extrinsic processes in RPC function by comparing clonal RPC lineages in fully dissociated clonal-density cell culture to those in intact explants [CBR03]. The remarkable finding of this study was that dissociated E16-17 rat RPC lineages produce very similar numbers and types of retinal neurons as their tissue-embedded counterparts, albeit without morphological or molecular markers of mature neurons. This observation provided strong evidence for the predominant importance of RPC-intrinsic processes in determining both mitotic and fate outcomes for late RPC lineages. The complex, spatially organised context of intact explanted tissue seemed to only be required for the maturation of neurons, and was not required to regulate proliferation or the initial commitment to an appropriate distribution of lineage outcomes. As correct cell numbers and types are the two most obvious and critical parameters that must be achieved for RPCs to produce a functional retina of the appropriate size, the suggestion that both are largely determined by intrinsic processes seemed a striking confirmation of Williams and Goldwitz’s much earlier suggestion [WG92], against the prevailing view of the day, that lineage had a greater role to play than cellular microenvironment in RPC contributions. Interestingly, Raff’s interpretation of their 2003 data was that RPCs were most likely stepping through a linear, programmed developmental sequence rather than undergoing shifts in the probabilities of variable outcomes over time. It is notable that this interpretation arises not from the data collected in their study, but from considerations of a single unusual clone reported by [TSC90], and by analogy with drosophila neuroblasts. In retrospect, these arguments for linear sequences of deterministic RPC outcomes do not seem particularly strong, and it is perhaps unsurprising that these

¹Co-culturing with P1 cells, did, however, significantly increase the proportion of E15 RPCs specified as rods, resulting in Raff’s suggestion here that both intrinsic and extrinsic factors are important.

studies are remembered mainly for highlighting the importance of RPC-intrinsic processes.

The Gomes study, with its detailed study of particular rat late-embryonic RPC lineages, thus seemed to solidify the notion that unpredictable RPC-intrinsic processes dominate lineage outcomes [GZC⁺11]. But this study, like its forebears originating in Raff's work, is concerned with a population of RPCs that is too old to produce RGCs. This is the essential point: the SMME does not try to explain the early appearance of RGCs in terms of some macromolecular mechanism, although this is the single most significant difference between the zebrafish RPC lineages studied by Harris and the rat lineages studied by Raff and Cayouette [CBR03, GZC⁺11]. It is only by assuming the unexplained linear temporal structure of RPC specification that the SMME models achieve their apparently good explanatory quality.

3.2 Implications of the SMME study for modelling CMZ RPCs

Having taken seriously the possibility that an adequate model of zebrafish retinogenesis already exists, and concluded in the negative, we may ask how to improve on this state of affairs. Firstly, lessons in statistical acumen must be learned. Careful model optimization is needed, and to have any confidence about our interpretation of the model, we must test alternatives that make fundamentally different assumptions about the causal structure of the phenomenon being explained by the models. Can we conclude that the approach used in Chapter 2 is adequate to our task? There are two broad elements to consider here: the appropriateness of the overall modelling approach represented by the SSM in relation to the hypotheses we wish to test, as well as the soundness of the statistical procedures.

Taking up the SSM, by far its most attractive features are its computational efficiency and the ease of producing a custom model to fit some particular case- the SMME models include some elements like the correlation of cell cycle lengths in mitotic sisters that greatly improve the temporal modelling of RPC lineage outcomes, for instance, which we will adopt in simulations presented below. The observations arising from the SMME strongly suggest that we would like to test hypotheses about RGC specification in order to find better models of RPC function. Carefully reconsidering the hypothesis of Neumann et al. [Neu00], that Shh from nearby RGCs induces cell cycle exit and RGC specification, would seem to be a high priority, for instance. Can such a scenario be represented in an SSM? One could model the probability of being within Shh induction range of an RGC in the early part of a lineage's life, for instance. Doubtless, a model that incorporated variable RPC specification outcomes determined on this basis could be made to fit data about as well as the variable mitotic mode or variable phase length models tested above. That said, it is not very clear that a highly abstract representation of signalling would constrain the inferred RGC production rate well. Moreover, while we determined that our deterministic mitotic mode model fit test data somewhat better than the stochastic model, the modest size of the calculated AIC differences suggests that comparing SSMs is not a particularly good way to distinguish even alternative hypotheses about the structure of processes the SSM models explicitly, like cell cycle length and mitotic mode.

We can therefore predict that we will be unable to test important models, including those involving documented macromolecular explanations of RGC specification, without the ability to represent some spatial information. Still, the computational benefits of the SSM are too appealing to leave out of the toolkit entirely, and the type of spatial information that is ultimately best used to constrain alternatives on processes like RGC fate commitment could be highly abstract. We would like to leave the door open for models of many forms, prioritising simpler ones. Taken together, this suggests that we need a very

general method of testing models of potentially very different structures against one another.

Turning then to our statistical procedures, are these adequate to our goals? We can broadly characterize the approach taken as estimating the local optimum of a loss function for model output, given the dataset; specifically, minimizing Akaike's information criterion by simultaneous perturbation stochastic approximation (SPSA). This procedure has some advantages. AIC is a well-understood measure, well-grounded in information theory, which penalizes superfluous model complexity in a consistent and rigorous way. SPSA is a widely used, well understood algorithm that can be applied to any reasonable euclidean parameter space; cases where parameter spaces are bounded (typical of biological models where negative parameter values are usually nonsensical) are explicitly accounted for, and so on. This procedure is better than many that are available.

Still, after the experience of the SMME model comparison, a certain uneasiness is warranted. The AIC calculation is, after all, based on a single estimate for the locally optimal parameterisation of the model. Relative AIC rankings, then, are strongly influenced by the "well depth" of the AIC surface at the local minimum in parameter space. We can easily imagine a case where a model with a very narrow range of parameter space that fits data very well appears to be better, by the AIC metric, than one that fits the data slightly more loosely, but over a much broader range of the parameter space. The first model is a "fragile" fit, probably depending heavily on the particular structure of the training dataset, while the second would likely be much more robust across a range of observations; still, in this case, AIC would lead us to select the fragile model over its robust counterpart. Indeed, blind interpretation of AIC rankings has lead to its use in ecology being described as a "cult" [BB20]. It is easy to imagine practitioners being reduced to "AIC hacking", in the same manner that "p hacking" occurs, in order to achieve some arbitrary value for a hypothesis.

Moreover, while SPSA is an eminently practical algorithm, useful in a wide variety of contexts, its statistical guarantee is only that it will almost-surely find the local minimum of the loss function. While this will be good enough for many applications, for highly parameterised models with complex loss function surfaces, there will be many local minima for SPSA to get "stuck" in that are nowhere near the global optimum². If we are evaluating hypotheses in order to make decisions about potentially years-long research projects, more certainty about the reliability of the method is necessary. Finally, while SPSA is requires much less computational effort than more global Monte Carlo parameter estimation techniques like simulated annealing or Hamiltonian Monte Carlo, it requires about as much application-specific tuning.

Fortunately, a complete system of Bayesian inference which addresses all of the problems mentioned above has been promulgated over the last 15 years: nested sampling. Originally introduced by John Skilling [Ski06], this use of this system rapidly became widespread in cosmology [Tro08, FHB09, HHHL19], where its generality and ability to cope with complex, high dimensional parameter spaces has been well proven. This system is discussed in detail in [Section 14.2.7](#). We have implemented two separate samplers for the exploration of parameter spaces using the overall nested sampling approach: `GMC_NS.jl`, described in [Chapter 7](#), and `BioMotifInference.jl`, described in [Chapter 10](#). `GMC_NS.jl` is a prototype general purpose Galilean Monte Carlo sampler intended to perform nested sampling of arbitrary tissue- or cell-level biological models, while `BioMotifInference.jl` is a highly specialized, well-tested, ad hoc sampler for evidence calculation and MAP estimation of independent component

²This is part of what is meant by "the curse of dimensionality", when speaking of the difficulty of sampling the loss function in high dimensional parameter spaces.

analysis models of genetic sequence emission. `GMC_NS.jl`, while less mature, is of greater general interest, and having it in hand allows us to broadly consider the features of CMZ models we might like to test, before proceeding to test the function of the package and to determine its general utility in tissue-scale models.

3.3 Desiridata for spatial CMZ models in a putative model comparison framework

The simulations presented in Chapter 2 consisted solely of abstract collections of lineages. The members of these model colonies have no activities beyond proliferation, and no functional attributes beyond their proliferative status³. Despite the underlying code consisting of relatively high performance C++, these simple models nonetheless occupied the local component of the cluster used in this work for some weeks. A simple approach to ranking basic models against a smallish dataset can thus approach the limits of what most labs are likely to be able to achieve with any given machine over a month or so. Because the introduction of explicit three-dimensional spatial simulation implies, perhaps, an order of magnitude more computational time, we may plausibly be limited to one inference based on model comparison per year with local resources. This constraint dominates all other considerations in the selection of the overall boundaries within which we intend to build models of the CMZ. In any environment where funding constraints limit the cloud-export of computational burden from the confines of the research institution, it is reasonable to proceed upwards in model computational expense from the cheaper-but-inadequate in search of the adequate-but-still-affordable. In this case, we move from the aspatial SSM into the realm of explicit spatial modelling, seeking the minimum model complexity required to compare hypotheses of interest to us.

3.3.1 Spatial dimension of the models: the "slice model"

Although decomposing the RPC population of the CMZ into a collection of unordered, independently-proliferating SSMs prevents us from assessing many interesting hypotheses, a computational model of the entire CMZ or retina may not be required. Because observations suggest that RPC lineages contribute to the retina in linear cohorts of neurons, any particular centrally-oriented slice of the CMZ annulus will be responsible for the generation of the neurons central to it. The retina can be represented by a series of these "slice units", lined up radially, like slices of pie. A complete "slice unit" would include the central-most larval remnant, contributed by embryonic retinogenesis, surrounded by the CMZ's more ordered neural contribution from the postembryonic period, and, peripherally, the CMZ itself. Depending on the hypotheses to be tested, the differentiated central retina may be mostly irrelevant, so the modelled slice may consist only of the CMZ and its interface with these central neurons. It is important to note that these slices are conceptually different from the linear cohorts generated from particular lineages, the so-called 'ArCCoS', which justify the slices conceptually [CHW11]. It is not necessarily the case that a slice model would consider only one RPC lineage; the slices are better thought of as spatial boxes that sample the CMZ and adjacent central neurons, the conceptual equivalent of the histological section through the eye⁴.

³I.e. the specified identity of post-proliferative cells in these models has no function within the model.

⁴The case of only one simulated lineage may still arise given thin enough sample boxes or old enough animals, but it is a limiting case and probably would not be the norm.

Slice models are especially attractive because the observed proliferative status, position, specified fate, etc. of simulated cells can be easily related to the summary statistics used to describe fixed sections of retinal tissue in this thesis and elsewhere. If our histological sections are of an appropriate width, the slice model can be selected to produce output that is directly comparable to observed histological results in central sections, where the assumption that adjacent cells derive from the same collection of lineages is justified. This is especially important for studying the postembryonic CMZ, which rapidly becomes optically inaccessible due to the increasing thickness and pigmentation of overlying tissue, so that live imaging often cannot be used⁵.

If the retina can be usefully thought of as a series of slice models, and the activity of the CMZ is basically homogenous around its circumference, it follows that the activity of the CMZ can be usefully represented with a single such model. Moreover, the slice need only include one portion of the retinal periphery, the orientation of which is irrelevant (i.e. the model parameterisation for the dorsal portion of a coronal slice is identical to the ventral). It may be erroneous to abstract such a slice from its context, because the modelled cells are in contact with adjacent slices. However, if the CMZ homogeneity premise is valid, this can easily be incorporated into the model by providing a layer of "ghost" cells on either side of slice whose parameters are determined by the slice itself⁶. That is, given the premises, a slice model which interacts with copies of itself is a complete model of CMZ-driven retinogenesis.

All this said, the zebrafish retina is a manifestly asymmetrical structure, with an optic nerve positioned ventro-temporally relative to the center of the optic cup. The CMZ itself is generally understood to be an asymmetric structure, with a larger dorsal than ventral population. This calls into question the assumption of CMZ homogeneity outlined above. It is nevertheless plausible that the appearance and maintenance of these structural features of the zebrafish retina could be explained with a set of slice models, for example, one for each of the dorsal, ventral, nasal, and temporal extrema. Ultimately, these considerations are only relevant if our objective is to compare model explanations for retinogenesis as a whole. If we restrict ourselves to the more modest goal of, say, ranking causal influences on the lineage outcomes of RPCs in the dorsal extremity of the CMZ, this could provide significant insight into the regulation of peripheral stem cells in other species. In this sense, a slice model could still be valuable even if it fails to explain aspects of tissue-level zebrafish retinogenesis.

3.3.2 Temporal resolution of the models

Another important consideration for any CMZ model comparison framework is the time-scale of any phenomena which are to be admitted as possible causal contributors to retinogenesis. While it is reasonable to think that proliferative events may be well-described with a model that operates on a scale of days and fractions thereof, and that such a model would be well suited to describing the full sweep of CMZ activity across the life of the organism, very few of the relevant macromolecular processes are likely to be well-described with a resolution more coarse than seconds or minutes. The implied difference in the number of calculations required to simulate any given time period is thus several orders of magnitude. A simplifying assumption of the temporal homogeneity of CMZ activity would allow us to abstract long developmental time frames; an explanation that pertains to a few hours can perhaps be extended without recalculation.

⁵It is worth noting that the spatial parameters of such models would pertain to fixed and not live retinas; it is probable that some scaling relation compensating for fixative shrinkage could allow the mixture of live imaging data in evidence calculations.

⁶This can be implemented in CHASTE with that framework's "ghost node" objects.

If an assumption of temporal homogeneity proves inadequate, the functional structure of the simulation will be determined by this consideration. To illustrate this, consider a case where we wish to incorporate measurements of eye pressure, or membrane tension across the retina, as inputs into the proliferative activity of the CMZ, by way of progenitor cortical tension [Win15]. This would permit assessing whether modelling tissue-mechanical inputs to cellular activities allows us to extract additional information from our observations. If such physical model elements are to be included, the functions which relate physical parametric data to the proliferative behaviour of CMZ progenitors must not operate on a time scale too short to reasonably cover the period of interest. Suppose we are mainly interested in explaining the assembly of functional units of the mature, specified retina by the CMZ, from the first division of the presumed distal stem cell responsible for the unit to the determination of the last neuron of its functional column. If this process takes days, finite element approximation of cortical tension with a resolution small enough to capture important details of relevant cellular movements (e.g. interkinetic nuclear migration) will probably consume too many computational resources to allow for effective application of Monte Carlo techniques, requiring thousands or millions of simulations per call of the model likelihood function.

3.4 Bayesian decisionmaking for structuring models under uncertainty

From the foregoing discussion, we can see that the extent to which model simplifications are justified, and the types of phenomena that could be explained with these models, depend heavily on considerations that can only be informed by observations. As has been demonstrated in Chapter 2, the growth of the CMZ population cannot be explained by models fitted to embryonic RPC activity. However, it remains unclear what sort of alternative structure is justified by observations, given our uncertainty about inferred parameters of the populations being measured. Because we have, in `GMC_NS.jl` a general method of comparing differently parameterised models against our observations, we can formulate this as a problem of decision theory, where we seek to select models from among alternatives on the basis of their total explanatory power for the observations, independent of the particular model structure or parameter values. Because this process also produces samples from the posterior distribution of the model's parameters, we are also able to account for our uncertainty on these parameters. This allows us to broadly answer the question of what sorts of hypotheses may be productively addressed by the conventional immunohistological techniques we have used thus far, and what changes to methodology or analysis may be necessary to produce data that adequately constrains models of interest.

Our general approach is to estimate model evidence over broad, uninformative prior distributions on model parameters. We assume as little as possible about the processes underlying our results. This leads us to begin with the exercise of testing the relative superiority of Normal and Log-Normal models of the data. The values used in these comparisons are the logarithm of the model evidence, $\log Z$, also understood as the logarithm of the marginal probability of the model. Likelihoods and probabilities are often compared in ratios; evidence is the same, and since the logarithm of a ratio is the logarithm of the numerator minus that of the denominator, this log-ratio, $\log Z_R$, may be calculated by subtracting the evidence of one model from another. It is important to note that raw evidence values do not have a

direct interpretation: these values depend on the size of the dataset tested⁷. On the other hand, logZR ratios are dimensionless; they arise from dividing logZ values from models explaining equivalent amounts of data. The logZR value can be interpreted directly as the number of orders of magnitude in favour of (positive values) or against (negative values) the numerator model in the ratio. We use two methods of estimating logZ values, nested sampling, as described above, as well as the Empirical Bayes method of linear regression implemented in `BayesianLinearRegression.jl`. The former provides for an estimate of the error on logZ calculations, and therefore allows the calculation of the significance of these results; these are generally presented as standard deviations of significance, following cosmological practice. This value can be understood as our estimate of the certainty we have about the selection of one model over the other. This general model-comparison logic is iterated to address most of the important hypotheses we test in the following chapters.

⁷That is, logZ values take data units. See [Ski12] for an extensive discussion of this issue.

Chapter 4

Bayesian periodization of postembryonic CMZ activity

4.1 Preliminaries: Calculation of Bayesian evidence supports independent Log-Normal modelling of CMZ parameters

To take up the question of how CMZ RPC activity evolves over time, we measured CMZ and retinal parameters in histological studies of cryosections of zebrafish eyes harvested over the first year of the animal's life. We wish to extract as much information as possible about the structure and time-evolution of the CMZ population as a whole, in order to know how to best model its constituent RPCs. The initial approach is to estimate parameters of the whole-eye CMZ from sample cryosections, and to use these estimates as the dataset which will inform our selection of appropriate models.

While it remains common to assume that population census data are Normally distributed, and so simply to calculate means and standard deviations as the statistical representation of the underlying population, it has long been known [Hea67] that Log-Normal distributions are usually better models of the outcomes produced by additive processes with small, variable steps (like population sizes or income distributions). We therefore start with the selection of appropriate models for the CMZ- and retina-level population measurements critical to the inferences that follow.

As a first pass at this question, we calculate the likelihood ratio for the hypothesis that the parameter measurements, and the calculated quantities derived from them, are Log-Normally distributed against the one that they are simply Normally distributed. These results are displayed in Table 4.1.

These results suggest that the organism-level population distribution of eye-level CMZ population counts, as assayed by PCNA immunostaining, is better modelled Log-Normally than Normally. This is true whether we test the primary per-section count measurements, or the estimated whole-annulus population, calculated as described in Section 11.1.4, even though this quantity is calculated using the Normally-distributed lens diameter¹. The most likely Log-Normal representations of the CMZ populations are about two orders of magnitude more likely than the Normal alternative. Additionally, although Normal models are slightly favoured for describing the population-level distributions of RPE

¹The apparent superiority of the Normal model for lens diameter may be due to the paucity of data at later time points; as described in Section 12.1.7, lenses are difficult to retain in this histological context.

Table 4.1: Likelihood ratio comparison between Normal and Log-Normal models of retinal population parameters

Parameter	\mathcal{N} logLH	Log- \mathcal{N} logLH	logLR
Sectional PCNA+ve	-285.611	-283.214	2.397
Lens diameter	-203.102	-203.854	-0.752
CMZ annular pop.(†)	-484.768	-482.733	2.035
RPE length	-368.232	-368.609	-0.377
CR thickness (†)	-240.858	-241.032	-0.174
CR volume (†)	-1091.615	-1091.146	0.469

\mathcal{N} : Normal distribution. logLH: logarithm of $p(D|M)$, the likelihood of the data given the model. logLR: logarithm of the likelihood ratio; positive ratios in favour of the log- \mathcal{N} model. Largest likelihoods are bolded. †: Calculated quantities. Sectional PCNA+ve: population of PCNA-positive CMZ RPCs per $14\mu\text{m}$ cryosection. CMZ annular pop: population of annular CMZ. RPE: retinal pigmented epithelium. CR: cellular retina. Methods in [Section 11.1.2.1](#) and [Section 12.1.7](#). Code in [Section 16.9.6](#).

length and cellular retina thickness, the derived cellular retina volume quantity is better modeled by a Log-Normal distribution. As the two calculated quantities will be the primary ones used in our inferences about population-level CMZ dynamics, we are most concerned with these measures; at this point, the Log-Normal distribution seems better for both.

We have emphasized the danger of relying overmuch on the parameterisation of single most-likely model fits in [Chapter 3](#), which is what the simple likelihood ratios above represent: the joint likelihood of the maximum a posteriori distribution fitted to the measurements taken from each age cohort. Since the choice of model describing the estimated annular population and retinal volume is critical to the success of later inferences, we used Galilean Monte Carlo-Nested Sampling (GMC-NS) to estimate the Bayesian evidence (the marginal probability of the data over all model parameterisations) for these hypotheses. Although it is very unlikely that GMC-NS will result in conclusions from the likelihood ratio, this simple test serves to prove the function of the `GMC_NS.jl` package, and demonstrate the inferential logic. Evidence estimates and ratios are presented in Table 4.2.

Table 4.2: Evidence favours Log-Normal models of retinal population parameters

Parameter	\mathcal{N} logZ	Log- \mathcal{N} logZ	logZR	σ significance
CMZ Population	-6127.3 ± 7.7	-1077.2 ± 1.1	5050.1 ± 7.8	650.2406109326922
Estimated Retinal Volume	-19516.0 ± 12.0	-2017.4 ± 1.1	17499.0 ± 12.0	1409.504095031284

\mathcal{N} : Normal distribution. logZ: logarithm of $p(D)$, the marginal likelihood of the data, or model evidence. logZR: evidence ratio; positive ratios in favour of the log- \mathcal{N} model. Largest evidence values bolded. CR: cellular retina. Methods in [Section 12.1.8](#). Code in [Section 16.9.4](#).

Unsurprisingly, full estimation of the Bayesian evidence for the Normal vs. Log-Normal hypotheses for our calculated parameters produces basically similar results to the rough calculation of likelihood ratio from the single-fit MAP models. There are approximately 5000 orders of magnitude more evidence for the Log-Normal model of interindividual variation in estimated CMZ annulus RPC population over time. This result has greater than 600 standard deviations of significance². However, there are approximately

²The typical standard for a "discovery" in particle physics is $>5\sigma$ [[Lyo13](#)]. Assuming Normally distributed error, the probability that the models were, in fact, equally good at 3 standard deviations of significance would be about a tenth of

17000 orders of magnitude more evidence for the Log-Normal model for the variability in the cellular retinal volume estimate, with 1400 standard deviations of significance. Given the likelihood ratios alone, we might have suspected that the Log-Normal model was more justified for modelling the population data than for the volume data. In fact, with the full estimation of the evidence, it becomes clear that the converse is true, demonstrating how evidence measurements can supply a fuller picture than maximum likelihood methods. In any case the evidence supplied by our observations best supports Log-Normal modelling of these parameters.

If between-individual variation in retinal CMZ population and retinal volume are both well-modelled Log-Normally, the question of their independence arises. It seems plausible that the size of the CMZ population would be roughly proportional to the overall volume of the retina, after postembryonic central specification, and establishment of the peripheral CMZ remnant. Moreover, since the growth of retinal volume over the life of the organism is driven primarily by the CMZ³, it seems likely that this volume is well correlated with the size of the CMZ. Since the manner in which we model the CMZ differ depending on our assessment of the dependence of these retinal parameters, we performed Bayesian model selection by the so-called *Empirical Bayes* method for linear regression, which provides for direct estimation of the evidence for models consisting of linear equations of variables [Bis06].

We find that individual CMZ population and retinal volume estimates are best described by uncorrelated models at all ages, with the exception of 23.0 dpf, where the evidence weakly favours correlation. It is interesting that the evidence in favour of non-correlation is weakest between 17 and 30 dpf, suggesting the correlation at 23.0 dpf is not spurious, but is related to CMZ activity at this time. These data are displayed in [Table 4.3](#). Of particular importance are the data for 3dpf embryos, as we intend to seed model retinae with CMZ populations and volumes drawn from these distributions. The data for these animals are plotted in [Figure 4.1](#). The general lack of correlation between CMZ population and retinal volume estimates may be due to the loss of information involved in the estimation calculations; on the other hand, it is more plausible that CMZ population should be associated with the rate of retinal volume growth rather than the volume of the retina itself, which suggests that the rate of retinal contribution from the CMZ is probably highest between 17 and 23 days. Because growth rate data are unavailable for single individuals, we cannot make this inference directly.

We conclude that interorganismal variability in the CMZ population and retinal volume estimates are best described by independent Log-Normal distributions. Because Log-Normal distributions are simply transformed Normal Gaussian distributions, we may model our uncertainty about their parameters with Normal-Gamma distributions over the mean and variance of the underlying Normal distribution of the Log-Normal population model ("the underlying"). That is, our prior and posterior belief about the relative likelihood of values of the mean of the underlying may be modelled with a Normal distribution, and our beliefs about its variance with a Gamma, such that our joint uncertainty is the product of the two distributions. This is explained in more detail in ???. A useful analytic feature of the Normal-Gamma prior is that the marginal posterior distribution of the mean, assuming an uninformative (ignorance) prior, is a location-scaled T distribution; this is so because the weighted sum of an infinite series of Normal

a percent (i.e. .001). At 10 standard deviations the figure is 7.62e–24. Assuming the sample is representative, we have nigh certainty about these results and no reason to pursue the matter further.

³One observes occasional proliferative clusters in the central retina throughout the life of the fish; these are typically ascribed to Müller glial repair processes. I am unaware of any estimate as to the relative contribution of these clusters vs. the CMZ. As we shall see, there is probably more turnover in the specified retina than previously believed. As a result, the relative contribution of these central clusters should probably be subject to statistical estimation; they may be more significant than mere lesion-repair sites.

Table 4.3: Evidence favours uncorrelated linear models of CMZ-population and retinal volume over time

Age (dpf)	Uncorrelated logZ	Correlated logZ	logZR
3.0	-87.651	-91.902	4.252
5.0	-90.0	-92.362	2.362
8.0	-90.918	-96.013	5.095
12.0	-91.183	-99.049	7.866
17.0	-94.818	-96.668	1.85
23.0	-103.386	-103.219	0.167
30.0	-103.511	-104.092	0.581
60.0	-115.025	-118.169	3.144
90.0	-113.533	-122.427	8.894
180.0	-116.778	-124.547	7.769
360.0	-121.016	-128.637	7.621

logZ: logarithm of $p(D)$, the marginal likelihood of the data, or model evidence. logZR: evidence ratio; positive ratios in favour of the uncorrelated model. Largest evidence values bolded. Methods in Section 12.1.8, Section 12.1.10. Code in Section 16.9.1.

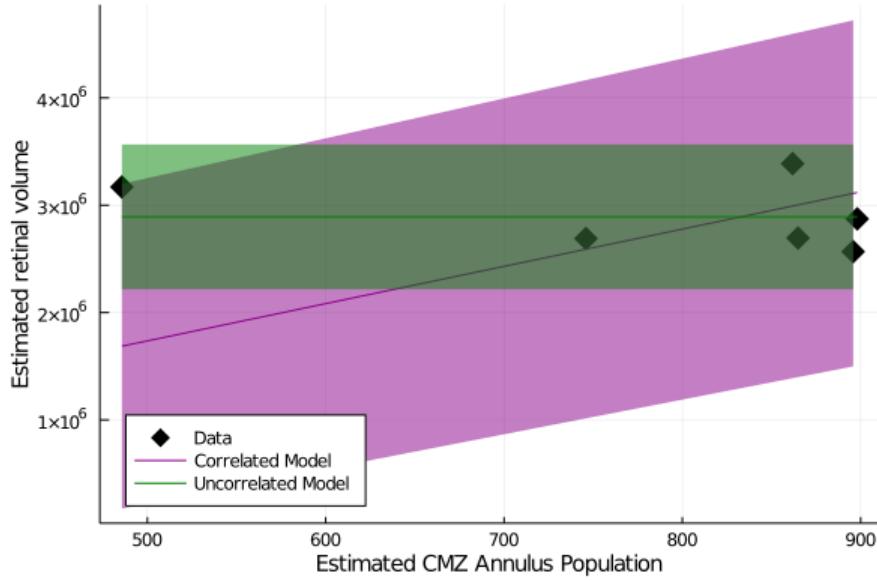


Figure 4.1: CMZ population and retinal volume estimates are uncorrelated at 3dpf
 Individual CMZ population estimate vs retinal volume estimate for 3dpf animals. Uncorrelated and correlated linear models of these variables are plotted as the mean \pm 95% CI of the predictive distribution of the fitted model. Methods in Section 12.1.8, Section 12.1.10. Code in Section 16.9.1.

distributions (i.e. the likelihood-weighted sum of all the Normal distributions that could underly the Log-Normal models), is a T distribution. T distributions are notably more resistant to outlier distortion than Normal distributions themselves are. Most of the descriptive statistics in the next section, therefore, calculate the credible interval for the posterior mean of the underlying by T distributions (with the correct change of variables by exponential transformation to produce the features of the correct Log-Normal distribution). Unfortunately, differences of T distributions are not, themselves, necessarily

T-distributed, so we have relied on Monte Carlo estimation of rates of change of the these posterior means over time. With our Log-Normal models selected, and the descriptive tool of the posterior mean T distribution in hand, we turn now to a survey of the zebrafish CMZ in the first year of life.

4.2 Survey of CMZ population and gross retinal contribution

If it is true that the majority of zebrafish retinogenesis occurs postembryonically, and that models trained on embryonic data do not describe this period well, as seen in Chapter 2, what characterises this CMZ-driven phase of retinogenesis? We begin answering this question by presenting our estimates of individual CMZ annulus population and retinal volume over the first year of life, in Figure 4.2, panels A and C.

The lack of growth observed in the first week of life suggests an initial quiescent period in the population history of the CMZ. Observations in Figure 5.2, which demonstrate that CMZ RPCs are proliferating too slowly to be labelled by a day’s pulse of a thymidine analogue at 5dpf in the wild-type and heterozygote siblings of npat mutants, also suggest this quiescence. On the other hand, we have good confidence that retinal volume continues to grow over this time; 99.56% of the marginal posterior mass of the mean 5dpf estimate is above the 3dpf mean, which may indicate this proliferative pause is too short to appear in the retinal volume data.

In any case, the first two to three weeks of life (magnified in lens insets in panels A and C) see a relatively slow build in both estimated CMZ annulus population and retinal volume compared to the explosion which follows immediately thereafter. While zebrafish are better staged by size than age [PEM⁺09], the onset of this explosive growth seems to come earlier than the typical metamorphic transition from larval to juvenile stages at 45dpf [SH14]. This raises the question of how the CMZ contributes to the retina during the critical period of exponential growth of the organism, between about 45 and 90dpf. It is plausible, for instance, that the growth of the CMZ population mainly reflects the increased output of stem cells, with RPCs specifying at some steady rate, or that the CMZ builds itself up for a wave of niche exit and specification somewhat later, similarly to the sequence of events in the embryonic central retina.

In order to ascertain this timing, we simulated the mean daily rate of CMZ annulus population and retinal volume change, by performing Monte Carlo difference operations between samples from the marginal posterior means of subsequent timepoints. These simulated mean rates and their associated confidence intervals are plotted in Figure 4.2 panels B and D. These show the time-structure of the phenomenon; the increase in simulated daily CMZ population growth rate occurs before the increase in retinal volume, but the CMZ population estimate does not begin to drop off until 60dpf, by which time the majority of volumetric growth is complete. This seems to substantiate some combination of the scenarios outlined above: there is an early buildup of CMZ population around 18-30dpf, before CMZ RPCs begin to make their primary contribution to the retina between 30-60dpf, which is characterised by much slower population growth and more rapid volumetric growth, implying steady and elevated specification and niche exit of RPCs. The greater uncertainty associated with our population estimates, relative to their magnitude, results in correspondingly less certainty about the size of the population growth rate spike. For instance, we calculate a >99% probability that the mean retinal volumetric rate growth peak at 60 dpf is at least 6-fold greater than the mean at 30dpf. By contrast, only about 97% of the posterior mean density of the population growth rate at the 23d peak ($230.2 \frac{\text{cells}}{d}$) is greater than

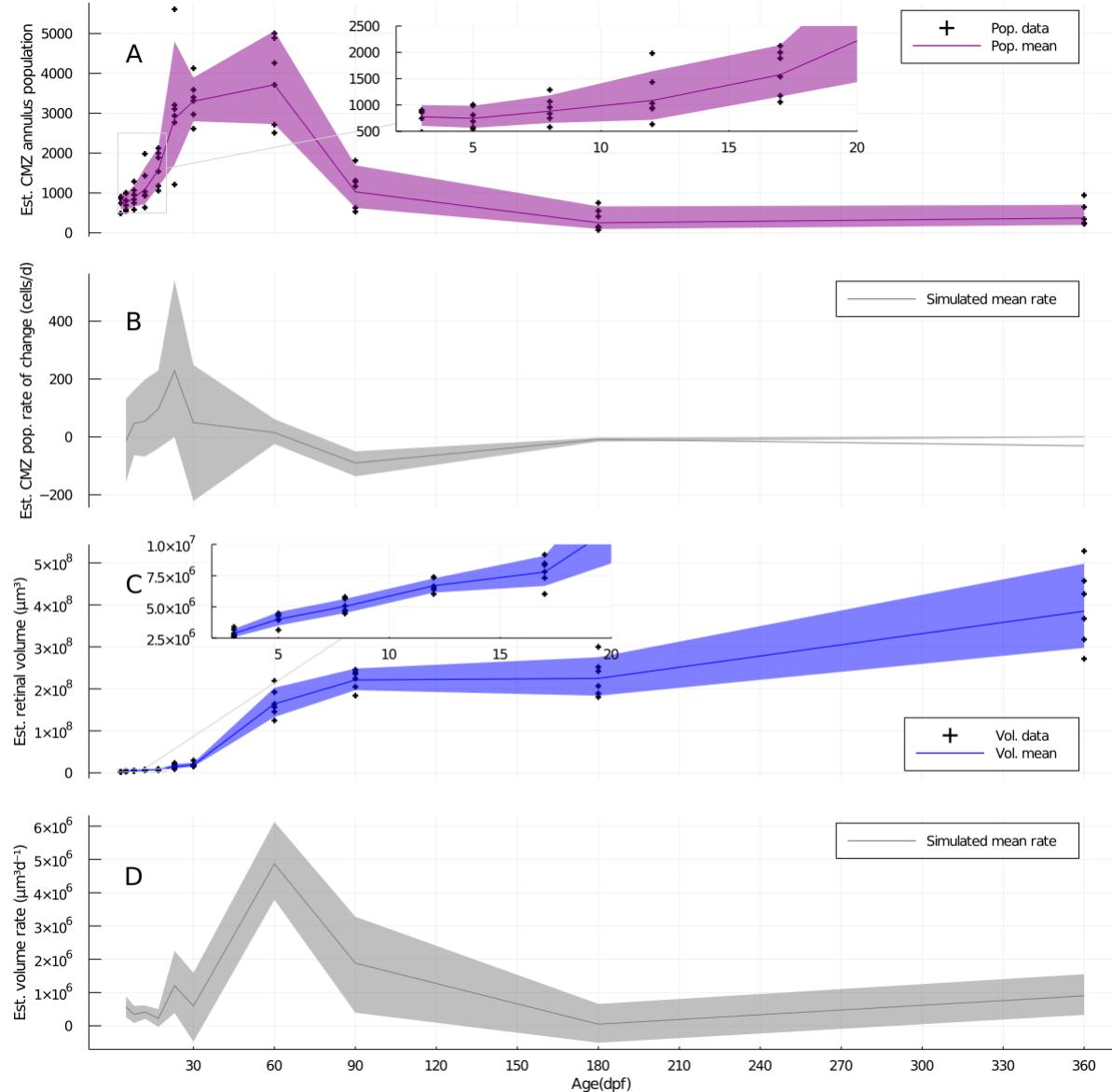


Figure 4.2: Population and activity of the CMZ over the first year of *D. rerio* life
 Panel A: Marginal posterior mean CMZ annulus population. Panel B: Marginal posterior mean retinal volume estimate. Insets in Panels A & B display data from 3-17 dpf. Panel C: Marginal posterior mean of the proliferative index of the CMZ annulus, assayed by specified retinal neurons with incorporated thymidine from an 8hr pulse at the indicated ages. Panel D: Mean daily rate of volumetric increase of the neural retina, calculated as the difference in volumes between two ages over the number of elapsed days. All means are displayed in a band representing the $\pm 95\%$ credible interval for the marginal posterior distribution of the mean. Methods in Section 12.1.8, Section 12.1.9. Code in Section 16.9.6.

the mean at 3dpf, which is a small negative value ($-5.5 \frac{\text{cells}}{\text{d}}$).

Subsequent to the bulk of the CMZ buildup and contribution to the retina, the population of the CMZ declines at about 39% the rate of its peak ascent, with an estimated $-90.1 \frac{\text{cells}}{\text{d}}$ by 90dpf. This thins out the CMZ population to below its initial postembryogenetic size, spread out over a much larger peripheral

annulus, for a much less dense mature CMZ. Interestingly, the 95% CI on the marginal posterior mean of estimated retinal volume at 180 dpf (ranging from 1.84e8 μm^3 to 2.75e8 μm^3) completely encompasses the 95% CI on volume at 90dpf (1.96e8 μm^3 -2.48e8 μm^3), while the 360dpf mean (3.85e8 μm^3) is greater than six standard deviations from the 180dpf mean (2.25e8 μm^3). This suggests a possible second period of quiescence from approximately 90-180dpf, followed by steady contribution to the retina without a buildup in the CMZ population subsequently.

Given this description, the ontogeny of the CMZ as a stem cell niche could be well-described by different phases of activity, with different rates of proliferation and specification. It is not immediately clear what sort of periodization is justified by the data. It seems plausible that as few as two phases could explain the data well enough: an initial phase of logarithmic growth, with short cell cycle time and lower exit rate of RPCs from the CMZ into the specified neural retina, followed by a second phase of decay with longer cycle time and higher exit rate. On the other hand, perhaps some of the data features noted above justify a more bespoke model that captures, for instance, the initial quiescent period, or the post-180dpf growth of the retina. Because this is straightforwardly a question of how much model structure is justified by our data, we may address it as a model selection problem, using the system of Bayesian inference provided by nested sampling, and it is to this we now turn.

4.3 Two-phase periodization of postembryonic CMZ activity by phased difference equation modelling

To perform our model selection task, we must simulate the time-evolution of CMZ population and retinal volume. This requires us to supply initial values for the size of the simulated CMZ populations and the volumes of the simulated retinae they are associated with. Given the findings presented in Figure 4.1, we are justified in initializing CMZ population and retinal volume by independent samples from the Log-Normal models of their interindividual variability at 3dpf, at the end of embryogenesis and the beginning of CMZ-driven retinal growth. In order to produce new, simulated values of CMZ population and retinal volume, we apply a system of difference equations as follows, where pop_n is the population at n dpf, CT is the mean cell cycle time of the population in hours, and ϵ is the proportion of the population at time $n - 1$ that exits cycle and contributes to the volume of the specified neural retina, and μ_{cv} is the mean volume per cell contributed to the retina in μm^3 :

$$p_n = p_{n-1} \cdot 2^{\frac{24}{CT}} - p_{n-1} \cdot \epsilon \quad (4.1)$$

$$v_n = v_{n-1} + p_{n-1} \cdot \epsilon \cdot \mu_{cv} \quad (4.2)$$

A model "phase" can then be defined by the CT and ϵ parameters it applies to update the population and volume, over the appropriate number of days. The full parameterisation of a model with p phases is given by p pairs of CT and ϵ values, and $p - 1$ phase transition times. μ_{cv} , which is taken to apply equally to all phases, is estimated from 3 dpf nuclear measurements, as described in Section 12.1.8. Given an initial population and volume sampled from the Log-Normal models of their interindividual distributions, Equation 4.1 and Equation 4.2 are applied to these values to produce simulated sample values at the times actually observed. Many such samples obtained by Monte Carlo can be used to estimate Log-Normal distributions for the model parameters, which can be used to score the model

against observations. By defining prior distributions over the model parameters, we may sample from the prior to initialize a model ensemble. The ensemble can then be compressed by nested sampling, moving each model-particle over the parameter space by Galilean Monte Carlo, as described in ???. Using typical procedures applied in nested sampling [Ski06], we estimated the Bayesian evidence, maximum a posteriori, and marginal posterior distributions on parameters for 2 and 3-phase models.

It is useful to begin with the maximum a posteriori model output, as this shows the major problem with this simple model; the model relationship between changes in CMZ population and changes in volume breaks down after 30dpf. That is, the later volume estimates are too large for the CMZ to produce, given the calculated cellular volume (μ_{cv}) at 3dpf. The models fit the early population and volume data quite well, but the population peak is dragged upward to produce more-likely volume output at later ages. While it is possible that the later CMZ contributes more volume per neuron to the cellular retina, it seems more likely that the volume approximation applies better to more-nearly spherical eyes at younger ages, than to the flattened eyes of later ages. Although we hoped that the estimated retinal volume data would constrain the ϵ exit rate parameters, this was not the case, as discussed below. When we tried floating μ_{cv} as a variable within the model, the MAP results were similar (data not shown), suggesting that a constant value for μ_{cv} across ages is the problem in achieving good model fits, not the particular value chosen. This reinforces the idea that the problem relates to the breakdown of the retinal volume estimate at later ages.

While this limitation prevents either model from explaining the combined estimate datasets very well, they are in this sense under the same constraint, and so a reasonable inference about the number of phases justified by the data is still possible. Evidence estimates for the 2-phase and 3-phase models, given these data, are presented in Table 4.4. There are greater than 3500 orders of magnitude more evidence for the 2-phase model; this result has over 200 standard deviations of significance. This reflects the expanded parameter space in the 3-phase model, which has 8 parameters, compared to the 2-phase models' 5. The additional flexibility afforded by the 3rd phase in fitting the later volume data is unable to overcome the evidentiary penalty associated with the larger parameter space. We conclude that the 2-phase hypothesis must be accepted.

Table 4.4: Evidence favours a 2-phase periodization of CMZ activity

2-phase logZ	3-phase logZ	logZR	σ Significance
-7147.3 ± 9.4	-10743.0 ± 13.0	3596.0 ± 16.0	227.624

logZ: logarithm of $p(D)$, the marginal likelihood of the data, or model evidence. logZR: evidence ratio; positive ratios in favour of the 2-phase model. Largest evidence value bolded. Methods in Section 12.1.11. Code in Section 16.9.5.

While the parameter estimates associated with these models are clearly unreliable, they are useful to inspect in order to demonstrate some properties of nested sampling, and for comparison to the simulations to follow. To begin with, we present the parameterization of the MAP model output displayed above in Table 4.5. Unsurprisingly, the selected 2-phase model begins with a first phase of rapid proliferation, with a CT of 14.5 hr, followed by a second, slower phase of 17.5 hr. The imputed exit rate ϵ is greater than 200% of the day's starting population in the first phase, suggesting that new cells exit the CMZ after about 12 hours, around one cycle, while the second phase exit rate is lower, with 160% of the day's starting population exiting the CMZ, again suggesting a residency time of about one cycle. The imputed phase transition age is about 23dpf. Due to the volume estimate problem noted above, it is reasonable

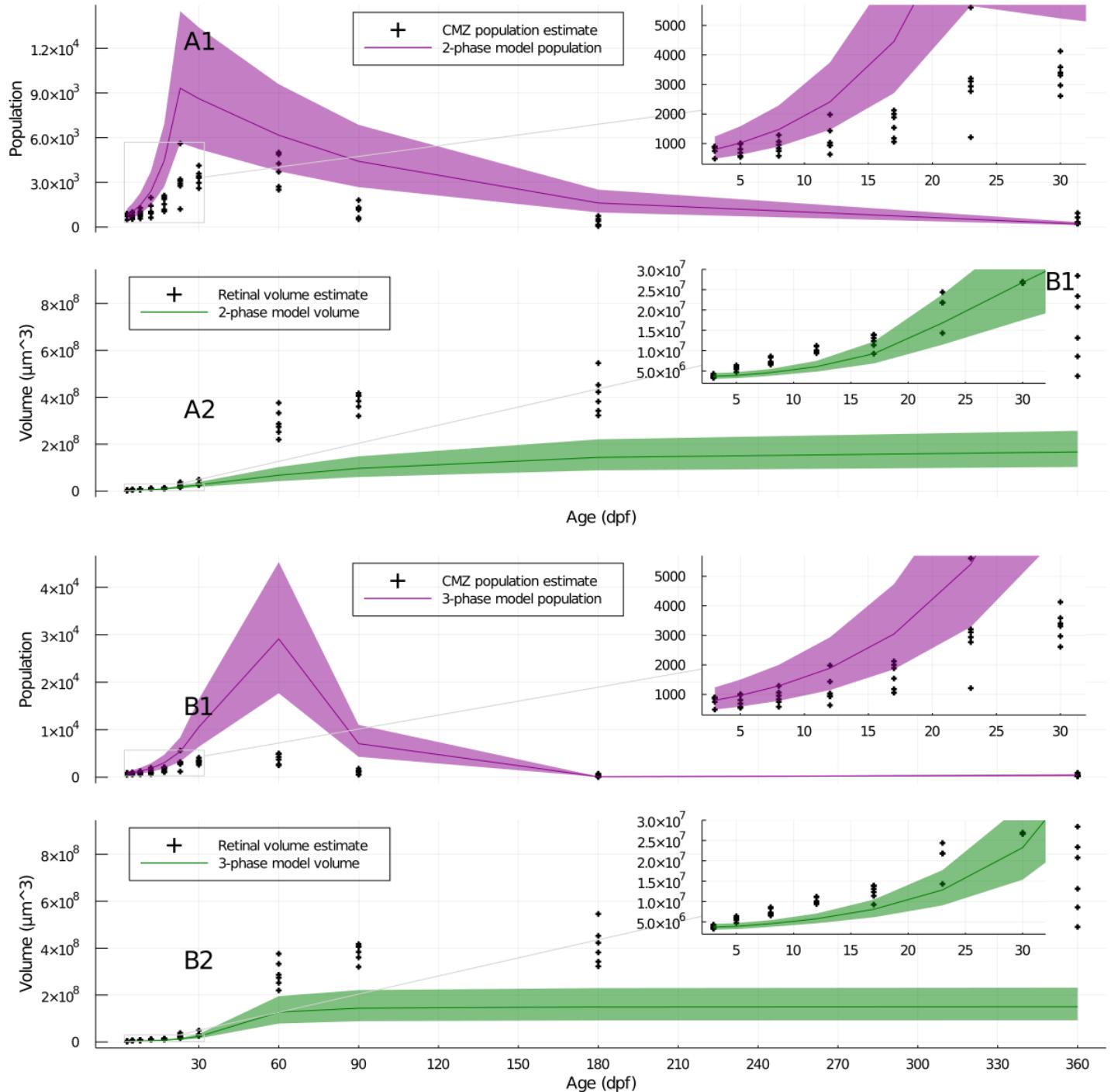


Figure 4.3: Maximum a posteriori output of periodization models

Population and volume estimates from observations (crosses) plotted with mean \pm 95% posterior mass model output, for the 2-phase model (A panels) and 3-phase model (B panels). A1,B1: population estimates. A2,B2: volume estimates. Insets provide magnified views of data from the first 30dpf. Methods in Section 12.1.11. Code in Section 16.9.5.

to believe that the CT estimates are likely too short, the ϵ estimates too high, all favoured in order to produce higher volume estimates at later ages.

Table 4.5: Maximum a posteriori parameter estimates for periodization models

Parameter	2-phase MAP	3-phase MAP
Phase 1 CT (h)	14.5	14.9
Phase 1 ϵ	2.02	1.95
Phase 2 CT (h)	17.5	75.6
Phase 2 ϵ	1.6	0.29
Phase 3 CT (h)	NA	68.4
Phase 3 ϵ	NA	0.27
Transition 1 age	23.2	47.9
Transition 2 age	NA	179.4

CT : cycle time. ϵ : niche exit rate. Methods in Section 12.1.11. Code in Section 16.9.5.

Because nested sampling naturally produces samples from the posterior, we can estimate posterior distributions using the evidence values for these samples. We performed this by kernel density estimation, specifically to investigate the extent to which the marginal posterior distributions for the selected model are polymodal; that is, the extent to which the evidence supports multiple hypotheses about the parameters of the two imputed phases. Kernel density estimates (KDEs) for marginal posterior distributions on the 2-phase models' parameters are presented in Figure 4.4.

These estimates reveal monomodal posterior distributions over the parameters. CT posteriors are well constrained to a small part of the sampling space (left panels, X axes, and top right panel), with the MAP parameter estimates given in Table 4.5 falling within the most dense regions. However, it is evident that the data do not constrain phase exit rates ϵ (left panels, Y axes) very well. That is, the marginal posterior probability distributions for these parameters are very widely spread. The marginal posterior distribution on the age at which the transition between phases occurs is plotted in the bottom right of Figure 4.4; the KDE suggests that the age of phase transition must be around 23 dpf.

We conclude that, while our global model of CMZ population and volumetric retinal contribution is too flawed to make good parameter estimates, a 2-phase model of this activity is far better substantiated by the evidence than a 3-phase model. We proceed on this basis, accepting the 2-phase model, and taking up the idea of the "slice model" introduced in Section 3.3.1, to investigate modelling the CMZ RPC population more concretely, directly from sectional observations, rather than from the calculated population and retinal volume estimates presented above.

4.4 Slice-model characterisation of asymmetrical CMZ population dynamics demonstrates anatomical homogeneity of proliferative schedule

In the course of the preceding investigations, it became apparent that the CMZ population asymmetry mentioned in Chapter 3 was not a static phenomenon, with the dorsal lobe of the CMZ annulus being consistently more populous than the ventral lobe, as generally implied by the sources covered in Chapter 1. Rather, both the extent and orientation of asymmetry seem to evolve over time. Sectional

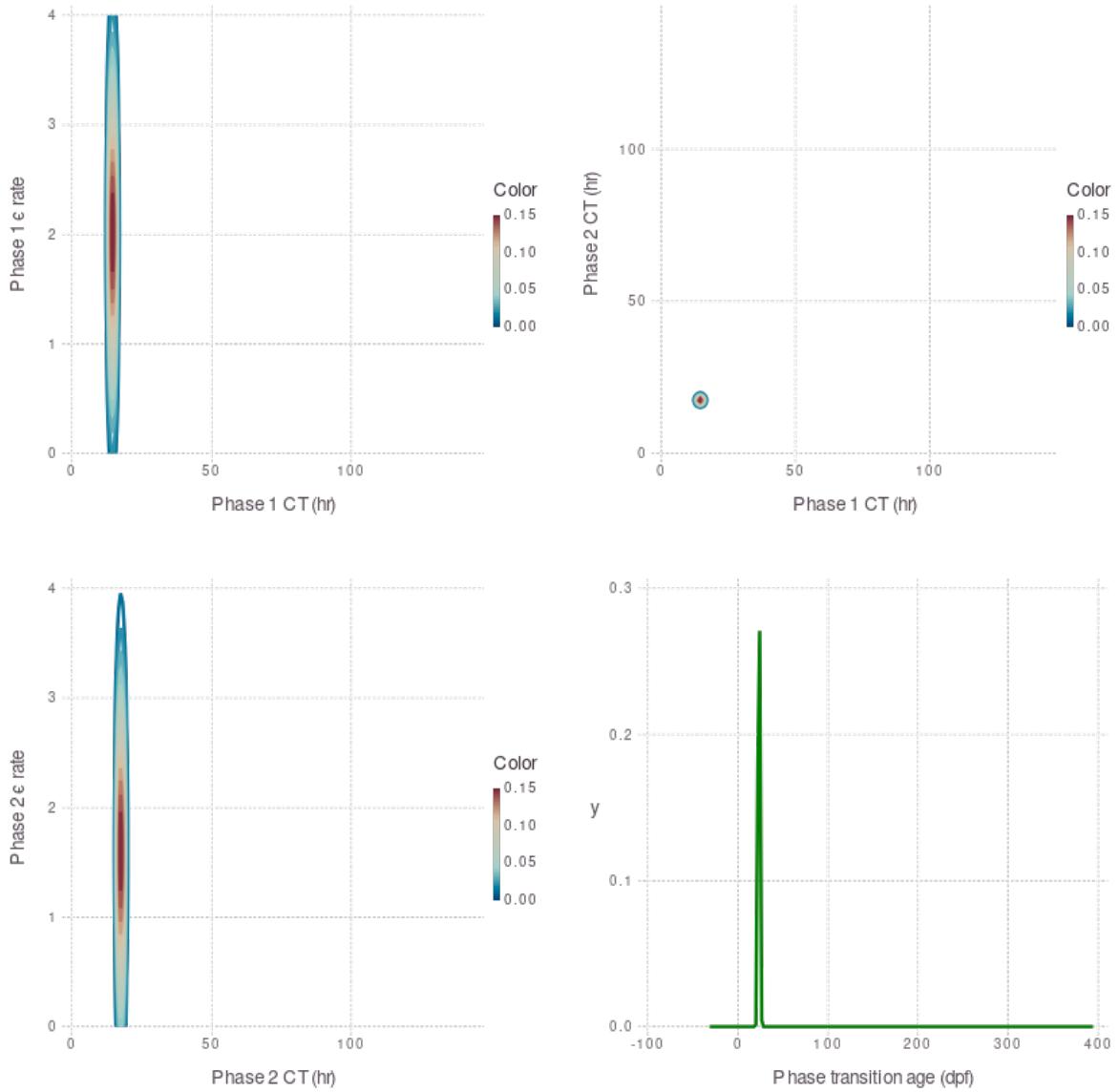


Figure 4.4: Kernel density estimates of marginal posterior parameter distributions, 2-phase model

Line height (bottom right panels) or color (other panels) indicates the estimated marginal posterior mass present at the indicated parameter value. Top left panel: Phase 1 ϵ exit rate vs Phase 1 CT cycle time (hr). Bottom left panel: Phase 2 ϵ exit rate vs Phase 2 CT cycle time (hr). Top right panel: Phase 2 CT cycle time (hr) vs Phase 1 CT cycle time (hr). Bottom right panel: Estimated marginal posterior mass vs. phase transition age (dpf) Methods in Section 12.1.12. Code in Section 16.9.5.

population totals for the dorsal and ventral CMZ are presented in Figure 4.5, Panel A, alongside the related intra-individual asymmetry ratio in Panel B. The initially pronounced dorsal population and reduced ventral population both seem to go through the overall boom-bust progression of CMZ population, but their relative proportion within individuals reverses itself over the period from 17-90dpf. We also observed a similar phenomenon occurring across the naso-temporal axis over the same time period

(Figure 12.2).

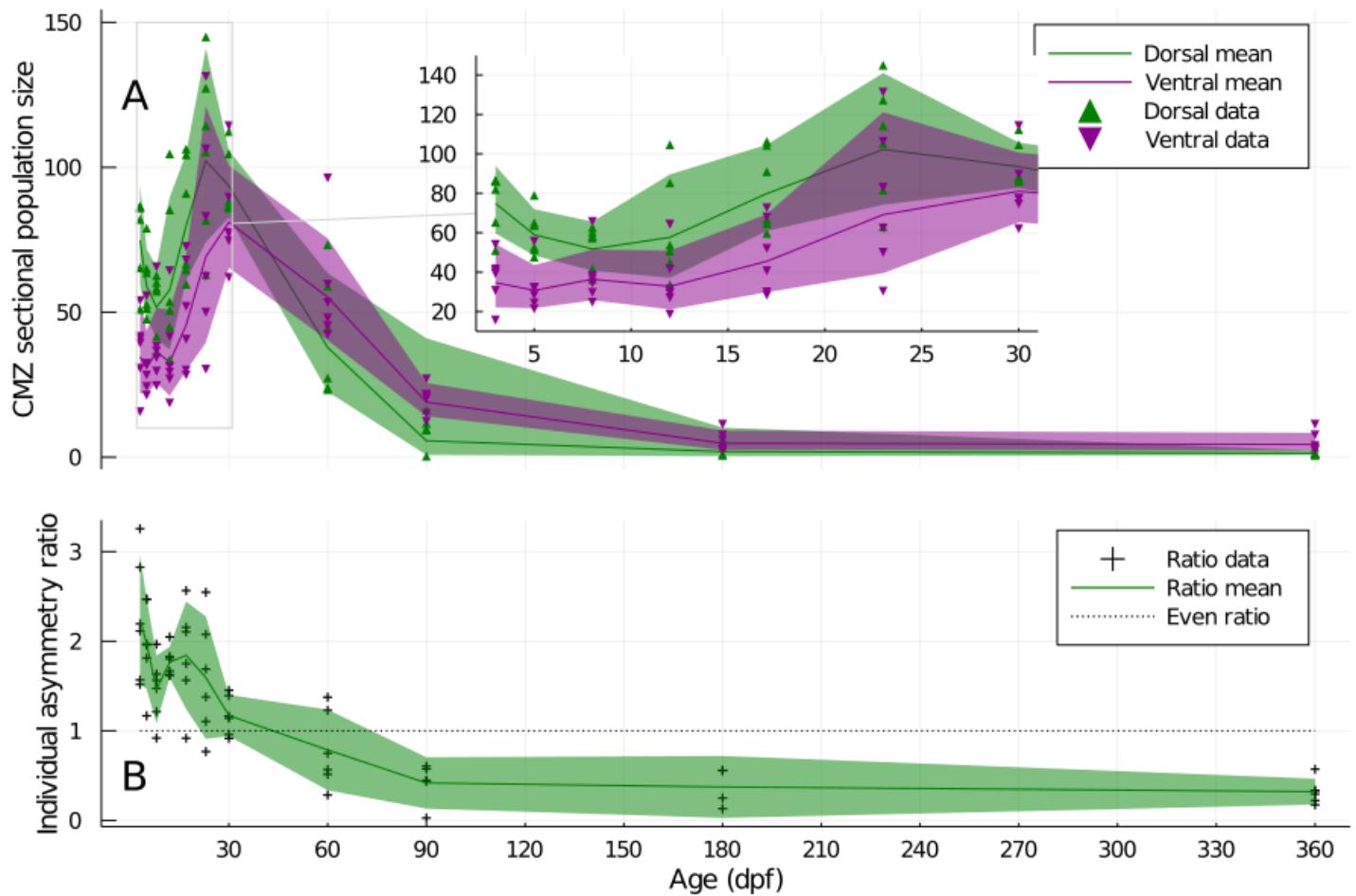


Figure 4.5: Developmental progression of dorso-ventral population asymmetry in the CMZ. Marginal posterior distribution of mean dorsal (D) and ventral (V) population size in $14\mu\text{m}$ coronal cryosections (panel A) or intra-individual D/V count asymmetry ratio (panel B), $\pm 95\%$ credible interval, $n=5$ animals per age. Data points represent mean counts from three central sections of an experimental animal's eye. Methods in Section 11.1.2.1. Code in Section 16.9.2.

Inspected closely, these data provide a possible rationale for the reversal of asymmetry in the proliferative dynamics of the niche itself: the sectional (or “slice”) population of the dorsal CMZ is increasing beyond its postembryonic minimum by 12dpf, while the ventral CMZ takes until 17dpf to exhibit a noticeable increase in size; moreover, the peak dorsal population is achieved by 23dpf, whilst ventrally the peak is only achieved at 30dpf. This suggests that the dorsal and ventral CMZ populations undergo similar, time-shifted processes of proliferation from different starting populations. If this is so, an explanation for this time-shifted phenomenon could have fundamental relevance to predicting and controlling the proliferative behaviour of peripheral RPCs and stem cells.

To test this hypothesis, we used a “slice model” of the CMZ, where the thickness of the slice is taken to be the same as the observed cryosection thickness ($14\mu\text{m}$). The population of the CMZ is modelled with a difference equation, as above, but with an additional exit term representing lateral,

circumferential contributions of the CMZ to the generation of new, adjacent “slices”. The value of this term is calculated from the difference in CMZ annulus diameter over the calculated time period, as implied by a power-law model of lens growth fitted to observations, discussed in Section 11.1.4. The resultant difference equation is Equation 4.3. Terms are as defined above, except that p_n is the sectional population at n dpf, and not the total CMZ annulus population; additionally, η is defined as the daily circumferential exit rate implied by the power-law model.

$$p_n = p_{n-1} \cdot 2^{\frac{24}{C\tau}} - p_{n-1} \cdot \epsilon - \eta \quad (4.3)$$

We reasoned that, if the phase transition occurs earlier in the dorsal CMZ than in the ventral CMZ, there should be some informational gain in separating these observations vs. a combined total sum for both lobes of the slice annulus⁴. In this case, the combination of two phase-shifted populations in the total model should produce a “fuzzy” peak relative to the separate modes. We therefore estimated the evidence, MAP, and posterior marginals for 2-phase models given the sectional sum, dorsal, and ventral populations. To focus on the most informative subset of the data for our hypothesis, we restricted this analysis to the population data within the first three months of life.

The slice model proves to have much greater success at explaining sectional counts than the whole-eye model does at explaining the annulus population estimates; maximum a posteriori model output is presented in Figure 4.6. In particular, all of the models adequately represent the early decline in sectional populations, arising from rapid early growth of the eye that exceeds the CMZs’ proliferative capacity, without further ado; there is no justification for the early quiescent period suggested above.

The hypothesis that there is a time-shift in the phase transition across the dorso-ventral axis is thoroughly refuted by these models, in two ways. First, the evidence estimates demonstrate that we are not justified in separating the dorsal and ventral populations. The total-population slice model receives greater than 500 orders of magnitude more evidence than the joint evidence for the separate dorsal and ventral models, with greater than 180 standard deviations of significance, as displayed in Table 4.6. It is interesting to note that the evidence for the dorsal model is substantially less than for either the total or ventral models. This may indicate a causal influence on the dorsal population that is neither in the model nor acting on the ventral population, or it may be an uninformative sampling effect.

Table 4.6: Evidence favours a combined slice model over separate dorsal and ventral models

Total logZ	Dorsal logZ	Ventral logZ	logZR	σ Significance
-547.6 ± 1.8	-673.5 ± 1.9	-405.4 ± 1.2	531.2 ± 2.9	182.214

logZ: logarithm of $p(D)$, the marginal likelihood of the data, or model evidence. logZR: evidence ratio; positive ratio in favour of the combined model. Largest evidence value bolded. Methods in Section 12.1.11. Code in Section 16.9.3.

A second indication that this hypothesis is unsupported are the MAP model parameter values, summarized in Table 4.7. The MAP phase transition age for the total slice model is functionally identical⁵ to that for the ventral model, and within two days of the MAP transition for the dorsal model. Additionally, the dorsal MAP transition is actually later than the ventral date, which further suggests the

⁴The “total” model is required to supply double the circumferential exit rate of the dorsal or ventral models, to reflect the requirement for this “full” slice to grow both lobes of the eye

⁵That is, both values round to 36 days in the likelihood function, which uses the discrete difference equation, with a step size of 1dpf, as indicated above.

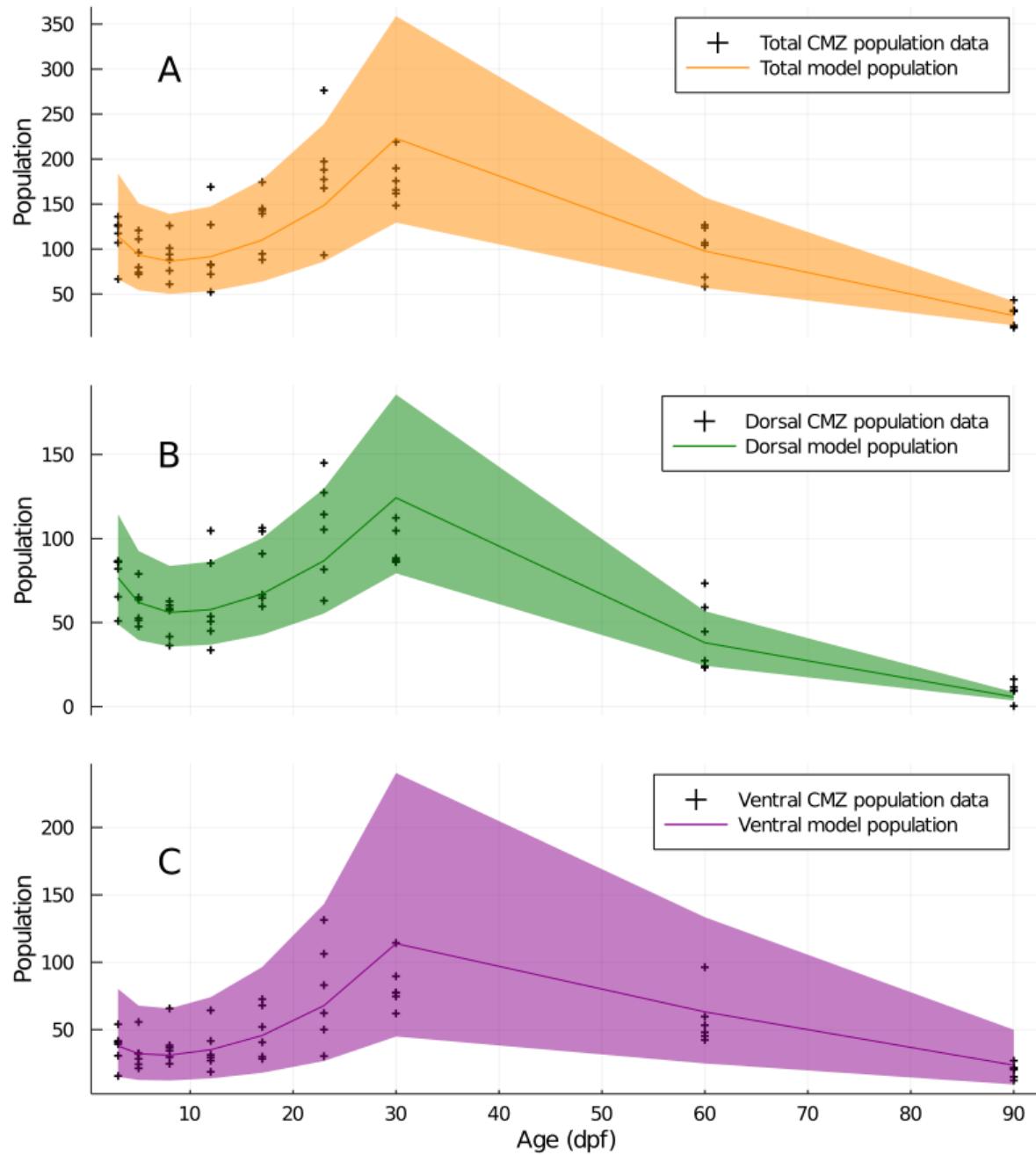


Figure 4.6: Maximum a posteriori output of total, dorsal, and ventral CMZ slice models
 Population and volume estimates from observations (crosses) plotted with mean \pm 95% posterior mass model output, for the total CMZ population model (panel A), dorsal CMZ population model (panel B), and ventral CMZ population model (panel C). Methods in Section 12.1.11. Code in Section 16.9.3.

original model-idea of a time-shifted late ventral phase change is unsupported by these data.

The MAP parameters for the total slice model confirm our supposition that those found for the 2-phase MAP in Figure 4.4 overstate cell cycle speed and exit rate. The total slice model gives *CTs* of

24.2 and 25.1 hours for the first and second phases, compared to 14.5 and 17.5 estimated previously. Moreover, the niche exit rates ϵ are estimated at .91 and .98 for the total slice model, compared to 2.02 and 1.6 for the 2-phase model. The values estimated for the slice model are likely to be more realistic, and suggest that the overall magnitude of the changes between phases is subtle. Interestingly, the MAP phase parameters differ markedly between the split dorsalventral models and the total slice model. Surprisingly, the dorsal estimates suggest a faster CT for the second phase. This may indicate that additional noise incurred from splitting the sectional CMZ population into dorsal and ventral lobes has a strong effect on parameter estimates. This may provide a practical reason to prefer combining these counts in slice models, beyond the calculations presented here.

Table 4.7: Maximum a posteriori parameter estimates for slice models

Parameter	Total MAP	Dorsal MAP	Ventral MAP
Phase 1 CT (h)	24.2	30.5	19.7
Phase 1 ϵ	0.91	0.65	1.23
Phase 2 CT (h)	25.1	19.5	30.1
Phase 2 ϵ	0.98	1.4	0.76
Transition age	36.3	37.7	36.0

CT : cycle time. ϵ : niche exit rate. Methods in Section 12.1.11. Code in Section 16.9.3.

The marginal posterior distributions of the total slice model, presented in Figure 4.7, are substantially more polymodal than those in Figure 4.4. This illustrates how data can support multiple hypotheses about model parameters to different degrees. Exit rate ϵ posteriors are no less constrained than the whole-eye model, suggesting the retinal volume estimate supplies little additional information on the rate at which RPCs leave the niche. However, the marginal posteriors on cycle length CT are less constrained than the whole-eye model, so the volume estimate may nonetheless narrow the range of credible CT values. This may be a rationale for pursuing better volumetric estimates for retinae from fish older than 30 dpf. The posterior distribution on the age of phase transition indicates that many values for this parameter retain some credibility. The MAP estimate does not occupy the most probable KDE kernel for this value. An important property of nested sampling is that the accuracy of evidence calculations is traded off against the accuracy of estimating the posterior distribution, as noted in Section 14.2.6. Since we have here prioritized evidence estimation, this is the cause of this discrepancy; the MAP models themselves have relatively little weight in the KDE estimates.

On the basis of this analysis, the best available hypothesis about the observed ontogeny of RPC population asymmetry across the dorsoventral axis is the pre-establishment of differential population size by asymmetrical progress of the embryonic specification wave, rather than differential proliferative schedules.

4.4.1 Cumulative thymidine labelling supports Galilean Monte Carlo Nested Sampling inference

To investigate the accuracy of the parameter estimates provided by the model studies above, we sought to confirm their plausibility by examining 3dpf CMZ RPCs cumulatively labelled with a 10.5 hour pulse of the thymidine analogue EdU. We used the Empirical Bayes approach to estimate the evidence for separate Nowakowski-style [NLM89] linear models for the dorsal and ventral CMZ, against a model for

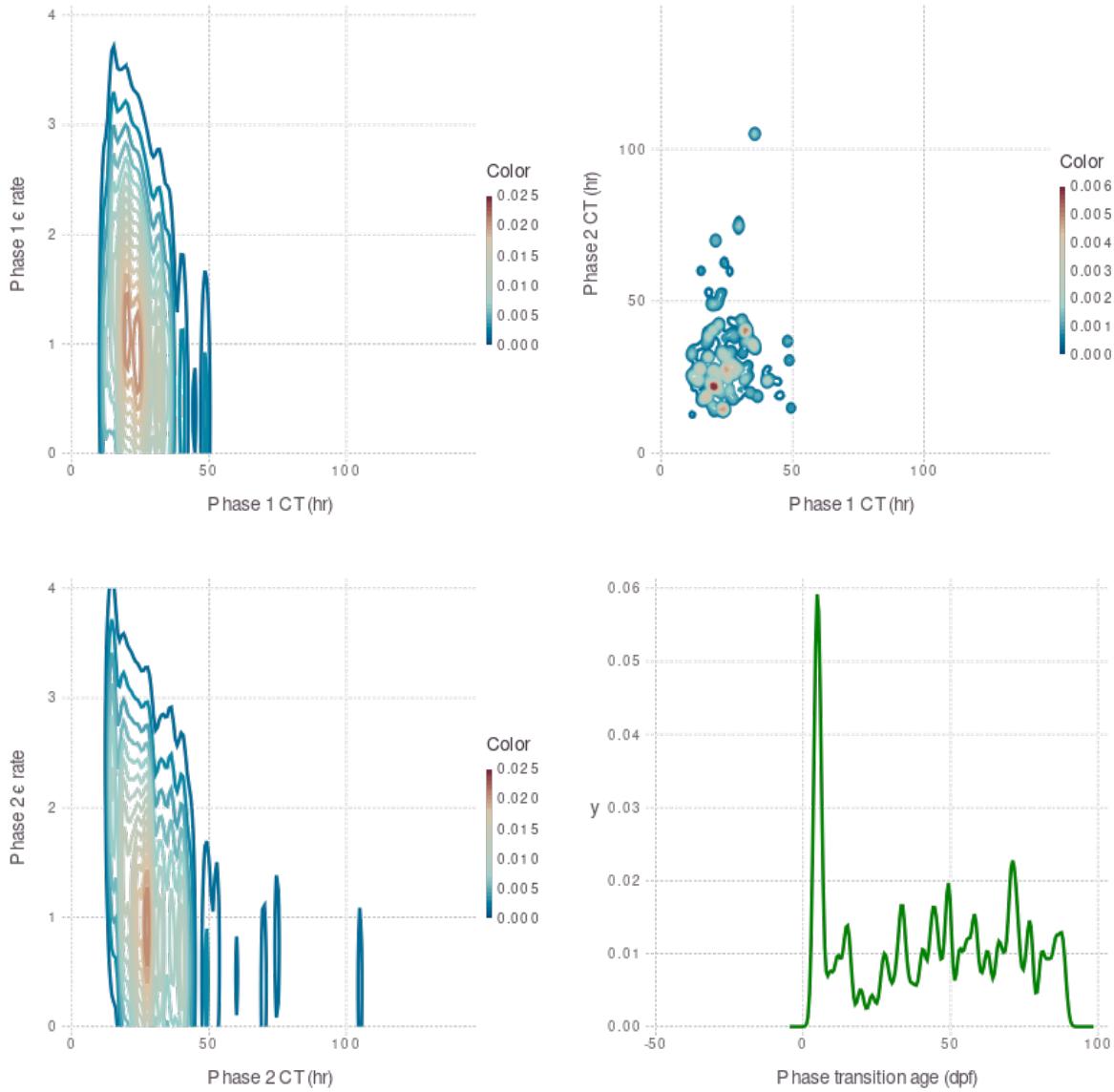


Figure 4.7: Kernel density estimates of marginal posterior parameter distributions, total slice model

Line height (bottom right panels) or color (other panels) indicates the estimated marginal posterior mass present at the indicated parameter value. Top left panel: Phase 1 ϵ exit rate vs Phase 1 CT cycle time (hr). Bottom left panel: Phase 2 ϵ exit rate vs Phase 2 CT cycle time (hr). Top right panel: Phase 2 CT cycle time (hr) vs Phase 1 CT cycle time (hr). Bottom right panel: Estimated marginal posterior mass vs. phase transition age (dpf) Methods in Section 12.1.12. Code in Section 16.9.3.

both subpopulations combined. While this model is inadequate for reasons described in ??, it can serve to substantiate the first phase cycle time parameter. These results are summarized in Table 4.8, with the relevant linear regressions displayed in Supplementary Figure 12.4.

Firstly, there are approximately 3 orders of magnitude of evidence in favour of the combined model vs. the joint evidence for separate models (ie. 26.165 vs. 22.980). This model provides no support

Table 4.8: Evidence favours whole-CMZ linear cycle models over separate D/V models

Model	Implied T_c (hr)	Implied T_s (hr)	logZ
Dorsal	14.7 ± 1.6	1.38 ± 0.76	7.778
Ventral	14.0 ± 1.2	0.8 ± 0.58	15.202
Combined	14.6 ± 1.1	1.25 ± 0.53	26.165

T_c : calculated cell cycle time. T_s : calculated s-phase length. logZ: logarithm of p(D), the marginal likelihood of the data, or model evidence. Largest evidence value bolded. Methods in Section 12.1.10. Code in Section 16.9.9.

for differing cell cycle characteristics across the D/V retinal axis of asymmetry at 3dpf. This confirms that the differing D/V MAP parameter estimates observed in the slice model are unsubstantiated; the evidence supports broadly similar cell cycle characteristics across anatomical axes in 3dpf CMZ RPCs. The Nowakowski-calculated cell cycle time T_c for the combined model, 14.6 ± 1.1 hr, includes the whole-eye MAP first phase TC , 14.5 hr, within one standard deviation, although not the first phase TC , 24.2 hr. This suggests the Nowakowski model has similar problems to the whole-eye models presented in Figure 4.3, substantially overstating cycle rate. Calculated S phase lengths are also unrealistically short. Finally, the data clearly diverge from a linear trend toward the end of the pulse (see Figure 12.4), showing the limitations of this model.

4.5 The CMZ contributes stably to each cellular layer with time-variable lineage composition

By labelling CMZ RPCs with the thymidine analogue EdU in a pulse at 3, 23, and 90 dpf, followed by histochemical analysis for known zebrafish retinal neural lineage markers after a 7 day chase, we investigated the possibility that RPC lineage outcomes change over the life of the organism. This hypothesis is of particular interest, as differences in the mosaic organisation of embryonically-contributed central retina and CMZ-contributed peripheral retina remain unexplained [ABS⁺10]. It may, moreover, have clinical significance, were quiescent peripheral stem cells to be entrained for regenerative medical purposes, as their lineage outcomes may be different than embryonic RPCs. We used antibodies raised against Pax6 and Isl2b to mark retinal ganglion cells (RGCs) of the ganglion cell layer and amacrine cells of the inner nuclear layer. Anti-glutamine synthetase (GS) and anti-PKC β were used to mark Müller glia (MG) and bipolar cell (BPC) populations of the INL. The unique flattened nuclear morphology of horizontal neurons was used to identify them. Lastly, the antibody Zpr1, directed against an unknown antigen present in photoreceptors with double cone morphology, was used to mark these cells.

Observations were collected in "staining groups", which combined histological markers; representative confocal micrographs from this study in animals pulsed at 23dpf are displayed in Figure 4.8, while data from all ages are plotted in Figure 4.9. It is worth noting that the relative position of the CMZ-contributed cohort is very different in older animals, with 7 days of chase time being just enough for the majority of the 90dpf cohort to be reliably located within the specified neural retina, as depicted in supplementary ???. Because we suspect that CMZ-contributed retinal cohorts may be subject to a process of attrition, and that this turnover might be higher near the CMZ, as discussed in Section 4.6, if this hypothetical turnover process has differential effects over time on specified retinal neurons of

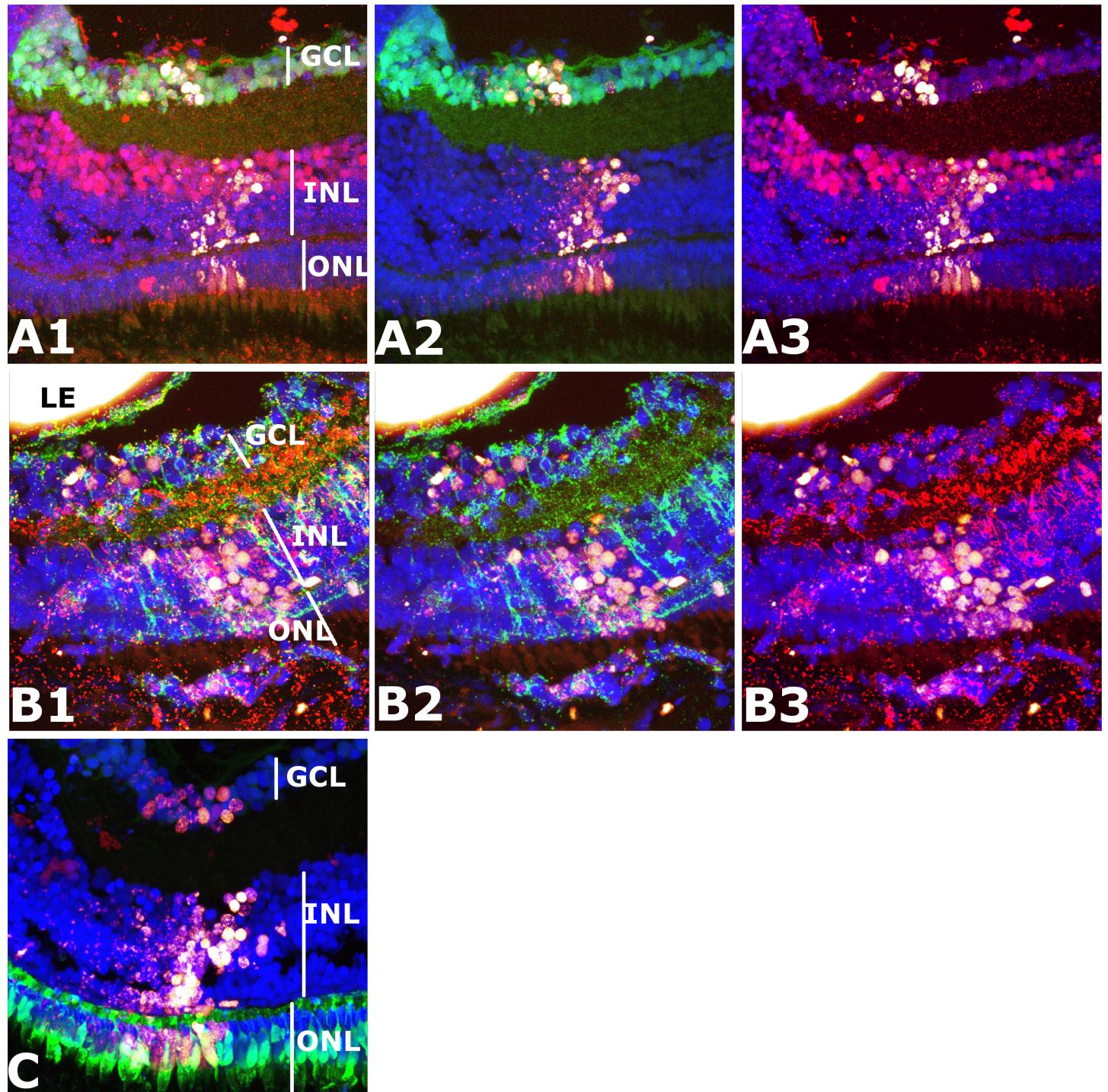


Figure 4.8: Representative 23dpf lineage marker confocal micrographs
Panel A1: RGC/Amacrine staining group. A2: Isl2b channel. A3: Pax6 channel.
Panel B1: MG/BPC staining group. B2: PKC β channel. B3: GS channel.
Panel C: Double cone staining group. Zpr1 channel.
GCL: Ganglion cell layer. INL: Inner nuclear layer. ONL: Outer nuclear layer. Methods in ??.

different lineages, results may not be directly comparable between ages. With that caveat, we proceed to the lineage data.

These data take the form of fractions of the thymidine-labelled cohort entering each of the three cellular layers (panel A of Figure 4.9), or the subfraction of the cohort within a given layer expressing a particular cellular marker (Panels B-I). While some variability is apparent in all of the measurements, it is unclear whether it is well-described as time-dependent in most cases. In order to address the question of whether CMZ layer or lineage outcomes differ over time, we needed to assess the joint evidence for separate models of each measurement at each age, against the evidence for a single model of the measurement for all of the assessed ages.

Because it is not obvious that these fractional measurements are better described Log-Normally as the underlying population counts are, we first assessed the joint evidence for Normal and Log-Normal models of the data, summarized in Supplementary Table 12.1. Our evidence supports Log-Normal modelling of all measurements aside from the INL and ONL, as well as INL-resident Pax6- and ONL-resident Zpr1-positive cells. We noted that the evidence estimates confirmed simple likelihood ratio tests in most cases, as summarized in Supplementary Section 12.4, with only INL totals, GS+, ONL total, and Zpr1+ results disagreeing with the evidence estimates. This demonstrates a case where full evidence estimation can produce differing results from maximum likelihood methods for multiple measurements. Without a clear fundamental justification for uniformly preferring one model or the other, we selected the best-supported model for each measurement. We estimated the evidence for an age-marginalized model, representing stable contribution to the layer or lineage over time, and compared this to the joint evidence for separate models at each age, representing a time-varying contribution model. These estimates are presented in Table 4.9.

Table 4.9: Evidence supports stable layer contributions with time-varying lineage contributions

Layer	Marker	Cell type	Stable logZ	Time-vary logZ	logZR	σ sign.
GCL	Cohort	All GCL cells	-53.473 ± 0.055	-63.93 ± 0.19	10.46 ± 0.2	52.8
GCL	Isl2b	RGC	-54.58 ± 0.9	-56.3 ± 0.76	1.7 ± 1.2	1.4
GCL	Pax6	Displaced am.	-31.09 ± 0.12	-35.39 ± 0.34	4.3 ± 0.36	12.0
GCL	Isl2b/Pax6	RGC subtype	-43.76 ± 0.76	-16.38 ± 0.18	-27.37 ± 0.78	34.9
INL	Cohort	All INL cells	-131.4 ± 1.4	-84.56 ± 0.91	-46.9 ± 1.7	27.8
INL	Pax6	Amacrine cell	-15.8 ± 0.024	-13.99 ± 0.24	-1.81 ± 0.24	7.5
INL	PKC β	Bipolar cell	-3.86 ± 0.2	8.68 ± 0.44	-12.54 ± 0.48	26.0
INL	GS	Müller glia	15.78 ± 0.25	14.18 ± 0.48	1.6 ± 0.54	3.0
INL	HM	Horizontal cell	10.79 ± 0.41	9.15 ± 0.37	1.65 ± 0.55	3.0
ONL	Cohort	All ONL cells	-79.87 ± 0.97	-128.68 ± 1.0	48.8 ± 1.4	35.1
ONL	Zpr1	Double cones	-54.66 ± 0.92	-42.25 ± 0.72	-12.4 ± 1.2	10.6

logZ: logarithm of p(D), the marginal likelihood of the data, or model evidence. Largest evidence values bolded. logZR: evidence ratio; positive values in favour of stable model. Methods in Section 12.1.4, Section 12.1.11. Code in Section 16.9.7.

These calculations demonstrate that there is evidence for time-varying contributions to the retina, in particular to the INL overall, and to amacrine, bipolar, and Müller glial fates. Additionally, time-varying models of Isl/Pax6+ RGCs in the GCL, and Zpr1+ double cones of the ONL are all well-supported.

All of these findings have between 3 and 35 standard deviations of significance. In the INL, the trend of the posterior mean for PKC β -positive bipolar neurons (panel F and GS-positive Müller glia of these lineages declines from the early postembryonic period (3dpf) to the late juvenile period (90dpf). Mean GCL Isl/Pax6-positive RGCs and ONL Zpr1-positive double cones also trend downward. By contrast, the posterior mean for Pax6-positive amacrine cells increases before declining. The trend of mean INL contributions is constant over this period, but variability increases markedly at 90dpf, justifying the time-varying model. These results show that CMZ RPCs do not contribute lineages in stereotypical proportions throughout the organism's lifetime; in fact, the proportions of particular lineages change over time, and contributions to the INL in particular become more variable. Since there are declining lineages in each of the three retinal layers, these lineages may be functionally related. However, we have no specific evidence that would implicate Isl2b/Pax6 double-positive RGCs in circuits with bipolar cells or double cones, for instance. In any case, this provides an explanation for the observation of a more-ordered retinal mosaic in later retinal contributions relative to the embryonic central remnant: since one or more sublineages in each layer are depleted in older fish, a different overall mosaic pattern as the neurons associate and pack together is expected. Based on the timing of these changes, we tentatively ascribe this differential lineage production to the second phase of CMZ contribution in our periodisation; the data do not have enough time resolution to test this with more precision.

Lastly, we investigated the possibility that there might be detectable differences in layer or lineage contributions across the dorso-ventral axis; we tested this by measuring the evidence for age-marginalized combined models of fractional contribution against age-marginalized models split along the dorso-ventral axis. We found no evidence to support this hypothesis. These results are presented in Summary Table 12.2.

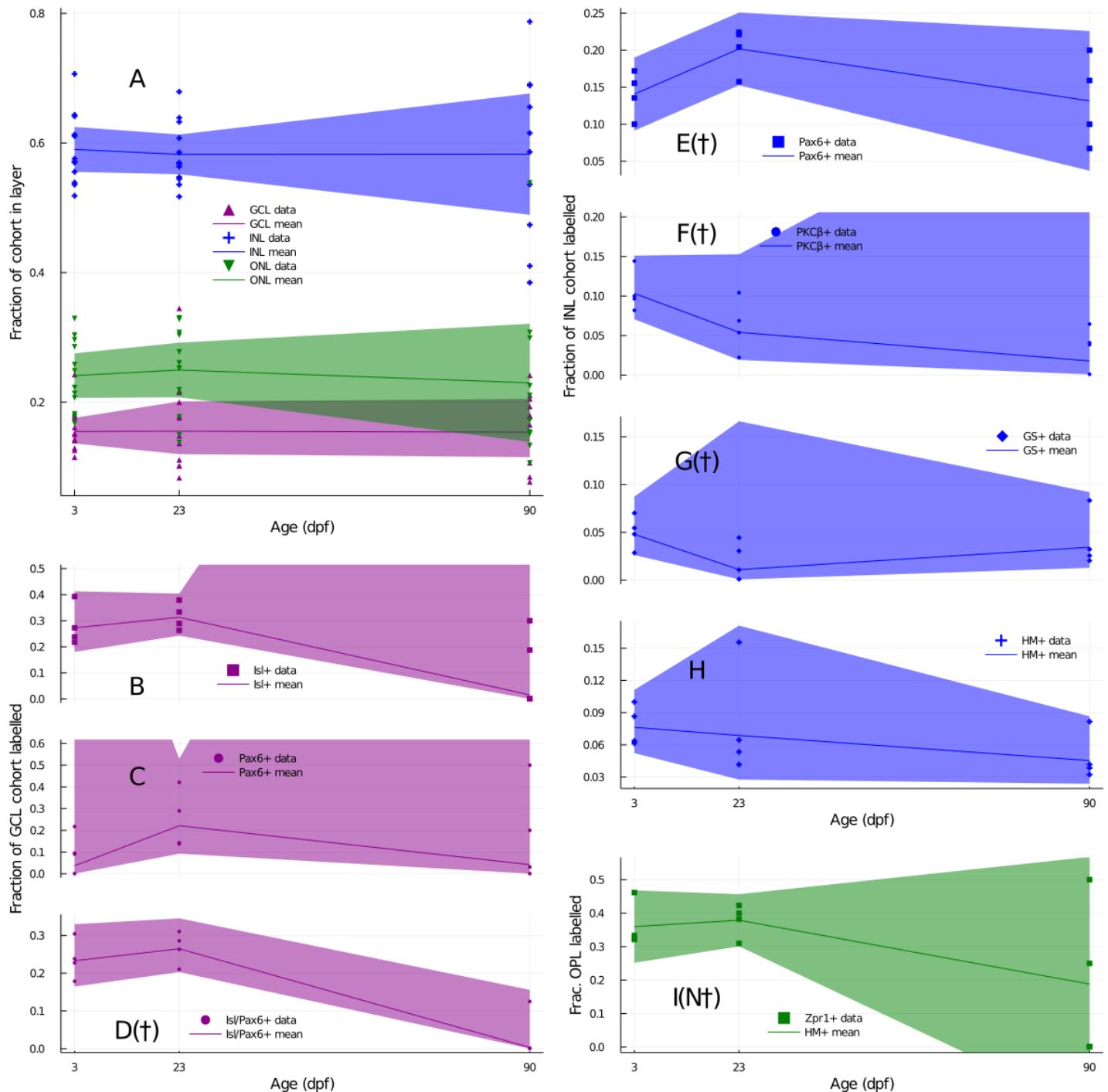


Figure 4.9: Second-phase declines in CMZ-contributed IslPax6+ RGCs, PKC β + bipolar neurons, and Zpr1+ double cones

Overall fraction of CMZ-contributed cohort entering cellular layers (Panel A), or fraction of the layer subcohort expressing the noted immunohistochemical marker of lineage. All mean values are presented as marginal posterior means \pm 95%CI.

λ : Fractional lineage contribution is modelled Log-Normally

\dagger : Evidence supports time-varying model of fractional lineage contribution

Magenta: GCL measurements; Blue: INL measurements; Green: ONL measurements

Methods in Section 12.1.4, Section 12.1.11. Code in Section 16.9.7.

4.6 Early retinal cohorts of the *D. rerio* retina are turned over at a low rate by 4C4-positive microglia

Recently, extensive neural death has been reported in older zebrafish retinae [VGV⁺18]. This was described as a “neurodegenerative pathology” and suggested as a model of age-related neurodegeneration. However, during our thymidine analogue pulse-chase studies, CMZ-contributed cohorts often appeared less numerous only a month or two after their entry into the neural retina, even in juveniles of 30–90 days of age. Moreover, we observed numerous 4C4-positive microglia associating with the CMZ, as displayed in Supplementary ??, which suggested that microglia may prune CMZ contributions. If neural retinal turnover occurs throughout the life of the organism, this would belie view that it should be treated as pathological, rather than constitutive. However, the thinning phenomenon could be explained by the changing morphology and geometry of the neural retina during this period. In particular, the neural retina thickens noticeably over this time, as displayed in Supplementary Figure 12.1. Although this increase is due, in large part, to the lengthening of photoreceptor outer segments, the inner nuclear layer is also significantly thickened. It is plausible that this process involves a compaction of the neurons along the coronal axis typically sampled, thus appearing to lose cells over time without this actually occurring.

In order to investigate this phenomenon, we administered 24 hr pulses of EdU to 1dpf embryos, and followed with 24 hr pulses of BrdU at 23dpf to mark a CMZ-contributed cohort near the height of its activity. By taking both coronal and transverse sections through animals at 30, 60, and 90 dpf, we sampled these cohorts from both morphological axes of the retina and counted labelled sectional totals. Data from the axes were combined to account for the possibility of the cohorts being compacted on the coronal plane and extended on the transverse one. In order to test the hypothesis of early turnover, we estimated the evidence for linearly correlated and uncorrelated models of cohort population over time, using the Empirical Bayes regression method. In effect, we suppose that if the addition of a time-dependent term in the correlated regression model is justified by the evidence at these early ages, this supports the notion that *D. rerio* retinal turnover is a lifelong phenomenon; if not, the apparent contraction of the cohorts is more likely a function of the alternative explanations noted above, or is occurring at too low a rate to be detected by these means. The results are summarized in Table 4.10, with the regressions plotted in Supplementary Figure 12.3.

Table 4.10: Evidence for linear regression models supports early cohort population stability

Measurement	Stable logZ	Declining logZ	logZR
1dpf Central Remnant	-212.953	-214.167	1.213
30dpf Cohort	-107.567	-110.827	3.261

logZ: logarithm of $p(D)$, the marginal likelihood of the data, or model evidence. Largest evidence values bolded. logZR: evidence ratio; positive values in favour of stable model. Methods in Section 12.1.3, Section 12.1.10. Code in Section 16.9.12.

In both cases, there is more evidence for a model of cohort population that is stable over time than one correlated with time, indicating that the additional model complexity implied by allowing turnover is unjustified for this early period. Despite this, we did find isolated instances where members of these cohorts were visibly being engulfed by 4C4-positive microglia; one such event is depicted in Figure 12.5. Moreover, we also observed TUNEL-positive nuclei in the central retina of *rys* siblings in the early

postembryonic period (Figure 5.6). This indicates that some level of turnover is indeed occurring during this earlier period. The evidence ratio in favour of the uncorrelated models is not overwhelming, which suggests that the best interpretation of the data is that the cohorts are not turned over at a high enough rate to be detectable in the early period, although the process does occur even at this early age. In order to confirm this finding, we followed with estimating the evidence for age-marginalized Log-Normal models of the population counts against age-differentiated models, representing time-constant and time-varying population models. This investigation proves to resolve any ambiguity: no time-varying model is justified by the available evidence, as summarized in Table 4.11.

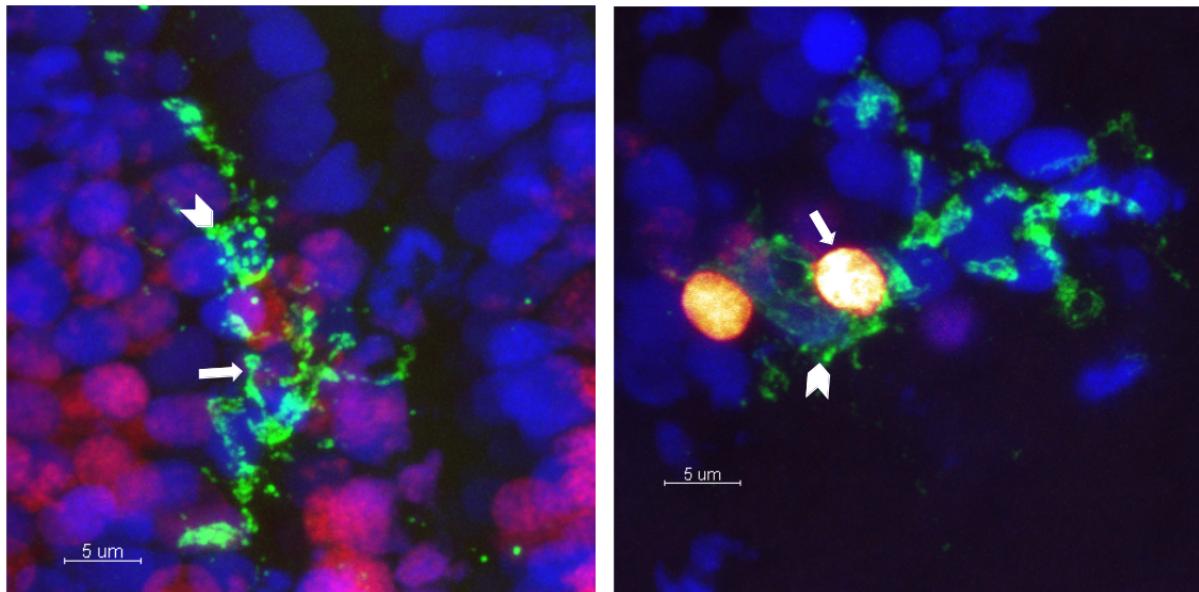


Figure 4.10: 4C4+ microglia associate with and engulf EdU-labelled CMZ contributions in the specified neural retina

Representative maximum intensity projections from confocal micrographs of 14 μ m coronal cryosections through 30dpf zebrafish eyes labelled at 23dpf with EdU.

Blue: Hoechst 33258 nuclear counterstain. Green: Microglia labelled with 4C4 antibody. Red-orange-yellow: Intensity scale of EdU staining, indicating cellular origin in the 23dpf CMZ.

Chevrons: microglial nuclei. Arrows: EdU-positive nuclei found within 4C4-labelled extent of microglial cell body and appendages.

Methods in Section 12.1.5.

Table 4.11: GMC-NS evidence estimates confirm Empirical Bayes analysis of early cohort stability

Cohort	Time-constant logZ	Time-varying logZ	logZR	σ Significance
Embryonic central remnant	-868.79 ± 0.79	-950.5 ± 1.5	81.7 ± 1.7	47.9
1 month CMZ	-399.78 ± 0.22	-404.05 ± 0.38	4.26 ± 0.43	9.8

logZ: logarithm of p(D), the marginal likelihood of the data, or model evidence. logZR: evidence ratio; positive ratios in favour of the 2-phase model. Largest evidence value bolded. Methods in Section 12.1.3, Section 12.1.11. Code in Section 16.9.11.

4.7 Summary: Two-phase periodisation of postembryonic CMZ activity & functional significance of CMZ activity

Our data is best explained by a 2-phase periodization of postembryonic CMZ activity, with an initial month-long phase of relatively rapid proliferation and lower rate of exit into the specified neural retina, followed by a second phase of slower proliferation and higher exit rate. We have established posterior distributions on likely parameter ranges that convey the uncertainty we have about them; these demonstrate that our estimates of cell cycle lengths are much more certain than of exit rates. The population of the CMZ displays an asymmetric structure which reverses over time; we have shown this is not due to time-shifting of the two proliferative phases across anatomical axes. The proportional layer composition of CMZ-driven contributions is stable over this time, and does not display variability over the dorsoventral axis. However, by estimating the evidence for time-stable and time-varying models of lineage contribution, we identified a decline in particular retinal lineage subtypes, particularly those of the INL, but including the GCL and ONL. This a potential explanation for the teleost postembryonic change in retinal mosaic pattern. Finally, we have shown that microglia-mediated turnover of retinal neurons is occurring at a rate too low to have a measurable effect on cohort sizes during this time.

These studies demonstrate that the niche history of the CMZ is not adequately explained by a “frozen” population of RPC progenitors, homeostatically recapitulating the program of development found in embryonic progenitors, as has been repeatedly advanced previously [HP98, WAR⁺16]. Instead, a month-long coordinated buildup in population is followed by a concerted slowing of proliferation and increase in the rate of retinal growth in the second month of life. This establishes that the postembryonic CMZ is under a separate regulatory regime from the embryonic RPCs which contribute the central, larval remnant. This period of RPC activity requires separate modelling and investigation. If, in *D. rerio*, an initially productive peripheral proliferative phase is followed by a second phase decline into quiescence, this regulatory pattern may be common across vertebrate species, and its mechanistic explanations must form the basis of any attempt to entrain endogenous mammalian peripheral retinal stem cells for regenerative medicine.

We are interested in identifying macromolecular factors involved in the control of proliferation and fate specification of CMZ RPCs. These analyses suggest that these may be most profitably investigated at different ages. If we take the phase transition times estimated in Section 4.4 to be the most reliable in this chapter, this would suggest that factors implicated in proliferative control would be best identified by assays bracketing the transition time at 35dpf; we would expect the rate of change of macromolecular correlates of cycle activity to be greatest around this time. There seems to be little spatial structure apparent in proliferative activity, and it would be unnecessary to break out these data by anatomical location, as the population size asymmetry is smallest at this time.

Overall, the data collected thus far provide the weakest support for hypotheses about RPC fate specification and niche exit. This emphasizes the sense in which proliferative and specificative activities of RPCs must be treated separately, since separate datasets, more carefully calibrated than the crude anatomical estimates used here, will be required to estimate parameters related to RPC contribution to the retina more precisely. While we were able to demonstrate an overall change in lineage subtype contribution, these pulse-chase data were not able to inform the analysis of niche exit rate. The use of saturating labelling concentrations and times in our lineage tracing experiments prevent gleaning more information about niche exit from them. The use of multiple, separately labelled thymidine analogues

administered in pulses separated by a day could be a cost-effective way to inform models of niche exit and retinal contribution of the later postembryonic CMZ. Ultimately, useful explanations of retinal lineage contribution are likely to require more explicit spatial representation in order to incorporate physical effects, signalling hypotheses, and the like.

In addition to discovering unique features of postembryonic CMZ activity, we have proved out a general logic for Bayesian evaluation and selection of arbitrary biological models, which can inform both future experiments as well as modelling and theoretical choices. For instance, the slice model proves to be a more successful way to combine population and morphological information to answer questions about CMZ population structure, than are abstract whole-population models of the CMZ. Given the relative ease of producing datasets for such models, we would likely do well to establish a multiple-label thymidine analogue experimental paradigm that uses this model form for analysis. From the foregoing analyses, we conclude that treating the CMZ population as a combined unit with shared proliferative parameter evolution is likely well justified, suggesting that much of the apparent complexity of the niche's population history can be usefully abstracted away.

Chapter 5

Mutant npat results in nucleosome positioning defects in *D. rerio* CMZ progenitors, blocking fate specification but not proliferation

5.1 Introduction

The zebrafish (*D. rerio*) circumferential marginal zone (CMZ), located in the retinal periphery, contains the retinal stem cells and progenitors responsible for the lifelong retinal neurogenesis observed in this cyprinid. Analogous to CMZs in other model organisms, such as *X. laevis* [PKVH98], it has been of particular interest to us since the discovery of quiescent stem cells at the mammalian retinal periphery [Tro00], as an understanding of the molecular regulation of this proliferative zone may shed light on whether these mammalian cells might be harnessed for the purpose of regenerative retinal medicine. While significant progress has been made in this direction [RBBP06], molecular lesions in a plethora of zebrafish mutants displaying defects in CMZ development and activity remain largely uncharacterised. We examine here one such microphthalmic line identified in an ENU screen, *rys* [WSM⁺05], characterised by Wehman et al. as a Class IIA CMZ mutant, with a small eyes (see Figure 5.1) and apparently paradoxically enlarged CMZ.

Mapping revealed the causative *rys* mutation in the zebrafish npat gene, the nuclear protein associated with the ataxia-telangiectasia locus in mammals [IYS⁺96]. Although npat is heretofore uncharacterised in zebrafish, its mammalian homologues, human NPAT and mouse Npat, have been extensively examined. These studies demonstrated that NPAT plays a critical role in coordinating events associated with the G1/S phase transition in proliferating cells [YWNH03]. S-phase entry requires tight co-ordination between the onset of genomic DNA synthesis and histone production, in order to achieve normal chromatin packaging and assembly. NPAT, found in the nucleus [STN⁺02] and localised, in a cell-cycle dependent manner, to histone locus bodies [GDL⁺09], induces S-phase entry [ZDI⁺98] and activates replication-dependent histone gene transcription by direct interaction with histone gene clusters [ZKL⁺00] in association with histone nuclear factor P (HiNF-P) [MXM⁺03]. The protein's effects

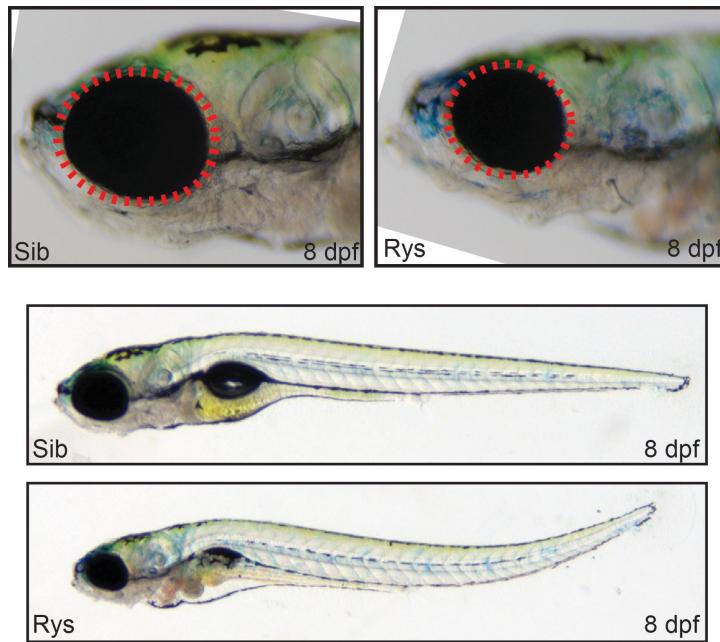


Figure 5.1: *rys* mutants exhibit a small-eye phenotype

8 dpf *rys* sibling (Sib) and mutant (Rys), head area (top panels), whole body (bottom panels). Red dashes highlight the overall reduced eye size in homozygous *rys* mutant animals.

on S-phase entry and histone transcription are associated with distinct domains at the C-terminus and N-terminus, respectively [WJH03]. NPAT is also known to associate with the histone acetyltransferase CBP/p300 [WIE04] and directs histone acetylation by this enzyme [HYSL11].

NPAT is involved in E2F transcriptional regulation [GBB⁺03] and is substrate of cyclin E/CDK2 [ZDI⁺98], although E2F-independent activation by cyclin D2/CDK4 in human ES cells has also been described [BGL⁺10]. The expression of NPAT protein peaks at the G1/S boundary [ZDI⁺98], as does its phosphorylation, which promotes its transcriptional activation of replication-dependent H2B [Ma00] and H4 [MGv⁺09] genes, while its effect on low, basal levels of H4 transcription is phosphorylation-independent [YWNH03]. Of particular interest, NPAT has recently been found to be required for CDK9 recruitment to replication-dependent histone genes [PJ10]; CDK9 and monoubiquitinated H2B are essential for proper 3' end processing of stem-loop histone transcripts [PSS⁺09]. NPAT and HiNF-P have also been found associated with the U7 snRNP complexes that perform this function [GDL⁺09]. The replication-dependent activities of NPAT are thought to be terminated by WEE1 phosphorylation of H2B, which excludes NPAT from histone clusters [MFKM12].

All of these studies were performed in tissue culture, perhaps due to the challenges associated with studying this critical protein *in vivo*; mouse embryos with provirally inactivated Npat arrest at the 8-cell stage, for instance [DFWV⁺97]. The availability of *rys*, a zebrafish npat mutant which develops well into the larval stage, is therefore of considerable interest, as it allows for the study of npat's function within complete tissues. We demonstrate here that npat is critical for fate specification of CMZ neural progenitors. We find substantial evidence for the role of *D. rerio* npat involvement with histone transcription, nucleosome positioning, and scheduling of mitotic activity; we suggest that the *rys* phenotype is brought about by a failure to coordinate the genomic states required to specify, independently of proliferative

capacity, in postembryonic zebrafish RPCs of the CMZ.

5.2 Results

5.2.1 The *rys* CMZ phenotype is characterised failure of RPCs to specify, altered nuclear morphology, aberrant proliferation and expanded early progenitor identity

rys has an enlarged CMZ [WSM⁺⁰⁵], but whether this is a consequence of more RPCs, larger RPCs, or other causes, is unknown. We examined niche ontogeny during the early life of *rys* animals using two histochemical markers of cycle activity: Proliferating Cell Nuclear Antigen (PCNA), which in *D. rerio* is expressed throughout the cell cycle; and the genomic incorporation of the thymidine analogue EdU over a 24 hour pulse, marking passage through S-phase during this time. These data are displayed in Figure 5.2. During this period, the PCNA positive population of the sibling CMZ declines (Panel A), with a corresponding decrease in proliferative activity as measured by the incorporation of EdU (Panel B). Whether or not the mutant *rys* CMZ population is enlarged by comparison depends on the age at which it is sampled. There is a 99.6% probability that the posterior mean RPC population in mutants is less than the sib mean at 4dpf, while there is a 99.8% probability of the 10dpf *rys* mean being greater than that of sibs. Therefore, the *rys* CMZ population is better described as achieving its peak periembryonic size later than siblings; its population is only numerically larger than sibs at later ages¹.

¹*rys* animals universally die by approximately 3 weeks of age.

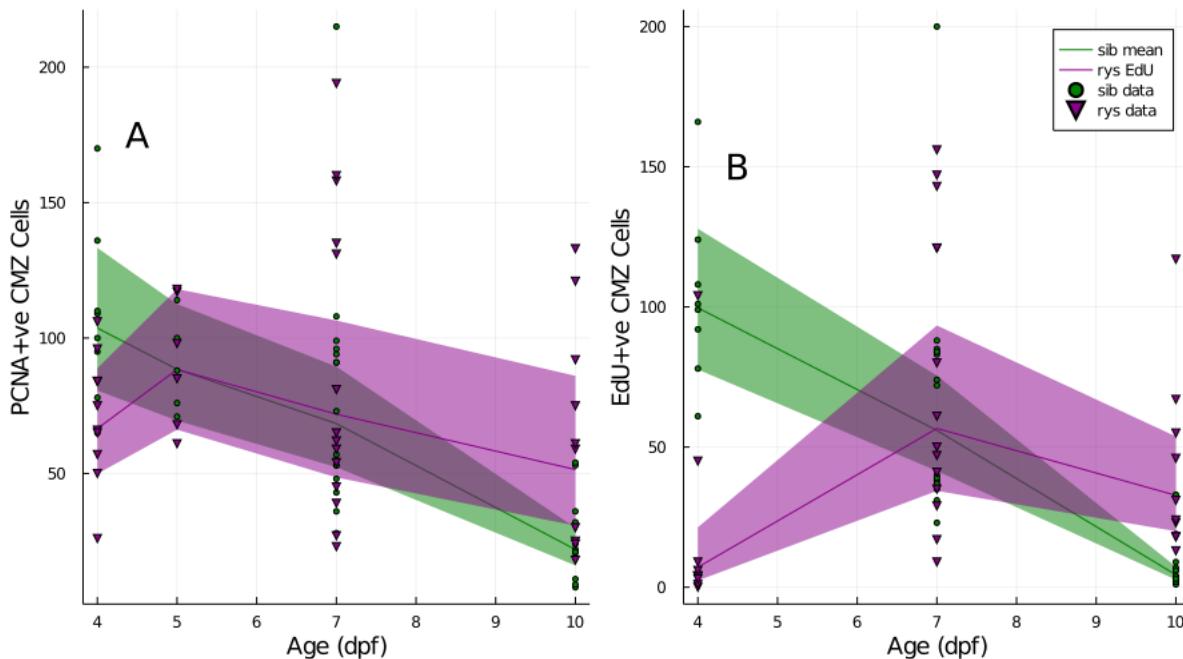


Figure 5.2: *rjs* CMZ populations start relatively small and quiescent, end abberantly large and proliferative

Panel A: Counts and estimated mean \pm 95% credible intervals of PCNA-positive cells in the peripheral CMZ of *rjs* (magenta) and their siblings (green). Counts obtained from one 14 μ m central coronal cryosection per individual. Panel B: As above, but for counts of double PCNA-,EdU-positive cells in the CMZ after a 24 hour pulse of EdU. Methods in Section 13.1.5. Code in Section 16.9.21.

Few *rjs* RPCs are actively cycling at 4dpf, by comparison to the rapidly proliferating sibling cells; a mean estimate of $96.2 \pm 3.4\%$ of sib RPCs are labelled during the 24 hr pulse at 4dpf, while only $24.1 \pm 37.2\%$ of *rjs* RPCs are. The situation is broadly reversed at 10dpf, with only $24.4 \pm 14.9\%$ of sib RPCs labelled, compared to $65.8 \pm 16.2\%$ of *rjs* RPCs. Confirming this late-stage increase in *rjs* thymidine analogue labelling represents bona fide mitotic activity, we found mitotic figures in labelled mutant RPCs at 10dpf, shown in Supplementary Figure 13.3. In *rjs* animals which have actively proliferating RPCs at early ages, the CMZ fails to contribute to the central neural retina. Confocal micrographs displaying *rjs* CMZ cohorts labelled with BrdU at 3dpf that have failed to enter the neural retina after 7 days of chase time are displayed in Figure 5.3.

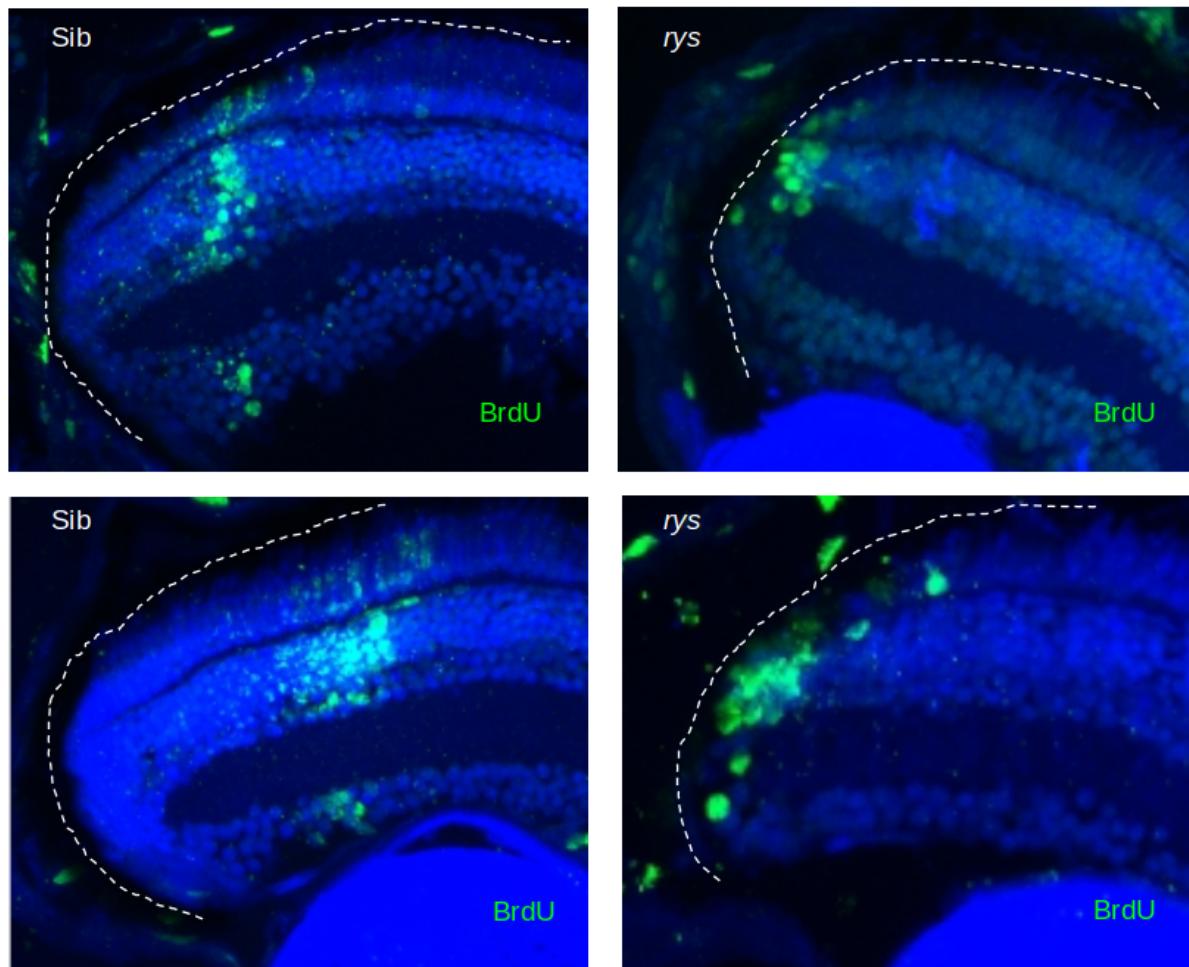


Figure 5.3: *rys* CMZ RPCs fail to contribute to the neural retina

14 μ m coronal cryosections through representative sib (left panels) and *rys* (right panels) eyes at 10dpf, 7 days after an 8hr BrdU pulse at 3dpf. Green: anti-BrdU labelling. Blue: Hoechst 33342 counterstain. Methods in [Section 13.1.6](#).

Examining 5dpf CMZ RPC nuclear number and morphology (Figure 5.4) suggests the enlarged appearance of *rys* CMZs is the failure to contribute to the neural retina, leading to *rys* RPCs contributing half as many central neurons as their siblings (Panel B), despite having similar overall populations at this age (Panel A). This appearance may be enhanced in the dorsal CMZ population, which tends to be more numerous in *rys* than sibs, although this tendency is reversed in the ventral population (Panels C and D). More significantly, *rys* nuclei are usually larger than their siblings (Panel E), as well as consistently less spherical (Panel F).

To rank these contributions to the *rys* nuclear phenotype, we calculated the Bayesian evidence (marginal probability) for combined Log-Normal (population measurements) and Normal (nuclear measurements) models of sib and *rys* data, against the joint evidence for separate models. These estimates are presented in [Table 5.1](#). Of the effects that differentiate *rys* from sibs (positive evidence ratio, logZR in [Table 5.1](#)), the largest is decreased nuclear sphericity, followed by decreased nuclear volume. These are followed by differences in dorsal and ventral CMZ populations, then by the mutant's decreased

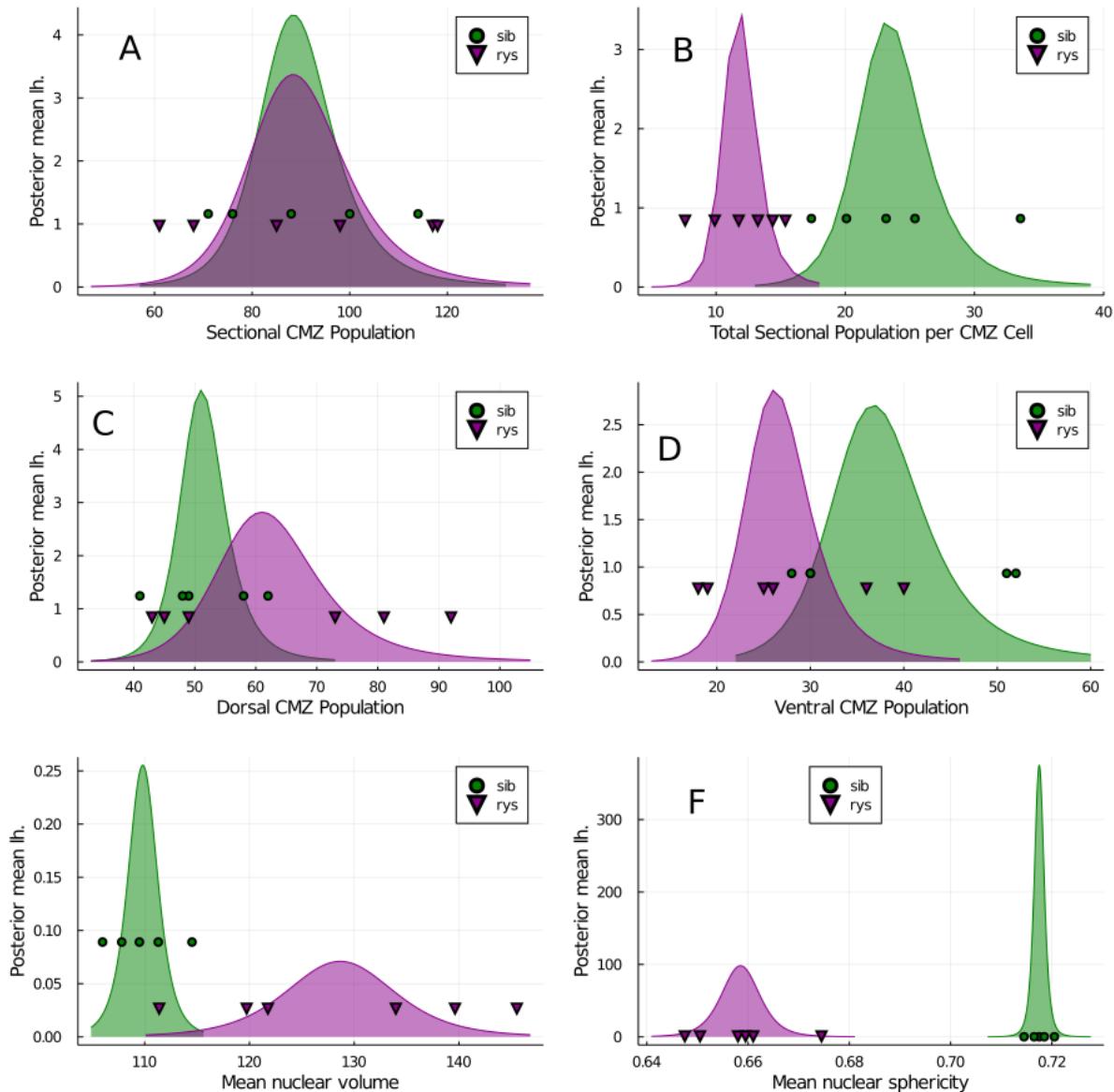


Figure 5.4: 7dpf *r ys* CMZ RPCs display enhanced asymmetry, increased volume, decreased sphericity, and relative but not absolute enlargement

All panels display sib observations as green circles and *r ys* as magenta triangles. Plotted behind the underlying observations are the calculated marginal posterior distributions of the mean, given log-Normal models of the population data and Normal models of the volume and sphericity data. The y-axis shows the relative likelihood of underlying mean values on the x-axis, given the data. Panel A: Total PCNA-positive RPC population per central coronal section. Panel B: Number of PCNA negative, specified central retinal neurons per PCNA positive RPC. Panels C and D: Dorsal and Ventral RPC populations per central coronal cryosection. Panel E: Mean volume of nuclei in a given individual's central coronal cryosection. Panel F: Mean sphericity of nuclei in a given individual's central coronal cryosection. Methods in Section 13.1.5. Code in Section 16.9.14.

number of central retinal neurons relative to the RPC population. These models suggest the nuclear

morphological changes are the most important contributors to the *rys* enlarged-CMZ phenotype.

Table 5.1: Evidence-based ranking of phenomenal contributors to *rys* phenotype

Parameter	Separate rys-sib logZ	Combined rys-sib logZ	logZR	σ sign.
Sectional CMZ pop.	-299.9 \pm 1.1	-203.4 \pm 1.0	-96.6 \pm 1.5	-65.4
Central pop./RPC	-56.11 \pm 0.27	-79.52 \pm 0.17	23.41 \pm 0.32	73.7
Dorsal CMZ pop.	-175.7 \pm 0.92	-228.4 \pm 1.4	52.7 \pm 1.6	32.2
Ventral CMZ pop.	-136.6 \pm 0.24	-167.15 \pm 0.13	30.55 \pm 0.27	113.8
Nuclear Volume	-164.57 \pm 0.22	-224.3 \pm 1.7	59.7 \pm 1.7	34.4
Nuclear Sphericity	-87.19 \pm 0.27	-265.5 \pm 2.3	178.3 \pm 2.3	78.4

logZ: logarithm of p(D), the marginal likelihood of the data, or model evidence. logZR: evidence ratio; positive ratios in favour of separate modelling of the sib and *rds* data. Largest evidence values bolded. Methods in [Section 13.1.7](#). Code in [Section 16.9.20](#).

Enlarged *rds* RPC nuclei have a billowy appearance suggestive of chromosomal disorganization. We used electron microscopy to examine the nuclear ultrastructure of RPC nuclei in *rds* and sibling CMZs. Representative electron micrographs are presented in [Figure 5.5](#). At 36000x magnification, the stretched and pancaked structure of *rds* nuclei is apparent, in contrast with the consistently teardrop-shaped nuclei of sibling RPCs. When the chromatin is imaged at 210000x magnification, it appears less electron-dense in *rds* mutants, with larger tracts of presumptive euchromatin, and less regular spacing of chromosomal material.

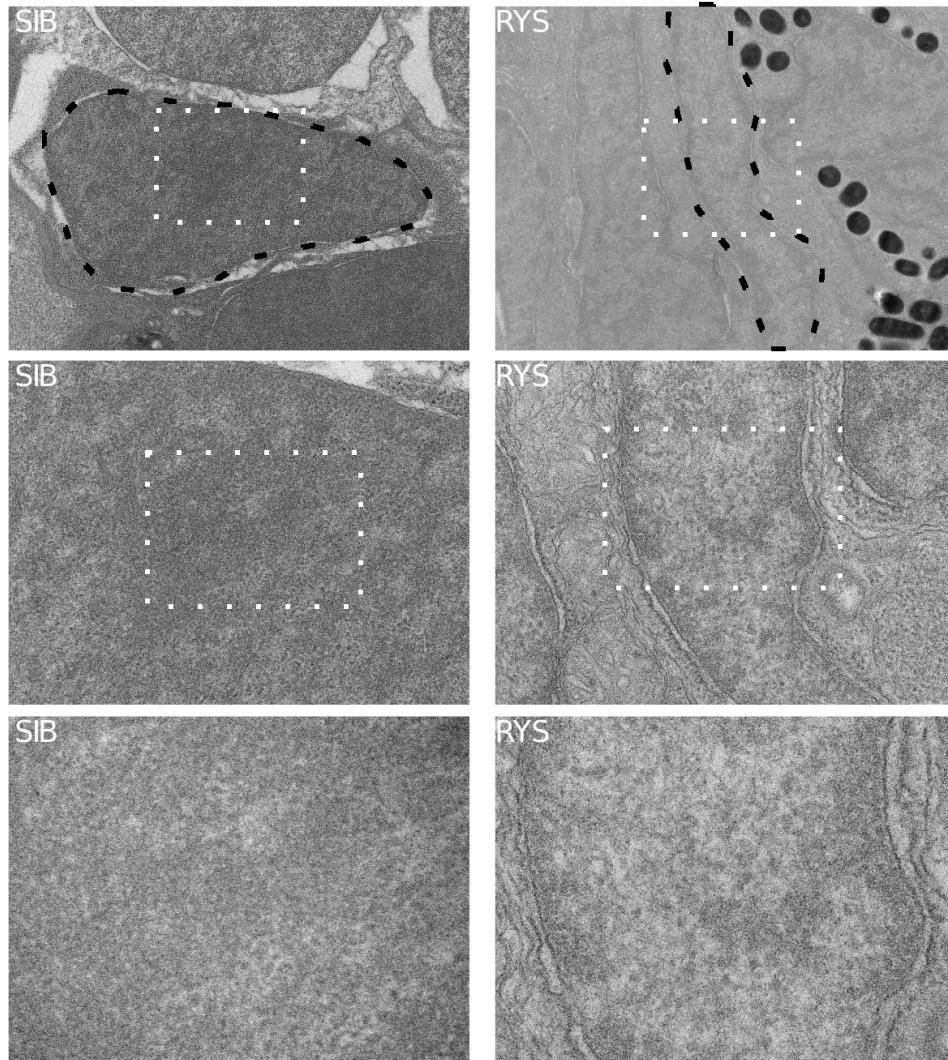


Figure 5.5: RPC nuclei of the *rys* CMZ display disorganized, loosely packed chromatin
 Representative electron micrographs of *rys* sibling and mutant CMZ RPC nuclei. Left panels: siblings. Right panels: *rys*. Top panels: 36000x magnification, general overview of the area around the nucleus, dashed black. Area displayed in middle panels dashed white. Middle panels: 110000x magnification nuclear detail. Area displayed in bottom panels dashed white. Bottom panels: 210000x magnification, chromosomal ultrastructure. Methods in [Section 13.1.11](#).

If RPCs in *rys* CMZs fail to enter the specified neural retina, but by 7dpf are becoming mitotically active, an explanation for the proliferative niche's population declining by 10dpf is required. We suspected *rys* RPCs may be apoptosing *in situ*. Although we did not detect pyknotic nuclear fragments in our EM investigations, it may be that the apoptotic events are too rare in *rys* to detect in this manner. We therefore assayed the presence of the apoptotic marker caspase-3 in *rys* and sib CMZs. As displayed in [Figure 5.6](#), caspase-3-positive nuclei can be detected in the *rys* CMZ at both 4 and 6 dpf (mean 6.5 ± 7.5 and 2.6 ± 1.1 cells, respectively) but are not found in sib CMZs, though both display a similar level of central apoptotic activity (4dpf *rys*: 1.25 ± 1.0 ; 4dpf sib: 1.4 ± 1.1 ; 6dpf *rys* 0.6 ± 0.9 ; 6dpf sib: 1.0 ± 1.4). The lack of debris observable in *rys* CMZs is likely attributable to the activity of 4C4-positive

microglia active in the area; we observed one such cell actively phagocytosing TUNEL-labelled *rys* RPC nuclear fragments in the CMZ, displayed in Supplementary Figure 13.4.

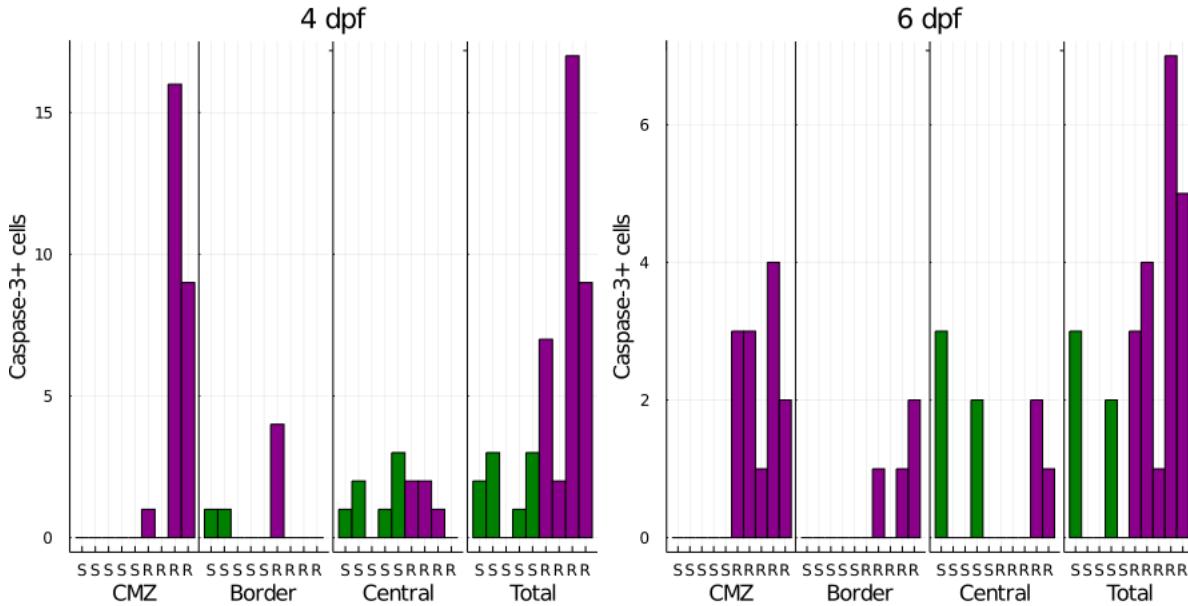


Figure 5.6: *rys* mutant CMZs have increased caspase-3 positive nuclei

Number of caspase-3 positive nuclei counted in CMZ, the border between the CMZ and specified central retina, the specified central neural retina, and the overall total, at 4 dpf (left panel) and 6 dpf (right panel). One central 20 µm section per sibling (S) and *rys* (R) larva. Methods in Section 13.1.9. Code in Section 16.9.16.

Suspecting that *rys* CMZ RPCs may maintain an early progenitor identity, we examined pax6a immunostaining of this population, which is normally restricted to putative progenitors in the peripheral and middle CMZ, as well as the retinal ganglion cell layer [RBBP06]. The Pax6-stained region was enlarged in *rys* CMZs relative to their siblings (Figure 5.7, center panels). As vsx2 is also known to be a marker of retinal progenitor cells in the CMZ [RBBP06], we also generated a transgenic vsx2::eGFP *rys* line, which produces eGFP expression in the utmost retinal periphery in siblings. Mutant fish from this line displayed substantially expanded eGFP expression in the CMZ (Figure 5.7, right panels).

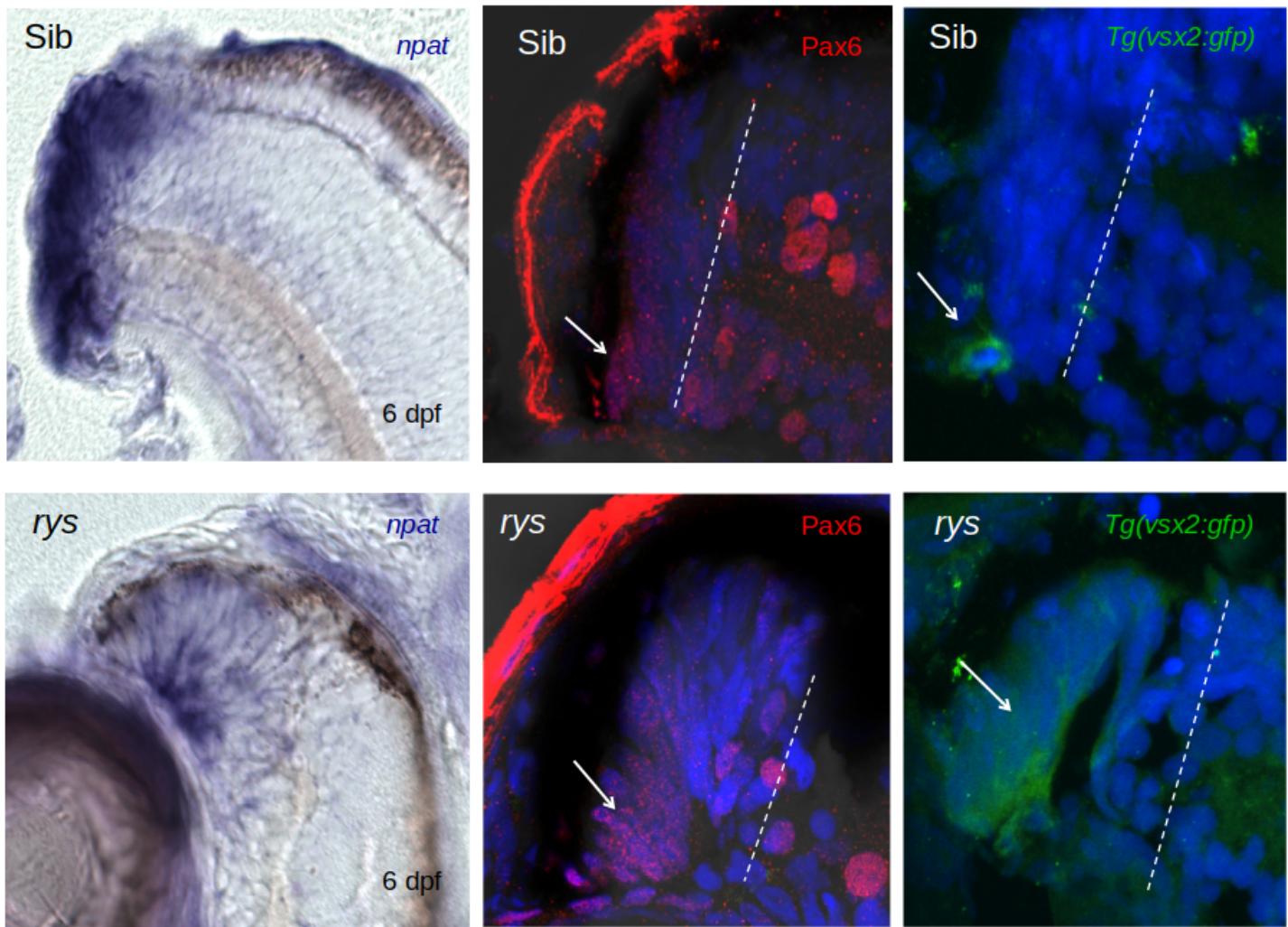


Figure 5.7: Mutant *rys* RPCs display expanded expression of early progenitor markers
 Representative transmitted light and confocal micrographs of *rys* sibling and mutant CMZ RPCs. Top panels: siblings. Bottom panels: *rys* mutants. Left panels: in-situ hybridization using npat probe (blue) on 20 μ m coronal cryosections. Middle panels: anti-Pax6 (red) on Hoechst 33342 nuclear counterstain. Right panels: anti-GFP (green) on Hoechst 33342 nuclear counterstain, in a Tg(vsx2:GFP) line introgressed into *rys*. Methods in Section 13.1.8, Section 13.1.10.

5.2.2 The microphthalmic zebrafish line *rys* is an npat mutant

We performed linkage mapping to identify the causative mutation responsible for the *rys* phenotype, followed by PCR analysis of the transcript products of candidate genes. This study revealed a single G>A transition at position 24862961 on chromosome 15 (NC_007126.5, Zv9), annotated as the first base of intron 9 in the zebrafish npat gene, in a canonical GU splice donor site.

We observed that this mutation reliably results in the retention of npat intron 8, and, less frequently, in the retention of both introns 8 and 11, in 6dpf *rys* mutants, shown in Figure 5.8. The predicted mutant protein is truncated by a stop codon at residue 283, which would preclude the translation of predicted phosphorylation sites and nuclear localisation signals cognate to those identified in human

NPAT [Ma00, STN⁺02], as displayed in Figure 5.9.

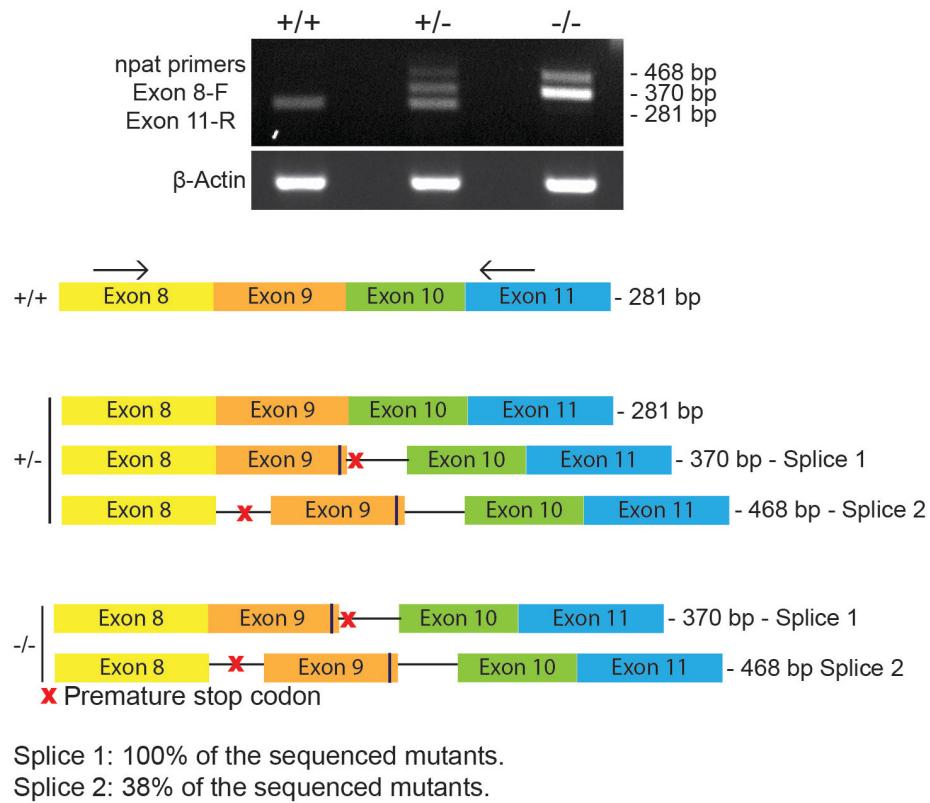


Figure 5.8: RT-PCR analysis reveals two aberrant intron retention variants in *ryst* mutant npat transcripts

Top panel: Agarose gel electrophoresis of mRNAs prepared from 3dpf homozygous wild type (+/+/), heterozygote (+/-), and homozygous mutant (-/-) animals, as identified by genomic PCR. Fragments were amplified from a forward primer sited in exon 8 and a reverse primer sited in exon 11. Bottom panel: exon/intron layout schematics of the putative transcripts detected. Methods in ??.

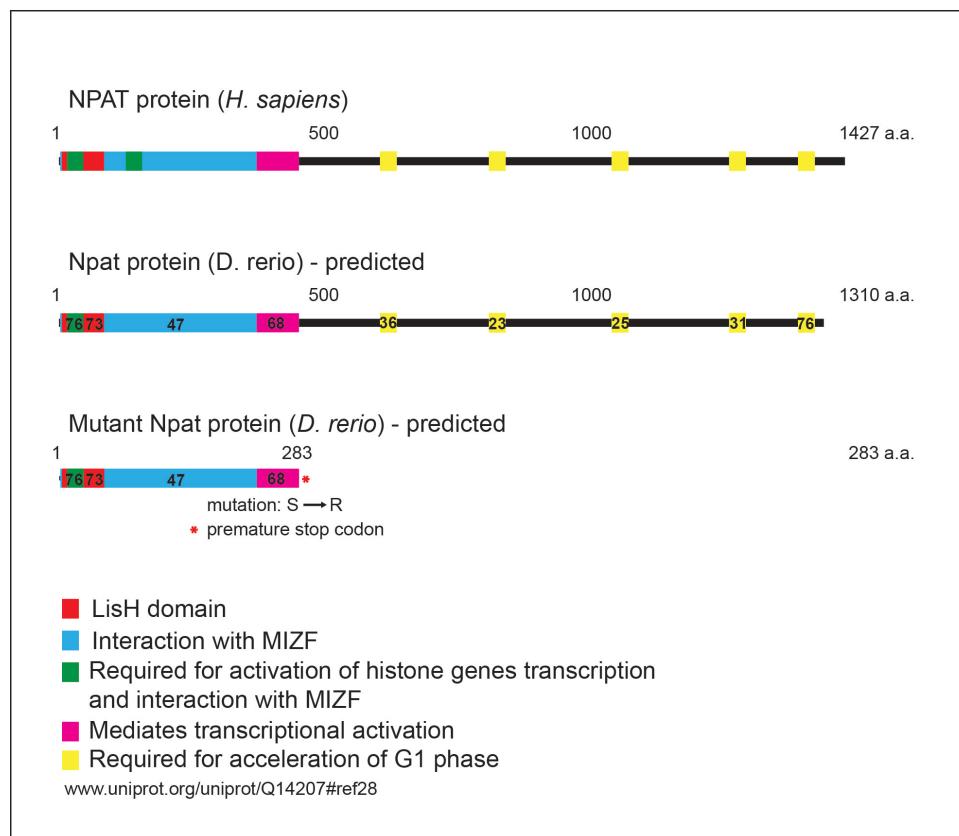


Figure 5.9: Functional domains of Human NPAT compared to predicted wild-type and *r ys* *Danio* npat

Proteins presented as linear domain schematics with number of amino acids along the horizontal axis.

Because *D. rerio* is a teleost known to have undergone genome duplication in an ancestral clade, we used the Synteny Database tool [CCP09] identify any possible npat duplicates. A duplicated gene could explain the lessened severity of the mutant *Danio* phenotype when compared to mammalian proviral inactivants. The results of this analysis are presented in Supplementary Figure 13.1. While npat is in the midst of a region which appears to be duplicated on chromosomes 5 and 15, relative to the homologous synteny run on *H. sapiens* chromosome 11, it is not, itself, duplicated.

We performed in situ hybridisation against npat transcript to confirm that the gene is indeed expressed in wild type CMZs; we found that npat expression is progressively restricted to the CMZ from 4 to 6 dpf in wild-type fish. A representative time-course of 20 µm cryosections through ISH-treated embryos is depicted in Figure 5.10, focusing on the retina at the times when it has formed. Although npat remains transcribed in both the specified GCL and amacrine-rich inner INL to a degree, it is most intensely expressed in the proliferative CMZ, as suggested by its cell cycle functions.

Given unusual chromatin ultrastructure in *r ys* CMZ RPCs, and npat's role in histone regulation, we investigated the transcriptional status of npat and its histone regulatory targets by RT-PCR. Homozygous *r ys* mutants overtranscribe npat by about 3-fold compared to their wild-type counterparts at both 6dpf and 8dpf. Sibling overabundance compared to wild-type declines from about 2.5-fold to 1.5-fold over this time period, as shown in Supplementary Figure 13.2.

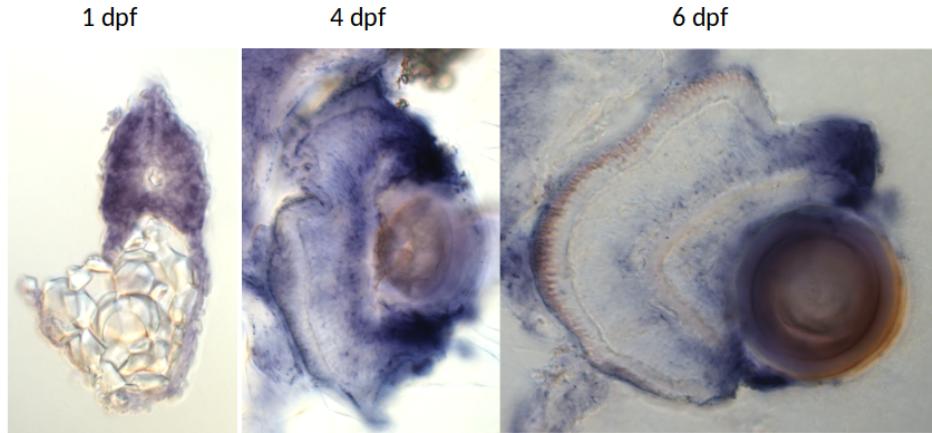


Figure 5.10: In situ hybridization reveals progressive restriction of npat expression to the CMZ

20µm sections of wild-type embryo (1dpf) and retinae (4 and 6 dpf), displaying progressive restriction of npat expression (blue) as assayed by in-situ hybridisation. Methods in Section 13.1.10.

Since mammalian NPAT regulates histone transcription and is critical for coordinating expression of replication-dependent histones required to package genomic DNA during S-phase [ZKL⁺00], we hypothesized that the altered nuclear morphology we observed in *rjs* CMZ RPCs may be a consequence of perturbed histone transcription. To test this, we performed qPCR on random-hexamer-primed cDNAs produced from 6 and 8dpf wild type, sibling, and *rjs* embryo mRNA extracts. These qPCR assays were performed using degenerate primers² directed toward zebrafish core histone gene families H2A, H2B, H3 and H4. As a role for NPAT in 3' end processing of histone transcripts has been identified [PJ10], we also set out to determine whether 3' end processing of histone transcripts is altered in *rjs*. By repeating these qPCR assays on oligo-dT-primed cDNAs, we examined the population of polyadenylated histone transcripts in isolation.

These results demonstrated that both mutant *rjs* and siblings overexpress histone transcripts to various degrees. In order to determine which histone families are most plausibly involved in the *rjs* mutant phenotype, we calculated the statistical significance for mean *rjs* transcript levels exceeding mean sibling transcript level or the WT standard (set to 1.0). To be implicated in the phenotype, mutant transcript levels should be higher than both siblings and wild-type animals. We used a cutoff of 2 SDs of significance for both the sibling and WT tests for a given transcript family. These values are presented in Table 5.3.

Both total and polyadenylated *rjs* H2A are overexpressed in mutants relative to both siblings and WT controls at 8 dpf, while total H2B is overexpressed at 6dpf and polyadenylated H2B at 8dpf, using the criterion of 2 SDs of significance on both tests. We note that the overabundances of polyadenylated H2A and H2B are approximately 2-20 fold greater than those observed for any other transcript pool or histone family.

The joint probability that mean *rjs* mutant total H2A is greater than the sib mean at both 6dpf and

²Zebrafish have notably populous histone clusters with numerous variants and pseudogenes not present in non-genomically-duplicated vertebrates, so degenerate primers are an appropriate way to survey the population of transcripts. A full catalogue has not been undertaken, to my knowledge.

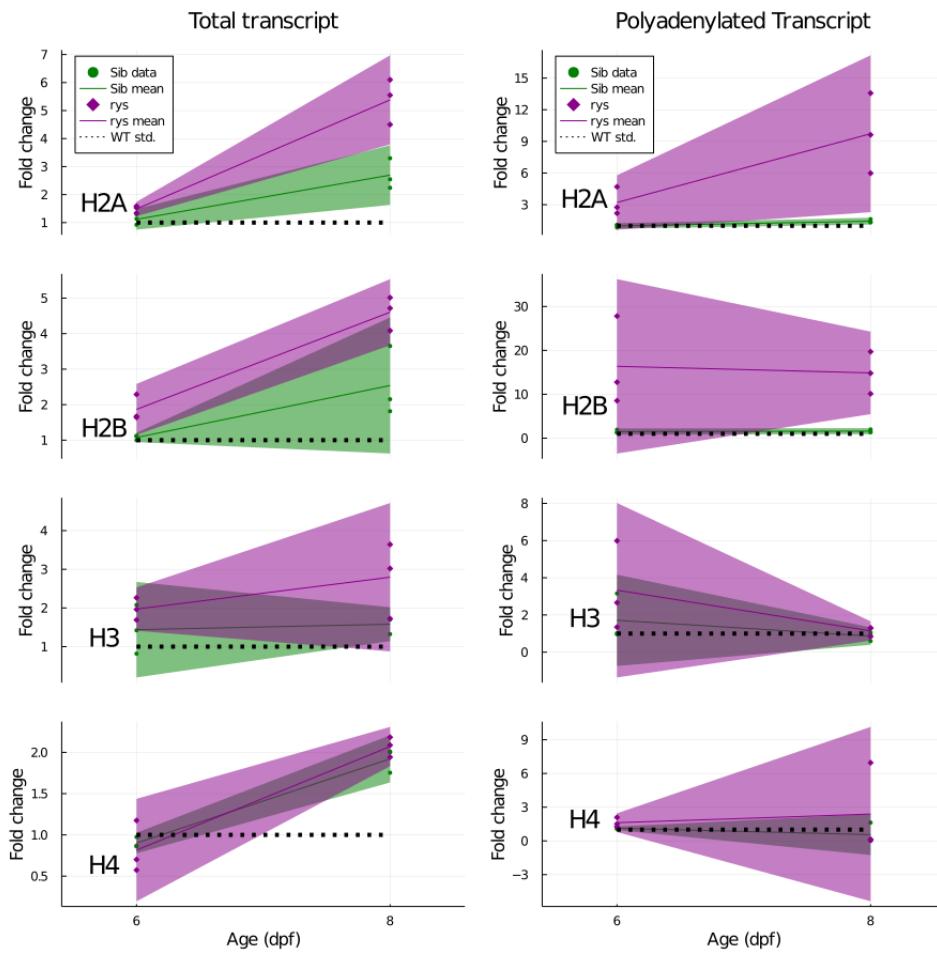


Figure 5.11: *ryst* overexpress total and polyadenylated core histone transcripts
qPCR results for degenerately-primed families of core histone total transcripts (left panels), and polyadenylated transcripts (right panels). From top to bottom, H2A, H2B, H3, and H4 family results are displayed in separate panels. Animal age in dpf on the x axis, fold change vs. wild-type standard on the y axis. Methods in ???. Code in Section 16.9.19.

8dpf to be $99.7\% \pm 5.0$; the same figure for H2B is $98.5\% \pm 5.1$. The joint probability that mean total H2A and H2B are elevated in *ryst* mutants compared to sibs at both ages is therefore $98.2\% \pm 7.1$. For the polyadenylated transcripts, the joint probability that the *ryst* mutant polyA H2A mean is greater than the sib mean at both ages is $94.1\% \pm 9.9$; the same figure for H2B is $92.6\% \pm 3.3$. The joint probability that mean polyadenylated H2A and H2B are elevated in *ryst* relative to sibs at both ages is then $87.1\% \pm 9.7$.

On the basis of these calculations, the most plausible causal contributors to the *ryst* phenotype are H2A and H2B family transcripts. While the relative magnitude of total H2A and H2B transcript overexpression is less than that of the polyadenylated pool, we have less uncertainty about these figures. Still, the polyadenylated transcript results suggest mutant npat increases polyadenylation of these transcripts, possibly as a result of a loss of regulation of this system. Unlike the total transcript pool, siblings retain tight, WT-like control over polyadenylated transcripts. *ryst* mutants display much more extensive

Table 5.2: Standard deviations of significance for *rys* mutant transcript > sib or WT

Transcript Family	Age (dpf)	Total		polyA	
		Sib mean σ	WT std σ	Sib mean σ	WT std σ
H2A	6	1.59	3.72	1.68	1.68
H2A	8	2.75	5.4	2.18	2.3
H2B	6	2.12	2.37	1.47	1.52
H2B	8	1.9	7.59	2.76	2.89
H3	6	0.77	3.39	0.6	0.97
H3	8	1.21	1.83	0.78	0.54
H4	6	-0.26	-0.58	1.15	1.46
H4	8	0.79	8.83	0.45	0.35

Table 5.3: Standard deviations of significance for differences between mean *rys* mutant transcript expression, relative to siblings or WT standard (1.0), at 6 and 8 dpf. Values for histone transcript pools with >2 SDs of significance for both sib and WT tests in bold. Methods in ???. Code in Section 16.9.19.

variability in polyA transcript expression, even where the mean is not elevated above siblings or WT controls.

We further investigated npat’s involvement by perturbing its transcription using morpholino injections of wild-type (AB strain) fish. As we do not suppose early morpholino transcriptional blockade will exactly replicate the cellular conditions of a genetic null some days after birth, particularly given the maternal contribution of npat transcript [HSK⁺13], we did not seek to recapitulate the *rys* phenotype. Rather, we sought to determine if any of *rys*’s constituent phenomena would appear after an early blockage of *rys* transcription, further substantiating the general involvement of npat in *rys*. We tested morpholinos directed both to the npat start codon and to the splice site affected in *rys*, alongside control morpholinos and uninjected animals. We used both a morpholino directed to the transcript ATG start site, as well as to the affected splice site.

The splice morpholino replicated the *rys* overexpression of both total and polyadenylated core histone transcript, while the ATG morpholino more narrowly replicated the effect on polyadenylated transcript (Figure 13.5). The ATG morpholino also produced some animals with small eyes by 72dpf, as shown in Figure 5.12. We also conducted an analysis of the nuclear morphological parameters measured in Figure 5.4 for *rys* mutants and siblings. For each of the ATG and splice morpholinos, we estimated the joint evidence for separate experimental morpholino and control morpholino models against a combined model. The evidence calculations are presented in Supplementary Table 13.1. We found substantial evidence in favour of separate models for the ATG and control morpholino effects on the number of central retinal neurons per proliferating RPC, suggesting that the ATG morpholino tends to decrease CMZ contribution to the specified retina. This result is displayed in Figure 5.13; the effect is more subtle than that displayed by mutants in Figure 5.4. These results establish that the identification of npat as the causative mutation in *rys* is plausible, as experimental perturbation to npat in WT fish can result in *rys* phenotypic phenomena, notably overexpression of core histone transcripts and small eyes arising from a decrease in the population of the central retina without a concomittant decrease in CMZ population.

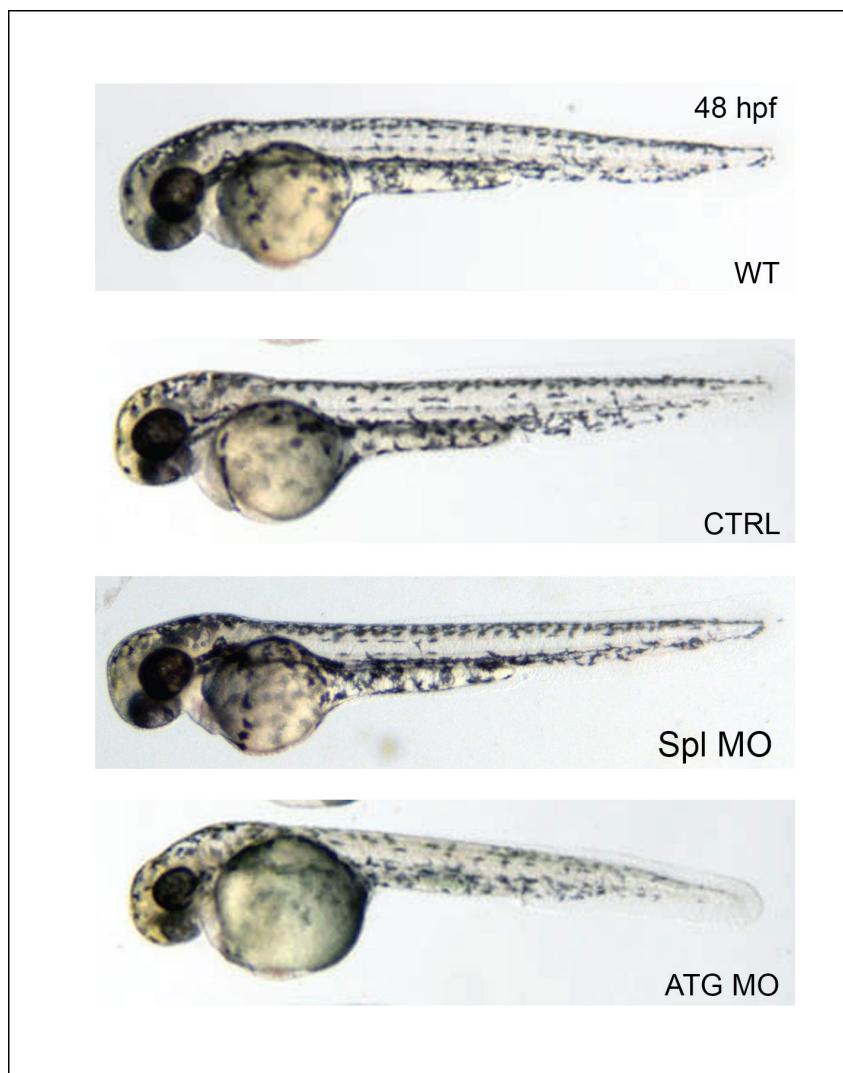


Figure 5.12: 48hpf embryos injected with an npat ATG-targeted morpholino display a small-eye phenotype

48 hpf wild-type embryos injected at the 1-2 cell stage with a control morpholino (CTRL), a morpholino targeting the npat splice site affected by the *rys* mutation (Spl MO), and a morpholino targeting the npat start codon (ATG Mo). Methods in [Section 13.1.3](#).

5.2.3 *rys* siblings and mutants have unique sets of nucleosome positions, best explained by different sequence preferences and increased sequence-dependent positioning in mutants

Perturbed histone expression in *rys* embryos suggests disrupted chromatin organisation as an explanation for the mutant nuclear phenotype, a possible consequence of altering the dynamic composition of the histone pool available for nucleosome formation during cell cycle. We therefore characterised nucleosome positioning in *rys* and wild-type siblings by micrococcal nuclease (MNase) digestion of pooled genomic DNA (gDNA) [CZ12]. Nucleosome-protected fragments from the MNase digests were sequenced and positions called as described previously [CXP⁺13].

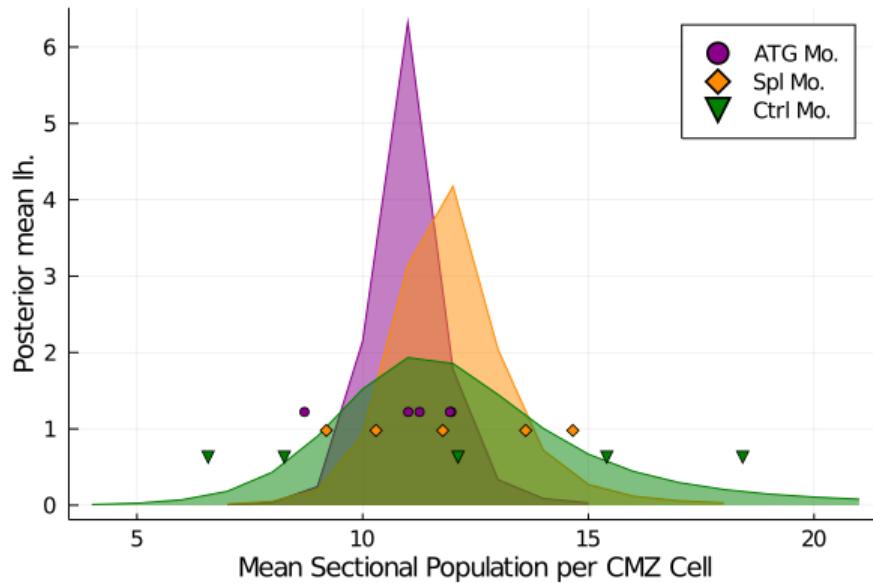


Figure 5.13: ATG morpholino perturbation of npat recapitulates decline in mean central retinal population relative to CMZ

Populations of 14 μ m central coronal cryosections from retinas of animals injected with control (green triangles), npat splice-directed (yellow diamonds), and npat start codon-directed (magenta circles), standardized by number of CMZ RPCs. Plotted behind the underlying observations are the calculated marginal posterior distributions of the mean, given log-Normal models of the data. The y-axis displays the relative likelihood of underlying mean values on the x-axis, given the data. Methods in [Section 13.1.3](#), [Section 13.1.5](#). Code in [Section 16.9.15](#).

We first examined the genomic disposition of nucleosome positions within wild-type siblings and *rys*. Nucleosome positions are distributed relatively evenly over sib genomic material, with only tiny deviations from a neutral assumption of a uniform distribution, as displayed in Fig. 5.14, panel A. Bulk *rys* chromatin displays minor differences from the proportions of positions found in each sib scaffold (panel B), with a tendency toward more even distribution of positions. Sib nucleosome positions are differentially occupied across scaffolds, with Chr 10 and scaffolds not yet mapped to chromosomes (NC) being notably more occupied than expected from scaffold length alone (panel C). Similarly to the distribution of positions, *rys* chromatin is more evenly occupied than sibs; scaffolds with positions that are more heavily occupied in sibs tend to be depleted in *rys* and vice versa (panel D).

By mapping the called nucleosome positions in *rys* to those in sibs, and calculating the number of bases the *rys* position is displaced from its mapped position, we characterised the translational displacement from the arrangement found in siblings. The probability distribution of this displacement parameter for the population of *rys* positions is displayed in [Figure 5.15](#). The notable bimodality of this distribution arises as a result of the inclusion of unmapped positions, to show the relative size of this population: 23% of positions are not mapped to any sibling position at all; these are located at 141, greater than the full length of a called nucleosome position in our pipeline. The other three-quarters of the positions are mapped to sibling positions, with 11% of overall *rys* positions coinciding entirely with the sibling positions, 19% displaced 10 bases or less from a sib position, and a further 21% displaced between 10 and 30 bases. The last quarter of *rys* positions display more extreme displacements, up to

the entire length of the position. The periodic multimodality observed in the displacement distance of *rys* positions relative to sibs is commonly observed in nucleosome positioning and arises from the 10-base periodicity of nucleosome contacts with DNA [WC17]. This distribution of population displacement suggests that the npat mutation in *rys* results both in a loss of translational control of nucleosomes that are in approximately the expected positions, most commonly by a nucleosome “roll” of one contact, as well as dysregulation of the chromatin remodelling processes which get nucleosomes to the correct positions to begin with, represented by the novel *rys* positions, with no mapped sibling counterpart.

We sought to identify position subpopulations that might represent those involved in the nuclear phenotype of mutant *rys* RPCs. Interestingly, not only are there *rys* nucleosome positions which are not found in siblings, but there is likewise a subset of sib positions which are never found to be occupied in mutant *rys*. Intriguingly, sib positions which are not observed in *rys* are compensated for quite evenly across the genome by new positions gained in the mutants, with a small excess of new *rys* positions on most chromosomes, as shown in Supplementary Figure 13.6. This result suggested that a particular subpopulation of wild type nucleosomes are mislocalised in *rys*. This could occur for a variety of reasons, which may not be reflected in the position sequences themselves. For instance, mutant npat could affect chromatin remodelling enzymes without this having a systematic effect on the primary sequence of positions. Mutant npat could also alter the pool of available histones in proliferating progenitors, resulting in altered physico-chemical interactions with DNA, which could manifest as a preponderance of nucleosomes with unusual sequence preferences. If so, this would explain the disappearance of a subset of positions in *rys* and the appearance of a new, similarly sized subset of positions (the ‘differential set’).

To distinguish between these different possibilities, we calculated the Bayesian evidence ratio for models of separate emission processes for sib and *rys* position sequences, against a combined process for both sets of sequences. If the effect of npat is unrelated to direct interaction between nucleosomes and DNA, the combined model should receive the most support, since any signals appearing in the separate models would be unrelated to the status of npat. On the other hand, if separate models receive more support, DNA sequence preferences are implicated in the differential positioning of mutant and sib nucleosomes. If the latter proves to be the case, the maximum a posteriori parameters of these models can be compared to reveal the specific changes in the prevalence and identity of detected sequence signals.

The emission model used was the Independent Component Analysis form described by Down and Hubbard [DH05], with a fixed number of independent, variable length position weight matrices related to observations by a Boolean mixing matrix. An observation is scored by the background likelihood of its sequence, given a model of genomic noise, convolved with a sequence-length- and cardinality-penalized score³ for each source which the mix matrix indicates is present in the observation. Therefore, we first needed to construct background models of *D. rerio*’s genomic “noise” from which repetitive sequence signals characteristic of nucleosome positions could be extracted. Following the suggestion of Down and Hubbard [DH05] that a principled approach to the selection of background models is to train and test a variety of them on relevant sequence, we used the Julia package BioBackgroundModels (presented in Chapter 9) to screen a panel of 1,2,4, and 6-state HMMs against 0th, 1st, and 2nd order encodings of samples from the zebrafish genome, partitioned grossly into exonic, periexonic, and intergenic sequences. We found that each of these partitions is best represented by 6-state HMMs trained on a 0th order genome encoding (i.e. the HMMs emit the 4 mononucleotides), as determined by model likelihood given

³That is, the additional score provided by a source to an observation is penalized both by the expected number of motif occurrences given an observation of that length, as well as by the number of sources which explain the observation in the model.

an independent test sample, displayed in Supplementary Figure 13.7.

We used the Julia library BioMotifInference (presented in Chapter 10) to sample from the posterior distributions for these models, given the differential set of *rys* nucleosome positions and the composite background model of genomic noise. We initialized separate ensembles from uninformative priors on the *rys* and *sib* data alone, as well as the combined *rys* and *sib* data, allowing for 8 independent PWM sources in all cases. We compressed three model ensembles to within 125 orders of magnitude between the maximum likelihood model sampled and the minimum ensemble likelihood⁴. This process produced the model evidence estimates summarized in Table 5.4, as well as maximum a posteriori samples for each of the ensembles. We estimate that there are greater than 360 orders of magnitude of evidence in favour of separate generative processes for the differential *sib* and *rys* than for a combined model, with an estimated 525 standard deviations of significance. This large evidentiary weight and significance give us statistical almost-certainty in selecting separate models for these sequences over a combined model. We present the MAP PWM source samples from the better-evidentiated separate *sib* and *rys* models in Figure 5.16 and Figure 5.17 respectively, while the inferred combined sources are available in Supplementary Figure 13.8.

Table 5.4: Evidence favours separate emission models for the *rys* mutant and sibling differential position sets

Sib logZ	<i>rys</i> logZ	Joint Sib/ <i>rys</i> logZ	Combined logZ	logZR
-1.117572e6 ± 0.43	-1.161415e6 ± 0.35	-2.278988e6 ± 0.56	-2.279356e6 ± 0.42	368.5 ± 0.7

Methods in Section 13.1.17. Code in Section 16.9.29.

BioMotifInference’s source detection was highly conservative. The background models were found to explain a majority of observed nucleosome positions adequately, without any PWM sources, in most models of the maximum a posteriori estimate, for both sibling and *rys* ensembles. In siblings, the top three sources are the only ones detected in more than 10% of observed sequences, suggesting that the influence of sequence preferences is weakly explanatory for these sites. By contrast, in the mutant *rys* positions, we found twenty-eight separate PWM sources detected in more than 10% of observations, in a variety of posterior modes. This indicates sequence preferences are more explanatory for the *rys* differential position set.

The CWG motifs detected in sibling sequences are commonly reported in nucleosome positions, and have been described as promoting nucleosome formation [Has03]. The majority of the sources detected in the various posterior modes were of this form, with rarer CT- and CA- dinucleotide repeats, as well as a rare ATGG repeat. *rys* positions have a much more diverse set of sources detectable above background genomic noise; notably, AG- dinucleotide repeats that are not found in *sib* sources at all. The CWG motifs also exhibit a preference for flanking A positions that is not evident in the sibling differential position set. CA- dinucleotide repeats are more commonly detected in *rys* positions, and the rare ATGG repeat is not found at all.

⁴That is, the convergence criterion was that the ensemble difference $\log(L_{max}) - \log(L_{min})$ be <125).

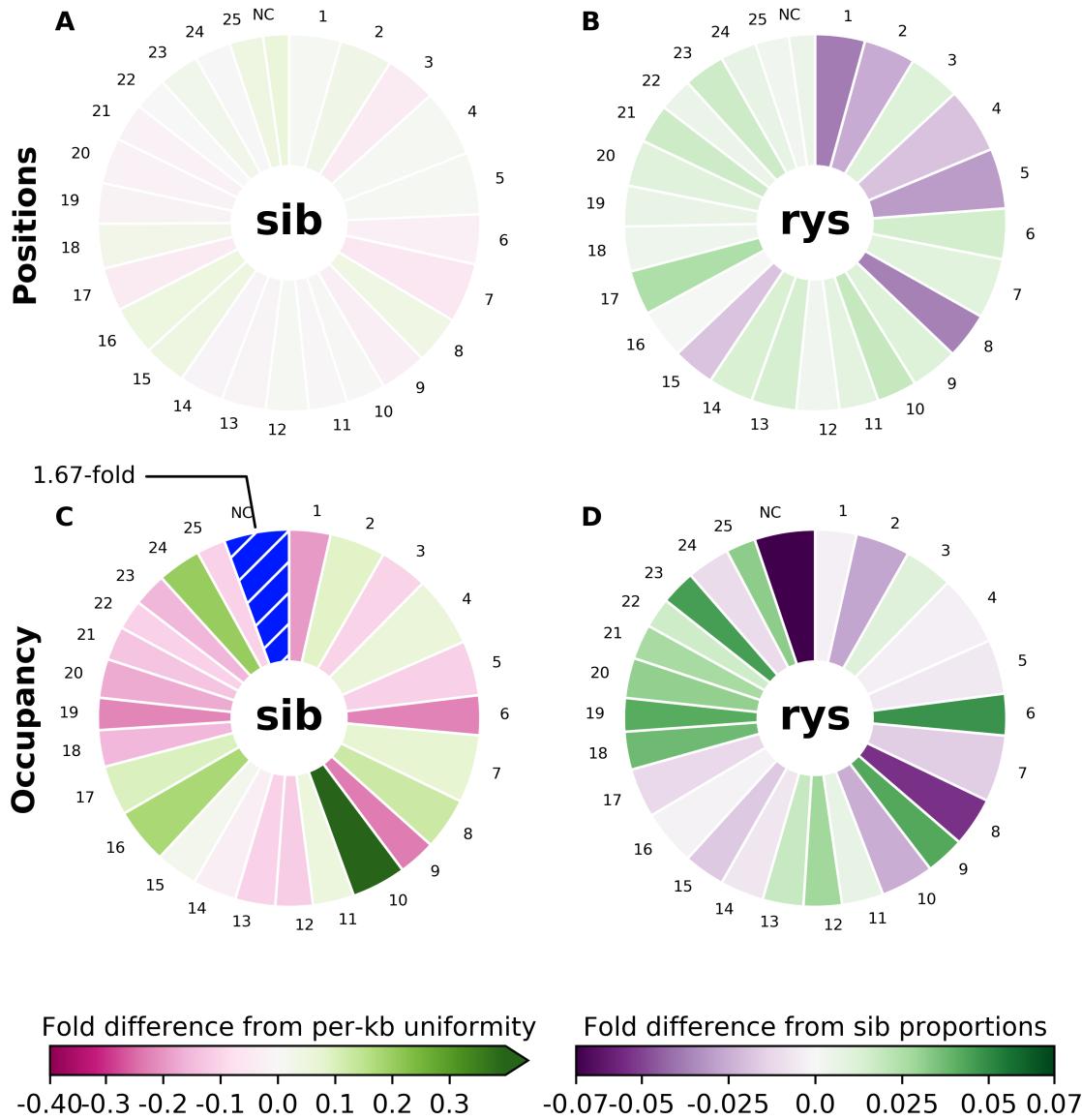


Figure 5.14: *rys* chromosomes are differentially enriched and depleted of nucleosome position density and occupancy.

Pie charts of nucleosome position density and occupancy by chromosome. Width of pie slices in panels A and B indicates the fraction of the total number of positions occurring in the numbered chromosomes and nonchromosomal scaffolds (NC). In panel A, depicting the sib genome, slices are colored according to the extent of deviation from an assumption of even distribution of nucleosomes across the genome. In panel B, depicting the *rys* genome, slices are colored according to the extent of deviation from the sib distribution. The width of slices in slices C and D indicate the fraction of the total nucleosome occupancy signal detected. Slice coloration depicts deviations from nucleosome occupancy distributions analogous to position distributions in A and B. Blue and white diagonal bars in the NC slice of panel C denote an out-of-scale positive deviation from the assumption of even distribution, i.e., sib NC scaffolds have 1.67-fold more of the total nucleosome occupancy signal than is expected from their length alone. Methods in Section 13.1.15. Code in Section 16.9.18.

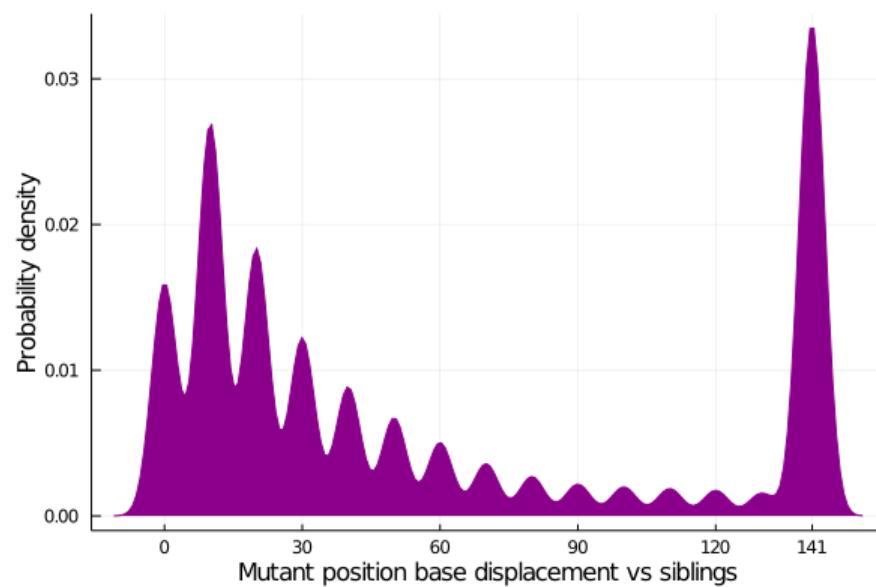


Figure 5.15: **PWM sources detected in sibling differential nucleosome positions.**
Probability distribution of *rys* position displacement distance from mapped sibling positions, in base pairs. Methods in Section 13.1.16. Code in Section 16.9.34.

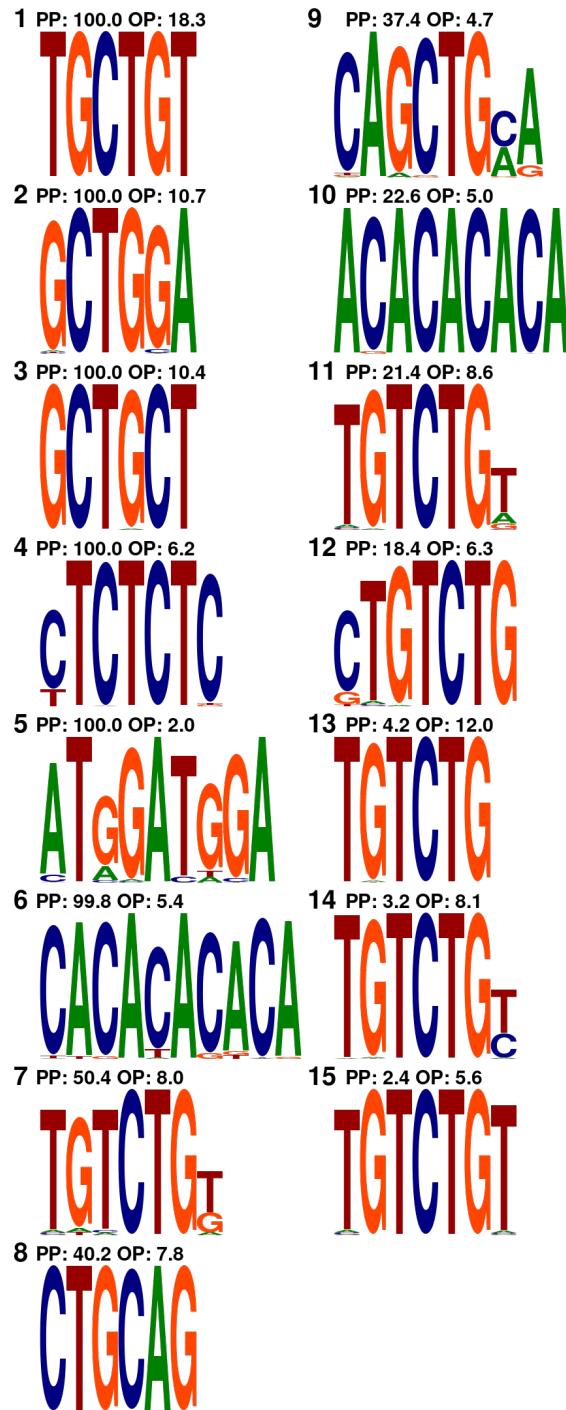
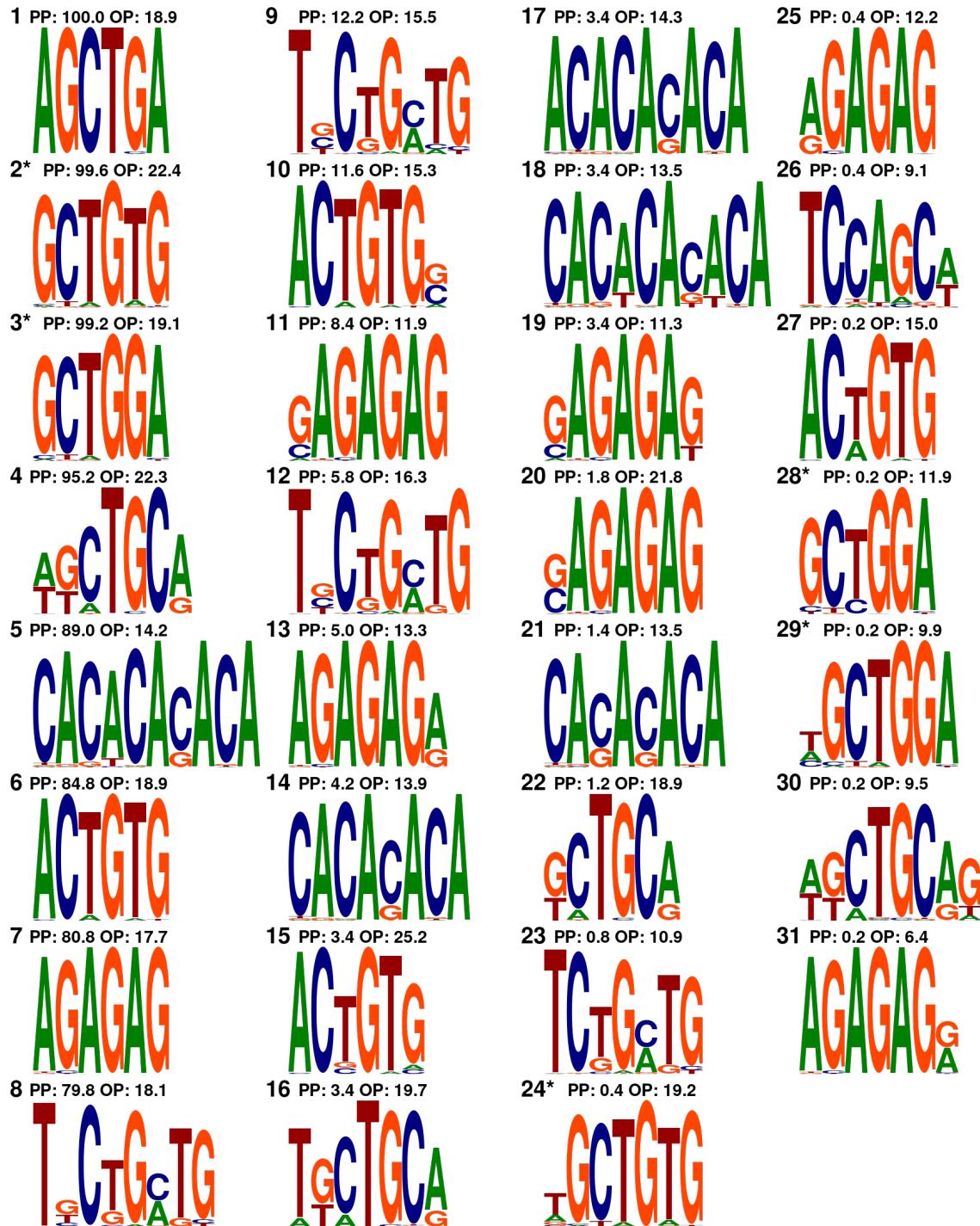


Figure 5.16: **PWM sources detected in sibling differential nucleosome positions.**
PWMS presented as sequence logos with letter height proportional to position informational contentemission probability.

PP: Posterior prevalence; proportion of models within the compressed maximum a posteriori ensemble which have this PWM as a signal source

OP: Observation prevalence; mean proportion of observed position sequences this sequence is used to explain, over all posterior samples

Methods in Section 13.1.17. Code in Section 16.9.29.

Figure 5.17: PWM sources detected in *rys* mutant differential sources.

PWMs presented as sequence logos with letter height proportional to position informational contentemission probability.

PP: Posterior prevalence; proportion of models within the compressed maximum a posteriori ensemble which have this PWM as a signal source

OP: Observation prevalence; mean proportion of observed position sequences this sequence is used to explain, over all posterior samples

Methods in Section 13.1.17. Code in Section 16.9.29.

5.3 Discussion

We began our investigations by revising the original description of the *rys* mutant CMZ phenotype. The apparently increased size of the CMZ in *rys* mutants, relative to siblings, is produced as an effect of one or more of the following phenomena:

1. Inappropriately retained RPCs in animals older than 7dpf
2. Reduced neural population of the specified central retina, relative to the CMZ
3. Expanded and spread-out RPC nuclei

The first two effects are consistent with RPCs failing to specify as particular retinal neural lineages, demonstrated by retention of early progenitor markers in these cells. *rys* RPCs are not, however, arrested in cell cycle. Unlike systems where elevated polyadenylated histone transcript levels resulting in cell cycle arrest [KKT⁺13], we find that the mitotic activity of these cells actually accelerates under an overabundance of polyadenylated H2A and H2B transcript. We therefore suggest that the *rys* phenotype is best characterised by chromatin disorganisation (phenomenon 3) and a failure of RPCs to correctly specify (which covers phenomena 1 and 2) as retinal neural subtypes. The enlarged appearance of the CMZ is a result of these effects, and not a general increase in the CMZ population, RPC cytoplasmic volume⁵, or other variables.

Moreover, in identifying npat as the lesioned gene underlying the *rys* phenotype, we nominate a plausible macromolecular mechanism underlying phenomenon 3 which could produce phenomena 1 and 2. Mutant npat's effects on cell-cycle dependent histone transcription and stability may result in aberrant nucleosome positioning within proliferating cells, by altering the pool of histone proteins available for nucleosome formation. We posit that this is what causes the observed loss of a subpopulation of sibling nucleosome positions in *rys*, together with the gain of novel, aberrant positions. When we investigated whether the sequences of these subpopulations were better modelled by separate emissions processes than a shared emissions process, we found substantial evidence in favour of separate emissions processes, suggesting that nucleosome localisation to unique *rys* positions occurs by different mechanisms than those unique to sibs. Interestingly, the PWM signals we detected in this differential nucleosome position set suggest some detail as to how changes in histone expression might result in the observed nuclear disorganisation of *rys*. The observation that the background models explain the positions unique to siblings better than those unique to mutant *rys* strongly suggests that sequence preference is less important in the process generating sibling positions than in the one generating mutant positions.

The relative importance of primary sequence in nucleosome positioning has been hotly debated; some have advocated for a nucleosome positioning code intrinsic to primary sequence [KMF⁺09], while others have disavowed the existence of any such code [ZMR⁺09]. In general, the nucleosome dynamics community has moved on from these discussions in favour of emphasis on rotational sequence preferences [TCM⁺07] and translational nucleosome positioning by *trans*-acting factors [KKZ⁺18]; a common interpretation of the earlier debate is that sequence preferences tend to dominate only at limiting concentrations of histones, when chromatin formation is rare [Poi13]. The fact that sequence preference is less explanatory for the sibling positions than for the mutant ones is suggestive against this background. It seems likely that the shifted and novel nucleosome positions observed in mutants reflects all of the following:

⁵That is, alone, without an increase in nucleus size. We did not directly measure this parameter.

1. The appearance of nucleosomes with aberrant subunit composition
2. A loss of translational control over *ryst* mutant nucleosomes by *trans*-acting factors
3. The limiting concentration of aberrantly-expressed nucleosomes, resulting in increased influence of “default” sequence-preference positioning

All of the above could plausibly be caused by disrupted histone expression arising from altered npat activity. As regulation of chromatin dynamics is known to be important for both replication timing [GTR⁺10] and cell identity and fate [SVT13], altered chromatin architecture could explain how mutated npat protein produces the observed shift in proliferative activity and failure of RPCs to specify, via altered histone expression. Moreover, chromatin density has recently been specifically implicated as fundamentally involved in the process by which pluripotent cells restrict gene expression to achieve their stable specified fates [GJH⁺17]; it may be this overall process which has been disrupted in mutant *ryst*.

It is possible that the sequence-level inference is compromised by the ad-hoc nature of the sampler used in our nested sampling procedure. For reasons explained in Chapter 10, the ICA model structure makes euclidean space representations difficult, and ensuring sampling is in detailed balance may not be possible, given changing PWM signal lengths. We suggest that it is appropriate to magnify our error estimate by several of magnitude as a consequence. A conservative estimate of 100-fold more error than estimated, this has the effect of reducing the significance of the result from 527 to 5.27 standard deviations, which is still above the typical 5σ significance typically accepted as a discovery in the physical sciences. We conclude that it is far more likely that the differential nucleosome populations found in *ryst* mutants and their phenotypically normal siblings are the result of separate generative processes. It is also possible that our identification of the differential subset of nucleosome positions with the disordered chromatin present in *ryst* RPCs is inaccurate. We have not conducted a full survey of *ryst* proliferative niches, and it is not clear how widely affected other cell types might be. Still, within the retina, only proliferative cells display the chromatin phenotype, and it is on this basis that we make the identification, which is the most parsimonious available.

There are a number of important lacunae in this explanation. It remains unclear what form of the npat protein is expressed, if any. The overall effect on the expressed pool of histones in RPCs also remains uncharacterised. It is likely thhat there are macromolecules involved in the *ryst* pathology which intermediate between the npat lesion and the observed chromatin phenotype which we have not examined. We suggest, however, that the overall effect on specification must be due to a failure of RPCs to organize chromatin for specification, and that, interestingly, this failure does not prevent proliferation. *ryst* therefore presents a useful model of the dissociability of proliferative and specificative behaviours in neural progenitors, which has recently been documented elsewhere in the developing *D. rerio* retina [ESY⁺17]. Given the methodological limitations we encountered in probing protein expression in *ryst* RPCs, the most interesting and productive avenues of research to pursue in *ryst* pertain to this chromatin organisation phenotype. Further experimentation is required to determine how particular changes in chromosome organisation (e.g. alterations in 3-dimensional chromatin organisation of gene expression, number of replication foci, etc.) produce specific features of the *ryst* phenotype.

The zebrafish *ryst* mutant model provides a unique opportunity to study the role of a human NPAT homologue in a complete, developing tissue. This has previously proven difficult using mouse Npat, proviral inactivation of which resulted in early embryonic arrest, prior to tissue formation [DFWV⁺97]. The survival and development of *ryst* mutant embryos beyond this stage may reflect the presence of

wild-type npat transcript contributed maternally [HSK⁺¹³]; *rys* mutants nevertheless do not survive beyond metamorphosis (~21dpf), so npat seems to be similarly obligatory for normal development in zebrafish, if over a longer timeframe. Still, we observed many differences between the function of npat in zebrafish and documented effects in other vertebrates, which require some explication.

As teleost fish are known to have undergone whole-genome duplication subsequent to their radiation from vertebrates, it is possible that zebrafish may have multiple npat paralogues. We have excluded this possibility by failing to identify any significantly similar CDS sequences in the Zv9 zebrafish genome using BLAST, and the synteny analysis in Figure 13.1. The zebrafish npat gene does have a substantially different genomic context from human NPAT, however: it is not associated with the eponymous ATM locus (which has been duplicated, and is present in this duplicate form elsewhere on chromosome 15). ATM's 5' position is, in zebrafish, occupied by hif1al, with the intergenic region lacking canonical E2F promoter sites. Of the 80 vertebrate genomes currently available from Ensembl, this organisation is shared only with the cave fish (*A. mexicanus*), with the human-like ATM-NPAT association preserved in all other species. This apparently evolutionarily novel genomic organisation for npat may be responsible for some of the differences in npat function we report here, relative to its homologues.

We identified alterations in histone mRNA transcription and 3' end processing as likely mechanistically involved in the *rys* phenotype. In both 6 and 8 dpf *rys* larvae as well as npat-morpholino treated 1dpf embryos, we observe increased abundances of total and polyadenylated histone transcripts. The mechanism by which these effects are produced in *rys* remains unclear. The increases in histone transcript abundance observed in both mutant and morpholino-treated animals are at odds with observations that NPAT knockouts display decreases in histone transcription [YWNH03], and that the destruction of CDK phosphorylation sites on NPAT protein, which would be the case in the putatively truncated *rys* npat protein, result in similar declines in histone transcript abundance [Ma00, MGv⁺⁰⁹]. This implies the role of zebrafish npat in regulating histone transcription differs from human NPAT. We are unable to determine from these data whether the overall increases in histone transcript abundance are a consequence of increased histone transcription, or of the greater stability of improperly polyadenylated histone transcript. The increased abundance of polyadenylated transcript in *rys* mutants and npat morpholino-treated embryos is consistent with the observed role of NPAT in recruiting CDK9 to replication-dependent histone gene clusters, known to be important in generating the normal stem-loop structure at the 3' end of these transcripts [PSS⁺⁰⁹], suggesting that this role is conserved in zebrafish. It is also possible that particular histone genes give rise to polyadenylated transcripts in zebrafish, as observed in a variety of cell lines [KKT⁺¹³]. Increased transcription of these particular genes might also account for the observed increase in polyadenylated histone transcript abundance. Although increased abundance of polyadenylated histone transcript has been associated with both cell cycle arrest and differentiation [KKT⁺¹³], we found that in the *rys* mutant CMZ, this was associated with altered cell cycle parameters, failure to differentiate, and ultimately, cell death by apoptosis. This suggests that the presence of polyadenylated histone transcripts are not directly related to particular cell cycle states or differentiated fates, but rather that a particular regime of coordination and control of the expression of polyadenylated and replication-dependent, stem-looped histone transcripts is required to achieve appropriate transitions between these states. In the case of *rys*, this seems to be related to the effect of altered histone expression on chromatin structure.

Chapter 6

Inferring and modelling specification dynamics in retinal progenitors

6.1 Specification as a function of non-proliferative chromatin dynamics

The npat mutant *rys* investigated in Chapter 5 provides support for the basic theme running through this thesis, that lineage commitment or specification can be, and often is, dissociable from the proliferative behaviour of retinal progenitors, as has been suggested by Engerer et al. [ESY⁺17]. *rys* illustrates this: RPCs undergo a final burst of proliferation before the death of the animal¹, but never contribute appropriately to the neural retina. Indeed, older *rys* larvae display a proliferative spillage of aberrant RPCs into the intraocular space between the lens and GCL; the progeny of these aberrant RPCs literally cannot be integrated into the tissue.

That this dissociability has fundamental implications for the quality of our inferences about RPCs can be gleaned from the basic problems encountered in each of the modelling studies. To begin with, the Stochastic Mitotic Mode Explanation models examined in Chapter 2 failed, in large part, because the underlying Simple Stochastic Models conflate proliferation and specification under the “mitotic mode” concept. This leads immediately to the introduction of model structure to explain specification outside of “mitotic mode”, assuming a phased temporal structure to RPC behaviour, without attempting to explain it. Lineage specification and niche exit processes need to be modelled separately from mitotic processes to differentiate their effects on outcomes. Next, proliferative data and morphological measurements in Chapter 4 fail to constrain the posterior distributions on the rate of niche exit in simple phased models, demonstrating that this parameter cannot be inferred with any certainty from overall population-level proliferative dynamics. To accurately model the behaviour of RPCs, data which provides accurate volumetric or temporal constraints on modelled specification or niche exit rate is of paramount importance. Finally, in *rys*, we encounter the problem of explaining RPCs that proliferate but fail to specify; we explain this phenomenon in terms of the effects of mutant npat on chromatin organization by way of effects on histone expression. This directs us to the relevant level of organization for explanation of RPC lineage outcomes: the progression of chromatin states that underlay long-term changes in gene

¹Likely, on this view, arising from systemic failure of progenitors to specify appropriately.

expression, which are required for the generation of the specified protein complement. Our inferential approach, while providing good evidence for the chromatin dynamics we believe arise from the effects of npat on the available histone pool, does not give us insight into how the appropriate progression of chromatin states might be usefully parameterised, beyond confirming that it is likely under the active control of *trans*-acting factors in the sibling case.

While a large number of techniques for studying aspects of chromatin structure exist, few suggest model parameterisation for capturing an overall “nuclear state”. That is, if we consider Waddington’s idea of a cell’s lineage state being a ball rolling down a canalized terrain, it remains unclear what sort of parameter space would allow us to infer where the ball is. Older ideas of cellular state space that rely exclusively on measures of protein expression are unable to address hypotheses about chromatin structure. It remains unclear what aspects of chromatin organisation are causally important in fate commitment, as little formal model comparison has taken place. One might, in the “-omics” enumerationist mode, propose a joint structure-sequence-expression space with thousands of variables drawn from the vast literature on chromatin organisation. Such a project is unlikely to succeed with reasonable compute budgets, even with the sophisticated Bayesian inference tools developed here, which can estimate the evidence for models with thousands of parameters across millions of observations.

Still, if it is not obvious how one might build a general model of the evolution of “nuclear state”, we may suggest some more limited approaches arising from the work presented in this thesis, that may make some headway here.

6.2 Future directions

rys provides a useful platform for investigating the processes required to organize the progression of chromatin through the sequence of states we presume to be required for progression through the series of stages implied by the studies presented in Chapter 2 and Chapter 4, and the associated shifts in RPC lineage contributions. Despite the evidence amassed for the involvement of npat effects on nucleosome positioning in *rys* mutants, many aspects of the suggested mechanism remain obscure. This arises, in large part, from the lack of reliable macromolecular tools for investigating npat and histone proteins in *D. rerio*. It would be useful to focus inquiry here on a small number of candidate histones. However, the large number of zebrafish histone genes, with no crystallographic data available, suggests that prediction of the involvement of particular histones would be difficult.

This situation is now rapidly changing, however. The AlphaFold algorithm has recently been recognized as a solution to the Critical Assessment of protein Structure Prediction (CASP) problem set in the CASP13 competition [AIQ19]. This is the first time protein folding has been adequately predicted *in silico* from sequence data alone. If it is possible to use this algorithm to predict *D. rerio* nucleosome structures from histone folding predictions, this may obviate the need for crystallographic data. Such structural predictions could be used with the posterior estimates of signals putatively arising from nucleosome-DNA contact produced in Figure 5.17 to, in turn, predict which nucleosome compositions are most likely to favour the detected sequences. This might be assisted by 3d pattern matching for DNA sequences [HPG07], or roll-and-slide modelling of the contact motifs [TCM⁺07]. Such a study would produce hypotheses that, if interesting enough to pursue *in vivo*, would supply estimates of how likely various histones are to be involved in the *rys* mutant phenotype, and in what regard, which would help prioritise resources for the development of assays for interrogating the specific transcript and protein

status of the most likely loci.

A more fundamental way of interrogating *rys* “nuclear state” might be provided by focusing on transcription itself, which has been proposed as a primary driver of chromatin organisation [CM18]. Such a project would likely require a transgenic line developed to monitor the transcription of one or more loci of interest in live cells, perhaps using bioorthogonally labelled transcripts, as has recently been reported in zebrafish [WCG⁺20]. This approach could possibly be used either to investigate the status and trafficking of npat and histone transcripts themselves, or to determine the impact of the npat lesion on the transcriptional shaping of chromatin organisation by tracking the transcription of progenitor markers, for instance.

Ultimately, it is likely that models that allow the prediction and control of RPC behaviour *in situ* in zebrafish retinae will treat the chromatin organisation of RPC nuclei as an integrator of multiple relevant sources of information, including physical forces, cytoskeletal dynamics, and extracellular signals. This type of functional organisation, in which biological “meaning” arises from the integration of salient cellular information by a combination of nonarbitrary physicochemical relations and arbitrary biological coding relations, is currently best described by the subdiscipline of biosemiotics [Hof08, Fav15, Hof15]. Of particular interest here is the clear-eyed and unobscurantist work of Marcello Barbieri, which may well provide the metaphysical framework necessary to plumb the depths of this topic [Bar15].

Part II

Software Technical Reports

Chapter 7

GMC_NS.jl: Nested sampling by Galilean Monte Carlo for biological models

`GMC_NS.jl` implements a basic Galilean Monte Carlo nested sampler [Ski12, Ski19] in pure Julia. It uses the updated Galilean reflection scheme of Skilling's later GMC publication [Ski19], which improves the algorithm's performance and preserves detailed balance. It uses the natural attrition of model-particles getting stuck in posterior modes to regulate the number of live particles in the model ensemble. This approach achieves the same end as algorithms which dynamically adjust the ensemble size based on parameters of the ensemble [FHB09, HHHL19], without any computational overhead or additional objective function. A minimum ensemble size may be supplied by the user, which is maintained by initializing a new trajectory isotropically from the position of an existing particle; this ensures that the ensemble may be sampled to convergence even if the number of trajectories started with is insufficient to guarantee this.

7.1 Implementation notes

The basic sampling scheme followed is, first, to initialize an ensemble of models with parameters sampled evenly from across the prior, then to compress the ensemble to the posterior by applying GMC moves to the least-likely model in the ensemble at a given iterate, constrained by its current likelihood (which is the ensemble's likelihood contour for that iterate). GMC tends to move model-particles across the parameter space very efficiently, particularly in the initial portion of compression across the uninformative bulk of the prior mass. That said, GMC particles may get "stuck" in widely separated minima, so there are instances when the least-likely particle has no valid GMC moves. In this case, the trajectory is not continued. Instead, the trajectory is removed from the ensemble and sampling continues, unless the number of particles in the ensemble would decline below the specified minimum. In this case, a new trajectory is begun by resampling from the remaining prior mass within the ensemble. This is accomplished by random "diffusive" proposals, in contrast to the ordinary, directed Galilean movements. This means a random particle remaining within the ensemble is selected, and isotropic velocity vectors

are sampled from this particle to find a starting location for the new trajectory (this vector is conferred to the new particle).

`GMC_NS.jl` follows the common convention of transforming parameter space to a unit-standardized hypersphere. The user is responsible for ensuring that the density of the prior is uniform, that is $\pi(x) = 1$, over the -1:+1 range of the hypersphere dimension. Utility functions `to_unit_ball` and `to_prior` which accept the prior and transformed positions and the relevant prior probability distribution, returning the unit hypersphere or prior coordinate, respectively. In future versions, this scheme is likely to be modified to a 0:1 unit hypercube, which does not require additional operations beyond obtaining the cumulative probability of a parameter value on the prior distribution. The user may also specify a sampling "box" to bound particles from illegal or nonsensical areas of the parameter space (eg. negative values for continuous distributions on positive-valued physical variables). The box reflects particles specularly in the same manner as the likelihood boundary, with the exception that because the orientation of the n th-dimensional box "side" is known (eg. is the plane at $n = 0$.), the reflection is performed by stopping the particle at the box side and giving it a new velocity vector identical to the old but with reversed sign in dimension n .

`GMC_NS.jl` attempts to maintain efficient GMC sampling by per-particle PID tuning of the GMC timestep. GMC treats models as particles defined by their parameter vectors, which give their positions in parameter space, as well as a velocity vector, normally chosen isotropically and only changed when the particle "reflects" off the ensemble's likelihood contour. In GMC, when a model-particle is to be moved through the parameter space (in this case, because it is the least likely particle in the ensemble), a new position is proposed by extending some distance along its velocity vector. This distance is determined by scaling the velocity vector by a "timestep" value, which defines the per-iterate rate at which the particle traverses the parameter space. As the model ensemble is compressed to the posterior, this timestep must decline fairly evenly in order for GMC to be efficient, as the amount of available parameter space declines rapidly. Additionally, GMC particles may enter convoluted regions of parameter space that form small likelihood-isthmuses to other, more likely regions. In order to deal with this, each trajectory is assigned its own PID tuner, which maintains an independent timestep for the trajectory, tuned to target a user-supplied unobstructed move rate (given as a floating point fraction in the range 0:1). In short, this will reduce the timestep if the particle is repeatedly reflecting off the likelihood boundary (in which case it is crossing the remaining prior mass without sampling much from within it, or it is in a highly convoluted area of the local likelihood surface), and extend the timestep if it repeatedly moves without encountering the boundary, so that particles that are closely sampling an open region without encountering the boundary begin to "speed up".

7.2 Ensemble, Model, and Model Record interfaces

In operation, `GMC_NS.jl` maintains an `GMC_NS_Engine` mutable struct in memory. The directory to which the sampler ought to save the ensemble is specified by its `path` field. `GMC_NS.jl` does not maintain calculated models in memory, instead serializing them to this directory. In order to track the positions and likelihoods of model-particles within the ensemble and as samples from the posterior, a `GMC_NS_Engine` maintains two vectors of `GMC_NS_Model_Records` as fields; `models` for live particles, and `posterior_samples` for the previous positions along trajectories. Observations against which models are being scored, prior distributions on model parameters, model constants (if any), and the sampling

box are specified by the `GMC_NS_Engine`'s `obs`, `priors`, `constants`, and `box` fields, respectively. The `GMC_NS_Engine` also specifies settings for the GMC sampler and the PID tuner. Sensible defaults for these values may be passed en masse to an ensemble constructor with the `GMC_DEFAULTS` constant exported by `GMC_NS.jl`.

Therefore, to use `GMC_NS.jl` with their model, the user must write a model-appropriate version of these three structs with the fields specified by the docstrings in the `/src/ensemble/GMC_NS_Engine.jl` file, as well as `/src/GMC_NS_Model.jl`. The user is responsible for an appropriate constructor and likelihood function for the model, any required parameter bounding functions, and so on. The user may optionally write a overloaded `Base.show(io::IO, m::GMC_NS_Model)` function for displaying the model, or a `Base.show(io::IO, m::GMC_NS_Model, e::GMC_NS_Engine)` function for displaying the model with observations. This function can be used with the package's `ProgressMeter` displays for on-line monitoring of the current most likely model.

7.3 Usage notes

7.3.1 Setting up for a run

Given an appropriately coded model, as outlined above, the user must define a number of important values for the sampler before a `GMC_NS_Engine` may be constructed. These values are stored in fields of the ensemble. Firstly, the user must prepare the observation data in whatever format is suitable for the model's likelihood function. Next, one must define a vector of `Distributions` on parameters of the model as the `prior` field of the ensemble. These must be univariate, as the CDF defines the position on that dimension. Some functions to produce univariate marginal distributions from multivariate Normal Gamma and Normal Inverse Gamma priors are available in `NGRefTools.jl`. Next, any constants used in the likelihood function must be available in the `constants` field of the ensemble. A `box` matrix with n -dimensional rows containing minimum and maximum values in the first and second columns defines the maximum extent of parameter space to be explored. The sampler expects the box in the transformed hypercoordinates mentioned above; a one-dimensional box across the entire prior would be `[-11]`. It is convenient to compose the matrix in the original parameter space and perform the transformation with a broadcast call to `to_unit_ball`, either in the ensemble constructor or in the calculation script.

7.3.2 PID tuner settings for GMC

As noted, GMC particle timesteps are governed by individual PID controllers. These controllers are defined by a number of values which must be supplied as `GMC_NS_Engine` fields. These are summarized below:

`GMC_Nmin` (Integer): The minimum number of active model-particles in the ensemble. After the initial sample from the prior, new particles will only be generated when required to maintain this value; otherwise, when a valid proposal cannot be found for the current least-likely trajectory (i.e. the PID tuner drives the timestep below `GMC_τ_death`), the nested sampling step consists of removing that particle to the posterior samples and moving on to the next live particle.

`GMC_τ_death` (Float): Value of τ timestep associated with a trajectory below which the particle is considered to be dead. This value is in hypersphere coordinates. It is reasonable to make it very small

for likelihood functions with low or no Monte Carlo noise; it should be somewhat larger for functions with significant MC noise.

GMC_init_ τ (Float): Initial value of the timestep for all trajectories. This applies to the particles assembled on ensemble construction; particles generated from diffusional resampling inherit the τ of their parent, but with the isotropic vector which generated the proposal for their current location.

GMC_tune_ μ (Integer): Length of tuner memory; the proposal acceptance rate α is calculated over the previous μ sampling steps for this trajectory.

GMC_tune_ α (Float): Target acceptance rate for proposals. Should generally be .8 or higher, depending on desired sampling density.

GMC_tune_PID (NTuple3,Float): kP, kI, and kD constants for the tuner. Keep in mind that the range of values for α is 0.:1.; given a **GMC_tune_** α constant of .8, the maximum error that will be encountered is likewise .8, so the values of the constants will generally be much smaller than 1. As with most PID applications, kP should be the largest value, and in this case generally determines the number of searches conducted before **GMC_** τ _death is reached.

GMC_timestep_ η (Float): If this value is >0., a normally distributed error with a standard deviation of $\tau \cdot \text{GMC_timestep}_\eta$ is applied to τ before calculating the GMC proposals, as suggested by [Ski12].

GMC_reflect_ η (Float): If this value is >0., it is used to perturb reflection vectors, as suggested by [Ski12]. It should be a small value.

GMC_exhaust_ σ (Float): Scale parameter applied to ensemble size to determine the maximum number of unsuccessful searches to perform before terminating sampling. Can usefully be fairly large.

7.3.3 Parallelization

GMC_NS.jl does not have an explicit parallelization scheme; the sampler proceeds linearly to the next least-likely particle and performs the appropriate Galilean trajectory sampling procedure. Parallelization may nonetheless be achieved at two levels; the likelihood function may be parallel according to the needs of the user, and individual nested sampling runs may be arbitrarily combined. That is, if one desires to parallelize a **GMC_NS.jl** job, it is best to think in terms of the total number of trajectories to be sampled, dividing this by the number of machines available to perform the sampling work, to obtain the number of particles to be sampled on each machine. The sampling runs can then be combined post hoc to achieve the desired final accuracy. In this scheme, the likelihood function is best parallelized by threading on a single machine.

7.3.4 Displays

If desired, parameters of the ensemble and most-likely model can be monitored on-line, while **GMC_NS.jl** is working. This is done by specifying a vector of function vectors (ie a `Vector{Vector{Function}}`). One such vector may be supplied to specify the displays to be shown above the `ProgressMeter`, one for those below, supplied as the `upper_displays` or `lower_displays` keyword arguments to `convergeensemble!()`. **GMC_NS.jl** will rotate the upper and lower displays to the next vector of display functions every `disp_rot_its` sampling iterates, supplied as another `converge_ensemble!()` keyword argument. Default display functions are available in `/src/utilities/progress_displays.jl` and are exported for easy composition of the function vectors.

7.3.5 Example use

`GMC_NS.jl` is supplied with two simple, but useful, statistical models by default. These are the `Normal_Model` and `LogNormal_Model` subtypes of `GMC_NS_Model`. Users may familiarize themselves with `GMC_NS.jl`'s operation by the use of these models. A simple demonstration is supplied in the `/test` directory of the package. The program is detailed with additional comments below. The demonstration involves converging an ensemble of 10 `Normal_Model` chains initialized from an inaccurate `NormalGamma` prior on data generated from a known `Normal` distribution.

```

1 #Import dependencies
2 using GMC_NS, Distributions, ConjugatePriors
3
4 #Generate some data from a Normal model with \mu 100. and \sigma 5.
5 sample_dist=Normal(100.,5.)
6 samples=rand(sample_dist, 10000)
7
8 #An inaccurate ConjugatePrior
9 prior=NormalGamma(300.,2e-3,1.,10.)
10
11 #Box for the two parameters of the Normal model.
12 #First column: parameter minimums.
13 #Second column: parameter maximums
14 #First row: \mu
15 #Second row: precision, ie 1/sqrt(\sigma)
16 #eps() is machine epsilon, ~1e-16; a small number
17 box=[0. 1000.
18      1/1000. 1/eps()]
19
20 #Start with the default sampler settings
21 #Index 1: Lower the minimum number of ensemble particles to 5
22 #Index 2: The minimum allowable particle timestep is set to a very small number; the
23   ↳ likelihood of the model parameterisation can be determined precisely and is well
24   ↳ behaved over the parameter space.
25 gmc=GMC_DEFAULTS
26 gmc[1]=5
27 gmc[2]=1.e-15
28
29 #Assemble the ensemble. Begin with 10 particles
30 e=Normal_Ensemble("NE_test", 10, samples, prior, box, gmc...)
31
32 #Define the upper displays to be rendered above the status line. Here we rotate a
   ↳ single display between three readouts.
33 uds=Vector{Vector{Function}}([[convergence_display],[evidence_display],[info_display]])

```

```

33 #Define the lower displays to be rendered below. We want to look at the most likely
   ↵ model in the ensemble plotted against the observations the whole time.
34 lds=Vector{Vector{Function}}([[model_obs_display]])
35
36 #Converge the ensemble, serializing to pwd/NE\test every 100 iterates and rotating
   ↵ the chosen displays every 1000 iterates.
37 converge_ensemble!(e,backup=(true,100),upper_displays=uds, lower_displays=lds,
   ↵ disp_rot_its=1000)

```

Typical output after executing this program is presented in Figure 7.1. The maximum a priori parameters may be read off the θ line of the `model_obs_display` below the status line. The algorithm estimates that the model from which the sample was drawn has a μ of ~ 100.29 and a precision of $\sim .045$, corresponding to a σ of ~ 4.7 ; the global optimum has been found despite the poor starting prior.

```
julia> converge_ensemble!(e, backup=(true,100), upper_displays=uds, lower_displays=lds, disp_rot_its=1000)
    Information History 4, i 2655, τ 0.00016045922271270535 ± 1.0e-155

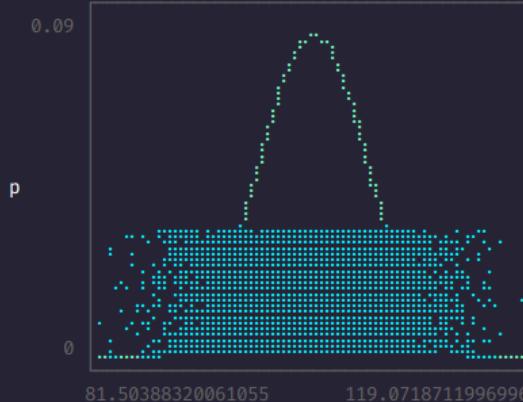
    Ensemble H
    Iterate
GMC-NS:: Converged. Time: 0:00:22 (5748 iterations). logZ: -31343.506034949176
Current MAP model
    Normal Model 1.19, log_Li -30390.369402920995

    Model
    Obs
    p
    X
    θ: [100.2878772001551, 0.04534653431580314]
    v: [0.17847619452553798, 0.4335501569005585]
    [ Info: Job done, sampled to convergence. Final logZ -31343.50451521636 ± 12.098247734502335
```

Figure 7.1: Example BMI.jl Output

Chapter 8

CMZNicheSims.jl: *D. rerio* CMZ RPC niche simulators

8.1 Introduction

`CMZNicheSims.jl` (hereafter `CNS.jl`) comprises a set of three simulators of zebrafish retinal progenitor cells (RPCs), representing the postembryonic Circumferential Marginal Zone niche at varying levels of abstraction. These simulators were used in [Chapter 4](#). This package extends `GMC_NS.jl`, described in [Chapter 7](#) and requires it. Prior distributions are specified by `Distributions.jl`, `ConjugatePriors.jl`, or other `Distributions`. The simulators are organized around the nested sampling procedures implemented in that package. This means that simulations are executed as sample chains from the simulations' parameter space in an ensemble. The basic operation of this package is accomplished by assembling an ensemble of models from the appropriate simulator submodule of `CNS.jl`, then elongating the sample chains of this ensemble by Galilean Monte Carlo using `GMC_NS.jl`'s `converge_ensemble` function. Note that `GMC_NS.jl`'s sampler settings are specified by fields of the `CNS.jl` model ensemble struct and not as arguments to `converge_ensemble`; even if default sampler settings are used, sampler operation should be understood before model priors are selected.

The three model ensemble types specified by `CNS.jl` are as follows:

- `CMZ_Ensemble`: Phased difference function models of CMZ population and overall retinal volume, intended to model total retinal CMZ population and its contributions to overall retinal volume over long time periods (months)
- `Slice_Ensemble`: Basic implementation of the “slice model” concept elaborated in [Chapter 3](#) for phased difference function CMZ models, uses a submodel of lens growth to determine “out-of-slice” growth contributions, intended to represent the population of a CMZ slice over long time periods (months)
- `Thymidine_Ensemble`: “Slice model” of proliferative CMZ population exposed to a pulse of thymidine analogue, intended to model cell cycle kinetics in the CMZ over short time periods (hours).

The operation of these simulators is described in more detail below. In general, the workflow is to assemble the ensemble, converge it, and to estimate the model evidence and posterior parameters from

the converged ensemble. For more on evidence and posterior estimation by `GMC_NS.jl`, see Chapter 7.

8.2 CMZ_Ensemble model and usage

A `CMZ_Ensemble` consists of `CMZ_Models` whose likelihood is assessed by Monte Carlo evaluation of a system of difference equations for simulated retinae, initialized from independent samples from the population and volume distributions specified in the ensemble constants vector. The update functions are specified in general by Equation 4.1 and Equation 4.2. However, the implementation of these functions in the model likelihood function (`CMZ_mc_1lh`, found in Section 16.5.3) differs from this presentation; the equations are not evaluated stepwise day-by-day, but are rather updated for the next model event, either an observation timepoint or a phase transition date. The specific equations used in this function are of the computationally efficient factorial form suggested by Langtangen [Lan12, p. 559], so that the equation for the CMZ population n days after some time 0 becomes:

$$p_n = p_0 \cdot (2^{\frac{24}{CT}} - \epsilon)^n$$

while the equation for retinal volume becomes:

$$v_n = v_0 + p_0 \cdot \epsilon \cdot \mu_{cv} \cdot \frac{1 - (2^{\frac{24}{CT}} - \epsilon)^{n-1}}{1 - (2^{\frac{24}{CT}} - \epsilon)}$$

After Monte Carlo iterative simulation of many retinae, the resultant simulated population and volume distributions at the observed time points are estimated by fitting `LogNormal Distributions`, after which the joint log likelihood of the observations given the simulation is calculated. The likelihood function is threaded so that the total number of Monte Carlo iterates for any given model parameter vector is divided equally between threads. For very large numbers of iterates, this can result in some load balancing inefficiency; in general, it is unnecessary to specify more than 5 million iterates in the ensemble constants vector (item 3 below). 1 million should be adequate for most purposes and allows the efficient sampling of ≥ 3 phase models on one performant machine.

A `CMZ_Ensemble` may be assembled by specifying the path of the folder in which its constituent model files will be serialized, the starting number of chains in the ensemble (a few thousand may reasonably be expected to converge within a day or two on a single high performance machine), the observations to estimate the models against, a vector of prior distributions, a vector of constants, a box matrix, and the sampler settings vectors. The parameter vectors must follow these guidelines:

1. Observations vector: Must be a vector of vector tuples. The first member of the tuple is the population observation vector (total CMZ population at the vector's date index, see 3), the second member is the date's total retinal volume observation vector (in cubicmicrometers). Ie. the observations vector takes the form of a `AbstractVector{<:Tuple{<:AbstractVector{<:Float64}, <:AbstractVector{<:Float64}}}`; the tuple of population and volume data found at index `x` corresponds from animals measured on date `constants_vec[1][x]`.
2. Prior vector: For each desired phase of the model, the prior vector must contain a distribution over that phase's mean population cycle time (in hours) and its mean exit rate ($\geq 0.$, may exceed 1.). Paired cycle time and exit rate for each phase is followed by one less phase transition time in days

than the number of phases. Ie. a two-phase prior vector might be, in pseudocode, [Phase1CT, Phase1ER, Phase2CT, Phase2ER, Phase1End].

3. Constants vector: A `Vector{<:Any}` containing, in order:
 - (a) T vector: Vector of integers or round floats, age in days of animals for each observations tuple
 - (b) Population distribution: `Distribution` defining the initial range of population values. Should normally be a fit from the initial population size observations
 - (c) Volume distribution: `Distribution` defining the initial range of retinal volume values. Should normally be a fit from the initial retinal volume observations
 - (d) Monte Carlo iterates: Integer specifying the number of retinae to be simulated at each point sampled from the parameter space. 1e6-5e6 is usually fine.
 - (e) Phases: Integer defining the number of phases specified by the prior vector
4. Box matrix: Two columns each of the same length as the prior, defining the minimum (column 1) and maximum (column 2) bounds for each parameter dimension to be sampled.

As an example, an ensemble with 3000 chains and using the default GMC sampler settings may be initialized and sampled as follows, where variable names correspond to the vectors described above:

```

1 e=CMZ_Ensemble(path, 3000, observations, prior, constants, box, GMC_DEFAULTS)
2 converge_ensemble!(e, backup=(true,50), mc_noise=.3)

```

The `mc_noise` parameter in this, and other models of the package, can be estimated by calculating the standard deviation of repeated evaluations of a parameter vector with a reasonably high likelihood, given the data vectors. The suggested value is roughly appropriate for 1e6 Monte Carlo iterates, given the data vectors used in Section 16.9.5.

8.3 Slice_Ensemble usage

A `Slice_Ensemble` consists of `Slice_Models`, whose likelihood is assessed by Monte Carlo evaluation of a hybrid system of a difference equation and a power-law equation, representing a simulated CMZ RPC population in a retinal slice of arbitrary thickness. The `Slice_Model` likelihood function uses the same factorial solution to calculate the CMZ population after time steps of n days as that described for `CMZ_Models`. However, these population values are penalized by a value determined by using the power-law model of lens growth to determine the change in CMZ circumference over this time period, and the per-slice contribution required to expand the CMZ by this amount, given the present slice population and slice thickness (see Section 16.5.5 for this calculation).

`Slice_Models` are broadly similar to `CMZ_Models`. The parameter vectors required to assemble a `Slice_Ensemble` differ from those mentioned for a `CMZ_Ensemble`, however, and are summarised below.

1. Observations vector: Must be a vector of vector tuples. The first member of the tuple is the population observation vector (total CMZ population at the vector's date index, see 3), the second

member is the date's total retinal volume observation vector (in cubicmicrometers). Ie. the observations vector takes the form of a `AbstractVector{<:Tuple{<:AbstractVector{<:Float64}, <:AbstractVector{<:Float64}}}}`; the tuple of population and volume data found at index `x` corresponds from animals measured on date `constants_vec[1][x]`.

2. Prior vector: For each desired phase of the model, the prior vector must contain a distribution over that phase's mean population cycle time (in hours) and its mean exit rate ($\geq 0.$, may exceed 1.). Paired cycle time and exit rate for each phase is followed by one less phase transition time in days than the number of phases. Ie. a two-phase prior vector might be, in pseudocode, `[Phase1CT, Phase1ER, Phase2CT, Phase2ER, Phase1End]`.
3. Constants vector: A `Vector{<:Any}` containing, in order:
 - (a) T vector: Vector of integers or round floats, age in days of animals for each observations tuple
 - (b) Population distribution: `Distribution` defining the initial range of population values. Should normally be a fit from the initial population size observations
 - (c) Lens model: `Lens_Model` defining the lens circumferential growth power law and the slice's sectional thickness.
 - (d) Monte Carlo iterates: Integer specifying the number of retinae to be simulated at each point sampled from the parameter space. `1e6-5e6` is usually fine.
 - (e) Phases: Integer defining the number of phases specified by the prior vector
4. Box matrix: Two columns each of the same length as the prior, defining the minimum (column 1) and maximum (column 2) bounds for each parameter dimension to be sampled.

As an example, an ensemble with 3000 chains and using the default GMC sampler settings may be initialized and sampled as follows, where variable names correspond to the vectors described above:

```

1 e=CMZ_Ensemble(path, 3000, observations, prior, constants, box, GMC_DEFAULTS)
2 converge_ensemble!(e, backup=(true,50), mc_noise=.3)

```

Chapter 9

BioBackgroundModels.jl: Parallel training of Hidden Markov Model zoos of genomic background noise

`BioBackgroundModels.jl` (`BBM.jl` hereafter) is a pure Julia package intended to automate the optimization and selection of large numbers (“zoos”) of Hidden Markov Models (HMMs), using sequence sampled from specified partitions of a given genome, on arbitrary collections of hardware. It is supplied with extensive utility functions for genomic sampling, high performance implementations of both the Baum-Welch and Churbanov-Winters algorithms for optimization of HMMs by expectation maximization (EM), as well as reporting functions for summarizing the results of the optimized model zoos. These tasks are necessary to select of models of genomic background noise, from which motif signals may be detected using a package such as `BioMotifInference.jl`.

HMMs generated by `BBM.jl` are standard ≥ 1 state HMMs that emit symbols corresponding to stretches of 1 or more base pairs. Following Down et al. [DH05], the number of bases encoded by an HMM symbol is denoted by the “order” of the HMM. 0th order HMM emit 4 symbols, one for each of the genomic nucleotides, while 1st order HMMs emit 16 symbols, one for each 2-base combination, and 2nd order HMMs emit 64 symbols, one for each 3-base combination, and so on. It is expected that users will be interested in comparing the explanatory value of a variety of background HMM state numbers and orders in selecting appropriate background models. These can all be optimized in one batch; because high state and, to a lesser extent, high order numbers impose much more significant memory costs, the use of the Churbanov-Winters linear memory algorithm is strongly recommended¹. A basic load balancing system has been provided to allow users to prefer particular machines for some optimization jobs over others, which permits the efficient use of clusters of dissimilar hardware in the batch context.

¹While the theoretical performance of the Churbanov-Winters algorithm is lower than Baum-Welch [CW08], the implementation herein is highly optimized and performant. For many use cases, it is both less memory-intensive and about as fast as Baum-Welch.

9.1 Genome partitioning and sampling

The optimization of an HMM zoo is performed against a sample of a “genome partition”, which defines a portion of a genome in relation to a genomic feature set. The required files to sample from a genome partition are:

- A whole-genome sequence in valid FASTA format (eg. a .fna or other appropriate nucleotide sequence file).
- An index for the genomic sequence file (eg. an .fna.fai file)
- A genomic feature file, annotating the genomic sequence, in GFF3 format

At present, BBM.jl offers a default strategy of sampling without replacement from exonic, periexonic, and intergenic partitions of genomes. This partition scheme is intended to differentiate between genomic sequences that are likely to differ in terms of the appropriateness of HMMs of different orders as background models. That is, the selective pressure imposed on genomic sequence by the codon code (exonic partition) and other higher-order functional features of gene organization (periexonic partition) are likely to favour different sorts of background HMM model than the simpler repetitive structure of intergenic material.

Sampling functions should normally be accessed through the API provided to the user. Firstly, sampling jobs may be set up by using the `setup_sample_jobs` function, which uses user-supplied paths to the files listed above, as well as user-specified partitioning variables, to divide the supplied genome into the partitions described and set up channels for these sampling jobs. For example:

```

1 using BioBackgroundModels
2 #FILE PATHS
3 danio_gff_path = "Danio_rerio.GRCz11.94.gff3"
4 danio_genome_path = "GCA_000002035.4_GRCz11_genomic.fna"
5 danio_gen_index_path = "GCA_000002035.4_GRCz11_genomic.fna.fai"
6
7 #CONSTANTS FOR GENOMIC SAMPLING
8 const sample_set_length = Int64(4e6)
9 const sample_window_min = 10
10 const sample_window_max = 3000
11 const perigenic_pad = 500
12
13 channels = setup_sample_jobs(danio_genome_path, danio_gen_index_path, danio_gff_path,
→ sample_set_length, sample_window_min, sample_window_max, perigenic_pad)

```

Here, `sample_set_length` defines the overall number of bases to be sampled from each partition, while `sample_window_min` and `sample_window_max` define the minimum and maximum length of each individual sample to be taken, without replacement, from the partitions. `sample_set_length` should be chosen to supply both training and test sets for background model selection; ie. it should be twice the desired length of the observation set in training. Lastly, `perigenic_pad` defines the number of bases up-

and down-stream of the first and last codon to consider, along with intronic material, as constituting the “perigenic” partition. This will include promoter elements, splicing signals, and the like.

Secondly, the partitions are sampled (in parallel, by worker, if desired), to produce dataframes of samples and relevant coordinate and strand orientation information:

```

1 using Distributed,Serialization
2 sample_output = "samples"
3 worker_pool = addprocs(3)
4 sample_record_dfs=execute_sample_jobs(channels, worker_pool)
5 serialize(sample_output, sample_record_dfs)

```

Here, one worker per partition is used to sample; lower numbers of workers will also work while larger numbers are of no benefit. In this example, the sample dataframes are serialized to the file “samples” for later use; they can also be used directly as described below.

9.2 Optimizing BHMMs by EM algorithms

Background HMM optimization by the expectation-maximization algorithms is performed by a similar two-step setup-and-execute functional pattern as genome sampling. Firstly, `setup_EM_jobs!` is used to transform supplied “job IDs” and the observations supplied by genome sampling into sets of EM chains to be elongated by the EM algorithm. `BBM.jl` “job IDs” take the form of `Chain_ID` structs, whose fields specify a string denoting the partition that forms the observations for the optimization, the number of states in the HMM, the order of the HMM’s emitted symbols, and an integer denoting the replicate number of the chain. In general, for any given partition, number of states, and emission order, three or more replicates are advised, to confirm that the converged values of any particular HMM are likely to be near the global optimum [YBW15]. That is, if replicates of some job ID chain all terminate with similar parameter values (see [Section 9.3](#) to examine this using `BBM.jl`), one such chain may reasonably be selected as a representative of the likely global optimum present in this region of the parameter space.

The helper function `split_obs_sets` is used to divide the genomic sample dataframes generated by the sampling API, described above, into training and test sets for optimization. The training set is supplied for the use of the EM algorithms, while the test set is used solely to calculate the final likelihoods of optimized models.

By default, `BBM.jl` initialises new HMM chains by sampling randomly from possible emission vectors, with strong priors on transition matrices with robust autotransition. Generally speaking, this tends to prevent chains from being optimized to trivial local minima with very short state residency times. This reflects our exclusive interest in those HMMs whose states represent stretches of nucleotides with shared biological significance; HMMs which tend to have state residency emission lengths of less than a few bases are of questionable biological relevance. If desired, the user may supply their own initialisation function as the `setup_EM_jobs!` keyword argument `init_function`, which defaults to `autotransition_init`. The default function should serve as the template for such user-defined initialisation functions.

`BBM.jl` operation is designed to be robust to most kinds of interruption, and the `setup_EM_jobs!` function may be used safely on existing sets of EM chains generated by `BBM`. This allows for resumption

from interruption, reconfiguration of hardware, decreasing the desired chain termination threshold, and so on. The dict containing existing serialized chains, if any, should be supplied to the setup function as the `chains` keyword variable to allow this.

```

1 using BioBackgroundModels, DataFrames, Distributed, Serialization
2
3 #FILE PATHS
4 hmm_output = "chains"
5 sample_output = "samples"
6
7 #JOB CONSTANTS
8 const replicates = 3 #repeat optimisation from this many seperately initialised
    → samples from the prior
9 const Ks = [1,2,4,6] #state #s to test
10 const order_nos = [0,1,2] #DNA kmer order #s to test
11 const delta_thresh=1e-5 #stopping/convergence criterion (log probability difference
    → btw subsequent EM iterates)
12 const max_iterates=50000
13
14 #PROGRAMATICALLY GENERATE Chain_ID Vector
15 job_ids=Vector{Chain_ID}()
16 for (obs_id, obs) in training_sets, K in Ks, order in order_nos, rep in 1:replicates
17     push!(job_ids, Chain_ID(obs_id, K, order, rep))
18 end
19
20 #SPLIT GENOME SAMPLES INTO TRAINING AND TEST SETS
21 sample_dfs = deserialize(sample_output)
22 training_sets, test_sets = split_obs_sets(sample_dfs)
23
24 #INTIIALIZE HMMS
25 if isfile(hmm_output) #if some results have already been collected, load them
26     hmm_results_dict = deserialize(hmm_output)
27 else #otherwise, pass a new results dict
28     hmm_results_dict = Dict{Chain_ID,Vector{EM_step}}()
29 end
30
31 em_jobset = setup_EM_jobs!(job_ids, training_sets; delta_thresh=delta_thresh,
    → chains=hmm_results_dict)
```

Once the EM jobset is set up, it is splatted into the `execute_EM_jobs!` function with any valid worker pool and the path for the Dict of EM chains to be serialized. All workers in the pool should be in `:master_worker` topology with the master process. BBM.jl workers will obtain new chains to elongate by EM until no more are available; the number of jobs, therefore, defines the useful upper limit for the

number of workers in the pool.

Load balancing is achieved by using BBM.jl’s LoadConfig struct, whose fields define acceptable ranges of states, orders, and optional vectors of blacklisted and whitelisted `Chain_IDs`. A Dict of LoadConfigs keyed by Integer defines the appropriate LoadConfig to use for any given worker’s integer id (ie. the output of `Distributed.myid()`).

The results of EM optimizations are serialized as they are “pulled off the wire”, so that they cannot be lost by the algorithm being interrupted, network failure etc. Consequently, `execute_EM_jobs!` does not return anything that needs to be serialized for subsequent analyses; results are always located at the path provided to the function and may be freely inspected by the tools described in Section 9.3 at any time during or after the optimization.

An example of `execute_EM_jobs!` usage, following from the setup example given above:

```

1 #SETUP LOAD BALANCING
2 local_config=LoadConfig(1:6,0:2)
3 remote_config=LoadConfig(1:4,0:1)
4
5 load_dict=Dict{Int64,LoadConfig}()
6
7 worker_pool=addprocs(no_local_processes, topology=:master_worker)
8 for worker in worker_pool
9     load_dict[worker]=local_config
10 end
11
12 remote_pool=addprocs([(remote_machine,no_remote_processes)], tunnel=true,
13   ↳ topology=:master_worker)
14 for worker in remote_pool
15     load_dict[worker]=remote_config
16 end
17
18 worker_pool=vcat(worker_pool, remote_pool)
19
20 #EXECUTE EM JOBS
21 execute_EM_jobs!(worker_pool, em_jobset..., hmm_output; load_dict=load_dict,
22   ↳ delta_thresh=delta_thresh, max_iterates=max_iterates)

```

9.3 BHMM analysis and display

A report generation and display API is provided in order to analyse the results of optimizing a zoo of background HMMs using BBM.jl. One function, `generate_reports`, serves to prepare all of the available reports on the zoo. Its use is straightforward:

```

1 chains=deserialize(hmm_output)
2 sample_dfs = deserialize(sample_output)
3 training_sets, test_sets = split_obs_sets(sample_dfs)
4
5 report_folders=generate_reports(chains, test_sets)
6 serialize(survey_folders, report_folders) #save reports

```

`generate_reports` creates a Dict of `Report_Folders`, keyed by partition id string. Each `Report_Folder` contains a `Partition_Report` with information about all HMMs trained on that partition, a `Replicate_Report` which enables the comparison of the replicates of the best model available for the partition, and a Dict of `Chain_Reports` keyed by `Chain_ID`, which give specific information concerning each chain produced for the partition. Examples of report output follow, derived from the model zoo training task whose results are summarised Figure 13.7. The contents of the reports on background models trained on the *D. rerio* “exonic” partition follow.

Figure 9.1 displays partial output from the `Partition_Report` associated with BHMM training on the exonic partition. The report identifies the partition in question, and, for each order of BHMM trained on the partition, displays a plot of the likelihood of the models vs. the number of states in the model. In this case, 0th and 2nd order models are displayed. The likelihood of a naive, 0th-order, 1-state model with equal emission probability for each symbol serves as a benchmark for BHMM likelihood; this value is plotted as a magenta line across the state axis. In this case, all 0th-order models are more likely descriptions of *D. rerio* exonic material than a naive model, while all 2nd-order models fall below this benchmark likelihood. This reflects the greatly expanded parameter space associated with 64 emission symbols. Thus, the additional exonic order capturable by 3mers is insufficient to offset the decreased probability of any given sequence using this many symbols.

Individual models are plotted as scatterplot points. Converged models are rendered in green, unconverged models in red². In this case, no unconverged models are present. The 0th-order models are tightly converged such that their likelihoods are not differentiable, while the 2nd-order models are more spread out across the larger parameter space. The intended use of this report is to perform a quick visual assessment of the state of the zoo trained on the specified partition, before proceeding to the `Replicate_Report` and particular `Chain_Reports`.

Figure 9.2 displays partial output from the exonic `Replicate_Report`. The chains displayed in the report are the most likely replicate set for the partition. The report consists of paired convergence plots for the autotransition probabilities and 2-dimensional symbol emission probabilities for each state and each replicate in the set. Because there is no guarantee that HMMs from the same optima will have the same ordering of states, similar states are identified by minimizing the Euclidean distance between state emission vectors across the trained replicates. In this case, replicate 1’s second state (red) is also identified with the second state of replicate 2 (green), but the sixth state of replicate 3 (blue).

The autotransition probability subplot displays the transition matrix diagonal probability for the designated state and replicates (that is, the probability that the HMM will stay in the state). If the HMMs are correctly converged on a global optimum, the autotransition probabilities for all chains should

²The reports API is agnostic about the state of the model zoo reported on; it can be used to monitor zoos before they are fully converged.

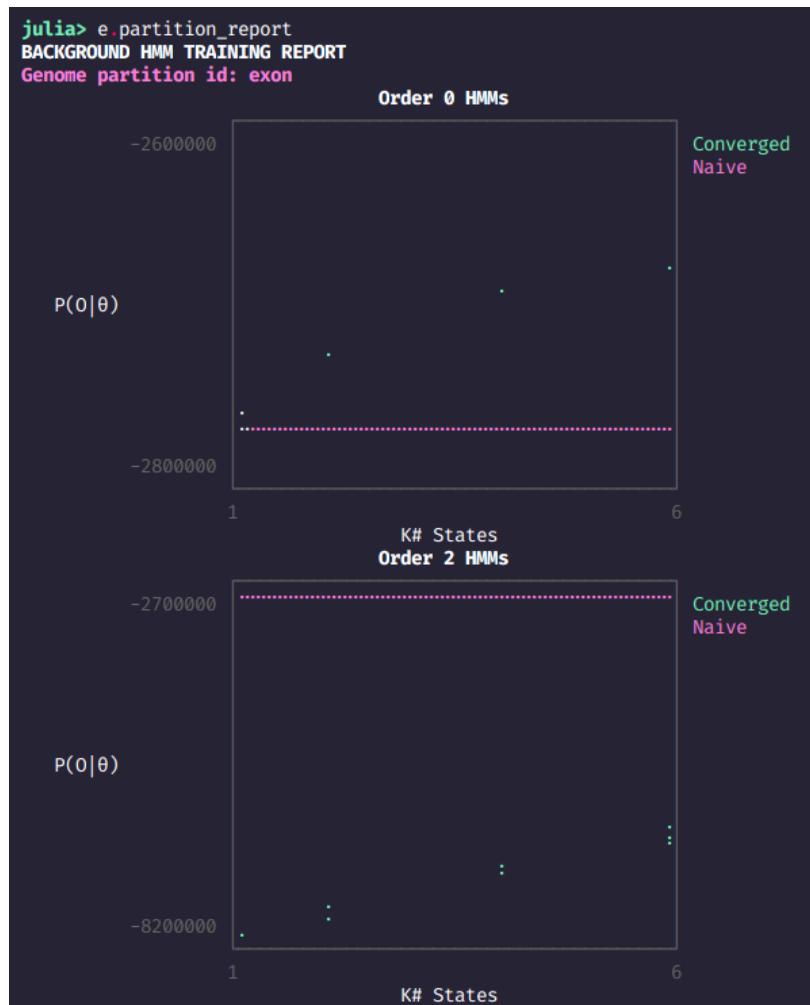


Figure 9.1: Example BBM.jl Partition Report

terminate at nearly the same value. Given that this probability will be stationary on converged chains, this can be visually confirmed by looking for substantial horizontal overlap of the chain autotransition probabilities for the state, as seen in Figure 9.2.

The symbol probability subplot displays the evolution of the probability of the first symbol emitted by the state plotted against the probability of the second symbol, providing a simple two-dimensional view into the convergence of the state's emission vector. If the chains are correctly converged on the global optimum, the plot's appearance should show chains from divergent areas of this two-dimensional parameter subset converging on the same values for the two symbols, as seen in Figure 9.2.

`Chain_Reports` for all HMMs trained on the partition are available in a Dict keyed by `Chain_ID`, found at the `chain_reports` field of the report folder. Example output for replicate 1 from the above-described replicate set is presented below. `Chain_Reports` consist of three major sections, described in turn.

The first `Chain_Report` section begins by stating the state number and order of the BHMM, as well as the genomic partition on which it was trained. This is followed by the likelihood of the model given

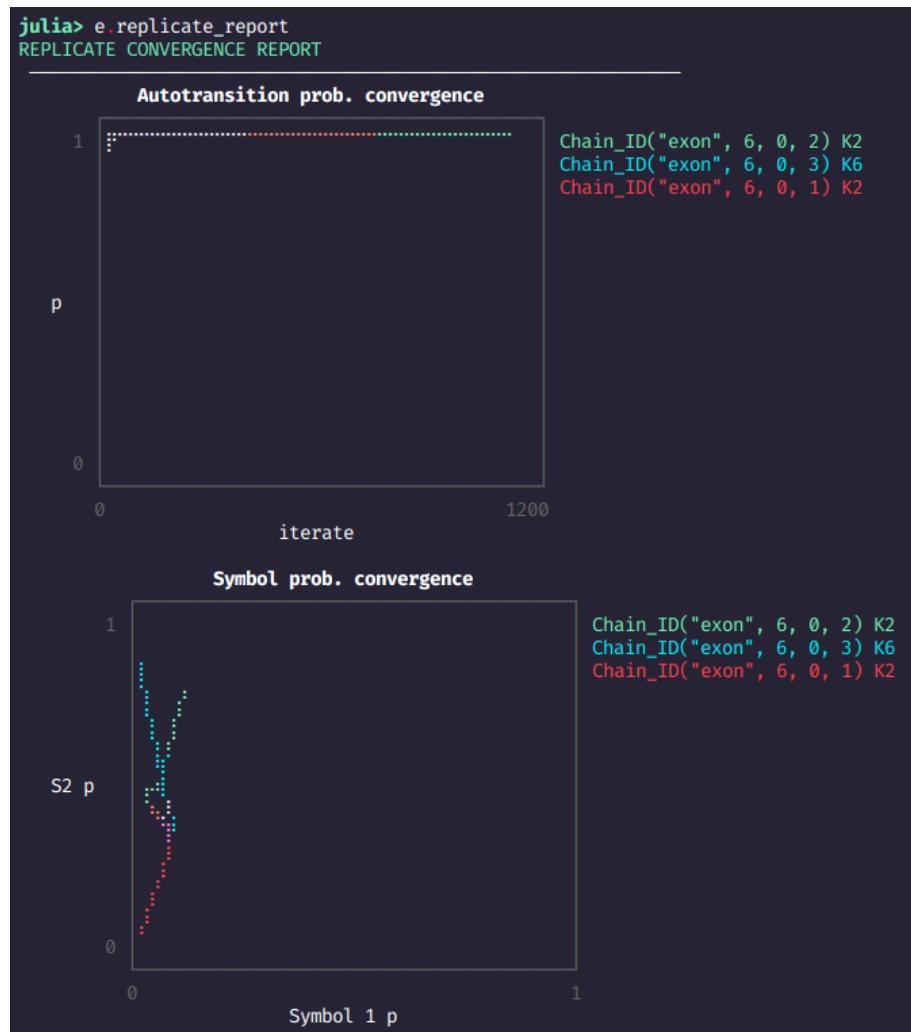


Figure 9.2: Example BBM.jl Replicate Report

the test observation set, compared to the naive model. The line stating these likelihoods will be green if the BHMM is better than the naive model, red if not. The general information concludes with the replicate number of the BHMM, as well as the convergence status of the chain and the final EM step size.

The section continues by stating the initial state probability vector a and the state transition matrix A for the converged BHMM. Emission vectors for the BHMMs' state are summarized by their informational content; symbols with $\geq .7$ bits of information are printed in bold capital characters, those with $.7$ information $\geq .25$ are printed in capital characters, those with $.25$ information $\geq .05$ are printed with lowercase characters, and those with less than $.05$ bits are not printed at all. In this case, state 2 emits adenine and cytosine symbols more frequently than guanine or thymidine, while this is reversed in state 4. The other states have similar enough emission probabilities for the four bases that they do not register on the informative symbols display. This gives a rough idea of what sort of information the BHMM states encode.

```
julia> e.chain_reports[Chain_ID("exon",6,0,1)]
Base.TTY(RawFD(0x000000017) open, 0 bytes waiting)BHMM EM Chain Results
6-state, 0th order BHMM
Trained on observation set "exon"
Test logP(0|θ): -2.677474495850883e6, greater than the naive model's -2.7733248445454747e6
Replicate 1
Converged with final step δ 0.0009887791238725185
Last θ:
Background BHMM
State Initial and Transition Probabilities
a: [0.3178632049327706, 0.08311610163606636, 0.14481102538589713, 0.1087121512373124, 0.09122496723393655, 0.2542734340135114]
A: 6×6 Array{Float64,2}:
 0.776466  0.00134197  0.000597762  9.3932e-17  1.12908e-29  0.221594
 4.9891e-18 0.993806  0.000114231  0.00035994  0.000666623  0.00505363
 0.00501082 2.53527e-23 0.993775  0.00119238  2.16171e-5   1.21753e-8
 4.75045e-22 1.85006e-24 9.91045e-118 0.979917  0.000395467  0.0196875
 7.04204e-5  0.000262805 0.00112526  0.000411873 0.996586  0.00154403
 0.174564  0.000300059 0.000513614  0.00213432  0.00127718  0.82121
INFORMATIVE SYMBOLS BY STATE
K1 <<< >>>
K2 <<< a c >>>
K3 <<< >>>
K4 <<< g t >>>
K5 <<< >>>
K6 <<< >>>
State Mean Feature Length (bp)
K1: 4.647
K2: 166.237
K3: 148.03
K4: 49.701
K5: 300.917
K6: 5.396
```

Figure 9.3: Example BBM.jl Chain Report - Part 1

The last part of this section presents an estimate of the mean feature length for each state, meaning the mean number of bases emitted from the state before transitioning to a new state (otherwise known as “state residency time” and similar concepts). This is estimated by Monte Carlo sampling of run lengths, given the autotransition probabilities for the BHMM. This is intended to give an idea of the biological relevance of the sequence features emitted by the BHMM’s states. Generally speaking, most “background noise” features, like intergenic CpG islands, are held to be on the order of hundreds of bases. Users should expect good BHMMs to have some states that tend to emit features around this size.

The second part of the `Chain_Report`, displayed in Figure 9.4, displays the evolution of the model’s likelihood and state autotransition probabilities over the chain iterates. This is primarily intended as a visual aid to the convergence tests presented in the third part of the report. Both the model likelihood and autotransition probabilities should be stationary over the last portion of the chain for it to be considered legitimately converged. This can be numerically confirmed in the third part of the report.

The third part of the `Chain_Report` displays the output of the Heidelberger and Welch convergence diagnostic [HW81], as implemented by `MCMCChains.jl` [Pf20]. Both the BHMM likelihood and the autotransition probability for each state are tested for stationarity. A chain from a good replicate set (as assessed by the `Replicate_Report`) that passes these diagnostic tests is reasonably considered properly converged on the global optimum for the partition samples used as the training set. If this is so, a green test-passing statement will be printed, if not, a red failure statement. The specific reason for failure can be determined by finding the parameters with a test value of 0. Taken together, these reports can

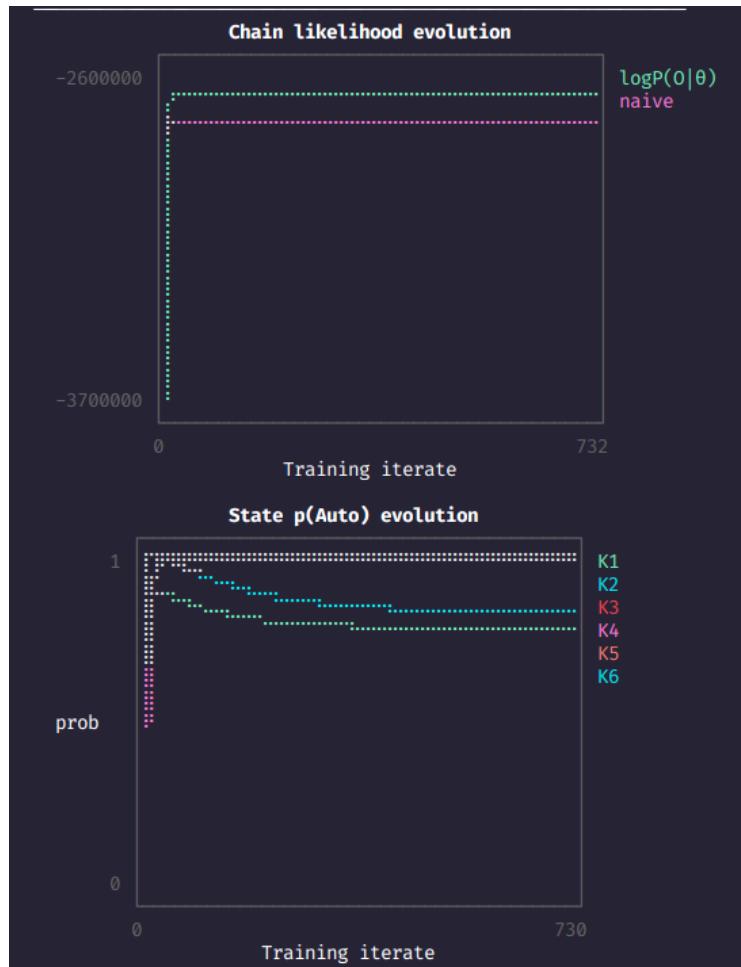


Figure 9.4: Example BBM.jl Chain Report - Part 2

Convergence Diagnostics						
Likelihood and autotransition probabilities converged and passing tests.						
Heidelberger and Welch Diagnostic - Chain 1						
parameters	burnin	stationarity	pvalue	mean	halfwidth	test
Symbol	Float64	Float64	Float64	Float64	Float64	Float64
$\log P(\mathbf{o} \theta)$	0.0000	1.0000	0.9391	-2686293.3768	6528.6612	1.0000
K1	0.0000	1.0000	0.2135	0.7959	0.0262	1.0000
K2	0.0000	1.0000	0.7116	0.9938	0.0002	1.0000
K3	0.0000	1.0000	0.2333	0.9936	0.0006	1.0000
K4	0.0000	1.0000	0.7631	0.9712	0.0161	1.0000
K5	0.0000	1.0000	0.1643	0.9966	0.0000	1.0000
K6	0.0000	1.0000	0.1011	0.8615	0.0501	1.0000

Figure 9.5: Example BBM.jl Chain Report - Part 3

be used to verify that the EM optimization is functioning properly, and that appropriately converged models are chosen to represent genomic background noise from the partition of interest.

Chapter 10

BioMotifInference.jl: Independent component analysis motif inference by nested sampling

10.1 Introduction

While many methods to detect overrepresented motifs in nucleotide sequences, only one is a rigorously validated system of statistical inference that allows us to calculate the evidence for these motif models, given genomic observations: nested sampling [Ski06]. Computational biologists almost immediately benefitted from Skilling's original publication, with the release of the Java-coded nMICA by Down et al. in 2005 [DH05]. Unfortunately, this code base is no longer being maintained. It is, moreover, desireable to take advantage of modern languages and programming techniques to improve the maintainability and productivity of important bioinformatic code. We therefore re-implemented Skilling's original algorithm, for use in inference of DNA motifs in Julia [BEKS15], as `BioMotifInference.jl`, hereafter `BMI.jl`.

In brief, `BMI.jl` is intended to estimate the evidence for Independent Component Analysis models of Position Weight Matrix signals on Hidden Markov Models of genomic background noise, and to estimate the maximum a priori parameters of detected signals. This allows the user to perform Bayesian model selection and comparison for generative hypotheses about genomic sequences of interest.

10.2 Implementation of the nested sampling algorithm

Skilling's nested sampling algorithm is accompanied with an almost-certain guarantee to converge an ensemble of models on the global optimum likelihood in the parameter space[Ski06]. Skilling acknowledges an important assumption underpinning this guarantee, which is that the sampling density (in effect, the size of the ensemble) be high enough that widely separated modes are populated by at least one model each. Although early suggestions for the generation of new models within the ensemble were generally to decorrelate existing models by diffusional Monte Carlo permutation, later work generally acknowledges that this can be highly inefficient [Ski12], particularly in large parameter spaces with many modes, of which the sequence parameter spaces explored in Chapter 5 are good examples. A number

of ways of addressing this problem have arisen in the cosmology and physics literature. These include improved sampling methods, such as sampling within an ellipsoidal hypersphere encompassing the positions of the ensemble models within the parameter space [FH08, FHB09] or Galilean Monte Carlo (GMC) [Ski12], as well as methods of increasing computational efficiency, such as dynamic adjustment of ensemble size [HHHL19].

Generally speaking, these methods are not good candidates for `BMI.jl` because the PWM representation of sequence signal in the ICA PWM model (IPM) is makes ordinary parameter space representation much less efficient. This is for at least four reasons:

1. Identical IPMs may be expressed with their vector of PWM sources in different orders, so the parameter space becomes increasingly degenerate with higher numbers of sources.
2. Closely equivalent repetitive signals can be represented by PWMs on opposite ends of the parameter space (e.g. ATAT vs TATA), reducing the efficiency of ellipsoidal sampling methods and introducing a further degeneracy.
3. If model likelihoods are being calculated on the reverse strand of observations, sources on opposite ends of the parameter space (ie. reverse complements) can also represent closely equivalent signals, introducing another source of degeneracy.
4. IPM sources may be of different lengths. Even if we can relate sources in different models to one another by some consistent distance rule, it is unclear how one would decide which dimensions of longer sources to project the parameters of shorter ones into. BioMotifInference does maintain an index for each source against its prior, but this is no guarantee that sources remain in some way "aligned". Rather, some type of alignment would have to be done, probably massively increasing computational cost to questionable benefit¹.

While it is possible to perform Markov Chain Monte Carlo in a manner that allows jumps between differently-dimensioned models in detailed balance, this applies only to diffeomorphic transformations [HG12]. PWM models are not merely matrices of unrelated parameters, but are an ordered sequence of 4-parameter discrete Categorical distributions. If the addition or removal of a discrete, indexed set of 4 parameters to an existing vector of such sets can be made reversible in this manner, the proof is beyond the scope of the present work². Therefore, BioMotifInference (BMI) is not likely in strict detailed balance over a well behaved parameter space, given its default permute functions and configuration. Permute functions are designed around pragmatic concerns associated with the flow of model information in the IPM ensemble, and are tuned for computational efficiency in producing permuted models that are more likely than the starting model. Despite this, we show that its evidentiary accumulation behaviour strongly resembles that of a nested sampler that *is* in detailed balance, suggesting that this problem is not fatal to the overall approach. Moreover, because of the extremely flexible nature of the sampler, the user may easily specify `BMI.jl`-compatible permute functions, should better ones for their application be apparent to them.

It should be noted that these considerations do not entirely preclude the operation of the `BMI.jl` in detailed balance over the prior. This can be achieved by the user specifying a single length for the PWM

¹Probably some combination of dimensional analysis and fast alignment algorithms could make some headway here, but it's not clear that it's necessary to do so.

²It is also likely to remain beyond my abilities. Please notify the author or submit a relevant pull request at <https://github.com/mmattocks/BioMotifInference.jl> if this is something you know how to do.

sources (rather than a range of permissible lengths), and using a permutation routine consisting solely of the functions `random_decorrelate` and `reinit_src`, along with any user-defined functions coded with the detailed balance stricture in mind. Operation in this fashion may improve the accuracy of both evidence estimates and the sources found in the maximum a posteriori (MAP) sample defined by the converged ensemble. That said, unless the supplied prior is unusually good, this mode of operation is only well suited to very small observation sets, on the order of kilobases. With uninformative priors and good background models, the vast majority of sources sampled from the prior will be significant likelihood penalties relative to the background model alone. Performing nested sampling on such an ensemble by diffusional techniques, in good detailed balance, will usually fail to produce models more likely than the background model within any reasonable compute budget, and will generally never converge. This feasibility constraint justifies the use of bespoke sampling routines for genome-scale datasets.

Like its predecessor `nMICA`, `BMI.jl` samples new models by applying one of a collection of permutation functions to an existing model. Additionally, although `BMI.jl` is supplied prior distributions on IPM parameters from which the initial ensemble is sampled, there is no way provided to calculate a posterior from the models generated by the nested sampling process, largely because of the identical source issue, 1. The primary outputs of interest are the estimation of the Bayesian evidence for model structure given the data, given as its logarithm, as well as the models in the final ensemble (which constitute samples from the MAP mode or modes).

Because the more conventional methods to address the inefficiency of nested sampling by Monte Carlo used in cosmological models described above are unavailable, `BMI.jl` uses an ad hoc method of adjusting the mixture of permute patterns that are applied to models. The basic permute logic (encoded by `Permute_Instruct` arguments to the `permute_IPM` function) is as follows:

1. Select a random model from the ensemble.
2. Apply a randomly selected permutation function from the instruction's function list according to the probability weights given to the functions by the instruction's weight vector.
3. Repeat 2 until a model more likely than the ensemble contour, given observations, is found, or the instruction's `func_limit` is reached.
4. If the `func_limit` is reached without a new model being found, return to 1 and repeat 2 until a model more likely than the ensemble's contour is found, or until the instruction's `model_limit` is reached.

10.3 Usage notes

10.3.1 Preparation of observation set

`BMI.jl` relies on `BioBackgroundModels.jl` both for selecting appropriate background models for motif inference, as well as to code observations and prepare an appropriate matrix of background scores. The selection of background models is covered in Chapter 9. The coding of observations requires the construction of a `DataFrame` containing a column of type `Vector{LongSequence{DNAAlphabet{2}}}`, the unambiguous DNA alphabet of `BioSequences.jl`. This is accomplished as follows:

```

1  using BioBackgroundModels, DataFrames, Serialization
2  obspath=/path/to/observations_dataframe
3  obs_df=deserialize(obspath)

```

Note that `observation_setup` must be supplied an `order` keyword argument of 0; while `BMI.jl` supports background HMMs of any order, signal motifs are 0th order. If the name of the sequence column of the observations `DataFrame` differs from “Seq”, a symbol specifying the column name should be passed as the `symbol` keyword argument.

To construct the background matrix, the observations must be masked by partition, and the positional likelihoods of the sequences calculated:

```

1  BioBackgroundModels.add_partition_masks!(obs_df, genome_gff_path, 500,
   ↳  (:chr,:seq,:start))
2
3  BHMM_dict = Dict{String,BHMM}()
4  reports_folder=deserialize(/path/to/BBM_Reports_Folder)
5  for (partition, folder) in reports_folder
6      BHMM_dict[partition]=folder.partition_report.best_model[2]
7  end

```

`add_partition_masks!` adds a mask column to the observations `DataFrame`, which is used by `BGHMM_likelihood_calc` to determine the correct background model to use for any given base in the observations sequence. It requires a path to a valid GFF3 annotation file for the genome. In order for the observations to be masked correctly, a tuple of column names containing the scaffold ID the observation is found on³, the sequence on the positive strand, and the start position of the sequence on the scaffold.

The calculation of background scores requires a `Dict` of `BBM.jl` background models keyed by the partition ID. In the example, this is constructed programmatically from a `Report_Folder`; see Chapter 9 for the generation of these reports.

10.3.2 IPM_Ensemble assembly

Once the coded observation set and the matrix of background scores are prepared, an `IPM_Ensemble` may be initialised by sampling from a prior. Priors must first be assembled. This process is executed as follows:

```

1  num_sources=5
2  source_length_range=3:10
3  uninformative_mixing_prior=.1
4  source_prior = BioMotifInference.assemble_source_priors(num_sources,
   ↳  Vector{Matrix{Float64}}())

```

³It must be ensured that this ID corresponds to the one used by the GFF3

```

5     mix_prior = (falses(0,0), uninformative_mixing_prior)
6
7     e = BioMotifInference.IPM_Ensemble(worker_pool, "/path/to/ensemble/",
→   ensemble_size, source_prior, (falses(0,0), mixing_prior), background_scores,
→   coded_obs, source_length_range, posterior_switch=true)

```

Here, an uninformative prior is assembled for 5 sources. If we wanted to supply some PWM samples create informative Dirichlet priors from, these would be supplied in the vector of matrices passed as the second positional argument to `assemble_source_priors`. Informative priors are produced by multiplying the PWM values by a fixed weight, which may be passed as the keyword argument `wt`.

An uninformative prior is supplied for the mixing matrix. The prior tuple consists of an empty `BitMatrix`; if an informative prior is desired, the appropriate `BitMatrix` for the informative sources is passed instead. Sources for which no informative prior is supplied have their mix vectors sampled from a fractional floating point success rate prior. Here, the `uninformative_mixing_prior` is .1, meaning that an average of 10% of observations will be initialized with each source.

Sampling and likelihood calculations may be performed in parallel by submitting a valid integer vector worker pool as the first positional argument of `IPM_Ensemble`, or conducted by the master process if this is omitted. Passing `true` for the keyword argument `posterior_switch` will ensure that the least likely models, discarded from the ensemble during the sampling procedure, are collected as samples from the posterior distribution. This is `false` by default, as no rigorous method for estimating posterior distributions is available.

10.3.3 Permute routine setup

In order to converge an assembled `IPM_Ensemble` by nested sampling, it is necessary to provide an initial `Permute_Instruct`, which determines sampler behaviour, and is tuned by the `Permute_Tuner` to prioritize permute functions which currently produce the largest positive particle displacements over the likelihood surface in parameter space, per computational time. Some application-specific tweaking is usually necessary. Depending on how the computation is to be parallelized, the weights assigned to network-intensive functions by the tuner, in particular, may benefit from being clamped. In order to convey how to set up an initial `Permute_Instruct` successfully, notes regarding the operation of the stock permute functions on `ICA_PWM_Models` (IPMs) is summarized below. In this discussion, “premature homogenisation” refers to the loss of sampling density in relevant parts of the posterior, so that the ensemble particles are trapped in some local optimum.

- `permute_source`: randomly permutes a single source in the IPM by altering the PWM weights or changing the length of the source. Distributions for the frequency and size of these permutations may be supplied. If the size of the perturbations is small, and the frequency of length changes is set to 0., this function is useful for finding sources that are slightly more probable than the current ones.
- `permute_mix`: flip a random number of mix matrix positions. Retains flips that make observations more probable. If the range of flip moves is kept small, this is a useful function for “polishing” the mix matrix, particularly of models with highly probable sources. Effective throughout the sampling process.

- **perm_src_fit_mix**: as `permute_source`, but the permuted source is supplied with a new mix vector which mixes the source solely into observations that it makes more probable. Efficient and effective throughout the sampling process.
- **fit_mix**: checks each source against each observation, mixes only those sources that make the observation more probable in isolation. By far the most important permute during the early sampling process, since it can quickly move improbable models sampled from uninformative priors into the portion of the parameter space with likelihoods greater than the background model. In effect, this allows the extraction of maximum information about the data from the initial sampling process, which typically produces many widely divergent sources. This prevents premature homogenisation of the ensemble after extensive sampling below the background model likelihood, and subsequent distortions of evidence and maximum a posteriori parameter estimates.
- **random_decorrelate**: perform the operations of `permute_source` and `permute_mix` on the same model. This is an inefficient permute in general, but it is useful for introducing new sources during the early part of a sampling run, in particular, working to prevent premature homogenisation.
- **shuffle_sources**: copy the source PWM from another model in the ensemble, leaving the original mix matrix. This can be an effective permute early in the sampling process. It is network intensive.
- **accumulate_mix**: randomly select a source from the model to be permuted. From another model in the ensemble, select the source which is most similar to the one selected in the original model. Add the two mixvectors together, such that the original source is now mixed in to any observations in the second model that had the similar source. This is an extremely effective permute when the ensemble begins to have many similar sources in different models, and often is the best way to produce models more likely than those produced by `fit_mix`. Network intensive. This is sometimes the last permute that can find model parameterisations more probable than those in a well-converged ensemble. Network intensive.
- **distance_merge**: Randomly select a source from the model to be permuted. From another model in the ensemble, select the most dissimilar source, and overwrite the selected source in the original model with the PWM and mixvector from this dissimilar source. This can be an effective permute throughout the sampling process. It tends to encourage the propagation of likely sources through the ensemble. Network intensive.
- **similarity_merge**: As `distance_merge`, except the most similar source is selected, and the original mixvector is retained. This tends to be less efficient than `distance_merge`, but can find new models throughout the sampling process. Network intensive.
- **reinit_src**: Resample a random source in the model to be permuted from the prior. This is a very inefficient permute, mostly useful in the early part of the sampling process to reintroduce sources from undersampled parts of the prior.
- **erode_model**: Trim a source's PWM in the model to be permuted to only those positions whose weight vectors express information above a threshold. This has the effect of generating high-likelihood short sources from longer ones with stretches of uninformative positions. It is mostly useful in the early part of the sampling process.

- `info_fill`: For a random source in the permute model, find the least informative PWM position, and set the probability of the most informative base to 1. This function assists in preventing premature homogenisation by rapidly increasing the likelihood of sources that are well-supported by observations but are unlikely to achieve appropriate high PWM weights by random permutation alone.

A `Permute_Instruct` requires a vector of the functions to be used in the permutation routine, a corresponding vector of their initial weights (ie. the frequency with which they will be called), a limit on the number of models to permute before the worker abandons sampling, and a limit on the number of functions to apply to any given model before selecting a new one to attempt permuting. The annotated example provided below also passes, as keyword arguments, vectors of minimum and maximum weights (`min_clmps` and `max_clmps`), as well as a vector of keyword arguments for the vector of functions (`args`).

```

1  funcvec=full_perm_funcvec #exported by BMI.jl
2  models_to_permute=10000
3  func_limit=25
4
5  #add two ``polishing functions'' to the function vector
6  push!(funcvec, BioMotifInference.perm_src_fit_mix)
7  push!(funcvec, BioMotifInference.random_decorrelate)
8
9  #all functions will receive a minimum of 1 in 100 function calls
10 min_clamps=fill(.01,length(funcvec))
11
12 #give some important functions a minimum of 10 in 100 function calls
13 min_clamps[2:3]=.1 #perm_src_fit_mix & permute_mix
14 min_clamps[8]=.1 #difference_merge
15
16 #no function will receive more than 50 in 100 function calls
17 max_clamps=fill(.5,length(funcvec))
18
19 #prevent network intensive functions from swamping the cluster
20 max_clamps[6:7]=.15 #shuffle_source and accumulate_mix
21 max_clamps[9]=.15 #similarity_merge
22
23 #evenly distributed initial weights
24 initial_weights= ones(length(funcvec))/length(funcvec)
25
26 #empty arguments vectors for all arguments in the funcvec
27 args=[Vector{Tuple{Symbol,Any}}]() for i in 1:length(funcvec)]
28
29 #add arguments for the ``polishing functions''
30 → args[end-1]=[(weight_shift_freq,0.), (length_change_freq,1.), (length_perm_range,1:1)]
```

```

31     args[end]=[(:iterates,50),(:source_permute_freq,.3),(:mix_move_range,1:10)]
32
33     #instantiate the Permute_Instruct
34     instruct = Permute_Instruct(funcvec, initial_weights, models_to_permute,
→   func_limit;min_clmps=min_clamps, max_clmps=max_clamps, args=args)

```

10.3.4 Nested sampling of IPM_Ensembles

If all of the above-described tasks are met, the `IPM_Ensemble` may be converged by nested sampling using the specified permutation routine. This can be performed in parallel; any number of permutation workers may be used. However, because the master process is responsible for disk access, many such workers performing permutation functions which frequently require the deserialization of other models in the ensemble (those noted as “network intensive” above) will rapidly reduce the computational efficiency of the cluster. This can be mitigated by clamping network intensive permute functions to a lower frequency of total function calls.

`BMI.jl` is supplied with a display system similar to, but less customisable than `GMC_NS.jl`. An example of display setup and nested sampling execution is presented below:

```

1 display_rotation=[true,10,1,[[:tuning_disp,:lh_disp,:src_disp],[:conv_plot,:liwi_disp,:ens_disp]]]
2
3 logZ = converge_ensemble!(e, instruct, worker_pool, backup=(true,25),
→   tuning_disp=true, lh_disp=true, src_disp=true, disp_rotate_inst=display_rotation)

```

Part III

Supplementary Materials

Chapter 11

Supplementary materials for Chapter 2

11.1 Materials and methods

11.1.1 Zebrafish husbandry

Zebrafish used in this study were of a wild type AB genetic background. Embryos were derived from pairwise crossings. Larvae were collected upon crossing and held in a dark incubator at 28°C. At 1dpf, embryos were treated with 100 μ l of bleach diluted in 170ml of embryo medium for 3 minutes, rinsed and dechorionated. Embryo medium was changed at 3dpf. After this, all animals were maintained at 28°C on a 14-hour light/10-hour dark cycle (light intensity of 300 lux) in an automated recirculating aquaculture system (Aquaneering). Animals were reared using standard protocols[[Wes00](#)]. Fish were sacrificed by tricaine overdose at the appropriate timepoints indicated in the text. All animal experiments were performed with the approval of the University of Toronto Animal Care Committee in accordance with guidelines from the Canadian Council for Animal Care (CCAC).

11.1.2 Proliferative RPC Histochemistry

11.1.2.1 Anti-PCNA histochemistry

In order to assay the number of proliferating cells in zebrafish CMZs, we used anti-PCNA histochemical labelling, as PCNA is, in zebrafish, detectable throughout the cell cycle in proliferating cells. After sacrifice as described above, fish (or their razor-decapitated heads, in the case of fish older than 30dpf) were fixed in 1:9 37% formaldehyde:95% ethanol at room temperature (RT) for 30 minutes, followed by overnight incubation in a fridge at 4°C. Subsequently, the samples were removed from fix and washed 3 times in PBS. They were then cryoprotected by successive 30 minute rinses at RT on a rocker in 5%, 13%, 17.5%, 22%, and 30% sucrose in PBS. Samples were allowed to incubate overnight at 4°C in 30% sucrose. The next day, samples were removed from the sucrose rinse and infiltrated with 2:1 30% sucrose:OCT compound (TissueTek) for 30 minutes at RT. Samples were then embedded for cryosectioning and frozen. All animals younger than 14dpf were embedded side-by-side in groups of 6 under a dissection microscope using a brass mold with milled 3mm channels for maintaining their precise axial orientation.

The cryosectioning block was completed by removing the frozen larval block from the mold and layering more sucrose:OCT mixture between the channels and on top of this filled-in block. Older animals were carefully oriented by hand under a dissection microscope. $14\mu\text{m}$ coronal cryosections were cut through the fish heads and collected on Superfrost slides (Fisherbrand). These were stored in a freezer at -20°C until staining.

Staining was begun by allowing the slides to dry briefly at RT. Sections were outlined with a PAP pen, then rehydrated in PBS for 30 minutes at RT. Subsequently, sections were blocked in 0.2% Triton X-100 + 2% goat serum in PBS for 30 minutes at RT. This was followed by incubation in mouse monoclonal (PC10) anti-PCNA primary antibody (Sigma), diluted 1:1000 in blocking solution, at 4°C overnight. The next day, primary antibody was removed, and slides were rinsed five times in PDT (PBS + 1% DMSO + 0.1% Tween-20). Cy5-conjugated goat-anti-mouse secondary antibody (Jackson Laboratories), diluted 1:100 in blocking solution, was applied to the sections, which were incubated at 37°C for 2 hours. Secondary antibody was removed, followed by five rinses in PDT as above. Sections were counterstained with Hoechst 33258 diluted to $100\ \mu\text{g/mL}$ in PBS for 15 minutes at RT. Counterstain was then removed, five rinses in PDT were performed as above, followed by a final rinse in PBS. Slides were then mounted in ProLong Gold antifade mounting medium (ThermoFisher Scientific), coverslipped, and sealed with clear nail polish. Slides were kept at 4°C until imaging.

11.1.2.2 Cumulative thymidine analogue labelling for estimation of CMZ RPC cell cycle length

In order to obtain an estimate of cell cycle length in CMZ RPCs at 3dpf, we used cumulative thymidine analogue labelling following the method of Nowakowski et al.[NLM89]. 3dpf zebrafish were divided into ten groups of n5 animals and held in 10 mM EdU for 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 7.5, 8.5, 9.5, and 10.5 hours, after which they were sacrificed as described above. Samples were processed as described under “Anti-PCNA histochemistry”, except that goat-anti-mouse Cy2-conjugated secondary antibody (Jackson Laboratories) was used to label anti-PCNA, after which the Alexa Fluor 647-conjugated azide from a Click-iT kit (ThermoFisher Scientific) was added to the sections for 30 minutes at room temperature to label EdU-bearing nuclei. This was followed by five PDT rinses as described above, and resumption of the protocol with counterstaining, mounting, etc. The raw data is available in `/empirical_data/cumulative_edu.xlsx`

11.1.2.3 Whole retina thymidine analogue labelling of post-embryonic CMZ contributions

We used thymidine analogue labelling as an indelible marker of CMZ contributions to the retina in Fig 2.5. Because thymidine analogues are incorporated into DNA during S-phase, postmitotic progeny of proliferating RPCs which take up the label may be detected at any point after this treatment. Dosing zebrafish with a thymidine analogue for a limited period of time ensures that the label is diluted to undetectable levels in cells which continue to proliferate. Repetitive dosing thus gives rise to labelled cohorts which represent a limited set of generations of cells which become postmitotic after the dose is provided. n=3 fish were held in 10mM BrdU (Sigma) diluted in facility water for 8 hours at 30, 60, 90, 120, and 150 days post fertilisation. 10 days after the last BrdU incubation, the fish were sacrificed as described above. One representative whole retina dissection is presented.

11.1.3 Confocal microscopy and image analysis

All histochemically processed samples (coronal sections of retinas and whole retinas) were imaged on a Leica TCS SP5 II laser confocal imaging microscope, using the Leica LAS AF software for acquisition. For coronal sections, central sections were identified by their position in the ribbon of cryosections. For Fig 2.7, the central section was imaged together with the flanking section on either side of it. Confocal data was analysed using Imaris Bitplane software (v. 7.1.0). Cell counting analyses were performed by segmenting the relevant channels using the Surfaces tool to produce counts of PCNA positive (Figs 2.7, S4 Fig) or PCNA/EdU double-positive (S4 Fig) nuclei. Lens diameters were measured in the DIC channel using the linear measurement tools, across the widest point of the section of spherical lens.

11.1.4 Estimation of CMZ annular population size

In order to estimate the total population of the CMZ in zebrafish eyes sampled throughout the first year of life, we counted PCNA-positive cells in the dorsal and ventral CMZ of central and flanking sections of these eyes as described above. We added the dorsal and ventral populations and took the average of the central and flanking section per-section populations to reduce the possibility of sampling error. We treated this as a sample of a torus mapped to the $14\mu\text{m}$ sphere segment of lens intersected by the average section. As the surface area of this zone is given by $S = \pi \times d_{lens} \times h_{section}$, and the total surface area of the lens by $A = \pi \times d_{lens}^2$, where d is the diameter of the lens and h the section thickness, we may calculate an estimate of the total population by $pop_{total} = \frac{pop_{section}}{S} * A$. We therefore obtained our annular CMZ population estimates using our measurements with the simplified formula $pop_{total} = \frac{pop_{section} \times d_{lens}}{h_{section}}$, calculating pop_{total} on a per-eye basis to account for covariance of the measurements, and subsequently averaging across $n=6$ eyes per sampled timepoint (3, 5, 8, 12, 17, 23, 30, 60, 90, 120, 180, and 360 dpf). These data are presented in Fig 2.7.

11.1.5 Modelling lens growth

To simulate the annular CMZ population, we wanted to be able to simulate the stem cells at the periphery of the retina occasionally undergoing symmetric divisions, so as to keep up the same density of stem cells in the first ring around the lens. We did this by normalising our lens diameter measurements to the 72hpf value, to produce a measure of relative increase in lens size over the first year of CMZ activity. We performed a linear regression of the logarithm of this relative increase vs the logarithm of time using the Python statsmodels library, using the constants derived from this regression for a power-law model of lens growth. This model was used to supply the `WanStemCellCycleModel` with a target population value as described below.

11.1.6 Estimation of 3dpf CMZ cell cycle length

To produce an estimate of the length of the cell cycle in 3dpf RPCs, we first took the total count of EdU-positive cells in dorsal and ventral CMZ from our cumulative labelling experiment described above, and divided by the total dorsal and ventral CMZ RPC population, as measured by the number of PCNA positive cells. This gave the labelled fraction measurements displayed in S4 Fig. Following Nowakowski et al. [NLM89], we performed an ordinary least squares linear regression on these data using the Python statsmodels library. We then calculated the total cell cycle time $T_c = \frac{1}{slope}$ and $T_s = T_c \times intercept_y$.

While this method is not suited for heterogenous populations of proliferating cells, its use is justified here, as the He SSM to which the estimate is being applied makes similar assumptions as Nowakowski et al., in particular the homogeneity of the population. It should not be considered more than a rough estimate.

11.1.7 CHASTE Simulations

11.1.7.1 Project code

All simulations were performed in an Ubuntu 16.04 environment using the CHASTE C++ simulation package version 2017.1 (git repository at <https://chaste.cs.ox.ac.uk/git/chaste.git>). The CHASTE package is a modular simulation suite for computational biology and has been described previously[MAB⁺13]. All of the code, simulator output, and empirical data used in this paper is available in the associated CHASTE project git repository at <http://github.com/mmattocks/SMME>, as well as in the supplementary archive [??](#). In brief, the approach taken was to encapsulate the SSMs examined here as separate concrete child classes inheriting from the CHASTE `AbstractSimpleCellCycleModel` class. An additional model, representing the simple immortal stem cell proposed in Wan et al.[WAR⁺16], was similarly produced. Each model is generic and provided with public methods to set their parameters and output relevant simulation outcomes (and per-lineage debug data, if necessary). While these may be used in the ordinary CHASTE unit test framework, permitting their use in any kind of cell-based simulation, we wrote standalone simulator executables (apps, in CHASTE parlance) to permit Python scripting of the simulator scenarios used in this study. This allows for the multi-threaded Monte Carlo simulations performed herein. Therefore, the Python fixtures available in the project’s `python_fixtures` directory were used to operate the single-lineage simulators (`GomesSimulator`, `HeSimulator`, and `BoijeSimulator`) and single-annular-CMZ simulator (`WanSimulator`) in `apps/`, including the `CellCycleModels` and related classes in `src/`; the simulators output their data into `python_fixtures/testoutput/`. These data, along with the empirical observations (both from the Harris groups’ papers and our own described herein) available in `empirical_data/`, were processed by the analytical Python scripts in `python_fixtures/figure_plots/` to produce all of the figures used in this study.

We have attempted to make the project fully reproducible and transparent. CHASTE uses the C++ boost implementation of the Mersenne Twister random number generator (RNG) to provide cross-platform reproducibility of simulations. All of our simulators make use of this feature, permitting user-specified ranges of seeds for the RNG. We have also used the numpy library’s RandomState Mersenne Twister container to provide reproducible results for the Python fixtures, notably the SPSA optimisation fixture. The output of the project code will, therefore, be identical every time it is run, on any platform.

It should be noted that none of the code Harris’ group used in their reports has been published. We have made every effort to replicate the functional logic of the models as closely as possible. Nonetheless, it is not possible to determine precisely why, for instance, the original He SSM parameterisation failed so notably in our implementation.

11.1.7.2 SPSA optimisation of models

In order to make a fair comparison between the He SSM and our deterministic mitotic mode alternative model, we coded an implementation of the SPSA algorithm described by Spall [Spa98]. This is available in `/python_fixtures/SPSA_fixture.py`. This algorithm, properly tuned, will converge almost-surely

on a Karush-Kuhn-Tucker optimum in the parameter space, given sufficient iterates, even with noisy output (eg. due to RNG noise from low numbers of Monte Carlo samples, permitting conservation of computational resources). It does so by approximating the loss function gradient around a point in parameter space, selecting two sampling locations at each iterate, then moving the next iterate's point in parameter space "downhill" along this gradient.

Our loss function was a modified AIC; we weighted the residual sum of squares from the PD-type mitotic probabilities by 1.5 in order to improve convergence, as the PD data are the most informative part of the dataset regarding the critical phase boundaries (PP mitoses occur in all 3 phases of the He model, DD occur in phases 2 and 3, PD mitoses occur only in phase 2). We also compared model induction count output (that is, panels A-C in Fig 2.4) up to count values of 1000 to penalise parameters producing very large lineage totals.

The values of the SPSA gain sequence constants were selected to be as large as possible without resulting in instability and failure to converge; this ensured that the algorithm stepped over the widest possible range of the parameter space in finding optima. The α and γ coefficients were initially set at the noise-tolerant suggested values of .602 and .101 respectively [Spa98]. 200 iterations were performed. Initially, each iterate involved 250 seeds of each induction timepoint for the count data (panels A-C in Fig 2.4) and 100 seeds of the entire 23-72 hr simulation time for mitotic mode rate data (panels D-F). At iterate 170, these were increased to 1000 and 250 seeds, respectively, decreasing RNG noise to a low level. For the last 10 iterations, RNG noise was reduced to close to nil by increasing the seed numbers to 5000 and 1250, respectively; α and γ were set to the asymptotically optimal 1 and $\frac{1}{6}$ for these iterates, as well. The particular seeds used were kept constant throughout in order to take advantage of the improved convergence rate this affords [KSN99].

Because the simple SPSA can assign any value to parameters, our implementation uses constraints, projecting nonsensical values for parameters (e.g. negative values, mitotic mode probabilities summing to > 1) back into legal parameter space. The use of constraints has no effect on the algorithm's ability to almost-surely converge within the given parameter space [Sad97]. As we felt that the deterministic mitotic mode models' "sister shift" should be relatively small in order to be biologically plausible, we also constrained this parameter such that 95% of sister shifts would be less than the mean length of the shortest phase.

The numerical results of the SPSA algorithm are available in `/python_fixtures/testoutput/SPSA/HeSPSAOutput`, alongside png images of output from each iterate, enabling the visualisation of the progress of the algorithm.

Monte Carlo simulations Subsequent to SPSA optimisation, the parameters derived from this procedure were used to perform Monte Carlo simulations of large numbers of individual lineages, using ranges of 10000 non-overlapping seeds for each of panels A, B, C, G, H, and I, and 1000 for each of panels D, E, and F of Fig 2.4 (using the SPSA-optimised parameter sets) and S1 Fig (using the original He et al. parameterisation). This was performed using `/python_fixtures/He_output_fixture.py`.

Similarly, 100 seeds were used in Monte Carlo fashion to simulate individual annular CMZ populations using `/python_fixtures/Wan_output_fixture.py`, which scripts the WanSimulator. Each of these simulations is initialised with an RPC population drawn from a normal distribution given the mean and standard deviation of the CMZ torus estimated from our 3dpf observations, as described above. Each RPC is given a TiL value randomly chosen from 0 (newly born from a stem cell) to 17hr (exiting the

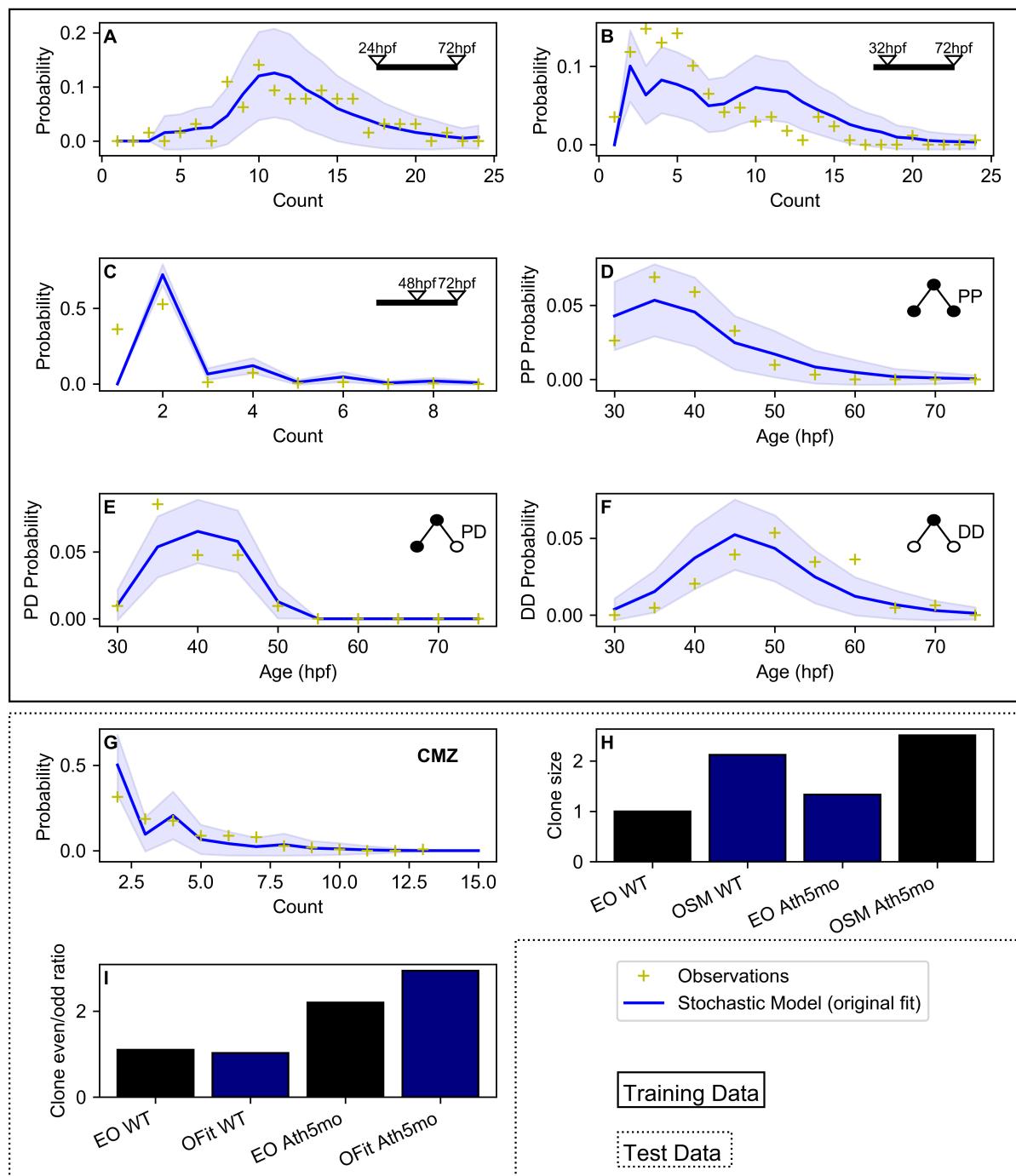
CMZ, “forced to differentiate”, in the terms of Wan et al.). A further population of stem cells, sized at $\frac{1}{10}$ of the RPC population, is added using the `WanStemCellCycleModel`. This stem population divides asymmetrically except when it falls under its target population value determined by the model of lens growth described above; as long as this condition holds, the stem cells will divide symmetrically to keep up their numbers.

Simulation data analysis Not all of the numerical data used in He et al. and Wan et al. has been published. As such, we reconstructed the lineage data from He et al.’s Figure 5C, used by He et al. to obtain their mitotic mode probabilities; our reconstructed values are available in `/empirical_data/empirical_lineages\`. We also reconstructed the He et al. WT and Ath5 morpholino data (Fig 2.4 panels H, I) and Wan et al. lineage count probabilities (panel G) from the relevant figures. These values are entered into `/python_fixtures/figure_plots/He_output_plot.py`, where they are used in the AIC calculations described below.

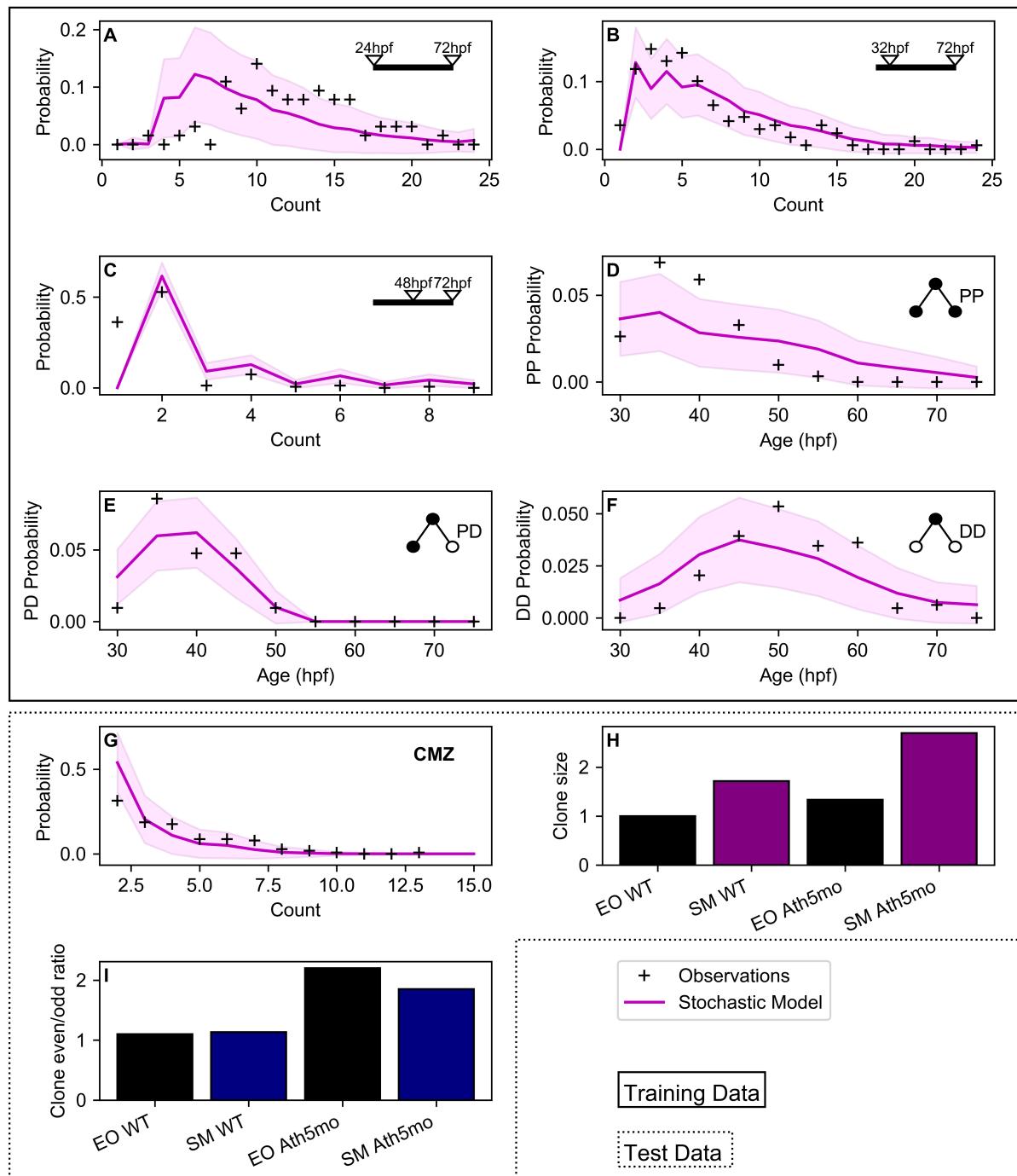
In order to assess the performance of the models used in our simulations, we used Akaike’s Information Criterion (AIC). This allows us to compare models with dissimilar numbers of parameters, as AIC trades off goodness-of-fit against parameterisation. The values reported in Table 2.1 were produced by `/python_fixtures/figure_plots/He_output_plot.py`.

The 95% CIs for the model output presented in this paper were estimated by repetitive sampling of the output data, using the same number of lineages observed empirically. 5000 such samples were performed. This provides a reasonable estimate of the confidence interval given the limited observational sample size.

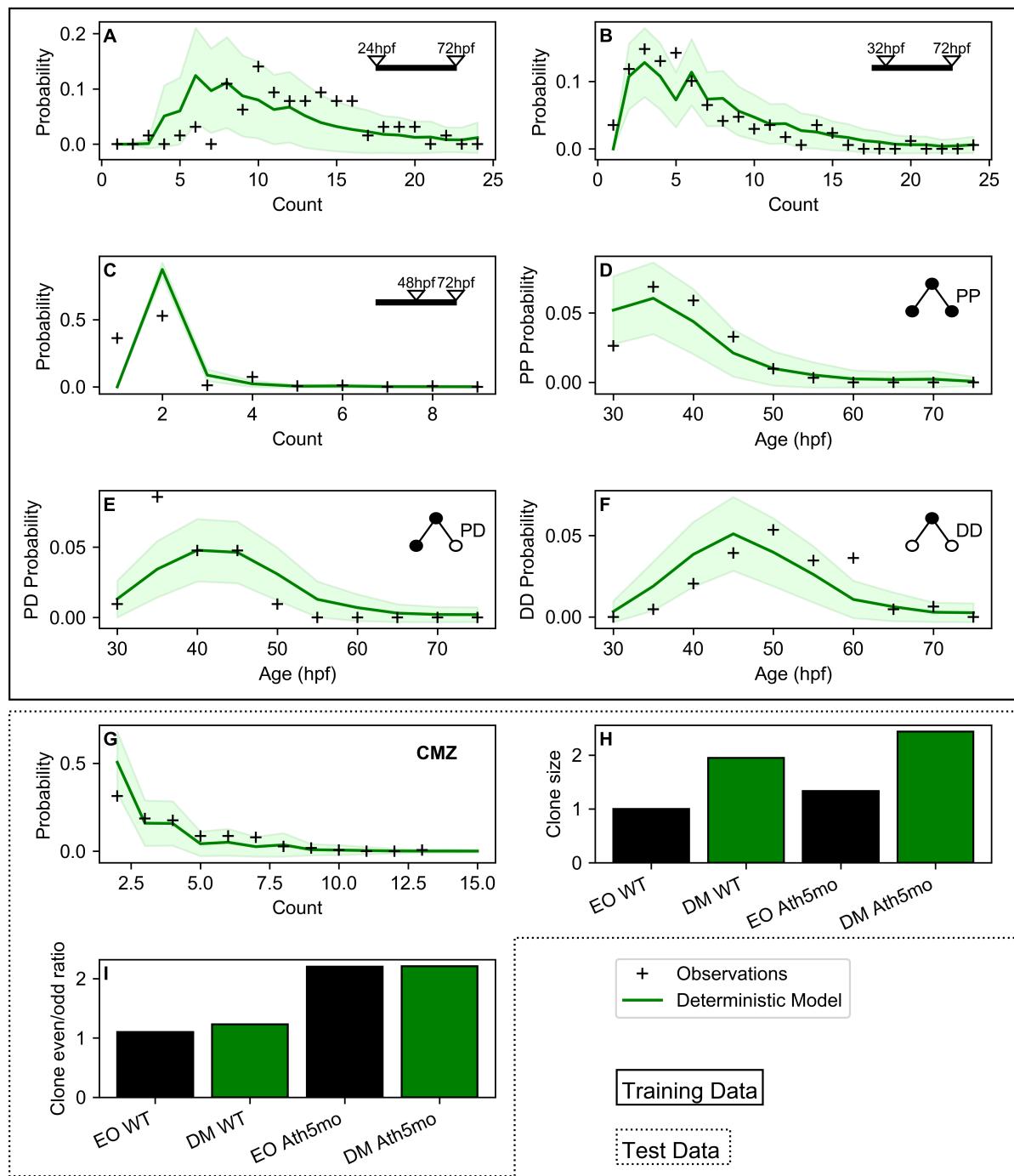
11.2 Supporting figures



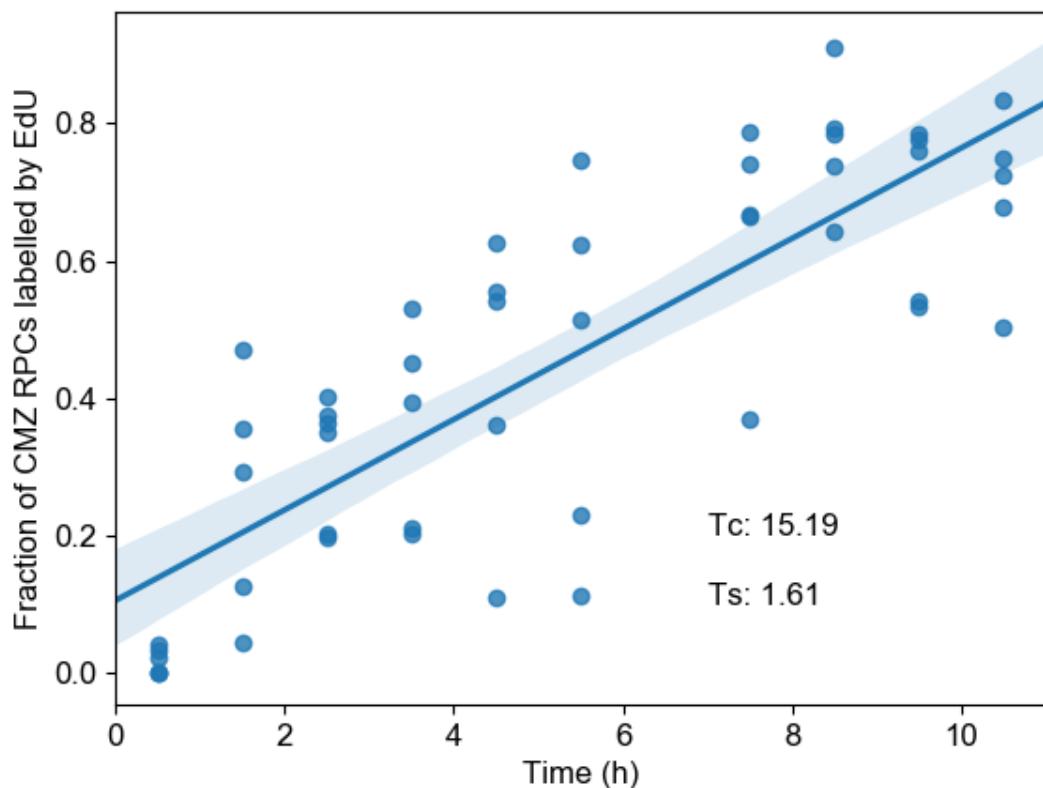
S1 Fig. He SSM original parameterisation model output. As Fig 2.4; model output uses the parameterisation given in He et al.[HZA⁺12]. Note significant deviation from observations in panel B, reflecting excess proliferative activity of modelled RPCs.



S2 Fig. SPSA-optimised He SSM model output. As Fig 2.4; stochastic model output displayed alone.



S3 Fig. SPSA-optimised deterministic mitotic mode model output. As Fig 2.4; deterministic model output displayed by itself.



S4 Fig. Cumulative EdU Labelling of 3dpf CMZ RPCs Fraction of PCNA-labelled CMZ RPCs which bear EdU label over time, as determined by histochemical labelling of central coronal cryosections of 3dpf zebrafish retinas. Dots represent results from individual retinas. Line is the ordinary least squares fit \pm 95% CI. Cell cycle length (Tc) and S-phase length (Ts) are estimated from this fit following the method of Nowakowski et al.[NLM89]

Chapter 12

Supplementary materials for Chapter 4

12.1 Materials and methods

12.1.1 Zebrafish husbandry

Zebrafish husbandry was performed as described in Section 11.1.1.

12.1.2 PCNA Immunohistochemistry

Anti-PCNA immunohistochemistry was performed as described in Section 11.1.2.1.

12.1.3 Thymidine analogue histochemistry

Both cumulative thymidine analogue histochemistry presented in Section 4.4.1, as well as the pulse-chase histochemistry used for the study of neural fate in Section 4.6 were performed by the methods described in Section 11.1.2.2 and Section 11.1.2.3, respectively, except that the data from the whole retinal labelling experiment derives from 14micrometre transverse and coronal cryosections (n=3-6 animals per axis, per age).

12.1.4 Lineage tracing immunohistochemistry

Embryos from wild-type AB crosses (staining groups 2 and 3) and *Tg(Isl2b:GFP)* crosses (staining group 1). The *Tg(Isl2b:GFP)* line was the kind gift of the late Dr. Chi-Bin Chien. Embryos were collected and reared as described in Section 11.1.1. Embryos were divided into three groups, to be pulsed with EdU at 3, 23, and 90 dpf and sacrificed after a 7 day chase period. In 3 and 23dpf larvae, animals were pulsed by allowing them to swim freely in a 10mM EdU solution for 2 hours. In 90dpf animals, 10microliters of 10mM EdU was injected intraperitoneally. After 7 days, animals were sacrificed and coronal cryosections were obtained as described in Section 11.1.2.1. These cryosections were divided into staining groups according to their genetic origin, as mentioned above.

Cryosections from all staining groups were allowed to dry briefly at room temperature (RT), before rehydration in PBS for 30 minutes at RT. Subsequently, sections were blocked in 0.2% Triton X-100 +

2% goat serum in PBS for 30 minutes at RT. Primary antibody incubations were as follows for each numbered staining group:

1. Covance rabbit anti-Pax6 primary antibody diluted 1:200 in blocking solution incubated at 4 overnight.
2. Chemicon mouse anti-GS primary antibody diluted 1:500 in blocking solution at 4 overnight.
3. ZIRC mouse anti-Zpr1 primary antibody diluted 1:400 in blocking solution at 4 overnight.

After these overnight incubations, primary antibodies were removed and all sections were rinsed five times in PDT (PBS + 1% DMSO + 0.1% Tween-20). Secondary antibody incubations were as follows for each numbered staining group:

1. Goat anti-rabbit Cy3-conjugated 2^o antibody diluted 1:100 in blocking solution at 37°C for 2 hr.
2. Goat anti-mouse Cy2-conjugated 2^o antibody diluted 1:100 in blocking solution at 37°C for 2 hr.
3. Goat anti-mouse Cy2-conjugated 2^o antibody diluted 1:100 in blocking solution at 37°C for 2 hr.

Staining group 2 was subsequently incubated with Santa Cruz rabbit anti-PKC β 1^o antibody diluted 1:100 in blocking solution at 4°C overnight. The next day, this second primary antibody was removed, and the group sections were rinsed in PDT as above. A further goat anti-mouse Cy2-conjugated 2^o antibody diluted 1:100 in blocking sol'n was applied at 37°C for 2 hr.

After each staining group's final secondary antibody was removed, slides were rinsed 3x in PDT, then stained for EdU with an Alexa 647 azide. After another three PDT rinses, 100 ug/mL Hoechst 33258 was applied for 15 minutes at RT. Five PDT rinses, after which all slides were mounted, slipped, and sealed.

12.1.5 4C4 immunohistochemistry

The 4C4 antibody was a generous gift of Dr. Pamela Raymond. It has been empirically determined to mark microglia [BB01]. It was used according to the methods described above with a Cy2 secondary antibody.

12.1.6 Confocal micrograph acquisition and processing

All confocal micrographs presented in Chapter 4 were acquired on a Leica TCS SP5 II microscope, operated using the LAS AF microscopy software (Leica). Data was assessed by using Bitplane Imaris software (v.). Specifically, the Imaris watershed segmentation algorithm was applied to the nuclear counterstain channel to segment cellular nuclei. Colocalisation of particular markers (ie. PCNA, EdU, or lineage markers) was assessed by thresholding signal from these channels with the segmented nuclear volumes to produce a subpopulation of colabelled nuclei. The segmented Imaris scenes and the associated settings are available in the thesis HDD archive.

12.1.7 Retinal measurements

The developmental morphology study presented in Figure 12.1 was conducted by using the LAS AF software measurement tools on transmitted light data collected in the process of conducting the confocal survey presented in Figure 4.2. Each measurement point is, therefore, likewise the average of the value three central coronal cryosections. Retinal thickness was the linear thickness of the entire cellular retina and its plexiform layers at the center of the section, and the layer thicknesses reflect segments along this line, with the addition of RPE thickness. RPE length was measured in approximating segments from the dorsal to the ventral extrema. Lens diameter was measured in any sections which retained the lens; this was rare at older ages, when the lens sections tend to separate readily as soon as the cryosection is rehydrated. Optic nerve diameter was measured at its thickest point.

12.1.8 Estimation of overall CMZ population and retinal volume

The overall CMZ population estimates were derived by the calculations presented in Section 11.1.4. The volume of the cellular retina was crudely estimated by subtracting the volume of two spheres. The circumference of the outer sphere is given by the RPE length plus the diameter of the lens. The radius of the inner sphere is the half total thickness of the cellular retina subtracted from radius of the outer sphere¹. The difference in volume between the two spheres is calculated; four-fifths this value is taken as the volume estimate in order to partially compensate for the flattening of the eye at older ages.

12.1.9 Monte Carlo estimation of CMZ population and retinal volume rates of change

In order to estimate the mean daily rate of change of the CMZ population and retinal volume estimates, we performed Monte Carlo difference operations between samples drawn from the estimated LogNormal distributions at subsequent timepoints. This allows us to empirically estimate the uncertainty on these rates, as the difference between two T-distributions is not, itself, T-distributed.

12.1.10 Evidence estimation by Empirical Bayes linear regression

Evidence comparisons presented without estimated standard deviations (σ) of significance are produced by the Empirical Bayes method of linear regression, as presented in Bishop's standard machine learning textbook [Bis06]. Calculations were performed using the Julia package `BayesianLinearRegression.jl`, which implements Bishop's algorithm in a high performance manner.

12.1.11 Evidence estimation by Galilean Monte Carlo nested sampling

Evidence comparisons presented with estimated standard deviations (σ) of significance are produced by converging ensembles of Galilean Monte Carlo sample chains, which collectively constitute a sample from the posterior in rigorous detailed balance [Ski19]. The ayesian evidence], or marginal probability of the model over the posterior sample is calculated by the ested sampling algorithm] [Ski06]. Calculations were performed by the use of the Julia packages `GMC_NS.jl` and `CMZNicheSims.jl`, written for this thesis

¹Halving serves as crude compensation for the lack of cellular retina on the lens' part of the sphere, as well as the thinning of the cellular retina toward the periphery

and presented in [Chapter 7](#) and [Chapter 8](#), respectively. Maximum a posteriori outputs are calculated from the most likely model parameterisation sampled during this process.

12.1.12 Estimation of posterior distributions of phase model parameters

Posterior distributions presented in [Figure 4.4](#) and [Figure 4.7](#) were produced by Kernel Density Estimation (KDE) [[Bis06](#), p. 122]. The KDE algorithm used was the implementation available in the Julia Robotics library `KernelDensityEstimate.jl`, described by Sudderth et al. [[SMFW10](#)]. Briefly, this uses multiscale nonparametric Gibbs sampling of supplied weighted “point clouds” to generate the kernel density estimate. Chains from `GMC_NS.jl` were used in this context by weighting the points in parameter space by their estimated evidence. It is important to note that, for this thesis, `GMC_NS.jl` was configured to prioritise the accuracy of evidence estimates over these posterior estimates; as a consequence, samples from around the maximum a posteriori have little weight in the KDE estimates presented herein [[HHHL18](#)]. Alternatives to this scheme are possible; one such algorithm is described by Higson et al. [[HHHL19](#)]

12.2 Supplementary Figures

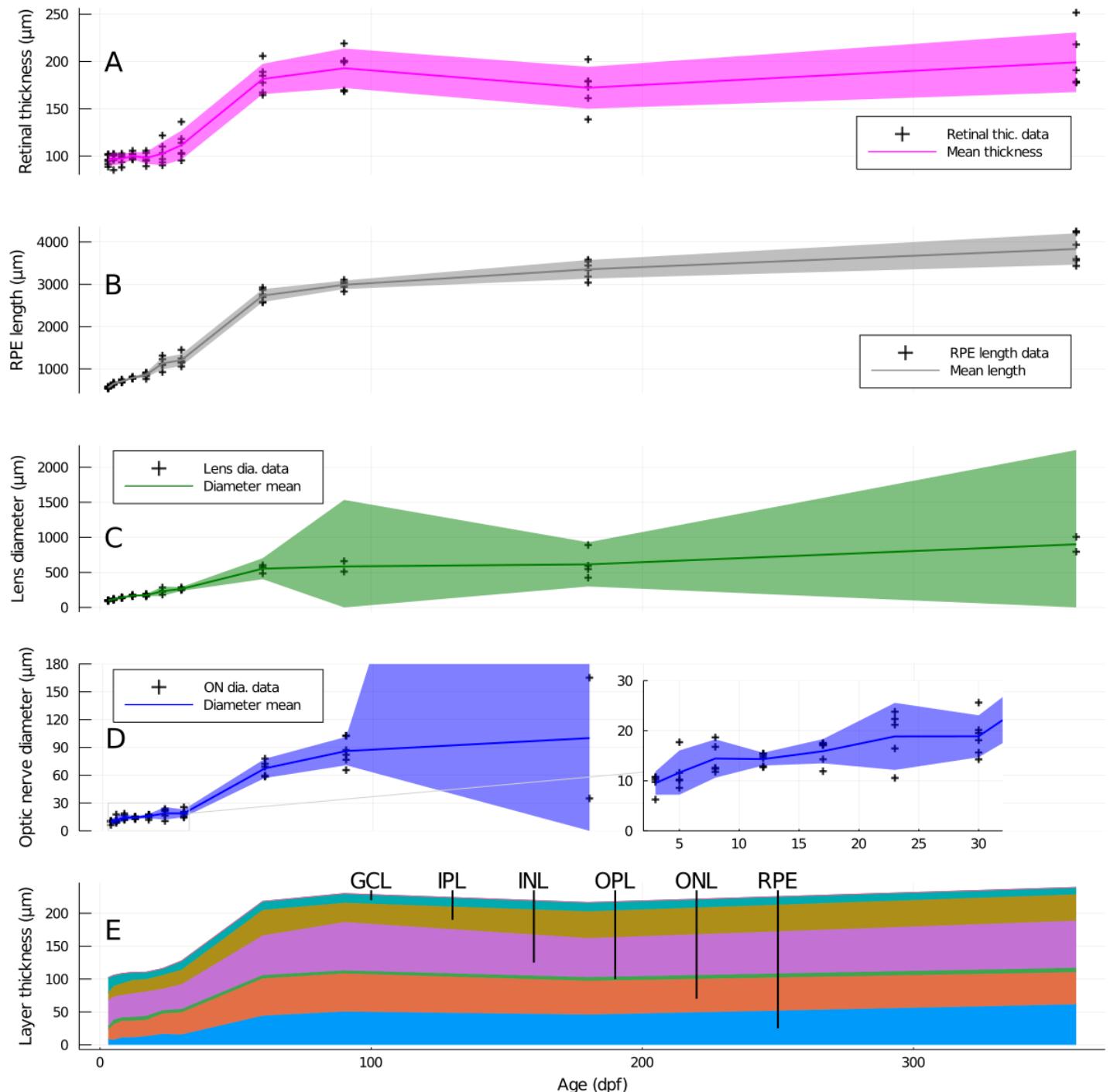


Figure 12.1: Developmental progression of naso-temporal population asymmetry in the CMZ.

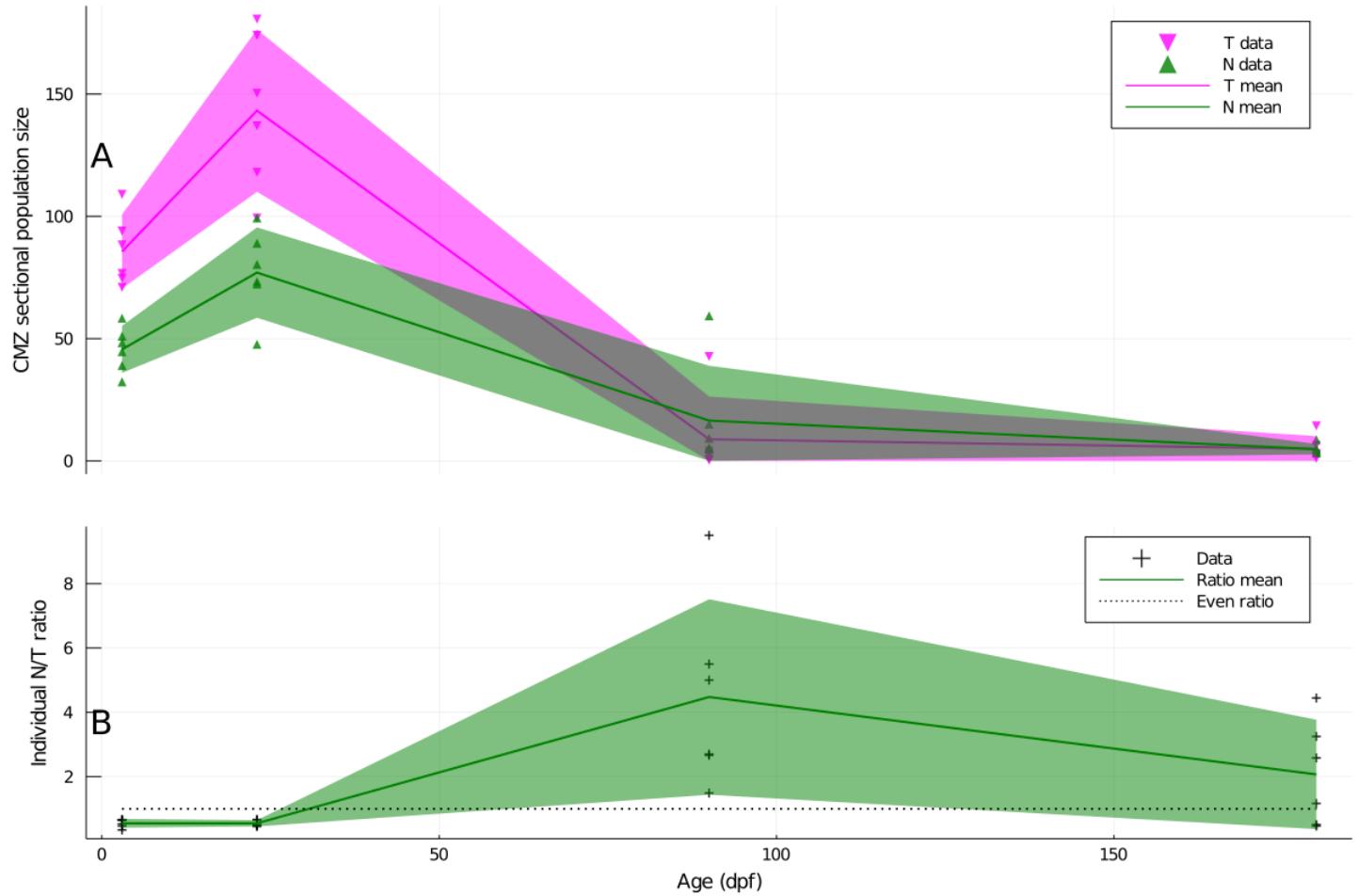


Figure 12.2: **Developmental progression of naso-temporal population asymmetry in the CMZ.**

Marginal posterior distribution of mean nasal (N) and temporal (T) population size in $14\mu\text{m}$ transverse cryosections (panel A) or intra-individual N/T count asymmetry ratio (panel B), $\pm 95\%$ credible interval, $n=6$ animals per age. Data points represent mean counts from three central sections of an experimental animal's eye.

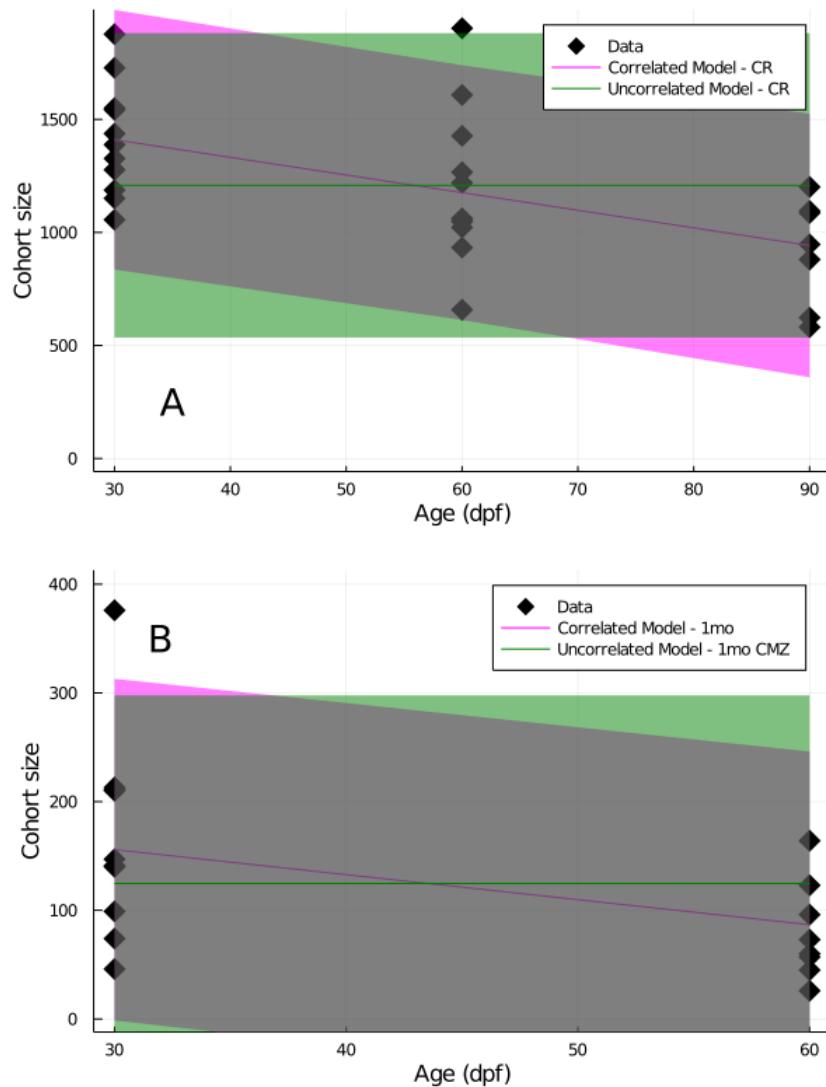


Figure 12.3: Linear regressions of temporally correlated and uncorrelated models of central retinal and 30dpf CMZ-contributed cohorts

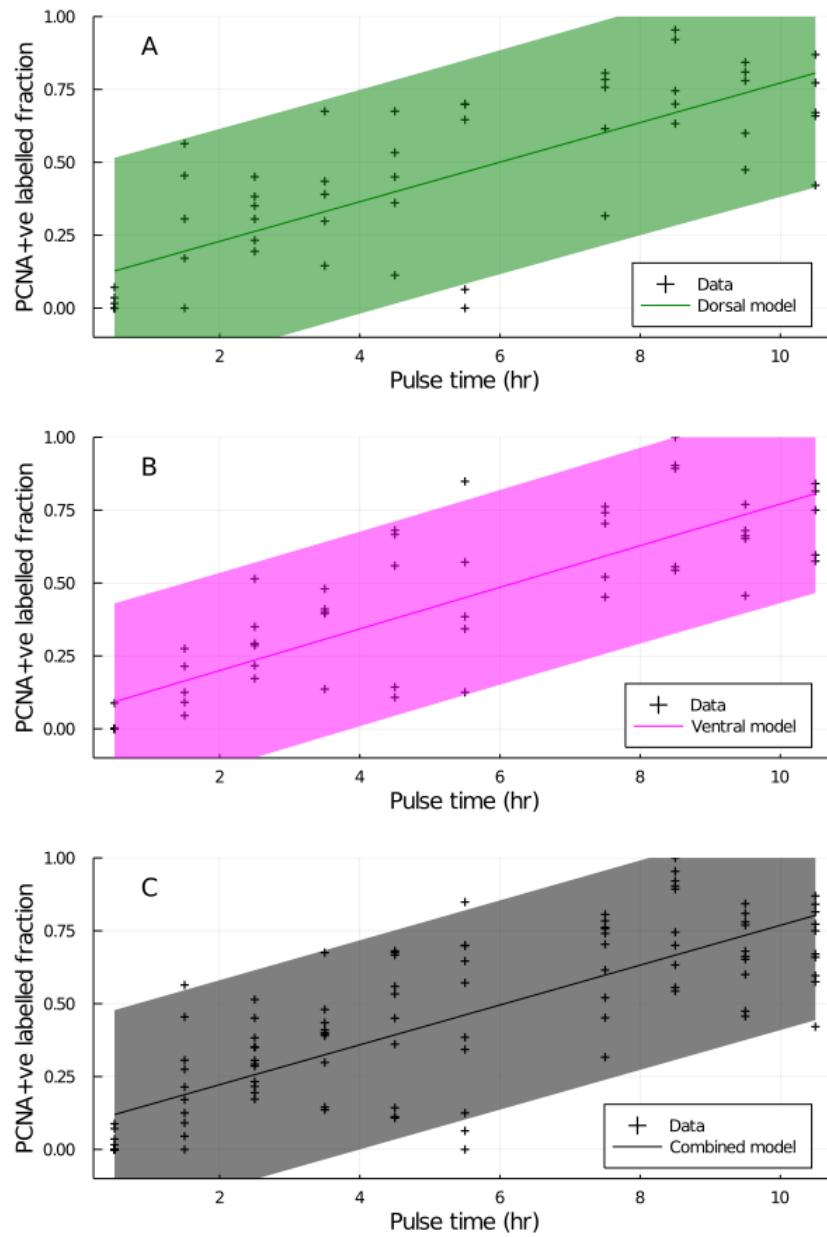


Figure 12.4: Linear regressions performed on cumulative labelling data from dorsal, ventral, and combined CMZ sectional populations

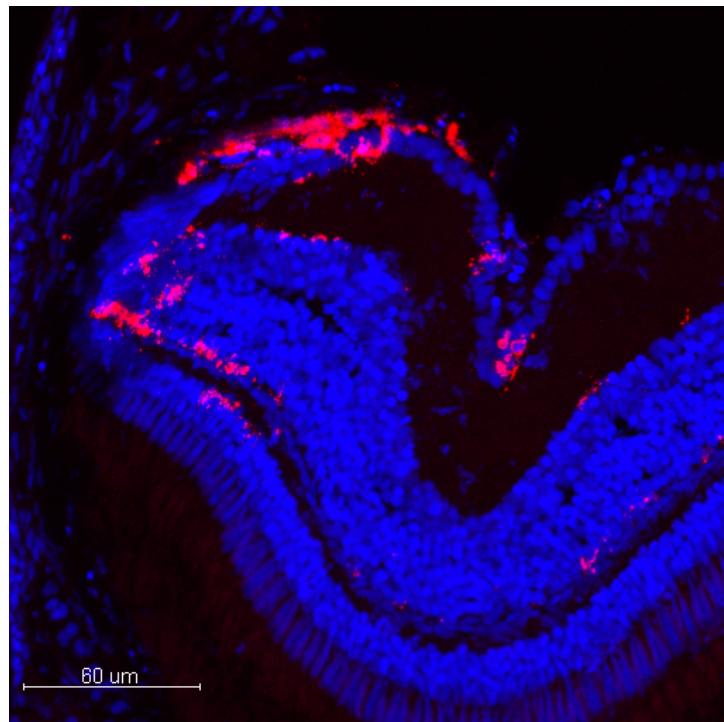


Figure 12.5: **4C4+ microglia are associated with the CMZ**

Representative maximum intensity projection from confocal micrographs of 14 μ m coronal cryosections through 90dpf zebrafish eyes.

Blue: Hoechst 33258 nuclear counterstain. Red: Microglia labelled with 4C4 antibody. Note extensive presence of 4C4+ microglia around the peripheral CMZ.

12.3 Evidence calculations for models of layer and lineage contribution

Table 12.1: Evidence for Normal vs. Log-Normal models of layer and lineage contribution

Layer	Marker	Cell type	\mathcal{N} logZ	Log- \mathcal{N} logZ	logZR	σ sign.
GCL	Cohort	All GCL cells	-92.81 ± 0.56	-63.93 ± 0.19	28.88 ± 0.6	48.5
GCL	Isl2b	RGC	-56.3 ± 0.76	-46.91 ± 0.35	9.38 ± 0.84	11.2
GCL	Pax6	Displaced am.	-110.7 ± 1.2	-35.39 ± 0.34	75.3 ± 1.2	62.1
GCL	Isl2b/Pax6	RGC subtype	-16.38 ± 0.18	-3.25 ± 0.6	13.13 ± 0.63	20.9
INL	Cohort	All INL cells	-84.56 ± 0.91	-286.89 ± 0.68	-202.3 ± 1.1	178.3
INL	Pax6	Amacrine cell	-13.99 ± 0.24	-18.5 ± 0.25	-4.52 ± 0.35	12.9
INL	PKC β	Bipolar cell	-13.66 ± 0.16	8.68 ± 0.44	22.34 ± 0.47	47.7
INL	GS	Müller glia	-12.36 ± 0.23	14.18 ± 0.48	26.54 ± 0.53	50.3
INL	HM	Horizontal cell	-16.44 ± 0.27	9.15 ± 0.37	25.59 ± 0.46	55.6
ONL	Cohort	All ONL cells	-128.68 ± 1.0	-171.66 ± 0.63	-43.0 ± 1.2	36.5
ONL	Zpr1	Double cones	-42.25 ± 0.72	-54.25 ± 0.37	-11.99 ± 0.81	14.9

logZ: logarithm of p(D), the marginal likelihood of the data, or model evidence. Largest evidence values bolded. logZR: evidence ratio; positive values in favour of Log- \mathcal{N} model.

Table 12.2: Evidence for combined vs. split models of layer and lineage contribution across the dorso-ventral axis

Layer	Marker	Cell type	Combined D-V logZ	Split D-V logZ	logZR	σ sign.
GCL	Cohort	All GCL cells	-53.473 ± 0.055	-184.34 ± 0.13	130.86 ± 0.14	911.5
GCL	Isl2b	RGC	-54.58 ± 0.9	-67.72 ± 0.94	13.1 ± 1.3	10.1
GCL	Pax6	Displaced am.	-31.09 ± 0.12	-46.41 ± 0.3	15.32 ± 0.33	46.9
GCL	Isl2b/Pax6	RGC subtype	-43.76 ± 0.76	-109.0 ± 1.3	65.3 ± 1.5	43.3
INL	Cohort	All INL cells	-131.4 ± 1.4	-184.4 ± 1.6	52.9 ± 2.1	25.0
INL	Pax6	Amacrine cell	-15.8 ± 0.024	-52.42 ± 0.69	36.62 ± 0.69	53.3
INL	PKC β	Bipolar cell	-3.86 ± 0.2	-19.81 ± 0.23	15.96 ± 0.31	51.9
INL	GS	Müller glia	15.78 ± 0.25	28.03 ± 0.41	-12.24 ± 0.48	-25.6
INL	HM	Horizontal cell	10.79 ± 0.41	-3.65 ± 0.19	14.45 ± 0.45	32.2
ONL	Cohort	All ONL cells	-79.87 ± 0.97	-158.2 ± 1.3	78.3 ± 1.6	48.5
ONL	Zpr1	Double cones	-54.66 ± 0.92	-64.27 ± 0.87	9.6 ± 1.3	7.6

logZ: logarithm of p(D), the marginal likelihood of the data, or model evidence. Largest evidence values bolded. logZR: evidence ratio; positive values in favour of combined model.

12.4 Likelihood ratio calculations for Normal and Log-Normal models of layer and lineage contribution

Layer	Marker	Cell type	\mathcal{N} MLE	Log- \mathcal{N} MLE	lhR
GCL	Cohort	All GCL cells	54.194	55.835	1.642
GCL	Isl2b	RGC	14.439	19.106	4.667
GCL	Pax6	Displaced am.	8.287	11.158	2.871
GCL	Isl2b/Pax6	RGC subtype	20.292	28.139	7.847
INL	Cohort	All INL cells	44.942	45.179	0.237
INL	Pax6	Amacrine cell	23.808	23.178	-0.63
INL	PKC β	Bipolar cell	27.243	26.119	-1.124
INL	GS	Müller glia	30.847	31.818	0.97
INL	HM	Horizontal cell	27.65	29.431	1.78
ONL	Cohort	All ONL cells	41.488	43.195	1.706
ONL	Zpr1	Double cones	13.256	18.483	5.227

logZ: logarithm of p(D), the marginal likelihood of the data, or model evidence. Largest evidence values bolded. logZR: evidence ratio; positive values in favour of stable model.

Chapter 13

Supplementary material for Chapter 5

13.1 Materials and methods

13.1.1 Zebrafish husbandry

Zebrafish husbandry was performed as described in Section 11.1.1.

13.1.2 Generation of transgenic *vsx2:eGFP rys* line

Figure 5.7 makes use of coronal cryosections of *Tg(vsx2:eGFP) rys* animals. Transgenesis was performed using reagents from the Tol2kit [KFG⁺07], which uses the Invitrogen Gateway plasmid construction system to assemble three-part constructs from donor plasmids. Briefly, the Gateway system uses BP clonase to introduce fragments of interest into sets of 3 entry vectors, which are recombined in predictable order by LR clonase. Tol2kit consists of a numbered, standardized set of preconstructed entry vectors intended for use in the construction of transposable Tol2 transgenesis vectors.

The construct of interest was built from a ~1.1kb fragment of the 5' promoter sequence of *vsx2* in the 5' entry vector, an eGFP expression cassette in the middle entry vector (Tol2kit #383), and a polyadenylation signal in the 3' entry vector (Tol2kit #302), recombined into pdestTol2pA2 (Tol2kit #394).

The *vsx2* promoter fragment was isolated by PCR amplification from the BAC (). BP clonase was bypassed in favour of restriction endonuclease sites inserted into the fragment's primers; this was necessitated by the instability of the ccd cassette in the BP vectors. The promoter fragment was amplified with a XhoI site 5' and a AgeI site 3'; the Tol2kit 5' Multiple Cloning Site entry vector (#228) was digested with SalI and XmaI in order to produce compatible sites for ligation. All restriction enzymes and the Quick Ligation kit were from NEB.

Injections into 1-2 cell embryos were conducted by standard techniques [Wes00]. Animals with stable expression of *vsx2* in postembryonic peripheral RPCs and in central retinal bipolar cells, as expected, were identified and bred out. These animals were subsequently introgressed into *rys* in order to produce *Tg(vsx2:eGFP) rys* animals.

13.1.3 Morpholino injections

Morpholino injections were performed by Maria Augusta Sartori by methods previously described [Won15].

13.1.4 Morphological photography

The photographs presented in Figure 5.1 were obtained with the transmitted light optics of a Zeiss fluorescent stereoscope (SteREO Lumar.V12), using ZEN Imaging software.

13.1.5 PCNA and EdU proliferative histochemistry

PCNA and EdU proliferative histochemistry giving rise to the data presented in Figure 5.2 was performed as described in Section 11.1.2.1 and Section 11.1.2.2, except that the length of the EdU pulse was 24 hours, and was not administered to 5dpf animals, which were set aside for the nuclear morphology study presented in Figure 5.4.

13.1.6 BrdU pulse-chase assay

The BrdU pulse-chase assay presented in Figure 5.3 was conducted by soaking 3dpf *rys* larvae in 10mmol BrdU for 8 hours. After a subsequent 7 day chase period, the 10dpf larvae were sacrificed and processed as described in Section 11.1.2.3.

13.1.7 Analysis of *rys* nuclear parameters by Galilean Monte Carlo Nested Sampling

The estimation of evidence for separate and combined mutant and sibling models of nuclear parameters presented in Table 5.1 was produced using the Julia package GMC_NS.jl, presented in Chapter 7.

13.1.8 Progenitor identity marker immunohistochemistry

13.1.9 Caspase-3 immunohistochemistry

Caspase-3 immunohistochemistry was performed by Monica Dixon.

13.1.10 In situ hybridization

In situ hybridization for npat transcript was performed by Monica Dixon.

13.1.11 Electron microscopy

Images presented in Figure 5.5 were produced from ultrathin sections for transmission electron microscopy, prepared as described previously [LDT12] by Monica Dixon and Audrey Darabie. These sections were imaged on a Hitachi H-7000 transmission electron microscope using AMT Image Capture Engine software.

13.1.12 RT-PCR analysis of *rys* npat expression

?? The RT-PCR analysis of *rys* npat expression presented in Figure 13.2 was conducted by Monica Dixon.

13.1.13 qPCR analysis of *rys* histone expression

?? The qPCR analysis of *rys* histone expression presented in Figure 5.11 was conducted by Maria Augusta Sartori.

13.1.14 *rys* MNase digestions and NGS

Two 10-animal pools each of 5dpf *rys* sib and *rys* animals had fresh nucleosome-protected MNase fragments produced using the Takara Biotech micrococcal nuclease kit. These digests were submitted to the SickKids TCAG facility for Illumina HiSeq 2500 NGS, with two lanes run per pool.

13.1.15 Nucleosome position calling

The nucleosome position calling pipeline used in Chapter 5 was automated using the Java machine learning framework KNIME [DB16] workspace incorporating KNIME4NGS nodes [HJH⁺17]. The pipeline is depicted in figureautoref. Node settings are largely default and are available in the `knime_workspace` thesis archive.

Briefly, FastQC (v0.11.8) [And18] was used to characterise the quality of the fastq sequence files describing the nucleosome-protected fragment pool from the MNase digestions described above. No sequence from any of the two duplicates of the two pools was rejected due to low quality. Sequences were found to be of uniformly high quality, with low adapter content. These results are available in the `fastqc` thesis git archive. TrimGalore (v0.5.0) [And18] was used to remove trailing adapter bases, using Adapter sequence: 'AGATCGGAAGAGC' (Illumina TruSeq, Sanger iPCR). The trim reports are available in the `thesis` git archive. Bowtie 2 [LS12] with default settings was used to map reads to the zebrafish genome (GRCz11). Nucleosome positions were called from the resulting sequence alignment map (SAM) files by using DANPOS2 (v2.2.2) [CXP⁺13]. As DANPOS2 is no longer supported, and the code contained errors preventing execution, a fixed version of this code has been uploaded to <https://github.com/mmattocks/DANPOS2fix> and filed in the `thesis` HDD archive, along with the called output positions.

The DANPOS nodes of the KNIME workflow are python nodes which execute DANPOS2 itself, as well as the nucleosome position analyses described below.

13.1.16 Analyses of *rys* nucleosome position disposition

Figure 5.14 was produced from the DANPOS2-called positions for the pooled *rys* sibling and mutant MNase-protected fragment datasets. For each numbered *D. rerio* chromosomal scaffold (1-25), as well as for scaffolds not assigned to any chromosome (NC), the number of sibling positions was divided by the total length of the scaffold. These values were compared to the naively expected number of positions per scaffold, determined by dividing the total number of positions per kilobase of genomic material, then multiplying the particular scaffold length. The per-chromosome relative number of positions, as well as fold-differences from the naive expected value, are displayed in pie chart format in Panel A. The Panel

C chart displays the result of normalizing these position values by relative occupancy, as determined by DANPOS2.

13.1.16.1 Background Hidden Markov modelling of *D. rerio* genomic sequence emission

The Julia package `BioBackgroundModels.jl` was used to perform background Hidden Markov Model selection on samples of *D. rerio* genomic material, in order to serve as a model of genomic noise for subsequent Independent Component Analysis (ICA) modelling of nucleosome positions, described below. The general strategy pursued was to first train a zoo of 3 replicates each of all HMMs with 1-6 states, emitting 0th-2nd order DNA kmers, on each of three broad partitions of the GRCz11 zebrafish genome, as described in Chapter 9, and initializing the zoo’s EM chains using the default `autotransition_init` function, which samples from an uninformative prior on state emission vectors, but a transition matrix prior heavily favouring state autotransition. This zoo was converged to an EM step likelihood delta of 1e-3 on 2e6 bp sampled without replacement from each of these partitions, then evaluated by testing against a further 2e6 bp sampled as a test set. The best model triplicate for each partition, in every case the 6 state, 0th order triplicate, was selected for further refinement by EM training on a new 8e6 bp sampled from the appropriate partitions. After this refinement process was complete, the triplicate chains were inspected using `BioBackgroundModels.jl`’s reports. The convergence of the triplicate on the same region of the parameter space, and the stationarity of the chains were verified before selecting the most likely models of the refined triplicate to represent *D. rerio* genomic background noise in the `BioMotifInference.jl` ICA models.

The code to sample GRCz11 for the initial zoo training task is available in Section 16.9.24, for the refinement task in Section 16.9.23. EM execution code is found in Section 16.9.25 and Section 16.9.27. Analysis code is given in Section 16.9.26 and Section 16.9.22.

13.1.17 Evidence and maximum a posteriori estimation of ICA models of *rys* nucleosome position sequence emission

The Julia package `BioMotifInference.jl`, presented in Chapter 10 was used to perform evidence and MAP estimation of Independent Component Analysis PWM source models of nucleosome position emission. The ICA models used had 8 PWM sources ranging from 3 to 10 positions in length. Model ensembles were initialized by sampling from uninformative source priors and an mixing prior of .07 (ie. for each source, mean 7% of observations will begin mixed with that source).

Observation sets comprised samples of 7092 nucleosome positions from each of the *rys* mutant and sibling differential position sets, corresponding to approximately 1 Mbp for each of the mutant and sibling sets, and 2 Mbp for the combined set.

Ensembles were converged to within 125 orders of magnitude likelihood compression.

The code used to produce the differential position set samples is available in ???. Ensembles are assembled in ???, and converged in ???.

13.2 Supplementary figures

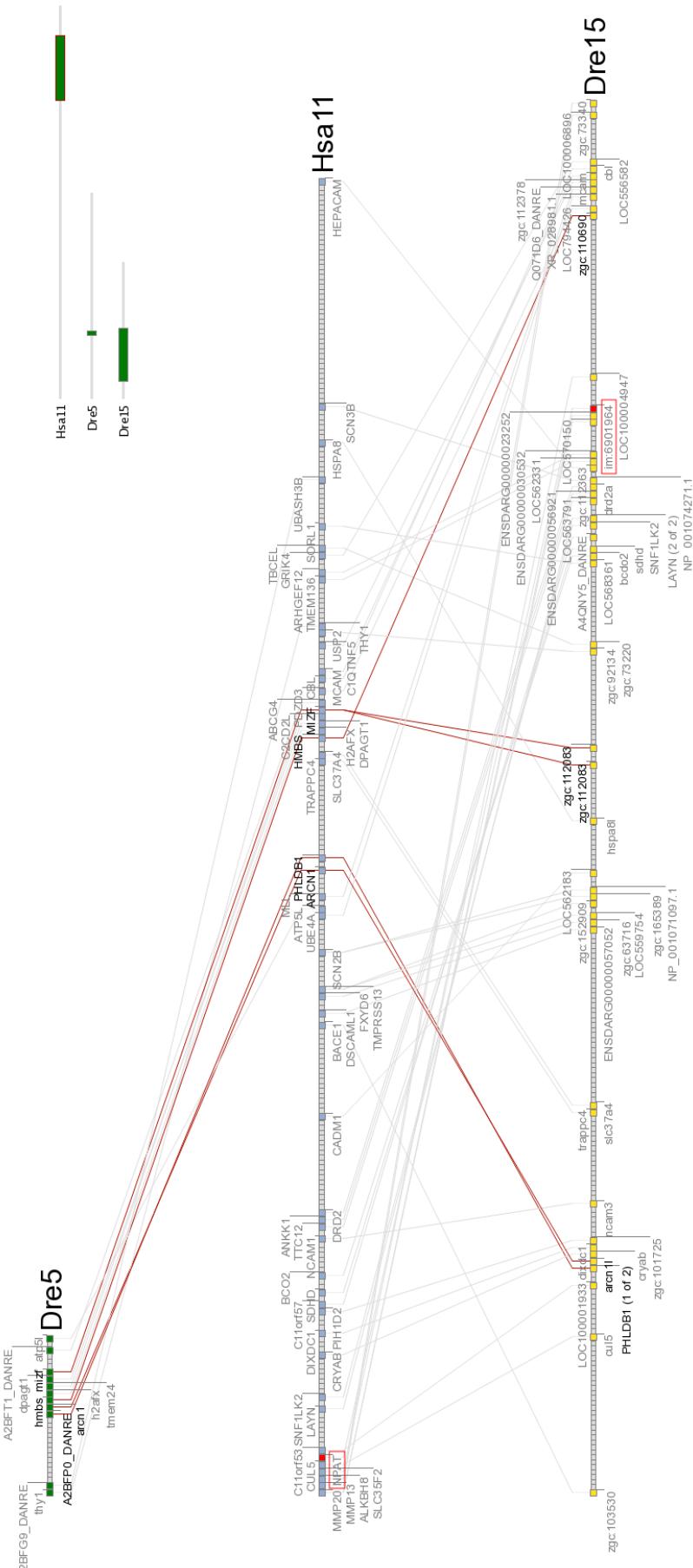


Figure 13.1: Synteny Database output for the syntenic region containing *D. rerio* npat

Dre#: *Danio rerio* chromosome # Hsa#: *Homo sapiens* chromosome #

Danio rerio chromosome 7 inset. *Homo sapiens* chromosome 7. The relative position of the displayed genomic neighbourhoods on their chromosome scaffolds is displayed inset, top right. *D. rerio* npat is highlighted with a red square, visible on the right of the selected Dre15 region, annotated “im6901964”. *H. sapiens* NPAT is highlighted similarly on the left of Hsa11. Landmarks of the duplication and rearrangement event that brackets the position of npat on Dre15 are highlighted with red lines.

On the scaffold of *H. sapiens* chromosome 11, NPAT occurs upstream of the highlighted duplication cluster bracketed by ARCN1 and MIZF. Zebrafish npat is located in the midst of this rearranged, duplicated cluster, but does not appear to have been duplicated itself; at least if it was, no parologue can be found by synteny or similarity analysis.

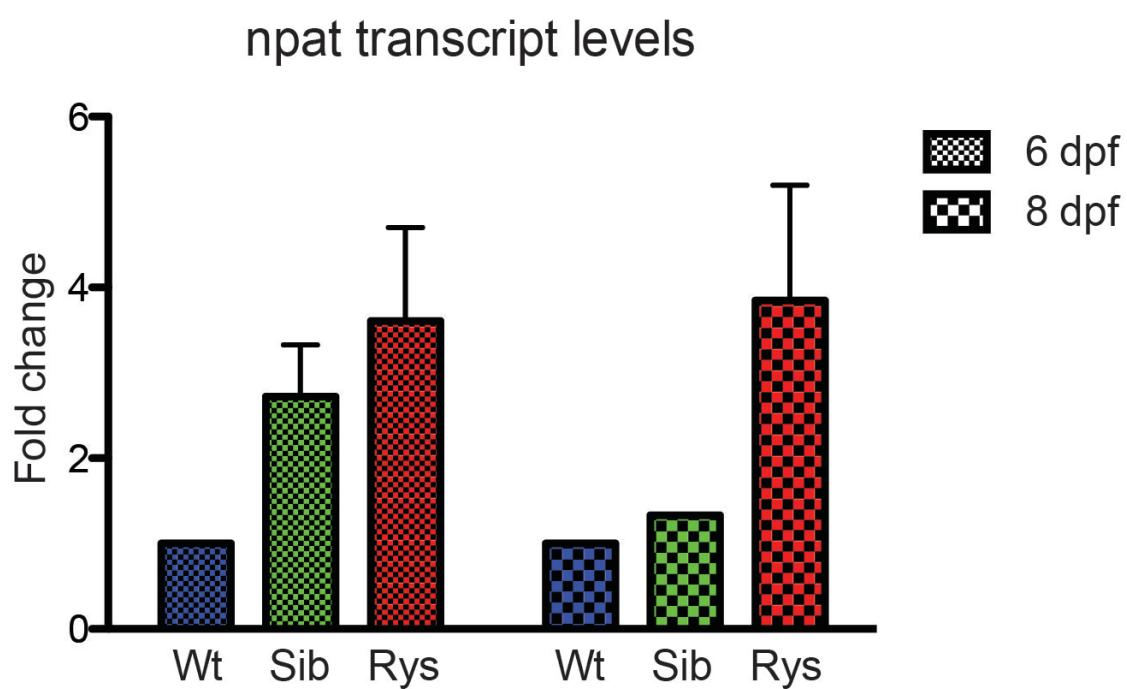


Figure 13.2: npat is overexpressed in rys

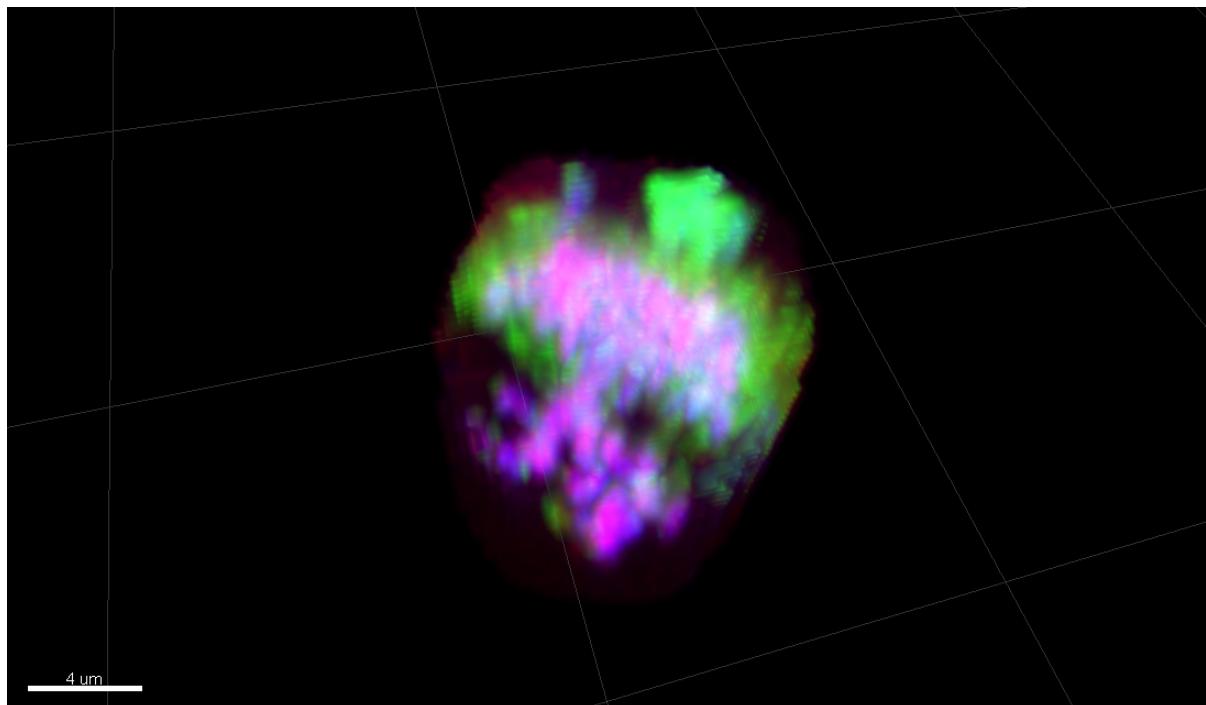


Figure 13.3: 10dpf mutant *rys* RPCs are mitotic

3d volume reconstruction of a complete mitotic figure in a *rys* mutant CMZ RPC at 10dpf, from a confocal micrograph of a 14μm coronal cryosection. Blue channel: Hoechst 33258; Green channel: PCNA; Red channel: EdU.

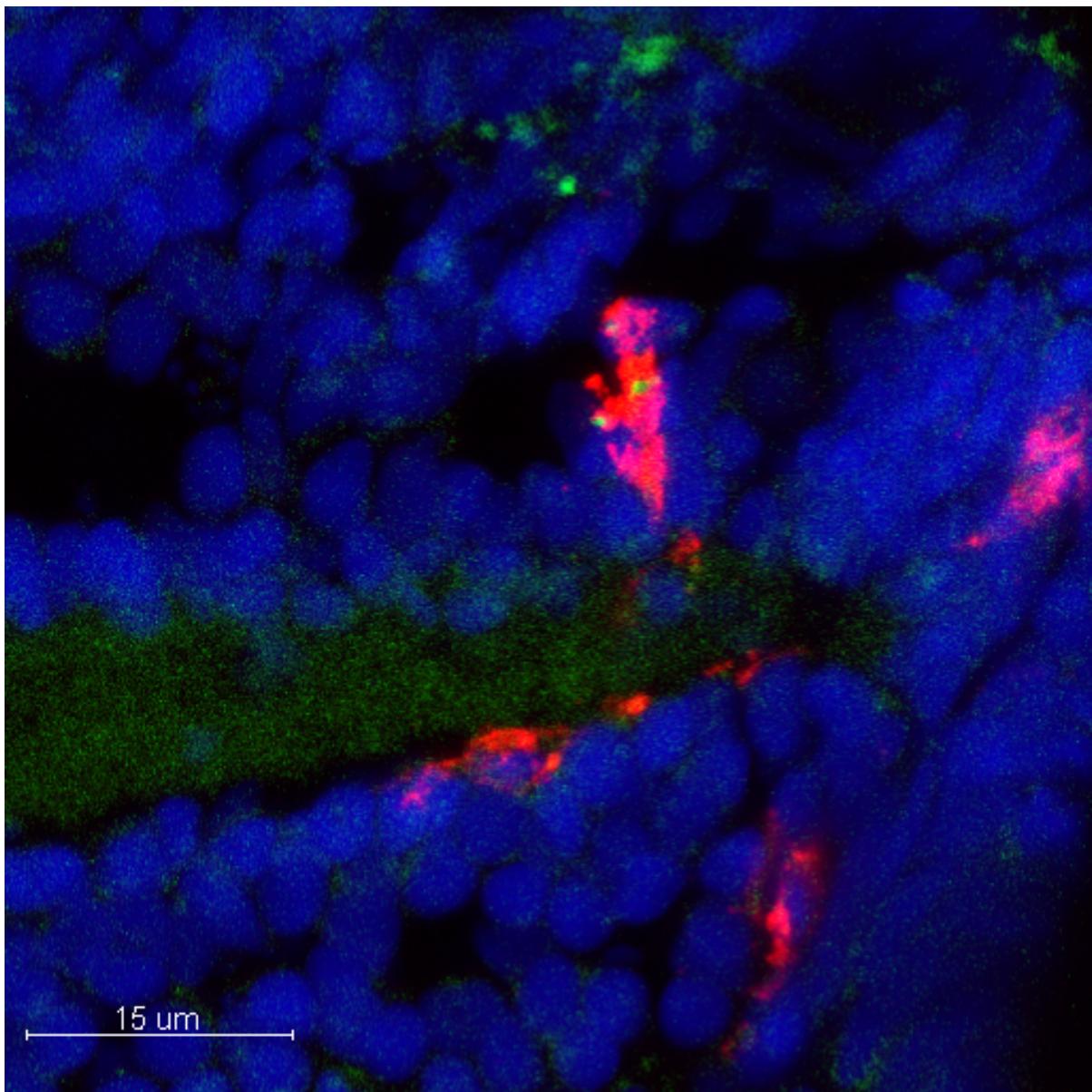


Figure 13.4: 4C4-positive microglia engulf *rys* mutant RPCs

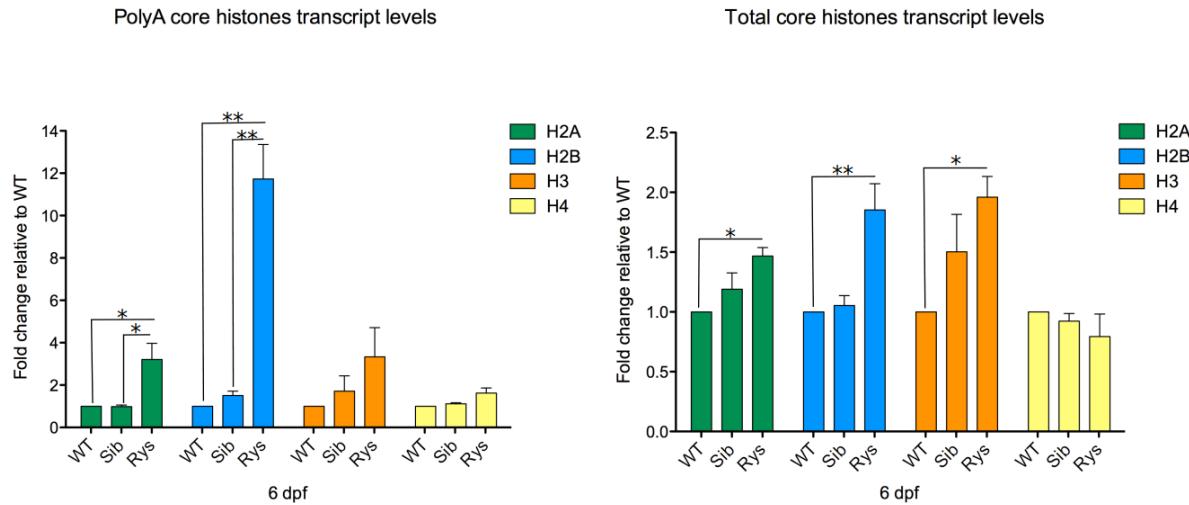


Figure 13.5: Morpholinos directed to npat result in histone transcript overexpression similar to *rys*

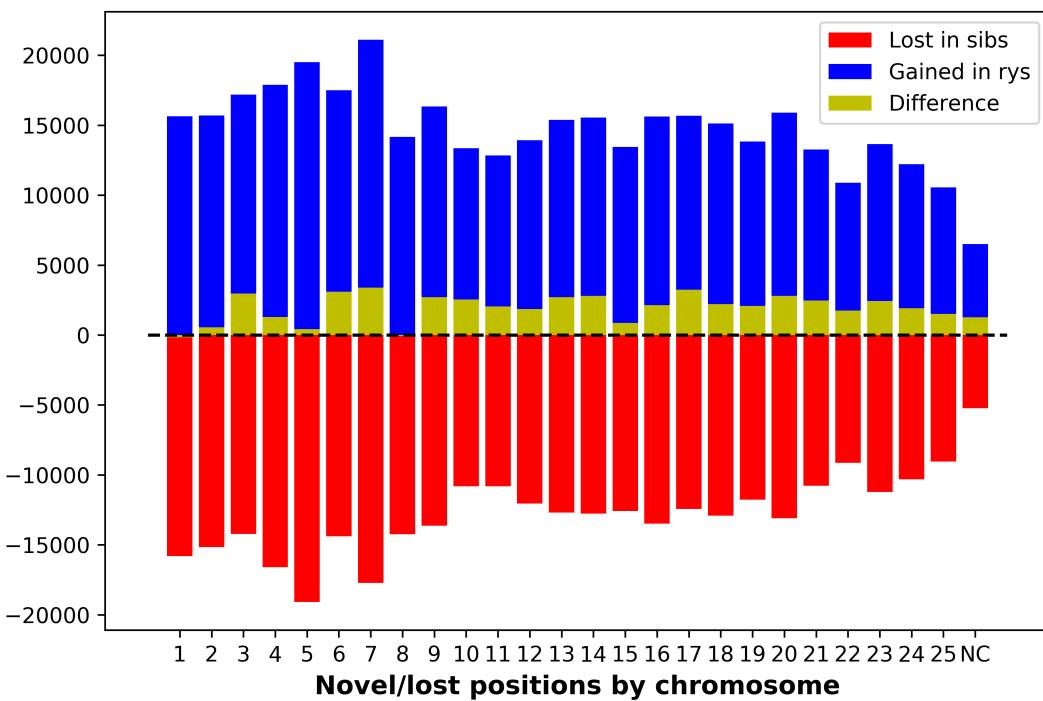


Figure 13.6: Novel nucleosome positions in *rys* occur in similar numbers to those lost from sibs.

Counts of positions found in sib but not *rys* (red bars, represented as negative numbers, as these are ‘lost’ in *rys*) and those found in *rys* but not sib (blue bars, ‘gained’). The magnitude of the difference between the counts is represented with a yellow bar.

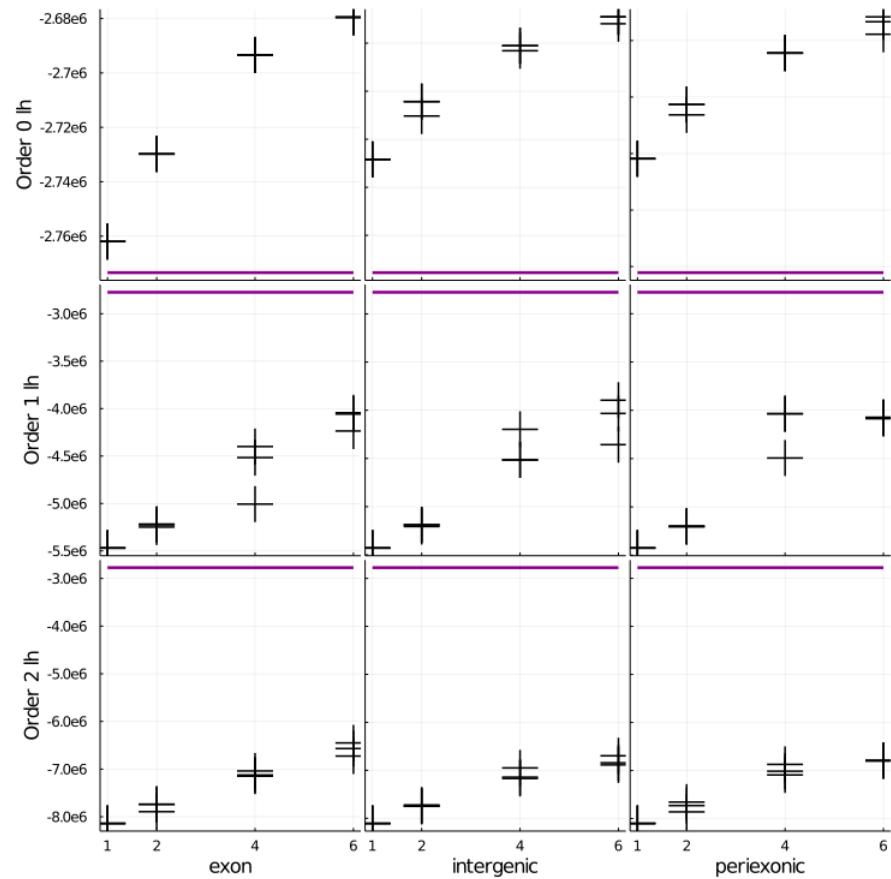


Figure 13.7: Test likelihoods for background HMMs trained on *D. rerio* genome partitions

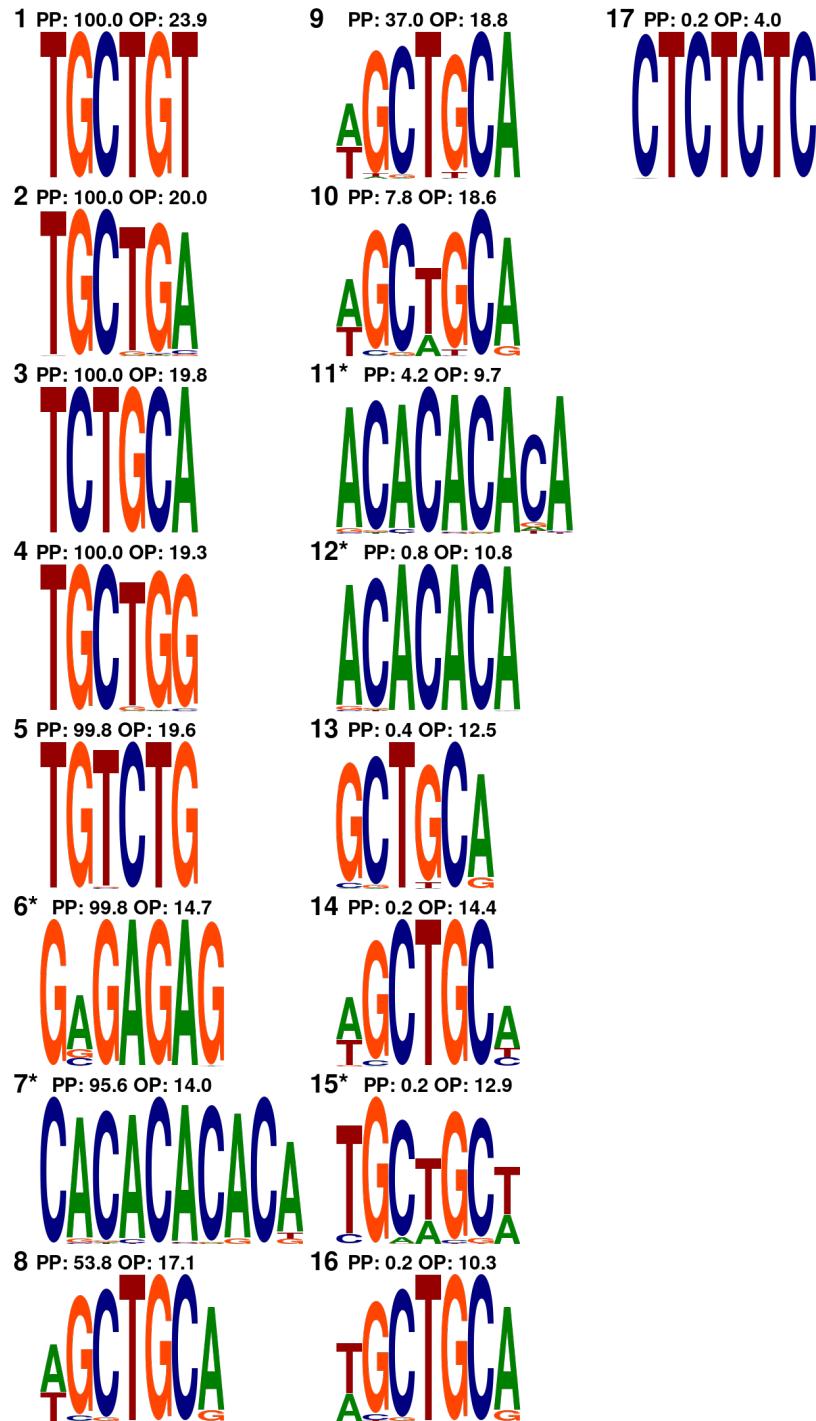


Figure 13.8: PWM sources detected in combined sib and rys differential sources.
 PWMs presented as sequence logos with letter height proportional to position informational contentemission probability.
 PP: Posterior prevalence; proportion of models within the compressed maximum a posteriori ensemble which have this PWM as a signal source
 OP: Observation prevalence; mean proportion of observed position sequences this sequence is used to explain, over all posterior samples

13.3 Supplementary tables

Table 13.1: Evidence for Normal vs. Log-Normal models of layer and lineage contribution

Morpholino	Measurement	Separate logZ	Combined logZ	logZR	σ sign.
ATG	CMZ pop.	-236.4 \pm 2.3	-198.3 \pm 2.2	-38.1 \pm 3.2	12.1
ATG	Total sectional pop.	-412.4 \pm 7.8	-225.3 \pm 4.9	-187.1 \pm 9.2	20.3
ATG	Sectional pop. per CMZ cell	-70.18 \pm 0.86	-78.0 \pm 0.86	7.8 \pm 1.2	6.4
ATG	Dorsal CMZ pop.	-169.5 \pm 1.4	-151.75 \pm 0.8	-17.8 \pm 1.6	11.2
ATG	Ventral CMZ pop.	-223.1 \pm 1.5	-165.3 \pm 1.0	-57.8 \pm 1.8	32.5
ATG	Nuclear volume	-290.6 \pm 1.8	-192.7 \pm 1.8	-97.9 \pm 2.5	39.6
ATG	Nuclear sphericity	-186.13 \pm 0.79	-215.66 \pm 0.57	29.53 \pm 0.97	30.3
Spl	CMZ pop.	-272.6 \pm 2.4	-197.0 \pm 2.6	-75.7 \pm 3.6	21.2
Spl	Total sectional pop.	-455.9 \pm 8.3	-205.6 \pm 4.7	-250.3 \pm 9.5	26.3
Spl	Sectional pop. per CMZ cell	-87.04 \pm 0.17	-66.22 \pm 0.29	-20.82 \pm 0.34	62.0
Spl	Dorsal CMZ pop.	-226.2 \pm 1.7	-171.0 \pm 1.1	-55.2 \pm 2.0	27.7
Spl	Ventral CMZ pop.	-234.97 \pm 0.81	-222.3 \pm 1.7	-12.7 \pm 1.9	6.8
Spl	Nuclear volume	-277.5 \pm 1.6	-223.2 \pm 2.1	-54.3 \pm 2.7	20.4
Spl	Nuclear sphericity	-94.3 \pm 1.1	-67.71 \pm 0.39	-26.6 \pm 1.1	23.2

logZ: logarithm of p(D), the marginal likelihood of the data, or model evidence. Largest evidence values bolded. logZR: evidence ratio; positive values in favour of stable model.

Chapter 14

Theoretical Appendix A: Model theory and statistical methods

14.1 Model Theory

This thesis is mainly concerned with a particular kind of scientific model: those that are subject to mathematically analysis, and tractable subjects for numerical simulation techniques. Most biological models do not fit this description, but increasing computing power has expanded the potential for, in particular, cellular and macromolecular explanations to be formalized and tested in this way. While doing so is sometimes derided as “physics envy”, the reality is more complex. Sometimes, simple heuristics are adequate biological explanation; much of differential diagnosis consists of reliable descriptive relations between qualitatively characterised signs in patients, and the presence of a pathogen. More often, the complexity of biological systems, and the sophistication of the instruments with which we probe them, give rise to observations which support more than one plausible causal explanation for the features of the data. The question of model comparison then immediately arises. Without numerical analysis, we may only make reference to the persistent use of the model in applied domains where the failure of the model to reflect reality results in its disuse. Because biological models are rarely required to succeed as engineering tools, for the prediction and control of outcomes of practical significance, an inability to analyse them numerically means that we are left only with rhetorical arguments about their relative explanatory value. This type of “model comparison” necessarily devolves into the type of anarchic Feyerabendian discursive chaos discussed in [Section 15.2.1](#). Feyerabend is correct to point out that scientific models can only be compared against one another, and that the selection of comparative criteria can never be “objective” in the sense of being fully independent of the contingent situation and goals of the observer. Nothing can be done about this; either we look for relatively-better criteria and relatively-better models as judged by those criteria, or we remain in the realm of pure metaphysical rhetoric, without investigating their implications, what Feyerabend describes as the realm of the “crank”:

[T]he distinction between the crank and the respectable thinker lies in the research that is done once a certain point of view is adopted. The crank usually is content with defending the point of view in its original, undeveloped, metaphysical form, and he is not prepared to test its usefulness in all those cases which seem to favor the opponent, or even admit that there exists a problem. It is this further investigation, the details of it, the knowledge

of the difficulties, of the general state of knowledge, the recognition of objections, which distinguishes the ‘respectable thinker’ from the crank. The original content of his theory does not. [Fey81, pp.199]

The support of complex biological systems for multiple explanations often gives rise to calls for explanatory pluralism [Bri10]. It should be emphasized that very differently parameterised models can be adequate explanations for the same system. This is particularly common for explanations at different descriptive depths. For instance, the models used in Chapter 4 make no reference to particular macromolecules, but we would still like to have macromolecular explanations of RPC function, particularly because these may supply us with means to intervene onto RPC proliferative and lineage outcomes. In other plural explanations, adequate, differently-parameterised models of the same system may be describing different aspects of the same level of organization. Pharmacological kinetic studies of G-protein association with receptors, and crystallographic studies of the same phenomenon, are a good example. These models allow experimenters to pursue different descriptive and interventional objectives. Indeed, as Nicholas Rescher has noted, attempts to synthesize many models into a single overarching explanation often result in descriptive chaos [Res00, p.65-6]. Rescher explains how the descriptive adequacy of some model in its local domain usually requires the blurring out of at least some pertinent details of the system that could have been included; it can be added that the computational tractability of the model requires the same.

If we virtually all accept this form of pluralism, we are still left with the cases where models are making contradictory claims about reality¹. Pluralism cannot coherently extend to abandoning the fundamental logical law of non-contradiction, without compromising the entire endeavour of scientific rationalism. We cannot accept logically contradictory notions about the structure of reality without precluding cognitive harmony arising from a broadly consistent view of the way the world works [Res05]. Given the complexity of biological systems, we have no option except to express models formally and to test them rigorously against one another. This is the task of model selection.

Model selection requires that we be able to score models against observational datasets encoding the phenomena they represent, to measure their quality. This requires the selection of a quality function. Loss functions, like AIC, used in Chapter 2, express the relative amount of information in the dataset lost by the model, given a set of parameters. Likelihood functions, used elsewhere, express the likelihood of the data given the model and parameters. The parameters of the model thus define an n-dimensional “parameter space”; the quality function expresses a hypersurface within this space. By sampling within this space, we may estimate the shape of the surface. This sampling information can be used in three ways: we may propose new, higher quality parameter space locations to sample from, in search of the highest quality parameterisation of the model (model optimisation); we may derive marginal likelihoods for particular parameter values (parameter estimation), or we may estimate the quality of the model over all parameterisations (evidence estimation). These topics are discussed below.

14.1.1 Bayesian Epistemological View on Model Comparison

The analyses presented in the data chapters express two views on how model comparison should be approached. The first, expressed in Chapter 2, is drawn from information theory, while the second, taken up in Chapter 4, is a relatively conventional Bayesian view, albeit with more sophisticated tools

¹That is, they have incompatible metaphysical content; they are therefore subject to counterinduction as explained in Section 15.2.1

and more computing power than has been available to Bayesians in the past. In the same way that frequentist analyses may be expressed as a subset of Bayesian analyses (i.e. they normally seek the maximum a priori model parameterisation and likelihood from uninformative priors), informational theoretical approaches to model comparison can be expressed as a subset of Bayesian model comparison theory. In fact, the loss function used in Chapter 2, Akaike Information Criterion, has been adapted to refer to a prior distribution, as the Bayesian Information Criterion [PB04]. The intent of these criteria is to take Occam’s Razor to the maximum-likelihood approach by penalizing the maximum-likelihood value (or maximum a priori score, in the case of BIC) by the number of free parameters.

The general approach of optimizing a model for a quality against a dataset, then penalizing the best model quality by the parameterisation of the model, allows us to overcome the most important problem with maximum-likelihood approaches to model selection: the requirement for models to be parametrically nested. Model nesting precludes counter-induction; we cannot compare the adequacy of models which express fundamentally different views of how the described system is parameterised, only whether adding more parameters improves a particular view. Escaping this limitation is what allows us to compare stochastic and deterministic mitotic mode models in Chapter 2; these models express different views of how reality is organised. Still, in important ways, this approach shares the basic problem of simply calculating the MLE: the score in no way accounts for the relative robustness of the model fits. That is, a model which is a terrible description of a dataset over most of its plausible parameter space, but an excellent one in a tiny region, can appear to be a better explanation than a model which is a broadly good description over the whole parameter space. Simply using the number of parameters to penalize the best model found in some sampling procedure fails to express the extent to which the inclusion of those parameters is justified by improving the overall likelihood of sampled models.

This leads us to what may be regarded as the completion of the Bayesian view on model selection², John Skilling’s system of Bayesian inference, nested sampling [Ski06, Ski12, Ski19]. By rearranging the usual presentation of Bayes’ rule, Skilling reveals how it specifies the computational inputs and outputs associated with the activities of model sampling, parameter estimation, and evidence estimation. Bayes’ rule is typically written as follows, where x is a logical proposition about the data (e.g. specific values for the cell cycle length and exit rates of the CMZ), and $Pr(a|b)$ denotes the probability of a given b:

$$Pr(x|data) = \frac{Pr(data|x)Pr(x)}{Pr(data)}$$

This can be read aloud as ”the posterior probability of the proposition, given the data, $Pr(x|data)$, is equal to the likelihood of the data, $Pr(data|x)$, given the proposition, multiplied by the prior probability of the proposition, $Pr(x)$, and divided by the marginal probability of the data over all such propositions, $Pr(data)$.” This gives the impression that the principal task in statistical analysis is the calculation of the posterior probability of a model, and gives rise to the treatment of the marginal probability, $Pr(data)$, as a mere normalizing constant. Skilling rearranges this to put computational inputs on the left, and outputs on the right:

$$Pr(x)Pr(data|x) = Pr(data)Pr(x|data)$$

²If nested sampling is not *the* completion of the Bayesian system, it is at least *a* complete Bayesian system.

This shows us that the evaluation of a model consists of supplying a prior probability for the proposition, $Pr(x)$, and a likelihood function to assess the probability of the data given that proposition, $(Pr(data|x))$. In return we receive, as computed output (over many propositions x) the total evidentiary mass of the data for this model, $(Pr(data))$, as well as the posterior parameter estimates, $Pr(x|data)$. Sample model parameters are drawn from the prior; the likelihood of this proposition about the data is calculated. By accumulating many such samples, the marginal probability of the model over all of these propositions (the evidence for the model) can be estimated. Because these samples may be weighted by their calculated likelihoods and position on the prior, we may also use them to estimate the marginal posterior probabilities of parameters of interest.

This encapsulates both the numerical procedures involved in model analysis, as well as the epistemological view implied by Bayesian statistics. That is, a model analysis is the joint product of the model and the data, which expresses our belief about the overall credibility of the model $(Pr(data))$ ³, as well as allowing us to estimate the distribution of credibility we should assign to various values for parameters of the model (propositions), $Pr(x|data)$. These are the two fundamental levels on which quantitative measurements of natural systems allow us to make inferences. We may distinguish between models of the systems in a general sense by their evidence, when applied to the same overall dataset. This allows us to counterinductively test contradictory descriptions of the structure of the phenomenon against one another, inferring which is a better map to the territory. The second level of inference is the ranking of propositions for the parameterisation of models achieved by the sampling procedure. This allows us to determine which particular propositions about the system are supported, given the model. Because the posterior distributions need not be unimodal, we can evaluate these modes as separate hypotheses that are supported to varying degrees by the data.

This view dispenses with problematic interpretations of model selection as being about finding the “true model” of reality, or of estimating the actual, objective probabilities inhering in things or processes (a view disputed in [Section 15.1](#)). Instead, we are guided to focus on the relative quality of models in explaining all of the relevant data we can gather; we may then evaluate the relative quality of specific propositions about the system within those models (i.e., the posterior distributions on model parameters) as we see fit.

14.1.2 Model sampling and optimization

There are now a fairly large number of widely-used techniques intended to sample the parameter space of a statistical model, given a quality function which scores the model. These have a variety of purposes. Most commonly, one wishes to “optimize” a model by finding the parameter vector which produces the best objective function result, given some dataset. This is often referred to as Maximum Likelihood Estimation, the product being a Maximum Likelihood Estimate, with MLE used to refer to these interchangeably. SPSA is used in this thesis to optimize cell-based models of RPC activity in [Chapter 2](#). The likelihood scores are penalized by the number of model parameters to produce an AIC score. MLE methods are broadly useful for many applications, with some tuning. SPSA is used in control systems, to maintain the consistency of modelled processes by estimating the inputs most likely to achieve a setpoint, for instance. More sophisticated applications of sampling involve the estimation of the distribution of quality values over the parameter space, rather than focusing solely on estimating the optimal value.

³ie. a better model gives higher marginal probability to observations than a worse one

The process of model sampling requires a type of algorithmic logic additional to the quality function, which is a means to generate proposed parameter vectors to be supplied to the function. Most effective sampling methods operate by performing an initial sample from some type of prior information⁴, and then iteratively generating new proposals from these initial ones. This iterative proposal generation forms a chain of linked positions within parameter space. Generally, new proposals are made considering information related only to the last accepted proposal⁵. Because the process is “memoryless” in the Markovian sense, and involves the iterative generation of models, it is often called Markov Chain Monte Carlo (MCMC).

Many algorithms for proposal generation exist, and the success of any sampling procedure depends on their properties. Most offer a physical interpretation of the surface formed by the quality function in parameter space. Basic random-walk proposal generation, like the original Metropolis-Hastings implementations of MCMC, is often too inefficient to be usable for high-dimensional parameter spaces, and has broadly been replaced by these physical sampling methods. SPSA, for instance, is a simple gradient method. New proposals are generated by bracketing an existing sample with a pair of samples that define an n -dimensional “slope” at the current location. By proposing a new location up-slope, we receive a new sample which is closer to the local optimum, and we may inexorably approach it in this way. Other, more sophisticated interpretations seek to fix problems with random-walk proposal generation by mechanical interpretations of proposal movement through parameter space, like Hamiltonian Monte Carlo, or thermodynamic interpretations, like simulated annealing. These methods are better suited to the estimation of the distribution of objective function values than an algorithm like SPSA.

In order to ensure the accuracy of any such estimated distribution across parameter space, the algorithm should, ideally, produce proposals in “detailed balance”, which refers to the physical concept of reversibility found in classical mechanics. The purpose of constraining proposal generation in this way is to guarantee that particular areas of the parameter space are not over- or under-sampled, distorting the final estimate, as a consequence of the manner in which the sampling algorithm generates proposals. Galilean Monte Carlo has the property of being in detailed balance.

14.1.3 Overfitting

Overfitting is among the most common problems in statistical modelling. This can arise from an unjustified excess of model structure relative to the information present in the data. This excess structure allows the model to capture measurement noise in the dataset the model is being scored against. This gives the appearance of a model which is highly explanatory, but which reflects the particular structure of the measurement errors in the training data better than the overall phenomenon. This problem will typically become apparent in cases of sequential inference; an overfit model will fail to explain new observations as well as it explained previous ones.

A second source of overfitting is the failure to properly account for uncertainty and prior information. For instance, if a unidimensional measurements are modelled with a Normal Gaussian distribution, the MLE Normal Gaussian (whose parameters arise from calculating the mean and standard deviation of the

⁴In the SPSA optimization performed in Chapter 2, this took the form of the original, poorly optimized fit for the stochastic model and a best guess for a related vector for the deterministic model. In subsequent nested sampling analyses, initialization is performed by sampling randomly from a defined prior distribution.

⁵Proposal rejection can occur for a variety of reasons, depending on how the parameter space is interpreted. In Galilean Monte Carlo, this occurs if proceeding along the specified velocity vector would produce a proposal less probable than the last one.

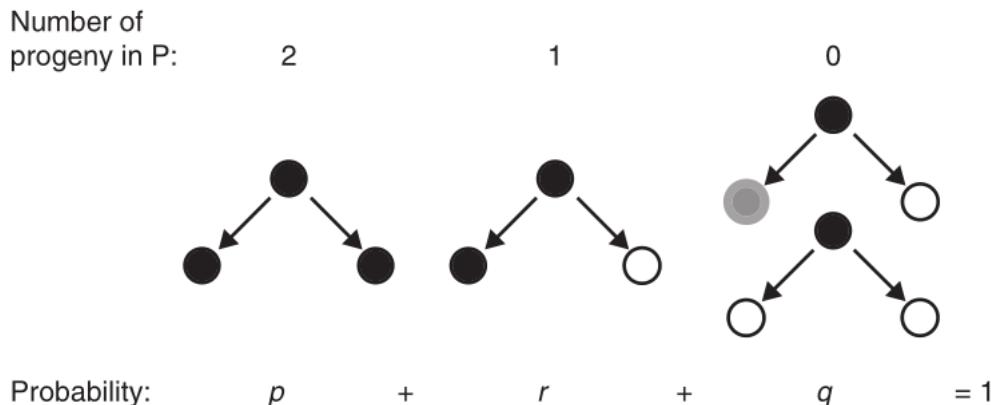


Figure 14.1: Simple stochastic stem cell model, representing probabilities of cell division events, excerpted from Fagan 2013 pg. 61. Black circles denote proliferative cells, while white and grey circles denote different types of postmitotic offspring. “Number of progeny in P” is the number of mitotic offspring produced by each type of division. The probability of each division type must sum to 1, as all possibilities are represented, granting that the division types are defined by the postdivisional mitotic history of the offspring.

measurements, given the assumption of Normality) can be an overfit to the data. In this simple example, this usually means that the MLE Normal is overly influenced by “outliers”. This is one case of the general overfitting problem seen in MLE methods [Gre17]. Accounting for available prior information is the best solution to this problem. As this thesis demonstrates, appropriate accounting for our uncertainty about the parameters of the Normal model, and comparison of the Normal case with appropriate alternatives that might better reflect the underlying causal structure that produces the “problem” data (in this case, Log-Normal models), both help in producing more accurate estimates of model parameters.

14.1.4 Simple Stochastic Models

The Simple Stochastic Model is schematically summarised in Figure 14.1. This is the basic structure of the great majority of formal models in the stem cell literature, derived from post-hoc analyses of populations taken to include stem and progenitor cells. The population-level approach is usually explicit, as no differentiation is made between types of proliferating cell- in general, no particular cell is identified with a stem cell, nor can any be identified from the necessarily retrospective population data used to infer the parameters of the model.

The central concept of the model is that divisions can be categorised by the number of progeny which remain mitotic after the division. It is important to note that a mitotic event cannot presently be categorized in this fashion except retrospectively. This must be kept in mind when analysing models of this type, as this categorisation does not necessarily imply that there is some mechanism by which the cell specifies the fate of offspring *at the time of mitosis*, although there is extensive evidence for the coupling of mitotic and specification processes at the molecular level.

In effect, then, the model compresses the process of fate specification into individual mitotic events. Since the primary distinction between cells in the model is simply whether they are proliferating or not, the model also elides any heterogeneity within the proliferating population. Beyond not identifying

particular cells as “stem cells”, this may make models derived from the SSM inappropriate for proliferative populations with a large degree of heterogeneity. One may think here of the classic idea of a small number of slowly proliferating “true” stem cells and a larger population of rapidly dividing “transit amplifying” progenitors- this type of internal structure within the proliferating population can only be represented by multiple, independent SSMs (as implemented in Chapter 4).

As Fagan notes, in the SSM, “relations among p, r, and q values entail general predictions about cell population size (growth, decrease, or ‘steady-state’), and equations that predict mean and standard deviation in population size, probability of [lineage] extinction, and features of steady-state populations are derived.”⁶[Fag13, p.60]

Typically, this type of model has been employed to describe population dynamics of proliferating cells in assays generating ostensibly *clonal* data, where a “clone” here refers to the population constituted by all of the offspring descended from some particular (usually “initial” and sometimes therefore taken for “stem”) proliferative cell. This population is the *lineage* generated by some particular dividing cell. The mitotic events which give rise to a lineage are represented as a Galton-Watson branching process, a stochastic process originally intended to model the lineage extinction of surnames. In the case of branching process models applied to proliferative cells, the random variable determines the mode of division of each cell within the lineage, with this mode being defined by the proliferative state (construed in the model as being either mitotic or postmitotic) of progeny. For any given division, a cell may produce two mitotic, one mitotic and one postmitotic, or two postmitotic progeny, and each of these division modes is given a defined (often, but not always, static) probability. Given these values, the history of a cell lineage may be simulated; the output of many of these simulations pooled together, in Monte Carlo fashion, allows the statistical properties of the dynamics of population of simulated cells to be estimated.

14.1.5 Systems of difference equations

Models defined by difference equations are used in Chapter 4. A difference equation is the discrete counterpart of a continuous differential equation. Both types of equations can be used to describe the time-evolution of a system, because they define the value of some model output value at a time t , given the value(s) of the output(s) at some time $t - p$, p time units in the past, plus the parameter vector for the model. In the models presented in this thesis, the simulation’s unit of resolution is the day, which motivates the use of the algorithmically simpler analytic solutions to difference equations. As implied above, the time-dependent value of a model output may depend on a value which is generated by another equation. This is the case for the model of estimated CMZ annulus population and retinal volume presented in Figure 4.2, where the volume estimate depends on resolving the value of the day’s starting population before evaluating the volume contribution of that population, given the exit rate. The slice models used to interrogate phase transition timing in Figure 4.6 are not fully systems of difference equations, since the population difference equation depends on evaluating a continuous power law model of lens growth.

⁶While Fagan refers to “stem cell” extinction, the model does not specifically define stem cells, nor does it imply intergenerational continuity, such that a particular intergenerationally identified stem cell should be said to have become extinct. The unit which survives or is made extinct is the lineage derived from some particular proliferative cell.

14.1.6 Independent Component Analysis models of sequence emission

The models of *rys* nucleosome position sequence emission presented in Chapter 5 are of a form that is more familiar to psychologists and signal processing engineers than molecular biologists. Independent Component Analysis (ICA) is a technique which is used to infer the independent contributions of multiple signal sources on a single multiplex channel; it can be thought of as an application of information theory. The most common example offered to illustrate this is the separation of the individual streams of speech produced by multiple speakers conversing in a noisy room. ICA can be used, in this context, to model the process by which a listener separates the independent signals of the speakers from background noises. In this thesis, the *D. rerio* genome is treated in this fashion. In effect, any given sample of genomic sequence may be treated as a multiplex channel produced by a host of independent causal processes. In this sense, each nucleosome position is a “noisy room”, with its own local structure of background noise and conversational participants.

The distinction between background noise and signal in an ICA model is made both conceptually, and in the numerical representation of these parts of the model. I have closely followed the early, pioneering example of Down et al. [DH05] in this regard. Down et al. use Hidden Markov Models optimized on promoter sequences as background models for promoter sequences in general, against which a foreground of Position Weight Matrix signals, representing binding motifs within the promoter region, is inferred by nested sampling. Similarly, in Chapter 5, separate HMMs were used to represent genomic sequences from the exonic, “periexonic” (introns and ± 500 bp intervals around the CDS), and intergenic sequences, against which the foreground of repetitive PWM structural signals is inferred, representing characteristic preferred contact points between the core nucleosome and genomic DNA. The HMM background models and s PWM signal models are linked in their description of a set of o observations by an $o \cdot s$ mixing matrix. While many implementations of source and background mixing are possible, in this case, Down et al.’s simple binary implementation is retained. Therefore, each observation o is associated with a “mix vector” of s binary values, indicating whether or not s source is to be scored in that observation.

The likelihood scoring function for this model is numerically equivalent to Down et al., and is available in full in Section 16.7.3. Briefly, for every observed sequence whose mix vector indicates a source is present, a score matrix is produced for that source and observation. This involves “scanning” the PWM down the length of the observation, offering a score for the entire PWM at each base (in the default reverse-complement operation of BioMotifInference.jl, this is repeated with the reverse complement of the motif, for motif detection on the reverse strand). This feature of likelihood function confers the “multiple uncounted” nature of the motif representation in these models. This means that multiple occurrences of a PWM source motif in a sequence can be detected, and that no particular number of occurrences must be specified. These features make this model particularly well suited to the detection of the repetitive structural signals inferred in Chapter 5. The PWM source score matrices are subsequently “woven” by combination with background scores, an expectation value for motif observation, given the length of the sequence, and a penalty for every source included in the observation, which prevents overfitting.

14.1.7 Position Weight Matrices

Position weight matrices (PWMs) were used to model signals arising from nucleosome contacts with their DNA positions in Chapter 5. For a sequence signal of length λ , the PWM which defines it is a $\lambda \cdot 4$ matrix, with each of the four columns representing the categorical probability weights of A,

C, G, and T base emission at the position represented by row λ^7 . The PWM is typically used in a frequentist context, without prior distributions over its parameters. However, in a Bayesian context, the PWM is easily interpreted as a λ -base length vector of discrete categorical distributions over base emission frequencies⁸. A computationally useful conjugate prior distribution for discrete categoricals is the Dirichlet distribution [Min00], and vectors of Dirichlets have been used as the priors for signals in BMI.jl models. Because of their conjugacy, Dirichlet priors can theoretically be updated to produce full posterior distributions over the PWM’s parameters. BMI.jl does not provide for this, for reasons related to the ICA PWM model implementation discussed in Chapter 10. The 4-parameter Dirichlet over the 4-category probability simplex can be usefully thought of as a density cloud in a 3-dimensional tetrahedron (i.e., an ordinary one with 4 vertices). The relative density within the tetrahedron expresses the prior (or posterior) probability of a particular categorical distribution at base λ , where the points of the tetrahedron represent categorical distributions with unit probabilities for one of the four bases, while points in the center of the tetrahedron represent categoricals with identical .25 probabilities of emitting each base.

14.1.8 Hidden Markov Models

Hidden Markov Models (HMMs) were used to model *D. rerio* genomic background noise in Chapter 5. An HMM is a state machine; it can be in one of a finite number of states at any given time. Each state is associated with the probability of some outcome obtaining, and the HMM generates a sequence of such outcomes by transitioning from state to state (or remaining in the same state, called autotransition). The states are “hidden” in the sense that their presence is inferred from the sequence of data upon which the model is trained, and not from direct observations of the states themselves. HMMs are, therefore, well-suited to modelling sequences of outcomes with obscure or irrelevant causal structures. A k -state HMM that emits s symbols is defined by an initial state probability vector of length k , a $k \cdot k$ state transition probability matrix, and the outcome probability vectors for each state. HMMs can be used in a variety of contexts, each of which is associated with different algorithmic procedures. For instance, the Viterbi algorithm can be used, given an HMM and a sequence of observations, to infer the most likely state the HMM would have resided in at each position in the sequence, had it been generated by that HMM. An example of this use is the inference of exon and intron features from an HMM trained on annotated CDSes [HSF97]. In this thesis, HMMs are optimized by an Expectation Maximization algorithm, and are subsequently used to score the likelihood of *rys* sibling and mutant nucleosome position sequences, given their genomic context.

14.2 Statistical Methods

14.2.1 Monte Carlo simulations

Monte Carlo refers to the repeated sampling of a statistical model’s output to estimate quantities related to it. Many of the simulations presented in this thesis involve two distinct uses of Monte Carlo techniques. The first use is the exploration of parameter spaces by Markov Chain Monte Carlo (MCMC), which is

⁷This base ordering is computationally significant, since it allows for trivial reverse complementing by reversing the matrix values across both dimensions.

⁸That is, a vector of nonparametric categorical distributions, themselves on a support of ACGT vector of the 4 nucleotide base emission frequencies. BMI.jl encodes the support as the integers 1234 for computational efficiency

the memoryless (Markovian) sequential sampling of parameter space. This is a means of exploring the parameter space by generating new parameter vector proposals from existing ones. These proposals can be generated by various means; in this thesis, Simultaneous Perturbation Stochastic Approximation, Galilean Monte Carlo, and an ad-hoc permutation routine are used. The second use of Monte Carlo is the estimation of variability in model outcomes, which involves repetitively sampling the same position in parameter space. This permits the direct estimation of the likelihood distribution of outcomes for models with variable output. Observations may then be scored by these distributions to determine their likelihood, given the model parameters.

14.2.2 Simultaneous Perturbation Stochastic Approximation (SPSA)

SPSA is a model optimization technique. It is a reliable method for finding the local optimum in parameter space, given an objective function and a sample starting position. It has been proven useful in a wide variety of engineering applications [KHI97, ZZLG08], not least because it is tolerant to measurement noise in the objective function. This makes it useful for applications where exact determination of the measurement function's value is not possible; these include on-line intervention into systems with real measurement noise [ZZLG08], as well as the calculation of model likelihoods by MonteCarlo.

14.2.3 Bayesian parameter estimation

14.2.3.1 Problems with frequentist inference using normal models of sample data

Typical biological practice is to report the mean and variance of a sample, assuming a normal distribution of the error around the mean. In other words, the sample is taken to be representative of a larger population; that population is modelled by a normal distribution with mean μ and variance σ ; the parameters of the maximum likelihood estimate (MLE) for the normal model are the values reported. A slightly more sophisticated approach is to report the standard error of the mean of the sampling distribution the sample is taken to be drawn from, if more than one sample can be obtained, although this usually plays no role in hypothesis testing (often conducted by t-test).

This approach has a number of defects which follow from one another. We are reporting MLE parameters without any account of our uncertainty about those parameters. There is no way to incorporate prior information we have about the parameters (even just to admit total ignorance about them). This leads to overfitting of our estimates to the sample data. Practically speaking, this means our estimate of the mean is stated too precisely, and the variance is too sensitive to outliers.

Additionally, the plain-sense interpretation of the estimates are often unclear. Means are usually reported plus-minus variance, $\mu \pm \sigma$, and σ is often erroneously interpreted as uncertainty about μ rather than an estimate of a second parameter, the variance of the normal population model. If the frequentist confidence interval for μ is reported, it is explicitly not understood as the interval in which we have e.g. 95% confidence that μ lies, but rather as the interval in which, in the case we repeat the experiment indefinitely, μ will be found in 95% of samples. Hypothesis tests are given similarly confusing interpretations involving long-run repeated experiments. These interpretations are widely, if not ubiquitously, misunderstood or ignored in favour of technically incorrect but comprehensible ones [?, ?].

14.2.3.2 The Bayesian approach to normal models of unknown mean and variance

Bayesian methods rectify these problems by understanding the normal model as a model of our information about the population and not of the population itself. This epistemological view of statistics is explicated in [??](#). Normal gaussian distributions are well-justified both by their ubiquitous success in parameter estimation and by information theoretic considerations [\[JBE03\]](#), and need not reflect the actual distribution of the population. However, we wish to express our uncertainty about the parameters of a normal gaussian distribution by giving further distributions over the mean m and variance of the normal distribution, with variance usually expressed as precision, $\lambda = 1/\sigma$. Typically, this is done by assuming normally distributed uncertainty on μ and gamma distributed uncertainty on λ , giving rise to a joint normal-gamma (NG) distribution [\[?\]](#):

An NG distribution may thus serve as a model of our prior information about the population being measured. Because my estimates are the first ones I have made about the relevant populations, and I have no specific guide as to the actual numbers of cells to expect, I have chosen to use the uninformative NG prior:

$$p(m, \lambda)$$

lies within that range, but is rather understood as the probability that, if the experiment were repeated indefinitely, 95

The appropriateness of the normal model is often in question because it is taken to represent some actually-existing population (which are often not well modelled by normal gaussians). Comparisons of these models using t-tests are given complex interpretations involving long-run rates of error

In Bayesian statistics, available information about a parameter is often modelled by a gaussian distribution over possible values of the parameter.

14.2.4 Normal and LogNormal models of population data

14.2.5 Empirical Bayes linear regression

14.2.6 Galilean Monte Carlo

14.2.7 Nested sampling

Nested sampling is an algorithmic process for estimating the quality of a model, given observations.

14.2.8 Expectation Maximization optimization of HMMs

In order to select appropriate background models for *D. rerio* genomic noise in [Chapter 5](#),

Chapter 15

Theoretical Appendix B: Metaphysical Arguments

15.1 Chance is not a valid explanation for biological phenomena; Randomness is a measure of property of sequences and not an explanation

Substantial portions of this document are dedicated to an examination of Harris' regular invocation of "stochastic" and related adjectives to describe the behaviour of RPCs. This is what lead me to characterise the theory primarily in those terms- the Stochastic Mitotic Mode Explanation. It may not be immediately obvious why this should be the case; most scientists assume that they know what words like "stochastic" and "random" mean well enough to use them in rigorous technical publications. We may not be aware that there has been a sprawling debate on the meaning of these terms since the earliest statistical formulations began to appear in the 19th century. However, even the simplest examples (as current today as they were in Laplace's time) reveal how difficult this topic can be.

If we consider the classic example of the coin flip, a process whose outcome we generally regard as being in some way "stochastic" or due to "chance", we immediately face the question of whether these descriptions refer to our inability to know the outcome of the process, or whether they refer to properties of the process itself. In other words, if we could specify the mechanics of the coin toss with sufficient precision, could we predict the outcome? This reflects two possible senses in which we may legitimately describe a process as "stochastic": referring to an epistemic dimension (we may describe some process as stochastic because we are unable to predict its outcome *a priori*), or referring to an ontological dimension (we describe the process as stochastic because this, in some way, describes how it *really is* independent of our knowledge of it).

Complicating matters is the sheer number of implications that we tend to associate with "stochasticity" and "randomness". We may be saying something about the causal structure of an event with deep metaphysical implications. It is common to distinguish between "deterministic" and "stochastic" processes, as though "stochastic" literally meant "indeterministic"- something like the Copenhagen interpretation of quantum physics. We may mean something about the apparent disorderliness of a series of outcomes of some process, with mathematical and information theoretical implications. What is an

apparently simple observation- cellular fate distribution in RPC lineages is “stochastic”, now seems to require at least a little clarification or interpretation. In general, we may say that stochasticity, for Harris, applies to at least the following entities:

1. The population-level phenomenal outcome of the RPC fate specification process (the phenomenon itself)
2. The overall behaviour of the macromolecular system whose operations produces these outcomes (the macromolecular mechanism itself)
3. The particular behaviour of some component of the macromolecular system, eg. stochastic expression of transcription factors (the macromolecules themselves)
4. The statistical generalisations used to characterise relevant aspects of the behaviour of the mechanism or the macromolecules

It is, moreover, hardly fair to expect Harris to be advancing a coherent theory about the ontological, objective basis of randomness or probability. Still, this leaves us in the awkward position of not knowing quite what the leading explanation for retinal formation is actually saying about its explanandum. The SMME is thus at risk of circularity- the explanandum (unpredictable variability in clonal outcomes) has as explanans a mechanistic explanation containing an abstract mathematical model tuned to produce this unpredictable variability. This may, in other words, turn out to be a convoluted case of *model overfitting*, if the “stochasticity” in question does not have a material biological referent. Before considering this, we need to define our terms more carefully to avoid the pervasive confusion mentioned above.

15.1.0.1 Chance versus Randomness

A commonplace belief is that randomness refers to outcomes produced by chance events. In an extensive and useful discussion, Antony Eagle reviews the evidence for this Commonplace Thesis, or (CT)[Eag18], drawing on discussions in the physics literature. Importantly, he notes that chance and randomness are not identical, and that one can conceivably exist without the other. This, in effect, disproves the (CT)- it is very difficult to imagine how the two concepts can be directly related in this productive fashion. I will attempt a brief summary of Eagle’s argument, with reference to Kolmogorov complexity:

Chance is mainly used to refer to processes. Exemplars are coin flips and die rolls. We can think of these as “single-case” probabilities that we take to inhere in the process. For instance, we may say that an evenly weighted coin has a .5 probability of returning a value of heads on a flip, even if it is only flipped once. That is, probabilities can be taken to be objective properties of individual instances of processes, and not only descriptions of the frequencies of the process’ outcomes over many repetitions. This is closely related to the logical concept of “possibility”. If something is possible, it has a chance of occurring. However, possibility is a logical binary; something is either possible or impossible. A “single-case” probability is understood as something like an objective feature of a system as a whole given its actual configuration and the relevant natural laws.

Randomness, by contrast, mainly refers to process *outcomes*. That is, randomness is a property of a series of outcomes of multiple instances of some process. It turns out to be challenging merely to define what a “random” binary sequence might be (perhaps generated by a series of coin flips). However, in general, we may say that a random sequence of outcomes is one that cannot be generated

by an description shorter than the sequence itself. That is, there is no set of rules that can generate a genuinely random sequence from a shorter sequence. In algorithmic information theory, the length of the ruleset required to produce some piece of information (like a sequence of measured outcomes) is called the Kolmogorov complexity of that object; if the Kolmogorov complexity of the object is equal to the object's length, the object definitionally has the property of algorithmic or Kolmogorov randomness¹.

Eagle produces numerous examples of the dissociability of these concepts, from which I have selected two concise illustrations:

Chance Without Randomness

...

A fair coin, tossed 1000 times, has a positive chance of landing heads more than 700 times. But any outcome sequence of 1000 tosses which contains more than 700 heads will be compressible (long runs of heads are common enough to be exploited by an efficient coding algorithm, and 1000 outcomes is long enough to swamp the constants involved in defining the universal prefix-free Kolmogorov complexity). So any such outcome sequence will not be random, even though it quite easily could come about by chance.

...

Randomness Without Chance

...

Open or dissipative systems, those which are not confined to a state space region of constant energy, are one much studied class [of the objects of deterministic classical physics- notably, biological systems are dissipative], because such systems are paradigms of chaotic systems ... the behaviour of a chaotic system will be intuitively random ... [t]he sensitive dependence on initial conditions means that, no matter how accurate our finite discrimination of the initial state of a given chaotic system is, there will exist states indiscriminable from the initial state (and so consistent with our knowledge of the initial state), but which would diverge arbitrarily far from the actual evolution of the system. No matter, then, how well we know the initial condition (as long as we do not have infinite powers of discrimination)², there is another state the system could be in for all we know that will evolve to a discriminably different future condition. Since this divergence happens relatively quickly, the system is unable to be predicted ... Just as before, the classical physical theory underlying the dynamics of these chaotic systems is one in which probability does not feature. [Eag18]

Therefore, the (CT) is untenable. Processes are “chancy”; collections of process outcomes, “trials”, or instantiations are “random”. It is tempting to say that Harris is explaining random fate outcomes with descriptions of chancy processes occurring internally to RPCs. Let us examine whether this is plausible.

15.1.0.2 Chance in molecular mechanisms

I turn first to consider what it might mean to describe the behaviour of a biological macromolecular system as “chancy”. Let us again distinguish between the ontological and epistemic dimensions of this description. There is a sense in which the operation of a macromolecular mechanism could be said to

¹The Kolmogorov complexity of a sequence can be estimated, contrary to common belief[LV08]. Minimum Message Length expresses a similar concept. More prosaically, one may simply compress a serialized representation of the sequence on one's hard drive with a reasonably good compression algorithm; the degree of compression achieved is a good indicator of the degree of non-random order available to shorten the sequence's description.

²Note that this condition defines chaotic randomness as an epistemic, rather than ontological, feature of complex systems- a being with infinite powers of discrimination *could* predict the evolution of a complex classical system with perfect accuracy.

be objectively chancy, and one in which the “chancy” outcome reflects our ignorance of some source of variability in the process.

Eagle proffers two common lines of argument in favour of chanciness as an objective property of processes. The first is the notion of the “single-case” probability mentioned above. The examples given are single coin flips, and the decay of single radioactive atoms, which are commonly taken to have chancy outcomes irrespective of anyone’s beliefs about them. As Eagle notes, this is closely related to frequentist ideas about stable processes, or trials:

It is the stable trial principle that has the closest connection with single-case chance, however. For in requiring that duplicate trials should receive the same chances, it is natural to take the chance to be grounded in the properties of that trial, plus the laws of nature. It is quite conceivable that the same laws could obtain even if that kind of trial has only one instance, and the very same chances should be assigned in that situation. But then there are well-defined chances even though that type of event occurs only once.

...

The upshot of this discussion is that chance is a *process* notion, rather than being entirely determined by features of the outcome to which the surface grammar of chance ascriptions assigns the chance. For if there can be a single-case chance of $\frac{1}{2}$ for a coin to land heads on a toss even if there is only one actual toss, and it lands tails, then surely the chance cannot be fixed by properties of the outcome ‘lands heads’, as that outcome does not exist. The chance must rather be grounded in features of the process that can produce the outcome: the coin-tossing trial, including the mass distribution of the coin and the details of how it is tossed, in this case, plus the background conditions and laws that govern the trial. Whether or not an event happens by chance is a feature of the process that produced it, not the event itself. The fact that a coin lands heads does not fix that the coin landed heads by chance, because if it was simply placed heads up, as opposed to tossed in a normal fashion, we have the same outcome not by chance. Sometimes features of the outcome event cannot be readily separated from the features of its causes that characterise the process by means of which it was produced.

[Eag18]

Examining the example of the coin toss, we find a fairly simple answer to the question posed earlier: if we knew enough about the mechanics of the toss, could we predict its outcome? The answer is yes, we can- the Bell Labs statistician Persi Diaconis has built a coin tossing machine that reliably produces heads or tails [Kes04]. We therefore know that tightly controlling the mechanics of a coin toss allows us to treat this system as entirely deterministic, without any significant element of chance in the outcome. A coin toss is only chancy when the human doing it does not have full control over the mechanical parameters of the process. Conceptually, there is no *a priori* reason why a coin-tosser should not be able to regularise the angular momentum of their thumb-flick by training with a strain gauge, place the coin on a stable surface allowing flicking, and achieve the same effect as the coin-tossing machine. In this case, Eagle’s suggestion that “[s]ometimes features of the outcome event cannot be readily separated from the features of its causes that characterise the process” seems obviously wrong- the “chancy” element of coin tossing is fully separable from the rest of the coin tossing process, and replaceable with a non-chancy component.

If the foregoing argument is correct, it seems that the coin toss is an example of the epistemic, rather than ontological, dimension of chance. The process appears to be chancy, or random, because the human tossing the coin is not able to precisely control the mechanical parameters of the process. Indeed, as

Diaconis notes, these epistemically-limited tossers do not actually produce unbiased random outcomes—human coin tosses come up as they were started slightly more often than with the obverse face [DHM07].

Eagle’s second example of an “objective single-case chance”, is the decay of a radioactive atom. This is a common method of making covert appeals to the second line of argument for objective chance, which is the existence of orthodox quantum theory. There is no known physical process whose parameters are thought to define the lifetime of individual radioactive atoms, in the way that there is a well-specified physical process that produces a particular coin toss outcome. Rather, this is an appeal to the Copenhagen theoretical principle that it is *a priori* impossible to predict the lifetimes of individual atoms. As appeals to quantum theory to ground “objective chance” in biological processes are becoming more common, let us consider whether a quantum theoretical explanation might plausibly underpin the “stochasticity” of RPC fate specification.

15.1.0.3 Can macromolecular chanciness be rooted in quantum indeterminacy?

Eagle suggests that, because the Copenhagen interpretation of quantum physics has wide currency among physicists, the theory’s implied indeterminacy of physical phenomena at the quantum level could ground “objective chance”. While common, this argument downplays the fact that quantum theory is not a homogenous scientific tradition. Unfortunately, a significant misrepresentation of the history of quantum theory has given rise to the impression that the Copenhagen theory is the unanimous or best articulation of quantum theory. We must briefly examine this misrepresentation before we can understand whether Eagle’s argument makes sense.

The conventional history of mid 20th-century quantum theory holds that John Stewart Bell, in the demonstration of his famous inequality, conclusively proved that deterministic (so-called “hidden variable”) theories of quantum mechanics were incorrect. As demonstrated (strangely, without any acknowledgement) in another section of the very pages Eagle’s argument appears in, this is a highly partisan and misleading view. Eminent Bohmian theorist Sheldon Goldstein conclusively demonstrates that Bell was an advocate of the deterministic Bohmian mechanical theory, and thought his famous inequality demonstrated that quantum phenomena could not be *local*, not that they could not be *deterministic*[Gol17].

Indeed, Bohmian quantum mechanics are fully deterministic, describe all of the same phenomena as Copenhagen, and in several cases resolve problems that orthodox quantum theory cannot [Gol17]. We are not, therefore, facing unanimous expert consensus that there is objective chance at the quantum level. We rather have a situation where physical phenomena are described by two different theory-sets, one of which takes its statistical generalisations to be descriptions of ontological indeterminacy (Copenhagen), and the other to reflect epistemic uncertainty about a determinate universe (Bohm). Moreover, there is no reason to prefer the Copenhagen approach, given that the Bohmian theory explains Copenhagen’s paradoxical results “without further ado”[Gol17]³, deals with empirically verified phenomena of physical and biological interest that Copenhagen does not (eg. electron tunneling), and was the preferred approach of the man who likely understood better than anyone his own results, J.S. Bell.

Therefore, Eagle’s argument is incorrect. There is no reason to suppose that the existence of quantum theoretical models that posit objective chance is good evidence for the *reality* of objective chance.

³Bizarrely, Copenhagen partisans claim that Bohmian mechanics is formally equivalent to the Copenhagen approach. If this is the case, chance is *clearly* a function of model choices and not of any underlying ontological reality. However, Bohmian mechanics is, in fact, substantially more complete than its Copenhagen equivalent, which, by Eagle’s (defective) logic, suggests reality is more likely to reflect the deterministic rather than the chancy approach.

Moreover, there are good reasons to suppose that the converse is true. In sum, then, we may say that there is no reason to consider Harris' argument to refer to *objective, ontological* chance, since the arguments for the existence of both single-case objective chance or quantum chance are weak and biologically irrelevant. Clearly, however, the *epistemic* dimension of chance is in play here.

15.1.0.4 Randomness in RPC fate specification

Having dealt with how the concept of chance might apply to Harris' SMME above, let us consider how the term "random" might relate to the process of RPC fate specification and differentiation. As introduced earlier, the technical meaning of "randomness" pertains to sequences of process outcomes. The process outcomes Harris is concerned with are the temporally-arranged fate outcomes of some particular RPC lineage. Therefore, we must ask whether these sequences meet any reasonable technical definition of "random".

Harris' own model proves that RPC fate outcomes are not algorithmically random. That is, the sequence of outcomes has a structure that can be meaningfully compressed by rules which produce typical RPC fate outcomes (Harris' mathematical models are such rule sets). One might object that Harris' meaning is that the particular rules which give rise to cellular fate "choice" in his models involve random number generation. In this case, the claim is trivially about the model and not about the sequence of outcomes that is actually observed in zebrafish eyes. Indeed, all of Harris' later models *axiomatically assume* a tripartite temporal structure to the differentiation process⁴. This is precisely the type of sequential bias which allows efficient compression of a non-random sequence of outcomes by an algorithm. Therefore, Harris himself concedes that RPC fate specification is not an algorithmically random process⁵.

We should further note that the question of how predictably ordered, i.e. non-random processes like fate specification arise in biological systems is a fundamental question of the biological sciences. It has long been recognised that classical and quantum physical systems which have algorithmically random initial conditions do not spontaneously evolve to a state of order. In many ways, then, it is the extent to which variable sequences of outcomes like RPC fate specification *depart* from algorithmic randomness which of interest when we are asking questions like "how does the ordered structure of a retina arise from RPC activity?"

15.1.0.5 Summary: "Stochastic" or "variable"?

Above, I argue that the RPC fate specification process is not objectively chancy (since objective chance is an empirically unsupported concept), nor random (since the sequence of RPC lineage outcomes is structured and therefore non-random). What, then, should we make of the argument that this process is "stochastic"? Let us consider the forceful argument of the great Bayesian statistician Edwin Thompson Jaynes:

"Belief in the existence of 'stochastic processes' in the real world; i.e. that the property of being 'stochastic' rather than 'deterministic' is a real physical property of a process, that exists independently of human information, is [an] example of the mind projection fallacy:

⁴That is, an early bias in RPC production is produced in these models by the a priori commitment to a "rule" which results in early RPC production.

⁵Having debunked the lay sense of "random" being equivalent to "chancy" above, there is no reason to consider these other, confused, non-technical definitions of randomness. They are neither logically defensible nor practically quantifiable, and therefore are of no scientific interest.

attributing one’s own ignorance to Nature instead. The current literature of probability theory is full of claims to the effect that a ‘Gaussian random process’ is fully determined by its first and second moments⁶. If it were made clear that this is only the defining property for an abstract mathematical model, there could be no objection to this; but it is always presented in verbiage that implies that one is describing an objectively true property of a real physical process. To one who believes such a thing literally, there could be no motivation to investigate the causes more deeply than noting the first and second moments, and so the real processes at work might never be discovered. This is not only irrational because one is throwing away the very information that is essential to understand the physical process; if carried into practice it can have disastrous consequences. Indeed, there is no such thing as a ‘stochastic process’ in the sense that the individual events have no specific causes.” [JBE03]

It is, thus, important to emphasize that the utility of stochastic modelling techniques should not be taken to suggest that on some level the modelled phenomenon is actually, i.e. irreducibly, random and without causal structure. When speaking of biological “randomness” or “stochasticity”, biologists rarely precisely define what is meant by these terms. This vagueness sometimes arises from, or results in, a theoretical deficit where properties of statistical models are understood to directly reflect the system being modelled; the scientist has failed to heed Korbzysky’s dictum insisting that “a map *is not* the territory it represents, but, if correct, it has a *similar structure* to the territory, which accounts for its usefulness” [Kor05] (italics in original). The “structural similarity” here is between the model’s outcomes and the collection of actually-observed population outcomes, *not* the underlying biological process giving rise to measured outcomes. I conclude by suggesting that this particular example applies to all such explanations in the biological sciences. There is presently no good reason to accept scientific explanations rooted in chance. As for explanations rooted in randomness, to be credible, these must actually estimate the degree of randomness present in the relevant outcomes, and explain departures from pure incompressibility.

15.2 Macromolecular mechanistic explanations in the Systems era

The data chapters of this thesis are concerned with two issues in scientific practice: the comparison of models with different structures, and their interpretation. I began my career in stem cell biology with a background in molecular pharmacology, and the particular explanatory worldview that training inscribed. For me, biological explanation was about elucidating mechanisms, which consisted of descriptions of macromolecules and their accessory small molecule messengers interacting. “Models,” in the sense of numerical simulations susceptible to formal statistical testing, were only the formal encoding of a body of knowledge that was first proved out at the bench, in interventional experiments. That these results could be encoded by a system of differential equations only confirmed the rigour of the original analysis which composed the mechanistic explanation in the first place. That engineers should bring their (allegedly) sophisticated numerical techniques into our laboratories, only to have their expert analyses confirm the plain-sense interpretations of the benchworkers producing the data, served to reinforce the sense that the pharmacological approach was fundamentally the correct one. It hardly ever entered our minds that model comparison was not occurring at all; it seemed that null hypotheses had already

⁶That is, the mean and the variance of the Normal Gaussian model are said to determine the process being modeled.

been slain by the flurry of t-tests and ANOVAe applied to the underlying datasets before the eggheads showed up.

That there were problems with this approach was already apparent by the beginning of my graduate education; Faculty of Medicine graduate pharmacology seminars in the early 2000s routinely included dark warnings about the collapse of antidepressant effect sizes over time, and the need for new statistical approaches. Reports that most biomedical research results are not replicable were met with a sort of palpable relief [Ioa05], if not much practical change; at least the growing welter of conflicting results could be explained this way, rather than risking interpretative collapse. Ultimately, the power of the macromolecular mechanism in pharmacology proved overstated; the productivity lull of the 2000-2010 era has been overcome not by rational mechanistic design of traditional small molecules, but by an influx of new classes of drugs, often with unknown or poorly characterised mechanisms of action [Mun19]. The arrival of new statistics has not helped matters much; serious Bayesian analyses tend to doubt whether medical pharmacological interventions are effective at all [Ste18]. Still, by the time I had left for greener pastures with the stem cell biologists at the new Department of Cell and Systems Biology, it was clear that the pharmacological view of biological explanation had won significant discursive battles.

A fascinating artefact of this discursive victory is present in the best available study on the use of mechanistic explanations in stem cell biology, Melinda Fagan's "Philosophy of stem cell biology: knowledge in flesh and blood". In concluding chapters outlining the future of stem cell biology, Fagan provides a diagrammatic summary of what she takes to be the field's consensus on its general direction into its future "Systems Biology" incarnation, which I reproduce here in Figure 15.1.

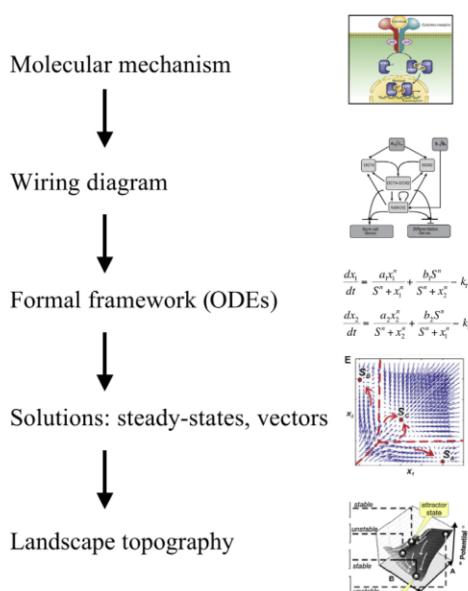


Figure 15.1: Cellular systems model-construction, excerpted from [Fag15, p.7].

The system of equations and subsequent steps are based on a 2-element wiring diagram.

The perspicacious reader will observe that this is nothing other than the pharmacological view I outline above: the practice of molecular biology is the elucidation of mechanisms, the practice of systems biology is the formalization of these mechanisms as systems of differential equations. What was most peculiar about the presence of this view is that it makes no account of the Simple Stochastic Model, which receives significant coverage elsewhere in Fagan's book. This was particularly important to me because I was trying to understand how to use Harris' models to explain my results. What Harris was doing was clearly intended to be both a mechanistic explanation, in the sense that it eventually nominated a pair of particular transcription factors as the causative agents, and also a Systems explanation, in the sense that it relied, for persuasive effect, on complex numerical modelling techniques. It was formulated in SSM terms, however, not as a SODE, and critically was about the metaphysical interpretation of a mathematical construct, not about the joint action of the named macromolecules, as Fagan suggests we should understand mechanistic explanations [Fag13].

Moreover, much of the sophisticated literature on mathematical models of stem cell variability are based on im-

interpretations of dynamical systems theory or chaos theory [FK12, HLZQ17] that deal with the macromolecular constituents of cells as bulk expression values; that is, a protein may be represented in one of these models as a coordinate on an dimension representing the amount of the protein expressed. It is extraordinarily rare for these models to be very concerned about crystal structures, phosphorylation states, or any of the other mechanistic details the pharmacological view is so concerned with. Other approaches rely on control systems theory [SK15, YSK15], many other gaily use technical information-theoretic terms like “noise” without any effort to define or measure it [CHB⁺08], still others treat the expression of macromolecules as the endpoints of tissue-mechanical forces [PLM⁺17], and so on. The actual variety of “systems” explanatory strategies found “in the field” is bewildering; to become proficient in even one is a years-long project. As I began to grasp the sheer number of technical and theoretical subdomains implicated in these explanations, the full force of Nicholas Reschers’ argument on this point became apparent:

The ramifications and implications of philosophical contentions do not respect a discipline’s taxonomic boundaries. And we all too easily risk losing sight of this interconnectedness when we pursue the technicalities of a narrow subdomain. In actuality, the stance we take on questions in one domain will generally have substantial implications and ramifications for very different issues in other, seeming unrelated domains. And this is exactly why systematization is so important in philosophy - because the way we *do* answer some questions will have limiting repercussions for the way we *can* answer others. We cannot emplace our philosophical convictions into conveniently delineated compartments in the comfortable expectation that what we maintain in one area of the field will have no unwelcome implications for what we are inclined to maintain in others. [Res05, p.97]

Indeed, the sudden injection of the philosophical contents of the “complexity sciences” into biological discourse felt like an invasion; suddenly, it was not very clear what a mechanism or a biological explanation might consist of after all. Michel Morange has argued persuasively that molecular biology is essentially mature [Mor03] and that “systems” explanations consist mainly in the complementation of ordinary molecular practice with sophisticated mathematical models [Mor08], while acknowledging the increasing space available for the entry of physical explanations in molecular biology [Mor11]. This account is appealing, but it belies the chaotic nature of the recent scientific scene, and cannot make sense of why there are so many contending, often quite incompatible, views on how to explain cellular and molecular biological phenomena and why so few of those views include any idea on *how those explanations might be tested against one another*.

15.2.1 The Feyerabendian modeller

Chapter 2 cites Paul Feyerabend in establishing that scientific theories exert their cognitive effects on us by making metaphysical claims about reality. This point informs the model-analysis in that chapter, highlighting the sense in which the entities to which the Stochastic Mitotic Mode Explanation (SMME) refer become progressively more abstract and restricted to the mitotic mode concept. Mitotic mode is not a physical existent, but rather an abstraction compounding the fate of multiple cells; it is in this sense plainly meta-physical. Feyerabend has a number of essential insights on scientific metaphysics, which, when judiciously understood, allow us to make sense of what is happening within stem cell biology and more broadly, in the “systems biology” era. The central, seminal insight of Paul Feyerabend’s *Against Method* is that the observed historical succession of scientific theories occurs

by counterpositional advancement of incompatible opposing theories, because only counterinductive comparisons *between* theories are capable of showing up their implicit assumptions and allowing them to be challenged. Feyerabend explains:

... it emerges that the evidence that might refute a theory can often be unearthed only with the help of an incompatible alternative: the advice (which goes back to Newton and which is still very popular today) to use alternatives only when refutations have already discredited the orthodox theory puts the cart before the horse. Also, some of the most important formal properties of a theory are found by contrast, and not by analysis. A scientist who wishes to maximize the empirical content of the views he holds and who wants to understand them as clearly as he possibly can must therefore introduce other views; that is, he must adopt a *pluralistic methodology*. He must compare ideas with other ideas rather than with 'experience' and he must try to improve rather than discard the views that have failed in the competition.[Fey93, p.20]

Feyerabend is interested in debunking the notion that science differs from other discursive social practices by showing that no universal method vouchsafes the progressive replacement of earlier, inferior theories with later, improved ones. He famously establishes that Galileo used a form of metaphysical propaganda, particularly in the ad hoc invocation of the now-discredited concept of circular inertia, to establish the credibility of the Copernican model against its orthodox Aristotlean competitor championed by the Catholic Church. Although we commonly think of the Copernican Revolution as the replacement of an obviously defective theological explanation by a properly formed theory from the empirical sciences, Feyerabend shows that this conceals the actual means by which Galileo makes his persuasive case for the (itself badly defective) Copernican model. Galileo's theory substantially contradicted the available evidence, erroneously asserted the reliability of some telescopic observations and discredited others⁷, made extensive use of ad hoc hypotheses, and was advanced by propagandistic and even dishonest means. Much of this was unavoidable. Ad hoc hypotheses are necessary for new theories because the auxiliary sciences associated with them have not been developed- scientific development is intrinsically uneven, obligating the use of these makeshift theoretical devices. Without a certain level of dishonesty and rhetorical sleight of hand on Galileo's part, the Copernican program would have succumbed to the greater development and argumentative weight of the scholastic tradition. As Feyerabend notes, if any of the typically suggested criteria for a universal scientific method were applied, the Church would have won the debate and we might still have an Aristotlean cosmology. Similar points can be made about aspects of Einstein or Bohr's scientific programmes, both scientists viewing themselves as outsiders attacking an established orthodoxy.

Because of his concern to attack what he sees as the overweening social influence of scientific and technical experts, Feyerabend puts a great deal of emphasis on the discursive process of establishing orthodoxy within a field, and the sense in which this is common to scientific and nonscientific domains. That is, the practice of the natural sciences are susceptible to the same irreducibly subjective and historically contingent social, political, economic, etc. forces as all other domains of human culture, and in this sense there is nothing special about scientific practice that shields its conclusions from error introduced by these means. These extra-scientific forces have significant effects on the forms scientific models take and the interpretations they are given, and it helps to remember this when reading modelling papers mainly published to keep the lights on. Still, I suggest that it is important not to interpret these

⁷Galileo asserted that comets are optical illusions, for instance, since their non-circular orbits disconfirm the Copernican system, which insists on the circularity of orbital motion.

arguments too pessimistically. Feyerabend genuinely wanted to promote what he called "Open Exchange" between different scientific and metaphysical traditions, centered around a noncoercive exchange of, in effect, models and standards for judging them. He was also wrong that there is nothing particular to the practice of natural sciences that is not present in other domains of human activity; statistical descriptions of collections of outcomes and of uncertainty, and a self-reinforcing dynamic of scaffolding methodological complexity, are unique to the modern natural sciences.

Ultimately, the value in Feyerabend's perspective is seeing that scientists routinely operate as epistemological anarchists, finding what works in their local institutional surroundings, and that we must expect that this will be the case. In other words, Fagan's (extremely carefully argued) conceptualization of the orthodox molecular mechanism, as found in stem cell biology, is useless for interpreting Harris' theories. This is so because Harris does not care about adhering to this standard of orthodoxy. The field has, in typical anarchic fashion, begun adapting models and numerical techniques from a panoply of engineering, physics, statistical mechanics, AI, etc. subdisciplines, more or less willy nilly, and often without any sort of analysis of the adequacy of the model relative to alternatives. Moreover, these models pertain to a variety of levels of biological organisation, from the tissue down to subcellular nuclear compartments, and frequently do not refer in any concrete way to biological macromolecules.

I argue that, from this point of view, the macromolecular mechanistic explanation Fagan reifies is, in fact, already dead. The era of painstaking, effector-by-effector construction of macromolecular pathways, to be virtually enshrined in their ultimate SODE incarnation, perhaps ultimately to be assembled into some Monodian model cell-as-cybernetic-factory, is over, if it ever existed. This is not to say that Morange is wrong- as he asserts, molecular biology is not dead, it has not been replaced by "systems biology". In practice, however, the traditional form of molecular biological explanation is rapidly being replaced by explanatory forms from other fields, and it is not always clear that this has been salutary. For Feyerabend, science, like other human social practices consists of a variety of interacting traditions with differing assumptions, methods, sensory interpretations, and so on. The development of science is thus "not the interaction of a practice with something different and external, *but the development of one tradition under the impact of others.*" [Fey93, p.232]. The concept of "systems biology" is fundamentally an artefact of the impact of advanced statistics and statistical mechanics on biology. The resolution to the question of how this impact is mediated will determine the institutional and intellectual landscape that results from it.

To have any agency in this process, molecular biologists must be able to assess the theoretical contents that are being imported into our field for ourselves. If we do not, we cannot assess the impact of the metaphysical commitments of these theories on the rest of our theorising, as Rescher persuasively insists that we must. Indeed, it was Feyerabend's perspective that led me to realise that there were at least three scientific orthodoxies hindering me from understanding the metaphysical contents of Harris' theories. These were the mechanism-to-SODE pipeline instilled by my pharmacological training and recapitulated by Fagan, the mendacious insistence on the actual reality of chanciness by Copenhagen theorists, and the broken and illogical statistical approaches commanded by frequentist statisticians. By realising that any conceptual element could appear in a mechanistic explanation, by rejecting the objective reality of chance, and embracing the original formulation of statistical orthodoxy suggested by LaPlace (that is, Bayesian statistics), I was able to counter-inductively show up the fundamental problems with Harris' theory.

Moreover, by the conscious adoption of Bayesian methods from cosmology to address local problems

in stem cell biology, I attempt to model Feyerabend's Open Exchange between two scientific traditions. My selection of Skilling's nested sampling system of inference is driven by my appreciation for its logical consistency, simplicity, deep roots in fundamental logic and information theory, and broad applicability to a wide range of problems. While Feyerabend is no doubt correct that, ultimately, the selection of criteria to distinguish between scientific theories must itself be subjective, the deep comportment of Skilling's system with the fundamental human drive toward cognitive harmony [Res05] compels me to suggest that it may serve as a near-universal yardstick of the adequacy of scientific models with respect to their underlying datasets in the not-too-distant future.

Chapter 16

Code Appendix

16.1 Code and Output Archive

16.2 SMME

16.2.1 setup_project.py

```
1 """Copyright (c) 2005-2017, University of Oxford.
2 All rights reserved.
3
4 University of Oxford means the Chancellor, Masters and Scholars of the
5 University of Oxford, having an administrative office at Wellington
6 Square, Oxford OX1 2JD, UK.
7
8 This file is part of Chaste.
9
10 Redistribution and use in source and binary forms, with or without
11 modification, are permitted provided that the following conditions are
12 met:
13 * Redistributions of source code must retain the above copyright notice,
14   this list of conditions and the following disclaimer.
15 * Redistributions in binary form must reproduce the above copyright
16   notice,
17   this list of conditions and the following disclaimer in the
18   documentation
19   and/or other materials provided with the distribution.
20 * Neither the name of the University of Oxford nor the names of its
21   contributors may be used to endorse or promote products derived from
22   this
23   software without specific prior written permission.
```

```
20
21 THIS SOFTWARE IS PROVIDED BY THE COPYRIGHT HOLDERS AND CONTRIBUTORS "AS
22 → IS"
23 AND ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, THE
24 IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR
25 → PURPOSE
26 ARE DISCLAIMED. IN NO EVENT SHALL THE COPYRIGHT HOLDER OR CONTRIBUTORS BE
27 LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, OR
28 CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO, PROCUREMENT OF
29 → SUBSTITUTE
30 GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR BUSINESS
31 → INTERRUPTION)
32 HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER IN CONTRACT,
33 → STRICT
34 LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE) ARISING IN ANY WAY
35 → OUT
36 OF THE USE OF THIS SOFTWARE, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH
37 → DAMAGE.
38 """
39
40 import os
41
42
43 def find_and_replace(filename, old_string, new_string):
44     # Read the file
45     f = open(filename, 'r')
46     file_contents = f.read()
47     f.close()
48
49     # Write to the file
50     f = open(filename, 'w')
51     f.write(file_contents.replace(old_string, new_string))
52     f.close()
53
54     return
55
56
57 def ask_for_response(question):
58     # Display the question
59     print(question)
60
61     # Define permitted yes/no answers
62     yes = {'yes', 'y', 'ye', ''}
```

```
56     no = {'no', 'n'}
```

```
57
58     # Take the lower case raw input
59     choice = raw_input().lower()
```

```
60
61     # Decide on the choice
62     if choice in yes:
63         return True
64     elif choice in no:
65         return False
66     else:
67         ask_for_response("Please respond with yes or no:")
```

```
68
69
70 def main():
71     # The absolute path to the project directory
72     path_to_project = os.path.dirname(os.path.realpath(__file__))
```

```
73
74     # Identify the name of the project
75     project_name = os.path.basename(path_to_project)
```

```
76
77     # Paths to the CMakeLists.txt files
78     base_cmakelists = os.path.join(path_to_project, 'CMakeLists.txt')
79     apps_cmakelists = os.path.join(path_to_project, 'apps',
80                                    'CMakeLists.txt')
80     test_cmakelists = os.path.join(path_to_project, 'test',
81                                    'CMakeLists.txt')
```

```
81
82     # Perform the find-and-replace tasks to update the template project
83     find_and_replace(base_cmakelists,
84                       'chaste_do_project(template_project', 'chaste_do_project(' +
85                       project_name)
84     find_and_replace(apps_cmakelists,
85                       'chaste_do_apps_project(template_project',
86                       'chaste_do_apps_project(' + project_name)
85     find_and_replace(test_cmakelists,
86                       'chaste_do_test_project(template_project',
87                       'chaste_do_test_project(' + project_name)
```

```
87
88     # Amend the components
89     components_list = []
```

```

90     if ask_for_response("Does this project depend on the cell_based
91         ↵ component? [Y/n] "):
92             components_list.append('cell_based')
93
94     if ask_for_response("Does this project depend on the crypt component?
95         ↵ [Y/n] "):
96             components_list.append('crypt')
97
98     if ask_for_response("Does this project depend on the heart component?
99         ↵ [Y/n] "):
100            components_list.append('heart')
101
102     # If the list is non-empty, replace the default components
103     if components_list:
104         components_string = ' '.join(components_list)
105         default_components = 'continuum_mechanics global io linalg mesh
106             ↵ ode pde'
107
108
109     if __name__ == "__main__":
110         main()

```

16.2.2 /apps/src/BoijeSimulator.cpp

```

1 #include <iostream>
2 #include <string>
3
4 #include <cxxtest/TestSuite.h>
5 #include "ExecutableSupport.hpp"
6 #include "Exception.hpp"
7 #include "PetscTools.hpp"
8 #include "PetscException.hpp"
9
10 #include "BoijeCellCycleModel.hpp"
11 #include "OffLatticeSimulationPropertyStop.hpp"

```

```
12
13 #include "AbstractCellBasedTestSuite.hpp"
14
15 #include "WildTypeCellMutationState.hpp"
16 #include "TransitCellProliferativeType.hpp"
17 #include "DifferentiatedCellProliferativeType.hpp"
18 #include "BoijeRetinalNeuralFates.hpp"
19
20 #include "CellsGenerator.hpp"
21 #include "HoneycombMeshGenerator.hpp"
22 #include "NodesOnlyMesh.hpp"
23 #include "NodeBasedCellPopulation.hpp"
24 #include "VertexBasedCellPopulation.hpp"
25
26 #include "ColumnDataWriter.hpp"
27
28 int main(int argc, char *argv[])
29 {
30     ExecutableSupport::StartupWithoutShowingCopyright(&argc, &argv);
31     //main() returns code indicating sim run success or failure mode
32     int exit_code = ExecutableSupport::EXIT_OK;
33
34     if (argc != 13)
35     {
36         ExecutableSupport::PrintError(
37             "Wrong arguments for simulator.\nUsage (replace<▷ with
38             → values, pass bools as 0 or 1):\n BoijeSimulator
39             → <directoryString> <filenameString>
40             → <outputModeUnsigned(0=counts,1=events,2=sequence)>
41             → <debugOutputBool> <startSeedUnsigned>
42             → <endSeedUnsigned> <endGenerationUnsigned>
43             → <phase2GenerationUnsigned> <phase3GenerationUnsigned>
44             → <pAtoh7Double(0-1)> <pPtf1aDouble(0-1)>
45             → <pngDouble(0-1)>",
46             true);
47         exit_code = ExecutableSupport::EXIT_BAD_ARGUMENTS;
48         return exit_code;
49     }
50
51     /*****
52     * SIMULATOR PARAMETERS
53     *****/
54
55     std::string directoryString, filenameString;
```

```

47     int outputMode; //0 = counts; 1 = mitotic mode events; 2 = mitotic
48         ↵ mode sequence sampling
49     bool debugOutput;
50     unsigned startSeed, endSeed, endGeneration, phase2Generation,
51         ↵ phase3Generation;
52     double pAtoh7, pPtf1a, png; //stochastic model parameters

53
54     //PARSE ARGUMENTS
55     directoryString = argv[1];
56     filenameString = argv[2];
57     outputMode = std::stoi(argv[3]);
58     debugOutput = std::stoul(argv[4]);
59     startSeed = std::stoul(argv[5]);
60     endSeed = std::stoul(argv[6]);
61     endGeneration = std::stoul(argv[7]);
62     phase2Generation = std::stoul(argv[8]);
63     phase3Generation = std::stoul(argv[9]);
64     pAtoh7 = std::stod(argv[10]);
65     pPtf1a = std::stod(argv[11]);
66     png = std::stod(argv[12]);

67     /*****
68     * PARAMETER/ARGUMENT SANITY CHECK
69     *****/
70
71     bool sane = 1;

72     if (outputMode != 0 && outputMode != 1 && outputMode != 2)
73     {
74         ExecutableSupport::PrintError(
75             "Bad outputMode (argument 3). Must be 0 (counts) 1
76                 ↵ (mitotic events) or 2 (sequence sampling)");
77         sane = 0;
78     }

79     if (endSeed < startSeed)
80     {
81         ExecutableSupport::PrintError("Bad start & end seeds (arguments,
82             ↵ 5, 6). endSeed must not be < startSeed");
83         sane = 0;
84     }

85     if (endGeneration <= 0)
86     {

```

```
86     ExecutableSupport::PrintError("Bad endGeneration (argument 7).  
87         ↪   endGeneration must be > 0");  
88     sane = 0;  
89 }  
90  
91 if (phase3Generation < phase2Generation)  
92 {  
93     ExecutableSupport::PrintError(  
94         "Bad phase2Generation or phase3Generation (arguments 8,  
95             ↪   9). phase3Generation must be > phase2Generation. Both  
96             ↪   must be >0");  
97     sane = 0;  
98 }  
99  
100 if (pAtoh7 < 0 || pAtoh7 > 1)  
101 {  
102     ExecutableSupport::PrintError("Bad pAtoh7 (argument 10). Must be  
103         ↪   0-1");  
104     sane = 0;  
105 }  
106  
107 if (pPtf1a < 0 || pPtf1a > 1)  
108 {  
109     ExecutableSupport::PrintError("Bad pPtf1a (argument 11). Must be  
110         ↪   0-1");  
111     sane = 0;  
112 }  
113  
114 if (png < 0 || png > 1)  
115 {  
116     ExecutableSupport::PrintError("Bad png (argument 12). Must be  
117         ↪   0-1");  
118     sane = 0;  
119 }  
120  
121 if (sane == 0)  
122 {  
123     ExecutableSupport::PrintError("Exiting with bad arguments. See  
124         ↪   errors for details");  
125     exit_code = ExecutableSupport::EXIT_BAD_ARGUMENTS;  
126     return exit_code;  
127 }  
128  
129  
130  
131
```

```

122 //*****
123 * SIMULATOR OUTPUT SETUP
124 *****/
125
126 //Set up singleton LogFile
127 LogFile* p_log = LogFile::Instance();
128 p_log->Set(0, directoryString, filenameString);
129
130 ExecutableSupport::Print("Simulator writing file " + filenameString +
131   " to directory " + directoryString);
132
133 //Log entry counter
134 unsigned entry_number = 1;
135
136 //Write appropriate headers to log
137 if (outputMode == 0) *p_log << "Entry\tSeed\tCount\n";
138 if (outputMode == 1) *p_log << "Time (hpf)\tSeed\tCellID\tMitotic
139   Mode (0=PP;1=PD;2=DD)\n";
140 if (outputMode == 2) *p_log << "Entry\tSeed\tSequence\n";
141
142 //Instance RNG
143 RandomNumberGenerator* p_RNG = RandomNumberGenerator::Instance();
144
145 //Initialise pointers to relevant singleton ProliferativeTypes and
146   Properties
147 MAKE_PTR(WildTypeCellMutationState, p_state);
148 MAKE_PTR(TransitCellProliferativeType, p_Mitotic);
149 MAKE_PTR(DifferentiatedCellProliferativeType, p_PostMitotic);
150 MAKE_PTR(RetinalGanglion, p_RGC_fate);
151 MAKE_PTR(AmacrineHorizontal, p_AC_HC_fate);
152 MAKE_PTR(ReceptorBipolar, p_PR_BC_fate);
153 MAKE_PTR(CellLabel, p_label);
154
155 //*****
156 * SIMULATOR SETUP & RUN
157 *****/
158
159 //iterate through supplied seed range, executing one simulation per seed
160 for (unsigned seed = startSeed; seed <= endSeed; seed++)
161 {
162     if (outputMode == 2) *p_log << entry_number << "\t" << seed <<
163       "\t"; //write seed to log - sequence written by
164       cellcyclemodel objects

```

```

160
161     //initialise pointer to debugWriter
162     ColumnDataWriter* debugWriter;
163
164     //initialise SimulationTime (permits cellcyclemodel setup)
165     SimulationTime::Instance()→SetStartTime(0.0);
166
167     //Reseed the RNG with the required seed
168     p_RNG→Reseed(seed);
169
170     //Initialise a HeCellCycleModel and set it up with appropriate
171     // TiL values
172     BoijeCellCycleModel* p_cycle_model = new BoijeCellCycleModel;
173
174     if (debugOutput)
175     {
176         //Pass ColumnDataWriter to cell cycle model for debug output
177         boost::shared_ptr<ColumnDataWriter> p_debugWriter(
178             new ColumnDataWriter(directoryString, filenameString
179             → + "DEBUG_" + std::to_string(seed), false, 10));
180         p_cycle_model→EnableModelDebugOutput(p_debugWriter);
181         debugWriter = &p_debugWriter;
182     }
183
184     //Setup lineages' cycle model with appropriate parameters
185     p_cycle_model→SetDimension(2);
186     p_cycle_model→SetPostMitoticType(p_PostMitotic);
187
188     //Setup vector containing lineage founder with the properly set
189     // up cell cycle model
190     std::vector<CellPtr> cells;
191     CellPtr p_cell(new Cell(p_state, p_cycle_model));
192     p_cell→SetCellProliferativeType(p_Mitotic);
193     p_cycle_model→SetModelParameters(phase2Generation,
194     → phase3Generation, pAtoh7, pPtf1a, png);
195     p_cycle_model→SetSpecifiedTypes(p_RGC_fate, p_AC_HC_fate,
196     → p_PR_BC_fate);
197     if (outputMode == 2)
198         → p_cycle_model→EnableSequenceSampler(p_label);
199     if (outputMode == 2) p_cell→AddCellProperty(p_label);
200     p_cell→InitialiseCellCycleModel();
201     cells.push_back(p_cell);
202
203

```

```

197     //Generate 1x1 mesh for single-cell colony
198     HoneycombMeshGenerator generator(1, 1);
199     MutableMesh<2, 2>* p_generating_mesh = generator.GetMesh();
200     NodesOnlyMesh<2> mesh;
201     mesh.ConstructNodesWithoutMesh(*p_generating_mesh, 1.5);

202
203     //Setup cell population
204     NodeBasedCellPopulation<2>* cell_population(new
205         ↪ NodeBasedCellPopulation<2>(mesh, cells));

206     //Setup simulator & run simulation
207     boost::shared_ptr<OffLatticeSimulationPropertyStop<2>>
208         ↪ p_simulator(
209             new
210                 ↪ OffLatticeSimulationPropertyStop<2>(*cell_population));
211     p_simulator->SetStopProperty(p_Mitotic); //simulation to stop if
212         ↪ no mitotic cells are left
213     p_simulator->SetDt(0.25);
214     p_simulator->SetEndTime(endGeneration);
215     p_simulator->SetOutputDirectory("UnusedSimOutput" +
216         ↪ filenameString); //unused output
217     p_simulator->Solve();

218     //Count lineage size
219     unsigned count = cell_population->GetNumRealCells();

220
221     if (outputMode == 0) *p_log << entry_number << "\t" << seed <<
222         ↪ "\t" << count << "\n";
223     if (outputMode == 2) *p_log << "\n";

224     //Reset for next simulation
225     SimulationTime::Destroy();
226     delete cell_population;
227     entry_number++;

228     if (debugOutput)
229     {
230         ↪ debugWriter->Close();
231     }

232 }

233 p_RNG->Destroy();

```

```

234     LogFile::Close();
235
236     return exit_code;
237 }
238 ;

```

16.2.3 /apps/src/GomesSimulator.cpp

```

1 #include <iostream>
2 #include <string>
3
4 #include <cxxtest/TestSuite.h>
5 #include "ExecutableSupport.hpp"
6 #include "Exception.hpp"
7 #include "PetscTools.hpp"
8 #include "PetscException.hpp"
9
10 #include "GomesCellCycleModel.hpp"
11 #include "OffLatticeSimulationPropertyStop.hpp"
12
13 #include "AbstractCellBasedTestSuite.hpp"
14
15 #include "WildTypeCellMutationState.hpp"
16 #include "TransitCellProliferativeType.hpp"
17 #include "DifferentiatedCellProliferativeType.hpp"
18 #include "GomesRetinalNeuralFates.hpp"
19
20 #include "CellsGenerator.hpp"
21 #include "HoneycombMeshGenerator.hpp"
22 #include "NodesOnlyMesh.hpp"
23 #include "NodeBasedCellPopulation.hpp"
24 #include "VertexBasedCellPopulation.hpp"
25
26 #include "ColumnDataWriter.hpp"
27
28 int main(int argc, char *argv[])
29 {
30     ExecutableSupport::StartupWithoutShowingCopyright(&argc, &argv);
31     //main() returns code indicating sim run success or failure mode
32     int exit_code = ExecutableSupport::EXIT_OK;
33
34     if (argc != 15)

```

```

35  {
36      ExecutableSupport::PrintError(
37          "Wrong arguments for simulator.\nUsage (replace <> with
38          → values, pass bools as 0 or 1):\n GomesSimulator
39          → <directoryString> <filenameString>
40          → <outputModeUnsigned(0=counts,1=events,2=sequence)>
41          → <debugOutputBool> <startSeedUnsigned>
42          → <endSeedUnsigned> <endTimeDoubleHours>
43          → <cellCycleNormalMeanDouble>
44          → <cellCycleNormalStdDouble> <pPPDouble(0-1)>
45          → <pPDDouble(0-1)> <pBCDouble(0-1)> <pACDouble(0-1)>
46          → <pMGDouble(0-1)>",
47          true);
48      exit_code = ExecutableSupport::EXIT_BAD_ARGUMENTS;
49      return exit_code;
50  }

51  //*****
52  * SIMULATOR PARAMETERS
53  *****/
54  std::string directoryString, filenameString;
55  int outputMode; //0 = counts; 1 = mitotic mode events; 2 = mitotic
56  → mode sequence sampling
57  bool debugOutput;
58  unsigned startSeed, endSeed;
59  double endTime;
60  double normalMu, normalSigma, pPP, pPD, pBC, pAC, pMG; //stochastic
61  → model parameters

62  //PARSE ARGUMENTS
63  directoryString = argv[1];
64  filenameString = argv[2];
65  outputMode = std::stoi(argv[3]);
66  debugOutput = std::stoul(argv[4]);
67  startSeed = std::stoul(argv[5]);
68  endSeed = std::stoul(argv[6]);
69  endTime = std::stod(argv[7]);
70  normalMu = std::stod(argv[8]);
71  normalSigma = std::stod(argv[9]);
72  pPP = std::stod(argv[10]);
73  pPD = std::stod(argv[11]);
74  pBC = std::stod(argv[12]);
75  pAC = std::stod(argv[13]);

```

```

67     pMG = std::stod(argv[14]);
68
69     /*****
70      * PARAMETER/ARGUMENT SANITY CHECK
71      *****/
72     bool sane = 1;
73
74     if (outputMode != 0 && outputMode != 1 && outputMode != 2)
75     {
76         ExecutableSupport::PrintError(
77             "Bad outputMode (argument 3). Must be 0 (counts) 1
78             ↳ (mitotic events) or 2 (sequence sampling)");
79         sane = 0;
80     }
81
82     if (endSeed < startSeed)
83     {
84         ExecutableSupport::PrintError("Bad start & end seeds (arguments,
85             ↳ 5, 6). endSeed must not be < startSeed");
86         sane = 0;
87     }
88
89     if (endTime <= 0)
90     {
91         ExecutableSupport::PrintError("Bad endTime (argument 7). endTime
92             ↳ must be > 0");
93         sane = 0;
94     }
95
96     if (normalMu <= 0 || normalSigma <= 0)
97     {
98         ExecutableSupport::PrintError("Bad cell cycle normal mean or std
99             ↳ (arguments 8, 9). Must be >0");
100        sane = 0;
101    }
102
103    if (pPP + pPD > 1 || pPP > 1 || pPP < 0 || pPD > 1 || pPD < 0)
104    {
105        ExecutableSupport::PrintError(
106            "Bad mitotic mode probabilities (arguments 10, 11). pPP +
107            ↳ pPD should be ≥0, ≤1, sum should not exceed 1");
108        sane = 0;
109    }

```

```

105
106     if (pBC + pAC + pMG > 1 || pBC > 1 || pBC < 0 || pAC > 1 || pAC < 0
107     ↪   || pMG > 1 || pMG < 0)
108 {
109     ExecutableSupport::PrintError(
110         "Bad specification probabilities (arguments 12, 13, 14).
111         ↪   pBC, pAC, pMG should be  $\geq 0$ ,  $\leq 1$ , sum should not
112         ↪   exceed 1");
113     sane = 0;
114 }
115
116 if (sane == 0)
117 {
118     ExecutableSupport::PrintError("Exiting with bad arguments. See
119         ↪   errors for details");
120     exit_code = ExecutableSupport::EXIT_BAD_ARGUMENTS;
121     return exit_code;
122 }
123
124 //Set up singleton LogFile
125    LogFile* p_log = LogFile::Instance();
126     p_log→Set(0, directoryString, filenameString);
127
128 ExecutableSupport::Print("Simulator writing file " + filenameString +
129     ↪   " to directory " + directoryString);
130
131 //Log entry counter
132     unsigned entry_number = 1;
133
134 //Write appropriate headers to log
135     if (outputMode == 0) *p_log << "Entry\tSeed\tCount\n";
136     if (outputMode == 1) *p_log << "Time (hpf)\tSeed\tCellID\tMitotic
137         ↪   Mode (0=PP;1=PD;2=DD)\n";
138     if (outputMode == 2) *p_log << "Entry\tSeed\tSequence\n";
139
140 //Instance RNG
141     RandomNumberGenerator* p_RNG = RandomNumberGenerator::Instance();

```

```

141 //Initialise pointers to relevant singleton ProliferativeTypes and
142   ↳ Properties
143     MAKE_PTR(WildTypeCellMutationState, p_state);
144     MAKE_PTR(TransitCellProliferativeType, p_Mitotic);
145     MAKE_PTR(DifferentiatedCellProliferativeType, p_PostMitotic);
146     MAKE_PTR(RodPhotoreceptor, p_RPh_fate);
147     MAKE_PTR(AmacrineCell, p_AC_fate);
148     MAKE_PTR(BipolarCell, p_BC_fate);
149     MAKE_PTR(MullerGlia, p_MG_fate);
150     MAKE_PTR(CellLabel, p_label);

151 /**
152 * SIMULATOR SETUP & RUN
153 */
154
155 //iterate through supplied seed range, executing one simulation per seed
156 for (unsigned seed = startSeed; seed ≤ endSeed; seed++)
157 {
158     if (outputMode == 2) *p_log << entry_number << "\t" << seed <<
159       ↳ "\t"; //write seed to log - sequence written by
160       ↳ cellcyclemodel objects
161
162     //initialise pointer to debugWriter
163     ColumnDataWriter* debugWriter;
164
165     //initialise SimulationTime (permits cellcyclemodel setup)
166     SimulationTime::Instance()→SetStartTime(0.0);
167
168     //Reseed the RNG with the required seed
169     p_RNG→Reseed(seed);
170
171     //Initialise a HeCellCycleModel and set it up with appropriate
172       ↳ TiL values
173     GomesCellCycleModel* p_cycle_model = new GomesCellCycleModel;
174
175     if (debugOutput)
176     {
177         //Pass ColumnDataWriter to cell cycle model for debug output
178         boost::shared_ptr<ColumnDataWriter> p_debugWriter(
179             new ColumnDataWriter(directoryString, filenameString
180               ↳ + "DEBUG_" + std::to_string(seed), false, 10));
181         p_cycle_model→EnableModelDebugOutput(p_debugWriter);
182         debugWriter = &p_debugWriter;

```

```

179     }
180
181     //Setup lineages' cycle model with appropriate parameters
182     p_cycle_model→SetDimension(2);
183     p_cycle_model→SetPostMitoticType(p_PostMitotic);
184
185     //Setup vector containing lineage founder with the properly set
186     // up cell cycle model
187     std::vector<CellPtr> cells;
188     CellPtr p_cell(new Cell(p_state, p_cycle_model));
189     p_cell→SetCellProliferativeType(p_Mitotic);
190     p_cycle_model→SetModelParameters(normalMu, normalSigma, pPP,
191     // pPD, pBC, pAC, pMG);
192     p_cycle_model→SetModelProperties(p_RPh_fate, p_AC_fate,
193     // p_BC_fate, p_MG_fate);
194     if (outputMode == 2)
195         p_cycle_model→EnableSequenceSampler(p_label);
196     if (outputMode == 2) p_cell→AddCellProperty(p_label);
197     p_cell→InitialiseCellCycleModel();
198     cells.push_back(p_cell);
199
200     //Generate 1x1 mesh for single-cell colony
201     HoneycombMeshGenerator generator(1, 1);
202     MutableMesh<2, 2>* p_generating_mesh = generator.GetMesh();
203     NodesOnlyMesh<2> mesh;
204     mesh.ConstructNodesWithoutMesh(*p_generating_mesh, 1.5);
205
206     //Setup cell population
207     NodeBasedCellPopulation<2>* cell_population(new
208         NodeBasedCellPopulation<2>(mesh, cells));
209
210     //Setup simulator & run simulation
211     boost::shared_ptr<OffLatticeSimulationPropertyStop<2>>
212         p_simulator(
213             new
214                 OffLatticeSimulationPropertyStop<2>(*cell_population));
215     p_simulator→SetStopProperty(p_Mitotic); //simulation to stop if
216     // no mitotic cells are left
217     p_simulator→SetDt(0.25);
218     p_simulator→SetEndTime(endTime);
219     p_simulator→SetOutputDirectory("UnusedSimOutput" +
220         filenameString); //unused output
221     p_simulator→Solve();

```

```

213
214     //Count lineage size
215     unsigned count = cell_population→GetNumRealCells();
216
217     if (outputMode == 0) *p_log << entry_number << "\t" << seed <<
218         "\t" << count << "\n";
219     if (outputMode == 2) *p_log << "\n";
220
221     //Reset for next simulation
222     SimulationTime::Destroy();
223     delete cell_population;
224     entry_number++;
225
226     if (debugOutput)
227     {
228         debugWriter→Close();
229     }
230
231
232     p_RNG→Destroy();
233     LogFile::Close();
234
235     return exit_code;
236 }
237 ;

```

16.2.4 /apps/src/HeSimulator.cpp

```

1 #include <iostream>
2 #include <string>
3
4 #include <cxxtest/TestSuite.h>
5 #include "ExecutableSupport.hpp"
6 #include "Exception.hpp"
7 #include "PetscTools.hpp"
8 #include "PetscException.hpp"
9
10 #include "HeCellCycleModel.hpp"
11 #include "OffLatticeSimulationPropertyStop.hpp"
12
13 #include "AbstractCellBasedTestSuite.hpp"

```

```
14
15 #include "WildTypeCellMutationState.hpp"
16 #include "TransitCellProliferativeType.hpp"
17 #include "DifferentiatedCellProliferativeType.hpp"
18
19 #include "CellsGenerator.hpp"
20 #include "HoneycombMeshGenerator.hpp"
21 #include "NodesOnlyMesh.hpp"
22 #include "NodeBasedCellPopulation.hpp"
23 #include "VertexBasedCellPopulation.hpp"
24
25 #include "ColumnDataWriter.hpp"
26
27 int main(int argc, char *argv[])
28 {
29     ExecutableSupport::StartupWithoutShowingCopyright(&argc, &argv);
30     //main() returns code indicating sim run success or failure mode
31     int exit_code = ExecutableSupport::EXIT_OK;
32
33     if (argc != 22 && argc != 20)
34     {
35         ExecutableSupport::PrintError(
```

```

36         "Wrong arguments for simulator.\nUsage (replace <> with
37         ← values, pass bools as 0 or 1):\nStochastic
38         ← Mode:\nHeSimulator <directoryString> <filenameString>
39         ← <outputModeUnsigned(0=counts,1=events,2=sequence)>
40         ← <deterministicBool=0>
41         ← <fixtureUnsigned(0=He;1=Wan;2=test)>
42         ← <founderAth5Mutant?Bool> <debugOutputBool>
43         ← <startSeedUnsigned> <endSeedUnsigned>
44         ← <inductionTimeDoubleHours>
45         ← <earliestLineageStartTime>
46         ← <latestLineageStartTime> <endTimeDoubleHours>
47         ← <mMitoticModePhase2Double> <mMitoticModePhase3Double>
48         ← <pPP1Double(0-1)> <pPD1Double(0-1)> <pPP1Double(0-1)>
49         ← <pPD1Double(0-1)> <pPP1Double(0-1)>
50         ← <pPD1Double(0-1)>\nDeterministic Mode:\nHeSimulator
51         ← <directoryString> <filenameString>
52         ← <outputModeUnsigned(0=counts,1=events,2=sequence)>
53         ← <deterministicBool=1>
54         ← <fixtureUnsigned(0=He;1=Wan;2=test)>
55         ← <founderAth5Mutant?Bool> <debugOutputBool>
56         ← <startSeedUnsigned> <endSeedUnsigned>
57         ← <inductionTimeDoubleHours>
58         ← <earliestLineageStartTime>
59         ← <latestLineageStartTime> <endTimeDoubleHours>
60         ← <phase1ShapeDouble(>0)> <phase1ScaleDouble(>0)>
61         ← <phase2ShapeDouble(>0)> <phase2ScaleDouble(>0)>
62         ← <phaseBoundarySisterShiftWidthDouble>\n",
63         true);
64     exit_code = ExecutableSupport::EXIT_BAD_ARGUMENTS;
65     return exit_code;
66 }
67
68 /*****
69  * SIMULATOR PARAMETERS
70 *****/
71 std::string directoryString, filenameString;
72 int outputMode; //0 = counts; 1 = mitotic mode events; 2 = mitotic
73     ← mode sequence sampling
74 bool deterministicMode, ath5founder, debugOutput;
75 unsigned fixture, startSeed, endSeed; //fixture 0 = He2012; 1 =
76     ← Wan2016
77 double inductionTime, earliestLineageStartTime,
78     ← latestLineageStartTime, endTime;

```

```
50     double mitoticModePhase2, mitoticModePhase3, pPP1, pPD1, pPP2, pPD2,
51     ↵ pPP3, pPD3; //stochastic model parameters
52     double phase1Shape, phase1Scale, phase2Shape, phase2Scale,
53     ↵ phaseSisterShiftWidth, phaseOffset;
54
55 //PARSE ARGUMENTS
56 directoryString = argv[1];
57 filenameString = argv[2];
58 outputMode = std::stoi(argv[3]);
59 deterministicMode = std::stoul(argv[4]);
60 fixture = std::stoul(argv[5]);
61 ath5founder = std::stoul(argv[6]);
62 debugOutput = std::stoul(argv[7]);
63 startSeed = std::stoul(argv[8]);
64 endSeed = std::stoul(argv[9]);
65 inductionTime = std::stod(argv[10]);
66 earliestLineageStartTime = std::stod(argv[11]);
67 latestLineageStartTime = std::stod(argv[12]);
68 endTime = std::stod(argv[13]);
69
70 if (deterministicMode == 0)
71 {
72     mitoticModePhase2 = std::stod(argv[14]);
73     mitoticModePhase3 = std::stod(argv[15]);
74     pPP1 = std::stod(argv[16]);
75     pPD1 = std::stod(argv[17]);
76     pPP2 = std::stod(argv[18]);
77     pPD2 = std::stod(argv[19]);
78     pPP3 = std::stod(argv[20]);
79     pPD3 = std::stod(argv[21]);
80 }
81 else if (deterministicMode == 1)
82 {
83     phase1Shape = std::stod(argv[14]);
84     phase1Scale = std::stod(argv[15]);
85     phase2Shape = std::stod(argv[16]);
86     phase2Scale = std::stod(argv[17]);
87     phaseSisterShiftWidth = std::stod(argv[18]);
88     phaseOffset = std::stod(argv[19]);
89 }
```

```

90     ExecutableSupport::PrintError("Bad deterministicMode (argument
91         ↳ 4). Must be 0 or 1");
92     exit_code = ExecutableSupport::EXIT_BAD_ARGUMENTS;
93     return exit_code;
94 }
95 /**
96 * PARAMETER/ARGUMENT SANITY CHECK
97 */
98 bool sane = 1;
99
100 if (outputMode != 0 && outputMode != 1 && outputMode != 2)
101 {
102     ExecutableSupport::PrintError(
103         "Bad outputMode (argument 3). Must be 0 (counts) 1
104             ↳ (mitotic events) or 2 (sequence sampling)");
105     sane = 0;
106 }
107 if (fixture != 0 && fixture != 1 && fixture != 2)
108 {
109     ExecutableSupport::PrintError("Bad fixture (argument 5). Must be
110         ↳ 0 (He), 1 (Wan), or 2 (validation/test)");
111     sane = 0;
112 }
113 if (ath5founder != 0 && ath5founder != 1)
114 {
115     ExecutableSupport::PrintError("Bad ath5founder (argument 6). Must
116         ↳ be 0 (wild type) or 1 (ath5 mutant)");
117     sane = 0;
118 }
119 if (endSeed < startSeed)
120 {
121     ExecutableSupport::PrintError("Bad start & end seeds (arguments,
122         ↳ 8, 9). endSeed must not be < startSeed");
123     sane = 0;
124 }
125 if (inductionTime ≥ endTime)
126 {

```

```
127     ExecutableSupport::PrintError("Bad latestLineageStartTime  
128         ↵ (argument 10). Must be <endTime(arg13)");  
129     sane = 0;  
130 }  
131 if (earliestLineageStartTime ≥ endTime || earliestLineageStartTime  
132     ↵ ≥ latestLineageStartTime)  
133 {  
134     ExecutableSupport::PrintError(  
135         "Bad earliestLineageStartTime (argument 11). Must be  
136             ↵ <endTime(arg13), <latestLineageStarTime (arg12)");  
137     sane = 0;  
138 }  
139 if (latestLineageStartTime > endTime)  
140 {  
141     ExecutableSupport::PrintError("Bad latestLineageStartTime  
142         ↵ (argument 12). Must be ≤ endTime(arg13)");  
143     sane = 0;  
144 }  
145  
146 if (deterministicMode == 0)  
147 {  
148     if (mitoticModePhase2 < 0)  
149     {  
150         ExecutableSupport::PrintError("Bad mitoticModePhase2  
151             ↵ (argument 14). Must be >0");  
152         sane = 0;  
153     }  
154     if (mitoticModePhase3 < 0)  
155     {  
156         ExecutableSupport::PrintError("Bad mitoticModePhase3  
157             ↵ (argument 15). Must be >0");  
158         sane = 0;  
159     }  
160     ExecutableSupport::PrintError(  
161         "Bad phase 1 probabilities (arguments 16, 17). pPP1 +  
162             ↵ pPD1 should be ≥0, ≤1, sum should not exceed  
163             ↵ 1");  
164     sane = 0;  
165 }
```

```
160     if (pPP2 + pPD2 > 1 || pPP2 > 1 || pPP2 < 0 || pPD2 > 1 || pPD2 <
161         → 0)
162     {
163         ExecutableSupport::PrintError(
164             "Bad phase 2 probabilities (arguments 18, 19). pPP2 +
165             → pPD2 should be  $\geq 0$ ,  $\leq 1$ , sum should not exceed
166             → 1");
167         sane = 0;
168     }
169     if (pPP3 + pPD3 > 1 || pPP3 > 1 || pPP3 < 0 || pPD3 > 1 || pPD3 <
170         → 0)
171     {
172         ExecutableSupport::PrintError(
173             "Bad phase 3 probabilities (arguments 20, 21). pPP3 +
174             → pPD3 should be  $\geq 0$ ,  $\leq 1$ , sum should not exceed
175             → 1");
176         sane = 0;
177     }
178     if (deterministicMode == 1)
179     {
180         if (phase1Shape  $\leq 0$ )
181         {
182             ExecutableSupport::PrintError("Bad phase1Shape (argument 14).
183                 → Must be  $> 0$ ");
184             sane = 0;
185         }
186         if (phase1Scale  $\leq 0$ )
187         {
188             ExecutableSupport::PrintError("Bad phase1Scale (argument 15).
189                 → Must be  $> 0$ ");
190             sane = 0;
191         }
192         if (phase2Shape  $\leq 0$ )
193         {
```

```

193         ExecutableSupport::PrintError("Bad phase1Scale (argument 17).
194             ↪ Must be >0");
195         sane = 0;
196     }
197     if (phaseSisterShiftWidth ≤ 0)
198     {
199         ExecutableSupport::PrintError("Bad phaseSisterShiftWidth
200             ↪ (argument 18). Must be >0");
201         sane = 0;
202     }
203     if (sane == 0)
204     {
205         ExecutableSupport::PrintError("Exiting with bad arguments. See
206             ↪ errors for details");
207         exit_code = ExecutableSupport::EXIT_BAD_ARGUMENTS;
208         return exit_code;
209     }
210
211 /*****
212 * SIMULATOR OUTPUT SETUP
213 *****/
214
215 //Set up singleton LogFile
216 LogFile* p_log = LogFile::Instance();
217 p_log→Set(0, directoryString, filenameString);
218
219 ExecutableSupport::Print("Simulator writing file " + filenameString +
220     ↪ " to directory " + directoryString);
221
222 //Log entry counter
223     unsigned entry_number = 1;
224
225 //Write appropriate headers to log
226     if (outputMode == 0) *p_log << "Entry\tInduction Time
227         ↪ (h)\tSeed\tCount\n";
228     if (outputMode == 1) *p_log << "Time (hpf)\tSeed\tCellID\tMitotic
229         ↪ Mode (0=PP;1=PD;2=DD)\n";
230     if (outputMode == 2) *p_log << "Entry\tSeed\tSequence\n";
231
232 //Instance RNG

```

```

230     RandomNumberGenerator* p_RNG = RandomNumberGenerator::Instance();
231
232 //Initialise pointers to relevant singleton ProliferativeTypes and
233     ↵ Properties
234     MAKE_PTR(WildTypeCellMutationState, p_state);
235     MAKE_PTR(TransitCellProliferativeType, p_Mitotic);
236     MAKE_PTR(DifferentiatedCellProliferativeType, p_PostMitotic);
237     MAKE_PTR(Ath5Mo, p_Morpholino);
238     MAKE_PTR(CellLabel, p_label);
239
240 //*****
241 * SIMULATOR SETUP & RUN
242 *****/
243
244 //iterate through supplied seed range, executing one simulation per seed
245 for (unsigned seed = startSeed; seed ≤ endSeed; seed++)
246 {
247     if (outputMode == 2) *p_log << entry_number << "\t" << seed <<
248         ↵ "\t"; //write seed to log - sequence written by
249         ↵ cellcyclemodel objects
250
251     //initialise pointer to debugWriter
252     ColumnDataWriter* debugWriter;
253
254     //initialise SimulationTime (permits cellcyclemodel setup)
255     SimulationTime::Instance()→SetStartTime(0.0);
256
257     //Reseed the RNG with the required seed
258     p_RNG→Reseed(seed);
259
260     //Initialise a HeCellCycleModel and set it up with appropriate
261     ↵ TiL values
262     HeCellCycleModel* p_cycle_model = new HeCellCycleModel;
263
264     if (debugOutput)
265     {
266         //Pass ColumnDataWriter to cell cycle model for debug output
267         boost::shared_ptr<ColumnDataWriter> p_debugWriter(
268             new ColumnDataWriter(directoryString, filenameString
269                 ↵ + "DEBUG_" + std::to_string(seed), false, 10));
270         p_cycle_model→EnableModelDebugOutput(p_debugWriter);
271         debugWriter = &p_debugWriter;
272     }

```



```

298     }
299     else if (fixture == 1) //Wan 2016-type fixture - each lineage
300         ↵ founder selected randomly across residency time, simulator
301         ↵ allowed to run until end of residency time
302         //passing residency time (as latestLineageStartTime) and endTime
303         ↵ separately allows for creation of "shadow CMZ" population
304         //this allows investigation of different assumptions about how
305         ↵ Wan et al.'s model output was generated
306     {
307         //generate random lineage start time from even random distro
308         ↵ across CMZ residency time
309         currTiL = p_RNG->ranf() * latestLineageStartTime;
310         currSimEndTime = std::max(.05, endTime - currTiL); //minimum
311         ↵ 1 timestep, prevents 0 timestep SimulationTime error
312         if (outputMode == 1) p_cycle_model->EnableModeEventOutput(0,
313             ↵ seed);
314     }
315     else if (fixture == 2) //validation fixture- all founders have
316         ↵ TiL given by induction time
317     {
318         currTiL = inductionTime;
319         currSimEndTime = endTime;
320     }
321
322     //Setup lineages' cycle model with appropriate parameters
323     p_cycle_model->SetDimension(2);
324     //p_cycle_model->SetPostMitoticType(p_PostMitotic);
325
326     if (!deterministicMode)
327     {
328         p_cycle_model->SetModelParameters(currTiL, mitoticModePhase2,
329             ↵ mitoticModePhase2 + mitoticModePhase3, pPP1,
330                 pPD1, pPP2, pPD2, pPP3,
331                     ↵ pPD3);
332     }
333     else
334     {
335         //Gamma-distribute phase3 boundary
336         double currPhase2Boundary = phaseOffset +
337             ↵ p_RNG->GammaRandomDeviate(phase1Shape, phase1Scale);
338         double currPhase3Boundary = currPhase2Boundary +
339             ↵ p_RNG->GammaRandomDeviate(phase2Shape, phase2Scale);
340     }
341 
```

```

329     p_cycle_model->SetDeterministicMode(currTil,
330         ↳ currPhase2Boundary, currPhase3Boundary,
331         ↳ phaseSisterShiftWidth);
332     }
333
334     //Setup vector containing lineage founder with the properly set
335     ↑ up cell cycle model
336     std::vector<CellPtr> cells;
337     CellPtr p_cell(new Cell(p_state, p_cycle_model));
338     p_cell->SetCellProliferativeType(p_Mitotic);
339     if (ath5founder = 1) p_cell->AddCellProperty(p_Morpholino);
340     if (outputMode = 2) p_cell->AddCellProperty(p_label);
341     p_cell->InitialiseCellCycleModel();
342     cells.push_back(p_cell);

343     //Generate 1x1 mesh for single-cell colony
344     HoneycombMeshGenerator generator(1, 1);
345     MutableMesh<2, 2>* p_generating_mesh = generator.GetMesh();
346     NodesOnlyMesh<2> mesh;
347     mesh.ConstructNodesWithoutMesh(*p_generating_mesh, 1.5);

348     //Setup cell population
349     NodeBasedCellPopulation<2>* cell_population(new
350         ↳ NodeBasedCellPopulation<2>(mesh, cells));

351     //Setup simulator & run simulation
352     boost::shared_ptr<OffLatticeSimulationPropertyStop<2>>
353         ↳ p_simulator(
354             new
355                 ↳ OffLatticeSimulationPropertyStop<2>(*cell_population));
356     p_simulator->SetStopProperty(p_Mitotic); //simulation to stop if
357     ↑ no mitotic cells are left
358     p_simulator->SetDt(0.05);
359     p_simulator->SetEndTime(currSimEndTime);
360     p_simulator->SetOutputDirectory("UnusedSimOutput" +
361         ↳ filenameString); //unused output
362     p_simulator->Solve();

363     //Count lineage size
364     unsigned count = cell_population->GetNumRealCells();
365

```

```

364     if (outputMode == 0) *p_log << entry_number << "\t" <<
365         inductionTime << "\t" << seed << "\t" << count << "\n";
366     if (outputMode == 2) *p_log << "\n";
367
368     //Reset for next simulation
369     SimulationTime::Destroy();
370     delete cell_population;
371     entry_number++;
372
373     if (debugOutput)
374     {
375         debugWriter->Close();
376     }
377
378
379     p_RNG->Destroy();
380     LogFile::Close();
381
382     return exit_code;
383 }
384 ;

```

16.2.5 /apps/src/WanSimDebug.cpp

```

1 #include <iostream>
2 #include <string>
3
4 #include <cxxtest/TestSuite.h>
5 #include "ExecutableSupport.hpp"
6 #include "Exception.hpp"
7 #include "PetscTools.hpp"
8 #include "PetscException.hpp"
9
10 #include "WanStemCellCycleModel.hpp"
11 #include "HeCellCycleModel.hpp"
12 #include "OffLatticeSimulation.hpp"
13
14 #include "AbstractCellBasedTestSuite.hpp"
15
16 #include "WildTypeCellMutationState.hpp"
17 #include "StemCellProliferativeType.hpp"

```

```
18 #include "TransitCellProliferativeType.hpp"
19 #include "DifferentiatedCellProliferativeType.hpp"
20
21 #include "CellsGenerator.hpp"
22 #include "HoneycombMeshGenerator.hpp"
23 #include "NodesOnlyMesh.hpp"
24 #include "NodeBasedCellPopulation.hpp"
25 #include "VertexBasedCellPopulation.hpp"
26
27 #include "CellProliferativeTypesCountWriter.hpp"
28
29
30 int main(int argc, char *argv[])
31 {
32     ExecutableSupport::StartupWithoutShowingCopyright(&argc, &argv);
33     //main() returns code indicating sim run success or failure mode
34     int exit_code = ExecutableSupport::EXIT_OK;
35
36     /*****
37     * SIMULATOR PARAMETERS
38     *****/
39     std::string directoryString, filenameString;
40
41     //PARSE ARGUMENTS
42     directoryString = argv[1];
43     filenameString = argv[2];
44
45     /*****
46     * SIMULATOR OUTPUT SETUP
47     *****/
48
49 //Set up singleton LogFile
50    LogFile* p_log = LogFile::Instance();
51     p_log->Set(0, directoryString, filenameString);
52
53     ExecutableSupport::Print("Simulator writing file " + filenameString +
54     → " to directory " + directoryString);
55
56 //Log entry counter
57     //unsigned entry_number = 1;
58
59 //Write appropriate header to log
```

```

59     *p_log <<
60     "Entry\tTotalCells\tStemCount\tProgenitorCount\tPostMitoticCount\n";
61
62 //Instance RNG
63     RandomNumberGenerator* p_RNG = RandomNumberGenerator::Instance();
64
65 //Initialise pointers to relevant singleton ProliferativeTypes and
66 //Properties
67     boost::shared_ptr<AbstractCellProperty>
68         → p_state(CellPropertyRegistry::Instance()→Get<WildTypeCellMutationState>());
69     boost::shared_ptr<AbstractCellProperty>
70         → p_Stem(CellPropertyRegistry::Instance()→Get<StemCellProliferativeType>());
71     boost::shared_ptr<AbstractCellProperty>
72         → p_Transit(CellPropertyRegistry::Instance()→Get<TransitCellProliferativeType>());
73     boost::shared_ptr<AbstractCellProperty>
74         → p_PostMitotic(CellPropertyRegistry::Instance()→Get<DifferentiatedCellProlife
75
76 //*****
77 // SIMULATOR SETUP & RUN
78 //*****
79
80 //initialise SimulationTime (permits cellcyclemodel setup)
81 SimulationTime::Instance()→SetStartTime(0.0);
82
83 std::vector<CellPtr> cells;
84
85 WanStemCellCycleModel* p_stem_model = new WanStemCellCycleModel;
86
87 boost::shared_ptr<ColumnDataWriter> p_debugWriter(
88     new ColumnDataWriter(directoryString, filenameString +
89     → "DEBUG_WAN", false, 10));
90
91 p_stem_model→SetDimension(2);
92 p_stem_model→EnableModelDebugOutput(p_debugWriter);
93 CellPtr p_cell(new Cell(p_state, p_stem_model));
94 p_cell→InitialiseCellCycleModel();
95 cells.push_back(p_cell);
96
97 //Generate 1x#cells mesh for abstract colony
98 HoneycombMeshGenerator generator(1, 1);
99 MutableMesh<2, 2>* p_generating_mesh = generator.GetMesh();
100 NodesOnlyMesh<2> mesh;

```

```
95     mesh.ConstructNodesWithoutMesh(*p_generating_mesh, 1.5);
96
97     //Setup cell population
98     boost::shared_ptr<NodeBasedCellPopulation<2>> cell_population(new
99         → NodeBasedCellPopulation<2>(mesh, cells));
100
101    → cell_population→AddCellPopulationCountWriter<CellProliferativeTypesCountWriter>
102
103    //Setup simulator & run simulation
104    boost::shared_ptr<OffLatticeSimulation<2>> p_simulator(
105        → new OffLatticeSimulation<2>(*cell_population));
106    p_simulator→SetDt(1);
107    p_simulator→SetOutputDirectory(directoryString + "/WanDebug");
108    p_simulator→SetEndTime(100);
109    p_simulator→Solve();
110
111    std::vector<unsigned> prolTypes =
112        → cell_population→GetCellProliferativeTypeCount();
113    unsigned realCells = cell_population→GetNumRealCells();
114    unsigned allCells = cell_population→GetNumAllCells();
115
116    Timer::Print("stem: " + std::to_string(prolTypes[0]) + " transit: " +
117        → std::to_string(prolTypes[1]) + " postmitotic: " +
118        → std::to_string(prolTypes[2]));
119    Timer::Print("realcells: " + std::to_string(realCells) + " allcells:
120        → " + std::to_string(allCells));
121
122    //Reset for next simulation
123    SimulationTime::Destroy();
124    cell_population.reset();
125
126    p_RNG→Destroy();
127    LogFile::Close();
128
129    return exit_code;
130 }
131 ;
```

16.2.6 /apps/src/WanSimulator.cpp

```
1 #include <iostream>
2 #include <string>
3
4 #include <cxxtest/TestSuite.h>
5 #include "ExecutableSupport.hpp"
6 #include "Exception.hpp"
7 #include "PetscTools.hpp"
8 #include "PetscException.hpp"
9
10 #include "WanStemCellCycleModel.hpp"
11 #include "HeCellCycleModel.hpp"
12 #include "OffLatticeSimulationPropertyStop.hpp"
13
14 #include "AbstractCellBasedTestSuite.hpp"
15
16 #include "WildTypeCellMutationState.hpp"
17 #include "StemCellProliferativeType.hpp"
18 #include "TransitCellProliferativeType.hpp"
19 #include "DifferentiatedCellProliferativeType.hpp"
20
21 #include "CellsGenerator.hpp"
22 #include "HoneycombMeshGenerator.hpp"
23 #include "NodesOnlyMesh.hpp"
24 #include "NodeBasedCellPopulation.hpp"
25 #include "VertexBasedCellPopulation.hpp"
26
27 #include "CellProliferativeTypesCountWriter.hpp"
28
29 int main(int argc, char *argv[])
30 {
31     ExecutableSupport::StartupWithoutShowingCopyright(&argc, &argv);
32     //main() returns code indicating sim run success or failure mode
33     int exit_code = ExecutableSupport::EXIT_OK;
34
35     if (argc != 23)
36     {
37         ExecutableSupport::PrintError(
```

```

38         "Wrong arguments for simulator.\nUsage (replace <> with
39         ← values, pass bools as 0 or 1):\n WanSimulator
40         ← <directoryString> <startSeedUnsigned>
41         ← <endSeedUnsigned> <cmzResidencyTimeDoubleHours>
42         ← <stemDivisorDouble>
43         ← <meanProgenitorPopualtion@3dpfDouble>
44         ← <stdProgenitorPopulation@3dpfDouble>
45         ← <stemGammaShiftDouble> <stemGammaShapeDouble>
46         ← <stemGammaScaleDouble> <progenitorGammaShiftDouble>
47         ← <progenitorGammaShapeDouble>
48         ← <progenitorGammaScaleDouble>
49         ← <progenitorSisterShiftDouble>
50         ← <mMitoticModePhase2Double> <mMitoticModePhase3Double>
51         ← <pPP1Double(0-1)> <pPD1Double(0-1)> <pPP1Double(0-1)>
52         ← <pPD1Double(0-1)> <pPP1Double(0-1)>
53         ← <pPD1Double(0-1)>",
54         true);
55     exit_code = ExecutableSupport::EXIT_BAD_ARGUMENTS;
56     return exit_code;
57 }
58
59 //*****
60 * SIMULATOR PARAMETERS
61 *****/
62 std::string directoryString;
63 unsigned startSeed, endSeed;
64 double cmzResidencyTime;
65 double stemDivisor, progenitorMean, progenitorStd;
66 double stemGammaShift, stemGammaShape, stemGammaScale,
67     → progenitorGammaShift, progenitorGammaShape,
68     → progenitorGammaScale, progenitorGammaSister;
69 double mitoticModePhase2, mitoticModePhase3, pPP1, pPD1, pPP2, pPD2,
70     → pPP3, pPD3; //stochastic He model parameters
71
72 //PARSE ARGUMENTS
73 directoryString = argv[1];
74 startSeed = std::stoul(argv[2]);
75 endSeed = std::stoul(argv[3]);
76 cmzResidencyTime = std::stod(argv[4]);
77 //starting cell number distributions
78 stemDivisor = std::stod(argv[5]);
79 progenitorMean = std::stod(argv[6]);
80 progenitorStd = std::stod(argv[7]);

```

```

64 //cycle duration params
65 stemGammaShift = std::stod(argv[8]);
66 stemGammaShape = std::stod(argv[9]);
67 stemGammaScale = std::stod(argv[10]);
68 progenitorGammaShift = std::stod(argv[11]);
69 progenitorGammaShape = std::stod(argv[12]);
70 progenitorGammaScale = std::stod(argv[13]);
71 progenitorGammaSister = std::stod(argv[14]);
72 //He model params
73 mitoticModePhase2 = std::stod(argv[15]);
74 mitoticModePhase3 = std::stod(argv[16]);
75 pPP1 = std::stod(argv[17]);
76 pPD1 = std::stod(argv[18]);
77 pPP2 = std::stod(argv[19]);
78 pPD2 = std::stod(argv[20]);
79 pPP3 = std::stod(argv[21]);
80 pPD3 = std::stod(argv[22]);

81 std::vector<double> stemOffspringParams = { mitoticModePhase2,
82   ↪ mitoticModePhase2 + mitoticModePhase3, pPP1, pPD1,
83   ↪ pPP2, pPD2, pPP3, pPD3,
84   ↪ progenitorGammaShift,
85   ↪ progenitorGammaShape,
86   ↪ progenitorGammaScale,
87   ↪ progenitorGammaSister
88   ↪ };

89 ****
90 * PARAMETER/ARGUMENT SANITY CHECK
91 ****
92 bool sane = 1;

93 if (endSeed < startSeed)
94 {
95     ExecutableSupport::PrintError("Bad start & end seeds (arguments,
96       ↪ 3, 4). endSeed must not be < startSeed");
97     sane = 0;
98 }
99 if (cmzResidencyTime < 0)
100 {
101     ExecutableSupport::PrintError("Bad CMZ residency time (argument
102       ↪ 5). cmzResidencyTime must be positive-valued");

```

```

100         sane = 0;
101     }
102
103     if (stemDivisor <= 0)
104     {
105         ExecutableSupport::PrintError("Bad stemDivisor (argument 6).
106             ↳ stemDivisor must be positive-valued");
107         sane = 0;
108     }
109
110     if (progenitorMean <= 0 || progenitorStd <= 0)
111     {
112         ExecutableSupport::PrintError("Bad progenitorMean or
113             ↳ progenitorStd (arguments 7,8). Must be positive-valued");
114         sane = 0;
115     }
116
117     if (stemGammaShift < 0 || stemGammaShape < 0 || stemGammaScale < 0)
118     {
119         ExecutableSupport::PrintError(
120             "Bad stemGammaShift, stemGammaShape, or stemGammaScale
121                 ↳ (arguments 9, 10, 11). Shifts must be ≥0, cycle
122                 ↳ shape and scale params must be positive-valued");
123         sane = 0;
124     }
125
126     if (progenitorGammaShift < 0 || progenitorGammaShape < 0 ||
127         ↳ progenitorGammaScale < 0 || progenitorGammaSister < 0)
128     {
129         ExecutableSupport::PrintError(
130             "Bad progenitorGammaShift, progenitorGammaShape,
131                 ↳ progenitorGammaScale, or progenitorGammaSisterShift
132                 ↳ (arguments 12,13,14,15). Shifts must be ≥0, cycle
133                 ↳ shape and scale params must be positive-valued");
134         sane = 0;
135     }
136
137     if (mitoticModePhase2 < 0)
138     {
139         ExecutableSupport::PrintError("Bad mitoticModePhase2 (argument
140             ↳ 14). Must be >0");
141         sane = 0;
142     }
143

```

```

134     if (mitoticModePhase3 < 0)
135     {
136         ExecutableSupport::PrintError("Bad mitoticModePhase3 (argument
137             ↳ 15). Must be >0");
138         sane = 0;
139     }
140     if (pPP1 + pPD1 > 1 || pPP1 > 1 || pPP1 < 0 || pPD1 > 1 || pPD1 < 0)
141     {
142         ExecutableSupport::PrintError(
143             "Bad phase 1 probabilities (arguments 16, 17). pPP1 +
144                 ↳ pPD1 should be ≥0, ≤1, sum should not exceed 1");
145         sane = 0;
146     }
147     if (pPP2 + pPD2 > 1 || pPP2 > 1 || pPP2 < 0 || pPD2 > 1 || pPD2 < 0)
148     {
149         ExecutableSupport::PrintError(
150             "Bad phase 2 probabilities (arguments 18, 19). pPP2 +
151                 ↳ pPD2 should be ≥0, ≤1, sum should not exceed 1");
152         sane = 0;
153     }
154     if (pPP3 + pPD3 > 1 || pPP3 > 1 || pPP3 < 0 || pPD3 > 1 || pPD3 < 0)
155     {
156         ExecutableSupport::PrintError(
157             "Bad phase 3 probabilities (arguments 20, 21). pPP3 +
158                 ↳ pPD3 should be ≥0, ≤1, sum should not exceed 1");
159         sane = 0;
160     }
161     if (sane == 0)
162     {
163         ExecutableSupport::PrintError("Exiting with bad arguments. See
164             ↳ errors for details");
165         exit_code = ExecutableSupport::EXIT_BAD_ARGUMENTS;
166         return exit_code;
167     }
168
169     /*****
170      * SIMULATOR SETUP & RUN
171      *****/
172
173     ExecutableSupport::Print("Simulator writing files to directory " +
174             ↳ directoryString);
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```

202     p_stem_model→SetModelParameters(stemGammaShift,
203     ↳ stemGammaShape, stemGammaScale, stemOffspringParams);
204
205     CellPtr p_cell(new Cell(p_state, p_stem_model));
206     p_cell→InitialiseCellCycleModel();
207     stems.push_back(p_cell);
208     cells.push_back(p_cell);
209 }
210
211 for (unsigned i = 0; i < numberProgenitors; i++)
212 {
213     double currTiL = p_RNG→ranf() * cmzResidencyTime;
214
215     HeCellCycleModel* p_prog_model = new HeCellCycleModel;
216     p_prog_model→SetDimension(2);
217     p_prog_model→SetModelParameters(currTiL, mitoticModePhase2,
218     ↳ mitoticModePhase2 + mitoticModePhase3, pPP1,
219     ↳ pPD1, pPP2, pPD2, pPP3,
220     ↳ pPD3);
221     p_prog_model→EnableKillSpecified();
222
223 }
224
225 //Generate 1x#cells mesh for abstract colony
226 HoneycombMeshGenerator generator(1, (numberProgenitors +
227   ↳ numberStem));
228 MutableMesh<2, 2>* p_generating_mesh = generator.GetMesh();
229 NodesOnlyMesh<2> mesh;
230 mesh.ConstructNodesWithoutMesh(*p_generating_mesh, 1.5);
231
232 //Setup cell population
233 boost::shared_ptr<NodeBasedCellPopulation<2>> cell_population(new
234   ↳ NodeBasedCellPopulation<2>(mesh, cells));
235
236   ↳ cell_population→AddCellPopulationCountWriter<CellProliferativeTypesCount>();
237
238 //Give Wan stem cells the population & base stem pop size
239 for (auto p_cell : stems)
240 {

```

```

238     WanStemCellCycleModel* p_cycle_model =
239         → dynamic_cast<WanStemCellCycleModel*>(p_cell→GetCellCycleModel());
240     p_cycle_model→EnableExpandingStemPopulation(numberStem,
241         → cell_population);
242 }
243
244 //Setup simulator & run simulation
245 boost::shared_ptr<OffLatticeSimulationPropertyStop<2>>
246     → p_simulator(
247         new
248             → OffLatticeSimulationPropertyStop<2>(*cell_population));
249     p_simulator→SetStopProperty(p_Transit); //simulation to stop if
250         → no RPCs are left
251     p_simulator→SetDt(1);
252     p_simulator→SetOutputDirectory(directoryString + "/Seed" +
253         → std::to_string(seed) + "Results");
254     p_simulator→SetEndTime(8568); // 360dpf - 3dpf simulation start
255         → time
256     p_simulator→Solve();
257
258 //Reset for next simulation
259 SimulationTime::Destroy();
260 cell_population.reset();
261 }
262
263 p_RNG→Destroy();
264
265 return exit_code;
266 }
267 ;

```

16.2.7 /python_fixtures/He_output_fixture.py

```

1 import multiprocessing
2 import os
3 import subprocess
4 import datetime
5
6 import numpy as np
7 from imageio.plugins._bsdf import BsdfSerializer
8
9 executable = '/home/main/chaste_build/projects/ISP/apps/HeSimulator'

```

```

10
11 if not(os.path.isfile(executable)):
12     raise Exception('Could not find executable: ' + executable)
13
14 #####
15 # SIMULATION PARAMETERS
16 #####
17
18 #Define start and end RNG seeds; determines:
19 #No. lineages per loss function run
20 #unique sequence of RNG results for each lineage
21 start_seed = 0
22 end_seed_counts = 9999
23 end_seed_events = 999
24 run_modes = [0,1,2] #1=deterministic mode, 0=refit stochastic mode,
    ↪ 2=original fit
25 debug_output = 0 #0=off;1=on
26
27 count_directory = "HeCounts"
28 count_filenames = ["induction", "wan", "ath5", "validate"]
29 event_directory = "HeModeEvent"
30 event_filenames = ["inductionMode", "validateMode"]
31 induction_times = [24, 32, 48]
32
33 #####
34 #GLOBAL MODEL PARAMETERS
35 #####
36
37 #Values defining different marker induction timepoints & relative start
    ↪ time of TiL counter
38 earliest_lineage_start_time = 23.0 #RPCs begin to enter "He model regime"
    ↪ nasally at 23hpf
39 latest_lineage_start_time = 39.0 #last temporal retinal RPC has entered
    ↪ "He model regime" at 39hpf
40 counts_end_time = 72.0
41 events_end_time = 80.0
42 wan_residency_time = 17.0
43
44 #####
45 # SPECIFIC MODEL PARAMETERS - THETAHAT
46 #####
47
48 #These parameters are the results of the SPSA optimisation fixture

```

```

49
50 #STOCHASTIC MITOTIC MODE
51 #HE ORIGINAL PARAMETERS
52 #mitotic mode per-phase probabilities
53 #3-phase mitotic mode time periodisation
54 o_mitotic_mode_phase_2 = 8 #These are phase lengths, so
55 o_mitotic_mode_phase_3 = 7 #Phase3 boundary = mmp2 + mmp3
56 o_phase_1_pPP = 1.0
57 o_phase_1_pPD = 0.0
58 o_phase_2_pPP = 0.2
59 o_phase_2_pPD = 0.4
60 o_phase_3_pPP = 0.2
61 o_phase_3_pPD = 0.0
62
63 #SPSA REFIT PARAMETERS
64 #mitotic mode per-phase probabilities
65 #3-phase mitotic mode time periodisation
66 mitotic_mode_phase_2 = 4.1483 #These are phase lengths, so
67 mitotic_mode_phase_3 = 11.6416 #Phase3 boundary = mmp2 + mmp3
68 phase_1_pPP = 1.0
69 phase_1_pPD = 0.0
70 phase_2_pPP = 0.1959
71 phase_2_pPD = 0.5168
72 phase_3_pPP = 0.2934
73 phase_3_pPD = 0.0
74 he_model_params = 15
75
76 #DETERMINISTIC MITOTIC MODE
77 #Phase boundary shift parameters
78 phase_1_shape = 3.7371
79 phase_1_scale = 1.8114
80 phase_2_shape = 2.5769
81 phase_2_scale = 1.6814
82 phase_sister_shift_widths = 1.6326
83 phase_offset = 1.2333
84 det_model_params = 12
85
86 original_theta_string = str(o_mitotic_mode_phase_2) + " " +
    ↵ str(o_mitotic_mode_phase_3) + " " + str(o_phase_1_pPP) + " " +
    ↵ str(o_phase_1_pPD) + " " + str(o_phase_2_pPP) + " " +
    ↵ str(o_phase_2_pPD) + " " + str(o_phase_3_pPP) + " " +
    ↵ str(o_phase_3_pPD)

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87 stochastic_theta_string = str(mitotic_mode_phase_2) + " " +
→   str(mitotic_mode_phase_3) + " " +str(phase_1_pPP) + " " +
→   str(phase_1_pPD) + " " + str(phase_2_pPP) + " " + str(phase_2_pPD) +
→   " " + str(phase_3_pPP) + " " + str(phase_3_pPD)
88 deterministic_theta_string = str(phase_1_shape) + " " +
→   str(phase_1_scale) + " " + str(phase_2_shape) + " " +
→   str(phase_2_scale) + " " + str(phase_sister_shift_widths) + " " +
→   str(phase_offset)

89
90 def main():
91
92     command_list = []
93
94     for m in range(0,len(run_modes)):
95         curr_list = []
96
97         run_mode_string = str(run_modes[m])
98         deterministic_mode = run_modes[m]
99         if run_modes[m] == 2:
100             deterministic_mode = 0
101         command_count = 0 #for iterating seed numbers
102         base_command = executable
103
104         #induction count commands
105         for i in range(0,len(induction_times)):
106
107             output_mode = 0 #0=lineage counts;1=mitotic event
→               logging;2=sequence sampling
108             fixture = 0 #0=He 2012;1=Wan 2016
109             curr_start_seed = start_seed + command_count *
→               (end_seed_counts+1)
110             curr_end_seed = curr_start_seed + end_seed_counts
111             ath5founder = 0
112
113             command = base_command+" "\n
114                 +count_directory+" "\n
115
→               +count_filenames[0]+str(induction_times[i])+SDMode+run_
→               "
→               "\n
116             +str(output_mode)+" "\n
117             +str(deterministic_mode)+" "\n
118             +str(fixture)+" "\n
119             +str(ath5founder)+" "

```

```

120             +str(debug_output)+" "\n
121             +str(curr_start_seed)+" "\n
122             +str(curr_end_seed)+" "\n
123             +str(induction_times[i])+" "\n
124             +str(earliest_lineage_start_time)+" "\n
125             +str(latest_lineage_start_time)+" "\n
126             +str(counts_end_time)+" "
127         curr_list.append(command)
128         command_count += 1
129
130     #wan command
131     output_mode = 0
132     fixture = 1
133     curr_start_seed = start_seed + command_count *
134         ↪ (end_seed_counts+1)
135     curr_end_seed = curr_start_seed + end_seed_counts
136     ath5founder = 0
137     wan_command = base_command+" \
138             +count_directory+" "\n
139             +count_filenames[1]+"SDMode"+run_mode_string+" "\n
140             +str(output_mode)+" "\n
141             +str(deterministic_mode)+" "\n
142             +str(fixture)+" "\n
143             +str(ath5founder)+" "\n
144             +str(debug_output)+" "\n
145             +str(curr_start_seed)+" "\n
146             +str(curr_end_seed)+" "\n
147             +str(0)+" "\n
148             +str(0)+" "\n
149             +str(wan_residency_time)+" "\n
150             +str(counts_end_time)+" "
151         curr_list.append(wan_command)
152         command_count += 1
153
154     #wan- 17 hr constraint- no "shadow RPCs"
155     curr_start_seed = start_seed + command_count *
156         ↪ (end_seed_counts+1)
157     curr_end_seed = curr_start_seed + end_seed_counts
158     wan_command = base_command+" \
159             +count_directory+" "\n
160             ↪ +count_filenames[1]+"NoShadSDMode"+run_mode_string+" "
161             "\n"

```

```
159          +str(output_mode)+" "\n"
160          +str(deterministic_mode)+" "\n"
161          +str(fixture)+" "\n"
162          +str(ath5founder)+" "\n"
163          +str(debug_output)+" "\n"
164          +str(curr_start_seed)+" "\n"
165          +str(curr_end_seed)+" "\n"
166          +str(0)+" "\n"
167          +str(0)+" "\n"
168          +str(wan_residency_time)+" "\n"
169          +str(wan_residency_time)+" "
170      curr_list.append(wan_command)
171      command_count += 1
172
173 #ath5 command
174 output_mode = 0
175 fixture = 2
176 curr_start_seed = start_seed + command_count *
177     ↪ (end_seed_counts+1)
177 curr_end_seed = curr_start_seed + end_seed_counts
178 ath5founder = 1
179
180 ath5_command = base_command+" "\n"
181         +count_directory+" "\n"
182         +count_filenames[2]+"SDMode"+run_mode_string+" "\n"
183         +str(output_mode)+" "\n"
184         +str(deterministic_mode)+" "\n"
185         +str(fixture)+" "\n"
186         +str(ath5founder)+" "\n"
187         +str(debug_output)+" "\n"
188         +str(curr_start_seed)+" "\n"
189         +str(curr_end_seed)+" "\n"
190         +str(0)+" "\n"
191         +str(earliest_lineage_start_time)+" "\n"
192         +str(latest_lineage_start_time)+" "\n"
193         +str(counts_end_time)+" "
194      curr_list.append(ath5_command)
195      command_count += 1
196
197 #validate counts command
198 curr_start_seed = start_seed + command_count *
199     ↪ (end_seed_counts+1)
200 curr_end_seed = curr_start_seed + end_seed_counts
```

```
200     ath5founder = 0
201
202     validate_command = base_command + " " \
203                         + count_directory + " " \
204                         + count_filenames[3] + "SDMode" + run_mode_string + " " \
205                         + str(output_mode) + " " \
206                         + str(deterministic_mode) + " " \
207                         + str(fixture) + " " \
208                         + str(ath5founder) + " " \
209                         + str(debug_output) + " " \
210                         + str(curr_start_seed) + " " \
211                         + str(curr_end_seed) + " " \
212                         + str(0) + " " \
213                         + str(earliest_lineage_start_time) + " " \
214                         + str(latest_lineage_start_time) + " " \
215                         + str(counts_end_time) + " "
216     curr_list.append(validate_command)
217     command_count += 1
218
219     #mitotic mode rate command
220     output_mode = 1
221     fixture = 0
222     curr_start_seed = start_seed + command_count *
223                     (end_seed_counts+1)
224     curr_end_seed = curr_start_seed + end_seed_events
225     mode_rate_command = base_command + " " \
226                         + event_directory + " " \
227                         + event_filenames[0] + "SDMode" + run_mode_string + " " \
228                         + str(output_mode) + " " \
229                         + str(deterministic_mode) + " " \
230                         + str(fixture) + " " \
231                         + str(ath5founder) + " " \
232                         + str(debug_output) + " " \
233                         + str(curr_start_seed) + " " \
234                         + str(curr_end_seed) + " " \
235                         + str(earliest_lineage_start_time) + " " \
236                         + str(earliest_lineage_start_time) + " " \
237                         + str(latest_lineage_start_time) + " " \
238                         + str(events_end_time) + " "
239     curr_list.append(mode_rate_command)
240     command_count += 1
241
```

```

242     #validate rate command
243     fixture = 2
244     curr_start_seed = start_seed + command_count *
245         ↵ (end_seed_counts+1)
246     curr_end_seed = curr_start_seed + end_seed_events
247     validate_rate_command = base_command+" \"\
248         +event_directory+" \"\
249         +event_filenames[1]+"SDMode"+run_mode_string+" \"\
250         +str(output_mode)+" \"\
251         +str(deterministic_mode)+" \"\
252         +str(fixture)+" \"\
253         +str(ath5founder)+" \"\
254         +str(debug_output)+" \"\
255         +str(curr_start_seed)+" \"\
256         +str(curr_end_seed)+" \"\
257         +str(0)+" \"\
258         +str(earliest_lineage_start_time)+" \"\
259         +str(latest_lineage_start_time)+" \"\
260         +str(events_end_time)+" "
261
262     curr_list.append(validate_rate_command)
263     command_count += 1
264
265     for i in range(0,len(curr_list)):
266         if run_modes[m] == 2:
267             curr_list[i] = curr_list[i] + original_theta_string
268         if run_modes[m] == 0:
269             curr_list[i] = curr_list[i] + stochastic_theta_string
270         if run_modes[m] == 1:
271             curr_list[i] = curr_list[i] + deterministic_theta_string
272
273     command_list = command_list + curr_list
274
275     # Use processes equal to the number of cpus available
276     cpu_count = multiprocessing.cpu_count()
277
278     print(command_list)
279
280     print("Starting simulations with " + str(cpu_count) + " processes")
281
282     # Generate a pool of workers
283     pool = multiprocessing.Pool(processes=cpu_count)

```

```

284     # Pass the list of bash commands to the pool, block until pool is
285     # complete
286     pool.map(execute_command, command_list, 1)
287
288 # This is a helper function for run_simulation that runs bash commands in
289 # separate processes
290 def execute_command(cmd):
291     print("Executing command: " + cmd)
292     return subprocess.call(cmd, shell=True)
293
294 if __name__ == "__main__":
295     main()

```

16.2.8 /python_fixtures/Kolmogorov_fixture.py

```

1 import multiprocessing
2 import os
3 import subprocess
4 import datetime
5 import numpy as np
6 from imageio.plugins._bsdf import BsdfSerializer
7
8 gomes_executable =
9     '/home/main/chaste_build/projects/ISP/apps/GomesSimulator'
10 he_executable = '/home/main/chaste_build/projects/ISP/apps/HeSimulator'
11 boije_executable =
12     '/home/main/chaste_build/projects/ISP/apps/BoijeSimulator'
13
14 empirical_data =
15     '/home/main/git/chaste/projects/ISP/empirical_data/empirical_lineages.csv'
16
17 if not(os.path.isfile(gomes_executable)):
18     raise Exception('Could not find executable: ' + gomes_executable)
19 if not(os.path.isfile(he_executable)):
20     raise Exception('Could not find executable: ' + he_executable)
21 if not(os.path.isfile(boije_executable)):
22     raise Exception('Could not find executable: ' + boije_executable)
23
24 #####
25 # SIMULATION PARAMETERS

```

```

23 #####
24
25 #Define start and end RNG seeds; determines:
26 #No. lineages per loss function run
27 #unique sequence of RNG results for each lineage
28
29 directory = "KolmogorovSequences"
30 empirical_sequences_name = "E0sequences"
31 output_mode = 2 #sequence sampler
32 debug_output = 0 #0=off;1=on
33 start_seed = 0
34 end_seed = 9999
35
36 #####
37 #GLOBAL MODEL PARAMETERS
38 #####
39
40 number_traversal_lineages = 60
41
42 #####
43 # SPECIFIC MODEL PARAMETERS
44 #####
45
46 #GOMES MODEL
47 g_end_time = 480
48 g_normal_mean = 3.9716
49 g_normal_sigma = .32839
50 g_pPP = .055
51 g_pPD = .221
52 g_pBC = .128
53 g_pAC = .106
54 g_pMG = .028
55
56 g_string = gomes_executable + " " + directory + " Gomes " +
   ↵ str(output_mode) + " " + str(debug_output) + " " + str(start_seed) +
   ↵ " " + str(end_seed) + " " + str(g_end_time) + " " +
   ↵ str(g_normal_mean) + " " + str(g_normal_sigma) + " " + str(g_pPP) + " "
   ↵ " " + str(g_pPD) + " " + str(g_pBC) + " " + str(g_pAC) + " " +
   ↵ str(g_pMG)
57
58 #HE MODEL - GENERAL
59 h_fixture = 2
60 h_ath5founder = 0

```

```

61 h_start_time = 0
62 h_end_time = 80
63
64 #HE MODEL - STOCHASTIC
65 h_deterministic_mode = 0
66 h_mitotic_mode_phase_2 = 8 #These are phase lengths, so
67 h_mitotic_mode_phase_3 = 7 #Phase3 boundary = mmp2 + mmp3
68 h_phase_1_pPP = 1.0
69 h_phase_1_pPD = 0.0
70 h_phase_2_pPP = 0.2
71 h_phase_2_pPD = 0.4
72 h_phase_3_pPP = 0.2
73 h_phase_3_pPD = 0.0
74
75 h_string = he_executable + " " + directory + " He " + str(output_mode) +
    " " + str(h_deterministic_mode) + " " + str(h_fixture) + " " +
    str(h_ath5founder) + " " + str(debug_output) + " " + str(start_seed)
    + " " + str(end_seed) + " " + str(h_start_time) + " " +
    str(h_start_time) + " " + str(h_start_time+1) + " " + str(h_end_time)
    + " " + str(h_mitotic_mode_phase_2) + " " +
    str(h_mitotic_mode_phase_3) + " " +str(h_phase_1_pPP) + " " +
    str(h_phase_1_pPD) + " " + str(h_phase_2_pPP) + " " +
    str(h_phase_2_pPD) + " " + str(h_phase_3_pPP) + " " +
    str(h_phase_3_pPD)
76
77 #HE MODEL -STOCHASTIC (REFIT)
78 hr_mitotic_mode_phase_2 = 4.1483 #These are phase lengths, so
79 hr_mitotic_mode_phase_3 = 11.6416 #Phase3 boundary = mmp2 + mmp3
80 hr_phase_1_pPP = 1.0
81 hr_phase_1_pPD = 0.0
82 hr_phase_2_pPP = 0.1959
83 hr_phase_2_pPD = 0.5168
84 hr_phase_3_pPP = 0.2934
85 hr_phase_3_pPD = 0.0
86

```

```

87 hr_string = he_executable + " " + directory + " HeRefit " +
→ str(output_mode) + " " + str(h_deterministic_mode) + " " +
→ str(h_fixture) + " " + str(h_ath5founder) + " " + str(debug_output) +
→ " " + str(start_seed) + " " + str(end_seed) + " " + str(h_start_time)
→ + " " + str(h_start_time) + " " + str(h_start_time+1) + " " +
→ str(h_end_time) + " " + str(hr_mitotic_mode_phase_2) + " " +
→ str(hr_mitotic_mode_phase_3) + " " + str(hr_phase_1_pPP) + " " +
→ str(hr_phase_1_pPD) + " " + str(hr_phase_2_pPP) + " " +
→ str(hr_phase_2_pPD) + " " + str(hr_phase_3_pPP) + " " +
→ str(hr_phase_3_pPD)

88
89 #HE MODEL - DETERMINISTIC ALTERNATIVE
90 d_deterministic_mode = 1
91 #Phase boundary shift parameters
92 d_phase_1_shape = 3.7371
93 d_phase_1_scale = 1.8114
94 d_phase_2_shape = 2.5769
95 d_phase_2_scale = 1.6814
96 d_phase_sister_shift_widths = 1.6326
97 d_phase_offset = 1.2333

98
99 d_string = he_executable + " " + directory + " Deterministic " +
→ str(output_mode) + " " + str(d_deterministic_mode) + " " +
→ str(h_fixture) + " " + str(h_ath5founder) + " " + str(debug_output) +
→ " " + str(start_seed) + " " + str(end_seed) + " " + str(h_start_time)
→ + " " + str(h_start_time) + " " + str(h_start_time+1) + " " +
→ str(h_end_time) + " " + str(d_phase_1_shape) + " " +
→ str(d_phase_1_scale) + " " + str(d_phase_2_shape) + " " +
→ str(d_phase_2_scale) + " " + str(d_phase_sister_shift_widths) + " " +
→ str(d_phase_offset)

100
101 #BOIJE MODEL
102 b_end_generation = 250
103 b_phase_2_generation = 3
104 b_phase_3_generation = 5
105 b_pAtoh7 = .32
106 b_pPtf1a = .3
107 b_png = .8

108

```

```

109 b_string = boije_executable + " " + directory + " Boije " +
→   str(output_mode) + " " + str(debug_output) + " " + str(start_seed) +
→   " " + str(end_seed) + " " + str(b_end_generation) + " " +
→   str(b_phase_2_generation) + " " + str(b_phase_3_generation) + " " +
→   str(b_pAtoh7) + " " + str(b_pPtf1a) + " " + str(b_png)

110
111 #setup the log file, appending to any existing results
112 e_filename =
→   "/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/"
→   +directory +"/" + empirical_sequences_name
113 os.makedirs(os.path.dirname(e_filename), exist_ok=True)

114
115 e_file = open(e_filename, "w")
116 e_file.write("Entry\tSequence\n")

117
118 def main():

119     command_list = []
120     command_list.append(g_string)
121     command_list.append(h_string)
122     command_list.append(hr_string)
123     command_list.append(d_string)
124     command_list.append(b_string)

125
126     # Use processes equal to the number of cpus available
127     cpu_count = multiprocessing.cpu_count()

128
129     print(command_list)

130
131     print("Starting simulations with " + str(cpu_count) + " processes")

132
133     # Generate a pool of workers
134     pool = multiprocessing.Pool(processes=cpu_count)

135
136     # Pass the list of bash commands to the pool, block until pool is
→       complete
137     pool.map(execute_command, command_list, 1)

138
139     traverse_lineages(empirical_data)

140
141
142 def traverse_lineages(data_filename):

143
144     mode_sequences = [None]*(end_seed+1)

```

```

145
146     #load lineage tracing data
147     e_data = np.loadtxt(data_filename, skiprows = 1, usecols = (0,1,2,3))
148
149     #reproducible RNG
150     p_RNG = np.random.RandomState(seed = 0)
151
152     k=0
153
154     for curr_seed in range (start_seed,end_seed+1):
155         random_lineage_number =
156             ↪ p_RNG.randint(1,number_traversal_lineages)
157         lineage_events =
158             ↪ np.array(e_data[np.where(e_data[:,0]==random_lineage_number)])
159
160         #find mitotic mode of first event and write to log
161         current_event = 1
162         mitotic_mode =
163             ↪ int(lineage_events[np.where(lineage_events[:,1]==current_event)][:,3])
164         mode_sequences[k] = f'{mitotic_mode:.0f}'
165
166         while mitotic_mode != 2:
167             if mitotic_mode == 1 and p_RNG.random_sample() < .5: break
168
169             child_events =
170                 ↪ lineage_events[np.where(lineage_events[:,2]==current_event)]
171             random_child_row =
172                 ↪ p_RNG.randint(0,np.ma.size(child_events,0))
173             current_event= child_events[random_child_row,1]
174             mitotic_mode = child_events[random_child_row,3]
175             mode_sequences[k] = mode_sequences[k] + f'{mitotic_mode:.0f}'

176             e_file.write(str(k+1)+"\t"+mode_sequences[k]+\n)
177             k += 1
178
179     return mode_sequences
180
181
182     # This is a helper function for run_simulation that runs bash commands in
183     ↪ separate processes
184     def execute_command(cmd):
185         print("Executing command: " + cmd)
186         return subprocess.call(cmd, shell=True)

```

```
182 if __name__ == "__main__":
183     main()
```

16.2.9 /python_fixtures/SPSA_fixture.py

```
1 import multiprocessing
2 import os
3 import subprocess
4 import datetime
5
6 import numpy as np
7 import matplotlib.pyplot as plt
8 from scipy.stats import bernoulli
9 from fileinput import filename
10 from statsmodels.sandbox.distributions.gof_new import a_st70_upp
11
12 executable = '/home/main/chaste_build/projects/ISP/apps/HeSimulator'
13
14 if not(os.path.isfile(executable)):
15     raise Exception('Could not find executable: ' + executable)
16
17 #####
18 # SPSA COEFFICIENTS
19 #####
20
21 a_s = .025
22 c_s = .1
23
24 a_d = .0019
25 c_d = .1
26
27 A = 20 #10% of expected # iterations
28 alpha = .602
29 gamma = .101
30
31 #####
32 # SPSA & AIC UTILITY VARS
33 #####
34
35 max_iterations = 199
```

```

36 number_comparisons = 3030 #total number of comparison points between
   ↵ model output and empirical data (1000 per induction time, 10 per rate
   ↵ mode)
37 number_comparisons_per_induction = 1000
38 error_samples = 5000 #number of samples to draw when estimating
   ↵ plausibility interval for simulations
39
40 #####
41 # SIMULATION PARAMETERS
42 #####
43
44 #Define start and end RNG seeds; determines:
45 #No. lineages per loss function run
46 #unique sequence of RNG results for each lineage
47 start_seed = 0
48 end_seed = 249
49 rate_end_seed = 99
50 directory_name = "SPSA"
51 file_name_he = "HeSPSA"
52 file_name_det = "DetSPSA"
53 log_name = "HeSPSAOutput"
54 deterministic_modes = [0, 1] #1=det. mode enabled
55 count_output_mode = 0 #0=lineage counts;1=mitotic event
   ↵ logging;2=sequence sampling
56 event_output_mode = 1
57 fixture = 0 #0=He 2012;1=Wan 2016
58 debug_output = 0 #0=off;1=on
59 ath5founder = 0 #0=no morpholino 1=ath5 morpholino
60
61 #####
62 #GLOBAL MODEL PARAMETERS
63 #####
64
65 #Values defining different marker induction timepoints & relative start
   ↵ time of TiL counter
66 earliest_lineage_start_time = 23.0 #RPCs begin to enter "He model regime"
   ↵ nasally at 23hpf
67 latest_lineage_start_time = 39.0 #last temporal retinal RPC has entered
   ↵ "He model regime" at 39hpf
68 induction_times = [ 24, 32, 48 ]
69 end_time = 72.0
70 rate_end_time = 80
71

```

```

72 #####
73 # SPECIFIC MODEL PARAMETERS - THETAHAT
74 #####
75
76 #STOCHASTIC MITOTIC MODE
77 #mitotic mode per-phase probabilities
78 #3-phase mitotic mode time periodisation
79 mitotic_mode_phase_2 = 8 #These are phase lengths, so
80 mitotic_mode_phase_3 = 7 #Phase3 boundary = mmp2 + mmp3
81 phase_1_pPP = 1.0
82 phase_1_pPD = 0.0
83 phase_2_pPP = 0.2
84 phase_2_pPD = 0.4
85 phase_3_pPP = 0.2
86 phase_3_pPD = 0.0
87 he_model_params = 15
88
89 #DETERMINISTIC MITOTIC MODE
90 #Phase boundary shift parameters
91 phase_1_shape = 3
92 phase_1_scale = 2
93 phase_2_shape = 2
94 phase_2_scale = 2
95 phase_sister_shift_widths = .25
96 phase_offset = 0
97 det_model_params = 13
98
99 #array "theta_spsa" is manipulated during SPSA calculations, these are
    # starting point references
100 stochastic_theta_zero = np.array([mitotic_mode_phase_2,
    # mitotic_mode_phase_3, phase_2_pPP, phase_2_pPD, phase_3_pPP ])
101 determinisitic_theta_zero = np.array([phase_1_shape, phase_1_scale,
    # phase_2_shape, phase_2_scale, phase_sister_shift_widths,
    # phase_offset])
102
103 #scales ak gain sequence for probability variables
104 prob_scale_vector = np.array([ 1, 1, .1, .1, .1])
105 shift_scale_vector = np.array([1, 1, 1, 1, 1, 3])
106
107 #####
108 # HE ET AL EMPIRICAL RESULTS
109 #####
110

```

```

111 count_bin_sequence = np.arange(1,32,1)
112 count_x_sequence = np.arange(1,31,1)
113 count_trim_value = 30
114
115 rate_bin_sequence = np.arange(30,85,5)
116 rate_x_sequence = np.arange(30,80,5)
117 rate_trim_value = 10
118
119 #Count probability arrays
120 raw_counts =
    ↳ np.loadtxt('/home/main/git/chaste/projects/ISP/empirical_data/empirical_counts.csv',
    ↳ skiprows=1, usecols=(3,4,5,6,7,8,9,10)) #collect the per-cell-type
    ↳ counts
121 raw_counts_24 = raw_counts[0:64,:]
122 raw_counts_32 = raw_counts[64:233,:]
123 raw_counts_48 = raw_counts[233:396,:]
124
125 prob_24,bin_edges =
    ↳ np.histogram(np.sum(raw_counts_24,axis=1),count_bin_sequence,density=True)
    ↳ #obtain probability density histogram for counts, retabulating by
    ↳ summing across types
126 prob_32,bin_edges =
    ↳ np.histogram(np.sum(raw_counts_32,axis=1),count_bin_sequence,density=True)
127 prob_48,bin_edges =
    ↳ np.histogram(np.sum(raw_counts_48,axis=1),count_bin_sequence,density=True)
128
129 #extend histogram to 1000 - allows large lineages of some iterates to be
    ↳ included in AIC comparison
130 prob_empirical_24 = np.concatenate([prob_24,
    ↳ np.zeros(int(number_comparisons_per_induction) - prob_24.size)])
131 prob_empirical_32 = np.concatenate([prob_32,
    ↳ np.zeros(int(number_comparisons_per_induction) - prob_32.size)])
132 prob_empirical_48 = np.concatenate([prob_48,
    ↳ np.zeros(int(number_comparisons_per_induction) - prob_48.size)])
133
134 count_prob_list = [prob_empirical_24, prob_empirical_32,
    ↳ prob_empirical_48]
135
136 #no. of lineages observed per induction timepoint/event group
137 lineages_sampled_24 = 64
138 lineages_sampled_32 = 169
139 lineages_sampled_48 = 163
140 lineages_sampled_events = 60

```

```

141 lineages_sampled_list =
142     ↳ [lineages_sampled_24,lineages_sampled_32,lineages_sampled_48]
143
144 #Mitotic mode rate probability arrays
145 raw_events =
146     ↳ np.loadtxt('/home/main/git/chaste/projects/ISP/empirical_data/empirical_lineages.
147     ↳ skiprows=1, usecols=(3,5,8))
148 observed_events = raw_events[np.where(raw_events[:,2]==1)] #exclude any
149     ↳ mitosis whose time was too early for recording
150
151 observed_PP = observed_events[np.where(observed_events[:,0]==0)]
152 observed_PD = observed_events[np.where(observed_events[:,0]==1)]
153 observed_DD = observed_events[np.where(observed_events[:,0]==2)]
154
155 histo_PP,bin_edges =
156     ↳ np.histogram(observed_PP,rate_bin_sequence,density=False)
157 histo_PD,bin_edges =
158     ↳ np.histogram(observed_PD,rate_bin_sequence,density=False)
159 histo_DD,bin_edges =
160     ↳ np.histogram(observed_DD,rate_bin_sequence,density=False)
161
162 #hourly per-lineage probabilities- NOT probability density function
163 prob_empirical_PP = np.array((histo_PP/lineages_sampled_events)/5)
164 prob_empirical_PD = np.array((histo_PD/lineages_sampled_events)/5)
165 prob_empirical_DD = np.array((histo_DD/lineages_sampled_events)/5)
166
167 event_prob_list = [prob_empirical_PP,prob_empirical_PD,prob_empirical_DD]
168
169 #setup the log file, appending to any existing results
170 log_filename =
171     ↳ "/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/"
172     ↳ +directory_name +"/" + log_name
173 os.makedirs(os.path.dirname(log_filename), exist_ok=True)
174
175 log = open(log_filename,"w")
176
177 #plotting utility stuff
178 #interactive plot mode
179 plt.ion()
180
181 #setup new iterate plot
182 fig, ((plt0, plt1, plt2),(plt3, plt4, plt5)) =
183     ↳ plt.subplots(2,3,figsize=(12,6))
184 plot_list = [plt0,plt1,plt2,plt3,plt4,plt5]

```

```

174
175
176 def main():
177     global theta_spsa,
178         ↳ a,c,file_name,scale_vector,end_seed,rate_end_seed,alpha,gamma
179
180     for m in range(0,len(deterministic_modes)):
181
182         #traceable RNG
183         p_RNG = np.random.RandomState(seed=786)
184
185         deterministic_mode = deterministic_modes[m]
186         if deterministic_mode == 0:
187             theta_spsa = stochastic_theta_zero
188             a = a_s
189             c = c_s
190             file_name = file_name_he
191             scale_vector = prob_scale_vector
192             now = datetime.datetime.now()
193             log.write("Began SPSA optimisation of He model @\n" +
194             ↳ str(datetime.datetime.now()) + "\n")
195             log.write("k\tphase2\tphase3\tPP2\tPD2\tPP3\n")
196             number_params = he_model_params
197             if deterministic_mode == 1:
198                 theta_spsa = determinisitic_theta_zero
199                 a = a_d
200                 c = c_d
201                 file_name = file_name_det
202                 scale_vector = shift_scale_vector
203                 now = datetime.datetime.now()
204                 log.write("Began SPSA optimisation of deterministic model
205                 ↳ @\n" + str(datetime.datetime.now()) + "\n")
206                 log.write("k\tP1Sh\tP1Sc\tP2Sh\tP2Sc\tSisterShift\tOffset\n")
207                 number_params = det_model_params
208
209                 #SPSA algorithm iterator k starts at 0
210                 k=0
211
212                 while k <= max_iterations:
213                     if k == 0:
214                         end_seed = 249
215                         rate_end_seed = 99

```

```

214     #@defined iterate, increase # of seed to decrease RNG noise
215     #> to low level
216     if k == 169:
217         end_seed = 999
218         rate_end_seed = 249
219
220     #@defined iterate, increase # of seeds to decrease RNG noise
221     #> to close to nil, switch to asymptotically optimal alpha
222     #> and gamma
223     if k == 189:
224         end_seed = 4999
225         rate_end_seed = 1249
226         alpha = 1
227         gamma = (1/6)
228
229     #write the parameter set to be evaluated to file
230     if deterministic_mode == 0: log.write(str(k) + "\t" +
231     #> str(theta_spsa[0]) + "\t" + str(theta_spsa[1]) + "\t" +
232     #> str(theta_spsa[2]) + "\t" + str(theta_spsa[3]) + "\t" +
233     #> str(theta_spsa[4]) + "\n")
234     if deterministic_mode == 1: log.write(str(k) + "\t" +
235     #> str(theta_spsa[0]) + "\t" + str(theta_spsa[1]) + "\t" +
236     #> str(theta_spsa[2]) + "\t" + str(theta_spsa[3]) + "\t" +
237     #> str(theta_spsa[4]) + "\t" + str(theta_spsa[5]) + "\n")
238
239     #populate the deltak perturbation vector with samples from a
240     #> .5p bernoulli +1 -1 distribution
241     delta_k = bernoulli.rvs(.5, size=theta_spsa.size,
242     #> random_state=p_RNG)
243     delta_k[delta_k == 0] = -1
244
245     ak = a / ((A + k + 1)**alpha) #calculate ak from gain
246     #> sequence
247     scaled_ak = ak * scale_vector #scale ak appropriately for
248     #> parameters expressed in hrs & percent
249
250     ck = c / ((k + 1)**gamma) #calculate ck from gain sequence
251     scaled_ck = ck * scale_vector
252
253     #Project theta_spsa into space bounded by ck to allow
254     #> gradient sampling at bounds of probability space

```

```

242     projected_theta = project_theta(theta_spsa, scaled_ck,
243                                     ↵ deterministic_mode)
244
245     #Calculate theta+ and theta- vectors for gradient estimate
246     theta_plus = projected_theta + scaled_ck * delta_k
247     theta_minus = projected_theta - scaled_ck * delta_k
248
249     AIC_gradient = evaluate_AIC_gradient(k, theta_plus,
250                                         ↵ theta_minus, deterministic_mode, number_params,
251                                         ↵ file_name)
252
253     ghat = ((AIC_gradient) / (2 * ck)) * delta_k
254
255     log.write("g0: " + str(ghat[0]) + "\n")
256
257     #update new theta_spsa
258     theta_spsa = theta_spsa - scaled_ak * ghat
259
260     #constrain updated theta_spsa
261     theta_spsa = project_theta(theta_spsa,
262                                 ↵ np.zeros(theta_spsa.size), deterministic_mode)
263
264     k+=1
265
266     #write final result and close log
267     if deterministic_mode == 0: log.write(str(k) + "\t" +
268                                         ↵ str(theta_spsa[0]) + "\t" + str(theta_spsa[1]) + "\t" +
269                                         ↵ str(theta_spsa[2]) + "\t" + str(theta_spsa[3]) + "\t" +
270                                         ↵ str(theta_spsa[4]) + "\n")
271     if deterministic_mode == 1: log.write(str(k) + "\t" +
272                                         ↵ str(theta_spsa[0]) + "\t" + str(theta_spsa[1]) + "\t" +
273                                         ↵ str(theta_spsa[2]) + "\t" + str(theta_spsa[3]) + "\t" +
274                                         ↵ str(theta_spsa[4]) + "\t" + str(theta_spsa[5]) + "\n")
275
276     log.close
277
278 def project_theta(theta, boundary, deterministic_mode):
279     # Required projection is onto unit triangle modified by boundary (ck
280     ↵ value)
281
282     # Values outside the lower bounds are reset first
283     # Then, for phase 2 probabilities, we project (PPa,PDa)→(PPb,PDb) such
284     ↵ that if( (PPa+ck) + (PDa+ck) >1), PPb+ck + PDb +ck = 1.
285
286     # This takes care of the upper bound

```

```

273
274     #project all negative parameters back into bounded space
275     if deterministic_mode == False:
276         for i in range(0, theta.size):
277             if i == 0:
278                 if theta[i] < boundary[i] + 4.0: theta[i] = boundary[i] +
279                     → 4.0 #project mitotic_mode_phase_2 to a minimum of 4-
280                     → below this param has no effect (due to refractory
281                     → period after first division)
282             else:
283                 if theta[i] < boundary[i]: theta[i] = boundary[i]
284
285             #if PP3 exceeds 1-boundary, project back to 1-boundary
286             if theta[4] > 1 - boundary[4]: theta[4] = 1 - boundary[4]
287
288             if theta[2] + theta[3] > 1 - boundary[2]: #if pPP2 + pPD2 gives a
289                 → total beyond the current boundary-reduced total of 1.0
290
291             if theta[2] > (1 - 2 * boundary[2]) and theta[2] ≤ theta[3]
292                 → - (1 - 2 * boundary[2]): # these (PP,PD) points will
293                 → project into negative PD space
294                 theta[2] = 1 - 2 * boundary[2]
295                 theta[3] = boundary[2]
296
297             elif theta[3] > (1 - 2 * boundary[2]) and theta[2] ≤
298                 → theta[3] - (1 - 2 * boundary[2]): # these (PP,PD) points
299                 → will project into negative PP space
300                 theta[3] = 1 - 2 * boundary[2]
301                 theta[2] = boundary[2]
302
303             else: # the (PP,PD) points can be projected onto the line PD
304                 → = -PP + 1 and modified by the boundary;
305                 v1 = [ 1, -1 ] # vector from (0,1) to (1,0)
306                 v2 = [ theta[2], theta[3] - 1 ] #vector from (0,1) to
307                     → (PP,PD)
308                 dot_product = np.dot(v1,v2)
309                 lengthv1 = 2
310                 theta[2] = (dot_product / lengthv1) - boundary[2]
311                 theta[3] = (1 - dot_product / lengthv1) - boundary[2]
312
313             if deterministic_mode == True:
314                 for i in range(0, theta.size-1): #exclude offset param

```

```

305         if theta[i] < boundary[i]: theta[i] = boundary[i] + 0.1
306             ↵ #prevents non→0 arguments for deterministic mode
307
308     #projects sister shift value such that 95% of sister shift values
309     ↵ will be less than smallest mean phase time
310     minPhase = min(theta[0]*theta[1],theta[2]*theta[3])
311     if theta[4] > minPhase/2 - boundary[4]: theta[4] = minPhase/2 -
312         ↵ boundary[4]
313
314     return theta
315
316
317 def evaluate_AIC_gradient(k, theta_plus, theta_minus, deterministic_mode,
318     ↵ number_params, file_name):
319     #Form the simulator commands for current thetas and deterministic
320     ↵ modes
321     command_list = []
322     base_command = executable
323
324     rate_settings = str(event_output_mode)+" "\
325         +str(deterministic_mode)+" "\
326         +str(fixture)+" "\
327         +str(ath5founder)+" "\
328         +str(debug_output)+" "\
329         +str(start_seed)+" "\
330         +str(rate_end_seed)+" "\
331         +str(earliest_lineage_start_time)+" "\
332         +str(earliest_lineage_start_time)+" "\
333         +str(latest_lineage_start_time)+" "\
334         +str(rate_end_time)+" "
335
336     count_settings_1 = str(count_output_mode)+" "\
337         +str(deterministic_mode)+" "\
338         +str(fixture)+" "\
339         +str(ath5founder)+" "\
340         +str(debug_output)+" "\
341         +str(start_seed)+" "\
342         +str(end_seed)+" "
343
344     count_settings_2 = str(earliest_lineage_start_time)+" "\
345         +str(latest_lineage_start_time)+" "\
346         +str(end_time)+" "
347
348     if deterministic_mode == 0:

```

```
343     stochastic_params_plus = str(theta_plus[0])+" "+\
344         +str(theta_plus[1])+" "+\
345         +str(phase_1_pPP)+" "+\
346         +str(phase_1_pPD)+" "+\
347         +str(theta_plus[2])+" "+\
348         +str(theta_plus[3])+" "+\
349         +str(theta_plus[4])+" "+\
350         +str(phase_3_pPD)

352
353     stochastic_params_minus = str(theta_minus[0])+" "+\
354         +str(theta_minus[1])+" "+\
355         +str(phase_1_pPP)+" "+\
356         +str(phase_1_pPD)+" "+\
357         +str(theta_minus[2])+" "+\
358         +str(theta_minus[3])+" "+\
359         +str(theta_minus[4])+" "+\
360         +str(phase_3_pPD)

361
362     command_rate_plus = base_command\
363         +" "+directory_name+" "+file_name+"RatePlus "+\
364         +rate_settings\
365         +stochastic_params_plus

366
367     command_rate_minus = base_command\
368         +" "+directory_name+" "+file_name+"RateMinus "+\
369         +rate_settings\
370         +stochastic_params_minus

371
372     command_list.append(command_rate_plus)
373     command_list.append(command_rate_minus)

374
375     for i in range(0,len(induction_times)):
376         command_plus = base_command\
377             +" "+directory_name+
378             " "+file_name+str(induction_times[i])+"Plus "+\
379             +count_settings_1\
380             +str(induction_times[i])+" "+\
381             +count_settings_2\
382             +stochastic_params_plus

383
384         command_minus = base_command\
```

```

384             + " "+directory_name+
385             ↵ "+file_name+str(induction_times[i])+"Minus "\n
386             +count_settings_1\
387             +str(induction_times[i])+ " "\n
388             +count_settings_2\
389             +stochastic_params_minus

390         command_list.append(command_plus)
391         command_list.append(command_minus)

392
393     if deterministic_mode == 1:

394
395         deterministic_params_plus = str(theta_plus[0])+" "\n
396             +str(theta_plus[1])+" "\n
397             +str(theta_plus[2])+" "\n
398             +str(theta_plus[3])+" "\n
399             +str(theta_plus[4])+" "\n
400             +str(theta_plus[5])

401
402         deterministic_params_minus = str(theta_minus[0])+" "\n
403             +str(theta_minus[1])+" "\n
404             +str(theta_minus[2])+" "\n
405             +str(theta_minus[3])+" "\n
406             +str(theta_minus[4])+" "\n
407             +str(theta_minus[5])

408
409         command_rate_plus = base_command\
410             +" "+directory_name+" "+ file_name+"RatePlus "\n
411             +rate_settings\
412             +deterministic_params_plus

413
414         command_rate_minus = base_command\
415             +" "+directory_name+" "+ file_name+"RateMinus "\n
416             +rate_settings\
417             +deterministic_params_minus

418
419         command_list.append(command_rate_plus)
420         command_list.append(command_rate_minus)

421
422     for i in range(0,len(induction_times)):
423         command_plus = base_command\
424             +" "+directory_name+
425             ↵ "+file_name+str(induction_times[i])+"Plus "\n

```

```

425             +count_settings_1\
426             +str(induction_times[i])+ " "\
427             +count_settings_2\
428             +deterministic_params_plus

429
430     command_minus = base_command\
431         + " "+directory_name+
432             ↳ " "+file_name+str(induction_times[i])+"Minus "\
433             +count_settings_1\
434             +str(induction_times[i])+ " "\
435             +count_settings_2\
436             +deterministic_params_minus

437     command_list.append(command_plus)
438     command_list.append(command_minus)

439
440
441 # Use processes equal to the number of cpus available
442 cpu_count = multiprocessing.cpu_count()

443
444 print("Starting simulations for iterate " + str(k) + " with " +
445       ↳ str(cpu_count) + " processes, " + str(end_seed+1) + " lineages
446       ↳ simulated for counts, " + str(rate_end_seed+1) + " lineages for
447       ↳ events")

448 log.flush() #required to prevent pool from jamming up log for some
449             ↳ reason

450
451 # Generate a pool of workers
452 pool = multiprocessing.Pool(processes=cpu_count)

453
454 # Pass the list of bash commands to the pool, block until pool is
455             ↳ complete
456 pool.map(execute_command, command_list, 1)

457
458 #these numpy arrays hold the individual RSS vals for timepoints +
459             ↳ rates
460 rss_plus = np.zeros(len(induction_times)+3)
461 rss_minus = np.zeros(len(induction_times)+3)

462
463 #clear the plots on the interactive figure
464 for i in range(0, len(plot_list)):
465     plt.sca(plot_list[i])

```



```

486     histo_rate_plus, bin_edges = np.histogram(mode_rate_plus[:,0],
487         ↵ rate_bin_sequence, density=False)
487     histo_rate_minus, bin_edges = np.histogram(mode_rate_minus[:,0],
488         ↵ rate_bin_sequence, density=False)

488
489     #hourly per-lineage probabilities
490     prob_histo_rate_plus = np.array(histo_rate_plus / ((rate_end_seed
491         ↵ + 1)*5))
491     prob_histo_rate_minus = np.array(histo_rate_plus /
492         ↵ ((rate_end_seed + 1)*5))

492
493     residual_plus = prob_histo_rate_plus - event_prob_list[i]
494     residual_minus = prob_histo_rate_minus - event_prob_list[i]

495
496     #PD RESIUDAL WEIGHTING
497     if i == 1:
498         rss_plus[i+3] = np.sum(1.5*np.square(residual_plus))
499         rss_minus[i+3] = np.sum(1.5*np.square(residual_minus))

500
501     else:
502         rss_plus[i+3] = np.sum(np.square(residual_plus))
503         rss_minus[i+3] = np.sum(np.square(residual_minus))

504
505     plotter(plot_list[i+3], mode_rate_plus[:,0],
506         ↵ mode_rate_minus[:,0],
507         ↵ event_prob_list[i],lineages_sampled_events, 1)

508
509     AIC_plus = 2 * number_params + number_comparisons *
510         ↵ np.log(np.sum(rss_plus))
511     AIC_minus = 2 * number_params + number_comparisons *
512         ↵ np.log(np.sum(rss_minus))

513     plt.show()

514     ↵ plt.savefig("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/"
515         ↵ +directory_name + "/" + "SDmode" + str(deterministic_mode) +
516         ↵ "iterate" + str(k) + ".png")

517
518     log.write("theta_plus:\n")
519     if deterministic_mode == 0: log.write(str(k) + "\t" +
520         ↵ str(theta_plus[0]) + "\t" + str(theta_plus[1]) + "\t" +
521         ↵ str(theta_plus[2]) + "\t" + str(theta_plus[3]) + "\t" +
522         ↵ str(theta_plus[4]) + "\n")

```

```

515     if deterministic_mode == 1: log.write(str(k) + "\t" +
516         str(theta_plus[0]) + "\t" + str(theta_plus[1]) + "\t" +
517         str(theta_plus[2]) + "\t" + str(theta_plus[3]) + "\t" +
518         str(theta_plus[4]) + "\t" + str(theta_plus[5]) +"\n")
519     log.write("theta_minus:\n")
520     if deterministic_mode == 0: log.write(str(k) + "\t" +
521         str(theta_minus[0]) + "\t" + str(theta_minus[1]) + "\t" +
522         str(theta_minus[2]) + "\t" + str(theta_minus[3]) + "\t" +
523         str(theta_minus[4]) + "\n")
524     if deterministic_mode == 1: log.write(str(k) + "\t" +
525         str(theta_minus[0]) + "\t" + str(theta_minus[1]) + "\t" +
526         str(theta_minus[2]) + "\t" + str(theta_minus[3]) + "\t" +
527         str(theta_minus[4]) + "\t" + str(theta_minus[5]) + "\n")
528
529     log.write("PositiveAIC: " + str(AIC_plus) + " NegativeAIC: " +
530         str(AIC_minus) + "\n")
531
532     AIC_gradient_sample = AIC_plus - AIC_minus;
533
534     return AIC_gradient_sample
535
536 #data plotter function for monitoring SPSA results
537 def plotter(subplot,plus,minus,empirical_prob,samples,mode):
538     global plt0,plt1,plt2,plt3,plt4,plt5, prob_histo_plus,
539         prob_histo_minus
540     bin_sequence = []
541     x_sequence = []
542     trim_value = 0
543
544     if mode == 0:
545         bin_sequence = count_bin_sequence
546         x_sequence = count_x_sequence
547         trim_value = count_trim_value
548         prob_histo_plus, bin_edges = np.histogram(plus, bin_sequence,
549             density=True)
550         prob_histo_minus, bin_edges = np.histogram(minus, bin_sequence,
551             density=True)
552
553     if mode == 1:
554         bin_sequence = rate_bin_sequence
555         x_sequence = rate_x_sequence
556         trim_value = rate_trim_value

```

```
544     histo_plus, bin_edges = np.histogram(plus, bin_sequence,
545         ↵ density=False)
546     histo_minus, bin_edges = np.histogram(minus, bin_sequence,
547         ↵ density=False)
548     prob_histo_plus = np.array(histo_plus / ((rate_end_seed + 1)*5))
549     prob_histo_minus = np.array(histo_plus / ((rate_end_seed + 1)*5))
550
551     trimmed_prob = empirical_prob[0:trim_value]
552
553     subplot.plot(x_sequence,trimmed_prob, 'k+')
554     plt.pause(0.0001)
555
556     interval_plus = sampler(plus,samples,bin_sequence)
557
558     subplot.plot(x_sequence,prob_histo_plus, 'g-')
559     plt.pause(0.0001)
560     subplot.fill_between(x_sequence, (prob_histo_plus - interval_plus),
561         ↵ (prob_histo_plus + interval_plus), alpha=0.2,
562         ↵ edgecolor='#008000', facecolor='#00FF00')
563     plt.pause(0.0001)
564
565     interval_minus = sampler(minus,samples,bin_sequence)
566
567     subplot.plot(x_sequence,prob_histo_minus, 'm-')
568     plt.pause(0.0001)
569     subplot.fill_between(x_sequence, (prob_histo_minus - interval_minus),
570         ↵ (prob_histo_minus + interval_minus), alpha=0.2,
571         ↵ edgecolor='#800080', facecolor='#FF00FF')
572     plt.pause(0.0001)
573
574     if subplot=plt0: plt0.set_ylim((0,.20))
575     if subplot=plt1: plt1.set_ylim(0,.20)
576     if subplot=plt2: plt2.set_ylim(0,.7)
577     if subplot=plt3: plt3.set_ylim(0, .30)
578     if subplot=plt4: plt4.set_ylim(0, .15)
579     if subplot=plt5: plt5.set_ylim(0, .35)
580
581 def sampler(data,samples,bin_sequence):
582
583     if len(data) == 0: #catches edge case w/ no entries for a mitotic
584         ↵ mode
585         data=[0]
```

```

580     base_sample=np.zeros((error_samples,len(bin_sequence)-1))
581
582     for i in range(0,error_samples):
583         new_data_sample = np.random.choice(data,samples)
584         new_histo_prob, bin_edges = np.histogram(new_data_sample,
585             ↪ bin_sequence, density=True)
586         base_sample[i,:] = new_histo_prob
587
588     sample_95CI = np.array(2 * (np.std(base_sample,0)))
589
590     return sample_95CI
591
592 # This is a helper function for run_simulation that runs bash commands in
593 # separate processes
594
595 def execute_command(cmd):
596     return subprocess.call(cmd, shell=True)
597
598 if __name__ == "__main__":
599     main()

```

16.2.10 /python_fixtures/Wan_output_fixture.py

```

1 import multiprocessing
2 import os
3 import subprocess
4 import datetime
5
6 import numpy as np
7 from openpyxl.styles.builtins import output
8 from sklearn.datasets.tests.test_svmlight_format import currdir
9
10 executable = '/home/main/chaste_build/projects/ISP/apps/WanSimulator'
11
12 if not(os.path.isfile(executable)):
13     raise Exception('Could not find executable: ' + executable)
14
15 ##########
16 # SIMULATION PARAMETERS
17 #####
18
19 #Define start and end RNG seeds; determines:
20 #No. simulated CMZs per run

```

```

21 #unique sequence of RNG results for each lineage
22 start_seed = 0
23 end_seed = 99 #total seeds should be divisible by # cores
24
25 output_directory = "WanOutput"
26
27 #####
28 #MODEL PARAMETERS
29 #####
30
31 #CMZ residency time
32 wan_residency_time = 17.0
33 #factor to divide 3dpf progenitor population by to obtain estimate of
34   ↳ stem cell population
34 stem_divisor = 10
35 #3dpf CMZ progenitor population mean and standard deviation
36 progenitor_mean = 792
37 progenitor_std = 160
38
39 #stem cell cycle parameters- cell cycle RV is a shifted gamma
40   ↳ distribution
40 stem_gamma_shift = 4
41 stem_gamma_shape = 6.5 #mean 30 hr stem cell time - in reality is
42   ↳ probably more like 60+
42 stem_gamma_scale = 4
43
44 progenitor_gamma_shift = 4 #default He et al. values, mean 6 hr cycle
45   ↳ time
45 progenitor_gamma_shape = 2
46 progenitor_gamma_scale = 1
47 progenitor_gamma_sister = 1
48
49 cmz_theta_string = str(wan_residency_time) + " " + str(stem_divisor) + "
50   ↳ " + str(progenitor_mean) + " " + str(progenitor_std) + " "
51   ↳ str(stem_gamma_shift) + " " + str(stem_gamma_shape) + " "
52   ↳ str(stem_gamma_scale) + " " + str(progenitor_gamma_shift) + " "
53   ↳ str(progenitor_gamma_shape) + " " + str(progenitor_gamma_scale) + " "
54   ↳ + str(progenitor_gamma_sister)
50
51 #STOCHASTIC MITOTIC MODE
52 #HE ORIGINAL PARAMETERS
53 #mitotic mode per-phase probabilities
54 #3-phase mitotic mode time periodisation

```

```

55 mitotic_mode_phase_2 = 8 #These are phase boundaries rather than lengths
  ↵ as in eg. He_output_fixture.py
56 mitotic_mode_phase_3 = 15
57 phase_1_pPP = 1.0
58 phase_1_pPD = 0.0
59 phase_2_pPP = 0.2
60 phase_2_pPD = 0.4
61 phase_3_pPP = 0.2
62 phase_3_pPD = 0.0
63
64 stochastic_theta_string = str(mitotic_mode_phase_2) + " " +
  ↵ str(mitotic_mode_phase_3) + " " +str(phase_1_pPP) + " " +
  ↵ str(phase_1_pPD) + " " + str(phase_2_pPP) + " " + str(phase_2_pPD) +
  ↵ " " + str(phase_3_pPP) + " " + str(phase_3_pPD)
65
66 def main():
67
68     command_list = []
69
70     # Use processes equal to the number of cpus available
71     cpu_count = multiprocessing.cpu_count()
72     seeds_per_command = (end_seed + 1) / cpu_count
73     base_command = executable + " " + output_directory
74
75     for i in range(0,cpu_count):
76         curr_start_seed = i * seeds_per_command
77         curr_end_seed = curr_start_seed + (seeds_per_command - 1)
78
79         command = base_command + " "\\
80             +str(curr_start_seed) + " "\\
81             +str(curr_end_seed) + " "\\
82             +cmz_theta_string + " "\\
83             +stochastic_theta_string
84
85     #command_list.append(command)
86
87     ↵ command_list.append("/home/main/chaste_build/projects/ISP/apps/WanSimulator"
     ↵ WanOutput 18.0 24.0 17.0 10 792 160 4 6.5 4 4 2 1 1 8 15 1.0 0.0
     ↵ 0.2 0.4 0.2 0.0")

```

```

88
    ↳ command_list.append( "/home/main/chaste_build/projects/ISP/apps/WanSimulator
    ↳ WanOutput 42.0 49.0 17.0 10 792 160 4 6.5 4 4 2 1 1 8 15 1.0 0.0
    ↳ 0.2 0.4 0.2 0.0" )

89
    ↳ command_list.append( "/home/main/chaste_build/projects/ISP/apps/WanSimulator
    ↳ WanOutput 51.0 57.0 17.0 10 792 160 4 6.5 4 4 2 1 1 8 15 1.0 0.0
    ↳ 0.2 0.4 0.2 0.0" )

90
    ↳ command_list.append( "/home/main/chaste_build/projects/ISP/apps/WanSimulator
    ↳ WanOutput 94.0 99.0 17.0 10 792 160 4 6.5 4 4 2 1 1 8 15 1.0 0.0
    ↳ 0.2 0.4 0.2 0.0" )

91
    ↳ command_list.append( "/home/main/chaste_build/projects/ISP/apps/WanSimulator
    ↳ WanOutput 58.0 64.0 17.0 10 792 160 4 6.5 4 4 2 1 1 8 15 1.0 0.0
    ↳ 0.2 0.4 0.2 0.0" )

92
    ↳ command_list.append( "/home/main/chaste_build/projects/ISP/apps/WanSimulator
    ↳ WanOutput 65.0 70.0 17.0 10 792 160 4 6.5 4 4 2 1 1 8 15 1.0 0.0
    ↳ 0.2 0.4 0.2 0.0" )

93
    ↳ command_list.append( "/home/main/chaste_build/projects/ISP/apps/WanSimulator
    ↳ WanOutput 71.0 74.0 17.0 10 792 160 4 6.5 4 4 2 1 1 8 15 1.0 0.0
    ↳ 0.2 0.4 0.2 0.0" )

94
print("Starting simulations with " + str(cpu_count) + " processes")

95
# Generate a pool of workers
pool = multiprocessing.Pool(processes=cpu_count)

96
97
# Pass the list of bash commands to the pool, block until pool is
    ↳ complete
98
pool.map(execute_command, command_list, 1)

99
100
101
102
103
104 # This is a helper function for run_simulation that runs bash commands in
    ↳ separate processes
105 def execute_command(cmd):
106     print("Executing command: " + cmd)
107     return subprocess.call(cmd, shell=True)

108
109
110 if __name__ == "__main__":

```

```
111     main()
```

16.2.11 /python_fixtures/diagram_utility_scripts/Gomes_He_cycle_plots.py

```
1 import os
2 import numpy as np
3 import matplotlib.pyplot as plt
4 from fileinput import filename
5 from scipy.stats import lognorm
6 from scipy.stats import gamma
7 from statsmodels.sandbox.distributions.gof_new import a_st70_upp
8
9 #Model parameters
10 gomes_normal_sigma = .32839
11 gomes_normal_mu = 3.9716
12
13 he_gamma_shape = 2
14 he_gamma_scale = 1
15 he_gamma_offset = 4
16
17 def main():
18     gomes_x_range = np.arange(1,151,1)
19     gomes_y = lognorm.pdf(gomes_x_range, gomes_normal_sigma, 0,
20                           np.exp(gomes_normal_mu))
21
22     he_x_range = np.arange(1,16,.1)
23     he_y = gamma.pdf(he_x_range, he_gamma_shape, he_gamma_offset,
24                       he_gamma_scale)
25
26     fig, (ax_g, ax_h) = plt.subplots(1,2,figsize=(6,2))
27
28     ax_g.plot(gomes_x_range,gomes_y, 'k-')
29     ax_h.plot(he_x_range, he_y, 'k-')
30
31     plt.sca(ax_g)
32     plt.xlabel("Cell cycle duration (h)")
33     plt.ylabel("Probability")
34
35     plt.sca(ax_h)
36     plt.xlabel("Cell cycle duration (h)")
37     plt.ylabel("Probability")
```

```

36
37     plt.tight_layout()
38     plt.savefig('/home/main/Desktop/utility.png', transparent=True)
39     plt.show()
40
41 if __name__ == "__main__":
42     main()

```

16.2.12 /python_fixtures/diagram_utility_scripts/He_Boije_signal_plots.py

```

1 import os
2 import numpy as np
3 import matplotlib.pyplot as plt
4 from fileinput import filename
5 from scipy.stats import lognorm
6 from scipy.stats import gamma
7 from statsmodels.sandbox.distributions.gof_new import a_st70_upp
8
9 #Model parameters
10 he_x = [0,8,15,25]
11 he_pp_y = [1,.2,.2,.2]
12 he_pd_y = [0,.4,0,0]
13 he_dd_y = [0,.4,.8,.8]
14
15 boije_x = [0,3,5,9]
16 boije_pAtoh7 = [0,.32,0,0]
17 boije_pPtf1a = [0,.3,0,0]
18 boije_pNg = [0,0,.8,.8]
19
20 def main():
21     fig, ((pp,pd,dd))= plt.subplots(1,3,figsize=(6,2),sharey=True)
22
23     fig2, ((pAtoh7),(pPtf1a),(pNg)) =
24         plt.subplots(3,1,figsize=(2,4),sharex=True)
25     pp.set_xlim((0,24))
26     pd.set_xlim((0,24))
27     dd.set_xlim((0,24))
28
29     pp.set_xticks([8,15])
30     pd.set_xticks([8,15])

```

```
31     dd.set_xticks([8,15])
32
33     pp.set_ylim((- .1,1.1))
34     pp.set_yticks(np.arange(0,1.2,.2))
35     pp.set_ylabel("Probability")
36     pp.set_xlabel("TiL (h)")
37     pp.axvline(8,linestyle='--',color='.1', alpha=.2)
38     pp.axvline(15,linestyle='--',color='.1', alpha=.2)
39     pp.step(he_x,he_pp_y, 'k-', where='post', linewidth=2)
40
41     pd.set_ylim((- .1,1.1))
42     pd.set_yticks(np.arange(0,1.2,.2))
43     pd.set_xlabel("TiL (h)")
44     pd.axvline(8,linestyle='--',color='.1', alpha=.2)
45     pd.axvline(15,linestyle='--',color='.1', alpha=.2)
46     pd.step(he_x,he_pd_y, 'k-', where='post', linewidth=2)
47
48     dd.set_ylim((- .1,1.1))
49     dd.set_yticks(np.arange(0,1.2,.2))
50     dd.set_xlabel("TiL (h)")
51     dd.axvline(8,linestyle='--',color='.1', alpha=.2)
52     dd.axvline(15,linestyle='--',color='.1', alpha=.2)
53     dd.step(he_x,he_dd_y, 'k-', where='post', linewidth=2)
54
55     pAtoh7.set_xlim((0,8))
56     pNg.set_xlim((0,8))
57     pPtf1a.set_xlim((0,8))
58
59     pAtoh7.set_xticks([3,5])
60     pNg.set_xticks([3,5])
61     pPtf1a.set_xticks([3,5])
62
63
64     pNg.set_xlabel("Generation")
65
66
67     pAtoh7.set_ylim((- .1,1.1))
68     pAtoh7.set_yticks(np.arange(0,1.25,.25))
69     pAtoh7.set_ylabel("Probability")
70     pAtoh7.axvline(3,linestyle='--',color='.1',alpha=.2)
71     pAtoh7.axvline(5,linestyle='--',color='.1',alpha=.2)
72     pAtoh7.step(boije_x,boije_pAtoh7, 'k-', where='post', linewidth=2)
73
```

```

74     pPtf1a.set_ylim((-1,1.1))
75     pPtf1a.set_yticks(np.arange(0,1.25,.25))
76     pPtf1a.set_ylabel("Probability")
77     pPtf1a.axvline(3,linestyle='--',color='.1',alpha=.2)
78     pPtf1a.axvline(5,linestyle='--',color='.1',alpha=.2)
79     pPtf1a.step(boije_x,boije_pPtf1a, 'k-', where='post', linewidth=2)

80
81     pNg.set_ylim((-1,1.1))
82     pNg.set_yticks(np.arange(0,1.25,.25))
83     pNg.set_ylabel("Probability")
84     pNg.axvline(3,linestyle='--',color='.1',alpha=.2)
85     pNg.axvline(5,linestyle='--',color='.1',alpha=.2)
86     pNg.step(boije_x,boije_pNg, 'k-', where='post', linewidth=2)

87
88
89     plt.tight_layout()
90     fig.savefig('Hemode.png', transparent=True)
91     fig2.savefig('Boijesignals.png', transparent=True)
92     plt.show()

93
94 if __name__ == "__main__":
95     main()

```

16.2.13 /python_fixtures/figure_plots/Cumulative_EdU.py

```

1 import os
2 import numpy as np
3 import statsmodels.api as sm
4 import pandas as pd
5 import matplotlib.pyplot as plt
6 import seaborn as sns
7
8 from PIL import Image
9 from io import BytesIO
10
11
12 #PLoS formatting stuff
13 plt.rcParams['font.size'] = 12
14 plt.rcParams['font.family'] = 'sans-serif'
15 plt.rcParams['font.sans-serif'] = ['Arial']
16
17 def main():

```

```

18 #Read & parse raw data file
19 raw_data =
20     → pd.read_excel('/home/main/git/chaste/projects/ISP/empirical_data/cumulative_e
21 #group data by dorsal/ventral
22 dv_grouped = raw_data.groupby('D/V')
23 #make new dataframe for totals and labelled fraction
24 totals_frame = dv_grouped.get_group('D')
25 #reset indices to ventral frame for addition operatinos
26 totals_frame =
27     → totals_frame.set_index(dv_grouped.get_group('V').index)
28 #sum D + V PCNA & EdU counts
29 totals_frame.PCNA = totals_frame.PCNA +
30     → dv_grouped.get_group('V').PCNA
31 totals_frame.EdU = totals_frame.EdU + dv_grouped.get_group('V').EdU
32 #create new column for labelled fraction
33 totals_frame['labelled_fraction'] = totals_frame.EdU /
34     → totals_frame.PCNA
35
36 #setup x for linear regression with y-intercept constant
37 X = totals_frame.Time
38 X = sm.add_constant(X)
39
40 model = sm.OLS(totals_frame.labelled_fraction, X).fit()
41 print(model.summary())
42 fit_results = model.params
43
44 tc = 1/fit_results[1]
45 ts = tc * fit_results[0]
46
47 sns.regplot(totals_frame.Time,totals_frame.labelled_fraction)
48
49 plt.text(7,.2,"Tc: " + f'{tc:.2f}')
50 plt.text(7,.1,"Ts: " + f'{ts:.2f}')
51
52 plt.xlabel("Time (h)")
53 plt.ylabel("Fraction of CMZ RPCs labelled by EdU")
54
55 plt.savefig("cumulative_edu.png")
56 png_memory = BytesIO()
57 plt.savefig(png_memory, format='png', dpi=600)
58 PILpng = Image.open(png_memory)
59 PILpng.save('cumulative_edu.tiff')
60 png_memory.close()

```

```
57
58     plt.show()
59
60 if __name__ == "__main__":
61     main()
```

16.2.14 /python_fixtures/figure_plots/He_output_plot.py

```
1 import os
2 import numpy as np
3 import matplotlib.pyplot as plt
4 import matplotlib.patches as patches
5 from fileinput import filename
6 from statsmodels.sandbox.distributions.gof_new import a_st70_upp
7
8 from PIL import Image
9 from io import BytesIO
10
11 #PLoS formatting stuff
12 plt.rcParams['font.size'] = 10
13 plt.rcParams['font.family'] = 'sans-serif'
14 plt.rcParams['font.sans-serif'] = ['Arial']
15
16 #AIC & Plotting utility params
17 count_seeds = 10000
18 event_seeds = 1000
19 error_samples = 5000 #number of samples to draw when estimating
    ↳ plausibility interval for simulations
20
21 he_model_params = 15
22 det_model_params = 13
23
24 #stats & line_plotter utility arrays
25 bin_sequence_24_32 = np.arange(1,26,1)
26 x_sequence_24_32 = np.arange(1,25,1)
27
28 bin_sequence_48 = np.arange(1,11,1)
29 x_sequence_48 = np.arange(1,10,1)
30
31 bin_sequence_wan = np.arange(2,17,1)
32 x_sequence_wan = np.arange(2,16,1)
33
```

```

34 bin_sequence_events = np.arange(30,85,5)
35 x_sequence_events = np.arange(30,80,5)
36
37 ##########
38 # HE ET AL EMPIRICAL RESULTS
39 #########
40
41 #Count probability arrays
42 raw_counts =
    ↵ np.loadtxt('/home/main/git/chaste/projects/ISP/empirical_data/empirical_counts.cs'
    ↵ skiprows=1, usecols=(3,4,5,6,7,8,9,10)) #collect the per-cell-type
    ↵ counts
43 raw_counts_24 = raw_counts[0:64,:]
44 raw_counts_32 = raw_counts[64:233,:]
45 raw_counts_48 = raw_counts[233:396,:]
46
47 prob_empirical_24,bin_edges =
    ↵ np.histogram(np.sum(raw_counts_24,axis=1),bin_sequence_24_32,density=True)
    ↵ #obtain probability density histogram for counts, retabulating by
    ↵ summing across types
48 prob_empirical_32,bin_edges =
    ↵ np.histogram(np.sum(raw_counts_32,axis=1),bin_sequence_24_32,density=True)
49 prob_empirical_48,bin_edges =
    ↵ np.histogram(np.sum(raw_counts_48,axis=1),bin_sequence_48,density=True)
50
51 #no. of lineages E0 per induction timepoint/event group
52 lineages_sampled_24 = 64
53 lineages_sampled_32 = 169
54 lineages_sampled_48 = 163
55 lineages_sampled_events = 60
56
57 #Mitotic mode rate probability arrays
58 raw_events =
    ↵ np.loadtxt('/home/main/git/chaste/projects/ISP/empirical_data/empirical_lineages.cs'
    ↵ skiprows=1, usecols=(3,5,8))
59 E0_events = raw_events[np.where(raw_events[:,2]==1)] #exclude any mitosis
    ↵ whose time was too early for recording
60
61 E0_PP = E0_events[np.where(E0_events[:,0]==0)]
62 E0_PD = E0_events[np.where(E0_events[:,0]==1)]
63 E0_DD = E0_events[np.where(E0_events[:,0]==2)]
64

```

```

65 prob_empirical_PP,bin_edges =
   ↵ np.histogram(EO_PP,bin_sequence_events,density=True)
66 prob_empirical_PD,bin_edges =
   ↵ np.histogram(EO_PD,bin_sequence_events,density=True)
67 prob_empirical_DD,bin_edges =
   ↵ np.histogram(EO_DD,bin_sequence_events,density=True)

68
69 empirical_fit_list =
   ↵ [prob_empirical_24,prob_empirical_32,prob_empirical_48,prob_empirical_PP,prob_emp

70
71 #mutant results
72 wt_mean_clone_size = 6
73 ath5_mean_clone_size = 8
74 wt_even_odd_ratio = 1.1
75 ath5_even_odd_ratio = 2.2

76
77 ##########
78 # WAN ET AL EMPIRICAL RESULTS
79 #####
80 #(derived from figure)
81 prob_wan =
   ↵ [.3143,.1857,.1757,.0871,.0871,.0786,.0271,.0179,.0071,0,0,.0071];
82 lineages_sampled_wan = 40 #not reported- approximation based on 95% CI

83
84 empirical_test_list = [prob_wan, wt_mean_clone_size,
   ↵ ath5_mean_clone_size, wt_even_odd_ratio, ath5_even_odd_ratio]

85
86 def main():
87     #LOAD & PARSE HE MODEL OUTPUT FILES
88     #original fit stochastic mitotic mode files
89     o_counts_24 =
       ↵ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
       ↵ skiprows=1, usecols=3)
90     o_counts_32 =
       ↵ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
       ↵ skiprows=1, usecols=3)
91     o_counts_48 =
       ↵ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
       ↵ skiprows=1, usecols=3)
92     o_events =
       ↵ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeM
       ↵ skiprows=1, usecols=(0,3))
93     o_events_PP = np.array(o_events[np.where(o_events[:,1]==0)])

```

```
94     o_events_PD = np.array(o_events[np.where(o_events[:,1]==1)])
95     o_events_DD = np.array(o_events[np.where(o_events[:,1]==2)])
96     o_counts_wan_no_shadow =
97         ↳ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
98             ↳ skiprows=1, usecols=3)
99
100    #refit stochastic mitotic mode files
101    s_counts_24 =
102        ↳ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
103            ↳ skiprows=1, usecols=3)
104    s_counts_32 =
105        ↳ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
106            ↳ skiprows=1, usecols=3)
107    s_counts_48 =
108        ↳ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
109            ↳ skiprows=1, usecols=3)
110    s_events =
111        ↳ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeM
112            ↳ skiprows=1, usecols=(0,3))
113    s_events_PP = np.array(s_events[np.where(s_events[:,1]==0)])
114    s_events_PD = np.array(s_events[np.where(s_events[:,1]==1)])
115    s_events_DD = np.array(s_events[np.where(s_events[:,1]==2)])
116    s_counts_wan_no_shadow =
117        ↳ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
118            ↳ skiprows=1, usecols=3)
119    s_counts_ath5 =
120        ↳ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
121            ↳ skiprows=1, usecols=3)
122    s_counts_validate =
123        ↳ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
124            ↳ skiprows=1, usecols=3)
125
126    #deterministic mitotic mode files
127    d_counts_24 =
128        ↳ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
129            ↳ skiprows=1, usecols=3)
```

```

114 d_counts_32 =
115     ↵ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
116     ↵ skiprows=1, usecols=3)
117 d_counts_48 =
118     ↵ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
119     ↵ skiprows=1, usecols=3)
120 d_events =
121     ↵ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeM
122     ↵ skiprows=1, usecols=(0,3))
123 d_events_PP = np.array(d_events[np.where(d_events[:,1]==0)])
124 d_events_PD = np.array(d_events[np.where(d_events[:,1]==1)])
125 d_events_DD = np.array(d_events[np.where(d_events[:,1]==2)])
126 d_counts_wan_no_shadow =
127     ↵ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
128     ↵ skiprows=1, usecols=3)
129 d_counts_ath5 =
130     ↵ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
131     ↵ skiprows=1, usecols=3)
132 d_counts_validate =
133     ↵ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeC
134     ↵ skiprows=1, usecols=3)

135 #calculate Ath5 clone size and even-odd ratios
136 o_wt_mean_clone_size = np.mean(o_counts_validate)
137 o_ath5_mean_clone_size = np.mean(o_counts_ath5)

138 s_wt_mean_clone_size = np.mean(s_counts_validate)
139 s_ath5_mean_clone_size = np.mean(s_counts_ath5)

140 d_wt_mean_clone_size = np.mean(d_counts_validate)
141 d_ath5_mean_clone_size = np.mean(d_counts_ath5)

142 o_wt_even_odd_ratio = len(np.where(o_counts_validate % 2 ==
143     ↵ 0)[0])/len(np.where(o_counts_validate % 2 == 1)[0])
144 o_ath5_even_odd_ratio = len(np.where(o_counts_ath5 % 2 ==
145     ↵ 0)[0])/len(np.where(o_counts_ath5 % 2 == 1)[0])

146 s_wt_even_odd_ratio = len(np.where(s_counts_validate % 2 ==
147     ↵ 0)[0])/len(np.where(s_counts_validate % 2 == 1)[0])
148 s_ath5_even_odd_ratio = len(np.where(s_counts_ath5 % 2 ==
149     ↵ 0)[0])/len(np.where(s_counts_ath5 % 2 == 1)[0])

```

```

140 d_wt_even_odd_ratio = len(np.where(d_counts_validate % 2 ==
141   ↵ 0)[0])/len(np.where(d_counts_validate % 2 == 1)[0])
d_ath5_even_odd_ratio = len(np.where(d_counts_ath5 % 2 ==
142   ↵ 0)[0])/len(np.where(d_counts_ath5 % 2 == 1)[0])

143 o_fit_output_list = [o_counts_24, o_counts_32, o_counts_48,
144   ↵ o_events_PP, o_events_PD, o_events_DD]
o_test_output_list = [o_counts_wan_no_shadow, o_wt_mean_clone_size,
145   ↵ o_ath5_mean_clone_size, o_wt_even_odd_ratio,
146   ↵ o_ath5_even_odd_ratio]

147 s_fit_output_list = [s_counts_24, s_counts_32, s_counts_48,
148   ↵ s_events_PP, s_events_PD, s_events_DD]
s_test_output_list = [s_counts_wan_no_shadow, s_wt_mean_clone_size,
149   ↵ s_ath5_mean_clone_size, s_wt_even_odd_ratio,
150   ↵ s_ath5_even_odd_ratio]

151 d_fit_output_list = [d_counts_24, d_counts_32, d_counts_48,
152   ↵ d_events_PP, d_events_PD, d_events_DD]
d_test_output_list = [d_counts_wan_no_shadow, d_wt_mean_clone_size,
153   ↵ d_ath5_mean_clone_size, d_wt_even_odd_ratio,
154   ↵ d_ath5_even_odd_ratio]

155 #SETUP FIGURES
156 #4 figures to be generated; 3 show output of individual model modes,
157   ↵ 4th overlays stochastic refit on deterministic fit
158 o_fig, ((o_24,o_32),(o_48,o_PP),(o_PD,o_DD),(o_wan_noshad,
159   ↵ o_ath5size),(o_ratio,o_unused)) =
160   ↵ plt.subplots(5,2,figsize=(7.5,8.75))
161 s_fig, ((s_24,s_32),(s_48,s_PP),(s_PD,s_DD),(s_wan_noshad,
162   ↵ s_ath5size),(s_ratio,s_unused)) =
163   ↵ plt.subplots(5,2,figsize=(7.5,8.75))
164 d_fig, ((d_24,d_32),(d_48,d_PP),(d_PD,d_DD),(d_wan_noshad,
165   ↵ d_ath5size),(d_ratio,d_unused)) =
166   ↵ plt.subplots(5,2,figsize=(7.5,8.75))
167 sd_fig, ((sd_24,sd_32),(sd_48,sd_PP),(sd_PD,sd_DD),(sd_wan_noshad,
168   ↵ sd_ath5size),(sd_ratio,sd_unused)) =
169   ↵ plt.subplots(5,2,figsize=(7.5,8.75))

170 #turn off unused axes
171
172   ↵ o_unused.axis('off');s_unused.axis('off');d_unused.axis('off');sd_unused.axis('off')

```

```
162 #set xlabel
163
164     → o_24.set_xlabel('Count');o_32.set_xlabel('Count');o_48.set_xlabel('Count');o_
165     → _PP.set_xlabel('Age (hpf)');o_PD.set_xlabel('Age
166     → (hpf)');o_DD.set_xlabel('Age (hpf)')
167
168
169     → s_24.set_xlabel('Count');s_32.set_xlabel('Count');s_48.set_xlabel('Count');s_
170     → _PP.set_xlabel('Age (hpf)');s_PD.set_xlabel('Age
171     → (hpf)');s_DD.set_xlabel('Age (hpf)')
172
173     → d_24.set_xlabel('Count');d_32.set_xlabel('Count');d_48.set_xlabel('Count');d_
174     → _PP.set_xlabel('Age (hpf)');d_PD.set_xlabel('Age
175     → (hpf)');d_DD.set_xlabel('Age (hpf)')
176
177 #set ylabel
178
179     → o_24.set_ylabel('Probability');o_32.set_ylabel('Probability');o_48.set_ylabel(
180     → o_PP.set_ylabel('PP Probability');o_PD.set_ylabel('PD
181     → Probability');o_DD.set_ylabel('DD Probability');
182     → o_at5size.set_ylabel('Clone size');o_ratio.set_ylabel('Clone
183     → even/odd ratio')
184
185     → s_24.set_ylabel('Probability');s_32.set_ylabel('Probability');s_48.set_ylabel(
186     → s_PP.set_ylabel('PP Probability');s_PD.set_ylabel('PD
187     → Probability');s_DD.set_ylabel('DD Probability');
188     → s_at5size.set_ylabel('Clone size');s_ratio.set_ylabel('Clone
189     → even/odd ratio')
190
191     → d_24.set_ylabel('Probability');d_32.set_ylabel('Probability');d_48.set_ylabel(
192     → d_PP.set_ylabel('PP Probability');d_PD.set_ylabel('PD
193     → Probability');d_DD.set_ylabel('DD Probability');
194     → d_at5size.set_ylabel('Clone size');d_ratio.set_ylabel('Clone
195     → even/odd ratio')
```

```

188     → sd_24.set_ylabel('Probability');sd_32.set_ylabel('Probability');sd_48.set_yla
189     sd_PP.set_ylabel('PP Probability');sd_PD.set_ylabel('PD
     → Probability');sd_DD.set_ylabel('DD Probability');
190     sd_ath5size.set_ylabel('Clone size');sd_ratio.set_ylabel('Clone
     → even/odd ratio')

191 #PLOT DATA
192 #plot lines, CIs, and bars
193 o_objects = line_plotter(o_24, o_counts_24, bin_sequence_24_32,
194     → x_sequence_24_32, count_seeds, lineages_sampled_24,
195     → prob_empirical_24, 'b-', '#000080', '#0000FF', 'y+', 0)
196 line_plotter(o_32, o_counts_32, bin_sequence_24_32, x_sequence_24_32,
197     → count_seeds, lineages_sampled_32, prob_empirical_32, 'b-',
198     → '#000080', '#0000FF', 'y+', 0)
199 line_plotter(o_48, o_counts_48, bin_sequence_48, x_sequence_48,
200     → count_seeds, lineages_sampled_48, prob_empirical_48, 'b-',
201     → '#000080', '#0000FF', 'y+', 0)
202 line_plotter(o_PP, o_events_PP[:,0], bin_sequence_events,
203     → x_sequence_events, event_seeds,
204     → lineages_sampled_events,prob_empirical_PP,'b-', '#000080',
205     → '#0000FF', 'y+', 1)
206 line_plotter(o_PD, o_events_PD[:,0], bin_sequence_events,
207     → x_sequence_events, event_seeds,
208     → lineages_sampled_events,prob_empirical_PD,'b-', '#000080',
209     → '#0000FF', 'y+', 1)
210 line_plotter(o_DD, o_events_DD[:,0], bin_sequence_events,
211     → x_sequence_events, event_seeds,
212     → lineages_sampled_events,prob_empirical_DD,'b-', '#000080',
213     → '#0000FF', 'y+', 1)
214 line_plotter(o_wan_noshad, o_counts_wan_no_shadow, bin_sequence_wan,
215     → x_sequence_wan, count_seeds, lineages_sampled_wan, prob_wan,
216     → 'b-', '#000080', '#0000FF', 'y+', 2)
217 o_ath5size.bar([0,1,2,3], [(wt_mean_clone_size/wt_mean_clone_size),
218     → (o_wt_mean_clone_size/wt_mean_clone_size),
219     → (ath5_mean_clone_size/wt_mean_clone_size),
220     → (o_ath5_mean_clone_size/wt_mean_clone_size)],color=['#000000','#000080','#000
221     → WT','OSM WT','EO Ath5mo','OSM Ath5mo'])
222 o_ratio.bar([0,1,2,3], [wt_even_odd_ratio, o_wt_even_odd_ratio,
223     → ath5_even_odd_ratio,
224     → o_ath5_even_odd_ratio],color=['#000000','#000080','#000000','#000080'],edgeco
225     → WT,'OFit WT','EO Ath5mo','OFit Ath5mo'])
226 plt.setp(o_ath5size.get_xticklabels(), rotation=25, ha='right')

```

```

204     plt.setp(o_ratio.get_xticklabels(), rotation=25, ha='right')
205
206     s_objects = line_plotter(s_24, s_counts_24, bin_sequence_24_32,
207         ↳ x_sequence_24_32, count_seeds, lineages_sampled_24,
208         ↳ prob_empirical_24, 'm-', '#800080', '#FF00FF', 'k+', 0)
209     line_plotter(s_32, s_counts_32, bin_sequence_24_32, x_sequence_24_32,
210         ↳ count_seeds, lineages_sampled_32, prob_empirical_32, 'm-',
211         ↳ '#800080', '#FF00FF', 'k+', 0)
212     line_plotter(s_48, s_counts_48, bin_sequence_48, x_sequence_48,
213         ↳ count_seeds, lineages_sampled_48, prob_empirical_48, 'm-',
214         ↳ '#800080', '#FF00FF', 'k+', 0)
215     line_plotter(s_PP, s_events_PP[:,0], bin_sequence_events,
216         ↳ x_sequence_events, event_seeds,
217         ↳ lineages_sampled_events,prob_empirical_PP,'m-', '#800080',
218         ↳ '#FF00FF', 'k+', 1)
219     line_plotter(s_PD, s_events_PD[:,0], bin_sequence_events,
220         ↳ x_sequence_events, event_seeds,
221         ↳ lineages_sampled_events,prob_empirical_PD,'m-', '#800080',
222         ↳ '#FF00FF', 'k+', 1)
223     line_plotter(s_DD, s_events_DD[:,0], bin_sequence_events,
224         ↳ x_sequence_events, event_seeds,
225         ↳ lineages_sampled_events,prob_empirical_DD,'m-', '#800080',
226         ↳ '#FF00FF', 'k+', 1)
227     line_plotter(s_wan_noshad, s_counts_wan_no_shadow, bin_sequence_wan,
228         ↳ x_sequence_wan, count_seeds, lineages_sampled_wan, prob_wan,
229         ↳ 'm-', '#800080', '#FF00FF', 'k+', 2)
230     s_ath5size.bar([0,1,2,3], [(wt_mean_clone_size/wt_mean_clone_size),
231         ↳ (s_wt_mean_clone_size/wt_mean_clone_size),(ath5_mean_clone_size/wt_mean_clone_
232         ↳ '#000000', '#800080'],edgecolor='#000000',tick_label=['EO WT','SM
233         ↳ WT','EO Ath5mo','SM Ath5mo'])
234     s_ratio.bar([0,1,2,3], [wt_even_odd_ratio, s_wt_even_odd_ratio,
235         ↳ ath5_even_odd_ratio,
236         ↳ s_ath5_even_odd_ratio],color=['#000000','#000080','#000000','#000080'],edgeco
237         ↳ WT,'SM WT','EO Ath5mo','SM Ath5mo'])
238     plt.setp(s_ath5size.get_xticklabels(), rotation=25, ha='right')
239     plt.setp(s_ratio.get_xticklabels(), rotation=25, ha='right')

240
241     d_objects = line_plotter(d_24, d_counts_24, bin_sequence_24_32,
242         ↳ x_sequence_24_32, count_seeds, lineages_sampled_24,
243         ↳ prob_empirical_24, 'g-', '#008000', '#00FF00', 'k+', 0)

```

```

220   line_plotter(d_32, d_counts_32, bin_sequence_24_32, x_sequence_24_32,
221     ↳ count_seeds, lineages_sampled_32, prob_empirical_32, 'g-',
222     ↳ '#008000', '#00FF00', 'k+', 0)
223   line_plotter(d_48, d_counts_48, bin_sequence_48, x_sequence_48,
224     ↳ count_seeds, lineages_sampled_48, prob_empirical_48, 'g-',
225     ↳ '#008000', '#00FF00', 'k+', 0)
226   line_plotter(d_PP, d_events_PP[:,0], bin_sequence_events,
227     ↳ x_sequence_events, event_seeds,
228     ↳ lineages_sampled_events, prob_empirical_PP, 'g-', '#008000',
229     ↳ '#00FF00', 'k+', 1)
230   line_plotter(d_PD, d_events_PD[:,0], bin_sequence_events,
231     ↳ x_sequence_events, event_seeds,
232     ↳ lineages_sampled_events, prob_empirical_PD, 'g-', '#008000',
233     ↳ '#00FF00', 'k+', 1)
234   line_plotter(d_DD, d_events_DD[:,0], bin_sequence_events,
235     ↳ x_sequence_events, event_seeds,
236     ↳ lineages_sampled_events, prob_empirical_DD, 'g-', '#008000',
237     ↳ '#00FF00', 'k+', 1)
238   line_plotter(d_wan_noshad, d_counts_wan_no_shadow, bin_sequence_wan,
239     ↳ x_sequence_wan, count_seeds, lineages_sampled_wan, prob_wan,
240     ↳ 'g-', '#008000', '#00FF00', 'k+', 2)
241   d_ath5size.bar([0,1,2,3], [(wt_mean_clone_size/wt_mean_clone_size),
242     ↳ (d_wt_mean_clone_size/wt_mean_clone_size),(ath5_mean_clone_size/wt_mean_clone_
243       ↳ WT', 'DM WT', 'EO Ath5mo', 'DM Ath5mo'])
244   d_ratio.bar([0,1,2,3], [wt_even_odd_ratio, d_wt_even_odd_ratio,
245     ↳ ath5_even_odd_ratio,
246     ↳ d_ath5_even_odd_ratio], color=['#000000', '#008000', '#000000', '#008000'], edgecolor='black')
247     ↳ WT', 'DM WT', 'EO Ath5mo', 'DM Ath5mo'])
248   plt.setp(d_ath5size.get_xticklabels(), rotation=25, ha='right')
249   plt.setp(d_ratio.get_xticklabels(), rotation=25, ha='right')
250
251
252   sd_objects_s = line_plotter(sd_24, s_counts_24, bin_sequence_24_32,
253     ↳ x_sequence_24_32, count_seeds, lineages_sampled_24,
254     ↳ prob_empirical_24, 'm-', '#800080', '#FF00FF', 'k+', 0)
255   sd_objects_d = line_plotter(sd_24, d_counts_24, bin_sequence_24_32,
256     ↳ x_sequence_24_32, count_seeds, lineages_sampled_24,
257     ↳ prob_empirical_24, 'g-', '#008000', '#00FF00', 'k+', 0)
258   line_plotter(sd_32, s_counts_32, bin_sequence_24_32,
259     ↳ x_sequence_24_32, count_seeds, lineages_sampled_32,
260     ↳ prob_empirical_32, 'm-', '#800080', '#FF00FF', 'k+', 0)

```

```

235   line_plotter(sd_32, d_counts_32, bin_sequence_24_32,
236     ↳ x_sequence_24_32, count_seeds, lineages_sampled_32,
237     ↳ prob_empirical_32, 'g-', '#008000', '#00FF00', 'k+', 0)
238   line_plotter(sd_48, s_counts_48, bin_sequence_48, x_sequence_48,
239     ↳ count_seeds, lineages_sampled_48, prob_empirical_48, 'm-',
240     ↳ '#800080', '#FF00FF', 'k+', 0)
241   line_plotter(sd_48, d_counts_48, bin_sequence_48, x_sequence_48,
242     ↳ count_seeds, lineages_sampled_48, prob_empirical_48, 'g-',
243     ↳ '#008000', '#00FF00', 'k+', 0)
244   line_plotter(sd_PP, s_events_PP[:,0], bin_sequence_events,
245     ↳ x_sequence_events, event_seeds,
246     ↳ lineages_sampled_events,prob_empirical_PP,'m-','#800080',
247     ↳ '#FF00FF', 'k+', 1)
248   line_plotter(sd_PP, d_events_PP[:,0], bin_sequence_events,
249     ↳ x_sequence_events, event_seeds,
250     ↳ lineages_sampled_events,prob_empirical_PP,'g-','#008000',
251     ↳ '#00FF00', 'k+', 1)
252   line_plotter(sd_PD, s_events_PD[:,0], bin_sequence_events,
253     ↳ x_sequence_events, event_seeds,
254     ↳ lineages_sampled_events,prob_empirical_PD,'m-','#800080',
255     ↳ '#FF00FF', 'k+', 1)
256   line_plotter(sd_PD, d_events_PD[:,0], bin_sequence_events,
257     ↳ x_sequence_events, event_seeds,
258     ↳ lineages_sampled_events,prob_empirical_PD,'g-','#008000',
259     ↳ '#00FF00', 'k+', 1)
260   line_plotter(sd_DD, s_events_DD[:,0], bin_sequence_events,
261     ↳ x_sequence_events, event_seeds,
262     ↳ lineages_sampled_events,prob_empirical_DD,'m-','#800080',
263     ↳ '#FF00FF', 'k+', 1)
264   line_plotter(sd_DD, d_events_DD[:,0], bin_sequence_events,
265     ↳ x_sequence_events, event_seeds,
266     ↳ lineages_sampled_events,prob_empirical_DD,'g-','#008000',
267     ↳ '#00FF00', 'k+', 1)
268   line_plotter(sd_wan_noshad, s_counts_wan_no_shadow, bin_sequence_wan,
269     ↳ x_sequence_wan, count_seeds, lineages_sampled_wan, prob_wan,
270     ↳ 'm-','#800080', '#FF00FF', 'k+', 2)
271   line_plotter(sd_wan_noshad, d_counts_wan_no_shadow, bin_sequence_wan,
272     ↳ x_sequence_wan, count_seeds, lineages_sampled_wan, prob_wan,
273     ↳ 'g-','#008000', '#00FF00', 'k+', 2)
274   sd_ath5size.bar([0,1,2,3,4,5], [(wt_mean_clone_size),
275     ↳ (s_wt_mean_clone_size),(d_wt_mean_clone_size),(ath5_mean_clone_size),(s_ath5_
276     ↳ WT','SM WT','DM WT', 'EO Ath5mo', 'SM Ath5Mo', 'DM Ath5mo')])

```

```

247     sd_ratio.bar([0,1,2,3,4,5], [wt_even_odd_ratio, s_wt_even_odd_ratio,
248     ↵ d_wt_even_odd_ratio, ath5_even_odd_ratio, s_ath5_even_odd_ratio,
249     ↵ d_ath5_even_odd_ratio],color=['#000000','#800080','#008000','#000000','#80008
250     ↵ WT','SM WT','DM WT', 'EO Ath5mo', 'SM Ath5Mo', 'DM Ath5mo'])
251
252 plt.setp(sd_ath5size.get_xticklabels(), rotation=25, ha='right')
253 plt.setp(sd_ratio.get_xticklabels(), rotation=25, ha='right')
254
255 #calculate AICs, write table to file
256 o_fit_AIC = calculate_fit_AIC(o_fit_output_list, empirical_fit_list,
257     ↵ he_model_params)
258 o_test_AIC = calculate_test_AIC(o_test_output_list,
259     ↵ empirical_fit_list, he_model_params)
260
261 s_fit_AIC = calculate_fit_AIC(s_fit_output_list, empirical_fit_list,
262     ↵ he_model_params)
263 s_test_AIC = calculate_test_AIC(s_test_output_list,
264     ↵ empirical_test_list, he_model_params)
265
266 d_fit_AIC = calculate_fit_AIC(d_fit_output_list, empirical_fit_list,
267     ↵ det_model_params)
268 d_test_AIC = calculate_test_AIC(d_test_output_list,
269     ↵ empirical_test_list, det_model_params)
270
271 AIC_table('AIC_table.tex', o_fit_AIC, o_test_AIC, s_fit_AIC,
272     ↵ s_test_AIC, d_fit_AIC, d_test_AIC)
273
274 #plot annotations
275 plot_annotater(s_fig,s_objects,'Stochastic Model')
276 plot_annotater(o_fig,o_objects,'Stochastic Model (original fit)')
277 plot_annotater(d_fig,d_objects, 'Deterministic Model')
278 plot_annotater(sd_fig,sd_objects_s, 'Stochastic Model', sd_objects_d,
279     ↵ 'Deterministic Model')
280
281 #export as .tiff, .png
282 o_fig.savefig('o_fig.png',dpi=600)
283 png_memory = BytesIO()
284 o_fig.savefig(png_memory, format='png', dpi=600)
285 PILpng = Image.open(png_memory)
286 PILpng.save('o_fig.tiff')
287 png_memory.close()
288
289 s_fig.savefig('s_fig.png',dpi=600)
290 png_memory = BytesIO()

```

```
279     s_fig.savefig(png_memory, format='png', dpi=600)
280     PILpng = Image.open(png_memory)
281     PILpng.save('s_fig.tiff')
282     png_memory.close()
283
284     d_fig.savefig('d_fig.png',dpi=600)
285     png_memory = BytesIO()
286     d_fig.savefig(png_memory, format='png', dpi=600)
287     PILpng = Image.open(png_memory)
288     PILpng.save('d_fig.tiff')
289     png_memory.close()
290
291     sd_fig.savefig('sd_fig.png',dpi=600)
292     png_memory = BytesIO()
293     sd_fig.savefig(png_memory, format='png', dpi=600)
294     PILpng = Image.open(png_memory)
295     PILpng.save('sd_fig.tiff')
296     png_memory.close()
297
298     plt.show()
299
300
301 def calculate_fit_AIC(output_list, empirical_list, number_params):
302     #function calculates AIC for He et al counts & event probability
303     #→ density (NOT per-lineage event probability)
304     #this numpy array holds the individual RSS vals for timepoints +
305     #→ events
306     rss = np.zeros(6)
307     number_comparisons = 0
308
309     for i in range(0,3):
310         output_counts = output_list[i]
311         empirical_prob = empirical_list[i]
312
313         output_histo,bin_edges = np.histogram(output_counts,
314             np.arange(1,len(empirical_prob)+2), density = False)
315         output_prob = np.array(output_histo/count_seeds)
316
317         residual = np.array(output_prob- empirical_prob)
318         number_comparisons += len(residual)
319         rss[i] = np.sum(np.square(residual))
320
321     for i in range (0,3):
```

```
319     output_events = output_list[i+3]
320     empirical_prob = empirical_list[i+3]
321
322     histo_events,bin_edges = np.histogram(output_events,
323         ↳ bin_sequence_events, density=True)
324     output_prob = np.array(histo_events/event_seeds)
325
326     residual = np.array(output_prob - empirical_prob)
327     number_comparisons += len(residual)
328     rss[i+3] = np.sum(np.square(residual))
329
330     AIC = 2 * number_params + number_comparisons * np.log(np.sum(rss))
331
332     return AIC
333
334 def calculate_test_AIC(output_list, empirical_list, number_params):
335     #function calculates AIC for He mutant data & Wan counts
336     rss=np.zeros(3)
337     number_comparisons = 0
338
339     #Wan data
340     for i in range (0,1):
341         output_counts = output_list[i]
342         empirical_prob = empirical_list[i]
343
344         output_histo,bin_edges = np.histogram(output_counts,
345             ↳ np.arange(2,len(empirical_prob)+3), density = False)
346         output_prob = np.array(output_histo/count_seeds)
347
348         residual = np.array(output_prob- empirical_prob)
349         number_comparisons += len(residual)
350         rss[i] = np.sum(np.square(residual))
351
352     #He data
353     for i in range(1,3):
354         output_measure = output_list[i]
355         empirical_measure = empirical_list[i]
356
357         residual = np.array(output_measure - empirical_measure)
358         number_comparisons += 1
359         rss[i] = np.sum(np.square(residual))
360
361     AIC = 2 * number_params + number_comparisons * np.log(np.sum(rss))
```

```
360
361     return AIC
362
363 #line plotter plots average counts or event event +- 2 standard
364 #    deviations (He et al's "95% probability interval")
365 # "mode" 1 gives correct hourly output for 5-hour event bins. mode 2
366 #    begins Wan plots at clone size 2, reflecting unavailable data
367 def line_plotter(subplot, data, bin_sequence, x_sequence, seeds, samples,
368 #    empirical_prob, line_colour_string, fill_edge_colour_string,
369 #    fill_face_colour_string, empirical_colour_string, mode):
370
371     legend_objects = []
372
373     prob_histo, bin_edges = np.histogram(data, bin_sequence,
374 #        density=True)
375
376     #estimate of 95% CI for empirical observations of model output from
377     #    repeated sampling of the data w/ the appropriate number of
378     #    empirically observed lineages
379     interval = sampler(data,samples,bin_sequence)
380     legend_objects.append(subplot.plot(x_sequence,prob_histo,
381 #        line_colour_string))
382     subplot.fill_between(x_sequence, (prob_histo - interval), (prob_histo
383 #        + interval), alpha=0.1, edgecolor=fill_edge_colour_string,
384 #        facecolor=fill_face_colour_string)
385
386     if mode == 0:
387
388         legend_objects.append(subplot.plot(np.arange(1,len(empirical_prob)+1),empirical_prob,
389 #            empirical_colour_string))
390
391     if mode == 1:
392
393         legend_objects.append(subplot.plot(np.arange(30,80,5),empirical_prob,
394 #            empirical_colour_string))
395
396     if mode == 2:
397
398         legend_objects.append(subplot.plot(np.arange(2,len(empirical_prob)+2,1),
399 #            empirical_prob, empirical_colour_string))
400
401     return legend_objects
402
```

```

387 def sampler(data,samples,bin_sequence):
388
389     if len(data) == 0: #catches edge case w/ no entries for a mitotic
        ↵ mode
390         data=[0]
391
392     base_sample=np.zeros((error_samples,len(bin_sequence)-1))
393
394     for i in range(0,error_samples):
395         new_data_sample = np.random.choice(data,samples)
396         new_histo_prob, bin_edges = np.histogram(new_data_sample,
            ↵ bin_sequence, density=True)
397         base_sample[i,:] = new_histo_prob
398
399     sample_95CI = np.array(2 * (np.std(base_sample,0)))
400
401     return sample_95CI
402
403 def plot_annotater(fig_to_ann, legend_objects_1, legend_objects_1_name,
    ↵ legend_objects_2=None, legend_objects_2_name=None):
404     #subplot labels
405     labels = ['A','B','C','D','E','F','G','H','I','']
406
407     for i, ax in enumerate(fig_to_ann.axes):
408         ax.text(.01,.95, labels[i], transform=ax.transAxes,
            ↵ fontweight='bold', va='top')
409
410         time_rectangle_x = .70
411         time_rectangle_y = .65
412         time_rectangle_width = .2
413         time_rectangle_height = .03
414         time_caret_half_width = .02
415         time_caret_height = np.sqrt(3)*(time_caret_half_width*2)
416
417         if i < 3:
418             #add the time bar and label 72 hr caret on appropriate plots
419
            ↵ ax.add_patch(plt.Rectangle((time_rectangle_x,time_rectangle_y),time_r
            ↵ color='k', transform=ax.transAxes))
420
            ↵ ax.add_patch(plt.Polygon(((time_rectangle_x+time_rectangle_width, time_
            ↵ fill=False, transform=ax.transAxes))

```

```
421     ↵ ax.text(time_rectangle_x+time_rectangle_width,time_rectangle_y+time_rect
        ↵ transform=ax.transAxes,
        ↵ fontsize='8',va='bottom',ha='center')

422
423 if i == 0:
424     #add 24hr caret
425
426     ↵ ax.add_patch(plt.Polygon(((time_rectangle_x,time_rectangle_y+time_rect
        ↵ fill=False, transform=ax.transAxes))

427
428 if i == 1:
429     #add 32hr caret
430
431     ↵ ax.add_patch(plt.Polygon(((time_rectangle_x+(8/48)*time_rectangle_wid
        ↵ fill=False, transform=ax.transAxes))

432
433 if i == 2:
434
435     ↵ ax.add_patch(plt.Polygon(((time_rectangle_x+(24/48)*time_rectangle_wi
        ↵ fill=False, transform=ax.transAxes))

436
437 parent_circle_coord = (.85,.8)
438 child_1_coord = (.8,.6)
439 child_2_coord = (.9,.6)
440 ellipse_width = .03
441 ellipse_height = 2.5*ellipse_width

442
443 if i == 3:
444     ↵ ax.add_patch(plt.Polygon((parent_circle_coord),child_1_coord),color=
```

```
445     ↪ ax.add_patch(plt.Polygon((parent_circle_coord),child_2_coord),color=
446     ↪ ax.add_patch(patches.Ellipse(parent_circle_coord,ellipse_width,ellipse_
     ↪ color='k', transform=ax.transAxes))
447     ↪ ax.add_patch(patches.Ellipse(child_1_coord,ellipse_width,ellipse_height,
     ↪ color='k', transform=ax.transAxes))
448     ↪ ax.add_patch(patches.Ellipse(child_2_coord,ellipse_width,ellipse_height,
     ↪ color='k', transform=ax.transAxes))
449 ax.text(.91, .7, 'PP', transform=ax.transAxes, va='center',
     ↪ ha='left')
450
451 if i == 4:
452     ↪ ax.add_patch(plt.Polygon((parent_circle_coord),child_1_coord),color=
453     ↪ ax.add_patch(plt.Polygon((parent_circle_coord),child_2_coord),color=
454     ↪ ax.add_patch(patches.Ellipse(parent_circle_coord,ellipse_width,ellipse_
     ↪ height,color='k', transform=ax.transAxes))
455     ↪ ax.add_patch(patches.Ellipse(child_1_coord,ellipse_width,ellipse_height,
     ↪ color='k', transform=ax.transAxes))
456     ↪ ax.add_patch(patches.Ellipse(child_2_coord,ellipse_width,ellipse_height,
     ↪ edgecolor='k', facecolor='w', transform=ax.transAxes))
457 ax.text(.91, .7, 'PD', transform=ax.transAxes, va='center',
     ↪ ha='left')
458
459
460 if i == 5:
461     ↪ ax.add_patch(plt.Polygon((parent_circle_coord),child_1_coord),color=
462     ↪ ax.add_patch(plt.Polygon((parent_circle_coord),child_2_coord),color=
463     ↪ ax.add_patch(patches.Ellipse(parent_circle_coord,ellipse_width,ellipse_
     ↪ height,color='k', transform=ax.transAxes))
464     ↪ ax.add_patch(patches.Ellipse(child_1_coord,ellipse_width,ellipse_height,
     ↪ edgecolor='k', facecolor='w', transform=ax.transAxes))
```



```

493         fill=False, color='k', linestyle=':',  

494         transform=fig_to_ann.transFigure,  

495         → figure=fig_to_ann))  

496  

497     fig_to_ann.subplots_adjust(wspace=0.4, hspace=0.3)  

498     fig_to_ann.tight_layout()  

499  

500 def AIC_table (filename, o_training_AIC, o_test_AIC, s_training_AIC,  

501   → s_test_AIC, d_training_AIC, d_test_AIC):  

502     #writes LaTeX tabular snippet with AIC values  

503     table_file = open(filename, 'w')  

504     table_file.write(r'\begin{tabular}{l|l|l}'+ '\n')  

505     table_file.write(r'\hline'+ '\n')  

506     table_file.write(r'{\bf Model} & {\bf Training AIC} & {\bf Test AIC}  

507     → \\ \thickhline'+ '\n')  

508     table_file.write(r'Stochastic (He fit) &  

509     → $'+f'{o_training_AIC:.2f}'+r'$ & $' + f'{o_test_AIC:.2f}' + r'$\\  

510     → \hline'+ '\n')  

511     table_file.write(r'Stochastic (SPSA fit) &  

512     → $'+f'{s_training_AIC:.2f}'+r'$ & $' + f'{s_test_AIC:.2f}' + r'$\\  

513     → \hline'+ '\n')  

514     table_file.write(r'Deterministic (SPSA fit) &  

515     → $'+f'{d_training_AIC:.2f}'+r'$ & $' + f'{d_test_AIC:.2f}' + r'$\\  

516     → \hline'+ '\n')  

517     table_file.write(r'\end{tabular}'+ '\n')  

518  

519 if __name__ == "__main__":  

520     main()

```

16.2.15 /python_fixtures/figure_plots/Kolmogorov_plot.py

```

1 import os  

2 import numpy as np  

3 import matplotlib.pyplot as plt  

4  

5 #AIC & Plotting utility params  

6 event_seeds = 1000  

7 error_samples = 5000 #number of samples to draw when estimating  

    → plausibility interval for simulations  

8  

9 def main():  

10     #LOAD & PARSE KOLMOGOROV COMPLEXITY FILES

```

```

11     gomes_kol =
12         np.loadtxt('/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/KolmogorovComplexity/gomes_kol.txt')
13         skiprows=1, usecols=1)
14     he_kol =
15         np.loadtxt('/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/KolmogorovComplexity/he_kol.txt')
16         skiprows=1, usecols=1)
17     he_refit_kol =
18         np.loadtxt('/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/KolmogorovComplexity/he_refit_kol.txt')
19         skiprows=1, usecols=1)
20     deterministic_kol =
21         np.loadtxt('/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/KolmogorovComplexity/deterministic_kol.txt')
22         skiprows=1, usecols=1)
23     boije_kol =
24         np.loadtxt('/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/KolmogorovComplexity/boije_kol.txt')
25         skiprows=1, usecols=1)
26     eo_kol =
27         np.loadtxt('/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/KolmogorovComplexity/eo_kol.txt')
28         skiprows=1, usecols=1)

29     data_sequence =
30         [gomes_kol,he_kol,he_refit_kol,deterministic_kol,boije_kol,eo_kol]
31
32     plt.violinplot(data_sequence, showextrema=True)
33
34     plt.ylabel ('Estimated Kolmogorov Complexity')
35
36     plt.show()

37
38 if __name__ == "__main__":
39     main()

```

16.2.16 /python_fixtures/figure_plots/Mitotic_rate_plot.py

```

1 import os
2 import numpy as np
3 import matplotlib.pyplot as plt
4
5 from PIL import Image
6 from io import BytesIO
7
8 #PLoS formatting stuff
9 plt.rcParams['font.size'] = 12

```

```

10 plt.rcParams['font.family'] = 'sans-serif'
11 plt.rcParams['font.sans-serif'] = ['Arial']

12
13 #AIC & Plotting utility params
14 event_seeds = 1000
15 error_samples = 5000 #number of samples to draw when estimating
    ↳ plausibility interval for simulations

16
17 bin_sequence_events = np.arange(30,85,5)
18 x_sequence_events = np.arange(30,80,5)

19
20 ######
21 # HE ET AL EMPIRICAL RESULTS
22 #####
23
24 rate_bin_sequence = np.arange(30,85,5)
25 rate_x_sequence = np.arange(30,80,5)
26 rate_trim_value = 10

27
28 #no. of lineages E0 per induction timepoint/event group
29 lineages_sampled_events = 60

30
31 #Mitotic mode rate probability arrays
32 raw_events =
    ↳ np.loadtxt('/home/main/git/chaste/projects/ISP/empirical_data/empirical_lineages.')
    ↳ skiprows=1, usecols=(3,5,8))
33 E0_events = raw_events[np.where(raw_events[:,2]==1)] #exclude any mitosis
    ↳ whose time was too early for recording

34
35 event_counts, bin_edges =
    ↳ np.histogram(E0_events,bin_sequence_events,density=False)
36 #hourly values
37 empirical_events_per_lineage =
    ↳ np.array(event_counts/(lineages_sampled_events*5))

38
39 def main():
    #LOAD & PARSE HE MODEL OUTPUT FILES
    #original fit stochastic mitotic mode files
40     o_events =
        ↳ np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeM")
        ↳ skiprows=1, usecols=(0))

41
42     #refit stochastic mitotic mode files

```

```

45     s_events =
46         np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeM
47         skiprows=1, usecols=(0))
48
49     #deterministic mitotic mode files
50     d_events =
51         np.loadtxt("/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/HeM
52         skiprows=1, usecols=(0))
53
54     original = line_plotter(o_events,bin_sequence_events,
55         x_sequence_events, event_seeds, lineages_sampled_events, 'b--',
56         '#000080', '#0000FF')
57     refit = line_plotter(s_events,bin_sequence_events, x_sequence_events,
58         event_seeds, lineages_sampled_events, 'm-', '#800080', '#FF00FF')
59     deterministic = line_plotter(d_events,bin_sequence_events,
60         x_sequence_events, event_seeds, lineages_sampled_events, 'g-',
61         '#008000', '#00FF00')
62
63     empirical = plt.plot(np.arange(30,80,5),empirical_events_per_lineage,
64         'k+')
65
66     plt.legend((original[0], refit[0], deterministic[0], empirical[0]),
67         ('SM (He fit)', 'SM (SPSA fit)', 'DM', 'Observed'))
68
69     plt.xlabel ('Age (hpf)')
70     plt.ylabel ('Probability of mitotic event per lineage per hour')
71
72     plt.savefig("per_lineage_mitotic_rate.png")
73     png_memory = BytesIO()
74     plt.savefig(png_memory, format='png', dpi=600)
75     PILpng = Image.open(png_memory)
76     PILpng.save('per_lineage_mitotic_rate.tiff')
77     png_memory.close()
78
79     plt.show()
80
81 #line plotter plots average counts or event event +- 2 standard
82     deviations (He et al's "95% probability interval")
83 # "mode" 1 gives correct hourly output for 5-hour event bins. mode 2
84     begins Wan plots at clone size 2, reflecting unavailable data

```

```
73 def line_plotter(data, bin_sequence, x_sequence, seeds, samples,
74     ↵ line_colour_string, fill_edge_colour_string,
75     ↵ fill_face_colour_string):
76
77
78     histo, bin_edges = np.histogram(data, bin_sequence, density=False)
79     prob_histo = np.array(histo / (seeds * 5))
80
81
82     #estimate of 95% CI for empirical observations of model output from
83     ↵ repeated sampling of the data w/ the appropriate number of
84     ↵ empirically observed lineages
85     interval = sampler(data,samples,bin_sequence)
86     line = plt.plot(x_sequence,prob_histo, line_colour_string)
87     plt.fill_between(x_sequence, (prob_histo - interval), (prob_histo +
88         ↵ interval), alpha=0.1, edgecolor=fill_edge_colour_string,
89         ↵ facecolor=fill_face_colour_string)
90
91
92     return line
93
94
95 def sampler(data,samples,bin_sequence):
96
97     if len(data) == 0: #catches edge case w/ no entries for a mitotic
98         ↵ mode
99         data=[0]
100
101
102     base_sample=np.zeros((error_samples,len(bin_sequence)-1))
103
104
105     for i in range(0,error_samples):
106         new_data_sample = np.random.choice(data,samples)
107         new_histo, bin_edges = np.histogram(new_data_sample,
108             ↵ bin_sequence, density=False)
109         new_histo_prob = np.array(new_histo/samples)
110         base_sample[i,:] = new_histo_prob
111
112
113     sample_95CI = np.array(2 * (np.std(base_sample,0)))
114
115
116     return sample_95CI
117
118
119 if __name__ == "__main__":
120     main()
```

16.2.17 /python_fixtures/figure_plots/Wan_output_plot.py

```
1 import os
2 import numpy as np
3 import pandas as pd
4 import matplotlib.pyplot as plt
5 import matplotlib.ticker as ticker
6
7 from PIL import Image
8 from io import BytesIO
9
10 #PLoS formatting stuff
11 plt.rcParams['font.size'] = 12
12 plt.rcParams['font.family'] = 'sans-serif'
13 plt.rcParams['font.sans-serif'] = ['Arial']
14
15 def main():
16     wan_output_dir =
17         '/home/main/git/chaste/projects/ISP/python_fixtures/testoutput/WanOutput'
18     wan_results_list = []
19     for root,dirs,files in os.walk(wan_output_dir):
20         for name in files:
21             if name == 'celltypes.dat':
22                 results = np.loadtxt(os.path.join(root,name), usecols=2)
23                 if len(results) == 8569:
24                     wan_results_list.append(results)
25
26     wan_sim_results = np.array(wan_results_list)
27     wan_sim_mean = np.mean(wan_sim_results, axis=0)
28     wan_sim_95CI = 2*np.std(wan_sim_results, axis=0)
29     wan_sim_x_seq = np.arange(72,8641,1)/24
30
31     empirical_pop_mean = np.array([792.0, 768.4, 906.1, 1159.7, 1630.0,
32         ↵ 3157.9, 3480.2, 4105.1, 1003.0, 477.2, 438.8088611111])
33     empirical_pop_95CI = 2*np.array([160.1, 200.1, 244.5, 477.6, 444.3,
34         ↵ 1414.3, 472.1, 1169.7, 422.8, 367.5, 294.8])
35     empirical_x_seq =
36         ↵ np.array([72,120,192,288,408,552,720,1440,2160,4320,8640])/24
37
38     fig, ax = plt.subplots(1,1)
39
40     empirical = plt.plot(empirical_x_seq, empirical_pop_mean, 'g-')
```

```

37     plt.fill_between(empirical_x_seq, (empirical_pop_mean -
38         ↵ empirical_pop_95CI), (empirical_pop_mean + empirical_pop_95CI),
39         ↵ alpha=0.1, edgecolor='#008000', facecolor='#00FF00')
40
41     wan_simulator = plt.plot(wan_sim_x_seq, wan_sim_mean, 'm-')
42     plt.fill_between(wan_sim_x_seq, (wan_sim_mean - wan_sim_95CI),
43         ↵ (wan_sim_mean + wan_sim_95CI), alpha=0.1, edgecolor='#800080',
44         ↵ facecolor='#FF00FF')
45
46     plt.ylim(bottom=0)
47     plt.xlim(left=0)
48
49     plt.ylabel("CMZ population size")
50     plt.xlabel("Retina age (dpf)")
51     ax.xaxis.set_major_locator(ticker.MultipleLocator(40))
52     ax.legend((empirical[0], wan_simulator[0]), ('Observed CMZ
53         ↵ population', '"Wan-type" simulated CMZ population'))
54
55     png_memory = BytesIO()
56     fig.savefig(png_memory, format='png', dpi=600)
57     PILpng = Image.open(png_memory)
58     PILpng.save('wan_fig.tiff')
59     png_memory.close()
60
61     plt.show()
62
63 if __name__ == "__main__":
64     main()

```

16.2.18 /src/BoijeCellCycleModel.cpp

```

1 #include "BoijeCellCycleModel.hpp"
2
3 BoijeCellCycleModel::BoijeCellCycleModel() :
4     AbstractSimpleCellCycleModel(), mOutput(false),
5         ↵ mEventStartTime(), mSequenceSampler(false),
6         ↵ mSeqSamplerLabelSister(
7             ↵ false), mDebug(false), mTimeID(), mVarIDs(),
8             ↵ mDebugWriter(), mGeneration(0), mPhase2gen(3),
9             ↵ mPhase3gen(
10                ↵ 5), mprobAtoh7(0.32), mprobPtf1a(0.30), mprobng(0.80),
11                ↵ mAtoh7Signal(false), mPtf1aSignal(false), mNgSignal(

```

```

7             false), mMitoticMode(0), mSeed(0), mp_PostMitoticType(),
8     ↳ mp_RGC_Type(), mp_AC_HC_Type(), mp_PR_BC_Type(),
9     ↳ mp_label_Type()
10    }
11
12 BoijeCellCycleModel::BoijeCellCycleModel(const BoijeCellCycleModel&
13   ↳ rModel) :
14     AbstractSimpleCellCycleModel(rModel), mOutput(rModel.mOutput),
15     ↳ mEventStartTime(rModel.mEventStartTime), mSequenceSampler(
16       rModel.mSequenceSampler),
17       ↳ mSeqSamplerLabelSister(rModel.mSeqSamplerLabelSister),
18       ↳ mDebug(rModel.mDebug), mTimeID(
19         rModel.mTimeID), mVarIDs(rModel.mVarIDs),
20         ↳ mDebugWriter(rModel.mDebugWriter), mGeneration(
21           rModel.mGeneration), mPhase2gen(rModel.mPhase2gen),
22           ↳ mPhase3gen(rModel.mPhase3gen), mprobAtoh7(
23             rModel.mprobAtoh7), mprobPtf1a(rModel.mprobPtf1a),
24             ↳ mprobng(rModel.mprobng), mAtoh7Signal(
25               rModel.mAtoh7Signal), mPtf1aSignal(rModel.mPtf1aSignal),
26               ↳ mNgSignal(rModel.mNgSignal), mMitoticMode(
27                 rModel.mMitoticMode), mSeed(rModel.mSeed),
28                 ↳ mp_PostMitoticType(rModel.mp_PostMitoticType),
29                 ↳ mp_RGC_Type(
30                   rModel.mp_RGC_Type), mp_AC_HC_Type(rModel.mp_AC_HC_Type),
31                   ↳ mp_PR_BC_Type(rModel.mp_PR_BC_Type), mp_label_Type(
32                     rModel.mp_label_Type)
33
34 {
35 }
```

23

```

24 AbstractCellCycleModel* BoijeCellCycleModel::CreateCellCycleModel()
25 {
26     return new BoijeCellCycleModel(*this);
27 }
```

28

```

29 void BoijeCellCycleModel::SetCellCycleDuration()
30 {
31
32     if
33     ↳ (mpCell->GetCellProliferativeType()->IsType<DifferentiatedCellProliferativeTy
34     {
35         mCellCycleDuration = DBL_MAX;
36     }
```



```

76     if (ptf1aRV < mprobPtf1a)
77     {
78         mPtf1aSignal = true;
79     }
80     if (ngRV < mprobng)
81     {
82         mNgSignal = true;
83     }
84 }
85
86 if (mGeneration > mPhase3gen) //if the cell is in the 3rd model
87   ↪ phase, only ng signal has a nonzero probability
87 {
88     ngRV = p_random_number_generator→ranf();
89     //roll a probability die for the ng signal
90     if (ngRV < mprobng)
91     {
92         mNgSignal = true;
93     }
94 }
95
96 /*****
97 * MITOTIC MODE & SPECIFICATION RULES
98 * -(additional asymmetric postmitotic rules specified in
99 ↪ InitialiseDaughterCell());
100 * *****/
100
101 if(mAtoh7Signal == true)
102 {
103     mMitoticMode = 1;
104 }
105
106 if (mPtf1aSignal == true && mAtoh7Signal == false) //Ptf1A alone
107   ↪ gives a symmetrical postmitotic AC/HC division
107 {
108     mMitoticMode = 2;
109     mpCell→SetCellProliferativeType(mp_PostMitoticType);
110     mpCell→AddCellProperty(mp_AC_HC_Type);
111 }
112
113 if (mPtf1aSignal == false && mAtoh7Signal == false && mNgSignal ==
114   ↪ true) //ng alone gives a symmetrical postmitotic PR/BC division
114 {

```

```
115     mMitoticMode = 2;
116     mpCell->SetCellProliferativeType(mp_PostMitoticType);
117     mpCell->AddCellProperty(mp_PR_BC_Type);
118 }
119
120 /*****
121 * Write mitotic event to file if appropriate
122 * mDebug: many files: detailed per-lineage info switch; intended for
123 * TestHeInductionCountFixture
124 * mOutput: 1 file: time, seed, cellID, mitotic mode, intended for
125 * TestHeMitoticModeRateFixture
126 * *****/
127
128 if (mDebug)
129 {
130     WriteDebugData(atoh7RV, ptf1aRV, ngRV);
131 }
132
133 if (mOutput)
134 {
135     WriteModeEventOutput();
136 }
137
138 /*****
139 * SEQUENCE SAMPLER
140 *****/
141 //if the sequence sampler has been turned on, check for the label &
142 // write mitotic mode to log
143 //50% chance of each daughter cell from a mitosis inheriting the
144 // label
145 if (mSequenceSampler)
146 {
147     if (mpCell->HasCellProperty<CellLabel>())
148     {
149         (*LogFile::Instance()) << mMitoticMode;
150         double labelRV = p_random_number_generator->ranf();
151         if (labelRV <= .5)
152         {
153             mSeqSamplerLabelSister = true;
154             mpCell->RemoveCellProperty<CellLabel>();
155         }
156     }
157 }
```

```
154         else
155     {
156         mSeqSamplerLabelSister = false;
157     }
158 }
159 else
160 {
161     //prevents lost-label cells from labelling their progeny
162     mSeqSamplerLabelSister = false;
163 }
164 }
165 }
166
167 void BoijeCellCycleModel::InitialiseDaughterCell()
168 {
169     //Asymmetric specification rules
170
171     if (mAtoh7Signal == true)
172     {
173         if (mPtf1aSignal == true)
174         {
175             mpCell->SetCellProliferativeType(mp_PostMitoticType);
176             mpCell->AddCellProperty(mp_AC_HC_Type);
177         }
178         else
179         {
180             mpCell->SetCellProliferativeType(mp_PostMitoticType);
181             mpCell->AddCellProperty(mp_RGC_Type);
182         }
183     }
184
185     ****
186     * SEQUENCE SAMPLER
187     ****
188     if (mSequenceSampler)
189     {
190         if (mSeqSamplerLabelSister)
191         {
192             mpCell->AddCellProperty(mp_label_Type);
193             mSeqSamplerLabelSister = false;
194         }
195         else
196         {
```

```
197         mpCell->RemoveCellProperty<CellLabel>();
198     }
199 }
200 }
201
202 void BoijeCellCycleModel::SetGeneration(unsigned generation)
203 {
204     mGeneration = generation;
205 }
206
207 unsigned BoijeCellCycleModel::GetGeneration() const
208 {
209     return mGeneration;
210 }
211
212 void
213     ← BoijeCellCycleModel::SetPostMitoticType(boost::shared_ptr<AbstractCellProperty>
214     ← p_PostMitoticType)
215 {
216     mp_PostMitoticType = p_PostMitoticType;
217
218     ← boost::shared_ptr<AbstractCellProperty>
219     ← p_AC_HC_Type,
220
221     ← boost::shared_ptr<AbstractCellProperty>
222     ← p_PR_BC_Type)
223 {
224
225     mp_RGC_Type = p_RGC_Type;
226     mp_AC_HC_Type = p_AC_HC_Type;
227     mp_PR_BC_Type = p_PR_BC_Type;
228 }
229
230
231 void BoijeCellCycleModel::SetModelParameters(unsigned phase2gen, unsigned
232     ← phase3gen, double probAtoh7, double probPtf1a,
233                                     double probng)
234 {
235
236     mPhase2gen = phase2gen;
237     mPhase3gen = phase3gen;
238     mprobAtoh7 = probAtoh7;
```

```
231     mprobPtf1a = probPtf1a;
232     mprobng = probng;
233 }
234
235 void BoijeCellCycleModel::EnableModeEventOutput(double eventStart,
236     → unsigned seed)
237 {
238     mOutput = true;
239     mEventStartTime = eventStart;
240     mSeed = seed;
241 }
242
243 void BoijeCellCycleModel::WriteModeEventOutput()
244 {
245     double currentTime = SimulationTime::Instance()→GetTime() +
246         → mEventStartTime;
247     CellPtr currentCell = GetCell();
248     double currentCellID = (double) currentCell→GetCellId();
249     (*LogFile::Instance()) << currentTime << "\t" << mSeed << "\t" <<
250         → currentCellID << "\t" << mMitoticMode << "\n";
251 }
252
253 void
254     → BoijeCellCycleModel::EnableSequenceSampler(boost::shared_ptr<AbstractCellProperty>
255         → label)
256 {
257     mSequenceSampler = true;
258     mp_label_Type = label;
259 }
260
261
262 void
263     → BoijeCellCycleModel::EnableModelDebugOutput(boost::shared_ptr<ColumnDataWriter>
264         → debugWriter)
265 {
266     mDebug = true;
267     mDebugWriter = debugWriter;
268
269     mTimeID = mDebugWriter→DefineUnlimitedDimension("Time", "h");
270     mVarIDs.push_back(mDebugWriter→DefineVariable("CellID", "No"));
271     mVarIDs.push_back(mDebugWriter→DefineVariable("Generation", "No"));
272     mVarIDs.push_back(mDebugWriter→DefineVariable("MitoticMode",
273         → "Mode"));
```

```

266     mVarIDs.push_back(mDebugWriter->DefineVariable("atoh7Set",
267         "Percentile"));
268     mVarIDs.push_back(mDebugWriter->DefineVariable("atoh7RV",
269         "Percentile"));
270     mVarIDs.push_back(mDebugWriter->DefineVariable("ptf1aSet",
271         "Percentile"));
272     mVarIDs.push_back(mDebugWriter->DefineVariable("ptf1aRV",
273         "Percentile"));
274     mVarIDs.push_back(mDebugWriter->DefineVariable("ngSet",
275         "Percentile"));
276     mVarIDs.push_back(mDebugWriter->DefineVariable("ngRV",
277         "Percentile"));

278     mDebugWriter->EndDefineMode();
279 }

280 void BoijeCellCycleModel::WriteDebugData(double atoh7RV, double ptf1aRV,
281     double ngRV)
282 {
283     double currentTime = SimulationTime::Instance()->GetTime();
284     CellPtr currentCell = GetCell();
285     double currentCellID = (double) currentCell->GetCellId();

286     mDebugWriter->PutVariable(mTimeID, currentTime);
287     mDebugWriter->PutVariable(mVarIDs[0], currentCellID);
288     mDebugWriter->PutVariable(mVarIDs[1], mGeneration);
289     mDebugWriter->PutVariable(mVarIDs[2], mMitoticMode);
290     mDebugWriter->PutVariable(mVarIDs[3], mprobAtoh7);
291     mDebugWriter->PutVariable(mVarIDs[4], atoh7RV);
292     mDebugWriter->PutVariable(mVarIDs[5], mprobPtf1a);
293     mDebugWriter->PutVariable(mVarIDs[6], ptf1aRV);
294     mDebugWriter->PutVariable(mVarIDs[7], mprobng);
295     mDebugWriter->PutVariable(mVarIDs[8], ngRV);
296     mDebugWriter->AdvanceAlongUnlimitedDimension();
297 }

298 ****
299 * UNUSED FUNCTIONS (required for class nonvirtuality, do not remove)
300 ****/
301
302 double BoijeCellCycleModel::GetAverageTransitCellCycleTime()
303 {
304     return mCellCycleDuration;

```

```

302 }
303
304 double BoijeCellCycleModel::GetAverageStemCellCycleTime()
305 {
306     return mCellCycleDuration;
307 }
308
309 void BoijeCellCycleModel::OutputCellCycleModelParameters(out_stream&
310             rParamsFile)
311 {
312     *rParamsFile << "\t\t\t<CellCycleDuration>" << mCellCycleDuration <<
313             "</CellCycleDuration>\n";
314
315     // Call method on direct parent class
316
317     ↵ AbstractSimpleCellCycleModel::OutputCellCycleModelParameters(rParamsFile);
318
319 // Serialization for Boost ≥ 1.36
320 #include "SerializationExportWrapperForCpp.hpp"
321 CHASTE_CLASS_EXPORT(BoijeCellCycleModel)

```

16.2.19 /src/BoijeCellCycleModel.hpp

```

1 #ifndef BOIJECELLCYCLEMODEL_HPP_
2 #define BOIJECELLCYCLEMODEL_HPP_
3
4 #include "AbstractSimpleCellCycleModel.hpp"
5 #include "RandomNumberGenerator.hpp"
6 #include "Cell.hpp"
7 #include "DifferentiatedCellProliferativeType.hpp"
8 #include "SmartPointers.hpp"
9 #include "ColumnDataWriter.hpp"
10 #include "LogFile.hpp"
11 #include "CellLabel.hpp"
12
13 #include "BoijeRetinalNeuralFates.hpp"
14
15
16 /*****
17 * BOIJE CELL CYCLE MODEL

```

```

18 * As described in Boije et al. 2015 [Boije2015] doi:
→ 10.1016/j.devcel.2015.08.011
19 *
20 * USE: By default, BoijeCellCycleModels are constructed with the
→ parameters reported in [Boije2015].
21 * In normal use, the model steps through three phases of probabilistic
→ transcription factor signalling.
22 * These signals determine the mitotic mode and the fate of offspring.
23 *
24 * NB: the Boije model is purely generational and assumes a generation
→ time of 1; time in Boije simulations
25 * thus represents generation number rather than proper time. Refactor for
→ use with simulations that refer
26 * to clocktime!!
27 *
28 * 2 per-model-event output modes:
29 * EnableModeEventOutput() enables mitotic mode event logging-all cells
→ will write to the singleton log file
30 * EnableModelDebugOutput() enables more detailed debug output, each seed
→ will have its own file written to
31 * by a ColumnDataWriter passed to it from the test
32 * (eg. by the SetupDebugOutput helper function in the project simulator)
33 *
34 * 1 mitotic-event-sequence sampler (only samples one "path" through the
→ lineage):
35 * EnableSequenceSampler() - one "sequence" of progenitors writes mitotic
→ event type to a string in the singleton log file
36 *
37 *****/
38
39 class BoijeCellCycleModel : public AbstractSimpleCellCycleModel
40 {
41     friend class TestSimpleCellCycleModels;
42
43 private:
44
45     /** Needed for serialization.*/
46     friend class boost::serialization::access;
47     /**
48      * Archive the cell-cycle model and random number generator, never
→ used directly - boost uses this.
49      *
50      * @param archive the archive

```

```
51     * @param version the current version of this class
52     */
53     template<class Archive>
54     void serialize(Archive & archive, const unsigned int version)
55     {
56         archive &
57             → boost::serialization::base_object<AbstractSimpleCellCycleModel>(*this);
58
59         SerializableSingleton<RandomNumberGenerator>* p_wrapper =
60             → RandomNumberGenerator::Instance()→GetSerializationWrapper();
61         archive & p_wrapper;
62         archive & mCellCycleDuration;
63         archive & mGeneration;
64     }
65
66     //Private write functions for models
67     void WriteModeEventOutput();
68     void WriteDebugData(double atoh7RV, double ptf1aRV, double ngRV);
69
70 protected:
71     //mode/output variables
72     bool mOutput;
73     double mEventStartTime;
74     bool mSequenceSampler;
75     bool mSeqSamplerLabelSister;
76     //debug writer stuff
77     bool mDebug;
78     int mTimeID;
79     std::vector<int> mVarIDs;
80     boost::shared_ptr<ColumnDataWriter> mDebugWriter;
81     //model parameters and state memory vars
82     unsigned mGeneration;
83     unsigned mPhase2gen;
84     unsigned mPhase3gen;
85     double mprobAtoh7;
86         double mprobPtf1a;
87         double mprobng;
88         bool mAtoh7Signal;
89         bool mPtf1aSignal;
90         bool mNgSignal;
91     unsigned mMitoticMode;
92     unsigned mSeed;
93     boost::shared_ptr<AbstractCellProperty> mp_PostMitoticType;
```

```
92     boost::shared_ptr<AbstractCellProperty> mp_RGC_Type;
93     boost::shared_ptr<AbstractCellProperty> mp_AC_HC_Type;
94     boost::shared_ptr<AbstractCellProperty> mp_PR_BC_Type;
95     boost::shared_ptr<AbstractCellProperty> mp_label_Type;
96
97     /**
98      * Protected copy-constructor for use by CreateCellCycleModel().
99      *
100     * The only way for external code to create a copy of a cell cycle
101    ← model
102    ← * is by calling that method, to ensure that a model of the correct
103    ← subclass is created.
104    ← * This copy-constructor helps subclasses to ensure that all member
105    ← variables are correctly copied when this happens.
106    ← *
107    ← * This method is called by child classes to set member variables for
108    ← a daughter cell upon cell division.
109    ← * Note that the parent cell cycle model will have had
110    ← ResetForDivision() called just before CreateCellCycleModel() is
111    ← called,
112    ← * so performing an exact copy of the parent is suitable behaviour.
113    ← Any daughter-cell-specific initialisation
114    ← * can be done in InitialiseDaughterCell().
115    ← *
116    ← * @param rModel the cell cycle model to copy.
117    ← */
118 BoijeCellCycleModel(const BoijeCellCycleModel& rModel);
119
120 public:
121
122 /**
123  * Constructor - just a default, mBirthTime is set in the
124  ← AbstractCellCycleModel class.
125  */
126 BoijeCellCycleModel();
127
128 /**
129  * SetCellCycleDuration() method to set length of cell cycle (default
130  ← 1.0 as Boije's model is purely generational)
131  */
132 void SetCellCycleDuration();
133
134 /**
135  *
```

```
126     * Overridden builder method to create new copies of
127     * this cell-cycle model.
128     *
129     * @return new cell-cycle model
130     */
131 AbstractCellCycleModel* CreateCellCycleModel();
132
133 /**
134  * Overridden ResetForDivision() method.
135  * Contains general mitotic mode logic
136  */
137 void ResetForDivision();
138
139 /**
140  * Overridden InitialiseDaughterCell() method.
141  * Used to implement asymmetric mitotic mode
142  */
143 void InitialiseDaughterCell();
144
145 /**
146  * Sets the cell's generation.
147  *
148  * @param generation the cell's generation
149  */
150 void SetGeneration(unsigned generation);
151
152 /**
153  * @return the cell's generation.
154  */
155 unsigned GetGeneration() const;
156
157 ****
158  * Model setup functions:
159  * Set TF signal probabilities with SetModelParameters
160  * Set Postmitotic and specification AbstractCellProperties w/ the
161  * relevant functions
162  * By default these are available in BoijeRetinalNeuralFates.hpp
163  *
164  * NB: The Boije model lumps together amacrine & horizontal cells,
165  * photoreceptors & bipolar neurons
166 ****/
```

```

166     void SetModelParameters(unsigned phase2gen = 3, unsigned phase3gen =
167     ↵ 5, double probAtoh7 = 0.32, double probPtf1a = 0.3, double probng
168     ↵ = 0.8);
169
170     void SetPostMitoticType(boost::shared_ptr<AbstractCellProperty>
171     ↵ p_PostMitoticType);
172
173     void SetSpecifiedTypes(boost::shared_ptr<AbstractCellProperty>
174     ↵ p_RGC_Type, boost::shared_ptr<AbstractCellProperty> p_AC_HC_Type,
175     ↵ boost::shared_ptr<AbstractCellProperty> p_PR_BC_Type);
176
177
178 //Functions to enable per-cell mitotic mode logging for mode rate &
179 //sequence sampling fixtures
180 //Uses singleton logfile
181
182     void EnableModeEventOutput(double eventStart, unsigned seed);
183
184     void EnableSequenceSampler(boost::shared_ptr<AbstractCellProperty>
185     ↵ label);
186
187
188 //More detailed debug output. Needs a ColumnDataWriter passed to it
189 //Only declare ColumnDataWriter directory, filename, etc; do not set
190 //up otherwise
191
192     void EnableModelDebugOutput(boost::shared_ptr<ColumnDataWriter>
193     ↵ debugWriter);
194
195
196 /**
197 * Overridden GetAverageTransitCellCycleTime() method.
198 *
199 * @return the average of mMinCellCycleDuration and
200 mMaxCellCycleDuration
201 */
202
203 double GetAverageTransitCellCycleTime();
204
205
206 /**
207 * Overridden GetAverageStemCellCycleTime() method.
208 *
209 * @return the average of mMinCellCycleDuration and
210 mMaxCellCycleDuration
211 */
212
213 double GetAverageStemCellCycleTime();
214
215
216 /**
217 * Overridden OutputCellCycleModelParameters() method.
218 *
219 * @param rParamsFile the file stream to which the parameters are
220 output

```

```

197     */
198     virtual void OutputCellCycleModelParameters(out_stream& rParamsFile);
199 };
200
201 #include "SerializationExportWrapper.hpp"
202 // Declare identifier for the serializer
203 CHASTE_CLASS_EXPORT(BoijeCellCycleModel)
204
205 #endif /*BOIJECELLCYCLEMODEL_HPP_*/

```

16.2.20 /src/BoijeRetinalNeuralFates.cpp

```

1 #include " ../../../../../projects/ISP/src/BoijeRetinalNeuralFates.hpp"
2
3 RetinalGanglion::RetinalGanglion(unsigned colour)
4     : AbstractCellProperty(),
5      mColour(colour)
6 {
7 }
8
9 RetinalGanglion::~RetinalGanglion()
10 {
11 }
12
13 unsigned RetinalGanglion::GetColour() const
14 {
15     return mColour;
16 }
17
18 AmacrineHorizontal::AmacrineHorizontal(unsigned colour)
19     : AbstractCellProperty(),
20      mColour(colour)
21 {
22 }
23
24 AmacrineHorizontal::~AmacrineHorizontal()
25 {
26 }
27
28 unsigned AmacrineHorizontal::GetColour() const
29 {
30     return mColour;

```

```

31 }
32
33 ReceptorBipolar::ReceptorBipolar(unsigned colour)
34     : AbstractCellProperty(),
35     mColour(colour)
36 {
37 }
38
39 ReceptorBipolar::~ReceptorBipolar()
40 {
41 }
42
43 unsigned ReceptorBipolar::GetColour() const
44 {
45     return mColour;
46 }
47
48 #include "SerializationExportWrapperForCpp.hpp"
49 // Declare identifier for the serializer
50 CHASTE_CLASS_EXPORT(RetinalGanglion)
51 CHASTE_CLASS_EXPORT(AmacrineHorizontal)
52 CHASTE_CLASS_EXPORT(ReceptorBipolar)

```

16.2.21 /src/BoijeRetinalNeuralFates.hpp

```

1 #ifndef BOIJERETINALNEURALFATES_HPP_
2 #define BOIJERETINALNEURALFATES_HPP_
3
4 #include <boost/shared_ptr.hpp>
5 #include "AbstractCellProperty.hpp"
6 #include "ChasteSerialization.hpp"
7 #include <boost/serialization/base_object.hpp>
8
9 class RetinalGanglion : public AbstractCellProperty
10 {
11 private:
12
13     /**
14      * Colour for use by visualizer.
15      */
16     unsigned mColour;
17

```

```
18  /** Needed for serialization.*/
19  friend class boost::serialization::access;
20  /**
21   * Archive the member variables.
22   *
23   * @param archive the archive
24   * @param version the current version of this class
25   */
26  template<class Archive>
27  void serialize(Archive & archive, const unsigned int version)
28  {
29      archive &
30      boost::serialization::base_object<AbstractCellProperty>(*this);
31      archive & mColour;
32  }
33
34 public:
35 /**
36  * Constructor.
37  *
38  * @param colour what colour cells with this property should be in
39  * the visualizer (defaults to 6)
40  */
41 RetinalGanglion(unsigned colour=3);
42 /**
43  * Destructor.
44  */
45 virtual ~RetinalGanglion();
46
47 /**
48  * @return #mColour.
49  */
50 unsigned GetColour() const;
51 };
52
53 class AmacrineHorizontal : public AbstractCellProperty
54 {
55 private:
56 /**
57  * Colour for use by visualizer.
```

```
59     */
60     unsigned mColour;
61
62     /** Needed for serialization.*/
63     friend class boost::serialization::access;
64     /**
65      * Archive the member variables.
66      *
67      * @param archive the archive
68      * @param version the current version of this class
69      */
70     template<class Archive>
71     void serialize(Archive & archive, const unsigned int version)
72     {
73         archive &
74             boost::serialization::base_object<AbstractCellProperty>(*this);
75         archive & mColour;
76     }
77
78 public:
79 /**
80  * Constructor.
81  *
82  * @param colour what colour cells with this property should be in
83  * the visualizer (defaults to 6)
84  */
85     AmacrineHorizontal(unsigned colour=4);
86
87 /**
88  * Destructor.
89  */
90     virtual ~AmacrineHorizontal();
91
92 /**
93  * @return #mColour.
94  */
95     unsigned GetColour() const;
96 };
97
98 class ReceptorBipolar : public AbstractCellProperty
99 {
```

```
100
101  /**
102   * Colour for use by visualizer.
103   */
104  unsigned mColour;
105
106  /** Needed for serialization.*/
107  friend class boost::serialization::access;
108  /**
109   * Archive the member variables.
110   *
111   * @param archive the archive
112   * @param version the current version of this class
113   */
114  template<class Archive>
115  void serialize(Archive & archive, const unsigned int version)
116  {
117      archive &
118          → boost::serialization::base_object<AbstractCellProperty>(*this);
119      archive & mColour;
120  }
121
122
123  /**
124   * Constructor.
125   *
126   * @param colour what colour cells with this property should be in
127   * the visualizer (defaults to 6)
128   */
129  ReceptorBipolar(unsigned colour=5);
130
131  /**
132   * Destructor.
133   */
134  virtual ~ReceptorBipolar();
135
136  /**
137   * @return #mColour.
138   */
139  unsigned GetColour() const;
140};
```

```
141 #include "SerializationExportWrapper.hpp"  
142 // Declare identifier for the serializer  
143 CHASTE_CLASS_EXPORT(RetinalGanglion)  
144 CHASTE_CLASS_EXPORT(amacrineHorizontal)  
145 CHASTE_CLASS_EXPORT(ReceptorBipolar)  
146  
147 #endif /* BOIJERETINALNEURALFATES_HPP_ */
```

16.2.22 /src/GomesCellCycleModel.cpp

```

1 #include "GomesCellCycleModel.hpp"
2 #include "GomesRetinalNeuralFates.hpp"
3
4 GomesCellCycleModel::GomesCellCycleModel() :
5     AbstractSimpleCellCycleModel(), mOutput(false),
6     ↳ mEventStartTime(), mSequenceSampler(false),
7     ↳ mSeqSamplerLabelSister(
8         false), mDebug(false), mTimeID(), mVarIDs(),
9         ↳ mDebugWriter(), mNormalMu(3.9716),
10        ↳ mNormalSigma(0.32839), mPP(
11            .055), mPD(.221), mpBC(.128), mpAC(.106), mpMG(.028),
12        ↳ mMitoticMode(), mSeed(), mp_PostMitoticType(),
13        ↳ mp_RPh_Type(), mp_BC_Type(), mp_AC_Type(),
14        ↳ mp_MG_Type(), mp_label_Type()
15    {
16 }
17
18 GomesCellCycleModel::GomesCellCycleModel(const GomesCellCycleModel&
19     ↳ rModel) :
20     AbstractSimpleCellCycleModel(rModel), mOutput(rModel.mOutput),
21     ↳ mEventStartTime(rModel.mEventStartTime), mSequenceSampler(
22         rModel.mSequenceSampler),
23         ↳ mSeqSamplerLabelSister(rModel.mSeqSamplerLabelSister),
24         ↳ mDebug(rModel.mDebug), mTimeID(
25             rModel.mTimeID), mVarIDs(rModel.mVarIDs),
26             ↳ mDebugWriter(rModel.mDebugWriter), mNormalMu(
27                 rModel.mNormalMu), mNormalSigma(rModel.mNormalSigma),
28                 ↳ mPP(rModel.mPP), mPD(rModel.mPD), mpBC(
29                     rModel.mpBC), mpAC(rModel.mpAC), mpMG(rModel.mpMG),
30                     ↳ mMitoticMode(rModel.mMitoticMode), mSeed(
31                         rModel.mSeed)
32

```

```

17             rModel.mSeed),
18             ↳ mp_PostMitoticType(rModel.mp_PostMitoticType),
19             ↳ mp_RPh_Type(rModel.mp_RPh_Type), mp_BC_Type(
20             rModel.mp_BC_Type), mp_AC_Type(rModel.mp_AC_Type),
21             ↳ mp_MG_Type(rModel.mp_MG_Type), mp_label_Type(
22             rModel.mp_label_Type)
23 {
24 }
25
26 AbstractCellCycleModel* GomesCellCycleModel::CreateCellCycleModel()
27 {
28     return new GomesCellCycleModel(*this);
29 }
30
31 /**
32 * CELL CYCLE DURATION RANDOM VARIABLE
33 */
34 RandomNumberGenerator* p_random_number_generator =
35     ↳ RandomNumberGenerator::Instance();
36
37 //Gomes cell cycle length determined by lognormal distribution with
38     ↳ default mean 56 hr, std 18.9 hrs.
39 mCellCycleDuration =
40     ↳ exp(p_random_number_generator->NormalRandomDeviate(mNormalMu,
41     ↳ mNormalSigma));
42
43 void GomesCellCycleModel::ResetForDivision()
44 {
45 /**
46 * Mitotic mode rules
47 */
48 RandomNumberGenerator* p_random_number_generator =
49     ↳ RandomNumberGenerator::Instance();
50
51 /**
52 * MITOTIC MODE RANDOM VARIABLE

```

```

52   *****/
53   //initialise mitoticmode random variable, set mitotic mode
54   // appropriately after comparing to mode probability array
55   double mitoticModeRV = p_random_number_generator->ranf();
56
57   if (mitoticModeRV > mPP && mitoticModeRV ≤ mPP + mPD)
58   {
59     mMitoticMode = 1;
60   }
61   if (mitoticModeRV > mPP + mPD)
62   {
63     mMitoticMode = 2;
64   }
65
66   /**
67   * Write mitotic event to file if appropriate
68   * mDebug: many files: detailed per-lineage info switch; intended for
69   // TestHeInductionCountFixture
70   * mOutput: 1 file: time, seed, cellID, mitotic mode, intended for
71   // TestHeMitoticModeRateFixture
72   * *****/
73
74   if (mDebug)
75   {
76     WriteDebugData(mitoticModeRV);
77   }
78
79   if (mOutput)
80   {
81     WriteModeEventOutput();
82   }
83
84   //set new cell cycle length (will be overwritten with DBL_MAX for DD
85   // divisions)
86   AbstractSimpleCellCycleModel::ResetForDivision();
87
88   /**
89   * Symmetric postmitotic specification rule
90   * -(asymmetric postmitotic rule specified in
91   // InitialiseDaughterCell());
92   * *****/
93
94   if (mMitoticMode == 2)

```

```

90  {
91      mpCell→SetCellProliferativeType(mp_PostMitoticType);
92      mCellCycleDuration = DBL_MAX;
93      /*****
94      * SPECIFICATION RANDOM VARIABLE
95      *****/
96      double specificationRV = p_random_number_generator→ranf();
97      if (specificationRV ≤ mpMG)
98      {
99          mpCell→AddCellProperty(mp_MG_Type);
100     }
101    if (specificationRV > mpMG && specificationRV ≤ mpMG + mpAC)
102    {
103        mpCell→AddCellProperty(mp_AC_Type);
104    }
105    if (specificationRV > mpMG + mpAC && specificationRV ≤ mpMG +
106        → mpAC + mpBC)
107    {
108        mpCell→AddCellProperty(mp_BC_Type);
109    }
110    if (specificationRV > mpMG + mpAC + mpBC)
111    {
112        mpCell→AddCellProperty(mp_RPh_Type);
113    }
114
115    *****
116    * SEQUENCE SAMPLER
117    *****/
118 //if the sequence sampler has been turned on, check for the label &
119 //→ write mitotic mode to log
120 //50% chance of each daughter cell from a mitosis inheriting the
121 //→ label
122 if (mSequenceSampler)
123 {
124     if (mpCell→HasCellProperty<CellLabel>())
125     {
126         (*LogFile::Instance()) << mMitoticMode;
127         double labelRV = p_random_number_generator→ranf();
128         if (labelRV ≤ .5)
129         {
130             mSeqSamplerLabelSister = true;
131             mpCell→RemoveCellProperty<CellLabel>();

```

```
130         }
131     else
132     {
133         mSeqSamplerLabelSister = false;
134     }
135 }
136 else
137 {
138     //prevents lost-label cells from labelling their progeny
139     mSeqSamplerLabelSister = false;
140 }
141 }
142 }
143
144 void GomesCellCycleModel::InitialiseDaughterCell()
145 {
146     if (mMitoticMode == 0)
147     {
148         //daughter cell's mCellCycleDuration is copied from parent; reset
149         //to new value from gamma PDF here
150         SetCellCycleDuration();
151     }
152
153     *****
154     * PD-type division
155     *****
156
157     if (mMitoticMode == 1)
158     {
159         RandomNumberGenerator* p_random_number_generator =
160             RandomNumberGenerator::Instance();
161         mpCell->SetCellProliferativeType(mp_PostMitoticType);
162         mCellCycleDuration = DBL_MAX;
163
164         *****
165         * SPECIFICATION RULES
166         *****
167         double specificationRV = p_random_number_generator->ranf();
168         if (specificationRV <= mpMG)
169         {
170             mpCell->AddCellProperty(mp_MG_Type);
171         }
172         if (specificationRV > mpMG && specificationRV <= mpMG + mpAC)
173         {
```

```
171         mpCell→AddCellProperty(mp_AC_Type);
172     }
173     if (specificationRV > mpMG + mpAC && specificationRV ≤ mpMG +
174         → mpAC + mpBC)
175     {
176         mpCell→AddCellProperty(mp_BC_Type);
177     }
178     if (specificationRV > mpMG + mpAC + mpBC)
179     {
180         mpCell→AddCellProperty(mp_RPh_Type);
181     }
182
183 if (mMitoticMode = 2)
184 {
185     RandomNumberGenerator* p_random_number_generator =
186         → RandomNumberGenerator::Instance();
187     //remove the fate assigned to the parent cell in
188     → ResetForDivision, then assign the sister fate as usual
189     mpCell→RemoveCellProperty<AbstractCellProperty>();
190     mpCell→SetCellProliferativeType(mp_PostMitoticType);
191
192     /*****
193     * SPECIFICATION RULES
194     *****/
195     double specificationRV = p_random_number_generator→ranf();
196     if (specificationRV ≤ mpMG)
197     {
198         mpCell→AddCellProperty(mp_MG_Type);
199     }
200     if (specificationRV > mpMG && specificationRV ≤ mpMG + mpAC)
201     {
202         mpCell→AddCellProperty(mp_AC_Type);
203     }
204     if (specificationRV > mpMG + mpAC && specificationRV ≤ mpMG +
205         → mpAC + mpBC)
206     {
207         mpCell→AddCellProperty(mp_BC_Type);
208     }
209     if (specificationRV > mpMG + mpAC + mpBC)
210     {
211         mpCell→AddCellProperty(mp_RPh_Type);
212     }
```

```

210     }
211
212     /*****
213     * SEQUENCE SAMPLER
214     *****/
215     if (mSequenceSampler)
216     {
217         if (mSeqSamplerLabelSister)
218         {
219             mpCell->AddCellProperty(mp_label_Type);
220             mSeqSamplerLabelSister = false;
221         }
222         else
223         {
224             mpCell->RemoveCellProperty<CellLabel>();
225         }
226     }
227 }
228
229 void GomesCellCycleModel::SetModelParameters(const double normalMu, const
230                                         → double normalSigma, const double PP,
231                                         → const double PD, const
232                                         → double pBC, const double
233                                         → pAC, const double pMG)
234 {
235     mNormalMu = normalMu;
236     mNormalSigma = normalSigma;
237     mPP = PP;
238     mPD = PD;
239     mpBC = pBC;
240     mpAC = pAC;
241     mpMG = pMG;
242 }
243
244 void
245     → GomesCellCycleModel::SetModelProperties(boost::shared_ptr<AbstractCellProperty>
246     → p_RPh_Type,
247                                         → boost::shared_ptr<AbstractCellProper
248                                         → p_AC_Type,
```



```

276     mSequenceSampler = true;
277     mp_label_Type = label;
278 }
279
280 void
281   ↵ GomesCellCycleModel :: EnableModelDebugOutput(boost :: shared_ptr<ColumnDataWriter>
282   ↵ debugWriter)
283 {
284
285     mDebug = true;
286     mDebugWriter = debugWriter;
287
288     mTimeID = mDebugWriter->DefineUnlimitedDimension("Time", "h");
289     mVarIDs.push_back(mDebugWriter->DefineVariable("CellID", "No"));
290     mVarIDs.push_back(mDebugWriter->DefineVariable("CycleDuration",
291       ↵ "h"));
292     mVarIDs.push_back(mDebugWriter->DefineVariable("PP", "Percentile"));
293     mVarIDs.push_back(mDebugWriter->DefineVariable("PD", "Percentile"));
294     mVarIDs.push_back(mDebugWriter->DefineVariable("Dieroll",
295       ↵ "Percentile"));
296     mVarIDs.push_back(mDebugWriter->DefineVariable("MitoticMode",
297       ↵ "Mode"));
298
299     mDebugWriter->EndDefineMode();
300 }
301
302
303 void GomesCellCycleModel :: WriteDebugData(double percentileRoll)
304 {
305     double currentTime = SimulationTime::Instance()->GetTime();
306     CellPtr currentCell = GetCell();
307     double currentCellID = (double) currentCell->GetCellId();
308
309     mDebugWriter->PutVariable(mTimeID, currentTime);
310     mDebugWriter->PutVariable(mVarIDs[0], currentCellID);
311     mDebugWriter->PutVariable(mVarIDs[1], mCellCycleDuration);
312     mDebugWriter->PutVariable(mVarIDs[2], mPP);
313     mDebugWriter->PutVariable(mVarIDs[3], mPD);
314     mDebugWriter->PutVariable(mVarIDs[4], percentileRoll);
315     mDebugWriter->PutVariable(mVarIDs[5], mMitoticMode);
316     mDebugWriter->AdvanceAlongUnlimitedDimension();
317 }
318
319 ****
320 * UNUSED FUNCTIONS (required for class nonvirtuality, do not remove)

```

```

314  *****/
315
316 double GomesCellCycleModel::GetAverageTransitCellCycleTime()
317 {
318     return (0.0);
319 }
320
321 double GomesCellCycleModel::GetAverageStemCellCycleTime()
322 {
323     return (0.0);
324 }
325
326 void GomesCellCycleModel::OutputCellCycleModelParameters(out_stream&
327   ↪ rParamsFile)
328 {
329     *rParamsFile << "\t\t\t<CellCycleDuration>" << mCellCycleDuration <<
330     ↪ "</CellCycleDuration>\n";
331
332     // Call method on direct parent class
333
334     ↪ AbstractSimpleCellCycleModel::OutputCellCycleModelParameters(rParamsFile);
335
336 // Serialization for Boost ≥ 1.36
337 #include "SerializationExportWrapperForCpp.hpp"
338 CHASTE_CLASS_EXPORT(GomesCellCycleModel)

```

16.2.23 /src/GomesCellCycleModel.hpp

```

1 #ifndef GOMESCELLCYCLEMODEL_HPP_
2 #define GOMESCELLCYCLEMODEL_HPP_
3
4 #include "AbstractSimpleCellCycleModel.hpp"
5 #include "RandomNumberGenerator.hpp"
6 #include "Cell.hpp"
7 #include "DifferentiatedCellProliferativeType.hpp"
8 #include "GomesRetinalNeuralFates.hpp"
9 #include "SmartPointers.hpp"
10 #include "ColumnDataWriter.hpp"
11 #include "LogFile.hpp"
12 #include "CellLabel.hpp"
13

```

```

14 /*****
15 * GOMES CELL CYCLE MODEL
16 * As described in Gomes et al. 2011 [Gomes2011] doi: 10.1242/dev.059683
17 *
18 * USE: By default, GomesCellCycleModels are constructed with the
19 *      parameter fit reported in [Gomes2011].
20 * Cell cycle length, mitotic mode, and postmitotic fate of cells are
21 *      determined by independent random variables
22 * PP = symmetric proliferative mitotic mode, both progeny remain mitotic
23 * PD = asymmetric proliferative mitotic mode, one progeny exits the cell
24 *      cycle and differentiates
25 * DD = symmetric differentiative mitotic mode, both progeny exit the
26 *      cell cycle and differentiate
27 *
28 * Change default model parameters with SetModelParameters(<params>);
29 * Set AbstractCellProperties for differentiated neural types with
30 *      SetModelProperties();
31 *
32 *
33 * 2 per-model-event output modes:
34 * EnableModeEventOutput() enables mitotic mode event logging-all cells
35 *      will write to the singleton log file
36 * EnableModelDebugOutput() enables more detailed debug output, each seed
37 *      will have its own file written to
38 * by a ColumnDataWriter passed to it from the test
39 * (eg. by the SetupDebugOutput helper function in the project simulator)
40 *
41 * 1 mitotic-event-sequence sampler (only samples one "path" through the
42 *      lineage):
43 * EnableSequenceSampler() - one "sequence" of progenitors writes mitotic
44 *      event type to a string in the singleton log file
45 *
46 *****/
47
48 class GomesCellCycleModel : public AbstractSimpleCellCycleModel
49 {
50     friend class TestSimpleCellCycleModels;
51
52 private:
53
54     /** Needed for serialization.*/
55     friend class boost::serialization::access;
56     /**

```

```

47     * Archive the cell-cycle model and random number generator, never
48     → used directly - boost uses this.
49
50     *
51     * @param archive the archive
52     * @param version the current version of this class
53     */
54
55     template<class Archive>
56     void serialize(Archive & archive, const unsigned int version)
57     {
58         archive &
59         → boost::serialization::base_object<AbstractSimpleCellCycleModel>(*this);
60
61         SerializableSingleton<RandomNumberGenerator>* p_wrapper =
62
63             → RandomNumberGenerator::Instance()→GetSerializationWrapper();
64         archive & p_wrapper;
65         archive & mCellCycleDuration;
66     }
67
68     //Private write functions for models
69     void WriteModeEventOutput();
70     void WriteDebugData(double percentile);
71
72 protected:
73     //mode/output variables
74     bool mOutput;
75     double mEventStartTime;
76     bool mSequenceSampler;
77     bool mSeqSamplerLabelSister;
78     //debug writer stuff
79     bool mDebug;
80     int mTimeID;
81     std::vector<int> mVarIDs;
82     boost::shared_ptr<ColumnDataWriter> mDebugWriter;
83     //model parameters and state memory vars
84     double mNormalMu;
85     double mNormalSigma;
86     double mPP;
87     double mPD;
88     double mpBC;
89     double mpAC;
90     double mpMG;
91     unsigned mMitoticMode;

```

```

87     unsigned mSeed;
88     boost::shared_ptr<AbstractCellProperty> mp_PostMitoticType;
89     boost::shared_ptr<AbstractCellProperty> mp_RPh_Type;
90     boost::shared_ptr<AbstractCellProperty> mp_BC_Type;
91     boost::shared_ptr<AbstractCellProperty> mp_AC_Type;
92     boost::shared_ptr<AbstractCellProperty> mp_MG_Type;
93     boost::shared_ptr<AbstractCellProperty> mp_label_Type;
94
95     /**
96      * Protected copy-constructor for use by CreateCellCycleModel().
97      *
98      * The only way for external code to create a copy of a cell cycle
99      * model
100     * is by calling that method, to ensure that a model of the correct
101     * subclass is created.
102     * This copy-constructor helps subclasses to ensure that all member
103     * variables are correctly copied when this happens.
104     *
105     * This method is called by child classes to set member variables for
106     * a daughter cell upon cell division.
107     * Note that the parent cell cycle model will have had
108     * ResetForDivision() called just before CreateCellCycleModel() is
109     * called,
110     * so performing an exact copy of the parent is suitable behaviour.
111     * Any daughter-cell-specific initialisation
112     * can be done in InitialiseDaughterCell().
113     */
114     GomesCellCycleModel(const GomesCellCycleModel& rModel);
115
116 public:
117
118     /**
119      * Constructor - just a default, mBirthTime is set in the
120      * AbstractCellCycleModel class.
121      */
122     GomesCellCycleModel();
123
124     /**
125      * SetCellCycleDuration() method to set length of cell cycle
126      * (lognormal distribution as specified in [Gomes2011])
127      */
128
129
130

```

```

121 void SetCellCycleDuration();
122
123 /**
124 * Overridden builder method to create new copies of
125 * this cell-cycle model.
126 *
127 * @return new cell-cycle model
128 */
129 AbstractCellCycleModel* CreateCellCycleModel();
130
131 /**
132 * Overridden ResetForDivision() method.
133 * Contains general mitotic mode logic
134 */
135 void ResetForDivision();
136
137 /** Overridden InitialiseDaughterCell() method. Used to implement
138 * asymmetric mitotic mode*/
139 void InitialiseDaughterCell();
140
141 /* Model setup functions
142 * Set lognormal cell cycle curve properties with *mean and std of
143 * corresponding NORMAL curve*
144 * Model requires valid AbstractCellProperties to assign postmitotic
145 * fate;
146 * Defaults are found in GomesRetinalNeuralFates.hpp
147 * (RodPhotoreceptor, AmacrineCell, BipolarCell, MullerGlia)
148 */
149
150 void SetModelParameters(const double normalMu = 3.9716, const double
151 * normalSigma = .32839, const double PP = .055,
152 * const double PD = .221, const double pBC =
153 * .128, const double pAC = .106, const
154 * double pMG =
155 * .028);
156
157 void SetModelProperties(boost::shared_ptr<AbstractCellProperty>
158 * p_RPh_Type,
159 * boost::shared_ptr<AbstractCellProperty>
160 * p_AC_Type,
161 * boost::shared_ptr<AbstractCellProperty>
162 * p_BC_Type,
163 * boost::shared_ptr<AbstractCellProperty>
164 * p_MG_Type);

```

```

154
155 //This should normally be a DifferentiatedCellProliferativeType
156 void SetPostMitoticType(boost::shared_ptr<AbstractCellProperty>
157   → p_PostMitoticType);
158
159 //Functions to enable per-cell mitotic mode logging for mode rate &
160   → sequence sampling fixtures
161 //Uses singleton logfile
162 void EnableModeEventOutput(double eventStart, unsigned seed);
163 void EnableSequenceSampler(boost::shared_ptr<AbstractCellProperty>
164   → label);
165
166 //More detailed debug output. Needs a ColumnDataWriter passed to it
167 //Only declare ColumnDataWriter directory, filename, etc; do not set
168   → up otherwise
169 void EnableModelDebugOutput(boost::shared_ptr<ColumnDataWriter>
170   → debugWriter);
171
172 //Not used, but must be overwritten lest GomesCellCycleModels be
173   → abstract
174 double GetAverageTransitCellCycleTime();
175 double GetAverageStemCellCycleTime();
176
177 /**
178 * Overridden OutputCellCycleModelParameters() method.
179 *
180 * @param rParamsFile the file stream to which the parameters are
181   → output
182 */
183 virtual void OutputCellCycleModelParameters(out_stream& rParamsFile);
184 };
185
186 #include "SerializationExportWrapper.hpp"
187 // Declare identifier for the serializer
188 CHASTE_CLASS_EXPORT(GomesCellCycleModel)
189
190
191 #endif /*GOMESCELLCYCLEMODEL_HPP_*/

```

16.2.24 /src/GomesRetinalNeuralFates.cpp

```

1 #include "GomesRetinalNeuralFates.hpp"
2

```

```
3 RodPhotoreceptor::RodPhotoreceptor(unsigned colour)
4     : AbstractCellProperty(),
5     mColour(colour)
6 {
7 }
8
9 RodPhotoreceptor::~RodPhotoreceptor()
10 {
11 }
12
13 unsigned RodPhotoreceptor::GetColour() const
14 {
15     return mColour;
16 }
17
18 AmacrineCell::AmacrineCell(unsigned colour)
19     : AbstractCellProperty(),
20     mColour(colour)
21 {
22 }
23
24 AmacrineCell::~AmacrineCell()
25 {
26 }
27
28 unsigned AmacrineCell::GetColour() const
29 {
30     return mColour;
31 }
32
33 BipolarCell::BipolarCell(unsigned colour)
34     : AbstractCellProperty(),
35     mColour(colour)
36 {
37 }
38
39 BipolarCell::~BipolarCell()
40 {
41 }
42
43 unsigned BipolarCell::GetColour() const
44 {
45     return mColour;
```

```

46 }
47
48 MullerGlia::MullerGlia(unsigned colour)
49     : AbstractCellProperty(),
50     mColour(colour)
51 {
52 }
53
54 MullerGlia::~MullerGlia()
55 {
56 }
57
58 unsigned MullerGlia::GetColour() const
59 {
60     return mColour;
61 }
62
63 #include "SerializationExportWrapperForCpp.hpp"
64 // Declare identifier for the serializer
65 CHASTE_CLASS_EXPORT(RodPhotoreceptor)
66 CHASTE_CLASS_EXPORT(AmacrineCell)
67 CHASTE_CLASS_EXPORT(BipolarCell)
68 CHASTE_CLASS_EXPORT(MullerGlia)

```

16.2.25 /src/GomesRetinalNeuralFates.hpp

```

1 #ifndef GOMESRETINALNEURALFATES_HPP_
2 #define GOMESRETINALNEURALFATES_HPP_
3
4 #include <boost/shared_ptr.hpp>
5 #include "AbstractCellProperty.hpp"
6 #include "ChasteSerialization.hpp"
7 #include <boost/serialization/base_object.hpp>
8
9 class RodPhotoreceptor : public AbstractCellProperty
10 {
11 private:
12
13     /**
14      * Colour for use by visualizer.
15      */
16     unsigned mColour;

```

```
17
18     /** Needed for serialization. */
19     friend class boost::serialization::access;
20
21     /**
22      * Archive the member variables.
23      *
24      * @param archive the archive
25      * @param version the current version of this class
26      */
27     template<class Archive>
28     void serialize(Archive & archive, const unsigned int version)
29     {
30         archive &
31             boost::serialization::base_object<AbstractCellProperty>(*this);
32         archive & mColour;
33     }
34
35 public:
36
37     /**
38      * Constructor.
39      *
40      * @param colour what colour cells with this property should be in
41      * the visualizer (defaults to 6)
42      */
43     RodPhotoreceptor(unsigned colour=3);
44
45     virtual ~RodPhotoreceptor();
46
47     /**
48      * @return #mColour.
49      */
50     unsigned GetColour() const;
51 };
52
53 class AmacrineCell : public AbstractCellProperty
54 {
55 private:
56
57     /**
```

```
58     * Colour for use by visualizer.  
59     */  
60     unsigned mColour;  
61  
62     /** Needed for serialization. */  
63     friend class boost::serialization::access;  
64     /**  
65      * Archive the member variables.  
66      *  
67      * @param archive the archive  
68      * @param version the current version of this class  
69      */  
70     template<class Archive>  
71     void serialize(Archive & archive, const unsigned int version)  
72     {  
73         archive &  
74             → boost::serialization::base_object<AbstractCellProperty>(*this);  
75         archive & mColour;  
76     }  
77  
78 public:  
79  
80     /**  
81      * Constructor.  
82      *  
83      * @param colour what colour cells with this property should be in  
84      * the visualizer (defaults to 6)  
85      */  
86     AmacrineCell(unsigned colour=4);  
87  
88     /**  
89      * Destructor.  
90      */  
91     virtual ~AmarineCell();  
92  
93     /**  
94      * @return #mColour.  
95      */  
96     unsigned GetColour() const;  
97 };  
98  
99 class BipolarCell : public AbstractCellProperty  
100 {
```

```
99 private:
100
101     /**
102      * Colour for use by visualizer.
103      */
104     unsigned mColour;
105
106     /** Needed for serialization.*/
107     friend class boost::serialization::access;
108     /**
109      * Archive the member variables.
110      *
111      * @param archive the archive
112      * @param version the current version of this class
113      */
114     template<class Archive>
115     void serialize(Archive & archive, const unsigned int version)
116     {
117         archive &
118             → boost::serialization::base_object<AbstractCellProperty>(*this);
119         archive & mColour;
120     }
121
122
123 public:
124
125     /**
126      * Constructor.
127      *
128      * @param colour what colour cells with this property should be in
129      * the visualizer (defaults to 6)
130      */
131     BipolarCell(unsigned colour=5);
132
133     /**
134      * Destructor.
135      */
136     virtual ~BipolarCell();
137
138     /**
139      * @return #mColour.
140      */
141     unsigned GetColour() const;
142 }
```

```
140
141 class MullerGlia : public AbstractCellProperty
142 {
143 private:
144
145     /**
146      * Colour for use by visualizer.
147      */
148     unsigned mColour;
149
150     /** Needed for serialization.*/
151     friend class boost::serialization::access;
152     /**
153      * Archive the member variables.
154      *
155      * @param archive the archive
156      * @param version the current version of this class
157      */
158     template<class Archive>
159     void serialize(Archive & archive, const unsigned int version)
160     {
161         archive &
162             boost::serialization::base_object<AbstractCellProperty>(*this);
163         archive & mColour;
164     }
165
166 public:
167
168     /**
169      * Constructor.
170      *
171      * @param colour what colour cells with this property should be in
172      * the visualizer (defaults to 6)
173      */
174     MullerGlia(unsigned colour=6);
175
176     /**
177      * Destructor.
178      */
179     virtual ~MullerGlia();
180
181     /**
182      * @return #mColour.
```

```

181     */
182     unsigned GetColour() const;
183 };
184 #include "SerializationExportWrapper.hpp"
185 // Declare identifier for the serializer
186 CHASTE_CLASS_EXPORT(RodPhotoreceptor)
187 CHASTE_CLASS_EXPORT(AmacrineCell)
188 CHASTE_CLASS_EXPORT(BipolarCell)
189 CHASTE_CLASS_EXPORT(MullerGlia)
190
191 #endif /* GOMESRETINALNEURALFATES_HPP_ */

```

16.2.26 /src/HeAth5Mo.cpp

```

1 #include "../..../..../projects/ISP/src/HeAth5Mo.hpp"
2
3 Ath5Mo::Ath5Mo(unsigned colour)
4     : AbstractCellProperty(),
5      mColour(colour)
6 {
7 }
8
9 Ath5Mo::~Ath5Mo()
10 {
11 }
12
13 unsigned Ath5Mo::GetColour() const
14 {
15     return mColour;
16 }
17
18 #include "SerializationExportWrapperForCpp.hpp"
19 // Declare identifier for the serializer
20 CHASTE_CLASS_EXPORT(Ath5Mo)

```

16.2.27 /src/HeAth5Mo.hpp

```

1 #ifndef HEATH5MO_HPP_
2 #define HEATH5MO_HPP_
3
4 #include <boost/shared_ptr.hpp>

```

```
5 #include "AbstractCellProperty.hpp"
6 #include "ChasteSerialization.hpp"
7 #include <boost/serialization/base_object.hpp>
8
9 class Ath5Mo : public AbstractCellProperty
10 {
11 private:
12
13     /**
14      * Colour for use by visualizer.
15      */
16     unsigned mColour;
17
18     /** Needed for serialization.*/
19     friend class boost::serialization::access;
20
21     /**
22      * Archive the member variables.
23      *
24      * @param archive the archive
25      * @param version the current version of this class
26      */
27     template<class Archive>
28     void serialize(Archive & archive, const unsigned int version)
29     {
30         archive &
31             boost::serialization::base_object<AbstractCellProperty>(*this);
32         archive & mColour;
33     }
34
35 public:
36
37     /**
38      * Constructor.
39      *
40      * @param colour what colour cells with this property should be in
41      * the visualizer (defaults to 6)
42      */
43     Ath5Mo(unsigned colour=6);
44
45     /**
46      * Destructor.
47      */
48     virtual ~Ath5Mo();
```

```

46
47     /**
48      * @return #mColour.
49      */
50     unsigned GetColour() const;
51 }
52
53
54 #include "SerializationExportWrapper.hpp"
55 // Declare identifier for the serializer
56 CHASTE_CLASS_EXPORT(Ath5Mo)
57 #endif /* HEATH5MO_HPP */

```

16.2.28 /src/HeCellCycleModel.cpp

```

1 #include "HeCellCycleModel.hpp"
2
3 HeCellCycleModel::HeCellCycleModel() :
4     AbstractSimpleCellCycleModel(), mKillSpecified(false),
5     mDeterministic(false), mOutput(false), mEventStartTime(
6         24.0), mSequenceSampler(false),
7     mSeqSamplerLabelSister(false), mDebug(false),
8     mTimeID(), mVarIDs(), mDebugWriter(), mTiLOffset(
9         0.0), mGammaShift(4.0), mGammaShape(2.0),
10    mGammaScale(1.0), mSisterShiftWidth(1),
11    mMitoticModePhase2(
12        8.0), mMitoticModePhase3(15.0), mPhaseShiftWidth(2.0),
13    mPhase1PP(1.0), mPhase1PD(0.0), mPhase2PP(0.2),
14    mPhase2PD(
15        0.4), mPhase3PP(0.2), mPhase3PD(0.0), mMitoticMode(0),
16    mSeed(0), mTimeDependentCycleDuration(false),
17    mPeakRateTime(), mIncreasingRateSlope(),
18    mDecreasingRateSlope(), mBaseGammaScale()
19 {
20     mReadyToDivide = true; //He model begins with a first division
21 }
22
23 HeCellCycleModel::HeCellCycleModel(const HeCellCycleModel& rModel) :
24     AbstractSimpleCellCycleModel(rModel),
25     mKillSpecified(rModel.mKillSpecified), mDeterministic(

```

```

15         rModel.mDeterministic), mOutput(rModel.mOutput),
16         ↳ mEventStartTime(rModel.mEventStartTime),
17         ↳ mSequenceSampler(
18             rModel.mSequenceSampler),
19             ↳ mSeqSamplerLabelSister(rModel.mSeqSamplerLabelSister),
20             ↳ mDebug(rModel.mDebug), mTimeID(
21                 rModel.mTimeID), mVarIDs(rModel.mVarIDs),
22                 ↳ mDebugWriter(rModel.mDebugWriter), mTiLOffset(
23                     rModel.mTiLOffset), mGammaShift(rModel.mGammaShift),
24                     ↳ mGammaShape(rModel.mGammaShape), mGammaScale(
25                         rModel.mGammaScale),
26                         ↳ mSisterShiftWidth(rModel.mSisterShiftWidth),
27                         ↳ mMitoticModePhase2(
28                             rModel.mMitoticModePhase2),
29                             ↳ mMitoticModePhase3(rModel.mMitoticModePhase3),
30                             ↳ mPhaseShiftWidth(
31                                 rModel.mPhaseShiftWidth), mPhase1PP(rModel.mPhase1PP),
32                                 ↳ mPhase1PD(rModel.mPhase1PD), mPhase2PP(
33                                     rModel.mPhase2PP), mPhase2PD(rModel.mPhase2PD),
34                                     ↳ mPhase3PP(rModel.mPhase3PP), mPhase3PD(
35                                         rModel.mPhase3PD), mMitoticMode(rModel.mMitoticMode),
36                                         ↳ mSeed(rModel.mSeed), mTimeDependentCycleDuration(
37                                             rModel.mTimeDependentCycleDuration),
38                                             ↳ mPeakRateTime(rModel.mPeakRateTime),
39                                             ↳ mIncreasingRateSlope(
40                                                 rModel.mIncreasingRateSlope),
41                                                 ↳ mDecreasingRateSlope(rModel.mDecreasingRateSlope),
42                                                 ↳ mBaseGammaScale(
43                                                     rModel.mBaseGammaScale)
44     {
45 }
46
47 AbstractCellCycleModel* HeCellCycleModel::CreateCellCycleModel()
48 {
49     return new HeCellCycleModel(*this);
50 }
51
52 void HeCellCycleModel::SetCellCycleDuration()
53 {
54     RandomNumberGenerator* p_random_number_generator =
55         ↳ RandomNumberGenerator::Instance();
56
57 /*****
58 *****/

```

```

40   * CELL CYCLE DURATION RANDOM VARIABLE
41   *****/
42
43   if (!mTimeDependentCycleDuration) //Normal operation, cell cycle
44     ↳ length stays constant
45   {
46     //He cell cycle length determined by shifted gamma distribution
47     ↳ reflecting 4 hr refractory period followed by gamma pdf
48     mCellCycleDuration = mGammaShift +
49     ↳ p_random_number_generator→GammaRandomDeviate(mGammaShape,
50     ↳ mGammaScale);
51
52   *****
53   * Variable cycle length
54   * Give -ve mIncreasingRateSlope and +ve mDecreasingRateSlope,
55   * cell cycle length linearly declines (increasing rate), then
56   ↳ increases, switching at mPeakRateTime
57   ****/
58   else
59   {
60     double currTime = SimulationTime::Instance()→GetTime();
61     if (currTime ≤ mPeakRateTime)
62     {
63       mGammaScale = std::max((mBaseGammaScale - currTime *
64         ↳ mIncreasingRateSlope), .000000000001);
65     }
66     if (currTime > mPeakRateTime)
67     {
68       mGammaScale = std::max(
69         ((mBaseGammaScale - mPeakRateTime *
70           ↳ mIncreasingRateSlope)
71           + (mBaseGammaScale + (currTime -
72             ↳ mPeakRateTime) * mDecreasingRateSlope)),
73           .000000000001);
74     }
75     mCellCycleDuration = mGammaShift +
76     ↳ p_random_number_generator→GammaRandomDeviate(mGammaShape,
77     ↳ mGammaScale);
78   }
79
80 }
81 }
82

```

```

73 void HeCellCycleModel::ResetForDivision()
74 {
75     /**************************************************************************
76     * TIME IN LINEAGE DEPENDENT MITOTIC MODE PHASE RULES
77     * ****
78     RandomNumberGenerator* p_random_number_generator =
79         → RandomNumberGenerator::Instance();
80
81     double currentTiL = SimulationTime::Instance()→GetTime() +
82         → mTiLOffset;
83
84     /*Rule logic defaults to phase 1 behaviour, checks for currentTiL >
85      → phaseBoundaries and changes
86      currentPhase and subsequently mMitoticMode as appropriate*/
87     unsigned currentPhase = 1;
88     mMitoticMode = 0;
89
90     //Check time in lineage and determine current mitotic mode phase
91     ****
92     * Phase boundary & deterministic mitotic mode rules
93     ****
94     if (currentTiL > mMitoticModePhase2 && currentTiL <
95         → mMitoticModePhase3)
96     {
97         //if current TiL is > phase 2 boundary time, set the currentPhase
98         → appropriately
99         currentPhase = 2;
100
101         //if deterministic mode is enabled, PD divisions are guaranteed
102         → unless this is an Ath5 morphant
103         if (mDeterministic)
104         {
105             mMitoticMode = 1; //0=PP;1=PD;2=DD
106             if (mpCell→HasCellProperty<Ath5Mo>()) //Ath5 morphants
107                 → undergo PP rather than PD divisions in 80% of cases
108             {
109                 double ath5RV = p_random_number_generator→ranf();
110                 if (ath5RV ≤ .8)
111                 {
112                     mMitoticMode = 0;
113                 }
114             }
115         }
116     }
117 }
```

```

109 }
110
111 if (currentTiL > mMitoticModePhase3)
112 {
113     //if current TiL is > phase 3 boundary time, set the currentPhase
114     //appropriately
115     currentPhase = 3;
116     if (mDeterministic)
117     {
118         //if deterministic mode is enabled, DD divisions are
119         //guaranteed
120         mMitoticMode = 2;
121     }
122 }
123
124 /**
125 * MITOTIC MODE RANDOM VARIABLE
126 *****/
127 //initialise mitoticmode random variable, set mitotic mode
128 //appropriately after comparing to mode probability matrix
129 double mitoticModeRV = p_random_number_generator->ranf(); //0-1
130 //evenly distributed RV
131
132 if (!mDeterministic)
133 {
134     //construct 3x2 matrix of mode probabilities arranged by phase
135     double modeProbabilityMatrix[3][2] = { { mPhase1PP, mPhase1PD },
136     { mPhase2PP, mPhase2PD }, { mPhase3PP,
137
138
139     //if the RV is > currentPhasePP && <= currentPhasePD, change
140     //mMitoticMode from PP to PD
141     if (mitoticModeRV > modeProbabilityMatrix[currentPhase - 1][0]
142         && mitoticModeRV
143             <= modeProbabilityMatrix[currentPhase - 1][0] +
144                 modeProbabilityMatrix[currentPhase - 1][1])
145     {
146         mMitoticMode = 1;
147         if (mpCell->HasCellProperty<Ath5Mo>()) //Ath5 morphants
148             //undergo PP rather than PD divisions in 80% of cases

```

```

141         {
142             double ath5RV = p_random_number_generator->ranf();
143             if (ath5RV <=.8)
144             {
145                 mMitoticMode = 0;
146             }
147         }
148     }
149     //if the RV is > currentPhasePP + currentPhasePD, change
150     //→ mMitoticMode from PP to DD
151     if (mitoticModeRV > modeProbabilityMatrix[currentPhase - 1][0] +
152         modeProbabilityMatrix[currentPhase - 1][1])
153     {
154         mMitoticMode = 2;
155     }
156 /**
157 * Write mitotic event to relevant files
158 */
159 if (mDebug)
160 {
161     WriteDebugData(currentTiL, currentPhase, mitoticModeRV);
162 }
163
164 if (mOutput)
165 {
166     WriteModeEventOutput();
167 }
168
169 //set new cell cycle length (will be overwritten with DBL_MAX for DD
170 //→ divisions)
171 AbstractSimpleCellCycleModel::ResetForDivision();
172 /**
173 * Symmetric postmitotic specification rule
174 * -(asymmetric postmitotic rule specified in
175 //→ InitialiseDaughterCell());
176 */
177 if (mMitoticMode == 2)
178 {
179     boost::shared_ptr<AbstractCellProperty> p_PostMitoticType =

```

```

179             → mpCell→rGetCellPropertyCollection().GetCellPropertyRegistry()→G
180             mpCell→SetCellProliferativeType(p_PostMitoticType);
181             mCellCycleDuration = DBL_MAX;
182
183         if(mKillSpecified)
184         {
185             mpCell→Kill();
186         }
187     }
188
189     /*****
190      * SEQUENCE SAMPLER
191      *****/
192     //if the sequence sampler has been turned on, check for the label &
193     → write mitotic mode to log
194     //50% chance of each daughter cell from a mitosis inheriting the
195     → label
196     if (mSequenceSampler)
197     {
198         if (mpCell→HasCellProperty<CellLabel>())
199         {
200             (*LogFile::Instance()) << mMitoticMode;
201             double labelRV = p_random_number_generator→ranf();
202             if (labelRV ≤ .5)
203             {
204                 mSeqSamplerLabelSister = true;
205                 mpCell→RemoveCellProperty<CellLabel>();
206             }
207             else
208             {
209                 mSeqSamplerLabelSister = false;
210             }
211         }
212         else
213         {
214             //prevents lost-label cells from labelling their progeny
215             mSeqSamplerLabelSister = false;
216         }
217     }
218 void HeCellCycleModel::Initialise()

```

```
219 {
220     boost::shared_ptr<AbstractCellProperty> p_Transit =
221
222         ↪ mpCell→rGetCellPropertyCollection().GetCellPropertyRegistry()→Get<T>
223     mpCell→SetCellProliferativeType(p_Transit);
224
225     if (mTiLOffset == 0) //the "regular" case, set cycle duration
226         ↪ normally
227     {
228         SetCellCycleDuration();
229     }
230
231     if (mTiLOffset < 0) //these cells are offspring of Wan stem cells
232     {
233         mReadyToDivide = false;
234         SetCellCycleDuration();
235     }
236
237     if (mTiLOffset > 0.0) //if the TiL is > 0, the first division has
238         ↪ already occurred
239     {
240         mReadyToDivide = false;
241
242         RandomNumberGenerator* p_random_number_generator =
243             ↪ RandomNumberGenerator::Instance();
244
245         /**
246             * This calculation "runs time forward" by subtracting
247             ↪ appropriately generated cell lengths from TiLOffset
248                 * Ultimately c is subtracted from a final cell length
249                 ↪ calculation to give the appropriate reduced cycle length
250             */
251
252         double c = mTiLOffset;
253         while (c > 0)
254         {
255             c = c - (mGammaShift +
256                 ↪ p_random_number_generator→GammaRandomDeviate(mGammaShape,
257                 ↪ mGammaScale));
258         }
259
260 }
```

```

252     mCellCycleDuration = (mGammaShift +
253         ↳ p_random_number_generator→GammaRandomDeviate(mGammaShape,
254             ↳ mGammaScale))
255         + c;
256     }
257
258 void HeCellCycleModel::InitialiseDaughterCell()
259 {
260     RandomNumberGenerator* p_random_number_generator =
261         ↳ RandomNumberGenerator::Instance();
262
263     /*****
264      * PD-type division & shifted sister cycle length & boundary
265      * adjustments
266      *****/
267
268     if (mMitoticMode == 1) //RPC becomes specified retinal neuron in
269         ↳ asymmetric PD mitosis
270     {
271         boost::shared_ptr<AbstractCellProperty> p_PostMitoticType =
272
273             ↳ mpCell→rGetCellPropertyCollection().GetCellPropertyRegistry()→G
274         mpCell→SetCellProliferativeType(p_PostMitoticType);
275         mCellCycleDuration = DBL_MAX;
276
277         if(mKillSpecified)
278         {
279             mpCell→Kill();
280         }
281
282         //daughter cell's mCellCycleDuration is copied from parent; modified
283         ↳ by a normally distributed shift if it remains proliferative
284     if (mMitoticMode == 0)
285     {
286         double sisterShift =
287             ↳ p_random_number_generator→NormalRandomDeviate(0,
288                 ↳ mSisterShiftWidth); //random variable mean 0 SD 1 by default
289         mCellCycleDuration = std::max(mGammaShift, mCellCycleDuration +
290             ↳ sisterShift); // sister shift respects 4 hour refractory
291             ↳ period

```



```

318                               double phase3PP, double
319                               ↵ phase3PD, double
320                               ↵ gammaShift, double
321                               ↵ gammaShape,
322                               double gammaScale, double
323                               ↵ sisterShift)
324 {
325     mTiLOffset = tiLOffset;
326     mMitoticModePhase2 = mitoticModePhase2;
327     mMitoticModePhase3 = mitoticModePhase3;
328     mPhase1PP = phase1PP;
329     mPhase1PD = phase1PD;
330     mPhase2PP = phase2PP;
331     mPhase2PD = phase2PD;
332     mPhase3PP = phase3PP;
333     mPhase3PD = phase3PD;
334     mGammaShift = gammaShift;
335     mGammaShape = gammaShape;
336     mGammaScale = gammaScale;
337     mSisterShiftWidth = sisterShift;
338 }
339
340 void HeCellCycleModel::SetDeterministicMode(double tiLOffset, double
341     ↵ mitoticModePhase2, double mitoticModePhase3,
342                               double phaseShiftWidth,
343                               ↵ double gammaShift, double
344                               ↵ gammaShape,
345                               double gammaScale, double
346                               ↵ sisterShift)
347 {
348     mDeterministic = true;
349     mTiLOffset = tiLOffset;
350     mMitoticModePhase2 = mitoticModePhase2;
351     mMitoticModePhase3 = mitoticModePhase3;
352     mPhaseShiftWidth = phaseShiftWidth;
353     mGammaShift = gammaShift;
354     mGammaShape = gammaShape;
355     mGammaScale = gammaScale;
356     mSisterShiftWidth = sisterShift;
357 }
358
359 void HeCellCycleModel::SetTimeDependentCycleDuration(double peakRateTime,
360     ↵ double increasingSlope,

```

```

352                               double
353                               ↵ decreasingSlope)
354
355     mTimeDependentCycleDuration = true;
356     mPeakRateTime = peakRateTime;
357     mIncreasingRateSlope = increasingSlope;
358     mDecreasingRateSlope = decreasingSlope;
359     mBaseGammaScale = mGammaScale;
360 }
361
362 void HeCellCycleModel::EnableKillSpecified()
363 {
364     mKillSpecified = true;
365 }
366
367 void HeCellCycleModel::EnableModeEventOutput(double eventStart, unsigned
368     ↵ seed)
369 {
370     mOutput = true;
371     mEventStartTime = eventStart;
372     mSeed = seed;
373 }
374
375 void HeCellCycleModel::WriteModeEventOutput()
376 {
377     double currentTime = SimulationTime::Instance()->GetTime() +
378         ↵ mEventStartTime;
379     CellPtr currentCell = GetCell();
380     double currentCellID = (double) currentCell->GetCellId();
381     (*LogFile::Instance()) << currentTime << "\t" << mSeed << "\t" <<
382         ↵ currentCellID << "\t" << mMitoticMode << "\n";
383 }
384
385 void HeCellCycleModel::EnableSequenceSampler()
386 {
387     mSequenceSampler = true;
388     boost::shared_ptr<AbstractCellProperty> p_label_type =
389
390         ↵ mpCell->rGetCellPropertyCollection().GetCellPropertyRegistry()->Get<C
391     mpCell->AddCellProperty(p_label_type);
392 }
393
394

```

```

389 void
400   → HeCellCycleModel::PassDebugWriter(boost::shared_ptr<ColumnDataWriter>
401   → debugWriter, int timeID,
402                                         std::vector<int> varIDs)
403 {
404   mDebug = true;
405   mDebugWriter = debugWriter;
406   mTimeID = timeID;
407   mVarIDs = varIDs;
408 }
409
410 void
411   → HeCellCycleModel::EnableModelDebugOutput(boost::shared_ptr<ColumnDataWriter>
412   → debugWriter)
413 {
414   mDebug = true;
415   mDebugWriter = debugWriter;
416
417   mTimeID = mDebugWriter->DefineUnlimitedDimension("Time", "h");
418
419   mVarIDs.push_back(mDebugWriter->DefineVariable("CellID", "No"));
420   mVarIDs.push_back(mDebugWriter->DefineVariable("TiL", "h"));
421   mVarIDs.push_back(mDebugWriter->DefineVariable("CycleDuration",
422     → "h"));
423   mVarIDs.push_back(mDebugWriter->DefineVariable("Phase2Boundary",
424     → "h"));
425   mVarIDs.push_back(mDebugWriter->DefineVariable("Phase3Boundary",
426     → "h"));
427   mVarIDs.push_back(mDebugWriter->DefineVariable("Phase", "No"));
428   mVarIDs.push_back(mDebugWriter->DefineVariable("MitoticModeRV",
429     → "Percentile"));
430   mVarIDs.push_back(mDebugWriter->DefineVariable("MitoticMode",
431     → "Mode"));
432   mVarIDs.push_back(mDebugWriter->DefineVariable("Label", "binary"));
433
434   mDebugWriter->EndDefineMode();
435 }
436
437 void HeCellCycleModel::WriteDebugData(double currentTiL, unsigned phase,
438   → double mitoticModeRV)
439 {
440   double currentTime = SimulationTime::Instance()->GetTime();
441   double currentCellID = mpCell->GetCellId();

```

```
422     unsigned label = 0;
423     if (mpCell->HasCellProperty<CellLabel>()) label = 1;
424
425     mDebugWriter->PutVariable(mTimeID, currentTime);
426     mDebugWriter->PutVariable(mVarIDs[0], currentCellID);
427     mDebugWriter->PutVariable(mVarIDs[1], currentTiL);
428     mDebugWriter->PutVariable(mVarIDs[2], mCellCycleDuration);
429     mDebugWriter->PutVariable(mVarIDs[3], mMitoticModePhase2);
430     mDebugWriter->PutVariable(mVarIDs[4], mMitoticModePhase3);
431     mDebugWriter->PutVariable(mVarIDs[5], phase);
432     if (!mDeterministic)
433     {
434         mDebugWriter->PutVariable(mVarIDs[6], mitoticModeRV);
435     }
436     mDebugWriter->PutVariable(mVarIDs[7], mMitoticMode);
437     if (mSequenceSampler)
438     {
439         mDebugWriter->PutVariable(mVarIDs[8], label);
440     }
441     mDebugWriter->AdvanceAlongUnlimitedDimension();
442 }
443
444 /*****
445 * UNUSED FUNCTIONS (required for class nonvirtuality, do not remove)
446 *****/
447
448 double HeCellCycleModel::GetAverageTransitCellCycleTime()
449 {
450     return (0.0);
451 }
452
453 double HeCellCycleModel::GetAverageStemCellCycleTime()
454 {
455     return (0.0);
456 }
457
458 void HeCellCycleModel::OutputCellCycleModelParameters(out_stream&
459             rParamsFile)
460 {
461     *rParamsFile << "\t\t\t<CellCycleDuration>" << mCellCycleDuration <<
462             "</CellCycleDuration>\n";
463
464     // Call method on direct parent class
```

```

463     ↳ AbstractSimpleCellCycleModel::OutputCellCycleModelParameters(rParamsFile);
464 }
465
466 // Serialization for Boost ≥ 1.36
467 #include "SerializationExportWrapperForCpp.hpp"
468 CHASTE_CLASS_EXPORT(HeCellCycleModel)

```

16.2.29 /src/HeCellCycleModel.hpp

```

1 #ifndef HECELLCYCLEMODEL_HPP_
2 #define HECELLCYCLEMODEL_HPP_
3
4 #include "AbstractSimpleCellCycleModel.hpp"
5 #include "RandomNumberGenerator.hpp"
6 #include "Cell.hpp"
7 #include "TransitCellProliferativeType.hpp"
8 #include "DifferentiatedCellProliferativeType.hpp"
9 #include "SmartPointers.hpp"
10 #include "ColumnDataWriter.hpp"
11 #include "LogFile.hpp"
12 #include "CellLabel.hpp"
13 #include "HeAth5Mo.hpp"
14
15 ****
16 * HE CELL CYCLE MODEL
17 * As described in He et al. 2012 [He2012] doi:
18 *   ↳ 10.1016/j.neuron.2012.06.033
19 *
20 * USE: By default, HeCellCycleModels are constructed with the parameter
21 *   ↳ fit reported in [He2012].
22 * In normal use, the model steps through three phases of mitotic mode
23 *   ↳ probability parameterisation.
24 * PP = symmetric proliferative mitotic mode, both progeny remain mitotic
25 * PD = asymmetric proliferative mitotic mode, one progeny exits the cell
26 *   ↳ cycle and differentiates
27 * DD = symmetric differentiative mitotic mode, both progeny exit the
28 *   ↳ cell cycle and differentiate
29 *
30 * Change default model parameters with SetModelParameters(<params>);
31 * Enable deterministic model alternative with
32 *   ↳ EnableDeterministicMode(<params>);

```

```

27  *
28  * 2 per-model-event output modes:
29  * EnableModeEventOutput() enables mitotic mode event logging-all cells
30  *   ↳ will write to the singleton log file
31  * EnableModelDebugOutput() enables more detailed debug output, each seed
32  *   ↳ will have its own file written to
33  * by a ColumnDataWriter passed to it from the test
34  * (eg. by the SetupDebugOutput helper function in the project simulator)
35  *
36  *
37  *****/
38
39 class HeCellCycleModel : public AbstractSimpleCellCycleModel
40 {
41     friend class TestSimpleCellCycleModels;
42
43 private:
44
45     /** Needed for serialization.*/
46     friend class boost::serialization::access;
47
48     * Archive the cell-cycle model and random number generator, never
49     * used directly - boost uses this.
50     *
51     * @param archive
52     * @param version the current version of this class
53     */
54     template<class Archive>
55     void serialize(Archive & archive, const unsigned int version)
56     {
57         archive &
58             ↳ boost::serialization::base_object<AbstractSimpleCellCycleModel>(*this);
59
60         SerializableSingleton<RandomNumberGenerator>* p_wrapper =
61
62             ↳ RandomNumberGenerator::Instance()→GetSerializationWrapper();
63         archive & p_wrapper;
64         archive & mCellCycleDuration;
65     }

```

```
63 //Private write functions for models
64 void WriteModeEventOutput();
65 void WriteDebugData(double currTiL, unsigned phase, double
66 → percentile);
67
68 protected:
69     //mode/output variables
70     bool mKillSpecified;
71     bool mDeterministic;
72     bool mOutput;
73     double mEventStartTime;
74     bool mSequenceSampler;
75     bool mSeqSamplerLabelSister;
76     //debug writer stuff
77     bool mDebug;
78     int mTimeID;
79     std::vector<int> mVarIDs;
80     boost::shared_ptr<ColumnDataWriter> mDebugWriter;
81     //model parameters and state memory vars
82     double mTiLOffset;
83     double mGammaShift;
84     double mGammaShape;
85     double mGammaScale;
86     double mSisterShiftWidth;
87     double mMitoticModePhase2;
88     double mMitoticModePhase3;
89     double mPhaseShiftWidth;
90     double mPhase1PP;
91     double mPhase1PD;
92     double mPhase2PP;
93     double mPhase2PD;
94     double mPhase3PP;
95     double mPhase3PD;
96     unsigned mMitoticMode;
97     unsigned mSeed;
98     bool mTimeDependentCycleDuration;
99     double mPeakRateTime;
100    double mIncreasingRateSlope;
101    double mDecreasingRateSlope;
102    double mBaseGammaScale;
103
104 /**
```

```
105     * Protected copy-constructor for use by CreateCellCycleModel().  
106     *  
107     * The only way for external code to create a copy of a cell cycle  
108     * model  
109     * is by calling that method, to ensure that a model of the correct  
110     * subclass is created.  
111     * This copy-constructor helps subclasses to ensure that all member  
112     * variables are correctly copied when this happens.  
113     *  
114     * This method is called by child classes to set member variables for  
115     * a daughter cell upon cell division.  
116     * Note that the parent cell cycle model will have had  
117     * ResetForDivision() called just before CreateCellCycleModel() is  
118     * called,  
119     * so performing an exact copy of the parent is suitable behaviour.  
120     * Any daughter-cell-specific initialisation  
121     * can be done in InitialiseDaughterCell().  
122     *  
123     * @param rModel the cell cycle model to copy.  
124     */  
125 HeCellCycleModel(const HeCellCycleModel& rModel);  
126  
127 public:  
128  
129     /**  
130     * Constructor - just a default, mBirthTime is set in the  
131     * AbstractCellCycleModel class.  
132     */  
133 HeCellCycleModel();  
134  
135     /**  
136     * SetCellCycleDuration() method to set length of cell cycle  
137     */  
138 void SetCellCycleDuration();  
139  
140     /**  
141     * Overridden builder method to create new copies of  
142     * this cell-cycle model.  
143     *  
144     * @return new cell-cycle model  
145     */  
146 AbstractCellCycleModel* CreateCellCycleModel();  
147  
148
```

```
140  /**
141   * Overridden ResetForDivision() method.
142   * Contains general mitotic mode logic
143   */
144  void ResetForDivision();

145 /**
146  * Overridden Initialise() method
147  * Used to give an appropriate mCellCycleDuration to cells w/ TiL
148  * offsets
149  * sets mReadytoDivide to false as appropriate
150  * Initialises as transit proliferative type
151  */
152 void Initialise();

153 /**
154  * Overridden InitialiseDaughterCell() method.
155  * Used to apply sister-cell time shifting (cell cycle duration,
156  * deterministic phase boundaries)
157  * Used to implement asymmetric mitotic mode
158  */
159 void InitialiseDaughterCell();

160 /*Model setup functions for standard He (SetModelParameters) and
161  * deterministic alternative (SetDeterministicMode) models
162  * Default parameters are from refits of He et al + deterministic
163  * alternatives
164  * He 2012 params: mitoticModePhase2 = 8, mitoticModePhase3 = 15,
165  * p1PP = 1, p1PD = 0, p2PP = .2, p2PD = .4, p3PP = .2, p3PD = 0
166  * gammaShift = 4, gammaShape = 2, gammaScale = 1, sisterShift = 1
167  */
168 void SetModelParameters(double tiLOffset = 0, double
169  * mitoticModePhase2 = 8, double mitoticModePhase3 = 15,
170  * double phase1PP = 1, double phase1PD = 0,
171  * double phase2PP = .2, double phase2PD =
172  * .4,
173  * double phase3PP = .2, double phase3PD = 0,
174  * double gammaShift = 4, double gammaShape
175  * = 2,
176  * double gammaScale = 1, double sisterShift =
177  * 1);
178 void SetDeterministicMode(double tiLOffset = 0, double
179  * mitoticModePhase2 = 8, double mitoticModePhase3 = 15,
```

```

171                     double phaseShiftWidth = 1, double
172                         ↵ gammaShift = 4, double gammaShape = 2,
173                         double gammaScale = 1, double sisterShift =
174                             ↵ 1);
175             void SetTimeDependentCycleDuration(double peakRateTime, double
176                 ↵ increasingSlope, double decreasingSlope);
177
178             //Function to set mKillSpecified = true; marks specified neurons for
179                 ↵ death and removal from population
180             //Intended to help w/ resource consumption for WanSimulator
181             void EnableKillSpecified();
182
183             //Functions to enable per-cell mitotic mode logging for mode rate &
184                 ↵ sequence sampling fixtures
185             //Uses singleton logfile
186             void EnableModeEventOutput(double eventStart, unsigned seed);
187             void EnableSequenceSampler();
188
189             //More detailed debug output. Needs a ColumnDataWriter passed to it
190             //Only declare ColumnDataWriter directory, filename, etc; do not set
191                 ↵ up otherwise
192             //Use PassDebugWriter if the writer is already enabled elsewhere (ie.
193                 ↵ in a Wan stem cell cycle model)
194             void EnableModelDebugOutput(boost::shared_ptr<ColumnDataWriter>
195                 ↵ debugWriter);
196             void PassDebugWriter(boost::shared_ptr<ColumnDataWriter> debugWriter,
197                 ↵ int timeID, std::vector<int> varIDs);
198
199             //Not used, but must be overwritten lest HeCellCycleModels be
200                 ↵ abstract
201             double GetAverageTransitCellCycleTime();
202             double GetAverageStemCellCycleTime();
203
204             /**
205             * Overridden OutputCellCycleModelParameters() method.
206             *
207             * @param rParamsFile the file stream to which the parameters are
208                 ↵ output
209             */
210             virtual void OutputCellCycleModelParameters(out_stream& rParamsFile);
211         };
212
213 #include "SerializationExportWrapper.hpp"

```

```

203 // Declare identifier for the serializer
204 CHASTE_CLASS_EXPORT(HeCellCycleModel)
205
206 #endif /*HECELLCYCLEMODEL_HPP_*/

```

16.2.30 /src/OffLatticeSimulationPropertyStop.cpp

```

1 #include "OffLatticeSimulationPropertyStop.hpp"
2
3 #include <boost/make_shared.hpp>
4
5 #include "CellBasedEventHandler.hpp"
6 #include "ForwardEulerNumericalMethod.hpp"
7 #include "StepSizeException.hpp"
8
9 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
10 OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::OffLatticeSimulationPropertyStop(
11     rCellPopulation,
12     bool deleteCellPopulationInDestructor,
13     bool initialiseCells
14 ) :
15     AbstractCellBasedSimulation<ELEMENT_DIM,SPACE_DIM>(rCellPopulation,
16     deleteCellPopulationInDestructor, initialiseCells),
17     p_property()
18 {
19     if (
20         !dynamic_cast<AbstractOffLatticeCellPopulation<ELEMENT_DIM,SPACE_DIM>>(&rCellPopulation)
21     ) {
22         EXCEPTION("OffLatticeSimulations require a subclass of
23             AbstractOffLatticeCellPopulation.");
24     }
25 }
26
27 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
28 bool OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::StoppingEventHasOccurred()
29 {
30     if(p_property->GetCellCount()<1){
31         return true;
32     }
33     else{

```

```
30             return false;
31         }
32     }
33
34 //Public access to Stopping Event bool
35 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
36 bool
37     → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::HasStoppingEventOccurred
38 {
39     return StoppingEventHasOccurred();
40 }
41 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
42 void
43     → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::SetStopProperty(boost::shared_p
44     → stopPropertySetting)
45 {
46     p_property = stopPropertySetting;
47 }
48 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
49 void
50     → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::AddForce(boost::shared_p
51     → pForce)
52 {
53     mForceCollection.push_back(pForce);
54 }
55 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
56 void
57     → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::RemoveAllForces()
58 {
59     mForceCollection.clear();
60 }
61 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
62 void
63     → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::AddCellPopulationBoundaryC
64     → pBoundaryCondition)
65 {
66     mBoundaryConditions.push_back(pBoundaryCondition);
67 }
```

```
65 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
66 void
67   → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::RemoveAllCellPopulationB
68 {
69   mBoundaryConditions.clear();
70 }
71
72 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
73 void
74   → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::SetNumericalMethod(boost
75   → SPACE_DIM> > pNumericalMethod)
76 {
77   mpNumericalMethod = pNumericalMethod;
78 }
79
80 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
81 const boost::shared_ptr<AbstractNumericalMethod<ELEMENT_DIM, SPACE_DIM> >
82   → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::GetNumericalMethod()
83   → const
84 {
85   return mpNumericalMethod;
86 }
87
88 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
89 const std::vector<boost::shared_ptr<AbstractForce<ELEMENT_DIM, SPACE_DIM>
90   → > >&
91   → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::rGetForceCollection()
92   → const
93 {
94   return mForceCollection;
95 }
96
97 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
98 void
99   → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::UpdateCellLocationsAndTo
```

```

99  {
100     // Store the initial node positions (these may be needed when
101     // applying boundary conditions)
102     std::map<Node<SPACE_DIM>*, c_vector<double, SPACE_DIM> >
103     old_node_locations;
104
105     for (typename AbstractMesh<ELEMENT_DIM, SPACE_DIM>::NodeIterator
106         node_iter =
107         this->mrCellPopulation.rGetMesh().GetNodeIteratorBegin();
108         node_iter !=
109         this->mrCellPopulation.rGetMesh().GetNodeIteratorEnd();
110         ++node_iter)
111     {
112         old_node_locations[&(*node_iter)] =
113             (node_iter)->rGetLocation();
114     }
115
116     // Try to update node positions according to the numerical method
117     try
118     {
119         mpNumericalMethod->UpdateAllNodePositions(present_time_step);
120         ApplyBoundaries(old_node_locations);
121
122         // Successful time step! Update time_advanced_so_far
123         time_advanced_so_far += present_time_step;
124
125         // If using adaptive timestep, then increase the
126         // present_time_step (by 1% for now)
127         if (mpNumericalMethod->HasAdaptiveTimestep())
128         {
129             ///\todo #2087 Make this a settable member variable
130             double timestep_increase = 0.01;
131             present_time_step =
132                 std::min((1+timestep_increase)*present_time_step,
133                         target_time_step - time_advanced_so_far);
134         }
135
136     }
137     catch (StepSizeException& e)
138     {
139         // Detects if a node has travelled too far in a single time
140         // step
141         if (mpNumericalMethod->HasAdaptiveTimestep())

```

```

132     {
133         // If adaptivity is switched on, revert node locations
134         → and choose a suitably smaller time step
135         RevertToOldLocations(old_node_locations);
136         present_time_step = std::min(e.GetSuggestedNewStep(),
137             → target_time_step - time_advanced_so_far);
138     }
139     else
140     {
141         // If adaptivity is switched off, terminate with an error
142         EXCEPTION(e.what());
143     }
144
145     CellBasedEventHandler::EndEvent(CellBasedEventHandler::POSITION);
146 }
147
148 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
149 void
150     → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::RevertToOldLocations(std::
151         → c_vector<double, SPACE_DIM> > oldNodeLoctions)
152 {
153     for (typename AbstractMesh<ELEMENT_DIM, SPACE_DIM>::NodeIterator
154         → node_iter =
155         → this→mrCellPopulation.rGetMesh().GetNodeIteratorBegin();
156         node_iter ≠
157         → this→mrCellPopulation.rGetMesh().GetNodeIteratorEnd();
158         ++node_iter)
159     {
160         (node_iter)→rGetModifiableLocation() =
161             → oldNodeLoctions[&(*node_iter)];
162     }
163 }
164
165 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
166 void
167     → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::ApplyBoundaries(std::map
168         → SPACE_DIM> > oldNodeLoctions)
169 {
170     // Apply any boundary conditions

```

```

163   for (typename
164     ↵ std::vector<boost::shared_ptr<AbstractCellPopulationBoundaryCondition<ELEMENT>>::iterator bcs_iter = mBoundaryConditions.begin();
165     ↵ bcs_iter != mBoundaryConditions.end();
166     ↵ ++bcs_iter)
167   {
168     (*bcs_iter)->ImposeBoundaryCondition(oldNodeLoctions);
169   }
170
171 // Verify that each boundary condition is now satisfied
172 for (typename
173   ↵ std::vector<boost::shared_ptr<AbstractCellPopulationBoundaryCondition<ELEMENT>>::iterator bcs_iter = mBoundaryConditions.begin();
174   ↵ bcs_iter != mBoundaryConditions.end();
175   ↵ ++bcs_iter)
176 {
177   if (!(*bcs_iter)->VerifyBoundaryCondition())
178   {
179     EXCEPTION("The cell population boundary conditions are
180     ↵ incompatible.");
181   }
182 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
183 void
184   ↵ OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::WriteVisualizerSetupFile
185 {
186   if (PetscTools::AmMaster())
187   {
188     for (unsigned i=0; i<this->mForceCollection.size(); i++)
189     {
190       ↵ this->mForceCollection[i]->WriteDataToVisualizerSetupFile(this->mpViz)
191     }
192
193   ↵ this->mrCellPopulation.WriteDataToVisualizerSetupFile(this->mpVizSetupFile)
194 }
195
196 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
```

```

197 void
198 {
199     // Clear all forces
200     for (typename AbstractMesh<ELEMENT_DIM, SPACE_DIM>::NodeIterator
201         node_iter =
202             this->mrCellPopulation.rGetMesh().GetNodeIteratorBegin();
203         node_iter !=
204             this->mrCellPopulation.rGetMesh().GetNodeIteratorEnd();
205         ++node_iter)
206     {
207         node_iter->ClearAppliedForce();
208     }
209
210     // Use a forward Euler method by default, unless a numerical method
211     // has been specified already
212     if (mpNumericalMethod == nullptr)
213     {
214         mpNumericalMethod =
215             boost::make_shared<ForwardEulerNumericalMethod<ELEMENT_DIM,
216             SPACE_DIM>>();
217
218         mpNumericalMethod->SetCellPopulation(dynamic_cast<AbstractOffLatticeCellPopul
219         mpNumericalMethod->SetForceCollection(&mForceCollection);
220     }
221
222     template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
223     void
224     OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::OutputAdditionalSimulati
225     rParamsFile)
226     {
227         // Loop over forces
228         *rParamsFile << "\n\t<Forces>\n";
229         for (typename
230             std::vector<boost::shared_ptr<AbstractForce<ELEMENT_DIM,SPACE_DIM>
231             >>::iterator iter = mForceCollection.begin();
232             iter != mForceCollection.end();
233             ++iter)
234         {
235             // Output force details
236             (*iter)->OutputForceInfo(rParamsFile);
237         }

```

```

228     *rParamsFile << "\t</Forces>\n";
229
230     // Loop over cell population boundary conditions
231     *rParamsFile << "\n\t<CellPopulationBoundaryConditions>\n";
232     for (typename
233         → std::vector<boost::shared_ptr<AbstractCellPopulationBoundaryCondition<ELEMENT>
234         → >>::iterator iter = mBoundaryConditions.begin();
235         iter ≠ mBoundaryConditions.end();
236         ++iter)
237     {
238         // Output cell boundary condition details
239         (*iter)→OutputCellPopulationBoundaryConditionInfo(rParamsFile);
240     }
241     *rParamsFile << "\t</CellPopulationBoundaryConditions>\n";
242
243     // Output numerical method details
244     *rParamsFile << "\n\t<NumericalMethod>\n";
245     mpNumericalMethod→OutputNumericalMethodInfo(rParamsFile);
246     *rParamsFile << "\t</NumericalMethod>\n";
247 }
248 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM>
249 void
250     → OffLatticeSimulationPropertyStop<ELEMENT_DIM,SPACE_DIM>::OutputSimulationParameter
251     → rParamsFile)
252 {
253     // No new parameters to output, so just call method on direct parent
254     → class
255     → AbstractCellBasedSimulation<ELEMENT_DIM,SPACE_DIM>::OutputSimulationParameter
256 }
257
258 // Explicit instantiation
259 template class OffLatticeSimulationPropertyStop<1,1>;
260 template class OffLatticeSimulationPropertyStop<1,2>;
261 template class OffLatticeSimulationPropertyStop<2,2>;
262 template class OffLatticeSimulationPropertyStop<1,3>;
263 template class OffLatticeSimulationPropertyStop<2,3>;
264 template class OffLatticeSimulationPropertyStop<3,3>;
265
266 // Serialization for Boost ≥ 1.36
267 #include "SerializationExportWrapperForCpp.hpp"
268 EXPORT_TEMPLATE_CLASS_ALL_DIMS(OffLatticeSimulationPropertyStop)

```

16.2.31 /src/OffLatticeSimulationPropertyStop.hpp

```
1 #ifndef OFFLATTICESIMULATIONPROPERTYSTOP_HPP_
2 #define OFFLATTICESIMULATIONPROPERTYSTOP_HPP_
3
4 #include "AbstractCellBasedSimulation.hpp"
5 #include "AbstractForce.hpp"
6 #include "AbstractCellPopulationBoundaryCondition.hpp"
7 #include "AbstractNumericalMethod.hpp"
8
9 #include "ChasteSerialization.hpp"
10 #include <boost/serialization/base_object.hpp>
11 #include <boost/serialization/set.hpp>
12 #include <boost/serialization/vector.hpp>
13
14 /**
15  * Run an off-lattice 2D or 3D cell-based simulation using an off-lattice
16  * cell population.
17  *
18  * In cell-centre-based cell populations, each cell is represented by a
19  * single node (corresponding to its centre), and connectivity is defined
20  * either by a Delaunay triangulation or a radius of influence. In
21  * vertex-
22  * based cell populations, each cell is represented by a polytope
23  * (corresponding to its membrane) with a variable number of vertices.
24  * Alternative cell populations may be defined by the user.
25  *
26  * The OffLatticeSimulation is constructed with a CellPopulation, which
27  * updates the correspondence between each Cell and its spatial
28  * representation
29  * and handles cell division (governed by the CellCycleModel associated
30  * with each cell). Once constructed, one or more Force laws may be
31  * passed
32  * to the OffLatticeSimulation object, to define the mechanical
33  * properties
34  * of the CellPopulation. Similarly, one or more CellKillers may be
35  * passed
36  * to the OffLatticeSimulation object to specify conditions in which
37  * Cells
38  * may die, and one or more CellPopulationBoundaryConditions to specify
39  * regions in space beyond which Cells may not move.
```

```

34  */
35 template<unsigned ELEMENT_DIM, unsigned SPACE_DIM = ELEMENT_DIM>
36 class OffLatticeSimulationPropertyStop : public
37   AbstractCellBasedSimulation<ELEMENT_DIM, SPACE_DIM>
38 {
39
40   /** Needed for serialization. */
41   friend class boost::serialization::access;
42   friend class TestOffLatticeSimulation;
43   friend class TestOffLatticeSimulationWithNodeBasedCellPopulation;
44
45 /**
46  * Save or restore the simulation.
47  *
48  * @param archive the archive
49  * @param version the current version of this class
50  */
51 template<class Archive>
52 void serialize(Archive & archive, const unsigned int version)
53 {
54   archive &
55     boost::serialization::base_object<AbstractCellBasedSimulation<ELEMENT_DIM,
56     SPACE_DIM>>(*this);
57   archive & mForceCollection;
58   archive & mBoundaryConditions;
59   archive & mpNumericalMethod;
60 }
61
62 protected:
63   boost::shared_ptr<AbstractCellProperty> p_property;
64   /** The mechanics used to determine the new location of the cells, a
65    * list of the forces. */
66   std::vector<boost::shared_ptr<AbstractForce<ELEMENT_DIM, SPACE_DIM>>
67     > mForceCollection;
68
69   /** List of boundary conditions. */
70
71   std::vector<boost::shared_ptr<AbstractCellPopulationBoundaryCondition<ELEMENT_
72     SPACE_DIM>>> mBoundaryConditions;
73
74   /** The numerical method to use in this simulation. Defaults to the
75    * explicit forward Euler method. */
76

```

```
69     boost::shared_ptr<AbstractNumericalMethod<ELEMENT_DIM, SPACE_DIM>>
70     mpNumericalMethod;
71
72     /**
73      * Overridden UpdateCellLocationsAndTopology() method.
74      *
75      * Calculate forces and update node positions.
76      */
77
78     virtual void UpdateCellLocationsAndTopology();
79
80     /**
81      * Sends nodes back to the positions given in the input map. Used
82      * after a failed step
83      * when adaptivity is turned on.
84      *
85      * @param oldNodeLoctions A map linking nodes to their old positions.
86      */
87     void RevertToOldLocations(std::map<Node<SPACE_DIM>*, c_vector<double,
88                               > oldNodeLoctions);
89
90     /**
91      * Applies any boundary conditions.
92      *
93      * @param oldNodeLoctions Mapping between node indices and old node
94      * locations
95      */
96     void ApplyBoundaries(std::map<Node<SPACE_DIM>*, c_vector<double,
97                           > oldNodeLoctions);
98
99     /**
100      * Overridden SetupSolve() method to clear the forces applied to the
101      * nodes.
102      */
103
104     virtual void SetupSolve();
105
106     /**
107      * Overridden WriteVisualizerSetupFile() method.
108      */
109
110     virtual void WriteVisualizerSetupFile();
111
112     bool StoppingEventHasOccurred();
113
114 public:
```

```

106
107  /**
108   * Constructor.
109   *
110  * @param rCellPopulation Reference to a cell population object
111  * @param deleteCellPopulationInDestructor Whether to delete the cell
112  * population on destruction to
113  *     * free up memory (defaults to false)
114  * @param initialiseCells Whether to initialise cells (defaults to
115  * true, set to false when loading
116  *     * from an archive)
117  */
118 OffLatticeSimulationPropertyStop(AbstractCellPopulation<ELEMENT_DIM,
119  * SPACE_DIM>& rCellPopulation,
120  *                                     bool
121  *                                     * deleteCellPopulationInDestructor
122  *                                     * = false, bool initialiseCells =
123  *                                     * true);
124
125
126
127
128 void SetStopProperty(boost::shared_ptr<AbstractCellProperty>
129  * stopPropertySetting);
130
131
132
133
134
135
136
137
138

```

```
139     */
140 void AddCellPopulationBoundaryCondition(
141     → boost::shared_ptr<AbstractCellPopulationBoundaryCondition<ELEMENT_DIM,
142     → SPACE_DIM> > pBoundaryCondition);
143 /**
144 * Method to remove all the cell population boundary conditions
145 */
146 void RemoveAllCellPopulationBoundaryConditions();
147 /**
148 * Set the numerical method to be used in this simulation (use this
149 → to solve the mechanics system).
150 *
151 * @param pNumericalMethod pointer to a numerical method object
152 */
153 void
154     → SetNumericalMethod(boost::shared_ptr<AbstractNumericalMethod<ELEMENT_DIM,
155     → SPACE_DIM> > pNumericalMethod);
156 /**
157 * @return the current numerical method.
158 */
159 const boost::shared_ptr<AbstractNumericalMethod<ELEMENT_DIM,
160     → SPACE_DIM> > GetNumericalMethod() const;
161 /**
162 * Overridden OutputAdditionalSimulationSetup() method.
163 *
164 * Output any force, boundary condition or numerical method
165 → information.
166 *
167 * @param rParamsFile the file stream to which the parameters are
168 → output
169 */
170 void OutputAdditionalSimulationSetup(out_stream& rParamsFile);
171 /**
172 * Overridden OutputSimulationParameters() method.
173 *
174 * @param rParamsFile the file stream to which the parameters are
175 → output
```

```

173     */
174     virtual void OutputSimulationParameters(out_stream& rParamsFile);
175
176     /**
177      * Directly access the forces attached to this simulation, to allow
178      * their manipulation after archiving.
179      *
180      * @return mForceCollection the vector of pointers to forces attached
181      * to this simulation
182      */
183     const std::vector<boost::shared_ptr<AbstractForce<ELEMENT_DIM,
184     >>& rGetForceCollection() const;
185
186 // Serialization for Boost ≥ 1.36
187 #include "SerializationExportWrapper.hpp"
188 EXPORT_TEMPLATE_CLASS_ALL_DIMS(OffLatticeSimulationPropertyStop)
189
190 namespace boost
191 {
192 namespace serialization
193 {
194 /**
195  * Serialize information required to construct an OffLatticeSimulation.
196 */
197 template<class Archive, unsigned ELEMENT_DIM, unsigned SPACE_DIM>
198 inline void save_construct_data(Archive & ar, const
199   OffLatticeSimulationPropertyStop<ELEMENT_DIM, SPACE_DIM> * t,
200                           const unsigned int file_version)
201 {
202     // Save data required to construct instance
203     const AbstractCellPopulation<ELEMENT_DIM, SPACE_DIM>*
204       p_cell_population = &(t->rGetCellPopulation());
205     ar & p_cell_population;
206 }
207
208 /**
209  * De-serialize constructor parameters and initialise an
210  * OffLatticeSimulation.
211 */
212 template<class Archive, unsigned ELEMENT_DIM, unsigned SPACE_DIM>
213 inline void load_construct_data(Archive & ar,
214   OffLatticeSimulationPropertyStop<ELEMENT_DIM, SPACE_DIM> * t,

```

```

209                         const unsigned int file_version)
210 {
211     // Retrieve data from archive required to construct new instance
212     AbstractCellPopulation<ELEMENT_DIM, SPACE_DIM>* p_cell_population;
213     ar >> p_cell_population;
214
215     // Invoke inplace constructor to initialise instance, middle two
216     // variables set extra
217     // member variables to be deleted as they are loaded from archive and
218     // to not initialise cells.
219     ::new (t) OffLatticeSimulationPropertyStop<ELEMENT_DIM,
220           SPACE_DIM>(*p_cell_population, true, false);
221 }
222 }
223 } // namespace
224
225 #endif /*OFFLATTICESIMULATIONPROPERTYSTOP_HPP_*/

```

16.2.32 /src/WanStemCellCycleModel.cpp

```

1 #include "WanStemCellCycleModel.hpp"
2
3 WanStemCellCycleModel::WanStemCellCycleModel() :
4     AbstractSimpleCellCycleModel(), mExpandingStemPopulation(false),
5     mPopulation(), mOutput(false), mEventStartTime(
6         72.0), mDebug(false), mTimeID(), mVarIDs(),
7         mDebugWriter(), mBasePopulation(), mGammaShift(4.0),
8         mGammaShape(
9             2.0), mGammaScale(1.0), mMitoticMode(0), mSeed(0),
10        mTimeDependentCycleDuration(false), mPeakRateTime(),
11        mIncreasingRateSlope(), mDecreasingRateSlope(),
12        mBaseGammaScale(), mHeParamVector(
13            { 8, 15, 1, 0, .2, .4, .2, 0, 4, 2, 1, 1 })
14    {
15    }
16
17 WanStemCellCycleModel::WanStemCellCycleModel(const WanStemCellCycleModel&
18      rModel) :
19     AbstractSimpleCellCycleModel(rModel),
20     mExpandingStemPopulation(rModel.mExpandingStemPopulation),
21     mPopulation(
22

```

```
13         rModel.mPopulation), mOutput(rModel.mOutput),
14         ↳ mEventStartTime(rModel.mEventStartTime), mDebug(
15             rModel.mDebug), mTimeID(rModel.mTimeID),
16             ↳ mVarIDs(rModel.mVarIDs),
17             ↳ mDebugWriter(rModel.mDebugWriter), mBasePopulation(
18                 rModel.mBasePopulation), mGammaShift(rModel.mGammaShift),
19                 ↳ mGammaShape(rModel.mGammaShape), mGammaScale(
20                     rModel.mGammaScale), mMitoticMode(rModel.mMitoticMode),
21                     ↳ mSeed(rModel.mSeed), mTimeDependentCycleDuration(
22                         rModel.mTimeDependentCycleDuration),
23                         ↳ mPeakRateTime(rModel.mPeakRateTime),
24                         ↳ mIncreasingRateSlope(
25                             rModel.mIncreasingRateSlope),
26                             ↳ mDecreasingRateSlope(rModel.mDecreasingRateSlope),
27                             ↳ mBaseGammaScale(
28                                 rModel.mBaseGammaScale),
29                                 ↳ mHeParamVector(rModel.mHeParamVector)
30     {
31 }
32
33 AbstractCellCycleModel* WanStemCellCycleModel::CreateCellCycleModel()
34 {
35     return new WanStemCellCycleModel(*this);
36 }
37
38 void WanStemCellCycleModel::SetCellCycleDuration()
39 {
40     RandomNumberGenerator* p_random_number_generator =
41         ↳ RandomNumberGenerator::Instance();
42
43     mCellCycleDuration = mGammaShift +
44         ↳ p_random_number_generator->GammaRandomDeviate(mGammaShape,
45             ↳ mGammaScale);
46
47     /*****
48     * CELL CYCLE DURATION RANDOM VARIABLE
49     *****/
50
51 //Wan stem cell cycle length determined by the same formula as He
52     ↳ RPCs
53 /*
54     if (!mTimeDependentCycleDuration) //Normal operation, cell cycle
55         ↳ length stays constant
56     {
```

```

41     //He cell cycle length determined by shifted gamma distribution
42     → reflecting 4 hr refractory period followed by gamma pdf
43     mCellCycleDuration = mGammaShift +
44     → p_random_number_generator→GammaRandomDeviate(mGammaShape,
45     → mGammaScale);
46     }*/
47
48 /**
49 * Variable cycle length
50 * Give -ve mIncreasingRateSlope and +ve mDecreasingRateSlope,
51 * cell cycle length linearly declines (increasing rate), then
52 * increases, switching at mPeakRateTime
53 */
54
55 /*
56 else
57 {
58 double currTime = SimulationTime::Instance()→GetTime();
59 if (currTime ≤ mPeakRateTime)
60 {
61     mGammaScale = std::max((mBaseGammaScale - currTime *
62     → mIncreasingRateSlope), .000000000001);
63     if (currTime > mPeakRateTime)
64     {
65         mGammaScale = std::max(((mBaseGammaScale - mPeakRateTime *
66     → mIncreasingRateSlope)
67     + (mBaseGammaScale + (currTime - mPeakRateTime) *
68     → mDecreasingRateSlope)),.000000000001);
69     }
70     Timer::Print("mGammaScale: " + std::to_string(mGammaScale));
71     mCellCycleDuration = mGammaShift +
72     → p_random_number_generator→GammaRandomDeviate(mGammaShape,
73     → mGammaScale);
74     */
75 }
76 */
77 }

78 void WanStemCellCycleModel::ResetForDivision()
79 {
80
81     mMitoticMode = 1; //by default, asymmetric division giving rise to He
82     → cell (mode 1)

```

```

74     if (mExpandingStemPopulation)
75     {
76         double currRetinaAge = SimulationTime::Instance()→GetTime() +
77             → mEventStartTime;
78         double lensGrowthFactor = .09256 * pow(currRetinaAge, .52728); // //
79         unsigned currentPopulationTarget = int(std::round(mBasePopulation
80             → * lensGrowthFactor));
81
82         unsigned currentStemPopulation =
83             (mPopulation→GetCellProliferativeTypeCount())[0];
84         if (currentStemPopulation < currentPopulationTarget)
85         {
86             mMitoticMode = 0; //if the current population is < target,
87             → symmetrical stem-stem division occurs (mode 0)
88         }
89     }
90
91     /*****
92     * Write mitotic event to relevant files
93     * *****/
94     if (mDebug)
95     {
96         WriteDebugData();
97     }
98
99     if (mOutput)
100    {
101        WriteModeEventOutput();
102    }
103
104 void WanStemCellCycleModel::Initialise()
105 {
106
107     boost::shared_ptr<AbstractCellProperty> p_Stem =
108
109         → mpCell→rGetCellPropertyCollection().GetCellPropertyRegistry()→Get<S
110     mpCell→SetCellProliferativeType(p_Stem);

```

```

111     SetCellCycleDuration();
112 }
113
114 void WanStemCellCycleModel::InitialiseDaughterCell()
115 {
116
117     if (mMitoticMode == 1)
118     {
119         /*****  

120          * RPC-fated cells are given HeCellCycleModel  

121          *****/  

122
123     double tiLOffset = -(SimulationTime::Instance()→GetTime());
124     //Initialise a HeCellCycleModel and set it up with appropriate
125     // TiL value & parameters
126     HeCellCycleModel* p_cycle_model = new HeCellCycleModel;
127     p_cycle_model→SetModelParameters(tiLOffset, mHeParamVector[0],
128                                     mHeParamVector[1], mHeParamVector[2],
129                                     mHeParamVector[3],
130                                     mHeParamVector[4],
131                                     mHeParamVector[5],
132                                     mHeParamVector[6],
133                                     mHeParamVector[7],
134                                     mHeParamVector[8],
135                                     mHeParamVector[9],
136                                     mHeParamVector[10],
137                                     mHeParamVector[11]);
138     p_cycle_model→EnableKillSpecified();
139
140     //if debug output is enabled for the stem cell, enable it for its
141     // progenitor offspring
142     if (mDebug)
143     {
144         p_cycle_model→PassDebugWriter(mDebugWriter, mTimeID,
145                                     mVarIDs);
146     }
147
148     mpCell→SetCellCycleModel(p_cycle_model);
149     p_cycle_model→Initialise();
150 }
151
152 else
153 {

```

```

144         SetCellCycleDuration();
145     }
146 }
147
148 void WanStemCellCycleModel::SetModelParameters(double gammaShift, double
149     ↪ gammaShape, double gammaScale,
150                     std::vector<double>
151     ↪ heParamVector)
152 {
153     mGammaShift = gammaShift;
154     mGammaShape = gammaShape;
155     mGammaScale = gammaScale;
156     mHeParamVector = heParamVector;
157 }
158
159 void WanStemCellCycleModel::EnableExpandingStemPopulation(int
160     ↪ basePopulation,
161                     boost::shared_ptr<AbstractPopulation>
162     ↪ p_population)
163 {
164     mExpandingStemPopulation = true;
165     mBasePopulation = basePopulation;
166     mPopulation = p_population;
167 }
168
169 void WanStemCellCycleModel::SetTimeDependentCycleDuration(double
170     ↪ peakRateTime, double increasingSlope,
171                     double
172     ↪ decreasingSlope)
173 {
174     mTimeDependentCycleDuration = true;
175     mPeakRateTime = peakRateTime;
176     mIncreasingRateSlope = increasingSlope;
177     mDecreasingRateSlope = decreasingSlope;
178     mBaseGammaScale = mGammaScale;
179 }
180
181 void WanStemCellCycleModel::EnableModeEventOutput(double eventStart,
182     ↪ unsigned seed)
183 {
184     mOutput = true;
185     mEventStartTime = eventStart;

```

```

179     mSeed = seed;
180 }
181
182 void WanStemCellCycleModel::WriteModeEventOutput()
183 {
184     double currentTime = SimulationTime::Instance()→GetTime() +
185         → mEventStartTime;
186     CellPtr currentCell = GetCell();
187     double currentCellID = (double) currentCell→GetCellId();
188     (*LogFile::Instance()) << currentTime << "\t" << mSeed << "\t" <<
189         → currentCellID << "\t" << mMitoticMode << "\n"; //
190 }
191
192 void
193     → WanStemCellCycleModel::EnableModelDebugOutput(boost :: shared_ptr<ColumnDataWriter>
194         → debugWriter)
195 {
196     mDebug = true;
197     mDebugWriter = debugWriter;
198
199     mTimeID = mDebugWriter→DefineUnlimitedDimension("Time", "h");
200     mVarIDs.push_back(mDebugWriter→DefineVariable("CellID", "No"));
201     mVarIDs.push_back(mDebugWriter→DefineVariable("TIL", "h"));
202     mVarIDs.push_back(mDebugWriter→DefineVariable("CycleDuration",
203         → "h"));
204     mVarIDs.push_back(mDebugWriter→DefineVariable("Phase2Boundary",
205         → "h"));
206     mVarIDs.push_back(mDebugWriter→DefineVariable("Phase3Boundary",
207         → "h"));
208     mVarIDs.push_back(mDebugWriter→DefineVariable("Phase", "No"));
209     mVarIDs.push_back(mDebugWriter→DefineVariable("MitoticModeRV",
210         → "Percentile"));
211     mVarIDs.push_back(mDebugWriter→DefineVariable("MitoticMode",
212         → "Mode"));
213     mVarIDs.push_back(mDebugWriter→DefineVariable("Label", "binary"));
214
215     mDebugWriter→EndDefineMode();
216 }
217
218
219 void WanStemCellCycleModel::WriteDebugData()
220 {
221     double currentTime = SimulationTime::Instance()→GetTime();
222     double currentCellID = mpCell→GetCellId();

```

```

213     mDebugWriter→PutVariable(mTimeID, currentTime);
214     mDebugWriter→PutVariable(mVarIDs[0], currentCellID);
215     mDebugWriter→PutVariable(mVarIDs[1], 0);
216     mDebugWriter→PutVariable(mVarIDs[2], mCellCycleDuration);
217     mDebugWriter→PutVariable(mVarIDs[3], 0);
218     mDebugWriter→PutVariable(mVarIDs[4], 0);
219     mDebugWriter→PutVariable(mVarIDs[5], 0);
220     mDebugWriter→PutVariable(mVarIDs[7], mMitoticMode);
221     mDebugWriter→AdvanceAlongUnlimitedDimension();
222 }
223 }

224 /**
225 * UNUSED FUNCTIONS (required for class nonvirtuality, do not remove)
226 *****/
227

228

229 double WanStemCellCycleModel::GetAverageTransitCellCycleTime()
230 {
231     return (0.0);
232 }
233

234 double WanStemCellCycleModel::GetAverageStemCellCycleTime()
235 {
236     return (0.0);
237 }
238

239 void WanStemCellCycleModel::OutputCellCycleModelParameters(out_stream&
240     ↪ rParamsFile)
241 {
242     *rParamsFile << "\t\t\t<CellCycleDuration>" << mCellCycleDuration <<
243     ↪ "</CellCycleDuration>\n";
244
245 // Call method on direct parent class
246
247     ↪ AbstractSimpleCellCycleModel::OutputCellCycleModelParameters(rParamsFile);
248 }
249

250 // Serialization for Boost ≥ 1.36
251 #include "SerializationExportWrapperForCpp.hpp"
252 CHASTE_CLASS_EXPORT(WanStemCellCycleModel)

```

16.2.33 /src/WanStemCellCycleModel.hpp

```

1 #ifndef WANSTEMCELLCYCLEMODEL_HPP_
2 #define WANSTEMCELLCYCLEMODEL_HPP_
3
4 #include "AbstractCellPopulation.hpp"
5 #include "AbstractSimpleCellCycleModel.hpp"
6 #include "RandomNumberGenerator.hpp"
7 #include "Cell.hpp"
8 #include "StemCellProliferativeType.hpp"
9 #include "SmartPointers.hpp"
10 #include "ColumnDataWriter.hpp"
11 #include "LogFile.hpp"
12
13 #include "HeCellCycleModel.hpp"
14
15 /*****
16  * WAN STEM CELL CYCLE MODEL
17  * A simple model standing in for the CMZ "stem cells" described in'
18  * Wan et al. 2016 [Wan2016]
19  *
20  * USE: By default, WanStemCellCycleModels consistently divide
21  *      ↳ asymmetrically.
22  * InitialiseDaughterCell() marks offspring for RPC fate
23  * So-marked cells are given HeCellCycleModels for their next division.
24  *
25  * Change default model parameters with SetModelParameters(<params>);
26  *
27  * 2 per-model-event output modes:
28  * EnableModeEventOutput() enables mitotic mode event logging-all cells
29  *      ↳ will write to the singleton log file
30  * EnableModelDebugOutput() enables more detailed debug output, each seed
31  *      ↳ will have its own file written to
32  * by a ColumnDataWriter passed to it from the test
33  * (eg. by the SetupDebugOutput helper function in the project simulator)
34  *
35  * 1 mitotic-event-sequence sampler (only samples one "path" through the
36  *      ↳ lineage):
37  * EnableSequenceSampler() - one "sequence" of progenitors writes mitotic
38  *      ↳ event type to a string in the singleton log file
39  *
40 *****/

```

```
37 class WanStemCellCycleModel : public AbstractSimpleCellCycleModel
38 {
39     friend class TestSimpleCellCycleModels;
40
41 private:
42
43     /** Needed for serialization. */
44     friend class boost::serialization::access;
45     /**
46         * Archive the cell-cycle model and random number generator, never
47         * used directly - boost uses this.
48         *
49         * @param archive the archive
50         * @param version the current version of this class
51         */
52     template<class Archive>
53     void serialize(Archive & archive, const unsigned int version)
54     {
55         archive &
56             boost::serialization::base_object<AbstractSimpleCellCycleModel>(*this);
57
58         SerializableSingleton<RandomNumberGenerator>* p_wrapper =
59
60             RandomNumberGenerator::Instance()→GetSerializationWrapper();
61         archive & p_wrapper;
62         archive & mCellCycleDuration;
63     }
64
65     //Private write functions for models
66     void WriteModeEventOutput();
67     void WriteDebugData();
68
69 protected:
70     //mode/output variables
71     bool mExpandingStemPopulation;
72     boost::shared_ptr<AbstractCellPopulation<2>> mPopulation;
73     bool mOutput;
74     double mEventStartTime;
75     //debug writer stuff
76     bool mDebug;
77     int mTimeID;
78     std::vector<int> mVarIDs;
79     boost::shared_ptr<ColumnDataWriter> mDebugWriter;
```

```

77     //model parameters and state memory vars
78     int mBasePopulation;
79     double mGammaShift;
80     double mGammaShape;
81     double mGammaScale;
82     unsigned mMitoticMode;
83     unsigned mSeed;
84     bool mTimeDependentCycleDuration;
85     double mPeakRateTime;
86     double mIncreasingRateSlope;
87     double mDecreasingRateSlope;
88     double mBaseGammaScale;
89     std::vector<double> mHeParamVector;

90
91 /**
92  * Protected copy-constructor for use by CreateCellCycleModel().
93  *
94  * The only way for external code to create a copy of a cell cycle
95  * model
96  * is by calling that method, to ensure that a model of the correct
97  * subclass is created.
98  * This copy-constructor helps subclasses to ensure that all member
99  * variables are correctly copied when this happens.
100  *
101  * This method is called by child classes to set member variables for
102  * a daughter cell upon cell division.
103  * Note that the parent cell cycle model will have had
104  * ResetForDivision() called just before CreateCellCycleModel() is
105  * called,
106  * so performing an exact copy of the parent is suitable behaviour.
107  * Any daughter-cell-specific initialisation
108  * can be done in InitialiseDaughterCell().
109  *
110  * @param rModel the cell cycle model to copy.
111  */
112 WanStemCellCycleModel(const WanStemCellCycleModel& rModel);

113
114 public:
115
116 /**
117  * Constructor - just a default, mBirthTime is set in the
118  * AbstractCellCycleModel class.
119  */

```

```
112     WanStemCellCycleModel();  
113  
114     /**  
115      * SetCellCycleDuration() method to set length of cell cycle  
116      */  
117     void SetCellCycleDuration();  
118  
119     /**pro  
120      * Overridden builder method to create new copies of  
121      * this cell-cycle model.  
122      *  
123      * @return new cell-cycle model  
124      */  
125     AbstractCellCycleModel* CreateCellCycleModel();  
126  
127     /**  
128      * Overridden ResetForDivision() method.  
129      ***/  
130     void ResetForDivision();  
131  
132     /**  
133      * Overridden Initialise() method.  
134      * Sets proliferative type as stem cell  
135      ***/  
136  
137     void Initialise();  
138  
139     /**  
140      * Overridden InitialiseDaughterCell() method.  
141      * Used to implement asymmetric mitotic mode  
142      * Daughter cells are initialised w/ He cell cycle model  
143      * */  
144     void InitialiseDaughterCell();  
145  
146     /*Model setup functions for standard He (SetModelParameters) and  
147      → deterministic alternative (SetDeterministicMode) models  
148      * Default parameters are from refits of He et al + deterministic  
149      ← alternatives  
150      * He 2012 params: mitoticModePhase2 = 8, mitoticModePhase3 = 15,  
151      ← p1PP = 1, p1PD = 0, p2PP = .2, p2PD = .4, p3PP = .2, p3PD = 0  
152      * gammaShift = 4, gammaShape = 2, gammaScale = 1, sisterShift = 1  
153      */
```

```

151 void SetModelParameters(double gammaShift = 4, double gammaShape = 2,
152   ↵ double gammaScale = 1, std::vector<double> heParamVector = { 8,
153   ↵ 15, 1, 0, .2, .4, .2, 0, 4, 2, 1, 1 });
152 void EnableExpandingStemPopulation(int basePopulation,
153   ↵ boost::shared_ptr<AbstractCellPopulation<2>> p_population);
153 void SetTimeDependentCycleDuration(double peakRateTime, double
154   ↵ increasingSlope, double decreasingSlope);

154
155 //Functions to enable per-cell mitotic mode logging for mode rate &
156   ↵ sequence sampling fixtures
156 //Uses singleton logfile
157 void EnableModeEventOutput(double eventStart, unsigned seed);

158
159 //More detailed debug output. Needs a ColumnDataWriter passed to it
160 //Only declare ColumnDataWriter directory, filename, etc; do not set
161   ↵ up otherwise
161 void EnableModelDebugOutput(boost::shared_ptr<ColumnDataWriter>
162   ↵ debugWriter);

162
163 //Not used, but must be overwritten lest WanStemCellCycleModels be
164   ↵ abstract
164 double GetAverageTransitCellCycleTime();
165 double GetAverageStemCellCycleTime();

166 /**
167 * Overridden OutputCellCycleModelParameters() method.
168 *
169 * @param rParamsFile the file stream to which the parameters are
170   ↵ output
171   */
172 virtual void OutputCellCycleModelParameters(out_stream& rParamsFile);
173 };

174
175 #include "SerializationExportWrapper.hpp"
176 // Declare identifier for the serializer
177 CHASTE_CLASS_EXPORT(WanStemCellCycleModel)

178
179 #endif /*WANSTEMCELLCYCLEMODEL_HPP_*/

```

16.3 NGRefTools

16.3.1 /src/LogNormalUtils.jl

```

1 function get_lognormal_params(desired_μ, desired_σ)
2     μ²=desired_μ^2
3     σ²=desired_σ^2
4     ln_μ=log(μ²/sqrt(μ²+σ²))
5     ln_σ=sqrt(log(1+(σ²/μ²)))
6     return ln_μ, ln_σ
7 end
8
9 function get_lognormal_desc(d::LogNormal)
10    return mean(d), std(d)
11 end

```

16.3.2 /src/MarginalTDist.jl

```

1 """
2     MarginalTDist(v,μ,σ)
3
4     Return a shifted, scaled T Distribution with degrees of freedom `v`, mean
5     ↪ `μ`, and standard deviation `σ`.
6
7     This is the marginal distribution of the posterior mean for an
8     ↪ uninformative (reference) Normal Gamma prior distribution. It is also
9     ↪ the distribution of the posterior predictive for m=1 new
10    ↪ observations.
11
12    MarginalTDist(v)           #Standard TDist with v dof
13    MarginalTDist(v,μ,σ)       #TDist with v dof shifted by μ, scaled by σ
14
15    params(d)                 #Return d.v, d.μ, d.σ
16    mean(d)                   #Return d.μ (median, mode are identical)
17    std(d)                    #Return d.σ
18    quantile(d,p)            #Return quantile at probability p
19
20
21    Reference: Kevin P. Murphy, Conjugate Bayesian Analysis of the Gaussian
22    ↪ Distribution. 2007.
23    ↪ https://www.cs.ubc.ca/~murphyk/Papers/bayesGauss.pdf
24 """
25
26 MarginalTDist=LocationScale{Float64,TDist{Float64}}

```

```

19
20 Distributions.dof(d::MarginalTDist) = d.ρ.ν
21
22 Distributions.std(d::MarginalTDist) = d.σ
23
24 """
25     fit(MarginalTDist, x; PP=false)
26
27 Assuming an uninformative (reference) Normal Gamma prior, return the
28     ↳ marginal posterior distribution of the mean of a Normal model of
29     ↳ observations `x`.
30
31 Equivalent to the frequentist sampling distribution of the MLE mean. If
32     ↳ `PP=true`, return the posterior predictive distribution for m=1
33     ↳ additional observations sampled from the marginal posterior mean
34     ↳ instead.
35
36 Example:
37
38 julia> PMM_MarginalTDist(rand(10))
39 MarginalTDist{Float64}(
40     t: TDist{Float64}(v=9.0)
41     μ: 0.4789484996068401
42     σ: 0.08491142725619677
43     )
44
45 Reference: Kevin P. Murphy, Conjugate Bayesian Analysis of the Gaussian
46     ↳ Distribution. 2007.
47     ↳ https://www.cs.ubc.ca/~murphyk/Papers/bayesGauss.pdf
48 """
49
50 function Distributions.fit(::Type{MarginalTDist},
51     ↳ x::AbstractVector{<:Real}; PP=false)
52     PP ? PPM_MTDist(x) : PMM_MTDist(x)
53 end
54
55 #Posterior marginal mean distribution
56 function PMM_MTDist(x)
57     n=length(x)
58     μ=mean(x)
59     ssr=sum((x.-μ).^2)
60     σ=sqrt(ssr/(n*(n-1)))
61     return MarginalTDist(μ,σ,TDist(n-1))
62 end
63

```

```

54
55 #Posterior predictive mean distribution
56 function PPM_MTDist(x)
57     n=length(x)
58     μ=mean(x)
59     ssr=sum((x.-μ).^2)
60     αn=(n-1)/2
61     βn=.5*ssr
62     return MarginalTDist(μ,sqrt((βn*(n+1))/(αn*n)),TDist(2*αn))
63 end
64
65 """
66 MTDist_MC_func(func, xs ... ; lower=.025, upper=.975, mc_its=1e6,
67   → summary=false)
68 Monte Carlo execute `func` with a random sample from a MarginalTDist
69   → fitted to each x in xs, over `mc_its` iterates, returning the
70   → calculated results (if `summary` is `true`) or the mean and quantiles
71   → specified by `lower` and `upper`.
72 Example:
73 julia> NGRefTools.MTDist_MC_func(*,[rand(10),rand(10)],summary=true)
74 (0.09491370397229557, 0.20712818357976473, 0.3385677491994645)
75 See also: [`fit(MarginalTDist,x)`](@ref)
76 """
77 function MTDist_MC_func(func::Function, xs; lower=.025, upper=.975,
78   → mc_its=1e7, summary=false)
79     dists=[fit(MarginalTDist,x) for x in xs]
80     results=Vector{Float64}()
81     for it in 1:mc_its
82       push!(results, func(rand.(dists)...))
83     end
84     summary ? (return quantile(results, lower), mean(results),
85       → quantile(results,upper)) : (return results)
86 end
87
88 function plot_logn_MTDist(xs, colors, markers, labels, xlabel, ylabel;
89   → args...)
90   plt=plot(;args...)
91   for (x,color) in zip(xs,colors)
92     min=floor(minimum(x)-.15*mean(x))

```

```

90         max=ceil(maximum(x)+.20*mean(x))
91         X=collect(min:max)
92         pmdist=fit(MarginalTDist, log.(x))
93         y=pdf(pmdist,log.(X))

94         → plot!(plt,X,y,ribbon=(y,zeros(length(y))),label=:none,color=color,xlabel=
95     end
96     for (x,color,marker,label) in zip(xs,colors, markers,labels)
97         pmdist=fit(MarginalTDist, log.(x))
98         min=floor(minimum(x)-.15*mean(x))
99         max=ceil(maximum(x)+.20*mean(x))
100        X=collect(min:max)
101        y=pdf(pmdist,log.(X))
102        scaty=[mean(y) for x in 1:length(x)]
103        plt=scatter!(plt,x,scaty,color=color, marker=marker, label=label)
104    end
105
106    return plt
107 end
108
109 function plot_n_MTDist(xs, colors, markers, labels, xlabel, ylabel;
110     ← args ... )
111     plt=plot(;args ... )
112     for (x,color) in zip(xs,colors)
113         min=(minimum(x)-.01*mean(x))
114         max=(maximum(x)+.01*mean(x))
115         X=collect(min:(max-min)/1000:max)
116         pmdist=fit(MarginalTDist, x)
117         y=pdf(pmdist,X)

118         → plot!(plt,X,y,ribbon=(y,zeros(length(y))),color=color,label=:none,xlabel=
119     end
120     for (x,color,marker,label) in zip(xs,colors, markers,labels)
121         pmdist=fit(MarginalTDist, x)
122         min=(minimum(x)-.01*mean(x))
123         max=(maximum(x)+.01*mean(x))
124         X=collect(min:max)
125         y=pdf(pmdist,X)
126         scaty=[mean(y) for x in 1:length(x)]
127         plt=scatter!(plt,x,scaty,color=color, marker=marker,label=label)
128     end
129
130     return plt

```

```

130 end

131
132 function mean_mass_comparator(x,y;labels=[ "x" , "y" ])
133     xdist=fit(MarginalTDist,x)
134     ydist=fit(MarginalTDist,y)
135     if mean(xdist)>mean(ydist)
136         frac=ccdf(xdist,mean(ydist))
137         println("$(round(frac*100,digits=1))% of the marginal posterior
138             ↪ mean density of $(labels[1]) is above the mean of
139             ↪ $(labels[2]).")
140     else
141         frac=cdf(xdist,mean(ydist))
142         println("$(round(frac*100,digits=1))% of the marginal posterior
143             ↪ mean density of $(labels[1]) is below the mean of
144             ↪ $(labels[2]).")
145     end
146 end

147
148 function log_mean_mass_comparator(x,y;labels=[ "x" , "y" ])
149     xdist=fit(MarginalTDist,log.(filter(n→!iszero(n),x)))
150     ydist=fit(MarginalTDist,log.(filter(n→!iszero(n),y)))
151     if mean(xdist)>mean(ydist)
152         frac=ccdf(xdist,mean(ydist))
153         println("$(round(frac*100,digits=1))% of the marginal posterior
154             ↪ mean density of $(labels[1]) is above the mean of
155             ↪ $(labels[2]).")
156     else
157         frac=cdf(xdist,mean(ydist))
158         println("$(round(frac*100,digits=1))% of the marginal posterior
159             ↪ mean density of $(labels[1]) is below the mean of
160             ↪ $(labels[2]).")
161     end
162 end

```

16.3.3 /src/NGRef.jl

```

1 """
2     fit(NormalGamma, x)
3
4 Return the posterior `NormalGamma` distribution on the unknown mean and
4     ↪ precision of a Normal model of data vector `x`, assuming an
4     ↪ uninformative (reference) prior.

```

```

5
6 Example:
7
8     julia> fit(NormalGamma,rand(10))
9     NormalGamma{Float64}(\mu=0.5052725306750604, nu=10.0, shape=4.5,
10    ↪ rate=0.5057485400844524)
11 Reference: Kevin P. Murphy, Conjugate Bayesian Analysis of the Gaussian
12    ↪ Distribution. 2007.
13    ↪ https://www.cs.ubc.ca/~murphyk/Papers/bayesGauss.pdf
14    """
15
16        function Distributions.fit(::Type{NormalGamma},x::AbstractVector)
17            n=length(x)
18            μ=mean(x)
19            ssr=sum((x.-μ).^2)
20            α=(n-1)/2
21            β=ssr/2
22            return NormalGamma(μ,n,α,β)
23        end
24
25
26        function Distributions.params(d::NormalGamma)
27            return d.mu, d.nu, d.shape, d.rate
28        end
29
30        function marginals(d::NormalGamma)
31            ↪ m_T=MarginalTDist(d.mu,sqrt(d.rate/(d.shape*d.nu)),TDist(2*d.shape))
32            m_Ga=Gamma(d.shape,1/d.rate)
33            return m_T,m_Ga
34        end

```

16.3.4 /src/NGRefTools.jl

```

1 module NGRefTools
2     using ConjugatePriors, Distributions, Plots
3     #import Makie:surface
4
5     include("NGRef.jl")
6     export marginals
7     include("NIGRef.jl")
8     #export NGplot
9     include("MarginalTDist.jl")

```

```

10   export MarginalTDist, MTDist_MC_func, mean_mass_comparator,
11     ↪ log_mean_mass_comparator, plot_n_MTDist, plot_logn_MTDist
11   include("LogNormalUtils.jl")
12   export get_lognormal_params, get_lognormal_desc
13 end # module

```

16.3.5 /src/NIGRef.jl

```

1 """
2     fit(NormalInverseGamma, x)
3
4 Return the posterior `NormalInverseGamma` distribution on the unknown
5   ↪ mean and variance of a Normal model of data vector `x`, assuming an
6   ↪ uninformative (reference) prior.
7
8 Example:
9
10 julia> fit(NormalInverseGamma, rand(10))
11 NormalInverseGamma{Float64}(mu=0.5052725306750604, nu=10.0,
12   ↪ shape=4.5, rate=0.5057485400844524)
13
14 Reference: Kevin P. Murphy, Conjugate Bayesian Analysis of the Gaussian
15   ↪ Distribution. 2007.
16   ↪ https://www.cs.ubc.ca/~murphyk/Papers/bayesGauss.pdf
17 """
18
19 function
20   ↪ Distributions.fit(::Type{NormalInverseGamma}, x::AbstractVector)
21   n=length(x)
22   Vn = 1/n
23   μ=mean(x)
24   an = -.5 + (n/2)
25   bn = .5 * (sum(x.^2)-(μ^2/Vn))
26   return NormalInverseGamma(μ,Vn,an,bn)
27 end
28
29 function Distributions.params(d::NormalInverseGamma)
30     return d.mu, d.v0, d.shape, d.scale
31 end
32
33 function marginals(d::NormalInverseGamma)
34   ↪ m_T=MarginalTDist(d.mu,sqrt(d.shape*d.v0/d.scale),TDist(2*d.shape))

```

```

28     m_Ga=InverseGamma(d.shape,d.scale)
29     return m_T,m_Ga
30 end
31
32 # function NGplot(d::NormalInverseGamma; grid=1000, upper=.95,
33 #   ↳ lower=.05)
34 #   marg_mean, marg_var = marginals(d)
35 #   xmax=quantile(marg_mean,upper)
36 #   xmin=quantile(marg_mean,lower)
37 #   ymax=quantile(marg_var,upper)
38 #   ymin=quantile(marg_var,lower)
39 #   xs=LinRange(xmin, xmax, grid)
40 #   ys=LinRange(ymin, ymax, grid)
41 #   zs=[pdf(d,x,y) for x in xs, y in ys]
42 #
43 #   return surface(xs,ys,zs)
# end

```

16.4 GMC_NS

16.4.1 /src/GMC_NS.jl

```

1 module GMC_NS
2     using Distributions, Serialization, UnicodePlots
3     import ProgressMeter: AbstractProgress, Progress, @showprogress,
4     #   ↳ next!, move_cursor_up_while_clearing_lines, printover,
4     #   ↳ durationstring
5     import Printf: @sprintf
6     import Random: rand
7     import Statistics: unscaled_covzm, det, eigen
8     import Serialization: serialize, deserialize
9     import LinearAlgebra: dot, normalize
10    import BioBackgroundModels: lps
11    import StatsFuns: logaddexp, logsumexp
12    import Base: show
13    import Measurements: measurement
14    import ConjugatePriors: NormalGamma, NormalInverseGamma
15    import NGRefTools: MarginalTDist
16    import KernelDensityEstimate: kde!

```

```

17 #GMC settings vector fields: [GMC_Nmin::Int64, GMC_τ_death::Float64,
18   ↵ GMC_init_τ::Float64, GMC_tune_μ::Int64, GMC_tune_α::Float64,
19   ↵ GMC_tune_PID::NTuple{3,Float64}, GMC_timestep_η::Float64,
20   ↵ GMC_reflect_η::Float64, GMC_exhaust_σ::Float64,
21   ↵ GMC_chain_κ::Int64]
22
23 GMC_DEFAULTS=Vector{Any}([50,1e-6,.5, 50,.8,(.175,1e-5,.1), .001,
24   ↵ .01, 10., typemax(Int64)])
25 export GMC_DEFAULTS
26
27 include("ensemble/GMC_NS_Engine.jl")
28 export GMC_NS_Engine
29 include("model/GMC_NS_Model.jl")
30 export GMC_NS_Model, GMC_NS_Model_Record
31 include("model/model_utilities.jl")
32 include("GMC/galilean_trajectory.jl")
33 include("utilities/t_Tuner.jl")
34 include("utilities/ellipsoid.jl")
35 include("nested_sampler/search_patterns.jl")
36 include("nested_sampler/nested_step.jl")
37 include("nested_sampler/converge_ensemble.jl")
38 export converge_ensemble!
39 include("utilities/coordinate_utils.jl")
40 export box_bound!, to_prior, to_unit_ball, box_reflect!
41 include("ensemble/ensemble_utilities.jl")
42 export ensemble_history, clean_ensemble_dir, measure_evidence,
43   ↵ reset_ensemble!, move_ensemble!, copy_ensemble, rewind_ensemble,
44   ↵ show_models, show_models_e, get_model, rectify_ensemble!,
45   ↵ posterior_kde
46 include("utilities/ns_progressmeter.jl")
47 include("utilities/progress_displays.jl")
48 export
49   ↵ tuning_display,evidence_display,convergence_display,info_display,lh_display,
50 include("utilities/stats.jl")
51 include("normal/Normal_Model.jl")
52 include("normal/Normal_Engine.jl")
53 export Normal_Engine
54 include("normal/LogNormal_Model.jl")
55 include("normal/LogNormal_Engine.jl")
56 export LogNormal_Engine
57
58 end # module

```

16.4.2 /src/GMC_NS_Model.jl

```

1      """
2      GMC_NS_Model_Record
3
4  Abstract supertype for records of serialised 'GMC_NS_Model's. Subtyped
5  record structs must implement the following fields as their interface
6  with 'GMC_NS':
7
8      struct Minimal_Record
9          trajectory::Integer #id assigned at construction, identifies
10         trajectory
11         i::Integer #id integer assigned at construction, identifies
12         position on trajectory
13         pos::Vector{<:AbstractFloat} #hypersphere position of
14         model-particle
15         path::String #model is serialized to ensembledir/trajectory.i
16         log_Li::AbstractFloat #log likelihood calculated by
17         'GMC_NS_Model' constructor
18         end
19
20      """
21
22      abstract type GMC_NS_Model_Record end
23
24
25      """
26      GMC_NS_Model_Record
27
28      Abstract supertype for models in 'GMC_NS_Ellement's. Subtyped model
29      structs must implement the following fields as their interface with
30      'GMC_NS':
31
32      mutable struct Minimal_Model
33          trajectory::Integer #id assigned at construction, identifies
34          trajectory
35          i::Integer #id integer assigned at construction, identifies
36          position on trajectory
37
38          θ::Vector{<:AbstractFloat} #model's parameter Vector
39          log_Li<:AbstractFloat #model's likelihood, calculated by
40          constructor
41
42          pos :: Vector{<:AbstractFloat}
43          v :: Vector{<:AbstractFloat} #model's velocity in hypersphere
44          coords

```

```

30     end
31 """
32 abstract type GMC_NS_Model end

```

16.4.3 /src/GMC/galilean_trajectory.jl

```

1 """
2     galilean_trajectory_sample!(m, e, tuner, cache)
3
4 Given 'm <: GMC_NS_Model', 'e <: GMC_NS_Engine', 'tuner <: GMC_Tuner',
5   and 'cache <: Union{Nothing,GMC_NS_Model}', return the next
6   model-sample from a Galilean trajectory through parameter space, as
7   well as any cached reflection model, obeying 'new_m.log_Li ≥
8   e.contour'.
9
10 Implements Skilling's 2019 update to the GMC algorithm. Briefly: proceed
11   "forward" along the trajectory's velocity vector unless the
12   likelihood contour is hit; in this case, attempt to reflect "east",
13   then "west", then "south". If a reflection is found, return the model
14   in the original position with the appropriate new velocity vector, as
15   well as the cached reflection model "forward" along this new vector.
16   If no model with likelihood greater than contour can be found, return
17   the original model (as long as the trajectory is alive it will be
18   retried at lower timestep τ).
19
20 Skilling, John. "Galilean and Hamiltonian Monte Carlo." In Proceedings,
21   33:8. Garching, Germany: MDPI, 2019.
22 """
23
24 function galilean_trajectory_sample!(m, e, tuner, cache)
25     t=m.trajectory; next_i=m.i+1; cache_i=m.i+2
26
27     e.GMC_timestep_η > 0. ? 
28         (τ=max(tuner.τ+rand(Normal(0,tuner.τ*e.GMC_timestep_η)),eps())) :
29         (τ=tuner.τ) #perturb τ if specified
30     d=τ*m.v #distance vector for given timestep
31     fwd_pos,adj_d,box_rflct=box_move(m.pos,d,e.box)
32
33     # println("=====NORTH=====")
34     # println(fwd_pos)
35     # println( to_prior.(fwd_pos,e.priors))
36     # println("==========")
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```

22     if !box_rflect #if we're not stopped by the sampling box
23         fwd_m=e.model_init(t, next_i, to_prior.(fwd_pos,e.priors),
24             ↵ fwd_pos, m.v, e.obs, e.constants ...) #try to proceed along
25             ↵ distance vector
26         if m.θ==fwd_m.θ #if forward motion is no longer making a
27             ↵ difference to the parameter vector (ie timestep is too
28             ↵ small), kill the trajectory and bail out
29             tuner.τ=e.GMC_τ_death
30             return m, nothing
31     end
32 else
33     fwd_m=m #placeholder, no effect on box reflection
34 end
35
36 if box_rflect || (fwd_m.log_Li < m.log_Li) #if no model is available
37     ↵ from the position along the distance vector, search for
38     ↵ reflections and store in cache
39     process_report!(tuner, false)
40
41     lhδ=lps(m.log_Li,-fwd_m.log_Li) #get the likelihood difference
42     ↵ between the two points
43     n=boundary_norm(adj_d,lhδ) #get the gradient normal for the
44     ↵ distance and lhδ
45     box_rflect ? (v[] = box_reflect(m.pos,e.box,m.v)) :
46     ↵ v[] = reflect(m.v,n,e.GMC_reflect_η) #get the reflected velocity
47     ↵ vector off the boundary "east", or off the sampling box
48     rd=τ*v[] #reflected distance given the timestep
49     east_pos,_=box_move(m.pos,rd,e.box)
50
51     # println("====EAST====")
52     # println(e.box)
53     # println(east_pos)
54     # println( to_prior.(east_pos,e.priors))
55     # println("====")
56
57     cache=e.model_init(t, cache_i, to_prior.(east_pos,e.priors),
58         ↵ east_pos, v[], e.obs, e.constants ...) #try going east, or
59         ↵ reflecting off the box
60     if cache.log_Li < m.log_Li && !box_rflect #no west reflection off
61         ↵ the sampling box
62         process_report!(tuner, false)
63
64     west_pos,_=box_move(m.pos,-rd,e.box)

```

```

52
53     # println("====WEST=====")
54     # println( to_prior.(west_pos,e.priors))
55     # println("=====")

56
57     cache=e.model_initλ(t, cache_i, to_prior.(west_pos,e.priors),
58     ↳ west_pos, -v[], e.obs, e.constants ... ) #if east
59     ↳ reflection fails, try west
60
61 end
62
63 if cache.log_Li < m.log_Li
64     process_report!(tuner, false)
65     south_pos,_=box_move(m.pos,-d,e.box)

66
67     # println("====SOUTH=====")
68     # println( to_prior.(south_pos,e.priors))
69     # println("=====")
70     cache=e.model_initλ(t, cache_i,
71     ↳ to_prior.(south_pos,e.priors),south_pos,-m.v, e.obs,
72     ↳ e.constants ... ) #if west or box reflection fails, try
73     ↳ south (reversed along the particle's vector)
74
75 end
76
77 cache.log_Li < m.log_Li && (process_report!(tuner, false)) #if
78     ↳ that fails wait for smaller t
79
80 end

81
82 if cache!=nothing && cache.log_Li ≥ m.log_Li #a reflection that
83     ↳ results in a model above contour exists, so return a model in
84     ↳ place with the appropriate velocity vector and the cached model
85     ↳ down the reflection vector
86     r_m=construct_reflection(m, next_i, cache.v)
87     return (r_m, cache)
88
89 else #if no reflections occurred or no reflected positions above the
90     ↳ contour were found - either forward search succeeded or the whole
91     ↳ search failed
92     cache!=nothing && (cache=nothing) #reset cache if it's been set
93     ↳ with a model below contour
94     fwd_m.i = next_i && fwd_m.log_Li ≥ m.log_Li ?
95     ↳ (process_report!(tuner, true); return fwd_m, cache) : (return
96     ↳ m, cache) #if the forward model is a new iterate and at or
97     ↳ above the contour, return that, otherwise return the original
98     ↳ model
99
100 end
101
102 end

```

```

79
80     function box_move(pos,d,box)
81         if !(all(box[:,1].<pos+d.<box[:,2])) #if not all
82             ↪   coordinates after move would be in the box
83             boundary_d=box.-pos #get distances to box
84             ↪   boundaries
85             b=isapprox.(pos,box) #find any boundaries that
86             ↪   have been exceeded due to numerical creep
87             boundary_d[b]=0 #correct these distances
88             boundary_t=boundary_d./d #get time to hit
89             ↪   boundaries, given particle displacement d
90
91
92
93         for idx in findall(x→x≥0.,boundary_t) #for any
94             ↪   boundaries the particle is riding (distance
95             ↪   0), give a small positive time if the
96             ↪   particle is headed into the boundary to allow
97             ↪   that dimension to be selected for bounding
98             (dim,bound)=(idx[1],idx[2])
99             boundary_t[idx]=0. && ((bound==1 && d[dim] <
100             ↪   0.) || (bound==2 && d[dim] > 0.)) &&
101             ↪   (boundary_t[idx]=eps())
102         end
103
104         ↪   first_b_idx=findfirst(isequal(minimum(boundary_t[findall(
105             ↪   #find the index of the first boundary that
106             ↪   would be hit
107             first_b_d=boundary_d[first_b_idx]
108             d=d.*((first_b_d/d[first_b_idx[1]]))
109         end
110
111         all(d.=0.) ? box_reflect=true : box_reflect=false
112
113         return pos+d, d, box_reflect
114     end
115
116     function box_reflect(pos,box,v)
117         b=isapprox.(pos,box)
118         low_idxs=[v[i]<0&&b[i,1] for i in 1:length(v)]
119         hi_idxs=[v[i]>0&&b[i,2] for i in 1:length(v)]
120
121         rv=copy(v)
122         rv[low_idxs]=-v[low_idxs]

```

```

109         rv[hi_idxs]=-v[hi_idxs]
110
111     end
112
113     function boundary_norm(v, lhδ)
114         b=lhδ./v
115         b[b.==Inf]=prevfloat(Inf) #rectify overflow
116         b[b.==0.]=rand([nextfloat(0.),prevfloat(0.)])
117         ↪ #rectify exact 0.
118         b[b.==-Inf]=nextfloat(-Inf) #rectify underflow
119         return normalize(b)
120     end
121
122     function reflect(v, n, η)
123         v[] = v - (2 * n * dot(n, v))
124         η > 0. ? (return r_perturb(v[], η)) : (return v[])
125     end
126
127     function r_perturb(v[], η)
128         return v[] * cos(η) +
129             ↪ rand(MvNormal(length(v[]), 1.)) * sin(η)
130     end

```

16.4.4 /src/ensemble/GMC_NS_Ensemble.jl

```

1 """
2
3 GMC_NS_Ensemble
4
5 Abstract supertype for model ensembles intended to be subjected to
6   ↪ Galilean Monte Carlo Nested Sampling ('GMC_NS'). Subtyped ensemble
7   structs must implement the following fields as their interface with
8   'GMC_NS':
9
10    mutable struct Minimal_Ensemble <: GMC_NS_Ensemble
11        path::String #ensemble records serialized here
12
13        models::Vector{<:GMC_NS_Model_Record} #records of model paths &
14        ↪ lhs
15        model_initλ::Function #GMC_NS_Model constructor
16
17        contour<:AbstractFloat #current log_Li[end], init
18        ↪ minimum([record.log_Li for record in models])

```

```

13      log_Li::Vector{<:AbstractFloat} #likelihood of lowest-ranked
14      ↵ model at iterate i, init [-Inf]
15          log_Xi::Vector{<:AbstractFloat} #amt of prior mass included in
16          ↵ ensemble contour at Li, init [0]
17              log_wi::Vector{<:AbstractFloat} #width of prior mass covered in
18              ↵ this iterate, init [-Inf]
19                  log_Liwi::Vector{<:AbstractFloat} #evidentiary weight of the
20                  ↵ iterate, init [-Inf]
21                      log_Zi::Vector{<:AbstractFloat} #ensemble evidence, init
22                      ↵ [typemin(FloatType)]
23                          Hi::Vector{<:AbstractFloat} #ensemble information, init [0]
24
25
26
27      obs::Any #observations formatted for GMC_NS_Model likelihood
28      ↵ Function
29          priors::Vector{<:Distribution} #prior distributions on
30          ↵ GMC_NS_Model parameters, formatted for model_initλ
31              constants::Vector #fixed parameters to be passed to GMC_NS_Model
32              ↵ constructor
33
34      sample_posterior::Bool #switch for posterior_samples collection
35      posterior_samples::Vector{<:GMC_NS_Model_Record}
36
37
38      #GMC tuning settings
39          GMC_tune_ι<:Integer #maximum number of initial τ tuning steps
40              GMC_tune_ρ<:AbstractFloat #use ensemble size*p GMC samples per
41              ↵ initial tuning step
42                  GMC_tune_μ<:Integer #tune τ on the basis of this number of the
43                  ↵ most recent steps
44                      GMC_tune_α<:AbstractFloat #tune τ such that the acceptance rate
45                      ↵ is between this value and 1.
46                          GMC_tune_β<:AbstractFloat #multiply τ by 1+β (sampling acceptance
47                          ↵ rate=1.) or 1-β (rate <α)
48
49
50      #GMC Sampler settings
51          GMC_timestep_η<:AbstractFloat #>0. to apply an perturbation term
52          ↵ randomly drawn from Normal(0, η*τ) to τ at each step (can assist
53          ↵ equilibration)
54              GMC_reflect_η<:AbstractFloat #>0. to apply perturbation to
55              ↵ reflection vectors, keep value small (can assist equilibration)
56                  GMC_exhaust_σ<:AbstractFloat #apply this scale parameter to the
57                  ↵ ensemble size to obtain the maximum number of GMC iterates to perform
58                  ↵ before terminating nested sampling
59                      GMC_chain_κ<:Integer #maximum chain length

```

```

39
40         t_counter<:Integer #counter for next instantiated trajectory
41     ↵ number
42   end
43
44 """"
45
46 """
47 """
48     show(e<:GMC_NS_Ensemble)
49     show(io,e<:GMC_NS_Ensemble)
50
51 Display the ensemble path, likelihood histogram, contour, maximum log
52   ↵ likelihood, and log evidence (marginal likelihood).
53 """
54 function Base.show(io::IO, e::GMC_NS_Ensemble; progress=false)
55     livec=[model.log_Li for model in e.models]
56     maxLH=maximum(livec)
57     printstyled(io, "$(typeof(e)) @ $(e.path)\n", bold=true)
58     msg = @sprintf "Contour: %3.6e MaxLH:%3.3e log Evidence:%3.6e"
59     ↵ e.contour maxLH e.log_Zi[end]
60     println(io, msg)
61     try
62         hist=UnicodePlots.histogram(livec, title="Ensemble Likelihood
63           ↵ Distribution")
64         show(io, hist)
65         println()
66         progress && return(nrows(hist.graphics)+6)
67     catch
68         printstyled("HISTOGRAM UNAVAILABLE", color=:green)
69         println()
70         progress && return 4
71     end
72 end
73 """

```

16.4.5 /src/ensemble/ensemble_utilities.jl

```

1 function ensemble_history(e::GMC_NS_Ensemble, bins=25)
2     !e.sample_posterior && throw(ArgumentError("This ensemble has no
3       ↵ posterior samples to show a history for!"))

```

```

3     livec=vcat([model.log_Li for model in e.models],[model.log_Li for
4         ↳ model in e.posterior_samples])
5     show(histogram(livec, nbins=bins))
6
7 function e_backup(e::GMC_NS_Ensemble, tuner::Dict{Int64,<:GMC_Tuner})
8     serialize(string(e.path,'/',"ens"), e)
9     serialize(string(e.path,'/',"tuner"), tuner)
10    end
11
12 function clean_ensemble_dir!(e::GMC_NS_Ensemble)
13     if e.sample_posterior
14         ens_paths=vcat(
15             [basename(m.path) for m in e.models],
16             [basename(m.path) for m in e.posterior_samples],
17             "ens", "tuner"
18         )
19     else
20         ens_paths=vcat(
21             [basename(m.path) for m in e.models],
22             "ens", "tuner"
23         )
24     end
25
26     for file in readdir(e.path)
27         !(file in ens_paths) && rm(e.path*'*file)
28     end
29 end
30
31 function complete_evidence(e::GMC_NS_Ensemble)
32     return final_logZ = logaddexp(e.log_Zi[end], (logsumexp([model.log_Li
33         ↳ for model in e.models] .+ (e.log_Xi[length(e.log_Li)] -
34         ↳ log(length(e.models)))))))
35 end
36
37 function measure_evidence(e::GMC_NS_Ensemble)
38     return
39         ↳ ms=measurement(complete_evidence(e),sqrt(abs(e.Hi[end])/length(e.models)))
40 end
41
42 function reset_ensemble!(e::GMC_NS_Ensemble)

```

```

41      ↵ Ni=Int64.(round.(collect(-1:-1:-length(e.log_Li)+1)./e.log_Xi[2:end]))
42 N=Ni[1]
43
44 new_models=Vector{typeof(e.models[1])}()
45
46 for traj in 1:N
47     if string(traj)*".1" in [basename(record.path) for record in
48         ↵ e.models]
49
50         ↵ push!(new_models,e.models[findfirst(isequal(string(traj)*".1"),
51         ↵ [basename(record.path) for record in e.models]]])
52     else
53
54         ↵ push!(new_models,e.posterior_samples[findfirst(isequal(string(traj)*"
55         ↵ [basename(record.path) for record in
56         ↵ e.posterior_samples])])
57     end
58 end
59
60 e.models=new_models
61 e.posterior_samples=Vector{typeof(e.models[1])}()
62
63 e.contour=minimum([record.log_Li for record in e.models])
64
65 e.log_Li=[e.log_Li[1]]
66 e.log_Xi=[e.log_Xi[1]]
67 e.log_wi=[e.log_wi[1]]
68 e.log_Liwi=[e.log_Liwi[1]]
69 e.log_Zi=[e.log_Zi[1]]
70 e.Hi=[e.Hi[1]]
71
72 e.t_counter=length(e.models)+1
73
74 clean_ensemble_dir(e, 0; ignore_warn=true)
75 isfile(e.path*"/tuner") && rm(e.path*"/tuner")
76 serialize(e.path*"/ens", e)
77
78 return e
79 end
80
81 function move_ensemble!(e::GMC_NS_Ensemble,path::String)
82     !isdir(path) && mkpath(path)

```

```

77 rectype=typeof(e.models[1])
78
79 for file in readdir(e.path)
80     mv(e.path*'*file, path*'*file)
81 end
82
83 for (n,model) in enumerate(e.models)
84     e.models[n]=rectype(model.trajectory, model.i, model.pos,
85     → path*'*basename(model.path), model.log_Li)
86 end
87 if e.sample_posterior
88     for (n,model) in enumerate(e.posterior_samples)
89         e.posterior_samples[n]=rectype(model.trajectory, model.i,
90         → model.pos, path*'*basename(model.path), model.log_Li)
91     end
92 end
93
94 rm(e.path)
95 e.path=path
96 serialize(e.path*"/ens",e)
97 return e
98 end
99
100 function copy_ensemble(e::GMC_NS_E ensemble, path::String)
101     new_e=deepcopy(e)
102     !isdir(path) && mkdir(path)
103     rectype=typeof(e.models[1])
104     for file in readdir(e.path)
105         cp(e.path*'*file, path*'*file)
106     end
107
108     for (n,model) in enumerate(e.models)
109         new_e.models[n]=rectype(model.trajectory, model.i, model.pos,
110         → path*'*basename(model.path), model.log_Li)
111     end
112     if e.sample_posterior
113         for (n,model) in enumerate(e.posterior_samples)
114             new_e.posterior_samples[n]=rectype(model.trajectory, model.i,
115             → model.pos, path*'*basename(model.path), model.log_Li)
116         end
117     end
118
119     new_e.path=path

```

```

116     serialize(new_e.path*"/ens",new_e)
117     return new_e
118 end
119
120 function rewind_ensemble!(e::GMC_NS_Ensemble,rewind_idx)
121     !e.sample_posterior && throw(ArgumentError("An ensemble not retaining
122         ↪ posterior samples cannot be rewound!"))
123     rewind_idx ≥ length(e.log_Li) && throw(ArgumentError("rewind_idx
124         ↪ must be less than the current iterate!"))
125
126
127     ↪ Ni=Int64.(round.(collect(-1:-1:-length(e.log_Li)+1)./e.log_Xi[2:end]))
128     insert!(Ni,1,Ni[1])
129
130     for it in length(e.log_Li)-1:-1:rewind_idx
131         last_post=pop!(e.posterior_samples)
132
133         if Ni[it]==Ni[it-1]
134             models_idx=findfirst(isequal(last_post.trajectory),
135                 ↪ [rec.trajectory for rec in e.models])
136             insert!(e.models, models_idx, last_post)
137             deleteat!(e.models, models_idx+1)
138         else
139             push!(e.models, last_post)
140         end
141
142         !(length(e.models)==Ni[it-1]) && throw(DomainError("it: $it
143             ↪ Ni[it]:$(Ni[it]) Ni[it-1]:$(Ni[it-1])"))
144     end
145
146     e.contour=e.log_Li[rewind_idx]
147     e.log_Li=e.log_Li[1:rewind_idx]
148     e.log_Xi=e.log_Xi[1:rewind_idx]
149     e.log_wi=e.log_wi[1:rewind_idx]
150     e.log_Liwi=e.log_Liwi[1:rewind_idx]
151     e.log_Zi=e.log_Zi[1:rewind_idx]
152     e.Hi=e.Hi[1:rewind_idx]
153
154     traj_nos=vcat([rec.trajectory for rec in e.models],[rec.trajectory
155         ↪ for rec in e.posterior_samples])
156
157     e.t_counter=maximum(traj_nos)

```

```

153     return e
154 end
155
156 function show_models(e::GMC_NS_Ensemble,idxs)
157     liperm=sortperm([model.log_Li for model in e.models],rev=true)
158     for idx in idxs
159         m=deserialize(e.models[liperm[idx]].path)
160         show(m)
161     end
162 end
163
164 function show_models_e(e::GMC_NS_Ensemble,idxs)
165     liperm=sortperm([model.log_Li for model in e.models],rev=true)
166     for idx in idxs
167         m=deserialize(e.models[liperm[idx]].path)
168         show(stdout,m,e)
169     end
170 end
171
172 function get_model(e::GMC_NS_Ensemble,no)
173     return deserialize(e.path*"/"*string(no))
174 end
175
176 #recalculate the ensemble's history from the succession of log_Li and
177 #→ log_Xi values
177 function recalculate_ensemble!(e::GMC_NS_Ensemble, wi_mode="trapezoidal")
178
179     ↳ Ni=Int64.(round.(collect(-1:-1:-length(e.log_Li)+1)./e.log_Xi[2:end]))
180     insert!(Ni,1,Ni[1])
181
182     for i in 1:length(e.log_Li)-1
183         j=i+1
184         if wi_mode=="trapezoidal"
185             e.log_wi[j]= logaddexp(e.log_Xi[i], - ((j+1)/Ni[j])) - log(2)
186             ↳ #log width of prior mass spanned by the last
187             ↳ step-trapezoidal approx
188         elseif wi_mode=="simple"
189             e.log_wi[j]= logaddexp(e.log_Xi[i], - e.log_Xi[j]/Ni[i]) #log
190             ↳ width of prior mass spanned by the last step-simple
191             ↳ approx
192         else
193             throw(ArgumentError("Unsupported wi_mode!"))
194         end

```

```

190     e.log_Liwi[j]=lps(e.log_Li[j],e.log_wi[j]) #log likelihood + log
191     ↪ width = increment of evidence spanned by iterate
192     e.log_Zi[j]=logaddexp(e.log_Zi[i],e.log_Liwi[j])      #log evidence
193     #information- dimensionless quantity
194     Hj=lps(
195         (exp(lps(e.log_Liwi[j],-e.log_Zi[j])) * e.log_Li[j]),
196         (exp(lps(e.log_Zi[i],-e.log_Zi[j])) *
197          ↪ lps(e.Hi[i],e.log_Zi[i])),
198         -e.log_Zi[j])
199     Hj ≡ -Inf ? (e.Hi[j]=0.) : (e.Hi[j]=Hj)
200   end
201 end
202
203 #get a posterior kernel density estimate from the ensemble
204 function posterior_kde(e::GMC_NS_Ensemble, scale=400., bw=1.)
205   !e.sample_posterior && throw(ArgumentError("This ensemble is not set
206   ↪ to collect posterior samples!"))
207
208   ↪ θ_mat=zeros(length(e.models[1].pos),length(e.models)+length(e.posterior_samples))
209   weight_vec=zeros(length(e.models)+length(e.posterior_samples))
210
211   for (n,mrec) in enumerate(e.posterior_samples)
212     m=deserialize(mrec.path)
213     θ_mat[:,n]=m.θ
214     weight_vec[n]=e.log_Liwi[n+1]
215   end
216
217   for (n,mrec) in enumerate(e.models)
218     m=deserialize(mrec.path)
219     p=length(e.posterior_samples)
220     θ_mat[:,n+p]=m.θ
221     weight_vec[n+p]=m.log_Li + lps(e.log_Xi[end], -length(e.models))
222   end
223
224   weight_vec-=maximum(weight_vec)-scale
225
226   return kde!(θ_mat,[bw],exp.(weight_vec))
227 end

```

16.4.6 /src/model/GMC_NS_Model.jl

```

1  """
2      GMC_NS_Model_Record
3
4  Abstract supertype for records of serialised 'GMC_NS_Model's. Subtyped
5  record structs must implement the following fields as their interface
6  with 'GMC_NS':
7
8      struct Minimal_Record
9          trajectory::Integer #id assigned at construction, identifies
10         trajectory
11         i::Integer #id integer assigned at construction, identifies
12         position on trajectory
13         pos::Vector{<:AbstractFloat} #hypersphere position of
14         model-particle
15         path::String #model is serialized to ensembledir/trajectory.i
16         log_Li::AbstractFloat #log likelihood calculated by
17         'GMC_NS_Model' constructor
18     end
19
20     """
21
22     abstract type GMC_NS_Model_Record end
23
24
25     """
26     GMC_NS_Model_Record
27
28  Abstract supertype for models in 'GMC_NS_Engineer's. Subtyped model
29  structs must NOT be mutable, and must not have inner constructors
30  that make field-by-field construction inaccessible. Subtyped model
31  structs must implement the following fields as their interface with
32  'GMC_NS', in order, and all additional fields must follow:
33
34      mutable struct Minimal_Model
35          trajectory::Integer #id assigned at construction, identifies
36          trajectory
37          i::Integer #id integer assigned at construction, identifies
38          position on trajectory
39
40          θ::Vector{<:AbstractFloat} #model's parameter Vector
41          log_Li<:AbstractFloat #model's likelihood, calculated by
42          constructor
43
44          pos::Vector{<:AbstractFloat}

```

```

29         v::Vector{<:AbstractFloat} #model's velocity in hypersphere
30     → coords
31   end
31 """
32 abstract type GMC_NS_Model end

```

16.4.7 /src/model/model_utilities.jl

```

1 function construct_reflection(m, i, r_v)
2     params=[getfield(m,pn) for pn in propertynames(m)]
3     params[2]=i
4     params[6]=r_v
5     return typeof(m)(params...)
6 end

```

16.4.8 /src/nested_sampler/converge_ensemble.jl

```

1 """
2     converge_ensemble!(e; max_iterates, backup, clean,
3     → converge_criterion, converge_factor, mc_noise, progargs)
4
5 Given 'e <: GMC_NS_Ensemble', recursively execute nested sampling steps
6     → ('nested_step!()') until ensemble convergence criterion is obtained,
7     → or 'max_iterates' is reached.
8
9 Convergence criteria specied by 'converge_criterion <: String':
10    → "standard" for evidence convergence, "compression" for ensemble
11    → likelihood range compression (can be used to improve MAP parameter
12    → estimation after evidence has converged). Factor to use specified by
13    → 'converge_factor <: AbstractFloat'. "Standard" defined in Skilling
14    → 2006.
15
16 For likelihood estimates generated by Monte Carlo model evaluation,
17    → 'mc_noise <: AbstractFloat' defines the expected standard deviation
18    → of likelihood in the MAP region of parameter space; this will be used
19    → as an alternative convergence criterion if the ensemble is converged
20    → to within this value.
21
22 Keyword arguments to GMC_NS_Progress are passed from progargs.

```

```

12 Skilling, John. "Nested Sampling for Bayesian Computations." In Proc.
13   → Valencia. Benidorm (Alicante, Spain): inference.org.uk, 2006.
14   → https://www.inference.org.uk/bayesys/Valencia.pdf.
13 """
14
14 function converge_ensemble!(e::GMC_NS_Engsemble;
15   → max_iterates=typemax(Int64), backup::Tuple{Bool, Integer}=(false, 0),
15   → clean::Tuple{Bool, Integer, Integer}=(false, 0, 0),
15   → converge_criterion::String="standard",
15   → converge_factor::AbstractFloat=.01, mc_noise::AbstractFloat=0.,
15   → progargs ... )
15   N = length(e.models); curr_it=length(e.log_Li)
16
17   if curr_it==1 || !isfile(e.path*"/tuner")
18     tuner_dict=get_tuner_dict(e)
19   else
20     tuner_dict=deserialize(e.path*"/tuner") #restore tuner from saved
21     → if any
21 end
22
23 meter = GMC_NS_Progress(e, 0.; start_it=curr_it, progargs ... )
24
25 converge_check = get_convfunc(converge_criterion)
26
27 reflect_cache = nothing #init cache for models precalculated in
28   → galilean reflection
29
30 cln_switch = clean[1] && !e.sample_posterior #ignore clean arguments
31   → if posterior samples are to be collected
32
33 while !converge_check(e, converge_factor, mc_noise) && (curr_it ≤
34   → max_iterates)
35   warn = nested_step!(e, tuner_dict, reflect_cache)
36   → warn == 1 && (@error "Failed to find new models, aborting at
37     → current iterate."; return e)
38   curr_it += 1
39
40   backup[1] && curr_it%backup[2] == 0 && e_backup(e, tuner_dict)
41     → #every backup interval, serialise the ensemble and tuner
42   cln_switch && curr_it%clean[2] == 0 &&
43     → clean_ensemble_dir(e, clean[3]) #every clean interval, remove
44     → old discarded models
45
46 update!(meter, converge_check(e, converge_factor, vals=true) ... )

```

```

40     end
41
42     if converge_check(e,converge_factor, mc_noise)
43         ms=measure_evidence(e)
44         @info "Job done, sampled to convergence. Final logZ $(ms.val) ±
45             $(ms.err)"
46
47         e_backup(e,tuner_dict) #final backup
48         cln_switch && clean_ensemble_dir(e,0) #final clean
49         return ms
50     elseif curr_it==max_iterates
51         @info "Job done, sampled to maximum iterate $max_iterates.
52             Convergence criterion not obtained."
53
54         e_backup(e,tuner_dict) #final backup
55         cln_switch && clean_ensemble_dir(e,0) #final clean
56         return e.log_Zi[end]
57     end
58
59     function evidence_converge(e, evidence_fraction,
60         → mc_noise=0.; vals=false)
61         mc_noise > 0. && noise_check(e,mc_noise) && return
62             → true
63
64         val=lps(findmax([model.log_Li for model in
65             → e.models])[1], e.log_Xi[end])
66         thresh=lps(log(evidence_fraction),e.log_Zi[end])
67
68         isinf(val)||isinf(thresh) && return false
69
70         vals ? (return val, thresh) : (return val

```

```

75         vals ? (return val, thresh) : (return val<thresh)
76     end
77
78     function get_convfunc(criterion)
79         if criterion == "standard"
80             return evidence_converge
81         elseif criterion == "compression"
82             return compress_converge
83         else
84             throw(ArgumentError("Convergence criterion
85             ↪ $criterion not supported! Try \"standard\" or
86             ↪ \"compression\"."))
87         end
88     end
89
90     #if the entire ensemble is within 99.7% of the noise
91     #→ distribution of its mean likelihood, it is converged
92     #→ to the extent possible given Monte Carlo noise
93     function noise_check(e, mc_noise)
94         mod_lhs=[m.log_Li for m in e.models]
95         std(mod_lhs) ≤ mc_noise ? (return true) : (return
96             ↪ false)
97     end

```

16.4.9 /src/nested_sampler/nested_step.jl

```

1 """
2     nested_step!(e<:GMC_NS_Ensemble)
3
4 Step and update ensemble e by sampling the e.contour-bounded prior mass
5   ↪ via Galilean Monte Carlo.
6 """
7
8 function nested_step!(e::GMC_NS_Ensemble, tuners::Dict{Int64,τ_PID},
9   ↪ cache::Union{GMC_NS_Model,Nothing})
10    N = length(e.models) #number of sample models/particles on the
11    ↪ posterior surface
12    i = length(e.log_Li) #iterate number, index for last values
13    j = i+1 #index for newly pushed values
14
15    while j < N
16        #push new particles
17        #sample from the contour-bounded prior
18        #update the ensemble
19    end
20
21    #compute log(Li) for the new particles
22    #compute the standard deviation of the log(Li) distribution
23    #check if the ensemble is converged
24    #if so, return true
25    #else, return false
26
27    return false
28
29 end

```

```

13     e.contour, least_likely_idx = findmin([model.log_Li for model in
14         ↪ e.models])
15     Li_model = e.models[least_likely_idx]
16     m = deserialize(Li_model.path)
17     t=tuners[Li_model.trajectory]

18     #SELECT NEW MODEL, SAVE TO ENSEMBLE DIRECTORY, CREATE RECORD AND PUSH
19     ↪ TO ENSEMBLE

20     cache==nothing ? (model_selected=false; cache_insert=false) :
21         ↪ (model_selected=true; cache_insert=true)
22     samples=1;
23         ↪ sample_limit=Int64(floor(length(e.models)*e.GMC_exhaust_σ))

24     while !model_selected && samples≤sample_limit
25         if t.τ > e.GMC_τ_death && m.i ≤ e.GMC_chain_κ #while the
26             ↪ particle's timestep is greater than the minimum to keep a
27             ↪ chain alive, and the chain is below the maximum allowed
28             ↪ length, sample by advancing it along a galilean trajectory
29             model_selected,cache =
30                 ↪ galilean_search!(m,e,t,least_likely_idx,samples,sample_limit,cache)
31                 ↪ #ensemble is modified by galilean_search! directly
32             elseif (N-1)<e.GMC_Nmin #if removing the particle would put the
33                 ↪ sample under the minimum size, resample by diffusion from an
34                 ↪ existing particle

35                 ↪ model_selected=diffusion_search!(e,tuners,least_likely_idx,N,e.GMC_τ_
36                 ↪ #ensemble modified by diffusion_search!
37                 samples+=1
38             else #if not, push to the posterior samples and let the
39                 ↪ trajectory chain die
40                 model_selected=true
41                 e.sample_posterior && push!(e.posterior_samples,
42                     ↪ e.models[least_likely_idx])#if sampling posterior, push
43                     ↪ the model record to the ensemble's posterior samples
44                     ↪ vector
45                 deleteat!(e.models, least_likely_idx) #remove least likely
46                     ↪ model record from the active particles in the ensemble
47             end
48         end

49     if cache_insert #in this case a cached precalculated reflection model
50         ↪ can be used

```

```

37     insert_reflection!(e, cache, least_likely_idx) #insert the model
38         ↵ into the ensemble
39     process_report!(t, true)
40     cache=nothing #reset the cache
41 end
42
43 samples=sample_limit && (return 1, cache) #failed to find a new
44         ↵ sample after sampling up to the limit, return an error code
45
46 #UPDATE ENSEMBLE QUANTITIES
47 push!(e.log_Li, minimum([model.log_Li for model in e.models])) #log
48         ↵ likelihood of the least likely model - the current ensemble ll
49         ↵ contour at Xi
50 push!(e.log_Xi, -i/N) #log Xi - crude estimate of the iterate's
51         ↵ enclosed prior mass
52 push!(e.log_wi, logaddexp(e.log_Xi[i], - ((j+1)/N)) - log(2)) #log
53         ↵ width of prior mass spanned by the last step-trapezoidal approx
54 push!(e.log_Liwi, lps(e.log_Li[j],e.log_wi[j])) #log likelihood + log
55         ↵ width = increment of evidence spanned by iterate
56 push!(e.log_Zi, logaddexp(e.log_Zi[i],e.log_Liwi[j]))    #log
57         ↵ evidence
58 #information- dimensionless quantity
59 Hj=lps(
60     (exp(lps(e.log_Liwi[j],-e.log_Zi[j])) * e.log_Li[j]),
61     (exp(lps(e.log_Zi[i],-e.log_Zi[j])) * lps(e.Hi[i],e.log_Zi[i])),
62     -e.log_Zi[j])
63 Hj == -Inf ? push!(e.Hi,0.) : push!(e.Hi, Hj) #prevent problems from
64         ↵ early strings of models with -Inf log likelihoods
65
66 return 0, cache
67 end

```

16.4.10 /src/nested_sampler/search_patterns.jl

```

1 function galilean_search!(m,e,t,llidx,samples,s_limit,cache)
2     new_m,cache=galilean_trajectory_sample!(m,e,t,cache)
3
4     while new_m.i==m.i && samples≤s_limit && t.τ > e.GMC_τ_death#failure
5         ↵ to find any new step, retune tau and try again until sample limit
6         ↵ reached or trajectory's tuner reaches taudeath
7         new_m,cache=galilean_trajectory_sample!(m,e,t,cache)
8         samples+=1

```

```

7     end
8
9     if new_m.i!=m.i
10        e.sample_posterior && push!(e.posterior_samples,
11            ↳ e.models[llidx])#if sampling posterior, push the model record
12            ↳ to the ensemble's posterior samples vector
13        rectype=typeof(e.models[1])
14        deleteat!(e.models, llidx) #remove least likely model record
15
16        new_model_record = rectype(new_m.trajectory,new_m.i, new_m.pos,
17            ↳ string(e.path,'/',new_m.trajectory,'.',new_m.i),
18            ↳ new_m.log_Li)
19        push!(e.models, new_model_record) #insert new model record
20        serialize(new_model_record.path, new_m)
21        move_cursor_up_while_clearing_lines(stdout,1)
22
23        return true, cache
24    else
25        return false, cache
26    end
27 end
28
29 function diffusion_search!(e, tuners, llidx, sample_limit, τ_limit)
30    sample=0; model_selected=false; new_m=0; src_traj=0
31    while !(sample > sample_limit) && !model_selected
32        sample +=1
33        mrec=rand(e.models)
34        model=deserialize(mrec.path)
35        randdir=rand(MvNormal(length(model.θ),1.))
36        τ=tuners[model.trajectory].τ
37        new_pos=model.pos+randdir*τ
38        box_bound!(new_pos,e.box)
39        new_m=e.model_initλ(e.t_counter, 1, to_prior.(new_pos,e.priors),
40            ↳ new_pos, randdir, e.obs, e.constants ... )
41
42        while τ*.7>τ_limit && new_m.log_Li<e.contour
43            τ*=.7
44            randdir=rand(MvNormal(length(model.θ),1.))
45            new_pos=model.pos+randdir*τ
46            box_bound!(new_pos,e.box)
47            new_m=e.model_initλ(e.t_counter, 1,
48                ↳ to_prior.(new_pos,e.priors), new_pos, randdir, e.obs,
49                ↳ e.constants ... )

```

```

43     end
44
45     new_m.log_Li > e.contour && (model_selected=true;
46         ↳ src_traj=model.trajectory)
47     end
48
49     if new_m.log_Li > e.contour
50         e.sample_posterior && push!(e.posterior_samples,
51             ↳ e.models[llidx])#if sampling posterior, push the model record
52             ↳ to the ensemble's posterior samples vector
53         rectype=typeof(e.models[1])
54         deleteat!(e.models, llidx) #remove least likely model record
55
56         new_model_record = rectype(new_m.trajectory,new_m.i, new_m.pos,
57             ↳ string(e.path,'/',new_m.trajectory,'.',new_m.i),
58             ↳ new_m.log_Li)
59         push!(e.models, new_model_record) #insert new model record
60         serialize(new_model_record.path, new_m)
61         tuners[e.t_counter]=τ_PID(e)
62         tuners[e.t_counter].τ=tuners[src_traj].τ
63         e.t_counter +=1
64         return true
65     else
66         return false
67     end
68 end
69
70 function ellipsoid_resample!(e,tdict,llidx,samples,s_limit)
71     posmat=zeros(length(e.models[1].pos),length(e.models))
72     for (n,rec) in enumerate(e.models)
73         posmat[:,n]=rec.pos
74     end
75     e_ellip=bounding_ellipsoid(posmat)
76
77     new_pos=sample_ellipsoid(e_ellip)
78     box_bound!(new_pos,e.box)
79     new_m=e.model_init!(e.t_counter, 1, to_prior.(new_pos,e.priors),
80         ↳ new_pos, [0.], e.obs, e.box, e.constants..., v_init=true)
81     samples+=1
82
83     while new_m.log_Li < e.contour && samples < s_limit
84         new_pos=sample_ellipsoid(e_ellip)
85         box_bound!(new_pos,e.box)

```

```

80         new_m=e.model_initλ(e.t_counter, 1, to_prior.(new_pos,e.priors),
81             ↵ new_pos, [0.], e.obs, e.box, e.constants..., v_init=true)
82         samples+=1
83     end
84
85     if new_m.log_Li > e.contour
86         e.sample_posterior && push!(e.posterior_samples,
87             ↵ e.models[llidx])#if sampling posterior, push the model record
88             ↵ to the ensemble's posterior samples vector
89         rectype=typeof(e.models[1])
90         deleteat!(e.models, llidx) #remove least likely model record
91
92         new_model_record = rectype(new_m.trajectory,new_m.i, new_m.pos,
93             ↵ string(e.path,'/',new_m.trajectory,'.',new_m.i),
94             ↵ new_m.log_Li)
95         push!(e.models, new_model_record) #insert new model record
96         serialize(new_model_record.path, new_m)
97         tdict[e.t_counter]=τ_PID(e)
98         e.t_counter +=1
99         return true
100    else
101        return false
102    end
103 end

```

16.4.11 /src/normal/LogNormal_Ensemble.jl

```

1 mutable struct LogNormal_Ensemble <: GMC_NS_Ensemble
2     path::String
3
4     model_initλ::Function
5     models::Vector{Normal_Record}
6
7     contour::Float64
8     log_Li::Vector{Float64}
9     log_Xi::Vector{Float64}
10    log_wi::Vector{Float64}
11    log_Liwi::Vector{Float64}
12    log_Zi::Vector{Float64}
13    Hi::Vector{Float64}
14
15    obs::Vector{Float64}

```

```

16     priors::Vector{<:Distribution}
17     constants::Vector{<:Real}
18     box::Matrix{Float64}
19
20     sample_posterior::Bool
21     posterior_samples::Vector{Normal_Record}
22
23     GMC_Nmin::Int64
24
25     GMC_τ_death::Float64
26     GMC_init_τ::Float64
27     GMC_tune_μ::Int64
28     GMC_tune_α::Float64
29     GMC_tune_PID::NTuple{3,Float64}
30
31     GMC_timestep_η::Float64
32     GMC_reflect_η::Float64
33     GMC_exhaust_σ::Float64
34     GMC_chain_κ::Int64
35
36     t_counter::Int64
37 end
38
39 LogNormal_Engsemble(path::String, no_models::Integer,
40   ↪ obs::AbstractVector{<:AbstractFloat}, prior, box, GMC_settings ... ;
41   ↪ sample_posterior::Bool=true) =
42
43 LogNormal_Engsemble(
44     path,
45     construct_lognormal_model,
46     assemble_lNMs(path, no_models, obs, prior, box, Vector{Real}()) ... ,
47     [-Inf], #L0 = 0
48     [0.], #ie exp(0) = all of the prior is covered
49     [-Inf], #w0 = 0
50     [-Inf], #Liwi0 = 0
51     [-1e300], #Z0 = 0
52     [0.], #H0 = 0,
53     obs,
54     length(prior)==1 ? ([GMC_NS.marginals(prior) ... ]) : (prior),
55     Vector{Real}(),
56     length(prior)==1 ? (to_unit_ball.(box,[GMC_NS.marginals(prior) ... ])) :
57     ↪ : (to_unit_ball.(box,prior)),
58     sample_posterior,
59     Vector{Normal_Record}(),
60     GMC_Nmin,
61     GMC_τ_death,
62     GMC_init_τ,
63     GMC_tune_μ,
64     GMC_tune_α,
65     GMC_timestep_η,
66     GMC_reflect_η,
67     GMC_exhaust_σ,
68     GMC_chain_κ,
69     GMC_Nmin,
70     GMC_τ_death,
71     GMC_init_τ,
72     GMC_tune_μ,
73     GMC_tune_α,
74     GMC_timestep_η,
75     GMC_reflect_η,
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77     GMC_chain_κ,
78     GMC_Nmin,
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1286   GMC_init_τ,
1287   GMC_tune_μ,

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91  @showprogress 1 "Assembling log-Normal Model ensemble..." for
92    → trajectory_no in 1:no_trajectories
93      model_path = string(path,'/',trajectory_no,'.',1)
94      if !isfile(model_path)
95        proposal=[rand.(prior) ... ]
96        pos=to_unit_ball.(proposal,marginals)
97        box_bound!(pos,box)
98        θvec=to_prior.(pos,marginals)
99
100       model = construct_lognormal_model(trajectory_no, 1, θvec,
101         → pos, [0.], obs, constants ... ; v_init=true)
102
103       serialize(model_path, model) #save the model to
104         → the ensemble directory
105       push!(ensemble_records,
106         → Normal_Record(trajectory_no,1,pos,model_path,model.log_Li))
107     else #interrupted assembly pick up from where we left off
108       model = deserialize(model_path)
109       push!(ensemble_records,
110         → Normal_Record(trajectory_no,1,model.pos,model_path,model.log_Li))
111   end
112 end
113
114 return ensemble_records, minimum([record.log_Li for record in
115   → ensemble_records])
116 end

```

16.4.12 /src/normal/LogNormal_Model.jl

```

1 #NOTE: LogNormal_Models reuse Normal_Records
2
3 struct LogNormal_Model <: GMC_NS_Model
4   trajectory::Integer #id integer assigned at construction
5   i::Integer #id of parent model, if any
6
7   θ::Vector{Float64} #model's parameter Vector
8   log_Li::Float64 #model's likelihood, calculated by constructor
9
10  pos::Vector{Float64}
11  v::Vector{Float64} #model's velocity in parameter space
12 end
13

```

```

14 function construct_lognormal_model(trajectory::Integer, i::Integer,
15   → θ::Vector{Float64}, pos::Vector{Float64}, v::Vector{Float64},
16   → obs::Vector{Float64}; v_init=false)
17   μ, λ=θ
18   lμ=log(μ^2/sqrt(μ^2 + inv(λ)))
19   lσ=sqrt(log(1+(inv(λ)/μ^2)))
20   mod_lnormal=LogNormal(lμ,lσ)
21   log_lh=lps(logpdf.(mod_lnormal, obs))
22   v_init && (v=rand(MvNormal(length(θ),1.)))
23 end
24
25 function Base.show(io::IO, m::LogNormal_Model; xsteps=100)
26   μ, λ=m.θ
27   lμ=log(μ^2/sqrt(μ^2 + inv(λ)))
28   lσ=sqrt(log(1+(inv(λ)/μ^2)))
29   n=LogNormal(lμ,lσ)
30
31   → X=[quantile(n,.025):(quantile(n,.975)-quantile(n,.025))/xsteps:quantile(n,.975)]
32   y=pdf.(n,X)
33
34   show(io, lineplot(X,y,xlabel="X",ylabel="p",title=title="LogNormal
35   → Model $(m.trajectory).$(m.i), log_Li $(m.log_Li)"))
36   println()
37   println("θ: $(m.θ)")
38   println("v: $(m.v)")
39 end

```

16.4.13 /src/normal/Normal_Ensemble.jl

```

1 mutable struct Normal_Ensemble <: GMC_NS_Ensemble
2   path::String
3
4   model_initλ::Function
5   models::Vector{Normal_Record}
6
7   contour::Float64
8   log_Li::Vector{Float64}
9   log_Xi::Vector{Float64}
10  log_wi::Vector{Float64}
11  log_Liwi::Vector{Float64}

```



```

53     length(prior)==1 ? (to_unit_ball.(box,[GMC_NS.marginals(prior) ... ]))
54     ↵   : (to_unit_ball.(box,prior)),
55     sample_posterior,
56     Vector{Normal_Record}(),
57     GMC_settings ... ,
58     no_models+1)
59
60 function Base.show(io::IO, m::Normal_Model, e::Normal_Ensemble;
61   ↵  xsteps=100, progress=true)
62   μ,λ=m.θ
63   σ=sqrt(1/λ)
64   n=Normal(μ,σ)
65   X=[μ-4σ:(μ+4σ)-(μ-4σ))/xsteps:μ+4σ ... ]
66   y=pdf.(n,X)
67
68   scattermin=y[Int(floor(xsteps/2)-floor(xsteps/3))]
69   scattermax=y[Int(floor(xsteps/2)-floor(xsteps/6))]
70
71   scatterrange=[scattermin:(scattermax-scattermin)/length(e.obs):scattermax ... ]
72   scattery=[rand(scatterrange) for i in 1:length(e.obs)]
73
74   xlims=(min(minimum(e.obs),minimum(X)),max(maximum(e.obs),maximum(X)))
75
76   plt=lineplot(X,y,xlabel="X",ylabel="p",title="Normal Model
77   ↵  $(m.trajectory).$(m.i), log_Li $(m.log_Li)", name="Model",
78   ↵  xlim=xlims)
79   scatterplot!(plt, e.obs, scattery, name="Obs")
80
81   progress && return nrows(plt.graphics)+8
82 end
83
84 function assemble_NM(path::String, no_trajectories::Integer, obs, prior,
85   ↵  box, constants)
86   ensemble_records = Vector{Normal_Record}()
87   !isdir(path) && mkpath(path)
88   length(prior)==1 ? (marginals=[GMC_NS.marginals(prior) ... ]) :
89   ↵   (marginals=prior)
90   box=to_unit_ball.(box,marginals)

```

```

89
90     @showprogress 1 "Assembling Normal Model ensemble..." for
91         trajectory_no in 1:no_trajectories
92             model_path = string(path,'/',trajectory_no,'.',1)
93             if !isfile(model_path)
94                 proposal=[rand.(prior)...]
95                 pos=to_unit_ball.(proposal,marginals)
96                 box_bound!(pos,box)
97                 θvec=to_prior.(pos,marginals)
98
99                 model = construct_normal_model(trajectory_no, 1, θvec, pos,
100                     [0.], obs, constants...; v_init=true)
101
102                     serialize(model_path, model) #save the model to
103                         ↪ the ensemble directory
104                         push!(ensemble_records,
105                             ↪ Normal_Record(trajectory_no,1,pos,model_path,model.log_Li)
106                         else #interrupted assembly pick up from where we left off
107                             model = deserialize(model_path)
108                             push!(ensemble_records,
109                                 ↪ Normal_Record(trajectory_no,1,model.pos,model_path,model.log_Li)
110                         end
111                     end
112
113                     return ensemble_records, minimum([record.log_Li for record in
114                         ↪ ensemble_records])
115                 end

```

16.4.14 /src/normal/Normal_Model.jl

```

1 mutable struct Normal_Record <: GMC_NS_Model_Record
2     trajectory::Int64
3     i::Int64
4     pos::Vector{Float64}
5     path::String
6     log_Li::Float64
7 end
8
9 struct Normal_Model <: GMC_NS_Model
10    trajectory::Integer #id integer assigned at construction
11    i::Integer #id of parent model, if any
12

```

```

13     θ::Vector{Float64} #model's parameter Vector
14     log_Li::Float64 #model's likelihood, calculated by constructor
15
16     pos::Vector{Float64}
17     v::Vector{Float64} #model's velocity in parameter space
18 end
19
20 function construct_normal_model(trajectory :: Integer, i :: Integer,
21     ↪ θ :: Vector{Float64}, pos :: Vector{Float64}, v :: Vector{Float64},
22     ↪ obs :: Vector{Float64}; v_init=false)
23     μ, λ = θ
24     mod_normal = Normal(μ, sqrt(1/λ))
25     log_lh = lps(logpdf.(mod_normal, obs))
26     v_init && (v = rand(MvNormal(length(θ), 1.)))
27
28     Normal_Model(trajectory, i, θ, log_lh, pos, v)
29 end
30
31 function Base.show(io :: IO, m :: Normal_Model; xsteps=100)
32     μ, λ = m.θ
33     σ = sqrt(1/λ)
34     n = Normal(μ, σ)
35     X = [μ - 4σ : ((μ + 4σ) - (μ - 4σ)) / xsteps : μ + 4σ ...]
36     y = pdf.(n, X)
37
38     show(io, lineplot(X, y, xlabel="X", ylabel="p", title="Normal Model
39     ↪ $(m.trajectory).$(m.i), log_Li $(m.log_Li)"))
40     println()
41     println("θ: $(m.θ)")
42     println("v: $(m.v)")
43 end

```

16.4.15 /src/utilities/coordinate_utils.jl

```

1 function to_prior(pos, prior)
2     return quantile(prior, .5+.5*pos)
3 end
4
5 function to_unit_ball(pos, prior)
6     return (cdf(prior, pos) - .5) / .5
7 end
8

```

```

9
10 function box_bound!(pos,box)
11     if !(all(box[:,1].<pos.<box[:,2]))
12         pos[pos.<box[:,1]]*=box[:,1][pos.<box[:,1]]
13         pos[pos.>box[:,2]]*=box[:,2][pos.>box[:,2]]
14     end
15 end

```

16.4.16 /src/utilities/ellipsoid.jl

```

1 # Represents an N-dimensional ellipsoid
2 struct Ellipsoid
3     ctr::Vector{Float64} # center coordinates
4     cov::Array{Float64, 2}
5     icov::Array{Float64, 2} # inverse of cov
6     vol::Float64
7 end
8
9 # Draw a random point from within a unit N-ball
10 function randnball(ndim)
11     z = randn(ndim)
12     r2 = 0.
13     for i=1:ndim
14         r2 += z[i]*z[i]
15     end
16     factor = rand()^(1. /ndim) / sqrt(r2)
17     for i=1:ndim
18         z[i] *= factor
19     end
20     return z
21 end
22
23 # proportionality constant depending on dimension
24 # for n even:      (2pi)^(n /2) / (2 * 4 * ... * n)
25 # for n odd : 2 * (2pi)^((n-1)/2) / (1 * 3 * ... * n)
26 function nbball_vol_factor(ndim::Int)
27     if ndim % 2 == 0
28         c = 1.
29         for i=2:2:ndim
30             c *= 2pi / i
31         end
32     return c

```

```
33     else
34         c = 2.
35         for i = 3:2:ndim
36             c *= 2pi / i
37         end
38         return c
39     end
40 end
41
42 function ellipsoid_volume(scaled_cov::Matrix{Float64})
43     ndim = size(scaled_cov, 1)
44     return nbball_vol_factor(ndim) * sqrt(det(scaled_cov))
45 end
46
47 # find the bounding ellipsoid of points x where
48 function bounding_ellipsoid(x::Matrix{Float64}, enlarge=1.0)
49
50     ndim, npoints = size(x)
51
52     ctr = mean(x, dims=2)[:, 1]
53     delta = x .- ctr
54     cov = unscaled_covzm(delta, 2)
55     icov = inv(cov)
56
57     # Calculate expansion factor necessary to bound each point.
58     # This finds the maximum of (delta_i' * icov * delta_i)
59     fmax = -Inf
60     for k in 1:npoints
61         f = 0.0
62         for j=1:ndim
63             for i=1:ndim
64                 f += icov[i, j] * delta[i, k] * delta[j, k]
65             end
66         end
67         fmax = max(fmax, f)
68     end
69
70     fmax *= enlarge
71     cov .=* fmax
72     icov .=* 1. /fmax
73     vol = ellipsoid_volume(cov)
74
75     return Ellipsoid(ctr, cov, icov, vol)
```

```

76 end

77

78 function sample_ellipsoid(ell::Ellipsoid)
79     ndim = length(ell.ctr)

80

81     # Get scaled eigenvectors (in columns): vs[:,i] is the i-th
82     # → eigenvector.
83     f = eigen(ell.cov)
84     v, w = f.vectors, f.values
85     for j=1:ndim
86         tmp = sqrt(abs(w[j]))
87         for i=1:ndim
88             v[i, j] *= tmp
89         end
90     end
91
92     return v*randnball(ndim) + ell.ctr
93 end

```

16.4.17 /src/utilities/ns_progressmeter.jl

```

1 const CONVERGENCE_MEMORY=500

2

3 mutable struct GMC_NS_Progress{T<:Real} <: AbstractProgress
4     interval::T
5     dt::AbstractFloat
6     start_it::Integer
7     counter::Integer
8     triggered::Bool
9     tfirst::AbstractFloat
10    tlast::AbstractFloat
11    tstop::AbstractFloat
12    printed::Bool      # true if we have issued at least one status
13    # → update
14    desc::AbstractString # prefix to the percentage, e.g. "Computing ... "
15    color::Symbol        # default to green
16    output::IO           # output stream into which the progress is
17    # → written
18    numprintedvalues::Integer # num values printed below progress in
19    # → last iteration
20    offset::Integer       # position offset of progress bar
21    # → (default is 0)

```

```

18
19     e::GMC_NS_Ensemble
20     #tuner::GMC_Tuner
21     top_m::GMC_NS_Model
22
23     mean_stp_time::AbstractFloat
24
25     upper_displays::Vector{Vector{Function}}
26     lower_displays::Vector{Vector{Function}}
27     display_rotate_iterates::Int64
28     upperidx::Integer
29     loweridx::Integer
30
31     convergence_history::Vector{Float64}
32     time_history::Vector{Float64}
33
34     function GMC_NS_Progress{T}(e::GMC_NS_Ensemble,
35                                     top_m::GMC_NS_Model,
36                                     #tuner::GMC_Tuner,
37                                     interval::T;
38                                     dt::Real=0.1,
39                                     desc::AbstractString="Nested Sampling::",
40                                     color::Symbol=:green,
41                                     output::IO=stdout,
42                                     offset::Int=0,
43                                     start_it::Int=1,
44                                     upper_displays=[[evidence_display]],
45                                     lower_displays=[[ensemble_display]],
46                                     disp_rot_its=0,
47                                     ) where T
48         tfirst = tlast = time()
49         printed = false
50         new{T}(interval,
51                 dt,
52                 start_it,
53                 start_it,
54                 false,
55                 tfirst,
56                 tlast,
57                 0.,
58                 printed,
59                 desc,
60                 color,

```

```

61         output,
62         0,
63         offset,
64         e,
65         #tuner,
66         top_m,
67         0.,
68         upper_displays,
69         lower_displays,
70         disp_rot_its,
71         1,
72         1,
73         zeros(CONVERGENCE_MEMORY),
74         zeros(CONVERGENCE_MEMORY))
75     end
76 end
77 """
78 """
79 GMC_NS_Progress(e, interval; kwargs...)
80
81 Return a GMC_NS.jl ProgressMeter for the given ensemble, to be updated at
82     'interval'. Important keyword arguments:
83
84     'upper_displays <: AbstractVector{<:AbstractVector{Function}}': define a
85         vector of above-status-line display functions to rotate through
86     'lower_displays <: AbstractVector{<:AbstractVector{Function}}': define a
87         vector of below-status-line display functions to rotate through
88     'disp_rot_its <: Integer': define the number of iterates to rotate to the
89         next upper and lower display function vectors
90
91 """
92
93 function GMC_NS_Progress(e::GMC_NS_Eensemle,
94     #tuner::GMC_Tuner,
95     interval::Real; dt::Real=0.1, desc::AbstractString="GMC-NS :: ",
96     color::Symbol=:green, output::IO=stderr, offset::Integer=0,
97     start_it::Integer=1,
98     upper_displays::AbstractVector{<:AbstractVector{Function}}=Vector{Vector{Fun
99     lower_displays::AbstractVector{<:AbstractVector{Function}}=Vector{Vector{Fun
100    disp_rot_its::Integer=0)
101    top_m = deserialize(e.models[findmax([model.log_Li for model in
102        e.models])[2]].path)
103
104    return GMC_NS_Progress{typeof(interval)}(e, top_m,

```

```

94     # tuner,
95     interval, dt=dt, desc=desc, color=color, output=output,
96     → offset=offset, start_it=start_it, upper_displays=upper_displays,
97     → lower_displays=lower_displays, disp_rot_its=disp_rot_its)
98 end
99
100
101
102 function update!(p::GMC_NS_Progress, val, thresh; options ... )
103     p.counter += 1
104
105     p.tstp=time()-p.tlast
106     popfirst!(p.time_history)
107     push!(p.time_history, p.tstp)
108
109     p.interval = val - thresh
110     popfirst!(p.convergence_history)
111     push!(p.convergence_history, p.interval)
112
113     string(p.top_m.trajectory,'.',p.top_m.i) !=
114     → basename(p.e.models[findmax([model.log_Li for model in
115     → p.e.models])[2]].path) &&
116     → (p.top_m=deserialize(p.e.models[findmax([model.log_Li for model
117     → in p.e.models])[2]].path))
118
119     updateProgress!(p; options ... )
120
121 end
122
123 function updateProgress!(p::GMC_NS_Progress; offset::Integer = p.offset,
124     → keep = (offset == 0))
125     p.offset = offset
126     t = time()
127     p.display_rotate_iterates > 0 && p.counter%p.display_rotate_iterates
128     → = 0 && rotate_displays(p)
129
130     if p.interval ≤ 0 && !p.triggered
131         p.triggered = true
132         if p.printed
133             p.triggered = true
134             dur = durationstring(t-p.tfirst)
135             msg = @sprintf "%s Converged. Time: %s (%d iterations). logZ:
136             → %s\n" p.desc dur p.counter p.e.log_Zi[end]
137
138         print(p.output, "\n" ^ (p.offset + p.numprintedvalues))

```

```

128     move_cursor_up_while_clearing_lines(p.output,
129         ↵ p.numprintedvalues)
130     length(p.upper_displays)>0 ? (upper_lines=display_dash(p,
131         ↵ p.upper_displays[p.upperidx])) : (upper_lines=0)
132     printover(p.output, msg, :magenta)
133     length(p.lower_displays)>0 ? (lower_lines=display_dash(p,
134         ↵ p.lower_displays[p.loweridx])) : (lower_lines=0)
135
136     p.numprintedvalues=upper_lines + lower_lines + 1
137
138     if keep
139         println(p.output)
140     else
141         print(p.output, "\r\u1b[A" ^ (p.offset +
142             ↵ p.numprintedvalues))
143     end
144 end
145 return
146 end
147
148 if t > p.tlast+p.dt && !p.triggered
149     p.counter < CONVERGENCE_MEMORY ?
150         ↵ mean_step_time=mean(p.time_history[end-(p.counter-1):end]) :
151
152             ↵ mean_step_time=mean(p.time_history)
153
154     msg = @sprintf "%s Iterate: %s Recent step time μ: %s Convergence
155         ↵ Interval: %g\n" p.desc p.counter hmss(mean_step_time)
156         ↵ p.interval
157
158     print(p.output, "\n" ^ (p.offset + p.numprintedvalues))
159     move_cursor_up_while_clearing_lines(p.output, p.numprintedvalues)
160     length(p.upper_displays)>0 ? (upper_lines=display_dash(p,
161         ↵ p.upper_displays[p.upperidx])) : (upper_lines=0)
162     printover(p.output, msg, p.color)
163     length(p.lower_displays)>0 ? (lower_lines=display_dash(p,
164         ↵ p.lower_displays[p.loweridx])) : (lower_lines=0)
165
166     p.numprintedvalues=upper_lines + lower_lines + 1
167     print(p.output, "\r\u1b[A" ^ (p.offset + p.numprintedvalues))
168
169 # Compensate for any overhead of printing. This can be
170 # especially important if you're running over a slow network
171 # connection.

```

```

161         p.tlast = t + 2*(time()-t)
162         p.printed = true
163     end
164 end
165     function rotate_displays(p)
166         p.upperidx < length(p.upper_displays) ? p.upperidx+=1
167             ↪ : p.upperidx=1
168         p.loweridx < length(p.lower_displays) ? p.loweridx+=1
169             ↪ : p.loweridx=1
170     end
171
172     function hmss(dt)
173         dt<0 ? (dt=-dt; prfx="-") : (prfx="")
174         isnan(dt) && return "NaN"
175         (h,r) = divrem(dt,60*60)
176         (m,r) = divrem(r, 60)
177         (isnan(h)||isnan(m)||isnan(r)) && return "NaN"
178         string(prfx,Int(h),":",Int(m),":",Int(ceil(r)))
179     end
180
181     function display_dash(p::GMC_NS_Progress,
182         ↪ funcvec::Vector{Function})
183         lines=0
184         for func in funcvec
185             lines+=func(p)
186         end
187         return lines
188     end

```

16.4.18 /src/utilities/progress_displays.jl

```

1 function tuning_display(p)
2     lines=show(p.output, p.tuner, progress=true)
3     println();
4     return lines
5 end
6
7 function convergence_display(p)
8     try

```

```
9      ↵ ciplot=lineplot([p.counter-(length(p.convergence_history))-1]:p.counter ...
10     ↵ p.convergence_history, title="Convergence Interval Recent ...
11     ↵ History", xlabel="Iterate",ylabel="CI", color=:yellow)
12     lines=nrows(ciplot.graphics)+5
13     show(p.output, ciplot); println()
14     return lines
15   catch
16     printstyled(p.output, "CONVERGENCE PLOT UNAVAILABLE. STANDBY\n",
17     ↵ bold=true, color=:yellow); println()
18     return 2
19   end
20 end
21
22 function evidence_display(p)
23   try
24     evplot=lineplot(p.e.log_Zi[2:end], title="Evidence History",
25     ↵ xlabel="Iterate", color=:red, name="Ensemble logZ")
26     lines=nrows(evplot.graphics)+5
27     show(p.output, evplot); println()
28     return lines
29   catch
30     printstyled(p.output, "EVIDENCE PLOT UNAVAILABLE. STANDBY\n",
31     ↵ bold=true, color=:red); println()
32     return 2
33   end
34 end
35
36 function info_display(p)
37   try
38     infoplot=lineplot(p.e.Hi[2:end], title="Information History",
39     ↵ xlabel="Iterate", color=:green, name="Ensemble H")
40     lines=nrows(infoplot.graphics)+5
41     show(p.output, infoplot); println()
42     return lines
43   catch
44     printstyled(p.output, "INFORMATION PLOT UNAVAILABLE. STANDBY\n",
45     ↵ bold=true, color=:green); println()
46     return 2
47   end
48 end
49
50 function lh_display(p)
```

```

44     try
45         lhplot=lineplot(p.e.log_Li[2:end], title="Contour History",
46             → xlabel="Iterate", color=:magenta, name="Ensemble logLH")
47         lines=nrows(lhplot.graphics)+5
48         show(p.output, lhplot); println()
49         return lines
50     catch
51         printstyled(p.output, "CONTOUR HISTORY UNAVAILABLE. STANDBY\n",
52             → bold=true, color=:magenta); println()
53     end
54 end
55
56 function liwi_display(p)
57     try
58         liwiplot=lineplot([max(2,p.counter-(CONVERGENCE_MEMORY-1)):p.counter ... ],
59             → title="Recent iterate evidentiary weight", xlabel="Iterate",
60             → name="Ensemble log Liwi", color=:cyan)
61         lines=nrows(liwiplot.graphics)+5
62         show(p.output, liwiplot); println()
63         return lines
64     catch
65         printstyled(p.output, "EVIDENTIARY HISTORY UNAVAILABLE.
66             → STANDBY\n", bold=true, color=:cyan); println()
67     end
68 end
69
70 function ensemble_display(p)
71     return lines=show(p.output, p.e, progress=true)
72 end
73
74 function model_display(p)
75     try
76         println("Current MAP model:")
77         return lines=show(p.output, p.top_m, progress=true)
78     catch
79         printstyled(p.output, "MODEL DISPLAY UNAVAILABLE\n", bold=true,
80             → color=:blue); println()
81     end
82 end
83
84 end

```

```

80
81 function model_obs_display(p)
82     try
83         println("Current MAP model")
84         return lines=show(p.output, p.top_m, p.e, progress=true)
85     catch
86         printstyled(p.output, "MODEL DISPLAY UNAVAILABLE\n", bold=true,
87                     color=:blue); println()
88         return 3
89     end
90 end

```

16.4.19 /src/utilities/stats.jl

```

1 function get_lognormal_params(desired_μ, desired_σ)
2     μ²=desired_μ^2
3     σ²=desired_σ^2
4     ln_μ=log(μ²/sqrt(μ²+σ²))
5     ln_σ=sqrt(log(1+(σ²/μ²)))
6     return ln_μ, ln_σ
7 end
8
9 function marginals(d::NormalInverseGamma)
10    → m_T=MarginalTDist(d.mu,sqrt((d.shape*inv(d.v0))/d.scale),TDist(2*d.shape))
11    m_Ga=InverseGamma(d.shape,d.scale)
12    return m_T,m_Ga
13 end
14
15 function marginals(d::NormalGamma)
16    m_T=MarginalTDist(d.mu,sqrt(d.rate/(d.shape*d.nu)),TDist(2*d.shape))
17    m_Ga=Gamma(d.shape,1/d.rate)
18    return m_T,m_Ga
19 end

```

16.4.20 /src/utilities/t_Tuner.jl

```

1 abstract type GMC_Tuner end
2
3 mutable struct τ_Tuner <: GMC_Tuner
4     τ::Float64

```

```

5     τ_history::Vector{Float64}
6     α::Float64
7     β::Float64
8     a::BitVector
9     memory::Integer
10
11    function τ_Tuner(τ1, e::GMC_NS_Ensemble)
12        new(τ1,
13             zeros(),
14             e.GMC_tune_α,
15             e.GMC_tune_β,
16             falses(),
17             e.GMC_tune_μ)
18    end
19 end
20
21 mutable struct τ_PID <: GMC_Tuner
22     τ::Float64
23     τ_history::Vector{Float64}
24     α::Float64
25     β::Float64
26
27     a::BitVector
28     a_history::BitVector
29     ?: Vector{Float64}
30     memory::Integer
31     Kp::Float64
32     Ki::Float64
33     Kd::Float64
34
35     ε::Float64
36     pterm::Float64
37     itemr::Float64
38     dterm::Float64
39
40    function τ_PID(e::GMC_NS_Ensemble)
41        new(e.GMC_init_τ,
42             zeros(),
43             e.GMC_tune_α,
44             1.,
45             falses(),
46             falses(),
47             Vector{Float64}(),
```

```

48         e.GMC_tune_μ,
49         e.GMC_tune_PID ... ,
50         0.,0.,0.,0.)
51     end
52 end
53
54 function process_report!(t::τ_PID, report::Bool)
55     push!(t.a_history, report)
56     length(t.a_history)>t.memory ? (t.a=t.a_history[end-t.memory:end]) :
57         (t.a=t.a_history)
58     push!(t.[],mean(t.a))
59     τ_update!(t)
60 end
61
62 function τ_update!(t::τ_PID)
63     push!(t.τ_history,t.τ)
64
65     t.ε=t.[][]
66     t.pterm=t.Kp * t.ε
67     !(t.τ==eps()) && (t.iterm+=(t.Ki * t.ε))
68
69     length(t.[]) > Int64(round(.1*t.memory)) ? (d =
70         t.[][]
71         - t.[])
72         : #d is positive
73         if acceptance is declining- in this case want smaller beta and
74         tau
75         d=0.
76     t.dterm=t.Kd * d
77
78     t.β=max(eps(), 1. + t.pterm + t.iterm - t.dterm)
79     t.τ=max(eps(), t.τ * t.β)
80 end
81
82
83
84 function init_tune(e::GMC_NS_Engine)
85     @info "Performing initial GMC timestep tuning on ensemble ... "
86     τ=1.; tuned=false; τd=1.1; accept=1.; it=1
87     while !tuned && it<e.GMC_tune_ι
88         test_report=false(0)
89         println(stdout, "τ: $τ, It: $it, Accept: $accept")
90
91         while
92             length(test_report)<Int64(floor(length(e.models)*e.GMC_tune_ρ))
93             m=deserialize(rand([m.path for m in e.models]))

```

```

86         candidate=galilean_trajectory_sample!(m,e,τ)
87
88         candidate.id==m.id ? push!(test_report,0) :
89             ↪ push!(test_report,1)
90         candidate=0
91         GC.gc()
92
93     last_accept=accept
94     accept=sum(test_report)/length(test_report)
95
96     if accept == 1.
97         τ*=τd
98         last_accept == 1. && (τd*=1+e.GMC_tune_β)
99         last_accept < e.GMC_tune_α &&
100            ↪ (τd=min(1.000001,1-e.GMC_tune_β*τd))
101     elseif accept < e.GMC_tune_α
102         τ/=τd
103         last_accept == 1. && (τd=min(1.000001,1-e.GMC_tune_β*τd))
104         last_accept < e.GMC_tune_α && (τd*=1+e.GMC_tune_β)
105     else
106         return τ
107     end
108
109     it+=1
110     move_cursor_up_while_clearing_lines(stdout, 1)
111   end
112   println("Done. τ=$τ")
113   return τ
114 end
115 function tune_τ!(t::τ_Tuner, step_report::BitVector)
116   push!(t.τ_history,t.τ)
117   t.a=vcat(t.a,step_report)
118   ls=length(t.a)
119   ls>t.memory && (t.a=t.a[ls-t.memory:end])
120
121   accept=sum(t.a)/ls
122   if accept==1.
123       t.τ=t.τ*(1+t.β)
124   elseif accept<t.α
125       t.τ=t.τ*(1-t.β)
126   end

```

```

127 end

128

129 function Base.show(io::IO, t::τ_PID; progress=false)
130     try
131         plot=lineplot(t.[], title="Recent Unobstructed Moves",
132                     xlabel="Proposals", ylabel="Rate", color=:white, name="Free
133                     moves")
134
135         lbls=plot.labels_left
136         (arrow="")
137         all(t.α .> [v for v in parse.(Float64,values(lbls))]) &&
138             (arrow="↑")
139         all(t.α .< [v for v in parse.(Float64,values(lbls))]) &&
140             (arrow="↓")
141
142         lineplot!(plot, [t.α for i in 1:length(t.[])], name="α setpoint
143                     *arrow, color=:red")
144
145         show(io, plot)
146         println()
147         printstyled("τ:$(t.τ), α:$(t.α), β:$(t.β)", bold=true)
148         println()
149         printstyled("Kp:$(t.Kp), Ki:$(t.Ki), Kd:$(t.Kd)", color=:green)
150         println()
151         printstyled("ptrm:$(t.pterm), itrm:$(t.iterm),
152                     dterm:$(t.dterm)", color=:magenta)
153         println()
154
155
156     function get_tuner_dict(e)
157         d=Dict{Int64,τ_PID}()
158         for rec in e.models
159             d[rec.trajectory]=τ_PID(e)
160         end
161         return d
162     end

```

16.4.21 /test/ensemble_tests.jl

```

1 @testset "GMC_NS_Ensemble tests ..." begin
2     sample_dist=Normal(100.,5.)
3     samples=rand(sample_dist, 10000)
4
5     n_chains=10
6
7     max_mu=1000
8     min_mu=eps()
9     max_lambda=100
10    min_lambda=1e-4
11
12    ln_max_mu=log(max_mu^2/sqrt(max_mu^2 + inv(max_lambda)))
13    ln_min_mu=log(min_mu^2/sqrt(min_mu^2 + inv(min_lambda)))
14    ln_max_lambda=1/log(1+(inv(max_lambda)/max_mu^2))
15    ln_min_lambda=1/log(1+(inv(min_lambda)/min_mu^2))
16
17    n_priors=[Uniform(min_mu,max_mu),Uniform(min_lambda,max_lambda)]
18    n_box=[min_mu max_mu;min_lambda max_lambda]
19    ln_priors=[Uniform(ln_min_mu,ln_max_mu),Uniform(ln_min_lambda,ln_max_lambda)]
20    ln_box=[ln_min_mu ln_max_mu;ln_min_lambda ln_max_lambda]
21
22    gmc=GMC_DEFAULTS
23    gmc[1]=3
24    gmc[2]=eps()
25    gmc[end]=1e4
26
27    ne=Normal_Ensemble("NE_test", n_chains, samples, n_priors, n_box,
28        → gmc ... )
28    lne=LogNormal_Ensemble("LNE_test", n_chains, samples, ln_priors,
29        → ln_box, gmc ... )
29
30    uds=Vector{Vector{Function}}(){}
31
32    lds=Vector{Vector{Function}}(){}
33
34    ne_ev = converge_ensemble!(ne,backup=(true,100),upper_displays=uds,
35        → lower_displays=lds)
35    lne_ev = converge_ensemble!(lne,backup=(true,100),upper_displays=uds,
36        → lower_displays=lds)
36
37    @test ne_ev > lne_ev

```

```

38
39     for e in [ne, lne]
40         @info "Testing detailed balance at $(e.path) ... "
41
42         @test issorted(e.log_Li)
43         @test all(e.log_Li.<0.)
44         @test issorted(e.log_Xi,rev=true)
45         @test all(e.log_Xi.≤0.)
46         @test all(e.log_wi.<0.)
47         @test all(e.log_Lawi.<0.)
48         @test all(e.log_Zi.<0.)

49
50     Nvec=Vector{Int64}()
51     for i in 2:length(e.log_Li)
52         Xi=e.log_Xi[i]
53         N=round(-(i-1)/Xi)
54         push!(Nvec, N)
55     end

56
57     @test all(e.GMC_Nmin.≤Nvec.≤n_chains)

58
59     for chain in 1:e.t_counter-1
60         ch_pfx=e.path*'*string(chain)*'.'
61         total_spls=0
62         while isfile(ch_pfx*string(total_spls+1))
63             total_spls+=1
64         end

65
66         lastm=deserialize(ch_pfx*"1")

67
68         for spl in 2:total_spls
69             splm=deserialize(ch_pfx*string(spl))

70
71             @test !(splm.θ!=lastm.θ && splm.v!=lastm.v)
72             @test !(splm.θ==lastm.θ && splm.v==lastm.v)
73             if splm.θ==lastm.θ
74                 @test splm.v!=lastm.v
75                 @test splm.log_Li==lastm.log_Li
76             elseif splm.v==lastm.v
77                 @test splm.θ!=lastm.θ
78                 @test splm.log_Li ≥ lastm.log_Li
79             else
80                 @error "Galilean trajectory generation is broken!"

```

```

81         end
82         lastm=splm
83     end
84 end
85
86 rm("NE_test", recursive=true)
87 rm("LNE_test", recursive=true)
88 end
89
90

```

16.4.22 /test/galilean_unit_tests.jl

```

1 @testset "Galilean trajectory unit tests ..." begin
2     #test box movement
3     pos=[.5,.5]
4     box=[0 1.
5         0 1.]
6     allow_d=[.1,.1]
7     box_d=[1.,1.]
8
9     allowed_pos, allowed_d=box_move(pos,allow_d,box)
10    @test allowed_pos==pos+allow_d
11    @test allowed_d == allow_d
12
13    boxed_pos, boxed_d=box_move(pos, box_d, box)
14    @test boxed_pos=[1.,1.]
15    @test boxed_d=[.5,.5]
16
17    box_reflect_v=box_reflect([.5,.9999999],box,[.1,.1])
18    @test box_reflect_v=[.1,-.1]
19
20    #test boundary norm finding
21    norm=1000
22    v=[.5,.5]
23    bn=boundary_norm(v, norm)
24    @test bn == normalize(norm./v)
25
26    #test reflection given norm
27    rv=reflect(v,bn,0.)
28    @test isapprox(rv, -v)
29

```

```

30      #test reflection perturbation
31      pv=r_perturb(v,.01)
32      @test v!=pv
33 end

```

16.4.23 /test/normal_demo.jl

```

1 using GMC_NS, Distributions, ConjugatePriors
2
3 sample_dist=Normal(100.,5.)
4 samples=rand(sample_dist, 10000)
5
6 prior=NormalGamma(300.,2e-3,1.,10.) #a lousy prior
7
8 box=[0. 1000.
9       1/1000. 1/eps())
10
11 gmc=GMC_DEFAULTS
12 gmc[1]=5
13 gmc[2]=eps()
14
15 e=Normal_Ensemble("NE_test", 10, samples, prior, box, gmc...)
16
17 uds=Vector{Vector{Function}}([[convergence_display],[evidence_display],[info_display]])
18
19 lds=Vector{Vector{Function}}([[model_obs_display]])
20
21 converge_ensemble!(e,backup=(true,100),upper_displays=uds,
22   ↳ lower_displays=lds, disp_rot_its=1000)

```

16.4.24 /test/runtests.jl

```

1 @info "Loading test packages ... "
2 using GMC_NS, Test, Distributions, Serialization
3 import GMC_NS:box_move, box_reflect, boundary_norm, reflect, r_perturb
4 import LinearAlgebra:normalize
5
6 @info "Beginning tests ... "
7 using Random
8 Random.seed!()
9

```

```

10 include("galilean_unit_tests.jl")
11 include("ensemble_tests.jl")
12
13 @info "Tests complete!"

```

16.5 CMZNicheSims

16.5.1 /src/CMZNicheSims.jl

```

1 module CMZNicheSims
2     using Distributions, GMC_NS, UnicodePlots
3     import ProgressMeter:
4         → @showprogress,move_cursor_up_while_clearing_lines
5     import BioBackgroundModels:lps
6     import StatsFuns:logsumexp
7     import Serialization:serialize,deserialize
8
9     include("thymidine_sim/thymidine_cell.jl")
10    include("thymidine_sim/thymidine_model.jl")
11    include("thymidine_sim/thymidine_ensemble.jl")
12    export Thymidine_Engine
13    include("thymidine_sim/thymidine_sim.jl")
14    include("thymidine_sim/thymidine_lh.jl")
15    include("CMZ_sim/CMZ_lh.jl")
16    include("CMZ_sim/CMZ_model.jl")
17    include("CMZ_sim/CMZ_ensemble.jl")
18    export CMZ_Engine
19    include("slice_pop_sim/lens_model.jl")
20    export Lens_Model
21    include("slice_pop_sim/slice_lh.jl")
22    include("slice_pop_sim/slice_model.jl")
23    include("slice_pop_sim/slice_ensemble.jl")
24    export Slice_Engine
25 end # module

```

16.5.2 /src/CMZ_sim/CMZ_ensemble.jl

```

1 mutable struct CMZ_Engine <: GMC_NS_Engine
2     path::String
3
4     model_initλ::Function

```

```

5   models :: Vector{CMZ_Record}
6
7   contour :: Float64
8   log_Li :: Vector{Float64}
9   log_Xi :: Vector{Float64}
10  log_wi :: Vector{Float64}
11  log_Liwi :: Vector{Float64}
12  log_Zi :: Vector{Float64}
13  Hi :: Vector{Float64}
14
15
16  → obs :: AbstractVector{<: Tuple{<: AbstractVector{<: Float64}, <: AbstractVector{<: Fl}}
17  priors :: Vector{<: Distribution}
18  constants :: Vector{<: Any}
19  #T, popdist, voldist, mc_its, phs
20  box :: Matrix{Float64}
21
22  sample_posterior :: Bool
23  posterior_samples :: Vector{CMZ_Record}
24
25  GMC_Nmin :: Int64
26
27  GMC_τ_death :: Float64
28  GMC_init_τ :: Float64
29  GMC_tune_μ :: Int64
30  GMC_tune_α :: Float64
31  GMC_tune_PID :: NTuple{3, Float64}
32
33  GMC_timestep_η :: Float64
34  GMC_reflect_η :: Float64
35  GMC_exhaust_σ :: Float64
36  GMC_chain_K :: Int64
37
38  t_counter :: Int64
39
40 CMZ_Engine(path :: String, no_models :: Integer,
41  → obs :: AbstractVector{<: Tuple{<: AbstractVector{<: Float64}, <: AbstractVector{<: Fl}
42  → priors :: AbstractVector{<: Distribution}, constants, box, GMC_settings;
43  → sample_posterior :: Bool = true) =
44 CMZ_Engine(
45     path,
46     construct_CMZ,

```

```

44     assemble_CMs(path, no_models, obs, priors, constants, box) ... ,
45     [-Inf], #L0 = 0
46         [0], #ie exp(0) = all of the prior is covered
47         [-Inf], #w0 = 0
48         [-Inf], #Liwi0 = 0
49         [-1e300], #Z0 = 0
50         [0], #H0 = 0,
51     obs,
52     priors,
53     constants, #T, popdist, voldist, mc_its, phs
54     box,
55     sample_posterior,
56     Vector{CMZ_Record}(),
57     GMC_settings ... ,
58     no_models+1)
59
60 function assemble_CMs(path::String, no_trajectories::Integer, obs,
61      $\leftarrow$  priors, constants, box)
62     ensemble_records = Vector{CMZ_Record}()
63     !isdir(path)  $\&\&$  mkpath(path)
64     phs=constants[6]
65     @showprogress 1 "Assembling CMZ_Model ensemble..." for trajectory_no
66      $\leftarrow$  in 1:no_trajectories
67     model_path = string(path,'/',trajectory_no,'.',1)
68     if !isfile(model_path)
69     proposal=rand.(priors)
70
71      $\leftarrow$  proposal[2*phs+1:(2*phs+1)+(phs-2)]=sort(proposal[2*phs+1:(2*phs+1)+(
72
73     pos=to_unit_ball.(proposal,priors)
74     box_bound!(pos,box)
75     θvec=to_prior.(pos,priors)
76
77     model = construct_CMZ(trajectory_no, 1, θvec, pos, [0.], obs,
78      $\leftarrow$  constants ... ; v_init=true)
79
80     serialize(model_path, model) #save the model to the ensemble
81      $\leftarrow$  directory
82     push!(ensemble_records, CMZ_Record(trajectory_no, 1,
83      $\leftarrow$  pos,model_path,model.log_Li))
84     else #interrupted assembly pick up from where we left off
85     model = deserialize(model_path)

```

```

80         push!(ensemble_records, CMZ_Record(trajectory_no, 1,
81             → model.pos,model_path,model.log_Li))
82     end
83   end
84
85   return ensemble_records, minimum([record.log_Li for record in
86             → ensemble_records])
87 end
88
89 function Base.show(io::IO, m::CMZ_Model, e::CMZ_Engagement; progress=false)
90     T=e.constants[1]
91
92     catpobs=vcat([e.obs[t][1] for t in 1:length(T)]...)
93     ymax=max(maximum(m.disp_mat[:, :, 1]),maximum(catpobs))
94     ymin=min(minimum(m.disp_mat[:, :, 1]),minimum(catpobs))
95
96     plt=lineplot(T,m.disp_mat[:, 2, 1],title="CMZ_Model
97             → $(m.trajectory).$(m.i), log_Li $(m.log_Li)",color=:green,name="μ
98             → pop", ylim=[ymin,ymax])
99     lineplot!(plt,T,m.disp_mat[:, 1, 1],color=:magenta,name="95% CI")
100    lineplot!(plt,T,m.disp_mat[:, 3, 1],color=:magenta)
101
102    Ts=vcat([[T[n] for i in 1:length(e.obs[n][1])] for n in
103             → 1:length(T)]...)
104    scatterplot!(plt,Ts, catpobs, color=:yellow, name="Obs")
105
106    catvobs=vcat([e.obs[t][2] for t in 1:length(T)]...)
107    ymax=max(maximum(m.disp_mat[:, :, 1]),maximum(catvobs))
108    ymin=min(minimum(m.disp_mat[:, :, 1]),minimum(catvobs))
109
110    plt=lineplot(T,m.disp_mat[:, 2, 2],color=:blue,name="μ vol",
111             → ylim=[ymin,ymax])
112    lineplot!(plt,T,m.disp_mat[:, 1, 2],color=:yellow,name="95% CI")
113    lineplot!(plt,T,m.disp_mat[:, 3, 2],color=:yellow)
114
115    Ts=vcat([[T[n] for i in 1:length(e.obs[n][2])] for n in
116             → 1:length(T)]...)
117    scatterplot!(plt,Ts, catvobs, color=:yellow, name="Obs")

```

```

116     show(io, plt)

117
118     println()
119     println("θ: $(m.θ)")
120     println("v: $(m.v)")

121
122     (progress && return nrows(plt.graphics)+27);
123 end

```

16.5.3 /src/CMZ_sim/CMZ_lh.jl

```

1 const MAXVAL=prevfloat(Inf)

2
3 function CMZ_mc_llh(popdist::Distribution, voldist::Distribution,
4   ← volconst::Float64, phase_ends::Vector{Float64},
4   ← pparams::Vector{Float64}, mc_its::Int64, T::Vector{<:AbstractFloat},
4   ← obs::Vector{<:Tuple{<:AbstractVector{<:Float64},<:AbstractVector{<:Float64}}})
4   times=length(T)
5   pends=floor.(phase_ends)
6   events=Int64.(sort(unique(vcat(T,pends))))
7   results=zeros(mc_its,length(events),2)

8
9   nt=Threads.nthreads()
10  Threads.@threads for t in 1:nt
11    t=nt ? (idxs=1+(t-1)*Int(floor(mc_its/nt)):mc_its) :
11      (idxs=(1+(t-1)*Int(floor(mc_its/nt))):t*Int(floor(mc_its/nt)))

12
13    phase=1
14    cycle_time,exit_rate=pparams[1+((phase-1)*2):2+((phase-1)*2)]
15
16    results[idxs,1,1]=rand.(popdist)
17    results[idxs,1,2]=rand.(voldist)

18
19    last_ph_ch=1;
20    next_event=2;
21    while next_event ≤ length(events)
22      n=events[next_event]-events[last_ph_ch]

23
24    pop_factor=max(eps(),(2^(24/cycle_time)-exit_rate))
25    pop_factor=1. && (pop_factor=pop_factor+eps())
26
27      ← vol_factor=volconst*exit_rate*(1-pop_factor^(n-1))/(1-pop_factor)

```

```

27
28     lastpops=view(results,idxs,last_ph_ch,1)
29     lastvols=view(results,idxs,last_ph_ch,2)
30
31
32         ↵ results[idxs,next_event,1]:=max.(min.(lastpops.*pop_factor^n,MAXVAL),
33
34         ↵ results[idxs,next_event,2]:=min.(lastvols.+(lastpops.*vol_factor),MAXVAL)
35
36         if events[next_event] in pends #after updating pop and vol to
37             ↵ t, update phase parameters for next event, make t_0 vol_0
38             ↵ and pop_0 the phase change vals
39             phase+=1; last_ph_ch=next_event
40             phase ≤ length(pparams)/2 &&
41                 ↵ ((cycle_time,exit_rate)=pparams[1+((phase-1)*2):2+((phase-1)*2)])
42         end #advance phase if necessary
43
44         next_event+=1
45     end
46 end
47
48 tidxs=[findfirst(t→t=time,events) for time in T]
49
50 pop_lns=Vector{LogNormal}(undef,times)
51 vol_lns=Vector{LogNormal}(undef,times)
52 pop_lns[1]=popdist
53 vol_lns[1]=voldist
54
55 Threads.@threads for t in 2:times
56     pop_lns[t]=fit(LogNormal,results[:,tidxs[t],1])
57     vol_lns[t]=fit(LogNormal,results[:,tidxs[t],2])
58 end
59
60 pop_lhs=Vector{Float64}(undef,times-1)
61 vol_lhs=Vector{Float64}(undef,times-1)
62
63 Threads.@threads for t in 1:times-1
64     pop_lhs[t]=lps(logpdf(pop_lns[t+1],obs[t+1][1]))
65     vol_lhs[t]=lps(logpdf(vol_lns[t+1],obs[t+1][2]))
66 end
67
68 log_lh=lps(lps(pop_lhs),lps(vol_lhs))

```

```

65 disp_mat=zeros(times,3,2)
66 Threads.@threads for t in 1:times
67
68     → disp_mat[t,:,:1]=[quantile(pop_lns[t],.025),mean(pop_lns[t]),quantile(pop_lns[t],.975)]
69     → disp_mat[t,:,:2]=[quantile(vol_lns[t],.025),mean(vol_lns[t]),quantile(vol_lns[t],.975)]
70 end
71 return log_lh, disp_mat
72 end

```

16.5.4 /src/CMZ_sim/CMZ_model.jl

```

25     log_lh,disp_mat=CMZ_mc_llh(popdist, voldist, volconst, phase_ends,
26     ↳ pparams, mc_its, T, obs)
27
28     v_init && (v=rand(MvNormal(length(θ),1.)))
29
30     CMZ_Model(trajectory, i, θ, log_lh, pos, v, disp_mat)
31 end

```

16.5.5 /src/slice_pop_sim/lens_model.jl

```

1 struct Lens_Model
2     factor::Float64
3     power::Float64
4     section::Float64
5 end
6
7 function circumferential_exit(lm,t1,t2,pops)
8     t1c=lm.factor*(t1^lm.power)
9     sliceno=t1c/lm.section
10
11    t2c=lm.factor*(t2^lm.power)
12    nc=t2c-t1c
13    psg=nc/sliceno
14    return (psg/lm.section).*pops
15 end
16
17 function Base.length(lm::Lens_Model)
18     return 1
19 end

```

16.5.6 /src/slice_pop_sim/slice_ensemble.jl

```

1 mutable struct Slice_Ensemble <: GMC_NS_Ensemble
2     path::String
3
4     model_initλ::Function
5     models::Vector{Slice_Record}
6
7     contour::Float64
8     log_Li::Vector{Float64}
9     log_Xi::Vector{Float64}

```

```

10    log_wi::Vector{Float64}
11    log_Liwi::Vector{Float64}
12    log_Zi::Vector{Float64}
13    Hi::Vector{Float64}

14
15    obs::AbstractVector{<:AbstractVector{<:Float64}}}
16    priors::Vector{<:Distribution}
17    constants::Vector{<:Any}
18    #T, popdist, lens_model, mc_its, phs
19    box::Matrix{Float64}

20
21    sample_posterior::Bool
22    posterior_samples::Vector{Slice_Record}

23
24    GMC_Nmin::Int64

25
26    GMC_tau_death::Float64
27    GMC_init_tau::Float64
28    GMC_tune_mu::Int64
29    GMC_tune_alpha::Float64
30    GMC_tune_PID::NTuple{3,Float64}

31
32    GMC_timestep_eta::Float64
33    GMC_reflect_eta::Float64
34    GMC_exhaust_sigma::Float64
35    GMC_chain_K::Int64

36
37    t_counter::Int64

38 end

39
40 Slice_Engine(path::String, no_models::Integer,
   ↳ obs::AbstractVector{<:AbstractVector{<:Float64}}},
   ↳ priors::AbstractVector{<:Distribution}, constants, box, GMC_settings;
   ↳ sample_posterior::Bool=true) =
41 Slice_Engine(
42     path,
43     construct_slice,
44     assemble_SMS(path, no_models, obs, priors, constants, box)...,
45     [-Inf], #L0 = 0
46     [0], #ie exp(0) = all of the prior is covered
47     [-Inf], #w0 = 0
48     [-Inf], #Liwi0 = 0
49     [-1e300], #Z0 = 0

```

```

50     [0], #H0 = 0,
51     obs,
52     priors,
53     constants, #T, popdist, lens_model, mc_its, phs
54     box,
55     sample_posterior,
56     Vector{Slice_Record}(),
57     GMC_settings ... ,
58     no_models+1)

59
60 function assemble_SMS(path::String, no_trajectories::Integer, obs,
61   ↳ priors, constants, box)
61   ensemble_records = Vector{Slice_Record}()
62   !isdir(path) && mkpath(path)
63   phs=constants[5]
64   @showprogress 1 "Assembling Slice_Model ensemble..." for
65     ↳ trajectory_no in 1:no_trajectories
66     model_path = string(path,'/',trajectory_no,'.',1)
67     if !isfile(model_path)
68       proposal=rand.(priors)

69
70     ↳ proposal[2*phs+1:(2*phs+1)+(phs-2)]=sort(proposal[2*phs+1:(2*phs+1)+(
71
72       pos=to_unit_ball.(proposal,priors)
73       box_bound!(pos,box)
74       θvec=to_prior.(pos,priors)

75
76       model = construct_slice(trajectory_no, 1, θvec, pos, [0.],
77         ↳ obs, constants ... ; v_init=true)

78       serialize(model_path, model) #save the model to the ensemble
79         ↳ directory
80       push!(ensemble_records, Slice_Record(trajectory_no, 1,
81         ↳ pos,model_path,model.log_Li))
82     else #interrupted assembly pick up from where we left off
83       model = deserialize(model_path)
84       push!(ensemble_records, Slice_Record(trajectory_no, 1,
85         ↳ model.pos,model_path,model.log_Li))
86     end
87   end

88   return ensemble_records, minimum([record.log_Li for record in
89     ↳ ensemble_records])

```

```

85 end
86
87
88 function Base.show(io::IO, m::Slice_Model, e::Slice_Ensemble;
89   ← progress=false)
90   T=e.constants[1]
91
92   catpobs=vcat([e.obs[t] for t in 1:length(T)] ... )
93   ymax=max(maximum(m.disp_mat[:, :]), maximum(catpobs))
94   ymin=min(minimum(m.disp_mat[:, :]), minimum(catpobs))
95
96   plt=lineplot(T,m.disp_mat[:,2],title="Slice_Model
97   ← $(m.trajectory).$(m.i), log_Li $(m.log_Li)",color=:green,name="μ
98   ← pop", ylim=[ymin,ymax])
99   lineplot!(plt,T,m.disp_mat[:,1],color=:magenta,name="95% CI")
100  lineplot!(plt,T,m.disp_mat[:,3],color=:magenta)
101
102  Ts=vcat([[T[n] for i in 1:length(e.obs[n])] for n in 1:length(T)] ... )
103  scatterplot!(plt,Ts, catpobs, color=:yellow, name="Obs")
104
105  show(io, plt)
106  println()
107  println("θ: $(m.θ)")
108  println("v: $(m.v)")

  (progress && return nrows(plt.graphics)+16);
end

```

16.5.7 /src/slice_pop_sim/slice_lh.jl

```

1 const MAXVAL=prevfloat(Inf)
2
3 function slice_mc_llh(popdist::Distribution, lm::Lens_Model,
4   ← phase_ends::Vector{Float64}, pparams::Vector{Float64}, mc_its::Int64,
5   ← T::Vector{Float64}, obs::Vector{Vector{Float64}})
6   times=length(T)
7   pends=floor.(phase_ends)
8   events=Int64.(sort(unique(vcat(T,pends))))
9   results=zeros(mc_its,length(events))
10
11  nt=Threads.nthreads()
12  Threads.@threads for t in 1:nt

```

```

11      t=nt ? (idxs=1+(t-1)*Int(floor(mc_its/nt)):mc_its) :
12      ↵ (idxs=(1+(t-1)*Int(floor(mc_its/nt))):t*Int(floor(mc_its/nt)))
13
14      phase=1
15      cycle_time,exit_rate=pparams[1+((phase-1)*2):2+((phase-1)*2)]
16
17      results[idxs,1,1]:=rand.(popdist)
18
19      next_event=2;
20      while next_event≤length(events)
21          n=events[next_event]-events[next_event-1]
22
23          pop_factor=max(eps(),(2^(24/cycle_time)-exit_rate))
24          pop_factor=1. && (pop_factor=pop_factor+eps())
25
26          lastpops=view(results,idxs,next_event-1)
27
28          results[idxs,next_event]:=min.(lastpops.*pop_factor^n,MAXVAL)
29
30          ↵ results[idxs,next_event]:=max.(view(results,idxs,next_event))-circumf
31
32          if events[next_event] in pends #after updating pop and vol to
33              ↵ t, update phase parameters for next event, make t, vol,
34              ↵ and pop. the phase change vals
35              phase+=1;
36              phase ≤ length(pparams)/2 &&
37                  ((cycle_time,exit_rate)=pparams[1+((phase-1)*2):2+((phase-1)*2)])
38          end #advance phase if necessary
39
40          next_event+=1
41      end
42  end
43
44  tidxs=[findfirst(t→t=time,events) for time in T]
45
46  pop_lns=Vector{LogNormal}(undef,times)
47  pop_lns[1]=popdist
48  Threads.@threads for t in 2:times
49      pop_lns[t]=fit(LogNormal,results[:,tidxs[t]])
50  end
51
52  pop_lhs=Vector{Float64}(undef,times-1)

```

```

49
50     Threads.@threads for t in 1:times-1
51         pop_lhs[t]=lps(logpdf(pop_lns[t+1],obs[t+1]))
52     end
53
54     log_lh=lps(pop_lhs)
55
56     disp_mat=zeros(times,3)
57     Threads.@threads for t in 1:times
58
59         → disp_mat[t,:]=[quantile(pop_lns[t],.025),mean(pop_lns[t]),quantile(pop_lns[t],.975)]
60     end
61
62     return log_lh, disp_mat
63 end

```

16.5.8 /src/slice_pop_sim/slice_model.jl

```

1 mutable struct Slice_Record <: GMC_NS_Model_Record
2     trajectory::Int64
3     i::Int64
4     pos::Vector{Float64}
5     path::String
6     log_Li::Float64
7 end
8
9 struct Slice_Model <: GMC_NS_Model
10    trajectory::Int64
11    i::Int64
12
13    θ::Vector{Float64}
14    log_Li::Float64
15
16    pos::Vector{Float64}
17    v::Vector{Float64}
18
19    disp_mat::Array{Float64} #matrix for mean & 95%CI plot of model
20    → output
21 end

```

```

22 function construct_slice(trajectory::Int64, i::Int64, θ::Vector{Float64},
23   → pos::Vector{Float64}, v::Vector{Float64},
24   → obs::Vector{Vector{Float64}}, T::Vector{Float64},
25   → popdist::Distribution, lens_model::Lens_Model, mc_its::Int64,
26   → phs::Int64; v_init=false)
27   pparams=θ[1:2*phs];phase_ends=θ[2*phs+1:(2*phs+1)+(phs-2)]
28
29   log_lh,disp_mat=slice_mc_llh(popdist, lens_model, phase_ends,
30   → pparams, mc_its, T, obs)
31
32   v_init && (v=rand(MvNormal(length(θ),1.)))
33
34   Slice_Model(trajectory, i, θ, log_lh, pos, v, disp_mat)
35 end

```

16.5.9 /src/thymidine_sim/thymidine_cell.jl

```

1 abstract type AbstractCell end
2
3 mutable struct LabelCell <: AbstractCell
4   time::Float64
5   tδ::Float64
6   s::Float64
7   label::Float64
8 end

```

16.5.10 /src/thymidine_sim/thymidine_ensemble.jl

```

1 mutable struct Thymidine_Engine <: GMC_NS_Engine
2   path::String
3
4   model_initλ::Function
5   models::Vector{Thymidine_Record}
6
7   contour::Float64
8   log_Li::Vector{Float64}
9   log_Xi::Vector{Float64}
10  log_wi::Vector{Float64}
11  log_Liwi::Vector{Float64}
12  log_Zi::Vector{Float64}
13  Hi::Vector{Float64}

```

```

14
15     obs::Vector{Vector{<:Integer}}
16     priors::Vector{<:Distribution}
17     constants::Vector{<:Any}
18     #T, pulse, mc_its, end_time
19     box::Matrix{Float64}

20
21     sample_posterior::Bool
22     posterior_samples::Vector{Thymidine_Record}

23
24     GMC_Nmin::Int64

25
26     GMC_tau_death::Float64
27     GMC_init_tau::Float64
28     GMC_tune_mu::Int64
29     GMC_tune_alpha::Float64
30     GMC_tune_PID::NTuple{3,Float64}

31
32     GMC_timestep_eta::Float64
33     GMC_reflect_eta::Float64
34     GMC_exhaust_sigma::Float64
35     GMC_chain_k::Int64

36
37     t_counter::Int64
38 end
39
40 Thymidine_Engineering(path::String, no_models::Integer,
41   ↳ obs::AbstractVector{<:AbstractVector{<:Integer}}),
42   ↳ priors::AbstractVector{<:Distribution}, constants, box, GMC_settings;
43   ↳ sample_posterior::Bool=true) =
44
45 Thymidine_Engineering(
46     path,
47     thymidine_constructor,
48     assemble_TMs(path, no_models, obs, priors, constants, box) ... ,
49     [-Inf], #L0 = 0
50       [0], #ie exp(0) = all of the prior is covered
51       [-Inf], #w0 = 0
52       [-Inf], #Liwi0 = 0
53       [-1e300], #Z0 = 0
54       [0], #H0 = 0,
55     obs,
56     priors,
57     constants, #T, pulse, mc_its, end_time

```

```

54     box,
55     sample_posterior,
56     Vector{Thymidine_Record}(),
57     GMC_settings ... ,
58     no_models+1)

59
60 function assemble_TMs(path::String, no_trajectories::Integer, obs,
61   → priors, constants, box)
62   ensemble_records = Vector{Thymidine_Record}()
63   !isdir(path) && mkpath(path)
64   @showprogress 1 "Assembling Thymidine_Model ensemble..." for
65   → trajectory_no in 1:no_trajectories
66   model_path = string(path,'/',trajectory_no,'.',1)
67   if !isfile(model_path)
68     proposal=rand.(priors)

69     pos=to_unit_ball.(proposal,priors)
70     box_bound!(pos,box)
71     θvec=to_prior.(pos,priors)

72     model = thymidine_constructor(trajectory_no, 1, θvec, pos,
73   → [0.], obs, constants... ; v_init=true)

74     serialize(model_path, model) #save the model to
75     → the ensemble directory
76     push!(ensemble_records,
77       → Thymidine_Record(trajectory_no, 1,
78       → pos,model_path,model.log_Li))
79   else #interrupted assembly pick up from where we left off
80     model = deserialize(model_path)
81     push!(ensemble_records,
82       → Thymidine_Record(trajectory_no, 1,
83       → model.pos,model_path,model.log_Li))
84   end
85
86   return ensemble_records, minimum([record.log_Li for record in
87   → ensemble_records])
88 end

89
90 function Base.show(io::IO, m::Thymidine_Model, e::Thymidine_Ensemble;
91   → progress=false)
92   T=e.constants[1]

```

```

87
88     catobs=vcat(e.obs ... )
89     ymax=max(maximum(m.disp_mat),maximum(catobs))
90     ymin=min(minimum(m.disp_mat),minimum(catobs))
91
92     plt=lineplot(T,m.disp_mat[:,2],title="Thymidine_Model
93     ↪ $(m.trajectory).$(m.i), log_Li $(m.log_Li)",color=:green,name="μ
94     ↪ count", ylim=[ymin,ymax])
95     lineplot!(plt,T,m.disp_mat[:,1],color=:magenta,name="95% CI")
96     lineplot!(plt,T,m.disp_mat[:,3],color=:magenta)
97
98
99     Ts=vcat([[T[n] for i in 1:length(e.obs[n])] for n in 1:length(T)] ... )
100    scatterplot!(plt,Ts, catobs, color=:yellow, name="Obs")
101
102    show(io, plt)
103    println()
104    println("θ: $(m.θ)")
105
106   function print_posterior(e::Thymidine_Ensemble, path::String=e.path)
107       MLEmod=deserialize(e.models[findmax([m.log_Li for m in
108         ↪ e.models])[2]].path)
109       θ=MLEmod.θ
110       n_pops=length(θ)/8
111       pparams=Vector{Tuple{LogNormal, LogNormal, Float64, Normal,
112         ↪ Float64}}}()
113       for pop in 1:n_pops
114           lpmu, lpσ², r, tcμ, tcσ², sμ, σ², sis_frac=
115           ↪ θ[Int64(1+((pop-1)*8)):Int64(8*pop)]
116           lpσ=sqrt(lpσ²); tcσ=sqrt(tcσ²); σ=sqrt(σ²)
117
118           ↪ push!(pparams,(LogNormal(lpmu,lpσ),LogNormal(tcμ,tcσ),r,Normal(sμ,σ),sis_
119       end
120
121       T, pulse, mc_its, end_time = e.constants
122
123
124       ↪ joint_DNPs=thymidine_mc_llh(pparams,Int64(1e6),end_time,pulse,T,e.obs,true)
125
126       max_ct=0
127       for DNP in joint_DNPs

```

```

123         max_ct<maximum(DNP.support) && (max_ct==maximum(DNP.support))
124     end
125     cts=LinRange(0,max_ct,max_ct+1)
126     probs=zeros(length(T),length(cts))
127     for (t,DNP) in enumerate(joint_DNPs)
128         for (ct, p) in zip(DNP.support,DNP.p)
129             ct_idx=findfirst(isequal(ct),cts)
130             ct_idx != nothing && (probs[t,ct_idx]=p)
131         end
132     end
133     catobs=vcat(e.obs ... )
134     ymax=Int64(round(maximum(catobs)*1.1))
135     ys=[0,ymax]
136     Ts=vcat([[T[n] for i in 1:length(e.obs[n])] for n in 1:length(T)] ... )
137
138     MAPout=Plots.heatmap(T,cts,transpose(probs),ylims=ys)
139     Plots.scatter!(MAPout,Ts,catobs,marker=:cross,markercolor=:green)
140
141     thetas=fill(zeros(0),length(theta))
142
143     for pidx in 1:length(e.posterior_samples)
144         prec=e.posterior_samples[pidx]
145         for t in 1:length(theta)
146             push!(thetas[t],prec.theta[t])
147         end
148     end
149
150
151
152
153 end

```

16.5.11 /src/thymidine_sim/thymidine_lh.jl

```

1 function thymidine_mc_llh(pparams, mc_its, end_time, pulse,
2   ↳ T::Vector{<:AbstractFloat}, obs::Vector{<:AbstractVector{<:Integer}}),
3   ↳ display=false)
4   n_pops=length(pparams); times=length(T)
5   popset_labelled=zeros(Int64,mc_its,n_pops,times)
6   max_labelled=maximum.(obs)

5
6   Threads.@threads for it in 1:mc_its

```

```

7      for p in 1:n_pops
8          pop_dist,tc_dist,r,s,sis_frac = pparams[p]
9          n_lineages=Int64(max(1,round(rand(pop_dist))))
10         plv=view(popset_labelled,it,p,:)

11
12         for l in 1:Int64(floor(n_lineages/2))
13             !all(max_labelled .<
14                 → sum(popset_labelled[it,:,:,:],dims=1)[1,:]) &&
15                 → sim_lin_pair!(plv, T, end_time, pulse, tc_dist, r, s,
16                 → sis_frac)
17             end
18
19         end
20     end
21
22     pop_DNPs=Matrix{DiscreteNonParametric}(undef,n_pops,times)
23     for p in 1:n_pops
24         Threads.@threads for t in 1:times
25
26             → pop_DNPs[p,t]=fit(DiscreteNonParametric,popset_labelled[:,p,t])
27         end
28     end
29
30     if n_pops > 1
31         joints=Vector{DiscreteNonParametric}(undef,times)
32         Threads.@threads for t in 1:times
33             joints[t]=joint_DNP_sum(pop_DNPs[:,t])
34         end
35     else
36         joints=pop_DNPs[1,:]
37     end
38
39     display && (return joints)

40     log_lhs=Vector{Float64}(undef,times)
41     Threads.@threads for t in 1:times
42         log_lhs[t]=lps(logpdf(joints[t],obs[t]))

```

```

43     end
44
45     log_lh=lps(log_lhs)
46
47     disp_mat=zeros(times,3)
48     Threads.@threads for t in 1:times
49
50         → disp_mat[t,:]=[quantile(joints[t],.025),mean(joints[t]),quantile(joints[t],
51     end
52     return log_lh, disp_mat
53 end
54
55 function joint_DNP_sum(cs)
56     npops=length(cs)
57     ct_supports=Vector{Vector{Int64}}()
58     for c in cs
59         push!(ct_supports,c.support[[c.p ... ].>0.])
60     end
61
62     possible_sums=collect(Iterators.product(ct_supports ... ))
63     possible_totals=unique(sum.(possible_sums))
64     total_mat=sum.(possible_sums)
65
66     probs=zeros(length(possible_totals))
67
68     Threads.@threads for (n,pt) in collect(enumerate(possible_totals))
69         psums=possible_sums[findall(t→t==pt,total_mat)]
70         probs[n]=logsumexp([lps([logpdf(cs[p],ps[p]) for p in 1:npops])
71                         → for ps in psums])
72     end
73
74     return DiscreteNonParametric(possible_totals, exp.(probs))
75 end
76

```

16.5.12 /src/thymidine_sim/thymidine_model.jl

```

1 mutable struct Thymidine_Record <: GMC_NS_Model_Record
2     trajectory :: Int64
3     i :: Int64

```

```

4      pos::Vector{Float64}
5      path::String
6      log_Li::Float64
7 end
8
9 struct Thymidine_Model <: GMC_NS_Model
10    trajectory::Int64
11    i::Int64
12
13    θ::Vector{Float64}
14    log_Li::Float64
15
16    pos::Vector{Float64}
17    v::Vector{Float64}
18
19    disp_mat::Matrix{Float64} #matrix for mean & 95%CI plot of model
20    → output
21 end
22
23 function thymidine_constructor(trajectory, i, θ, pos, v, obs, T, pulse,
24   → mc_its, end_time; v_init=false)
25   mod(length(θ),8)≠0 && throw(ArgumentError("θ must contain 8 values
26   → per lineage population!"))
27   pulse<0 && throw(ArgumentError("pulse length must be ≥ 0!"))
28
29   n_pops=length(θ)/8
30   pparams=Vector{Tuple{LogNormal, LogNormal, Float64, Normal,
31     → Float64}}}()
32   for pop in 1:n_pops
33     lpmu, lpσ², r, tcμ, tcσ², sμ, σ², sis_frac=
34     → θ[Int64(1+((pop-1)*8)):Int64(8*pop)]
35     lpσ=sqrt(lpσ²); tcσ=sqrt(tcσ²); σ=sqrt(σ²)
36
37     → push!(pparams,(LogNormal(lpmu,lpσ),LogNormal(tcμ,tcσ),r,Normal(sμ,σ),sis_
38   end
39
40   log_lh,disp_mat=thymidine_mc_llh(pparams, mc_its, end_time, pulse, T,
41   → obs)
42
43   v_init && (v=rand(MvNormal(length(θ),1.)))
44
45   Thymidine_Model(trajectory, i, θ, log_lh, pos, v, disp_mat)
46 end

```

16.5.13 /src/thymidine_sim/thymidine_sim.jl

```

1 const DETECTION_THRESHOLD=.01
2
3 function sim_lin_pair!(plv, T, end_time, pulse, Tc, g1, s, sis_frac)
4     tδ1, s1 = cycle_model(g1, Tc, s)
5     tδ2, s2 = (1+rand([-1,1])*sis_frac).*tδ1, s1)
6
7     mint=-min(tδ1,tδ2)
8     mint=0. ? (birth_time=mint) : (birth_time=rand(Uniform(mint,0.)))
9
10    cells1=[LabelCell(birth_time,tδ1,s1,0.)]
11    cells2=[LabelCell(birth_time,tδ2,s2,0.)]
12
13    for cellvec in [cells1,cells2]
14        ci=1
15        while ci≤length(cellvec)
16            cycle_sim!(plv, ci, cellvec, T, end_time, pulse, Tc,g1,s,
17                         ↳ sis_frac)
18            ci+=1
19        end
20    end
21
22
23 function sim_lineage!(plv, T, end_time, pulse, Tc, g1, s, sis_frac)
24     tδ1, s1 = cycle_model(g1, Tc, s)
25     birth_time=rand(Uniform(-tδ1,0.))
26     cells=[LabelCell(birth_time,tδ1,s1,0.)]
27
28     ci=1
29     while ci≤ length(cells)
30         cycle_sim!(plv, ci, cells, T, end_time, pulse, Tc, g1, s,
31                         ↳ sis_frac)
32         ci+=1
33     end
34 end
35 function cycle_sim!(plv, ci::Int64, cells::Vector{LabelCell},
36                         ↳ T::Vector{Float64}, end_time::Float64, pulse::Float64, Tc::LogNormal,
37                         ↳ g1::Float64, sd::Normal, sis_frac::Float64)

```

```

36     n_times=length(T);
37
38     cell=cells[ci]
39     t=cell.time; tδ=tδ; s=s; l=cell.label
40
41     first_cycle=true
42
43     while t<end_time
44         tδ,s=0.,0.
45         first_cycle ? (tδ=tδ;s=s;first_cycle=false) : ((tδ,
46             ↵ s)=cycle_model(g1, Tc, sd))
47
48         s_start=t+g1
49         s_end=s_start+s
50         cycle_mitosis=t+tδ
51         cycle_events=[s_start,s_end,cycle_mitosis]
52
53         oldl=l; cycle_label=false
54         if (s_start ≤ pulse && s_end ≥ 0) #some s-phase overlaps with
55             ↵ the pulse in this case
56             cycle_label=true
57             l=update_label(l, s, s_start, s_end, pulse)
58         end
59
60         tidxs=t.<T.<cycle_mitosis
61         n_idxssum(tidxs)
62         (length(tidxs)>0 && (oldl > DETECTION_THRESHOLD || l >
63             ↵ DETECTION_THRESHOLD))
64
65         if n_idxss>0 && (oldl > DETECTION_THRESHOLD || l >
66             ↵ DETECTION_THRESHOLD)
67             cycle_label ? (label_outcomes=[oldl,-Inf,l]) :
68                 ↵ (label_outcomes=[oldl,oldl,oldl])
69             cubuf=falses(n_idxss)
70             for (n,timept) in enumerate(T[tidxs])
71                 count_labelled_at_T!(n,cubuf,timept, cycle_events,
72                     ↵ label_outcomes, oldl, s_start, s, pulse)
73             end
74             plv[tidxs]+=cubuf
75         end
76
77         l/=2 #mitosis halves label for the daughters
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92

```

```

73     tδ2, s2 = (1+rand([-1,1])*sis_frac).*(tδ, s)
74     tδ2 < s_end && (tδ2=s_end) #sister cycle shouldn't be shorter
    ↵   than the end of s phase for sibling- prevents large sis_frac
    ↵   from causing infinitely proliferative loops
75
76     cycle_mitosis < end_time &&
    ↵   push!(cells,LabelCell(cycle_mitosis,tδ2,s2,l))
77     t+=tδ
78     #println("t(tδ)")
79   end
80 end
81
82 function cycle_model(g1, Tc, s_dist)
83   cycle_time=rand(Tc)
84   s=max(eps(),rand(s_dist))
85   tδ=max(g1 + s, cycle_time)
86   return tδ, s
87 end
88
89 function update_label(label, s, s_start, s_end, pulse)
90   s_overlap=min(pulse,s_start+s_end)-max(0,s_start)
91   nuc_label_frac=s_overlap/s
92   return min(nuc_label_frac+label,1.) #label before mitosis time
    ↵   will be what's been accumulated from past + this cycle's s
    ↵   phase
93 end
94
95 function count_labelled_at_T!(n, cubuf, timept, cycle_events,
    ↵   label_outcomes, oldl, s_start, s, pulse)
96   outcome_idx=timept.<cycle_events
97   timept_label=label_outcomes[outcome_idx][1]
98   timept_label=-Inf &&
    ↵   (timept_label=partial_label(timept,oldl,s_start,s,pulse))
99   timept_label ≥ DETECTION_THRESHOLD && (cubuf[n]=true)
100 end
101
102 function partial_label(timept, label, s_start, s, pulse)
103   s_overlap=min(pulse,timept)-max(0.,s_start)
104   nuc_label_frac=s_overlap/s
105   return min(nuc_label_frac+label,1.)
106 end

```

16.6 BioBackgroundModels

16.6.1 /src/BioBackgroundModels.jl

```

1 module BioBackgroundModels
2
3 import Base:copy,size
4 import Distances: euclidean
5 import Distributions:Univariate,Dirichlet,Categorical,logpdf,isprobvec
6 import Distributed: RemoteChannel, myid, remote_do, rmprocs
7 import HMMBase: AbstractHMM, assert_hmm, istransmat
8 import MCMCChains: Chains, ChainDataFrame, heideldiag
9 import Printf: @sprintf
10 import Random: rand, shuffle
11 import Serialization: serialize
12 import StatsFuns: logsumexp, logaddexp
13 import Statistics: mean
14 import UnicodePlots: lineplot,lineplot!, scatterplot,scatterplot!
15 using BioSequences, DataFrames, FASTX, GFF3, ProgressMeter
16
17 include("BHMM/BHMM.jl")
18 export BHMM
19 include("EM/chain.jl")
20 export Chain_ID, EM_step
21 include("API/genome_sampling.jl")
22 export setup_sample_jobs, execute_sample_jobs
23 include("API/EM_master.jl")
24 export setup_EM_jobs!, execute_EM_jobs!
25
26 include("reports/chain_report.jl")
27 include("reports/partition_report.jl")
28 include("reports/replicate_convergence.jl")
29 include("API/reports.jl")
30 export generate_reports
31
32 include("EM/baum-welch.jl")
33 include("EM/churbanov.jl")
34 include("utilities/load_balancer.jl")
35 export LoadConfig
36 include("EM/EM_converge.jl")
37 include("genome_sampling/partition_masker.jl")
38 include("genome_sampling/sequence_sampler.jl")
39 include("likelihood_funcs/bg_lh_matrix.jl")

```

```

40 export BGHMM_likelihood_calc
41 include("likelihood_funcs/hmm.jl")
42 export obs_lh_given_hmm
43 include("utilities/observation_coding.jl")
44 include("utilities/BBG_analysis.jl")
45 include("utilities/BBG_progressmeter.jl")
46 include("utilities/HMM_init.jl")
47 include("utilities/model_display.jl")
48 include("utilities/utilities.jl")
49 export split_obs_sets
50 include("utilities/log_prob_sum.jl")
51 export lps
52 end # module

```

16.6.2 /src/API/EM_master.jl

```

1 #function to setup an BHMM chains dictionary and RemoteChannel for
  ↳ learning jobs, given a vector of state #s, order_nos, replicates to
  ↳ train, the dictionary to fill, the RemoteChannel and the training
  ↳ sequences
2 #resumes any existing non-converged chains, otherwise initialises hmms
  ↳ for new chains given provided constants
3 function setup_EM_jobs!(job_ids::Vector{Chain_ID},
  ↳ obs_sets::Dict{String,Vector{LongSequence{DNAAlphabet{2}}}};
  ↳ delta_thresh::Float64=1e-3,
  ↳ chains::Dict{Chain_ID,Vector{EM_step}}=Dict{Chain_ID,Vector{EM_step}}(),
  ↳ init_function::Function=autotransition_init)
  ↳ #argument checking
  ↳ length(job_ids) < 1 && throw(ArgumentError("Empty job id vector!"))
  ↳ length(obs_sets) < 1 && throw(ArgumentError("Empty observation
    ↳ sets!"))

7   no_input_hmms = length(job_ids)
8   input_channel= RemoteChannel(()→Channel{Tuple}(no_input_hmms*3))
  ↳ #channel to hold BHMM learning jobs
9   output_channel= RemoteChannel(()→Channel{Tuple}(Inf)) #channel to
  ↳ take EM iterates off of

11  code_dict = code_job_obs(job_ids, obs_sets)
13
14  @showprogress 1 "Setting up HMMs ... " for id in job_ids #for each
  ↳ jobid, add an initial BHMM to input_channel for EM_workers

```

```

15     if haskey(chains, id) && length(chains[id]) > 0 #true if resuming
16         ← from incomplete chain
17         chain_end=chains[id][end]
18         if !chain_end.converged || (chain_end.converged &&
19             ← chain_end.delta > delta_thresh)#push the last hmm iterate
20             ← for nonconverged chains to the input channel with coded
21             ← observations and values for chain resumption
22             put!(input_channel, (id, chain_end.iterate,
23                 ← chain_end.hmm, chain_end.log_p, code_dict[(id.obs_id,
24                     ← id.order)]))
25         else #skip any jobs that have converged from previous runs
26             no_input_hmms -= 1
27         end
28
29     else
30         hmm = init_function(id.K, id.order, id.obs_id) #initialise
31             ← first BHMM in chain
32         chains[id] = Vector{EM_step}() #initialise the relevant chain
33         obs=code_dict[(id.obs_id,id.order)]
34         lh=obs_lh_given_hmm(obs,hmm,linear=false)
35         put!(input_channel, (id, 1, hmm, lh, obs))
36     end
37     return no_input_hmms, chains, input_channel, output_channel
38 end
39
40 function execute_EM_jobs!(worker_pool::Vector{Int64},
41     ← no_input_hmms::Integer, chains::Dict{Chain_ID,Vector{EM_step}},
42     ← input_channel::RemoteChannel, output_channel::RemoteChannel,
43     ← chains_path::String; load_dict=Dict{Int64,LoadConfig}(),
44     ← EM_func::Function=linear_step, delta_thresh=1e-3, max_iterates=5000,
45     ← verbose=false)
46     #argument checking
47     length(worker_pool) < 1 && throw(ArgumentError("Worker pool must
48         ← contain one or more worker IDs!"))
49     no_input_hmms < 1 && throw(ArgumentError("Zero input HMMs reported,
50         ← likely continuing from chains already converged beyond default
51         ← delta_thresh for setup_EM_jobs"))
52     length(chains) < 1 && throw(ArgumentError("No chains supplied, likely
53         ← job set from setup_EM_jobs passed incorrectly"))
54     !isready(input_channel) && throw(ArgumentError("BHMM input channel
55         ← has no contents, likely job set from setup_EM_jobs already
56         ← executed"))

```

```

41 #SEND BHMM FIT JOBS TO WORKERS
42 if isready(input_channel) > 0
43     @info "Fitting HMMs .."
44     #WORKERS FIT HMMS
45     for worker in worker_pool
46         if worker in keys(load_dict)
47             remote_do(EM_converge!, worker, input_channel,
48                         → output_channel, no_input_hmms,
49                         → load_config=load_dict[worker], EM_func=EM_func,
50                         → delta_thresh=delta_thresh, max_iterates=max_iterates,
51                         → verbose=verbose)
52         else
53             remote_do(EM_converge!, worker, input_channel,
54                         → output_channel, no_input_hmms, EM_func=EM_func,
55                         → delta_thresh=delta_thresh, max_iterates=max_iterates,
56                         → verbose=verbose)
57         end
58     end
59 else
60     @warn "No input HMMs (all already converged?), skipping
61         fitting .."
62 end

63 #GET LEARNT HMMS OFF REMOTECHANNEL, SERIALISE AT EVERY ITERATION,
64     → UPDATE PROGRESS METERS
65 job_counter=no_input_hmms
66 learning_meters=Dict{Chain_ID, ProgressHMM}()
67 overall_meter=Progress(no_input_hmms, "Overall batch progress:")

68 while job_counter > 0
69     wait(output_channel)
70     workerid, jobid, iterate, hmm, log_p, delta, converged, steptime
71         → = take!(output_channel)
72     #either update an existing ProgressHMM meter or create a new one
73         → for the job
74     if haskey(learning_meters, jobid) && iterate > 2
75         update!(learning_meters[jobid], delta, steptime)
76     else
77         offset = workerid - 1
78         if iterate ≤ 2
79             learning_meters[jobid] = ProgressHMM(delta_thresh,
80                         → "$jobid on Wk $workerid:", offset, 2)
81         end
82     end
83 end

```

```

71         update!(learning_meters[jobid], delta, steptime)
72     else
73         learning_meters[jobid] = ProgressHMM(delta_thresh,
74             ↳ "$jobid on Wk $workerid:", offset, iterate)
75         update!(learning_meters[jobid], delta, steptime)
76     end
77
78     #push the hmm and related params to the results_dict
79     push!(chains[jobid], EM_step(iterate, hmm, log_p, delta,
80         ↳ converged))
81     #try to serialize the results; catch interrupts and other errors
82     ↳ to prevent corruption
83     try
84         serialize(chains_path, chains)
85     catch e
86         @warn "Serializing failed!"
87         println(e)
88     end
89
90     #decrement the job counter, update overall progress meter, and
91     ↳ save the current results dict on convergence or max iterate
92     if converged || iterate == max_iterates
93         job_counter -= 1
94         ProgressMeter.update!(overall_meter,
95             ↳ (no_input_hmms-job_counter))
96         if !isready(input_channel) #if there are no more jobs to be
97             ↳ learnt, retire the worker
98             workerid!=1 && rmprocs(workerid)
99         end
100     end
101
102     #count converged & unconverged jobs, report results
103     converged_counter = 0
104     unconverged_counter = 0
105     for (id, chain) in chains
106         chain[end].converged = true ? (converged_counter += 1) :
107             (unconverged_counter += 1)
108     end
109
110     @info "Background HMM batch learning task complete,
111         ↳ $converged_counter converged jobs, $unconverged_counter jobs
112         ↳ failed to converge in $max_iterates iterates since job start."

```

 105 end

16.6.3 /src/API/genome_sampling.jl

```

1 #function to partition genome and set up Distributed RemoteChannels so
  ↵ partitions can be sampled simultaneously
2 function setup_sample_jobs(genome_path::String,
  ↵ genome_index_path::String, gff3_path::String,
  ↵ sample_set_length::Integer, sample_window_min::Integer,
  ↵ sample_window_max::Integer, perigenic_pad::Integer;
  ↵ deterministic::Bool=false)
3   #argument checking
4   !ispAth(genome_path) && throw(ArgumentError("Bad genome path!"))
5   !ispAth(genome_index_path) && throw(ArgumentError("Bad genome index
  ↵ path!"))
6   !ispAth(gff3_path) && throw(ArgumentError("Bad gff3 path!"))
7   sample_set_length < 1 && throw(ArgumentError("Sample set length must
  ↵ be a positive integer!"))
8   sample_window_min < 1 || sample_window_max < 1 &&
  ↵ throw(ArgumentError("Sample window minimum and maximum bounds
  ↵ must be positive integers!"))
9   sample_window_min ≥ sample_window_max && throw(ArgumentError("Sample
  ↵ window minimum size must be smaller than maximum size"))
10  perigenic_pad < 0 && throw(ArgumentError("Perigenic pad must be 0 or
  ↵ positive!"))

11
12  coordinate_partitions = partition_genome_coordinates(gff3_path,
  ↵ perigenic_pad)
13  sample_sets = DataFrame[]
14  input_sample_jobs =
  ↵ RemoteChannel(()→Channel{Tuple}(length(coordinate_partitions)))
  ↵ #channel to hold sampling jobs
15  completed_sample_jobs =
  ↵ RemoteChannel(()→Channel{Tuple}(length(coordinate_partitions)))
  ↵ #channel to hold completed sample dfs
16  for (partitionid, partition) in coordinate_partitions
    add_metacoordinates!(partition)
    put!(input_sample_jobs, (genome_path, genome_index_path,
      ↵ partition, partitionid, sample_set_length, sample_window_min,
      ↵ sample_window_max, deterministic))
17  end
18  progress_channel = RemoteChannel(()→Channel{Tuple}(20))

```

```

21     return (input_sample_jobs, completed_sample_jobs, progress_channel,
22             ↵ sample_set_length)
23
24 function
25     ↵ execute_sample_jobs(channels::Tuple{RemoteChannel,RemoteChannel,RemoteChannel,Int}
26                             ↵ worker_pool::Vector{Int64}; partitions::Integer=3)
27         input_sample_channel, completed_sample_channel, progress_channel,
28         ↵ sample_set_length = channels
29
30     #argument checking
31     length(worker_pool) < 1 && throw(ArgumentError("Worker pool must
32             ↵ contain one or more worker IDs!"))
33     !isready(input_sample_channel) && throw(ArgumentError("Input sample
34             ↵ channel not ready, likely channel set from setup_sample_jobs
35             ↵ passed incorrectly"))
36     sample_set_length < 1 && throw(ArgumentError("Sample set length must
37             ↵ be a positive integer, likely channel set from setup_sample_jobs
38             ↵ passed incorrectly"))
39
40     #send sampling jobs to workers
41     if isready(input_sample_channel) > 0
42         @info "Sampling.."
43         #WORKERS SAMPLE
44         for (n, worker) in enumerate(worker_pool)
45             if n ≤ partitions #no more workers than partitions to be
46                 ↵ used
47                 remote_do(get_sample_set, worker, input_sample_channel,
48                         ↵ completed_sample_channel, progress_channel)
49             end
50         end
51     else
52         @error "No sampling jobs!"
53     end
54
55     #progress meters for sampling
56     sampling_meters=Dict{String, Progress}()
57     overall_sampling_meter=Progress(partitions, "Overall sampling
58             ↵ progress:")
59     completed_counter = 0
60     ProgressMeter.update!(overall_sampling_meter, completed_counter)
61     sampling_offset = ones(Bool, partitions)

```

```

52     #collect progress updates while waiting on completion of sampling
53     → jobs
54     while completed_counter < partitions
55         wait(progress_channel)
56         partition_id, progress = take!(progress_channel)
57         if haskey(sampling_meters, partition_id)
58             ProgressMeter.update!(sampling_meters[partition_id],
59             → progress)
60         else
61             offset = findfirst(sampling_offset)[1]
62             sampling_meters[partition_id] = Progress(sample_set_length,
63             → "Sampling partition $partition_id:", offset)
64             ProgressMeter.update!(sampling_meters[partition_id],
65             → progress)
66             sampling_offset[offset] = false
67         end
68     end
69
70     #collect sample dfs by partition id when ready
71     sample_record_dfs = Dict{String,DataFrame}()
72     collected_counter = 0
73     while collected_counter < partitions
74         wait(completed_sample_channel)
75         partition_id, sample_df = take!(completed_sample_channel)
76         sample_record_dfs[partition_id] = sample_df
77         collected_counter += 1
78         @info "Partition $partition_id completed sampling..."
79     end
80
81     return sample_record_dfs
82 end

```

16.6.4 /src/API/reports.jl

```

1 struct Report_Folder
2     partition_id::String
3     partition_report::Partition_Report

```

```

4      replicate_report::Replicate_Report
5      chain_reports::Dict{Chain_ID,Chain_Report}
6  end
7
8  function generate_reports(chains::Dict{Chain_ID,Vector{EM_step}},
9    ↳ test_sets::Dict{String,Vector{LongSequence{DNAAlphabet{2}}}})
10   #check chains dict for problems
11
12   length(chains)==0 && throw(ArgumentError("Empty chains dict!"))
13   any(chain→length(chain)<2, values(chains)) &&
14     ↳ throw(ArgumentError("Some chains are too short (<2 EM steps)!")
15     ↳ "Probably not all chains have been operated on by EM workers yet.
16     ↳ Try EM_converge first!"))
17   unconv=sum(!chain[end].converged for chain in values(chains))
18   unconv > 0 && @warn "Not all chains are converged to the selected
19     ↳ step delta."
20   length(test_sets)==0 && throw(ArgumentError("Empty test_sets dict!"))
21
22   chain_reports = report_chains(chains, test_sets)
23   partition_reports = report_partitions(chain_reports)
24
25   report_folders=Dict{String, Report_Folder}()
26
27   for part_report in partition_reports
28     rep_report=report_replicates(part_report.best_repset, chains)
29     chain_subset=Dict{Chain_ID,Chain_Report}()
30     for id in keys(chain_reports)
31       id.obs_id=part_report.partition_id &&
32         ↳ (chain_subset[id]=chain_reports[id])
33     end
34
35     ↳ report_folders[part_report.partition_id]=Report_Folder(part_report.partit
36     ↳ part_report, rep_report, chain_subset)
37   end
38
39   return report_folders
40 end

```

16.6.5 /src/BHMM/BHMM.jl

```

1 """
2     BHMM([a::AbstractVector{T}, ]A::AbstractMatrix{T},
3         ↳ B::AbstractVector{<:Categorical}) where F where T
4 Build an BHMM with transition matrix `A` and observations distributions
5         ↳ `B`.
6 If the initial state distribution `a` is not specified, a uniform
7         ↳ distribution is assumed.
8 Observations distributions can be of different types (for example
9         ↳ `Normal` and `Exponential`).
10 However they must be of the same dimension (all scalars or all
11         ↳ multivariates).
12 # Example
13 ````julia
14 hmm = BHMM([0.9 0.1; 0.1 0.9], [Normal(0,1), Normal(10,1)])
15 `````
16 """
17 struct BHMM{T} <: AbstractHMM{Univariate}
18     a::AbstractVector{T}
19     A::AbstractMatrix{T}
20     B::AbstractVector{<:Categorical}
21     partition::String
22     BHMM{T}(a, A, B, partition="") where {T} = assert_BHMM(a, A, B) &&
23         ↳ new(a, A, B, partition)
24 end
25
26 """
27     assert_BHMM(a, A, B)
28 Throw an `ArgumentError` if the initial state distribution and the
29         ↳ transition matrix rows does not sum to 1,

```

```

30 and if the observations distributions does not have the same dimensions.
31 """
32 function assert_BHMM(a::AbstractVector,
33                         A::AbstractMatrix,
34                         B::AbstractVector{<:Categorical})
35     !isprobvec(a) && throw(ArgumentError("Initial state vector a is not a
36                             ↳ valid probability vector!"))
37     !istransmat(A) && throw(ArgumentError("Transition matrix A is not
38                             ↳ valid!"))
39     !all([length(d.p) for d in B] .== length(B[1].p)) &&
40                             ↳ throw(ArgumentError("All distributions must have the same
41                             ↳ dimensions"))
42     !(length(a) == size(A,1) == length(B)) && throw(ArgumentError("Length
43                             ↳ of initial state vector a, dimension of transition matrix A, and
44                             ↳ number of distributions B are not the same!"))
45     return true
46 end
47
48 function Base.show(io::IO, hmm::BHMM)
49     println(io, "Background BHMM")
50     println(io, "State Initial and Transition Probabilities")
51     print(io, "a: ")
52     show(io, hmm.a)
53     println(io)
54     print(io, "A: ")
55     display(hmm.A)
56     println(io)
57     println(io, "INFORMATIVE SYMBOLS BY STATE")
58     for (n,d) in enumerate(hmm.B)
59         print(io, "K$n ")
60         print_emitters(d)
61     end
62 end

```

16.6.6 /src/EM/EM_converge.jl

```

1 function EM_converge!(hmm_jobs :: RemoteChannel,
2   ↳ output_hmms :: RemoteChannel, no_models :: Integer;
3   ↳ load_config :: LoadConfig=LoadConfig(1:typemax(Int64)-1,
4   ↳ 0:typemax(Int64)-1), EM_func :: Function=linear_step,
5   ↳ delta_thresh=1e-3, max_iterates=5000, verbose=false)
6   ↳ while isready(hmm_jobs)
7     ↳ workerid = myid()
8     ↳ jobid, start_iterate, hmm, job_norm, observations =
9       ↳ load_balancer(no_models, hmm_jobs, load_config)
10    ↳ jobid == 0 && break #no valid job for this worker according to
11      ↳ load_table entry
12
13    ↳ start_iterate > max_iterates - 1 && throw(ArgumentError("BHMM
14      ↳ chain $jobid is already longer ($start_iterate iterates) than
15      ↳ specified max_iterates!"))
16    ↳ curr_iterate = start_iterate
17
18    ↳ #mask calculations here rather than ms_mle_step to prevent
19      ↳ recalculation every iterate
20    ↳ #build array of observation lengths
21
22    ↳ obs_lengths = [findfirst(iszero, observations[o,:])-1 for o in
23      ↳ 1:size(observations,1)] #mask calculations here rather than
24      ↳ mle_step to prevent recalculation every iterate
25    ↳ EM_func=bw_step && (observations=transpose(observations))
26
27    ↳ start_iterate = 1 && put!(output_hmms, (workerid, jobid,
28      ↳ curr_iterate, hmm, job_norm, 0.0, false, 0.0)); #on the first
29      ↳ iterate return the initial BHMM immediately
30    ↳ verbose && @info "Fitting BHMM on Wk $workerid, start iterate
31      ↳ $start_iterate, $jobid with $(size(hmm)[1]) states and
32      ↳ $(size(hmm)[2]) symbols ... "
33
34    ↳ curr_iterate += 1
35
36    ↳ start=time()
37    ↳ new_hmm, last_norm = EM_func(hmm, observations, obs_lengths)
38    ↳ delta = abs(lps(job_norm, -last_norm))
39    ↳ put!(output_hmms, (workerid, jobid, curr_iterate, new_hmm,
40      ↳ last_norm, delta, false, time()-start))

```

```

26     for i in curr_iterate:max_iterates
27         start=time()
28         new_hmm, norm = EM_func(new_hmm, observations, obs_lengths)
29         curr_iterate += 1
30         delta = abs(lps(norm, -last_norm))
31         if delta < delta_thresh
32             put!(output_hmms, (workerid, jobid, curr_iterate,
33             ↪ new_hmm, norm, delta, true, time()-start))
34             verbose && @info "$jobid converged after
35             ↪ $(curr_iterate-1) EM steps"
36             break
37         else
38             put!(output_hmms, (workerid, jobid, curr_iterate,
39             ↪ new_hmm, norm, delta, false, time()-start))
40             verbose && @info "$jobid EM step $(curr_iterate-1) delta
41             ↪ $delta"
42             last_norm = norm
43         end
44     end
45 end
46

```

16.6.7 /src/EM/baum-welch.jl

```

1 """
2     ms_mle_step(hmm::AbstractHMM{F}, observations) where F
3
4 Perform one step of the EM (Baum-Welch) algorithm.
5
6 # Example
7 """
8 hmm, log_likelihood = ms_mle_step(hmm, observations)
9 """
10 """
11
12 function bw_step(hmm::BHMM, observations, obs_lengths)
13     lls = bw_llhs(hmm, observations)
14     log_α = messages_forwards_log(hmm.a, hmm.A, lls, obs_lengths)
15     log_β = messages_backwards_log(hmm.A, lls, obs_lengths)
16     log_A = log.(hmm.A)
17

```

```

18 K,Tmaxplus1,O = size(lls) #the last T value is the 0 end marker of
  ↵ the longest T
19
20 #transforms to cut down log_ξ, log_γ assignment times
21 lls = permutedims(lls, [2,3,1]) # from (K,T,O) to (T,O,K)
22 log_α = permutedims(log_α, [2,3,1])
23 log_β = permutedims(log_β, [2,3,1])
24
25 # E-step
26 log_ξ = fill(-Inf, Tmaxplus1,O,K,K)
27 log_γ = fill(-Inf, Tmaxplus1,O,K)
28 log_pobs = zeros(O)
29
30 @inbounds for o = 1:O
31     log_pobs[o] = logsumexp(lps.(log_α[1,o,:], log_β[1,o,:]))
32 end
33
34 Threads.@threads for idx in [(i,j,o) for i=1:K, j=1:K, o=1:O]
35     i,j,o = idx[1],idx[2],idx[3]
36     obsl = obs_lengths[o]
37     @inbounds for t = 1:obsl-1 #log_ξ & log_γ calculated to T-1 for
      ↵ each o
38         log_ξ[t,o,i,j] = lps(log_α[t,o,i], log_A[i,j], log_β[t+1,o,j],
          ↵ lls[t+1,o,j], -log_pobs[o])
39         log_γ[t,o,i] = lps(log_α[t,o,i], log_β[t,o,i], -log_pobs[o])
40     end
41     t=obsl #log_ξ @ T = 0
42     log_ξ[t,o,i,j] = 0
43     log_γ[t,o,i] = lps(log_α[t,o,i], log_β[t,o,i], -log_pobs[o])
44 end
45
46 ξ = similar(log_ξ)
47 ξ .= exp.(log_ξ)
48 Σk_ξ = sum(ξ, dims=[3,4])
49 nan_mask = Σk_ξ .== 0
50 Σk_ξ[nan_mask] .= Inf #prevent NaNs in dummy renorm
51 ξ ./= Σk_ξ #dummy renorm across K to keep numerical creep from
  ↵ causing isprobvec to fail on new new_A during hmm creation
52
53 γ = similar(log_γ)
54 γ .= exp.(log_γ)
55 Σk_γ = sum(γ, dims=3)
56 Σk_γ[nan_mask[:, :, :, :]] .= Inf #prevent NaNs in dummy renorm

```

```

57     γ .= Σk_γ #dummy renorm
58
59     # M-step
60     new_A = zeros(K,K)
61     for i=1:K, j=1:K
62         Σotξ_vec = zeros(0)
63         Σotγ_vec = zeros(0)
64         Threads.@threads for o in 1:O
65             Σotξ_vec[o] = sum(ξ[1:obs_lengths[o]-1,o,i,j])
66             Σotγ_vec[o] = sum(γ[1:obs_lengths[o]-1,o,i])
67         end
68         new_A[i,j] = sum(Σotξ_vec) / sum(Σotγ_vec)
69     end
70     new_A .= sum(new_A, dims=2) #dummy renorm
71     new_a = (sum(γ[1,:,:], dims=1)./sum(sum(γ[1,:,:], dims=1)))[1,:]
72     new_a .= sum(new_a) #dummy renorm
73
74     obs_mask = .!nan_mask
75     obs_collection = observations[obs_mask[:, :]]
76
77     B = Categorical[]
78     @inbounds for (i, d) in enumerate(hmm.B)
79         γ_d = γ[:, :, i]
80         push!(B, t_categorical_mle(Categorical, d.support[end],
81             → obs_collection, γ_d[obs_mask[:, :]]))
82     end
83
84     return typeof(hmm)(new_a, new_A, B), lps(log_pobs)
85 end
86
87 function bw_llhs(hmm, observations)
88     lls = zeros(length(hmm.B), size(observations)...)
89     Threads.@threads for d in 1:length(hmm.B)
90         lls[d,:,:] = logpdf.(hmm.B[d], observations)
91     end
92     return lls
93 end
94
95 #Multisequence competent log implementations of forward
# and backwards algos
96
97 function messages_forwards_log(init_distn, trans_matrix,
98     → log_likelihoods, obs_lengths)
99     log_alphas = zeros(size(log_likelihoods))
100    log_trans_matrix = log.(trans_matrix)

```

```

96         log_alphas[:,1,:] = log.(init_distn) .+
97             ↪ log_likelihoods[:,1,:]
98         Threads.@threads for o in 1:size(log_likelihoods)[3]
99             @inbounds for t in 2:obs_lengths[o], j in
100                 ↪ 1:size(log_likelihoods)[1]
101
102                 ↪ log_alphas[j,t,o]=logsumexp(log_alphas[:,t-1,o]
103                 ↪ .+ log_trans_matrix[:,j]) +
104                 ↪ log_likelihoods[j,t,o]
105             end
106         end
107         return log_alphas
108     end
109
110     function messages_backwards_log(trans_matrix,
111         ↪ log_likelihoods, obs_lengths)
112         log_betas = zeros(size(log_likelihoods))
113         log_trans_matrix = log.(trans_matrix)
114         Threads.@threads for o in 1:size(log_likelihoods)[3]
115             @inbounds for t in obs_lengths[o]-1:-1:1
116                 tmp = view(log_betas, :, t+1, o) .+
117                     ↪ view(log_likelihoods, :, t+1, o)
118                 @inbounds for i in 1:size(log_likelihoods)[1]
119                     log_betas[i,t,o] =
120                         ↪ logsumexp(view(log_trans_matrix, i,:)
121                         ↪ .+ tmp)
122                 end
123             end
124         end
125         return log_betas
126     end
127
128     #subfunc derived from Distributions.jl categorical.jl
129     ↪ fit_mle, threaded
130     function t_categorical_mle(::Type{<:Categorical},
131         ↪ k::Integer, x::AbstractArray{T}, w::AbstractArray{F})
132         ↪ where T<:Integer where F<:AbstractFloat
133
134             ↪ Categorical(t_pnormalize!(t_add_categorical_counts!(zeros(k),
135             ↪ x, w)))
136         end
137
138         t_pnormalize!(v::Vector) = (v ./= sum(v); v)
139
140

```

```

125     function t_add_categorical_counts!(h::Vector{F},
126         x::AbstractArray{T}, w::AbstractArray{F}) where
127             T<:Integer where F<:AbstractFloat
128                 n = length(x)
129                 if n ≠ length(w)
130                     throw(DimensionMismatch("Inconsistent array
131                         → lengths."))
132                 end
133                 hlock = ReentrantLock()
134                 Threads.@threads for i=1:n
135                     @inbounds xi = x[i]
136                     @inbounds wi = w[i]
137                     lock(hlock)
138                     h[xi] += wi    # cannot use @inbounds, as no
139                         → guarantee that x[i] is in bound
140                     unlock(hlock)
141                 end
142                 return h
143             end
144         end

```

16.6.8 /src/EM/chain.jl

```

1 struct Chain_ID
2     obs_id::AbstractString
3     K::Integer
4     order::Integer
5     replicate::Integer
6     Chain_ID(obs_id,K,order,replicate) =
7         → assert_chain_id(K,order,replicate) &&
8         → new(obs_id,K,order,replicate)
9 end
10
11 function assert_chain_id(K, order, replicate)
12     K < 1 && throw(ArgumentError("Chain_ID K (# of hmm states) must be a
13         → positive integer!"))
14     order < 0 && throw(ArgumentError("Chain_ID symbol order must be zero
15         → or a positive integer!"))
16     replicate < 1 && throw(ArgumentError("Chain_ID replicate must be a
17         → positive integer!"))
18     return true
19 end
20
21

```

```

16 struct EM_step
17     iterate::Integer
18     hmm::BHMM
19     log_p::AbstractFloat
20     delta::AbstractFloat
21     converged::Bool
22
23     → EM_step(iterate,hmm,log_p,delta,converged)=assert_step(iterate,hmm,log_p)
24     → && new(iterate, hmm, log_p, delta, converged)
25 end
26
27 function assert_step(iterate, hmm, log_p)
28     iterate < 1 && throw(ArgumentError("EM_step iterate number must be a
29     → positive integer!"))
30     assert_hmm(hmm.a, hmm.A, hmm.B)
31     log_p > 0.0 && throw(ArgumentError("EM_step log probability value
32     → must be 0 or negative!"))
33     return true
34 end

```

16.6.9 /src/EM/churbanov.jl

```

1 function linear_step(hmm, observations, obs_lengths)
2     O,T = size(observations);
3     a = transpose(log.(hmm.a)); A = log.(hmm.A) #less expensive to
4         → transpose transmatrix in the backwards_sweep than to transpose
5         → here and take a ranged view ie view(A,m:m,:) is more expensive
6         → than transpose(view(A,m,:))
7     N = length(hmm.B); Γ = length(hmm.B[1].support);
8     mask=observations.≠0
9     #INITIALIZATION
10    βoi_T = zeros(O,N); βoi_t = zeros(O,N) #log betas at T initialised as
11        → zeros
12    Eoyim_T = fill(-Inf,O,Γ,N,N); Eoyim_t = fill(-Inf,O,Γ,N,N)
13    for m in 1:N, i in 1:N, y in 1:Γ, o in 1:O
14        observations[o, obs_lengths[o]] = y && m == i && (Eoyim_T[o, y,
15            → i, m] = 0)
16    end
17    Tijm_T = fill(-Inf,O,N,N,N); Tijm_t = fill(-Inf,O,N,N,N) #Ti,j(T,m) =
18        → 0 for all m; in logspace
19
20    #RECURRENCE

```

```

15     ↵   βoi_T,Tijm_T,Eoyim_T=backwards_sweep!(hmm,A,N,Γ,βoi_T,βoi_t,Tijm_T,Tijm_t,Eoy
16
17 #TERMINATION
18 lls = c_llhs(hmm,observations[:,1])
19 α1om = lps.lls,a #first position forward msgs
20 log_pobs = [c_lse(lps.(α1om[o,:], βoi_T[o,:])) for o in 1:O]
21
22 Toij = [c_lse([lps(Tijm_T[o,i,j,m], α1om[o,m], -log_pobs[o]) for m in
23     ↵ 1:N]) for o in 1:O, i in 1:N, j in 1:N] #terminate Tijs with
24     ↵ forward messages
25 Eoiy = [c_lse([lps(Eoyim_T[o,y,i,m], α1om[o,m], -log_pobs[o]) for m
26     ↵ in 1:N]) for o in 1:O, i in 1:N, y in 1:Γ] #terminate Eids with
27     ↵ forward messages
28
29 #INTEGRATE ACROSS OBSERVATIONS AND SOLVE FOR NEW BHMM PARAMS
30 #INITIAL STATE DIST
31 a_o=α1om.+βoi_T.-c_lse.(eachrow(α1om.+βoi_T)) #estimate a for each o
32 obs_penalty=log(0) #broadcast subtraction to normalise log prob vals
33     ↵ by obs number
34 new_a=c_lse.(eachcol(a_o))-obs_penalty #sum over obs and normalise
35     ↵ by number of obs
36 #TRANSITION MATRIX
37 new_A = [c_lse(view(Toij,:,:i,j)) for i in 1:N, j in
38     ↵ 1:N]-c_lse.(eachcol(c_lse.([view(Toij,o,i,:) for o in 1:O, i in
39     ↵ 1:N])))
40 #EMISSION MATRIX
41 new_b=[c_lse(view(Eoiy,:,:i,y)) for i in 1:N, y in
42     ↵ 1:Γ]-c_lse.(eachcol(c_lse.([view(Eoiy,o,i,:) for o in 1:O, i in
43     ↵ 1:N])))
44 new_B=[Categorical(exp.(new_b[i,:])) for i in 1:N]
45
46 return BHMM(exp.(new_a), exp.(new_A), new_B, hmm.partition),
47     ↵ lps(log_pobs)
48 end
49 #LINEAR_STEP SUBFUNCS
50 function backwards_sweep!(hmm, A, N, Γ, βoi_T, βoi_t,
51     ↵ Tijm_T, Tijm_t, Eoyim_T, Eoyim_t, observations, mask,
52     ↵ obs_lengths)
53     for t in maximum(obs_lengths)-1:-1:1
54         last_β=copy(βoi_T)
55         lls = c_llhs(hmm,observations[:,t+1])
56         omask = findall(mask[:,t+1])

```

```

44         βoi_T[omask,:] .+= view(lls,omask,:)
45 Threads.@threads for m in 1:N
46     βoi_t[omask,m] =
47         → c_lse.(eachrow(view(βoi_T,omask,:).+transpose(view(A,
48             for j in 1:N, i in 1:N
49                 Tijm_t[omask, i, j, m] .=
50                     → c_lse.(eachrow(lps.(view(Tijm_T,omask,i,j,:),
51                         → view(lls,omask,:),
52                         → transpose(view(A,m,:)))))
53                 i==m && (Tijm_t[omask, i, j, m] .=
54                     → logaddexp.(Tijm_t[omask, i, j, m],
55                         → lps.(last_β[omask,j],
56                         → A[m,j],lls[omask,j])))
57             end
58         for i in 1:N, y in 1:Γ
59             Eoyim_t[omask, y, i, m] .=
60                 → c_lse.(eachrow(lps.(view(Eoyim_T,omask,y,i,:)),view(
61                     if i==m
62                         symmask =
63                             → findall(observations[:,t]==y)
64                         Eoyim_t[symmask, y, i, m] .=
65                             → logaddexp.(Eoyim_t[symmask, y, i,
66                                         → m], βoi_t[symmask,m])
67                     end
68                 end
69             end
70             βoi_T=copy(βoi_t); Tijm_T=copy(Tijm_t); Eoyim_T =
71                 → copy(Eoyim_t);
72         end
73         return βoi_T, Tijm_T, Eoyim_T
74     end
75
76     #logsumexp
77     function c_lse(X::AbstractArray{T}; dims=:) where
78         {T<:Real}
79         u=maximum(X)
80         u isa AbstractArray || isfinite(u) || return float(u)
81         return u + log(sum(x → exp(x-u), X))
82     end
83
84     #log likelihoods
85     function c_llhs(hmm, observation)
86         lls = zeros(length(observation),length(hmm.B))
87     end
88
89
90
91
92
93

```

```

74         Threads.@threads for d in 1:length(hmm.B)
75             lls[:,d] = logpdf.(hmm.B[d], observation)
76         end
77     return lls
78 end

```

16.6.10 /src/genome_sampling/partition_masker.jl

```

1 function get_partition_code_dict(dict_forward::Bool=true)
2     if dict_forward == true
3         return partition_code_dict =
4             Dict("intergenic"⇒1, "periexonic"⇒2, "exon"⇒3)
5     else
6         return code_partition_dict = Dict(1⇒"intergenic",
7             2⇒"periexonic", 3⇒"exon")
8     end
9 end
10
11 function make_padded_df(position_fasta::String, gff3_path::String,
12     ← genome_path::String, genome_index_path::String, pad::Integer)
13     position_reader = FASTA.Reader(open((position_fasta), "r"))
14     genome_reader = open(FASTA.Reader, genome_path,
15         ← index=genome_index_path)
16     scaffold_df = build_scaffold_df(gff3_path)
17     position_df = DataFrame(SeqID = String[], Start=Int[], End=Int[],
18         ← PadSeq = LongSequence[], PadStart=Int[], RelStart=Int[],
19         ← SeqOffset=Int[])
20     scaffold_seq_dict = build_scaffold_seq_dict(genome_path,
21         ← genome_index_path)
22
23     for entry in position_reader
24         scaffold = FASTA.identifier(entry)
25
26         if scaffold ≠ "MT"
27             desc_array = split(FASTA.description(entry))
28             pos_start = parse(Int, desc_array[2])
29             pos_end = parse(Int, desc_array[4])
30             scaffold_end = scaffold_df.End[findfirst(isequal(scaffold),
31                 ← scaffold_df.SeqID)]
32
33             pad_start=max(1, pos_start-pad)
34             pad_length= pos_start - pad_start

```

```

27         seq_offset = pad - pad_length
28         padded_seq = fetch_sequence(scaffold, scaffold_seq_dict,
29             → pad_start, pos_end, '+')
30
31     if !hasambiguity(padded_seq)
32         push!(position_df, [scaffold, pos_start, pos_end,
33             → padded_seq, pad_start, pad_length, seq_offset])
34     end
35 end
36
37 close(position_reader)
38 close(genome_reader)
39 return position_df
40
41 function add_partition_masks!(position_df::DataFrame, gff3_path::String,
42     → perigenic_pad::Integer=500,
43     → columns::Tuple{Symbol,Symbol,Symbol}=(:SeqID, :PadSeq, :PadStart))
44     partitions=["exon", "periexonic", "intergenic"]
45     partition_coords_dict = partition_genome_coordinates(gff3_path,
46         → perigenic_pad)
47     partitioned_scaffolds =
48         → divide_partitions_by_scaffold(partition_coords_dict)
49     maskcol = [zeros(Int64,0,0) for i in 1:size(position_df,1)]
50     position_df.MaskMatrix=maskcol
51
52     @Threads.threads for entry in eachrow(position_df)
53         scaffold = entry[columns[1]]
54         maskLength = length(entry[columns[2]])
55         seqStart = entry[columns[3]]
56
57         scaffold_coords_dict = Dict{String,DataFrame}()
58
59         for ((partition, part_scaffold), df) in partitioned_scaffolds
60             if scaffold == part_scaffold
61                 scaffold_coords_dict[partition] = df
62             end
63         end
64
65         entry.MaskMatrix=mask_sequence_by_partition(maskLength, seqStart,
66             → scaffold_coords_dict)
67     end

```

```

63
64 end
65 #add_partition_masks!() SUBFUNCTIONS
66 function
67   → divide_partitions_by_scaffold(partition_coords_dict::Dict{String,
68   → DataFrame})
69   scaffold_coords_dict = Dict{Tuple{String, String}, DataFrame}()
70   for (partition_id, partition_df) in
71     → partition_coords_dict
72     for scaffold_subframe in groupby(partition_df,
73       → :SeqID)
74       scaffold_id = scaffold_subframe.SeqID[1]
75       scaffold_df = copy(scaffold_subframe)
76       scaffold_coords_dict[(partition_id,
77       → scaffold_id)] = scaffold_df
78     end
79   end
80   return scaffold_coords_dict
81 end
82
83 function mask_sequence_by_partition(maskLength::Integer,
84   → seqStart::Integer, scaffold_coords_dict::Dict{String,
85   → DataFrame})
86   partition_code_dict = get_partition_code_dict()
87   seqMask = zeros(Integer, (maskLength, 2))
88   position = seqStart
89   while position ≤ seqStart+maskLength
90     position_partition, partition_extent,
91     → position_strand =
92     → find_position_partition(position,
93     → scaffold_coords_dict)
94
95     partition_code =
96     → partition_code_dict[position_partition]
97     mask_position = position - seqStart + 1
98
99     → seqMask[mask_position:min(maskLength,mask_position
100    → + partition_extent),1] .= partition_code
101    if position_strand == '+'
102      → seqMask[mask_position:min(maskLength,mask_position
103      → + partition_extent),2] .= 1

```

```

90         elseif position_strand == '-'
91             ↪ seqMask[mask_position:min(maskLength,mask_position
92                 ↪ + partition_extent),2] *= -1
93         else
94
95             ↪ seqMask[mask_position:min(maskLength,mask_position
96                 ↪ + partition_extent),2] *= 0
97         end
98
99         position += partition_extent + 1
100     end
101
102     return seqMask
103 end
104
105 function find_position_partition(position::Integer,
106     ↪ partition_dict::Dict{String, DataFrame})
107     foundPos = false
108     position_partition_id = ""
109     three_prime_extent = 0
110     sample_strand = 0
111     for (partition_id, partition) in partition_dict
112         hitindex = findfirst(x→x≥position,
113             ↪ partition.End)
114         if hitindex ≠ nothing
115             if position ≥ partition.Start[hitindex]
116                 foundPos=true
117                 position_partition_id = partition_id
118                 three_prime_extent =
119                     ↪ partition.End[hitindex] - position
120                 if partition_id == "exon" || partition_id
121                     ↪ == "perixonic"
122                     sample_strand =
123                         ↪ partition.Strand[hitindex]
124                 end
125             end
126         end
127     end
128
129     if foundPos == false
130         throw(DomainError("Position $position not found
131             ↪ among partition coordinates!"))
132     end

```

```

123         else
124             return position_partition_id, three_prime_extent,
125                 ↵ sample_strand
126         end
127     end
128 #function to partition a genome into coordinate sets of:
129 #merged exons
130 #"periexonic" sequences (genes with 5' and 3' boundaries projected
131   ↵ -/+perigenic_pad bp, minus exons) - includes promoter elements,
132   ↵ introns, 3' elements
133 #intergenic sequences (everything else)
134 #given a valid gff3
135 function partition_genome_coordinates(gff3_path::String,
136   ↵ perigenic_pad::Integer=500)
137     # construct dataframes of scaffolds and metacoordinates
138     scaffold_df = build_scaffold_df(gff3_path)
139
140     #partition genome into intragenic, periexonic, and exonic coordinate
141       ↵ sets
142     #assemble exonic featureset
143     exon_df = DataFrame(SeqID = String[], Start = Integer[], End =
144       ↵ Integer[], Strand=Char[])
145     build_feature_df!(gff3_path, "CDS", "MT", exon_df)
146
147     #project exon coordinates onto the scaffold bitwise, merging
148       ↵ overlapping features and returning the merged dataframe
149     merged_exon_df = DataFrame(SeqID = String[], Start = Integer[], End =
150       ↵ Integer[], Strand=Char[])
151     @showprogress 1 "Partitioning exons..." for scaffold_subframe in
152       ↵ DataFrames.groupby(exon_df, :SeqID) # for each scaffold subframe
153       ↵ that has exon features
154       scaffold_id = scaffold_subframe.SeqID[1] #get the scaffold id
155       scaffold_bitarray = init_scaffold_bitarray(scaffold_id,
156         ↵ scaffold_df, false, stranded=true) #init a stranded bitarray
157         ↵ of scaffold length
158
159       ↵ project_features_to_bitarray!(scaffold_subframe,scaffold_bitarray)
160       ↵ #project features as Trues on bitarray of falses
161     merged_subframe = get_feature_df_from_bitarray(scaffold_id,
162       ↵ scaffold_bitarray) #get a feature df from the projected
163       ↵ bitarray

```

```

149      append!(merged_exon_df,merged_subframe) #append the merged
150      → scaffold df to the overall merged df
151
152      end
153
154      #assemble gene featureset
155      gene_df = DataFrame(SeqID = String[], Start = Integer[], End =
156      → Integer[], Strand = Char[])
157      build_feature_df!(gff3_path, "gene", "MT", gene_df)
158
159      perigenic_pad > 0 && add_pad_to_coordinates!(gene_df, scaffold_df,
160      → perigenic_pad) #if a perigenic pad is specified (to capture
161      → promoter/downstream elements etc in the periexonic set), apply it
162      → to the gene coords
163
164      #build intergenic coordinate set by subtracting gene features from
165      → scaffold bitarrays
166      intergenic_df = DataFrame(SeqID = String[], Start = Integer[], End =
167      → Integer[])
168      @showprogress 1 "Partitioning intergenic regions ... " for
169      → scaffold_subframe in DataFrames.groupby(scaffold_df, :SeqID) #
170      → for each scaffold subframe that has exon features
171      scaffold_id = scaffold_subframe.SeqID[1] #get the scaffold id
172      scaffold_bitarray = init_scaffold_bitarray(scaffold_id,
173      → scaffold_df, true) #init a bitarray of scaffold length
174      if any(isequal(scaffold_id),gene_df.SeqID) #if any genes on the
175      → scaffold
176      scaffold_genes=gene_df[findall(isequal(scaffold_id),
177      → gene_df.SeqID), :] #get the gene rows by finding the
178      → scaffold_id
179
180      → subtract_features_from_bitarray!(scaffold_genes,scaffold_bitarray)
181      → #subtract the gene positions from the scaffold bitarray
182
183      end
184      intragenic_subframe = get_feature_df_from_bitarray(scaffold_id,
185      → scaffold_bitarray) #get a feature df from the projected
186      → bitarray
187      append!(intergenic_df,intragenic_subframe) #append the merged
188      → scaffold df to the overall merged df
189
190      end
191
192      #build periexonic set by projecting gene coordinates onto the
193      → scaffold bitwise, then subtracting the exons

```

```

172 periexonic_df = DataFrame(SeqID = String[], Start = Integer[], End =
173   ↪ Integer[], Strand=Char[])
174 @showprogress 1 "Partitioning periexonic regions..." for
175   ↪ gene_subframe in DataFrames.groupby(gene_df, :SeqID) # for each
176   ↪ scaffold subframe that has exon features
177   scaffold_id = gene_subframe.SeqID[1] #get the scaffold id
178   scaffold_bitarray = init_scaffold_bitarray(scaffold_id,
179     ↪ scaffold_df, false, stranded=true) #init a bitarray of
180   ↪ scaffold length
181   project_features_to_bitarray!(gene_subframe,scaffold_bitarray)
182   ↪ #project features as Trues on bitarray of falses
183   if any(isequal(scaffold_id),merged_exon_df.SeqID)
184     scaffold_exons=merged_exon_df[findall(isequal(scaffold_id),
185       ↪ merged_exon_df.SeqID), :]
186
187     ↪ subtract_features_from_bitarray!(scaffold_exons,scaffold_bitarray)
188   end
189
190   periexonic_subframe = get_feature_df_from_bitarray(scaffold_id,
191     ↪ scaffold_bitarray) #get a feature df from the projected
192   ↪ bitarray
193   append!(periexonic_df,periexonic_subframe) #append the merged
194   ↪ scaffold df to the overall merged df
195
196 end

197
198 #partition_genome_coordinates() SUBFUNCTIONS
199 #BITARRAY SCAFFOLD REPRESENTATION SUBFUNCTIONS
200 function init_scaffold_bitarray(scaffold_id::String,
201   ↪ scaffold_df, value::Bool; stranded::Bool=false)
202   scaffold_length =
203     ↪ scaffold_df.End[findfirst(isequal(scaffold_id),
204       ↪ scaffold_df.SeqID)]
205   if stranded
206     value ? (return rscaffold_bitarray =
207       ↪ trues(scaffold_length,2)) : (return
208       ↪ rscaffold_bitarray = falses(scaffold_length,2))
209   else
210     value ? (return rscaffold_bitarray =
211       ↪ trues(scaffold_length,1)) : (return
212       ↪ rscaffold_bitarray = falses(scaffold_length,1))

```

```

196         end
197     end
198
199     function
200         → project_features_to_bitarray!(scaffold_feature_sf :: SubDataFrame,
201         → scaffold_bitarray :: BitArray)
202         @inbounds for item in eachrow(scaffold_feature_sf)
203             scaffold_bitarray[item.Start:item.End,1] = [true for
204                 → base in item.Start:item.End]
205             if size(scaffold_bitarray)[2] == 2 #if the bitarray
206                 → is stranded
207                 if item.Strand == '+'
208                     scaffold_bitarray[item.Start:item.End,2] =
209                         → [true for base in item.Start:item.End]
210             end
211         end
212     end
213
214     function
215         → subtract_features_from_bitarray!(scaffold_feature_sf :: DataFrame,
216         → scaffold_bitarray :: BitArray)
217         @inbounds for item in eachrow(scaffold_feature_sf)
218             scaffold_bitarray[item.Start:item.End,1] = [false for
219                 → base in item.Start:item.End]
220         end
221
222     function get_feature_df_from_bitarray(scaffold_id :: String,
223         → scaffold_bitarray :: BitArray)
224         size(scaffold_bitarray)[2] == 2 ? scaffold_feature_df =
225             → DataFrame(SeqID = String[], Start = Integer[], End =
226                 → Integer[], Strand=Char[]) : scaffold_feature_df =
227             → DataFrame(SeqID = String[], Start = Integer[], End =
228                 → Integer[])
229
230         new_feature_start = findnext(view(scaffold_bitarray,:,:1),
231             → 1)
232         while new_feature_start ≠ nothing # while new features
233             → are still found on the bitarray
234                 if size(scaffold_bitarray)[2] == 2 #if stranded,
235                     → get strand info

```

```

222             scaffold_bitarray[new_feature_start,2] == 1 ?
223                 new_feature_strand = '+'
224                 new_feature_strand = '-'
225             end
226         if
227             → findnext(!eval,view(scaffold_bitarray,:,:1),new_feature_start)
228             → ≠ nothing
229             new_feature_end =
230                 → findnext(!eval,view(scaffold_bitarray,:,:1),new_feature_start)
231                 → #find next false after feature start and
232                 → subtract 1 for feature end
233             else
234                 new_feature_end = size(scaffold_bitarray)[1]
235                     → #if none is found, the end of the feature
236                     → is the end of the scaffold
237             end
238             size(scaffold_bitarray)[2] == 2 ?
239                 → push!(scaffold_feature_df,[scaffold_id,
240                 → new_feature_start, new_feature_end,
241                 → new_feature_strand]) :
242                 → push!(scaffold_feature_df,[scaffold_id,
243                 → new_feature_start, new_feature_end]) #push
244                 → stranded feature info as appropriate
245             new_feature_start =
246                 → findnext(view(scaffold_bitarray,:,:1),
247                 → new_feature_end+1)
248             end
249         return scaffold_feature_df
250     end

```

16.6.11 /src/genome_sampling/sequence_sampler.jl

```

1 #####SAMPLING FUNCTIONS#####
2 #function for a Distributed worker to produce a set of samples of given
3 # parameters from genomic sequences
4 function get_sample_set(input_sample_jobs :: RemoteChannel,
5                         completed_sample_jobs :: RemoteChannel,
6                         progress_updates :: RemoteChannel)
7     while isready(input_sample_jobs)
8         genome_path, genome_index_path, partition_df, partitionid,
9             sample_set_length, sample_window_min, sample_window_max,
10            deterministic = take!(input_sample_jobs)

```

```

6
7     stranded::Bool = get_strand_dict()[partitionid]
8     scaffold_sequence_record_dict::Dict{String,LongSequence} =
9         → build_scaffold_seq_dict(genome_path, genome_index_path)
10
11    sample_df =
12        → DataFrame(SampleScaffold=String[], SampleStart=Integer[], SampleEnd=Integer[])
13    metacoordinate_bitarray = trues(partition_df.MetaEnd[end])
14    sample_set_counter = 0
15
16    while sample_set_counter < sample_set_length #while we don't yet
17        → have enough sample sequence
18        sample_scaffold::String, sample_Start::Integer,
19            → sample_End::Integer, sample_metaStart::Integer,
20            → sample_metaEnd::Integer, sample_sequence::LongSequence,
21            → strand::Char = get_sample(metacoordinate_bitarray,
22                → sample_window_min, sample_window_max, partition_df,
23                → scaffold_sequence_record_dict; stranded=stranded,
24                → deterministic=deterministic)
25        push!(sample_df,[sample_scaffold, sample_Start, sample_End,
26            → sample_sequence, strand]) #push the sample to the df
27        sample_length = sample_End - sample_Start + 1
28        sample_set_counter += sample_length #increase the counter by
29            → the length of the sampled sequence
30        metacoordinate_bitarray[sample_metaStart:sample_metaEnd] =
31            → [false for base in 1:sample_length] #mark these residues
32            → as sampled
33        put!(progress_updates, (partitionid,
34            → min(sample_set_counter,sample_set_length)))
35    end
36    put!(completed_sample_jobs,(partitionid, sample_df))
37
38 end
39
40 #get_sample_set() SUBFUNCTIONS
41 #function defining whether partitions respect stranding
42     → upon fetching sequence (ie is the sequence fetched in
43     → the feature strand orientation, or are we agnostic
44     → about the strand we sample?)
45 function get_strand_dict()
46     return
47         → Dict("exon"⇒true,"periexonic"⇒true,"intergenic"⇒false)
48 end
49

```

```

30 #function to obtain a dict of scaffold sequences from a
31   ↵ FASTA reader
32
33 function build_scaffold_seq_dict(genome_fa, genome_index)
34   genome_reader = open(FASTA.Reader, genome_fa,
35     ↵ index=genome_index)
36   seq_dict::Dict{String, LongSequence} =
37     ↵ Dict{String,FASTA.Record}()
38   @inbounds for record in genome_reader
39     id = FASTA.identifier(record)
40
41       ↵ seq_dict[rectify_identifier(id)]=FASTA.sequence(record)
42   end
43   close(genome_reader)
44   return seq_dict
45 end
46
47
48 # function to convert scaffold ID from that observed by
49   ↵ the masked .fna to the more legible one observed by
50   ↵ the GRCz11 GFF3
51
52 function rectify_identifier(scaffold_id::String)
53   if length(scaffold_id) ≥ 4 && scaffold_id[1:4] ==
54     ↵ "CM00" #marks chromosome scaffold
55     chr_code = scaffold_id[5:10]
56     chr_no = "$(Int((parse(Float64,chr_code)) -
57       ↵ 2884.2))"
58     return chr_no
59   else
60     return scaffold_id
61   end
62 end
63
64
65 #function to produce a single sample from a
66   ↵ metacoordinate set and the feature df
67
68 function get_sample(metacoordinate_bitarray::BitArray,
69   ↵ sample_window_min::Integer,
70   ↵ sample_window_max::Integer, partition_df::DataFrame,
71   ↵ scaffold_seq_dict::Dict{String,LongSequence};
72   ↵ stranded::Bool=false, deterministic::Bool=false)
73   proposal_acceptance = false
74   sample_metaStart = 0
75   sample_metaEnd = 0
76   sample_Start = 0
77   sample_End = 0

```

```

60             sample_sequence = LongSequence{DNAAlphabet{2}}("")  

61             sample_scaffold = ""  

62             strand = nothing  

63  

64             while proposal_acceptance == false  

65                 available_indices =  

66                     → findall(metacoordinate_bitarray) #find all  

67                     → unsampled indices  

68                 window = "FAIL"  

69                 start_index,feature_metaStart,feature_metaEnd,  

70                     → feature_length = 0,0,0,0  

71                 strand = '0'  

72                 while window == "FAIL"  

73                     start_index = rand(available_indices)  

74                         → #randomly choose from the unsampled  

75                         → indices  

76                     feature_metaStart, feature_metaEnd, strand =  

77                         → get_feature_params_from_metacoord(start_index,  

78                         → partition_df, stranded)  

79                     feature_length =  

80                         → length(feature_metaStart:feature_metaEnd)  

81                         → #find the metaboundaries of the feature  

82                         → the index occurs in  

83                     if feature_length ≥ sample_window_min #don't  

84                         → bother finding windows on features  

85                         → smaller than min  

86                     window =  

87                         → determine_sample_window(feature_metaStart,  

88                         → feature_metaEnd, start_index,  

89                         → metacoordinate_bitarray,  

90                         → sample_window_min, sample_window_max)  

91                         → #get an appropriate sampling window  

92                         → around the selected index, given the  

93                         → feature boundaries and params  

94                 end  

95             end  

96  

97             sample_scaffoldid, sample_scaffold_start,  

98                 → sample_scaffold_end =  

99                 → meta_to_feature_coord(window[1],window[2],partition_df)

```

```

80         proposal_sequence =
81             ↳ fetch_sequence(sample_scaffoldid,
82                             ↳ scaffold_seq_dict, sample_scaffold_start,
83                             ↳ sample_scaffold_end, strand;
84                             ↳ deterministic=deterministic) #get the
85                             ↳ sequence associated with the sample window
86
87
88
89
90     end
91
92     return sample_scaffold, sample_Start, sample_End,
93             ↳ sample_MetaStart, sample_MetaEnd,
94             ↳ sample_sequence, strand
95
96
97
98
99
100
101
102
103
#function to find a valid sampling window
function
    ↳ determine_sample_window(feature_MetaStart::Integer,
    ↳ feature_MetaEnd::Integer, metacoord::Integer,
    ↳ metacoord_bitarray::BitArray,
    ↳ sample_window_min::Integer,
    ↳ sample_window_max::Integer)
        window_start = 0
        window_end = 0
        feature_size = feature_MetaEnd - feature_MetaStart +
            ↳ 1
        feature_sampled = any(!eval,
            ↳ metacoord_bitarray[feature_MetaStart:feature_MetaEnd])
#attempt to construct the sample window by taking the
    ↳ whole feature
if !feature_sampled && feature_size <
    ↳ sample_window_max && feature_size >
    ↳ sample_window_min
        window_start = feature_MetaStart

```

```

104         window_end = feature_metaEnd
105         return window_start, window_end
106     else #if this fails, build the biggest window we can
107         ← from the sampling point, within the feature
108         featurepos = metacoord - feature_metaStart + 1
109         next_sampled_index = findnext(!eval,
110             ← metacoord_bitarray[feature_metaStart:feature_metaEnd],
111             ← featurepos)
112         prev_sampled_index = findprev(!eval,
113             ← metacoord_bitarray[feature_metaStart:feature_metaEnd],
114             ← featurepos)
115         next_sampled_index ≡ nothing ? (window_end =
116             ← feature_metaEnd) : (window_end =
117             ← next_sampled_index + feature_metaStart - 1)
118         prev_sampled_index ≡ nothing ? (window_start =
119             ← feature_metaStart) : (window_start =
120             ← prev_sampled_index + feature_metaStart - 1)
121         windowsize = window_end - window_start + 1
122         #check to see if this window is bigger than the
123         ← min, if not, return a failure code
124         windowsize < sample_window_min && return "FAIL"
125         #check to see if this window is bigger than the
126         ← max, if so, trim it before returning,
127         ← removing as evenly as possible around the
128         ← metacoordinate
129         if windowsize > sample_window_max
130             bases_to_trim = windowsize -

```

```

131                     end
132             return window_start, window_end
133         end
134     end
135
136
137     # function to check for repetitive stretches or
138     #→ degenerate bases in proposal sequence
139     function mask_check(proposal_sequence::LongSequence)
140         proposal_acceptance = true
141         if hasambiguity(proposal_sequence) ||
142             → isrepetitive(proposal_sequence,
143             → (length(proposal_sequence) ÷ 10))
144             proposal_acceptance = false
145         end
146         return proposal_acceptance
147     end
148
149
150 ######SHARED SEQUENCE FETCHER#####
151 # function to get proposal sequence from dict of scaffold sequences,
152 #→ given coords and scaffold id
153 function fetch_sequence(scaffold_id::String,
154     → scaffold_seq_dict::Dict{String, LongSequence},
155     → proposal_start::Integer, proposal_end::Integer, strand::Char;
156     → deterministic=false)
157     if strand == '0' #unstranded samples may be returned with no
158         → preference in either orientation
159         deterministic ? strand = '+' : (rand(1)[1] ≤ .5 ? strand = '+' :
160             → strand = '-')
161     end
162     if strand == '+'
163         proposal_sequence =
164             → scaffold_seq_dict[scaffold_id][proposal_start:proposal_end]
165     elseif strand == '-'
166         proposal_sequence =
167             → reverse_complement(scaffold_seq_dict[scaffold_id][proposal_start:proposal_end])
168     else
169         throw(ArgumentError("Invalid sample code! Must be '+', '-' , or
170             → '0' (random strand)"))
171     end
172
173     return proposal_sequence
174 end

```

```

162
163 #####SHARED BASIC COORDINATE SUBFUNCTIONS#####
164 # function to push scaffold ID, start, and end points of given
165 #       ↳ featuretype to supplied dataframe
166 function build_feature_df!(GFF3_path::String, feature_type::String,
167   ↳ scaffold_exclusion::String, feature_df::DataFrame)
168   reader = open(GFF3.Reader, GFF3_path) # access the GFF3
169   @inbounds for record in reader # iterate over Gff3 records
170     if GFF3.isfeature(record) # if the record is a feature
171       if GFF3.featuretype(record) == feature_type #if the features
172         ↳ is of the requested type, get the following info
173         seqID = GFF3.seqid(record)
174         if seqID != scaffold_exclusion
175           seq_start = GFF3.seqstart(record)
176           seq_end = GFF3.seqend(record)
177           if feature_type == "CDS" || feature_type == "gene"
178             seq_strand = convert(Char, GFF3.strand(record))
179             push!(feature_df, [seqID, seq_start, seq_end,
180               ↳ seq_strand])
181           else
182             push!(feature_df, [seqID, seq_start, seq_end]) #
183               ↳ push relevant info to the df
184           end
185         end
186       end
187     end
188     close(reader)
189   end
190
191   #function to assemble dataframe of scaffold coords + metacoords given
192   ↳ gff3
193   function build_scaffold_df(gff3_path)
194     scaffold_df = DataFrame(SeqID = String[], Start = Integer[], End =
195       ↳ Integer[])
196     build_feature_df!(gff3_path, "supercontig", "MT", scaffold_df)
197     build_feature_df!(gff3_path, "chromosome", "MT", scaffold_df)
198     add_metacoordinates!(scaffold_df)
199     return scaffold_df
200   end
201
202   #function to add pad to either side of some featureset

```

```

197 function add_pad_to_coordinates!(feature_df::DataFrame,
198   → scaffold_df::DataFrame, pad_size::Integer;
199   → col_symbols::Array{Symbol}=[:Start, :End])
200   pad_start_array = zeros(Integer,size(feature_df,1))
201   pad_end_array = zeros(Integer,size(feature_df,1))
202   feature_df[!, col_symbols[1]] = [max(feature_df.Start[i]-pad_size,1)
203     → for i in 1:size(feature_df,1)] #truncate pads at beginning and
204     → end of scaffolds
205   feature_df[!, col_symbols[2]] =
206     → [min(feature_df.End[i]+pad_size,scaffold_df.End[findfirst(isequal(feature_df.
207       → .Start, scaffold_df.Start))],1)
208     → for i in 1:size(feature_df,1)]
209 end
210
211
212 ####SHARED METACOORDINATE FUNCTIONS####
213 # function to add a metacoordinate column to a dataframe of scaffold
214   → positions, allowing sampling across all scaffolds
215 function add_metacoordinates!(feature_df::DataFrame)
216   meta_start_array = Integer[]
217   meta_end_array = Integer[]
218   metaposition = 1
219   @inbounds for feature in eachrow(feature_df)
220     push!(meta_start_array, (metaposition))
221     metaposition += feature.End - feature.Start
222     push!(meta_end_array, (metaposition))
223     metaposition += 1
224   end
225   feature_df.MetaStart = meta_start_array # metacoordinate contains
226     → 'start' metaposition across all genomic material
227   feature_df.MetaEnd = meta_end_array # metacoordinate contains 'end'
228     → metaposition across all genomic material
229 end
230
231
232 # function to convert metacoordinate set to scaffold-relative coordinates
233 function meta_to_feature_coord(meta_start::Integer, meta_end::Integer,
234   → feature_df::DataFrame)
235   feature_row = get_feature_row_index(feature_df, meta_start)
236   seqid = feature_df.SeqID[feature_row]
237   scaffold_start = feature_df.Start[feature_row] + (meta_start -
238     → feature_df.MetaStart[feature_row])
239   scaffold_end = feature_df.End[feature_row] -
240     → (feature_df.MetaEnd[feature_row] - meta_end)
241   return seqid, scaffold_start, scaffold_end
242 end
243

```

```

228
229 #function to obtain the feature boundaries and strand of the feature that
  ↳ a metacoordinate falls within
230 function get_feature_params_from_metacoord(metacoordinate::Integer,
  ↳ feature_df::DataFrame, stranded::Bool)
231     feature_row = get_feature_row_index(feature_df, metacoordinate)
232     feature_metaStart = feature_df.MetaStart[feature_row]
233     feature_metaEnd = feature_df.MetaEnd[feature_row]
234     stranded ? feature_strand = feature_df.Strand[feature_row] :
  ↳ feature_strand = '0'
235     return feature_metaStart, feature_metaEnd, feature_strand
236 end
237
238 #function obtain the index of a feature given its metacoordinate
239 function get_feature_row_index(feature_df::DataFrame,
  ↳ metacoordinate::Integer)
240     total_feature_bases = feature_df.MetaEnd[end]
241     if metacoordinate == total_feature_bases #edge case of metacoord at
  ↳ end of range
        return size(feature_df,1) #last index in df
242     else
243         index =
244             findfirst(end_coord→end_coord>metacoordinate, feature_df.MetaEnd)
  ↳ #find the index of the feature whose end metacoord is > the
  ↳ query metacoord
245         if index > 1 && metacoordinate == feature_df.MetaEnd[index-1] #if
  ↳ the metacoordinate is the last base of the previous feature
246             if metacoordinate ≥ feature_df.MetaStart[index-1] &&
  ↳ metacoordinate ≤ feature_df.MetaEnd[index-1] #confirm
  ↳ that the metacoord is in the previous feature
                  return index-1 #return the previous feature index
247             end
248         elseif metacoordinate ≥ feature_df.MetaStart[index] &&
  ↳ metacoordinate ≤ feature_df.MetaEnd[index] #else confirm
  ↳ that the metacoordinate is in the found feature
                  return index #return the found feature index
249     else
250         throw(DomainError("Unexpected metacoordinate $metacoordinate
  ↳ in partition of $total_feature_bases bases, with feature
  ↳ start $(feature_df.MetaStart[index]), end
  ↳ $(feature_df.MetaEnd[index]))")
251     end
252 end
253
254 end

```

 255 end

16.6.12 /src/likelihood_funcs/bg_lh_matrix.jl

```

1 #function to obtain positional likelihoods for a sequence under a given
2   ↳ BHMM.
3 function get_BGHMM_symbol_lh(seq::AbstractMatrix, hmm::BHMM)
4   @assert size(seq)[1] == 1
5   (seq=Array(transpose(seq))) # one sequence at a time only
6   symbol_lhs = zeros(length(seq))
7   length_mask = [length(seq)-1]
8
9   lls = bw_llhs(hmm, seq) #obtain log likelihoods for sequences and
10    ↳ states
11  log_α = messages_forwards_log(hmm.a, hmm.A, lls, length_mask) #get
12    ↳ forward messages
13  log_β = messages_backwards_log(hmm.A, lls, length_mask) # get
14    ↳ backwards messages
15
16  #calculate observation probability and γ weights
17  K,Tmaxplus1,Strand = size(lls) #the last T value is the 0 end marker
18    ↳ of the longest T
19
20  #transforms to cut down log_ξ, log_γ assignment times
21  lls = permutedims(lls, [2,1,3]) # from (K,T) to (T,K)
22  log_α = permutedims(log_α, [2,1,3])
23  log_β = permutedims(log_β, [2,1,3])
24
25  log_γ = fill(-Inf, Tmaxplus1,K)
26  log_pobs = logsumexp(lps.(log_α[1,:], log_β[1,:]))
27
28  @inbounds for i = 1:K, t = 1:length_mask[1]
29      log_γ[t,i] = lps(log_α[t,i],log_β[t,i],-log_pobs)
30  end
31
32  for t in 1:length_mask[1]
33      symbol_lh::AbstractFloat = -Inf #ie log(p=0)
34      for k = 1:K #iterate over states
35          state_symbol_lh::AbstractFloat = lps(log_γ[t,k],
36            ↳ log(hmm.B[k].p[seq[t]])) #state symbol likelihood is
37            ↳ the γ weight * the state symbol probability (log
38            ↳ implementation)

```

```

31         symbol_lh = logaddexp(symbol_lh, state_symbol_lh) #sum
32             ↵ the probabilities over states
33     end
34     symbol_lhs[t] = symbol_lh
35   end
36
37   return symbol_lhs[1:end-1] #remove trailing index position
38 end
39
40 #function to calculate BGHMM from an observation set and a dict of BGHMMs
41 function BGHMM_likelihood_calc(observations::DataFrame, BGHMM_dict::Dict,
42   ↵ code_partition_dict = get_partition_code_dict(false); symbol=:PadSeq)
43   lh_matrix_size = ((findmax(length.(collect(values(observations[!,,
44   ↵ symbol]))))[1]), length(observations[!, symbol]))
45   BGHMM_lh_matrix = zeros(lh_matrix_size) #T, Strand, 0
46
47   #@showprogress 1 "Writing frags to matrix.."
48   Threads.@threads for (jobid, frag) in BGHMM_fragments
49     (frag_start, o, partition, strand) = jobid
50
51     partition_BGHMM::BHMM =
52       ↵ BGHMM_dict[code_partition_dict[partition]]
53     no_symbols = length(partition_BGHMM.B[1].p)
54     order = Int(log(4,no_symbols) - 1)
55
56     order_seq = get_order_n_seqs([frag], order)
57     coded_seq = code_seqs(order_seq)
58
59     subseq_symbol_lh = get_BGHMM_symbol_lh(coded_seq,
60       ↵ partition_BGHMM)
61
62     if strand == -1
63       subseq_symbol_lh = reverse(subseq_symbol_lh)
64     end #positive, unstranded frags are inserted as-is
65     BGHMM_lh_matrix[frag_start:frag_start+length(frag)-1,o] =
66       ↵ subseq_symbol_lh
67   end
68
69   return BGHMM_lh_matrix

```

```

67 end

68

69 function fragment_observations_by_BGHMM(seqs :: AbstractVector,
70   ↳ masks :: AbstractVector)
71   likelihood_jobs = Vector{Tuple{Tuple, LongSequence{DNAAlphabet{2}}}}()
72   @showprogress 1 "Fragmenting observations by partition ... " for (o,
73     ↳ obs_seq) in enumerate(seqs)
74     mask = masks[o]
75     frags = Vector{LongSequence{DNAAlphabet{2}}}()

76     frag = LongSequence{DNAAlphabet{2}}()

77     frag_end=0
78     frag_start = 1

79     while frag_start < length(obs_seq) # while we're not at the
80       ↳ sequence end
81       curr_partition = mask[frag_start,1] #get the partition code
82       ↳ of the frag start
83       curr_strand = mask[frag_start,2] #get the strand of the frag
84       ↳ start

85       #JOBID COMPOSED HERE
86       jobid = (frag_start, o, curr_partition, curr_strand) #compose
87       ↳ an identifying index for this frag

88       findnext(!isequal(curr_partition),mask[:,1],frag_start) ≠
89         ↳ nothing ? frag_end =
90         ↳ findnext(!isequal(curr_partition),mask[:,1],frag_start)
91         ↳ -1 : frag_end = length(obs_seq) #find the next position
92         ↳ in the frag that has a different partition mask value
93         ↳ from hte current one and set that position-1 to frag end,
94         ↳ alternately frag end is end of the overall sequence
95       frag = obs_seq[frag_start:frag_end] #get the frag bases
96       if curr_strand == -1 #if the fragment is reverse stranded
97         ↳ reverse_complement!(frag) #use the reverse complement
98         ↳ sequence
99       end

100      push!(likelihood_jobs,(jobid, frag)) #put the frag in the
101      ↳ jobs vec
102      frag_start = frag_end + 1 #move on
103    end

```

```

95     end
96     return likelihood_jobs
97 end

```

16.6.13 /src/likelihood_funcs/hmm.jl

```

1 function obs_lh_given_hmm(observations, hmm; linear=true)
2     linear ? (return linear_likelihood(observations, hmm)) : (return
3         ↳ bw_likelihood(Matrix(transpose(observations)), hmm))
4 end
5
6 function bw_likelihood(observations, hmm)
7     obs_lengths = [findfirst(iszero, observations[:,o])-1 for o in
8         ↳ 1:size(observations,2)]
9
10    lls = bw_llhs(hmm, observations)
11    log_α = messages_forwards_log(hmm.a, hmm.A, lls, obs_lengths)
12    log_β = messages_backwards_log(hmm.A, lls, obs_lengths)
13
14    O = size(lls)[3] #the last T value is the 0 end marker of the longest
15    ↳ T
16    log_pobs = zeros(O)
17    Threads.@threads for o in 1:O
18        log_pobs[o] = logsumexp(lps.(log_α[:,1,o], log_β[:,1,o]))
19    end
20
21    return sum(log_pobs)
22 end
23
24 function linear_likelihood(observations,hmm)
25     O= size(observations,1);
26     obs_lengths = [findfirst(iszero,observations[o,:])-1 for o in
27         ↳ 1:size(observations,1)] #mask calculations here rather than
28         ↳ mle_step to prevent recalculation every iterate
29
30     a = transpose(log.(hmm.a)); A = log.(hmm.A)
31     N = length(hmm.B)
32     mask=observations.≠0
33     #INITIALIZATION
34     βoi_T = zeros(0,N); βoi_t = zeros(0,N) #log betas at T initialised as
35         ↳ zeros

```

```

31 #RECURRENCE
32 βoi_T = backwards_lh_sweep!(hmm, A, N, βoi_T, βoi_t, observations,
33   ↵ mask, obs_lengths)
34
35 #TERMINATION
36 lls = c_llhs(hmm,observations[:,1])
37 α1om = lls .+ a #first position forward msgs
38
39 return lps([logsumexp(lps.(α1om[o,:], βoi_T[o,:])) for o in 1:o])
40 end
41
42           #LINEAR_STEP SUBFUNCS
43 function backwards_lh_sweep!(hmm, A, N, βoi_T, βoi_t,
44   ↵ observations, mask, obs_lengths)
45   for t in maximum(obs_lengths)-1:-1:1
46     last_β=copy(βoi_T)
47     lls = c_llhs(hmm,observations[:,t+1])
48     omask = findall(mask[:,t+1])
49     βoi_T[omask,:] .+= view(lls,omask,:)
50     Threads.@threads for m in 1:N
51       βoi_t[omask,m] =
52         ↵ logsumexp.(eachrow(view(βoi_T,omask,:).+transpose(view(
53           ↵ βoi_T=copy(βoi_t)
54         end
55         ↵ return βoi_T
56       end
57     end

```

16.6.14 /src/reports/chain_report.jl

```
1 struct Chain_Report
2     id::Chain_ID
3     final_hmm::BHMM
4     test_lh::AbstractFloat
5     naive_lh::AbstractFloat
6     final_delta::AbstractFloat
7     state_run_lengths::AbstractVector{AbstractFloat}
8     convergence_values::Chains
9     convergence_diagnostic :: ChainDataFrame
10    converged :: Bool
11 end
12
13 function Base.show(io :: IO, report :: Chain_Report)
```

```

14 nominal_dict=Dict(0=>"th",1=>"st",2=>"nd",3=>"rd",4=>"th",5=>"th")
15 haskey(nominal_dict,report.id.order) ?
16   → (nom_str=nominal_dict[report.id.order]) : (nom_str="th")
17 println(io, "BHMM EM Chain Results\n"; bold=true)
18 println(io, "$(report.id.K)-state, $(report.id.order)$nom_str order
19   → BHMM")
20 println(io, "Trained on observation set \$(report.id.obs_id)\n")
21 report.test_lh > report.naive_lh ? (lh_str="greater";
22   → lh_color=:green) : (lh_str="less"; lh_color=:red)
23 println(io, "Test logP(0|θ): $(report.test_lh), $lh_str than the
24   → naive model's $(report.naive_lh)\n"; color=lh_color)
25 println(io, "Replicate $(report.id.replicate)")
26 report.converged ? println(io, "Converged with final step δ
27   → $(report.final_delta)") : println(io, "Failed to converge!")
28 println(io, "-----")
29   → -----
30   → -----
31   → -----
32   → -----
33   → -----
34   → zidx=findfirst(!iszero,report.convergence_values["logP(0|θ)"].data)[1]
35   → lh_vec=Vector([report.convergence_values["logP(0|θ)"].data ... ][zidx:end])
36   → lh_plot=lineplot([zidx:length(lh_vec)+zidx-1 ... ],lh_vec;title="Chain
37   → likelihood evolution", xlabel="Training iterate",
38   → xlim=(0,length(lh_vec)+zidx+1), ylim=
39   → (floor(minimum(lh_vec),sigdigits=2),ceil(maximum(lh_vec),sigdigits=2)),
40   → name="logP(0|θ)")
41 lineplot!(lh_plot,[report.naive_lh for i in 1:length(lh_vec)],
42   → color=:magenta,name="naive")
43 show(io, lh_plot)
44 println(io, "\n")
45 k1vec=Vector([report.convergence_values["K1"].data ... ])

```

```

40     k_plot=lineplot(k1vec, title="State p(Auto) evolution",
41         ← xlabel="Training iterate", ylabel="prob",
42         ← name="K1",ylim=(0,1),xlim=(0,length(k1vec)))
43     for k in 2:report.id.K
44         kvec=Vector([report.convergence_values["K$k"].data ... ])
45         lineplot!(k_plot,kvec,name="K$k")
46     end
47     show(io,k_plot)
48     println(io)
49
50     printstyled(io, "\nConvergence Diagnostics\n",bold=true)
51     if report.convergence_diagnostic.name == "short"
52         printstyled(io, "Convergence diagnostics unavailable for chains
53             ← <10 steps!\n", color=:yellow)
54     elseif report.convergence_diagnostic.name == "error"
55         printstyled(io, "Convergence diagnostics errored! Zeros in
56             ← autotransition matrix?\n", color=:red)
57     else
58         all(Bool.(report.convergence_diagnostic.nt.stationarity)) &&
59             all(Bool.(report.convergence_diagnostic.nt.test)) ?
60                 printstyled(io, "Likelihood and autotransition probabilites
61                     ← converged and passing tests.\n", color=:green) :
62                     printstyled(io, "Not all parameters converged or passing
63                         ← tests!\n",color=:red)
64         display(report.convergence_diagnostic)
65     end
66   end
67 end
68
69 function latex_report(report::Chain_Report)
70
71 end
72
73 function report_chains(chains::Dict{Chain_ID,Vector{EM_step}},
74     ← test_sets::Dict{String,Vector{LongSequence{DNAAlphabet{2}}}});
75     ← naive_hmm=BHMM(ones(1,1),[Categorical(4)])
76     job_ids=[id for (id,chain) in chains] # make list of chain_ids
77     partitions=unique([id.obs_id for id in job_ids]) #get list of unique
78         ← partitions represented among chain_ids
79     naive_order = Integer(log(4,length(naive_hmm.B[1].support))-1)
80     for partition in partitions #check that all partitions are available
81         ← for testing and make sure codes for naive hmm are generated by
82         ← adding job_ids

```

```

68     !(partition in keys(test_sets)) &&
69         → throw(ArgumentError("Observation set $partition required by
70             → chains for testing not present in test sets!"))
71
72     push!(job_ids,
73         → Chain_ID(partition,length(naive_hmm.a),naive_order, 1))
74 end
75
76 code_dict=code_job_obs(job_ids, test_sets) #code all the obs sets
77     → that are necessary ot perform our tests
78 naive_lhs=Dict{String,Float64}() #construct dict of naive likelihoods
79     → for all partitions to be tested prior to individual tests
80 @showprogress 1 "Testing naive hmm..." for
81     → ((partition,order),obs_set) in code_dict
82     order=naive_order &&
83         → (naive_lhs[partition]=obs_lh_given_hmm(code_dict[(partition,naive_order)])
84 end
85
86 reports=Dict{Chain_ID,Chain_Report}()
87 @showprogress 1 "Testing chains..." for (id, chain) in chains
88     chains_array=zeros(length(chain),1+id.K)
89     for (n,step) in enumerate(chain)
90         chains_array[n,1]=step.log_p
91         chains_array[n,2:end]=get_diagonal_array(step.hmm)
92     end
93     convergence_values=Chains(chains_array,[ "logP(0|θ)",
94         → ["K"*string(i) for i in 1:id.K] ... ])
95     if length(chain) ≥ 10
96         try
97             convergence_diagnostic=heideldiag(convergence_values)[1]
98         catch
99             → convergence_diagnostic=ChainDataFrame("error",(:;a⇒[1.]))
100         end
101     else
102         convergence_diagnostic=ChainDataFrame("short",(:;a⇒[1.]))
103     end
104
105     id.K > 1 ?
106         → (mean_run_lengths=sim_run_lengths(get_diagonal_array(chain[end].hmm),1000
107         → : (mean_run_lengths=[Inf])
108         test_lh=obs_lh_given_hmm(code_dict[(id.obs_id,id.order)],
109         → chain[end].hmm)

```

```

98     reports[id]=Chain_Report(id, chain[end].hmm, test_lh,
99         ← naive_lhs[id.obs_id], chain[end].delta, mean_run_lengths,
100        ← convergence_values, convergence_diagnostic,
101        ← chain[end].converged)
102 end
103
104 function get_diagonal_array(hmm::BHMM)
105     k = length(hmm.a)
106     diagonal = zeros(k)
107     @inbounds for i in 1:k
108         diagonal[i] = hmm.A[i,i]
109     end
110     return diagonal
111 end
112
113 #function to simulate run lengths for vector of diagonal values
114 function sim_run_lengths(diagonal_value::AbstractArray, samples::Integer)
115     mean_run_lengths = zeros(length(diagonal_value))
116     for (i, value) in enumerate(diagonal_value)
117         if value == 0.
118             mean_run_lengths[i]=0.
119         elseif value ==1.
120             mean_run_lengths[i]=Inf
121         else
122             runlengths = zeros(Integer, samples)
123             for s in 1:samples
124                 run = true
125                 runlength = 0
126                 while run
127                     runlength += 1
128                     if rand(1)[1] > value
129                         run = false
130                     end
131                 end
132                 runlengths[s] = runlength
133             end
134             mean_run_lengths[i] = mean(runlengths)
135         end
136     end
137     return mean_run_lengths

```

138 end

16.6.15 /src/reports/partition_report.jl

```

1 struct Order_Report
2     converged_K::Vector{Float64}
3     converged_lh::Vector{Float64}
4     failed_K::Vector{Float64}
5     failed_lh::Vector{Float64}
6 end
7
8 struct Partition_Report
9     partition_id::String
10    naive_lh::Float64
11    orddict::Dict{Integer,Order_Report}
12    best_model::Tuple{Chain_ID,BHMM}
13    best_repset::Vector{Chain_ID}
14 end
15
16 function Base.show(io::IO, report::Partition_Report)
17     printstyled(io, "BACKGROUND HMM TRAINING REPORT\n", bold=true)
18     printstyled(io, "Genome partition id: $(report.partition_id)\n",
19                 ↪ bold=true, color=:magenta)
20
21     for (order, ordreport) in report.orddict
22         if length(ordreport.failed_lh)>0
23             ordplot=scatterplot(ordreport.converged_K,
24                                 ↪ ordreport.converged_lh; name="Converged", title="Order
25                                 ↪ $order HMMs", xlabel="K# States", ylabel="P(O|θ)",
26                                 ↪ color=:green, xlim=(1,maximum(ordreport.converged_K)),
27                                 ↪ ylim=(floor(minimum(ordreport.converged_lh)),minimum(ordreport.fai
28             length(ordreport.failed_K) ≥ 1 && scatterplot!(ordplot,
29                 ↪ ordreport.failed_K, ordreport.failed_lh;
30                 ↪ name="Unconverged",color=:red)
31             lineplot!(ordplot, [report.naive_lh for i in
32                 ↪ 1:maximum(ordreport.converged_K)],
33                 ↪ name="Naive",color=:magenta)
34             show(ordplot)
35             println()
36
37         else

```

```

28         ordplot=scatterplot(ordreport.converged_K,
29             ←  ordreport.converged_lh; name="Converged", title="Order
30             $order HMMs", xlabel="K# States", ylabel="P(O|θ)",
31             ←  color=:green, xlim=(1,maximum(ordreport.converged_K)),
32             ←  ylim=(floor(min(minimum(ordreport.converged_lh),report.naive_lh),sigd
33             lineplot!(ordplot, [report.naive_lh for i in
34                 ←  1:maximum(ordreport.converged_K)],
35                 ←  name="Naive",color=:magenta)
36             show(ordplot)
37             println()
38         end
39     end
40 end
41
42 function report_partitions(chain_reports::Dict{Chain_ID,Chain_Report})
43     partitions=Vector{String}()
44     orders=Vector{Integer}()
45     Ks=Vector{Integer}()
46     reps=Vector{Integer}()
47     for id in keys(chain_reports)
48         !in(id.obs_id, partitions) && push!(partitions, id.obs_id)
49         !in(id.order, orders) && push!(orders, id.order)
50         !in(id.K, Ks) && push!(Ks, id.K)
51         !in(id.replicate, reps) && push!(reps, id.replicate)
52     end
53
54     reports=Vector{Partition_Report}()
55     for partition in partitions
56         best_lh,best_rep=-Inf,Chain_ID("empty",1,0,1)
57         naive_lh=1.
58         orddict=Dict{Integer,Order_Report}()
59         for order in orders
60             conv_statevec=Vector{Float64}()
61             fail_statevec=Vector{Float64}()
62             conv_lh_vec=Vector{Float64}()
63             fail_lh_vec=Vector{Float64}()
64             for (id,report) in chain_reports
65                 if id.obs_id==partition && id.order==order
66                     report.test_lh > best_lh &&
67                         (best_lh=report.test_lh;best_rep=id)
68                     naive_lh=1. && (naive_lh=chain_reports[id].naive_lh)
69                     chain_reports[id].converged ?
70                         (push!(conv_statevec,id.K);

```

```

63         push!(conv_lh_vec,chain_reports[id].test_lh)) :
64             ↳ (push!(fail_statevec,id.K);
65             push!(fail_lh_vec,chain_reports[id].test_lh))
66     end
67
68         ↳ orddict[order]=Order_Report(conv_statevec,conv_lh_vec,fail_statevec,f
69     end
70     best_model=(best_rep,chain_reports[best_rep].final_hmm)
71     best_repset=[Chain_ID(partition, best_rep.K, best_rep.order, rep)
72         ↳ for rep in reps]
73
74         ↳ push!(reports,Partition_Report(partition,naive_lh,orddict,best_model,best_
75     end
76     return reports
77 end

```

16.6.16 /src/reports/replicate_convergence.jl

```

1 struct Replicate_Report
2     ids::Vector{Chain_ID} #replicates
3     state_vecs::Dict{Chain_ID,Matrix{Float64}} #vectors of autotransition
4         ↳ probabilities evolving by iterate, concatenated to matrices,
5         ↳ indexed by Chain_ID
6     emission_arrays::Dict{Chain_ID,Array{Float64}}
7         ↳ #iterate*symbol*state_vecs
8     sorted_id1_states::Vector{Integer}
9     sort_dicts::Dict{Chain_ID,Dict{Integer, Integer}}#emission channels
10        ↳ sorted on the basis of euclidean closeness to ids[1]
11     sorted_symbols::Dict{Integer,Vector{Integer}}
12
13     Replicate_Report(ids,state_vecs,emission_arrays,
14         ↳ sorted_id1_states,sort_dicts,sorted_symbols) =
15         ↳ assert_repreport(ids) &&
16         ↳ new(ids,state_vecs,emission_arrays,sorted_id1_states,sort_dicts,sorted_symbols)
17 end
18
19 function assert_repreport(ids::Vector{Chain_ID})
20     Ks=Vector{Int64}()
21     orders=Vector{Int64}()
22     replicates=Vector{Int64}()

```

```

17     obsids=Vector{String}()
18
19     for id in ids
20         push!(Ks,id.K)
21         push!(orders,id.order)
22         push!(obsids,id.obs_id)
23         push!(replicates, id.replicate)
24     end
25
26     length(unique(Ks)) > 1 && throw(ArgumentError("Replicate set includes
27     ↳ chains with different K state numbers! All chains must have HMMs
28     ↳ with the same number of states."))
29     length(unique(orders)) > 1 && throw(ArgumentError("Replicate set
30     ↳ includes chains with different order coding numbers! All chains
31     ↳ must have HMMs with the same order coding."))
32     !allunique(replicates) && throw(ArgumentError("Replicate set includes
33     ↳ chains with the same replicate #. Replicates should be unique!"))
34
35     return true
36   end
37
38   function
39     ↳ report_replicates(repset::Vector{Chain_ID},chains::Dict{Chain_ID,Vector{EM_step}})
40     assert_repreport(repset) #check that the repset will pass its
41     ↳ constructor before doing the work
42     emission_arrays=Dict{Chain_ID,AbstractArray{AbstractFloat}}()
43     state_arrays=Dict{Chain_ID,AbstractArray{AbstractFloat}}()
44
45     for id in repset
46       emission_array=zeros(length(chains[id]),4^(id.order+1),id.K)
47       state_array=zeros(length(chains[id]),id.K)
48       for (it, step) in enumerate(chains[id])
49         for k in 1:id.K
50           emission_array[it,:,k]=step.hmm.B[k].p
51           state_array[it,k]=step.hmm.A[k,k]
52         end
53       end
54       emission_arrays[id]=emission_array
55       state_arrays[id]=state_array
56     end
57
58     println(repset)
59     println(emission_arrays)

```

```

53    println(state_arrays)
54
55    ↵  sort_dicts,sorted_id1_states,sorted_symbols=sort_emitters_by_distance!(repset
56
57    return
58      ↵  Replicate_Report(repset,state_arrays,emission_arrays,sorted_id1_states,
59      ↵  sort_dicts,sorted_symbols)
60 end
61
62 function sort_emitters_by_distance!(repset,emission_arrays)
63   id1=repset[1]
64   length(repset)==1 && (return
65     ↵  Dict{Chain_ID,Dict{Integer,Integer}}(),[1:id1.K...],[1:4^id1.order+1])
66
67   final_emissions =
68     ↵  zeros(length(repset),size(emission_arrays[id1])[2:3]...)
69   for (n,id) in enumerate(repset)
70     final_emissions[n,:,:] = emission_arrays[id][end,:,:]
71   end
72
73   distances=zeros(length(repset)-1,id1.K,id1.K)
74   for rep in 1:length(repset)-1
75     for k in 1:id1.K, j in 1:id1.K
76       ↵  distances[rep,k,j]=euclidean(final_emissions[1,:,:k],final_emissions[1
77     end
78   end
79
80   sortdicts=Dict{Chain_ID,Dict{Integer,Integer}}()
81   sorted_id1_states=Vector{Integer}()
82   sorted_symbols=Dict{Integer,Vector{Integer}}()
83   for rep in 1:length(repset)-1
84     sortdicts[repset[rep+1]]=Dict{Integer,Integer}()
85   end
86
87   for i in 1:id1.K
88     id1_mindist_K=findmin([sum([minimum(distances[rep,k,:]) for rep
89       ↵  in 1:length(repset)-1]) for k in 1:id1.K])[2]#find the state
90       ↵  from the first replicate that has minimum cumulative distance
91       ↵  to the closest state in the other replicates, excluding
92       ↵  already-chosen states by spiking their values
93     push!(sorted_id1_states,id1_mindist_K)
94     for rep in 1:length(repset)-1

```

```

86         rep_k=findmin(distances[rep,id1_mindist_K,:])[2]
87         sortdicts[repset[rep+1]][id1_mindist_K]=rep_k
88         distances[rep,:,:rep_k] *= 1.0 #mask
89         distances[1,id1_mindist_K,:] *= 1.0 #mask
90     end
91
92
93     → symbol_distances = sum(hcat([euclidean.(final_emissions[1,:,:id1_mindist_K],
94     → for (n,id) in enumerate(repset[2:end])) ... ), dims=2)
95     sorted_symbols[id1_mindist_K] = sortperm(symbol_distances[:,1])
96 end
97
98
99
100 function Base.show(io::IO, report::Replicate_Report;
101   → top_states::Integer=report.ids[1].K, top_symbols::Integer=2)
102   printstyled(io, "REPLICATE CONVERGENCE REPORT\n", color=:green)
103   println(" -----")
104   → -----
105   → -----
106   for i in 1:top_states
107     id=report.ids[1]
108     id1_state=report.sorted_id1_states[i]
109     itmax=maximum([size(report.state_vecs[id],1) for id in
110       → report.ids])
111
112     autoplt=lineplot(report.state_vecs[id][:,id1_state],
113       title="Autotransition prob. convergence",
114       name="$(report.ids[1]) K$id1_state",
115       xlim=(0,ceil(itmax, sigdigits=2)),
116       ylim=(0,1),
117       xlabel="iterate",
118       ylabel="p")
119
120     → symplot=lineplot(report.emission_arrays[id][:,report.sorted_symbols[id1_st

```

```
121         ylim=(0,1),  
122         xlabel="Symbol 1 p",  
123         ylabel="S2 p")  
124  
125     for (n,id) in enumerate(report.ids)  
126         if n > 1  
127             lineplot!(autoplt,  
128                     → report.state_vecs[id][:,report.sort_dicts[id][id1_state]],  
129                     name="$(report.ids[n])"  
130                     → K$(report.sort_dicts[id][id1_state]))  
131             lineplot!(symplot,  
132                     → report.emission_arrays[id][:,report.sorted_symbols[id]]  
133                     → report.emission_arrays[id][:,report.sorted_symbols[id]]  
134                     name="$(report.ids[n])"  
135                     → K$(report.sort_dicts[id][id1_state]))  
136         end  
137     end  
138  
139     show(autoplt)  
140     println("\n")  
141     show(symplot)  
142     println("\n")  
143 end  
144 end
```

16.6.17 /src/utilities/BBG analysis.jl

```
1 #function to produce matrix of hmm chain parameter coordinate evolution-
  ↵ selects 3 matched emission parameters from 3+ state HMMs, one per
  ↵ state for 3 states. Matching is performed by minimizing euclidean
  ↵ distance between the state emission probability vectors B for the
  ↵ states to be matched (as EM-optimised replicates will have similar
  ↵ states organized in different orders)

2

3 function chain_3devo_coords(chains :: Vector{Vector{Any}})
  ↵ length(chains) ≤ 0 && throw(ArgumentError, "Argument must be vector of
  ↵ more than one hmm chain")
  ↵ coords=[Vector{Tuple{AbstractFloat, AbstractFloat, AbstractFloat}}]()
  ↵ for i in 1:length(chains)]
```

```

7   for step in chains[1]
8     hmm=step[2]
9     length(hmm.B)<3 && throw(ArgumentError, "3- or greater state hmms
10    ↪ required")
11    push!(coords[1], (hmm.B[1].p[1],hmm.B[2].p[1],hmm.B[3].p[1]))
12  end
13
14  for (c, chain) in enumerate(chains[2:end])
15    ref_idxs=Vector{Integer}()
16
17    ↪ ref_vecs=[chains[1][end][2].B[1].p,chains[1][end][2].B[2].p,chains[1][end]
18    end_hmm=chain[end][2]
19    length(end_hmm.B)<3 && throw(ArgumentError, "3- or greater state
20    ↪ hmms required")
21
22    for vec in ref_vecs
23      euclideans=Vector{AbstractFloat}()
24      for d in 1:length(end_hmm.B)
25        push!(euclideans, euclidean(vec, end_hmm.B[d].p))
26      end
27      min_euc_idx = findmin(euclideans)[2]
28      while min_euc_idx in ref_idxs
29        euclideans[min_euc_idx]=1.
30        min_euc_idx = findmin(euclideans)[2]
31      end
32      push!(ref_idxs,findmin(euclideans)[2])
33    end
34
35    for step in chain
36      hmm=step[2]
37      push!(coords[c+1],
38        ↪ (hmm.B[ref_idxs[1]].p[1],hmm.B[ref_idxs[2]].p[1],hmm.B[ref_idxs[3]].p
39      end
40    end
41
42  return coords
43 end

```

16.6.18 /src/utilities/BBG_progressmeter.jl

```

1 #UTILITY PROGRESSMETER, REPORTS WORKER NUMBER AND CURRENT ITERATE
2 mutable struct ProgressHMM{T<:Real} <: ProgressMeter.AbstractProgress

```

```

3   thresh::T
4   dt::AbstractFloat
5   val::T
6   counter::Integer
7   triggered::Bool
8   tfirst::AbstractFloat
9   tlast::AbstractFloat
10  printed::Bool      # true if we have issued at least one status
11    → update
12  desc::AbstractString # prefix to the percentage, e.g. "Computing ... "
13  color::Symbol        # default to green
14  output::IO           # output stream into which the progress is
15    → written
16  numprintedvalues::Integer # num values printed below progress in
17    → last iteration
18  offset::Integer       # position offset of progress bar
19    → (default is 0)
20  steptime::AbstractFloat
21
22 function ProgressHMM{T}(thresh;
23                           dt::Real=0.1,
24                           desc::AbstractString="Progress: ",
25                           color::Symbol=:green,
26                           output::IO=stderr,
27                           offset::Integer=0,
28                           start_it::Integer=1) where T
29
30   tfirst = tlast = time()
31   printed = false
32   new{T}(thresh, dt, typemax(T), start_it, false, tfirst, tlast,
33         → printed, desc, color, output, 0, offset, 0.0)
34 end
35
36 ProgressHMM(thresh::Real, dt::Real=0.1, desc::AbstractString="Progress:
37   → ",
38   color::Symbol=:green, output::IO=stderr;
39   offset::Integer=0, start_it::Integer=1) =
40     → ProgressHMM{typeof(thresh)}(thresh, dt=dt, desc=desc,
41     → color=color, output=output, offset=offset,
42     → start_it=start_it)
43
44 ProgressHMM(thresh::Real, desc::AbstractString, offset::Integer=0,
45   → start_it::Integer=1) = ProgressHMM{typeof(thresh)}(thresh, desc=desc,
46   → offset=offset, start_it=start_it)

```

```

35
36 function update!(p::ProgressHMM, val, steptime; options ... )
37     p.val = val
38     p.counter += 1
39     p.steptime = steptime
40     updateProgress!(p; options ... )
41 end
42
43 function updateProgress!(p::ProgressHMM; showvalues = Any[], valuecolor =
44     :blue, offset::Integer = p.offset, keep = (offset == 0))
45     p.offset = offset
46     t = time()
47     if p.val ≤ p.thresh && !p.triggered
48         p.triggered = true
49         if p.printed
50             p.triggered = true
51             dur = ProgressMeter.durationstring(t-p.tfirst)
52             msg = @sprintf "%s Time: %s (%d iterations)" p.desc dur
53             → p.counter
54             print(p.output, "\n" ^ (p.offset + p.numprintedvalues))
55             ProgressMeter.move_cursor_up_while_clearing_lines(p.output,
56             → p.numprintedvalues)
57             ProgressMeter.printover(p.output, msg, p.color)
58             ProgressMeter.printvalues!(p, showvalues; color = valuecolor)
59             if keep
60                 println(p.output)
61             else
62                 print(p.output, "\r\u1b[A" ^ (p.offset +
63                 → p.numprintedvalues))
64             end
65         end
66         return
67     end
68     if t > p.tlast+p.dt && !p.triggered
69         elapsed_time = t - p.tfirst
70         msg = @sprintf "%s (convergence: %g ⇒ %g, iterate: %g, step
71             → time: %s)" p.desc p.val p.thresh p.counter hmss(p.steptime)
72         print(p.output, "\n" ^ (p.offset + p.numprintedvalues))
73         ProgressMeter.move_cursor_up_while_clearing_lines(p.output,
74             → p.numprintedvalues)
75         ProgressMeter.printover(p.output, msg, p.color)
76         ProgressMeter.printvalues!(p, showvalues; color = valuecolor)
77     end

```

```

72     print(p.output, "\r\u1b[A" ^ (p.offset + p.numprintedvalues))
73     # Compensate for any overhead of printing. This can be
74     # especially important if you're running over a slow network
75     # connection.
76     p.tlast = t + 2*(time()-t)
77     p.printed = true
78   end
79 end
80
81   function hmss(dt)
82     isnan(dt) && return "NaN"
83     (h,r) = divrem(dt,60*60)
84     (m,r) = divrem(r, 60)
85     (isnan(h)||isnan(m)||isnan(r)) && return "NaN"
86     string(Int(h),":",Int(m),":",Int(ceil(r)))
87   end

```

16.6.19 /src/utilities/HMM_init.jl

```

1 function autotransition_init(K::Integer, order::Integer,
2   ← partition::String="")
3   a = rand(Dirichlet(ones(K)/K)) #uninformative prior on initial
4   ← state probabilities
5   A = strong_autotrans_matrix(K)
6   no_emission_symbols = Int(4^(order+1)) #alphabet size for the
7   ← order
8   emission_dists = [generate_emission_dist(no_emission_symbols) for
9   ← i in 1:K]
10  #generate the BHMM with the appropriate transition matrix and
11  ← emissions distributions
12  hmm = BHMM(a, A, emission_dists, partition)
13
14  #function to construct BHMM transition matrix with strong
15  ← priors on auto-transition
16  function strong_autotrans_matrix(states::Integer,
17    ← prior_dope::AbstractFloat=(states*250.0),
18    ← prior_background::AbstractFloat=.1)
19    transition_matrix=zeros(states,states)
20    for k in 1:states
21      dirichlet_params = fill(prior_background, states)
22      dirichlet_params[k] = prior_dope

```

```

15         transition_matrix[k,:] =
16             ↳ rand(Dirichlet(dirichlet_params))
17     end
18     return transition_matrix
19 end
20
21 #function to construct BHMM state emission distribution from
22     ↳ uninformative dirichlet over the alphabet size
23 function generate_emission_dist(no_emission_symbols,
24     ↳ prior=Dirichlet(ones(no_emission_symbols)/no_emission_symbols))
25     return Categorical(rand(prior))
26 end

```

16.6.20 /src/utilities/load_balancer.jl

```

1 struct LoadConfig
2     k_range::UnitRange
3     o_range::UnitRange
4     blacklist::Vector{Chain_ID}
5     whitelist::Vector{Chain_ID}
6     function LoadConfig(k_range, o_range; blacklist=Vector{Chain_ID}(),
7         ↳ whitelist=Vector{Chain_ID}())
8         assert_loadconfig(k_range, o_range) && new(k_range, o_range,
9             ↳ blacklist, whitelist)
10    end
11 end
12
13 function assert_loadconfig(k_range,o_range)
14     k_range[1] < 1 && throw(ArgumentError("Minimum value of LoadConfig
15         ↳ k_range is 1!"))
16     o_range[1] < 0 && throw(ArgumentError("Minimum value of LoadConfig
17         ↳ o_range is 0!"))
18     return true
19 end
20
21 #subfunc to handle balancing memory load on dissimilar machines in
22     ↳ cluster
23 function load_balancer(no_models::Integer, hmm_jobs::RemoteChannel,
24     ↳ config::LoadConfig, timeout::Integer=300)
25     jobid::Chain_ID, start_iterate::Integer, hmm::BHMM,
26     ↳ job_norm::AbstractFloat, observations::Matrix = take!(hmm_jobs)

```

```

21     starttime=time()
22     while (jobid.K < config.k_range[1] || jobid.K > config.k_range[end]
23       → || jobid.order < config.o_range[1] || jobid.order >
24       → config.o_range[end] || jobid in config.blacklist ||
25       → (length(config.whitelist)>0 && !(jobid in config.whitelist))) &&
26       → (time()-starttime ≤ timeout) #while a job prohibited by load
27       → table, keep putting the job back and drawing a new one
28         put!(hmm_jobs, (jobid, start_iterate, hmm, job_norm,
29           → observations))
30         jobid, start_iterate, hmm, job_norm, observations =
31           → take!(hmm_jobs)
32     end
33     (time()-starttime > timeout) ? (return 0,0,0,0,0) : (return jobid,
34       → start_iterate, hmm, job_norm, observations)
35   end

```

16.6.21 /src/utilities/log_prob_sum.jl

```

1      #subfuncs to handle sums of log probabilities that may
2       → include -Inf (ie p=0), returning -Inf in this case
3       → rather than NaNs
4       function lps(adjuvants)
5         prob = sum(adjuvants) ; isnan(prob) ? - Inf : prob
6       end
7
7       function lps(base, adjuvants ... )
8         prob = base+sum(adjuvants) ; isnan(prob) ? -Inf :
9           → prob
10      end

```

16.6.22 /src/utilities/model_display.jl

```

1 cs_dict=Dict('A'⇒:green, 'C'⇒:blue, 'G'⇒:yellow, 'T'⇒:red)
2
3 function print_emitters(state::Categorical) #print informative symbols
4   → from the state's emission distribution, if any
5   order=Integer(log(4,length(state.p))-1)
6   alphabet=CompoundAlphabet(ACGT,order)
7   bits=log(2,length(state.p)) #higher order hmms express more
8     → information per symbol

```

```

7     dummy_p=state.p.+10^-99 #prevent -Inf or NaN from zero probability
   ↵ symbols
8     infoscore=(bits+sum(x*log(2,x) for x in dummy_p))
9     infovec=[x*infoscore for x in dummy_p]
10    infovec./=bits
11    print("<<< ")
12    for (symbol,symbol_prob) in enumerate(infovec)
13        if symbol_prob ≥ .05
14            str=string(alphabet.integers[symbol])
15            if symbol_prob ≥ .7
16                for char in str
17                    printstyled(stdout, char; bold=true,
   ↵ color=cs_dict[char])
18            end
19            elseif .7 > symbol_prob ≥ .25
20                for char in str
21                    printstyled(stdout, char; color=cs_dict[char])
22                end
23            elseif .25 > symbol_prob
24                for char in str
25                    printstyled(stdout, lowercase(char);
   ↵ color=cs_dict[char])
26                end
27            end
28            print(" ")
29        end
30    end
31    println(">>>")
32 end

```

16.6.23 /src/utilities/observation_coding.jl

```

1 #KMER ORDER/SEQUENCE INTEGER CODING UTILITIES
2 #higher order DNA alphabet
3 struct CompoundAlphabet
4     symbols::Dict{Mer{DNAAlphabet{2}}, Integer}
5     integers::Dict{Integer,Mer{DNAAlphabet{2}}}
6     #build a CompoundAlphabet for DNA of some order_no
7     function CompoundAlphabet(alphabet::Tuple, order_no::Integer)
8         symbols = Dict{Mer{DNAAlphabet{2}}, Integer}()
9         integers = Dict{Integer,Mer{DNAAlphabet{2}}}()
10

```

```

11     tuples = Array{Tuple}
12     if order_no > 0
13         tuples = collect(Iterators.product([alphabet for order in
14             ← 0:order_no] ... ))
15     else #0th order compound product of an alphabet is that alphabet
16         tuples = collect(Iterators.product(alphabet))
17     end
18
19     @inbounds for index in eachindex(tuples)
20         tuple_seq = Mer{DNAAlphabet{2}}(collect(tuples[index]))
21         integers[index] = tuple_seq
22         symbols[tuple_seq] = index
23     end
24
25     new(symbols,integers)
26 end
27
28
29 struct N_Order_ntSequence
30     alphabet::CompoundAlphabet
31     seq_lengths::Vector{Integer}
32     order_kmers::Vector{Vector{Mer{DNAAlphabet{2}}}}
33 end
34
35
36 function code_job_obs(job_ids::Vector{Chain_ID},
37     ← obs_sets::Dict{String,Vector{LongSequence{DNAAlphabet{2}}}})
38     code_jobs=Vector{Tuple{String,Integer}}(){}
39     for id in job_ids #assemble a vector of observation encoding jobs
40         code_job=(id.obs_id, id.order)
41         !(code_job in code_jobs) && push!(code_jobs, code_job)
42     end
43
44     code_dict = Dict{Tuple{String,Integer}, AbstractArray}(){}
45     @showprogress 1 "Encoding observations ..." for (obs_id, order) in
46         ← code_jobs #build the appropriate sample sets once
47         order_seqs = get_order_n_seqs(obs_sets[obs_id],order) #get the
48         ← kmer sequences at the appropriate order
49         coded_seqs = code_seqs(order_seqs, sorted=true) #numerically code
49         ← the sequences in trainable format
50         code_dict[(obs_id, order)] = coded_seqs
51     end

```

```

49     return code_dict
50 end
51
52 #from a vector of LongSequences, get
53 function get_order_n_seqs(seqs::Vector{LongSequence{DNAAlphabet{2}}}),
54     ↪ order_no::Integer, base_tuple::Tuple=ACGT)
55     kmer_vecs = Vector{Vector{Mer{DNAAlphabet{2}}}}()
56     length_vec = Vector{Integer}()
57     window = order_no + 1
58
59     for seq in seqs
60         kmer_vec = Vector{Mer{DNAAlphabet{2}}}()
61         @inbounds for (i, kmer) in
62             ↪ collect(each(Mer{DNAAlphabet{2}},window),seq))
63             push!(kmer_vec, kmer)
64         end
65
66         push!(kmer_vecs, kmer_vec)
67         push!(length_vec, length(seq))
68     end
69
70     return NordSeqs = N_Order_ntSequence(CompoundAlphabet(base_tuple,
71         ↪ order_no), length_vec, kmer_vecs)
72 end
73
74 #convert tuple kmers to symbol codes
75 function code_seqs(input::N_Order_ntSequence, offsets::Vector=[0 for i in
76     ↪ 1:length(input.order_kmers)]; sorted::Bool=false)
77     symbol_no=length(input.alphabet.symbols)
78     if symbol_no ≤ typemax(UInt8)
79         integer_type = UInt8
80     elseif typemax(UInt8) < symbol_no < typemax(UInt16)
81         integer_type = UInt16
82     else
83         integer_type = UInt32
84     end
85
86     alphabet = input.alphabet
87     output = zeros(integer_type, length(input.order_kmers),
88         ↪ (maximum([length(seq) for seq in input.order_kmers])+1)) #leave 1
89         ↪ missing value after the longest sequence for indexing sequence
90         ↪ length in CLHMM messages
91     sorted && (sort_idxs = sortperm(input.seq_lengths,rev=true))

```

```
85     sorted ? input_seqs = input.order_kmers[sort_idxs] : input_seqs =
86             ↵   input.order_kmers
87
88     for (i, seq) in enumerate(input_seqs)
89         for t in 1:length(seq)
90             curr_kmer = seq[t]
91             curr_code = alphabet.symbols[curr_kmer]
92             output[i,t+offsets[i]]=curr_code
93         end
94     end
95     return output
96 end
```

16.6.24 /src/utilities/utilities.jl

```
1    """
2 Utility functions for learning and using background genomic hidden markov
3     models
4 """
5
6 #function to split random sample dataframe into training and test sets
7     (divide total sequence length by half)
8
9 function split_obs_sets(sample_dfs::Dict{String,DataFrame})
10    training_sets = Dict{String,Vector{LongSequence{DNAAlphabet{2}}}}()
11    test_sets = Dict{String,Vector{LongSequence{DNAAlphabet{2}}}}()
12
13    for (partition_id, partition) in sample_dfs
14        partition = partition[shuffle(1:size(partition, 1)),:] #shuffle
15        # the samples to avoid any effect from sampling without
16        # replacement
17        partition.sampleLength = (partition.SampleEnd -
18            partition.SampleStart) .+ 1
19        midway = sum(partition.sampleLength)÷2
20        split_index = 0
21        counter = 0
22
23        while split_index == 0
24            counter += 1
25            length_sum = sum(partition.sampleLength[1:counter])
26            if length_sum > midway
27                split_index = counter
28            end
29        end
30    end
```

```

23     training_sets[partition_id] =
24         ↳ partition.SampleSequence[1:split_index-1]
25     test_sets[partition_id] =
26         ↳ partition.SampleSequence[split_index:end]
27   end
28   return training_sets, test_sets
29 end

```

16.6.25 /test/ref_fns.jl

```

1 #slow algo written in mimicry of Churbanov & Winters
2 function old_linear(hmm, observations, obs_lengths)
3     O = size(observations)[2]
4     a = log.(hmm.A); a = log.(hmm.a)
5     N = length(hmm.B); B = length(hmm.B[1].support); b =
6         ↳ [log(hmm.B[m].p[y]) for m in 1:N, y in 1:B]
7     α1oi = zeros(O,N); β1oi = zeros(O,N); Eoi = zeros(O,N,B); Toij =
8         ↳ zeros(O,N,N); Aoi = zeros(O,N); log_pobs=zeros(O); yt=0
9
10    for o in 1:O
11        #INITIALIZATION
12        T = obs_lengths[o]; βoi_T = zeros(N) #log betas at T initialised
13        ↳ as zeros
14        EiT = fill(-Inf,B,N,N); TijT = fill(-Inf,N,N,N) #Ti,j(T,m) = 0
15        ↳ for all m; in logspace
16
17        @inbounds for m in 1:N, i in 1:N, y in 1:B
18            observations[T, o] = y && m == i ? (EiT[y, i, m] = 0) :
19                (EiT[y, i, m] = -Inf) #log Ei initialisation
20        end
21
22        #RECURRANCE
23        @inbounds for t in T-1:-1:1
24            βoi_t = similar(βoi_T); Tijt = similar(TijT); Eit =
25                ↳ similar(EiT)
26            Γ = observations[t+1,o]; yt = observations[t,o]
27            Γ=0 && println("hooo !! $o $t")
28            for m in 1:N
29                βoi_t[m] = logsumexp([lps(a[m,j], b[j,Γ], βoi_T[j]) for j
30                    ↳ in 1:N])
31                for i in 1:N
32                    for j in 1:N
33

```

```

27         Tijt[i, j, m] = logsumexp([lps(a[m,n], TijT[i, j,
28             ↵ n], b[n,Γ]) for n in 1:N])
29         i==m && (Tijt[i, j, m] = logaddexp(Tijt[i, j, m],
30             ↵ lps(βoi_T[j], a[m,j], b[j, Γ])))
31     end
32     for y in 1:B
33         Eit[y, i, m] = logsumexp([lps(b[n,Γ], a[m,n],
34             ↵ EiT[y, i, n]) for n in 1:N])
35         i==m && y==yt && (Eit[y, i, m] = logaddexp(Eit[y,
36             ↵ i, m], βoi_t[m])))
37     end
38     end
39
40     βoi_T = βoi_t; TijT = Tijt; EiT = Eit
41 end
42
43 #TERMINATION
44
45     Γ = observations[1,o]
46     α1oi[o,:]= [lps(a[i], b[i, Γ]) for i in 1:N]
47     β1oi[o,:]= βoi_T
48     log_pobs[o]= logsumexp(lps.(α1oi[o,:], βoi_T[:]))
49     Eoi[o,:,:]= [logsumexp([lps(EiT[y,i,m], a[m], b[m,yt]) for m in
50             ↵ 1:N]) for i in 1:N, y in 1:B]
51     Toij[o,:,:,:]= [logsumexp([lps(TijT[i,j,m], a[m], b[m,yt]) for m
52             ↵ in 1:N]) for i in 1:N, j in 1:N]
53 end
54
55 #INTEGRATE ACROSS OBSERVATIONS AND SOLVE FOR NEW BHMM PARAMS
56
57     obs_penalty=log(0)
58     a_o=α1oi.+β1oi.-logsumexp.(eachrow(α1oi.+β1oi))
59     new_a=logsumexp.(eachcol(a_o))-obs_penalty
60
61     a_int = Toij.-logsumexp.([Toij[o,i,:] for o in 1:O, i in 1:N])
62     new_a = logsumexp.([a_int[:,i,j] for i in 1:N, j in
63             ↵ 1:N])-obs_penalty
64
65     e_int=Eoi.-logsumexp.([Eoi[o,j,:] for o in 1:O, j in 1:N])
66     new_b=logsumexp.([e_int[:,j,d] for d in 1:B, j in 1:N])-obs_penalty
67     new_D=[Categorical(exp.(new_b[:,i])) for i in 1:N]
68
69
70     return typeof(hmm)(exp.(new_a), exp.(new_a), new_D), lps(log_pobs)
71 end

```

```

63
64 #HMMBase
65 function mouchet_mle_step(hmm::BHMM, observations) where F
66     # NOTE: This function works but there is room for improvement.
67
68     log_likelihoods = mouchet_log_likelihoods(hmm, observations)
69
70     log_α = mouchet_messages_forwards_log(hmm.a, hmm.A, log_likelihoods)
71     log_β = mouchet_messages_backwards_log(hmm.A, log_likelihoods)
72     log_A = log.(hmm.A)
73
74     normalizer = logsumexp(log_α[1,:] + log_β[1,:])
75
76     # E-step
77
78     T, K = size(log_likelihoods)
79     log_ξ = zeros(T, K, K)
80
81     @inbounds for t = 1:T-1, i = 1:K, j = 1:K
82         log_ξ[t,i,j] = log_α[t,i] + log_A[i,j] + log_β[t+1,j] +
83             ↳ log_likelihoods[t+1,j] - normalizer
84     end
85
86     ξ = exp.(log_ξ)
87     ξ ./= sum(ξ, dims=[2,3])
88
89     # M-step
90     new_A = sum(ξ[1:end-1,:,:], dims=1)[1,:,:] #index fix-MM
91     new_A ./= sum(new_A, dims=2)
92
93     new_a = exp.((log_α[1,:] + log_β[1,:]) .- normalizer)
94     new_a ./= sum(new_a)
95
96     # TODO: Cleanup/optimize this part
97     γ = exp.((log_α .+ log_β) .- normalizer)
98
99     B = Categorical[]
100    for (i, d) in enumerate(hmm.B)
101        # Super hacky ...
102        # https://github.com/JuliaStats/Distributions.jl/issues/809
103        push!(B, fit_mle(Categorical, permutedims(observations), γ[:,i]))
104    end

```

```
105     typeof(hmm)(new_a, new_A, B), normalizer
106 end
107
108
109 function mouchet_log_likelihoods(hmm, observations)
110     hcat(map(d → logpdf.(d, observations), hmm.B) ... )
111 end
112
113
114
115 function mouchet_messages_forwards_log(init_distn, trans_matrix,
116   ↪ log_likelihoods)
116   # OPTIMIZE
117   log_alphas = zeros(size(log_likelihoods))
118   log_trans_matrix = log.(trans_matrix)
119   log_alphas[1,:] = log.(init_distn) .+ log_likelihoods[1,:]
120   @inbounds for t = 2:size(log_alphas)[1]
121       for i in 1:size(log_alphas)[2]
122           log_alphas[t,i] = logsumexp(log_alphas[t-1,:] .+
123             ↪ log_trans_matrix[:,i]) + log_likelihoods[t,i]
124       end
125   end
126   log_alphas
126 end
127
128 function mouchet_messages_backwards_log(trans_matrix, log_likelihoods)
129   # OPTIMIZE
130   log_betas = zeros(size(log_likelihoods))
131   log_trans_matrix = log.(trans_matrix)
132   @inbounds for t = size(log_betas)[1]-1:-1:1
133       tmp = view(log_betas, t+1, :) .+ view(log_likelihoods, t+1, :)
134       @inbounds for i in 1:size(log_betas)[2]
135           log_betas[t,i] = logsumexp(view(log_trans_matrix, i, :) .+
136             ↪ tmp)
137       end
138   end
139   log_betas
139 end
```

16.6.26 /test/runtests.jl

```

1 using
  ↵ BioBackgroundModels,BioSequences,DataFrames,Distributed,Distributions,FASTX,Progr
2 import BioBackgroundModels: autotransition_init, load_balancer,
  ↵ CompoundAlphabet, get_order_n_seqs, code_seqs, code_job_obs,
  ↵ make_padded_df, add_partition_masks!, partition_genome_coordinates,
  ↵ divide_partitions_by_scaffold, mask_sequence_by_partition,
  ↵ find_position_partition, setup_sample_jobs, rectify_identifier,
  ↵ add_metacoordinates!, meta_to_feature_coord, get_feature_row_index,
  ↵ get_strand_dict, build_scaffold_seq_dict,
  ↵ get_feature_params_from_metacoord, determine_sample_window,
  ↵ fetch_sequence, get_sample_set, get_sample, assert_hmm, linear_step,
  ↵ get_BGHMM_symbol_lh,make_padded_df,add_partition_masks!,BGHMM_likelihood_calc,lin
  ↵ t_add_categorical_counts!
3 import Serialization: deserialize
4 include("synthetic_sequence_gen.jl")
5 include("ref_fns.jl")
6
7 #JOB FILEPATHS
8 #GFF3 feature database, FASTA genome and index paths
9 Sys.islinux() ? genome = (@__DIR__) * "/synthetic.fna" : genome =
  ↵ (@__DIR__) * "\\synthetic.fna"
10 Sys.islinux() ? index = (@__DIR__) * "/synthetic.fna.fai" : index =
  ↵ (@__DIR__) * "\\synthetic.fna.fai"
11 Sys.islinux() ? gff = (@__DIR__) * "/synthetic.gff3" : gff = (@__DIR__)
  ↵ * "\\synthetic.gff3"
12 Sys.islinux() ? posfasta = (@__DIR__) * "/syntheticpos.fa" : posfasta =
  ↵ (@__DIR__) * "\\syntheticpos.fa"
13 !isfile(genome) && print_synthetic_fasta(genome)
15 !isfile(index) && print_synthetic_index(index)
16 !isfile(gff) && print_synthetic_gff(gff)
17 !isfile(posfasta) && print_synthetic_position(posfasta)
18
19 Random.seed!(1)
20
21 @testset "BHMM/BHMM.jl constructors" begin
22     good_a = [.20,.30,.20,.30]
23     bad_probvec_a = [.25,.25,.25,.27]
24     good_A = fill(.25,4,4)
25     bad_probvec_A = deepcopy(good_A)
26     bad_probvec_A[:,3] *= .27

```

```

27     bad_size_A = fill(.25,5,4)
28     good_D = Categorical([.25,.25,.25,.25])
29     bad_size_D = Categorical([.2,.2,.2,.2,.2])
30     good_Dvec = [good_D for i in 1:4]
31     bad_size_Dvec =[good_D, good_D, good_D, bad_size_D]
32     bad_length_Dvec=[good_D for i in 1:5]

33
34     @test_throws ArgumentError BHMM(bad_probvec_a, good_A, good_Dvec)
35     @test_throws ArgumentError BHMM(good_a, bad_probvec_A, good_Dvec)
36     @test_throws ArgumentError BHMM(good_a, bad_size_A, good_Dvec)
37     @test_throws ArgumentError BHMM(good_a, good_A, bad_size_Dvec)
38     @test_throws ArgumentError BHMM(good_a, good_A, bad_length_Dvec)

39
40     hmm=BHMM(good_a, good_A, good_Dvec)
41     @test typeof(hmm)==BHMM{Float64}
42     @test hmm.a==good_a
43     @test hmm.A==good_A
44     @test hmm.B==good_Dvec
45     @test size(hmm) == (4,4)

46
47     hmm_copy=copy(hmm)
48     @test hmm.a==hmm_copy.a
49     @test hmm.A==hmm_copy.A
50     @test hmm.B==hmm_copy.B

51
52     hmm2=BHMM(good_A, good_Dvec)
53     @test hmm2.a==[.25,.25,.25,.25]
54     @test hmm2.A==good_A
55     @test hmm2.B==good_Dvec

56 end

57
58 @testset "BHMM/chain.jl Chain_ID and EM_step constructors" begin
59     obs_id="test"
60     K,zero_k,neg_K=4,0,-1
61     order,badorder=0,-1
62     replicate,zero_rep,neg_rep=1,0,-1

63
64     @test_throws ArgumentError Chain_ID(obs_id, zero_k, order, replicate)
65     @test_throws ArgumentError Chain_ID(obs_id, neg_K, order, replicate)
66     @test_throws ArgumentError Chain_ID(obs_id, K, badorder, replicate)
67     @test_throws ArgumentError Chain_ID(obs_id, K, order, zero_rep)
68     @test_throws ArgumentError Chain_ID(obs_id, K, order, neg_rep)

69

```

```

70     id=Chain_ID(obs_id, K, order, replicate)
71     @test typeof(id)=Chain_ID
72     @test id.obs_id==obs_id
73     @test id.K==K
74     @test id.order==order
75     @test id.replicate==replicate
76
77     good_A = fill(.25,4,4)
78     good_D = Categorical([.25,.25,.25,.25])
79     good_Dvec = [good_D for i in 1:4]
80     hmm=BHMM(good_A,good_Dvec)
81     log_p=-.001
82
83     @test_throws ArgumentError EM_step(0,hmm,0.0,0.0,true)
84     @test_throws ArgumentError EM_step(-1,hmm,0.0,0.0,true)
85     @test_throws ArgumentError EM_step(1,hmm,1.0,0.0,true)
86
87     steppe=EM_step(1,hmm,log_p,0.0,false)
88     @test typeof(steppe)=EM_step
89     @test steppe.iterate==1
90     @test steppe.hmm==hmm
91     @test steppe.log_p==log_p
92     @test steppe.delta==0.0
93     @test steppe.converged==false
94 end
95
96 @testset "utilities/log_prob_sum.jl Log probability summation functions" begin
97     @test isapprox(lps([.1, .1, .1]), .3)
98     @test lps([-Inf, .1, .1]) == -Inf
99     @test isapprox(lps(.1, .1, .1), .3)
100    @test lps(-Inf, .1, .1) == -Inf
101    @test lps(.1, -Inf, .1) == -Inf
102 end
103
104 @testset "utilities/HMM_init.jl initialization functions" begin
105     K=4
106     order=2
107     hmm=autotransition_init(K,order)
108     @test typeof(hmm)==BHMM{Float64}
109     @test size(hmm)==(4,64)
110 end
111

```

```

112 @testset "utilities/load_balancer.jl EM_converge load balancing structs
113   ↪ and functions" begin
114     @test_throws ArgumentError LoadConfig(0:5,1:5)
115     @test_throws ArgumentError LoadConfig(1:5,-1:5)
116
117     ↪ job_ids=[Chain_ID("test",6,2,1),Chain_ID("test",4,2,1),Chain_ID("test",2,0,1)]
118
119     K_exclusive_config=LoadConfig(1:1,0:0)
120     O_exclusive_config=LoadConfig(1:6,3:4)
121     blacklist_config=LoadConfig(1:6,0:2,blacklist=job_ids)
122
123     ↪ whitelist_config=LoadConfig(1:6,0:2,whitelist=[Chain_ID("test",6,2,2)])
124     high_config=LoadConfig(4:6,0:2,whitelist=job_ids)
125
126     no_input_hmms, chains, input_channel, output_channel =
127       ↪ setup_EM_jobs!(job_ids,obs_sets)
128     @test load_balancer(no_input_hmms, input_channel, K_exclusive_config,
129       ↪ 3) = (0,0,0,0,0)
130
131     no_input_hmms, chains, input_channel, output_channel =
132       ↪ setup_EM_jobs!(job_ids,obs_sets)
133     @test load_balancer(no_input_hmms, input_channel, O_exclusive_config,
134       ↪ 3) = (0,0,0,0,0)
135
136     no_input_hmms, chains, input_channel, output_channel =
137       ↪ setup_EM_jobs!(job_ids,obs_sets)
138     @test load_balancer(no_input_hmms, input_channel, blacklist_config,
139       ↪ 3) = (0,0,0,0,0)
140
141     no_input_hmms, chains, input_channel, output_channel =
142       ↪ setup_EM_jobs!(job_ids,obs_sets)
143     high_set=load_balancer(no_input_hmms, input_channel, high_config)
144
145     @test high_set[1]=Chain_ID("test",6,2,1)
146     @test high_set[2]=1

```

```

142 @test typeof(high_set[3])=BHMM{Float64}
143 @test
144     ↪ high_set[4]=obs_lh_given_hmm(high_set[5],high_set[3],linear=false)
145 @test typeof(high_set[5])=Matrix{UInt8}
146 end
147
148 @testset "utilities/observation_coding.jl Order coding structs and
149     ↪ functions" begin
150     compound_alphabet=CompoundAlphabet(ACGT, 2)
151     @test
152         ↪ typeof(compound_alphabet.symbols)=Dict{Mer{DNAAlphabet{2}},Integer}
153     @test length(compound_alphabet.symbols)=64
154     @test compound_alphabet.symbols[Mer{DNAAlphabet{2}}("AAA")]=1
155     @test compound_alphabet.symbols[Mer{DNAAlphabet{2}}("TTT")]=64
156
157     test_seqs =
158         ↪ [BioSequences.LongSequence{DNAAlphabet{2}}("ACGTACGTACGTACGT"),BioSequences.L
159     target0= [1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 0; 4 4 4 4 4 4 4 4 0 0 0 0 0
160         ↪ 0 0 0 0 0]
161     target2= [37 58 15 20 37 58 15 20 37 58 15 20 37 58 0; 64 64 64 64 64
162         ↪ 0 0 0 0 0 0 0 0 0]
163
164     order0_seqs = get_order_n_seqs(test_seqs,0)
165     @test typeof(order0_seqs)=BioBackgroundModels.N_Order_ntSequence
166     for (mer, code) in CompoundAlphabet(ACGT,0).symbols
167         @test order0_seqs.alphabet.symbols[mer]==code
168     end
169     @test order0_seqs.seq_lengths==[16,7]
170     @test order0_seqs.order_kmers[1][1]==Mer{DNAAlphabet{2}}("A")
171     code0_seqs = code_seqs(order0_seqs)
172     @test target0 == code0_seqs
173
174     order2_seqs = get_order_n_seqs(test_seqs,2)
175     @test typeof(order2_seqs)=BioBackgroundModels.N_Order_ntSequence
176     for (mer, code) in CompoundAlphabet(ACGT,2).symbols
177         @test order2_seqs.alphabet.symbols[mer]==code
178     end
179     @test order2_seqs.seq_lengths==[16,7]
180     @test order2_seqs.order_kmers[1][1]==Mer{DNAAlphabet{2}}("ACG")
181     code2_seqs = code_seqs(order2_seqs)
182     @test target2 == code2_seqs
183
184     bw_o0_seqs = get_order_n_seqs(test_seqs[end:-1:1], 0)

```

```

179     sorted_code_seqs = code_seqs(bw_o0_seqs, sorted=true)
180     @test target0 == sorted_code_seqs
181
182     obs_sets=Dict("test1"⇒test_seqs,
183                   "test2"⇒test_seqs)
184
185     → job_ids=[Chain_ID("test1",4,0,1),Chain_ID("test2",4,0,1),Chain_ID("test1",2,2)
186
187     code_dict=code_job_obs(job_ids,obs_sets)
188     @test ("test1",0) in keys(code_dict)
189     @test ("test1",2) in keys(code_dict)
190     @test ("test2",0) in keys(code_dict)
191     @test !(("test2",2) in keys(code_dict))
192     @test code_dict[("test1",0)]==target0
193     @test code_dict[("test1",2)]==target2
194     @test code_dict[("test2",0)]==target0
195
196     #test type selection for high order numbers
197     o5_nos=get_order_n_seqs(test_seqs,5)
198     o5_codes=code_seqs(o5_nos)
199     @test typeof(o5_codes)==Matrix{UInt16}
200
201     o7_nos=get_order_n_seqs(test_seqs,7)
202     o7_codes=code_seqs(o7_nos)
203     @test typeof(o7_codes)==Matrix{UInt32}
204
205 end
206
207 @testset "genome_sampling/partition_masker.jl functions" begin
208     synthetic_seq =
209         → BioSequences.LongSequence{DNAAlphabet{2}}(generate_synthetic_seq())
210     position_start = 501
211     position_length=141
212     position_pad=350
213     perigenic_pad = 250
214
215     position_df = make_padded_df(posfasta, gff, genome, index,
216         → position_pad)
217     add_partition_masks!(position_df, gff, perigenic_pad)
218
219     @test position_df.SeqID[1]=="1"

```

```

217  @test length(position_df.PadSeq[1]) == position_pad+position_length
218  ↳   ==
219  ↳   length(position_df.PadStart[1]:position_df.PadStart[1]+position_length+positi
220  ↳   = position_df.End[1]-position_df.Start[1]+1+position_pad =
221  ↳   size(position_df.MaskMatrix[1])[1]
222  @test
223  ↳   synthetic_seq[position_df.PadStart[1]:position_df.End[1]]==position_df.PadSeq
224
225  mm = position_df.MaskMatrix[1]
226  idx=1#pos 151
227  @test sum(mm[idx:idx+99,1] .== 1)==length(mm[idx:idx+99,1])
228  idx+=100#pos 251
229  @test sum(mm[idx:idx+259,1] .== 2)==length(mm[idx:idx+259,1])
230  idx+=260 #pos 511
231  @test sum(mm[idx:idx+59,1] .== 3)==length(mm[idx:idx+59,1])
232  idx+=60#pos 571
233  @test sum(mm[idx:idx+29,1] .== 2)==length(mm[idx:idx+29,1])
234  idx+=30#pos601
235  @test sum(mm[idx:idx+40,1] .== 3)==length(mm[idx:idx+40,1])
236
237  #test exception
238  position_df = make_padded_df(posfasta, gff, genome, index,
239  ↳   position_pad)
240
241  partition_coords_dict=partition_genome_coordinates(gff,
242  ↳   perigenic_pad)
243
244  ↳   partitioned_scaffolds=divide_partitions_by_scaffold(partition_coords_dict)
245  scaffold_coords_dict = Dict{String,DataFrame}()
246  scaffold="1"
247  for ((partition, part_scaffold), df) in partitioned_scaffolds
248      if scaffold == part_scaffold
249          scaffold_coords_dict[partition] = df
250      end
251  end
252
253  @test_throws DomainError
254  ↳   mask_sequence_by_partition(1001,151,scaffold_coords_dict)
255 end
256
257 @testset "genome_sampling/sequence_sampler.jl functions &
258 ↳   API/genome_sampling.jl interface" begin
259     #rectifier tests

```

```

250 @test rectify_identifier("1")=="1"
251
252 partitions = 3 #exonic, periexonic, intragenic
253 sample_set_length=100
254 min_sample_window=5
255 max_sample_window=25
256 perigenic_pad=250
257 syn_intergenic_starts = [1]
258 syn_intergenic_ends = [250]
259 syn_periexonic_starts = [251,571,661,761,861,961]
260 syn_periexonic_ends = [510,600,700,800,900,1000]
261 syn_periexonic_strands = ['-', '-', '--', '--', '--', '--']
262 syn_exonic_starts = [511,601,701,801,901]
263 syn_exonic_ends = [570,660,760,860,960]
264 syn_exonic_strands = ['+', '--', '--', '--', '--']
265 partition_lengths =
266   ↳ Dict("exon"⇒5*60, "intergenic"⇒250, "periexonic"⇒450)
267
268 # "Testing sequence sampler fns ... "
269 synthetic_seq =
270   ↳ BioSequences.LongSequence{DNAAlphabet{2}}(generate_synthetic_seq())
271
272 # "Partitioning synthetic genome coordinates ... "
273 coordinate_partitions = partition_genome_coordinates(gff,
274   ↳ perigenic_pad)
275
276 @test coordinate_partitions["intergenic"].Start =
277   ↳ syn_intergenic_starts
278 @test coordinate_partitions["intergenic"].End = syn_intergenic_ends
279
280 @test coordinate_partitions["periexonic"].Start =
281   ↳ syn_periexonic_starts
282 @test coordinate_partitions["periexonic"].End = syn_periexonic_ends
283 @test coordinate_partitions["periexonic"].Strand =
284   ↳ syn_periexonic_strands
285
286 @test coordinate_partitions["exon"].Start = syn_exonic_starts
287 @test coordinate_partitions["exon"].End = syn_exonic_ends
288 @test coordinate_partitions["exon"].Strand = syn_exonic_strands
289
290 # "Checking sampling functions at all synthetic indices ... "

```

```

286     input_test_channel, completed_test_channel, progress_channel,
287     ↵ setlength = setup_sample_jobs(genome, index, gff,
288     ↵ sample_set_length, min_sample_window, max_sample_window,
289     ↵ perigenic_pad; deterministic=true)
290     while isready(input_test_channel)
291         genome_path, genome_index_path, partition_df, partitionid,
292         ↵ sample_set_length, sample_window_min, sample_window_max,
293         ↵ deterministic = take!(input_test_channel)
294
295         for feature in eachrow(partition_df)
296             seqid, scaffold_start, scaffold_end =
297                 ↵ meta_to_feature_coord(feature.MetaStart, feature.MetaEnd,
298                 ↵ partition_df)
299             @test scaffold_start == feature.Start && scaffold_end ==
300                 ↵ feature.End
301         end
302
303         stranded = get_strand_dict()[partitionid]
304         @test typeof(stranded) == Bool
305
306         scaffold_sequence_record_dict = build_scaffold_seq_dict(genome,
307             ↵ index)
308         @test scaffold_sequence_record_dict["1"] == synthetic_seq
309
310         partition_length = partition_df.MetaEnd[end]
311         @test partition_length == partition_lengths[partitionid]
312
313         metacoordinate_bitarray = trues(partition_df.MetaEnd[end])
314
315         for bitindex in findall(metacoordinate_bitarray)
316             feature_metaStart, feature_metaEnd, strand =
317                 ↵ get_feature_params_from_metacoord(bitindex, partition_df,
318                 ↵ stranded)
319             @test 1 ≤ feature_metaStart < feature_metaEnd ≤
320                 ↵ length(metacoordinate_bitarray)
321             @test feature_metaStart in partition_df.MetaStart
322             @test feature_metaEnd in partition_df.MetaEnd
323             if partitionid == "periexonic"
324                 @test strand == '-'
325             elseif partitionid == "exon"
326                 @test strand in ['-','+']
327             end
328             feature_length = length(feature_metaStart:feature_metaEnd)

```

```

317         window = determine_sample_window(feature_metaStart,
318                                         → feature_metaEnd, bitindex, metacoordinate_bitarray,
319                                         → sample_window_min, sample_window_max) #get an appropriate
320                                         → sampling window around the selected index, given the
321                                         → feature boundaries and params
322
323     @test 1 ≤ feature_metaStart ≤ window[1] <
324         → window[1]+sample_window_min-1 <
325         → window[1]+sample_window_max-1 ≤ window[2] ≤
326         → feature_metaEnd ≤ length(metacoordinate_bitarray)
327
328     sample_scaffoldid, sample_scaffold_start, sample_scaffold_end
329         → = meta_to_feature_coord(window[1],window[2],partition_df)
330
331     @test sample_scaffoldid = "1"
332
333     @test 1 ≤ sample_scaffold_start ≤
334         → sample_scaffold_start+sample_window_min ≤
335         → sample_scaffold_end ≤
336         → min(sample_scaffold_start+sample_window_max,1000) ≤ 1000
337
338
339     strand = '-' ?
340         → target_seq=reverse_complement(synthetic_seq[sample_scaffold_start:sample_scaffold_end])
341         → :
342
343     target_seq =
344         → synthetic_seq[sample_scaffold_start:sample_scaffold_end]
345
346
347     proposal_sequence = fetch_sequence(sample_scaffoldid,
348                                         → scaffold_sequence_record_dict, sample_scaffold_start,
349                                         → sample_scaffold_end, strand; deterministic=deterministic)
350                                         → #get the sequence associated with the sample window
351
352
353     @test proposal_sequence == target_seq
354
355 end
356
357
358 # "Verifying sampling channels ... "
359
360
361 input_sample_channel, completed_sample_channel, progress_channel,
362     → setlength = setup_sample_jobs(genome, index, gff,
363     → sample_set_length, min_sample_window, max_sample_window,
364     → perigenic_pad; deterministic=true)
365 get_sample_set(input_sample_channel, completed_sample_channel,
366     → progress_channel)
367
368 #collect sample dfs by partition id when ready
369 collected_counter = 0

```

```

339     sample_record_dfs = Dict{String,DataFrame}()
340     while collected_counter < partitions
341         wait(completed_sample_channel)
342         partition_id, sample_df = take!(completed_sample_channel)
343         sample_record_dfs[partition_id] = sample_df
344         collected_counter += 1
345     end
346
347     #get_sample entire feature selection
348     coordinate_partitions = partition_genome_coordinates(gff,
349     ↳ perigenic_pad)
350     partition_id="exon"
351     partition=coordinate_partitions[partition_id]
352     add_metacoordinates!(partition)
353     metacoordinate_bitarray=true(partition.MetaEnd[end])
354     scaffold_sequence_record_dict = build_scaffold_seq_dict(genome,
355     ↳ index)
356
357     #test fetch_sequence strand char ArgumentError
358     @test_throws ArgumentError fetch_sequence("1",
359     ↳ Dict{String,LongSequence}("1"⇒LongSequence{DNAAlphabet{2}}("AAAAAAAAAAAAAA"))
360     ↳ 1, 5, 'L')
361
362     #test get_feature_row_index ArgumentError
363     @test_throws DomainError get_feature_row_index(partition,0)
364
365     @test length(get_sample(metacoordinate_bitarray, 1, 250, partition,
366     ↳ scaffold_sequence_record_dict)[6])=60
367
368     synthetic_seq =
369     ↳ BioSequences.LongSequence{DNAAlphabet{2}}(generate_synthetic_seq())
370     for (partid, df) in sample_record_dfs
371         for sample in eachrow(df)
372             target_seq =
373             ↳ synthetic_seq[sample.SampleStart:sample.SampleEnd]
374             strand = sample.Strand
375             if sample.Strand == '-'
376                 target_seq = reverse_complement(target_seq)
377             end
378             @test sample.SampleSequence == target_seq
379         end
380     end
381
382 
```

```

375 #execute_sample_jobs API
376 wkpool=addprocs(1)
377 @everywhere using BioBackgroundModels
378
379 channels = setup_sample_jobs(genome, index, gff, sample_set_length,
380   → min_sample_window, max_sample_window, perigenic_pad;
381   → deterministic=true)
380 records=execute_sample_jobs(channels, wkpool)
381
382 rmprocs(wkpool)
383 end
384
385 @testset "likelihood_funcs/hmm.jl & bg_lh_matrix.jl functions" begin
386     pvec = [.4,.3,.2,.1]
387     trans = ones(1,1)
388     B = [Categorical(pvec)]
389     hmm = BHMM(trans, B)
390
391     testseq=zeros(Int64,1,5)
392     testseq[1:4] = [1,2,3,4]
393     @test isapprox(get_BGHMM_symbol_lh(testseq, hmm)[1], log.(pvec[1]))
394     @test
395         → isapprox(obs_lh_given_hmm(testseq,hmm),obs_lh_given_hmm(testseq,hmm,linear=false))
396     @test isapprox(sum(get_BGHMM_symbol_lh(testseq,hmm)),
397         → obs_lh_given_hmm(testseq,hmm))
396     @test isapprox(sum(get_BGHMM_symbol_lh(testseq,hmm)),
398         → obs_lh_given_hmm(testseq,hmm,linear=false))
399
400     pvec=[.25,.25,.25,.25]
401     trans = [.9 .1
402               .1 .9]
403     B = [Categorical(pvec), Categorical(pvec)]
404     hmm = BHMM(trans, B)
405
406     @test
407         → isapprox(obs_lh_given_hmm(testseq,hmm),obs_lh_given_hmm(testseq,hmm,linear=false))
408     @test isapprox(sum(get_BGHMM_symbol_lh(testseq,hmm)),
409         → obs_lh_given_hmm(testseq,hmm))
410     @test isapprox(sum(get_BGHMM_symbol_lh(testseq,hmm)),
411         → obs_lh_given_hmm(testseq,hmm,linear=false))
412
413     testseq=zeros(Int64,1,1001)
414     testseq[1,1:1000]=rand(1:4,1000)

```

```

410
411     @test isapprox(sum(get_BGHMM_symbol_lh(testseq,
412         → hmm)),obs_lh_given_hmm(testseq,hmm))
413
414     Dex = [Categorical([.3, .1, .3, .3]),Categorical([.15, .35, .35,
415         → .15])]
416     Dper = [Categorical([.15, .35, .35, .15]),Categorical([.4, .1, .1,
417         → .4])]
418     Dint = [Categorical([.4, .1, .1, .4]),Categorical([.45, .05, .05,
419         → .45])]
420     BGHMM_dict = Dict{String,BHMM}()
421     BGHMM_dict["exon"] = BHMM(trans, Dex)
422     BGHMM_dict["perixonic"] = BHMM(trans, Dper)
423     BGHMM_dict["intergenic"] = BHMM(trans, Dint)
424
425     position_length=141;perigenic_pad=250;
426     position_df = make_padded_df(posfasta, gff, genome, index,
427         → position_length)
428
429     reader=open(FASTA.Reader, genome, index=index)
430     seq=FASTA.sequence(reader["CM002885.2.1"])
431     @test position_df.Start[1]==501
432     @test position_df.End[1]==641
433     @test position_df.PadSeq[1]==seq[360:641]
434     @test position_df.PadStart[1]==360
435     @test position_df.RelStart[1]==141
436     @test position_df.SeqOffset[1]==0
437
438     offset_position_df = make_padded_df(posfasta, gff, genome, index,600)
439     @test offset_position_df.Start[1]==501
440     @test offset_position_df.End[1]==641
441     @test offset_position_df.PadSeq[1]==seq[1:641]
442     @test offset_position_df.PadStart[1]==1
443     @test offset_position_df.RelStart[1]==500
444     @test offset_position_df.SeqOffset[1]==100
445
446     add_partition_masks!(position_df, gff, perigenic_pad)
447     @test all(position_df.MaskMatrix[1][1:151,1]==2)
448     @test all(position_df.MaskMatrix[1][152:211,1]==3)
449     @test all(position_df.MaskMatrix[1][212:241,1]==2)
450     @test all(position_df.MaskMatrix[1][242:end,1]==3)
451     @test all(position_df.MaskMatrix[1][1:151,2]==-1)
452     @test all(position_df.MaskMatrix[1][152:211,2]==1)

```

```

448 @test all(position_df.MaskMatrix[1][212:end,2].== -1)
449
450 lh_matrix=BGHMM_likelihood_calc(position_df,BGHMM_dict)
451 @test size(lh_matrix)=(position_length*2,1)
452
453
454     → periexonic_frag=reverse_complement!(LongSequence{DNAAlphabet{2}})(seq[360:510])
455 pno=get_order_n_seqs([periexonic_frag],0)
456 pcode=code_seqs(pno)
457 plh=get_BGHMM_symbol_lh(pcode, BGHMM_dict["periexonic"])
458 @test isapprox(lh_matrix[1:151,1], reverse(plh))
459
460 exonic_frag=LongSequence{DNAAlphabet{2}}(seq[511:570])
461 eno=get_order_n_seqs([exonic_frag],0)
462 ecode=code_seqs(eno)
463 elh=get_BGHMM_symbol_lh(ecode, BGHMM_dict["exon"])
464 @test isapprox(lh_matrix[152:211,1],elh)
465 end
466
467 @testset "EM/baum-welch.jl MS_HMMBase equivalency and functions" begin
468     A = [.5 .5
469             .5 .5]
470     B = [Categorical(ones(4)/4), Categorical([.7,.1,.1,.1])]
471     hmm = BHMM(A, B)
472     log_A = log.(hmm.A)
473
474     obs = zeros(UInt8, 22,1)
475     obs[1:21] = [4,3,3,2,3,2,1,1,2,3,3,3,4,4,2,3,2,3,4,3,2]
476     obs_lengths=[21]
477
478     lls = BioBackgroundModels.bw_llhs(hmm,obs)
479     m_lls = mouchet_log_likelihoods(hmm,obs[1:21])
480
481     K,Tmaxplus1,0 = size(lls)
482     T=Tmaxplus1-1
483
484     #TEST LOG LIKELIHOOD FN
485     @test transpose(lls[:,1:end-1,1]) == m_lls
486
487     log_α = BioBackgroundModels.messages_forwards_log(hmm.a, hmm.A, lls,
488             → obs_lengths)
489     m_log_α = mouchet_messages_forwards_log(hmm.a, hmm.A, m_lls)
490
491

```

```

489 #TEST FORWARD MESSAGES FN
490 @test transpose(log_α[:,1:end-1,1]) = m_log_α
491
492 log_β = BioBackgroundModels.messages_backwards_log(hmm.A, lls,
493   ↳ obs_lengths)
493 m_log_β = mouchet_messages_backwards_log(hmm.A, m_lls)
494
495 #TEST BACKWARDS MESSAGES FN
496 @test isapprox(transpose(log_β[:,1:end-1,1]), m_log_β)
497
498 lls = permutedims(lls, [2,3,1]) # from (K,T,O) to (T,O,K)
499 log_α = permutedims(log_α, [2,3,1])
500 log_β = permutedims(log_β, [2,3,1])
501
502 # "Testing obs probability calcs ... "
503 #TEST OBSERVATION PROBABILITY CALC
504 normalizer = logsumexp(m_log_α[1,:]+m_log_β[1,:])
505 log_pobs = logsumexp(lps.(log_α[1,1,:], log_β[1,1,:]))
506
507 @test isapprox(normalizer, log_pobs)
508
509 # "Testing ξ, γ calcs ... "
510
511 log_ξ = fill(-Inf, Tmaxplus1, 0, K, K)
512 log_γ = fill(-Inf, Tmaxplus1, 0, K)
513
514 m_log_ξ = zeros(T, K, K)
515
516 for t = 1:T-1, i = 1:K, j = 1:K
517   m_log_ξ[t,i,j] = m_log_α[t,i] + log_A[i,j] + m_log_β[t+1,j] +
518     ↳ m_lls[t+1,j] - normalizer
519 end
520
521 for i = 1:K, j = 1:K, o = 1:O
522   obsl = obs_lengths[o]
523   for t = 1:obsl-1 #log_ξ & log_γ calculated to T-1 for each o
524     log_ξ[t,o,i,j] = lps(log_α[t,o,i], log_A[i,j], log_β[t+1,o,j],
525       ↳ lls[t+1,o,j], -log_pobs[o])
526     log_γ[t,o,i] = lps(log_α[t,o,i], log_β[t,o,i], -log_pobs[o])
527   end
528   t=obsl #log_ξ @ T = 0
529   log_ξ[t,o,i,j] = 0
530   log_γ[t,o,i] = lps(log_α[t,o,i], log_β[t,o,i], -log_pobs)

```

```

529     end
530
531     ξ = exp.(log_ξ)
532     k_ξ = sum(ξ, dims=[3,4])
533     nan_mask = k_ξ .== 0; k_ξ[nan_mask] .= Inf #prevent NaNs in dummy
      ↵ renorm arising from multiple sequences indexing
534     ξ ./= k_ξ #dummy renorm across K to keep numerical creep from
      ↵ causing isprobvec to fail on new new_A during hmm creation
535
536     m_ξ = exp.(m_log_ξ)
537     m_ξ ./= sum(m_ξ, dims=[2,3])
538
539     #check equivalency of xi calc methods
540     @test isapprox(reshape(ξ,Tmaxplus1,K,K)[1:21,:,:],m_ξ)
541
542     γ = exp.(log_γ)
543     k_γ = sum(γ, dims=3); k_γ[nan_mask[:, :, :]] .= Inf #prevent NaNs in
      ↵ dummy renorm
544     γ ./= k_γ
545
546     m_γ = exp.((m_log_α .+ m_log_β) .- normalizer)
547
548     #check equivalency of gamma calculations
549     @test isapprox(reshape(γ,Tmaxplus1,K)[1:21,:,:],m_γ)
550
551     # "Testing initial and transition matrix calcs ... "
552
553     m_new_A = sum(m_ξ[1:end-1,:,:], dims=1)[1,:,:]
554     m_new_A ./= sum(m_new_A, dims=2)
555
556     new_A = zeros(K,K)
557     for i=1:K, j=1:K
558         Σotξ_vec = zeros(0)
559         Σotγ_vec = zeros(0)
560         for o in 1:0
561             Σotξ_vec[o] = sum(ξ[1:obs_lengths[o]-1,o,i,j])
562             Σotγ_vec[o] = sum(γ[1:obs_lengths[o]-1,o,i])
563         end
564         new_A[i,j] = sum(Σotξ_vec) / sum(Σotγ_vec)
565     end
566     new_A ./= sum(new_A, dims=[2]) #dummy renorm
567
568     #check equivalency of transition matrix calculations

```

```

569 @test isapprox(m_new_A,new_A)
570
571 m_new_a = exp.((m_log_α[1,:]+m_log_β[1,:]) .- normalizer)
572 m_new_a ./= sum(m_new_a)
573
574 new_a = (sum(y[1,:,:], dims=1)./sum(sum(y[1,:,:], dims=1)))[1,:]
575 new_a ./= sum(new_a) #dummy renorm
576
577 @test isapprox(m_new_a,new_a)
578
579 # "Testing distribution calcs ... "
580
581 F=Univariate
582 m_D = Distribution{F}[]
583 for (i, d) in enumerate(hmm.B)
584     # Super hacky ...
585     # https://github.com/JuliaStats/Distributions.jl/issues/809
586     push!(m_D, fit_mle(eval(typeof(d).name.name),
587         ↳ permutedims(obs[1:21]), m_y[:,i]))
588 end
589
590 obs_mask = .!nan_mask
591 obs_collection = obs[obs_mask[:, :]]
592
593 B = Distribution{F}[]
594 @inbounds for (i, d) in enumerate(hmm.B)
595     # Super hacky ...
596     # https://github.com/JuliaStats/Distributions.jl/issues/809
597     γ_d = y[:, :, i]
598     push!(B, fit_mle(eval(typeof(d).name.name), obs_collection,
599         ↳ γ_d[obs_mask[:, :]]))
600     #slowest call by orders of magnitude
601 end
602
603 #test maximization equivalency
604 for (d, dist) in enumerate(B)
605     @test isapprox(dist.support, m_D[d].support)
606     @test isapprox(dist.p, m_D[d].p)
607 end
608
609 # "Testing mle_step ... "
610
611

```

```

609  #verify that above methods independently produce equivalent output,
610  #→ and that this is true of multiple identical obs, but not true of
611  #→ different obs sets
612  mouchet_hmm = mouchet_mle_step(hmm, obs[1:21])
613
614  dblobs = zeros(UInt8, 22,2)
615  dblobs[1:21,1] = [4,3,3,2,3,2,1,1,2,3,3,3,4,4,2,3,2,3,4,3,2]
616  dblobs[1:21,2] = [4,3,3,2,3,2,1,1,2,3,3,3,4,4,2,3,2,3,4,3,2]
617  dblobs_lengths=[21,21]
618  dbl_hmm = BioBackgroundModels.bw_step(hmm, dblobs, dblobs_lengths)
619
620  otherobs = dblobs
621  otherobs[1:21,2] = [1,2,3,4,1,2,3,4,1,2,3,4,1,2,3,4,1,2,3,4,1]
622  other_hmm = BioBackgroundModels.bw_step(hmm, otherobs,
623  #→ dblobs_lengths)
624
625  for n in fieldnames(typeof(new_hmm[1]))
626      if n == :B
627          for (d, dist) in enumerate(new_hmm[1].B)
628              @test dist.support == mouchet_hmm[1].B[d].support
629              @test isapprox(dist.p,mouchet_hmm[1].B[d].p)
630              @test dist.support == dbl_hmm[1].B[d].support
631              @test isapprox(dist.p,dbl_hmm[1].B[d].p)
632              @test dist.support == other_hmm[1].B[d].support
633              @test !isapprox(dist.p, other_hmm[1].B[d].p)
634      end
635  elseif n == :partition
636      @test getfield(new_hmm[1],n) == getfield(mouchet_hmm[1],n)
637      @test getfield(new_hmm[1],n) == getfield(dbl_hmm[1],n)
638      @test getfield(new_hmm[1],n) == getfield(other_hmm[1],n)
639  else
640      @test isapprox(getfield(new_hmm[1],n),
641      #→ getfield(mouchet_hmm[1],n))
642      @test isapprox(getfield(new_hmm[1],n),
643      #→ getfield(dbl_hmm[1],n))
644      @test !isapprox(getfield(new_hmm[1],n),
645      #→ getfield(other_hmm[1],n))
646  end
647
648  @test new_hmm[2] == mouchet_hmm[2] ≠ dbl_hmm[2] ≠ other_hmm[2]

```

```

646
647 # "Testing fit_mle! ... "
648
649 # "test fit_mle! function"
650 input_hmms= RemoteChannel(()→Channel{Tuple}(1))
651 output_hmms = RemoteChannel(()→Channel{Tuple}(30))
652 chainid=Chain_ID("Test",2,0,1)
653 put!(input_hmms, (chainid, 2, hmm, 0.0, transpose(obs)))
654 BioBackgroundModels.EM_converge!(input_hmms, output_hmms, 1;
655     → EM_func=BioBackgroundModels.bw_step, max_iterates=4,
656     → verbose=true)
657 wait(output_hmms)
658 workerid, jobid, iterate, hmm3, log_p, epsilon, converged =
659     → take!(output_hmms)
660 @test jobid == chainid
661 @test iterate == 3
662 @test assert_hmm(hmm3.a, hmm3.A, hmm3.B)
663 @test size(hmm3) == size(hmm) == (2,4)
664 @test log_p < 1
665 @test log_p == obs_lh_given_hmm(transpose(obs),hmm, linear=false)
666 @test converged == false
667 wait(output_hmms)
668 workerid, jobid, iterate, hmm4, log_p, epsilon, converged =
669     → take!(output_hmms)
670 @test jobid == chainid
671 @test iterate == 4
672 @test assert_hmm(hmm4.a, hmm4.A, hmm4.B)
673 @test size(hmm4) == size(hmm) == (2,4)
674 @test log_p < 1
675 @test log_p == obs_lh_given_hmm(transpose(obs),hmm3,linear=false)
676 @test converged == false
677
678 # "Test convergence.. "
679 obs=zeros(UInt8, 101, 2)
680 for i in 1:size(obs)[2]
681     obs[1:100,i]=rand(1:4,100)
682 end
683 input_hmms= RemoteChannel(()→Channel{Tuple}(1))
684 output_hmms = RemoteChannel(()→Channel{Tuple}(30))
685 put!(input_hmms, (chainid, 2, hmm, 0.0, transpose(obs)))
686 BioBackgroundModels.EM_converge!(input_hmms, output_hmms, 1;
687     → EM_func=BioBackgroundModels.bw_step, delta_thresh=.05,
688     → max_iterates=100, verbose=true)

```

```

683     wait(output_hmms)
684     workerid, jobid, iterate, hmm4, log_p, epsilon, converged =
685         → take!(output_hmms)
686     while isready(output_hmms)
687         workerid, jobid, iterate, hmm4, log_p, epsilon, converged =
688             → take!(output_hmms)
689     end
690     @test converged==1
691
692     #test t_add_categorical_counts DimensionMismatch
693     @test_throws DimensionMismatch t_add_categorical_counts!(zeros(4),
694         → zeros(UInt8,2,3), zeros(2,2))
695
696 end
697
698
699 @testset "EM/churbanov.jl Baum-Welch equivalency and functions" begin
700     # "Setting up for MLE function tests.."
701     A = fill((1/6),6,6)
702     B = [Categorical(ones(4)/4),
703         → Categorical([.7,.05,.15,.1]),Categorical([.15,.35,.4,.1]),
704         → Categorical([.6,.15,.15,.1]),Categorical([.1,.4,.4,.1]),
705         → Categorical([.2,.2,.3,.3])]
706     hmm = BHMM(A, B)
707     log_A = log.(hmm.A)
708
709     obs = zeros(Int64,1,250)
710     obs[1:249] = rand(1:4,249)
711     obs_lengths=[249]
712     # "Testing mle_step ... "
713
714     #verify that above methods independently produce equivalent output,
715     → and that this is true of multiple identical obs, but not true of
716     → different obs sets
717     mouchet_hmm = mouchet_mle_step(hmm, obs[1:249])
718
719     new_hmm = linear_step(hmm, obs, obs_lengths)
720
721     ms_sng = BioBackgroundModels.bw_step(hmm, Array(transpose(obs)),
722         → obs_lengths)
723
724     dblobs = zeros(Int64, 2,250)
725     dblobs[1,1:249] = obs[1:249]
726     dblobs[2,1:249] = obs[1:249]

```

```

717     dbllobs_lengths=[249,249]
718     dbl_hmm = linear_step(hmm, dbllobs, dbllobs_lengths)
719
720
721     ms_dbl =BioBackgroundModels.bw_step(hmm,
722         → Array(transpose(dbllobs)),dblobs_lengths)
723
724     otherobs = deepcopy(dbllobs)
725     otherobs[2,1:249] = rand(1:4,249)
726     if otherobs[1,1]==otherobs[2,1]
727         otherobs[1,1]=1&&(otherobs[2,1]==2)
728         otherobs[1,1]=2&&(otherobs[2,1]==3)
729         otherobs[1,1]=3&&(otherobs[2,1]==4)
730         otherobs[1,1]=4&&(otherobs[2,1]==1)
731     end
732
733     other_hmm = linear_step(hmm, otherobs, dbllobs_lengths)
734
735     ms_hmm = BioBackgroundModels.bw_step(hmm,
736         → Array(transpose(otherobs)),dblobs_lengths)
737
738     for n in fieldnames(typeof(new_hmm[1]))
739         if n == :B
740             for (d, dist) in enumerate(new_hmm[1].B)
741                 @test dist.support==mouchet_hmm[1].B[d].support
742                 @test isapprox(dist.p,mouchet_hmm[1].B[d].p)
743                 @test dist.support==ms_sng[1].B[d].support
744                 @test isapprox(dist.p,ms_sng[1].B[d].p)
745                 @test dist.support==dbl_hmm[1].B[d].support
746                 @test isapprox(dist.p,dbl_hmm[1].B[d].p)
747                 @test dist.support==ms_dbl[1].B[d].support
748                 @test isapprox(dist.p,ms_dbl[1].B[d].p)
749                 @test dist.support==other_hmm[1].B[d].support
750                 @test ms_hmm[1].B[d].support==other_hmm[1].B[d].support
751                 @test !isapprox(dist.p, other_hmm[1].B[d].p)
752                 @test isapprox(ms_hmm[1].B[d].p, other_hmm[1].B[d].p)
753             end
754         elseif n == :partition
755             @test getfield(new_hmm[1],n) == getfield(mouchet_hmm[1],n)
756             @test getfield(new_hmm[1],n) == getfield(dbl_hmm[1],n)
757             @test getfield(new_hmm[1],n) == getfield(other_hmm[1],n)
758         else

```

```

758         @test isapprox(getfield(new_hmm[1],n),
759                         getfield(mouchet_hmm[1],n))
760
761         @test isapprox(getfield(new_hmm[1],n),
762                         getfield(dbl_hmm[1],n))
763
764         @test isapprox(getfield(new_hmm[1],n), getfield(ms_dbl[1],n))
765
766     end
767
768 end
769
770 @test ms_sng[2] == new_hmm[2] == mouchet_hmm[2] != dbl_hmm[2] ==
771     → ms_dbl[2] != other_hmm[2] == ms_hmm[2]
772
773 # "Testing fit_mle! ... "
774
775 #test fit_mle! function
776 input_hmms= RemoteChannel(()→Channel{Tuple}(1))
777 output_hmms = RemoteChannel(()→Channel{Tuple}(30))
778 chainid=Chain_ID("Test",6,0,1)
779 put!(input_hmms, (chainid, 2, hmm, 0.0, obs))
780 BioBackgroundModels.EM_converge!(input_hmms, output_hmms, 1;
781     → max_iterates=4, verbose=true)
782 wait(output_hmms)
783 workerid, jobid, iterate, hmm3, log_p, delta, converged =
784     → take!(output_hmms)
785 @test jobid == chainid
786 @test iterate == 3
787 @test BioBackgroundModels.assert_hmm(hmm3.a, hmm3.A, hmm3.B)
788 @test size(hmm3) == size(hmm) == (6,4)
789 @test log_p < 1
790 @test log_p == linear_likelihood(obs, hmm)
791 @test converged == false
792 wait(output_hmms)
793 workerid, jobid, iterate, hmm4, log_p, delta, converged =
794     → take!(output_hmms)
795 @test jobid == chainid
796 @test iterate == 4

```

```

793     @test BioBackgroundModels.assert_hmm(hmm4.a, hmm4.A, hmm4.B)
794     @test size(hmm4) == size(hmm) == (6,4)
795     @test log_p < 1
796     @test log_p == linear_likelihood(obs, hmm3)
797     @test converged == false

798
799     # "Test convergence.."
800     obs=zeros(Int64, 4, 1001)
801     for o in 1:size(obs)[1]
802         obsl=rand(100:1000)
803         obs[o,1:obsl]=rand(1:4,obsl)
804     end
805     input_hmms= RemoteChannel(()→Channel{Tuple}(1))
806     output_hmms = RemoteChannel(()→Channel{Tuple}(Inf))
807     put!(input_hmms, (chainid, 2, hmm, 0.0, obs))
808     BioBackgroundModels.EM_converge!(input_hmms, output_hmms, 1;
809         → delta_thresh=.05, max_iterates=100, verbose=true)
810     wait(output_hmms)
811     workerid, jobid, iterate, hmm4, log_p, delta, converged =
812         → take!(output_hmms)
813     while isready(output_hmms)
814         workerid, jobid, iterate, hmm4, log_p, delta, converged =
815             → take!(output_hmms)
816     end
817     @test converged==1
818
819     @testset "API/EM_master.jl and reports.jl API tests" begin
820         #CONSTANTS FOR BHMM LEARNING
821         replicates = 4 #repeat optimisation from this many seperately
822             → initialised samples from the prior
823         Ks = [1,2,4,6] #mosaic class #s to test
824         order_nos = [0,1,2] #DNA kmer order #s to test
825         sample_set_length=100
826         min_sample_window=5
827         max_sample_window=25
828         perigenic_pad=250
829
830         wkpool=addprocs(2)
831         @everywhere using BioBackgroundModels

```

```

830     channels = setup_sample_jobs(genome, index, gff, sample_set_length,
831         ↳ min_sample_window, max_sample_window, perigenic_pad;
832         ↳ deterministic=true)
833     sample_record_dfs=execute_sample_jobs(channels, wkpool)
834     training_sets, test_sets = split_obs_sets(sample_record_dfs)
835
836
837     job_ids=Vector{Chain_ID}()
838     for (obs_id, obs) in training_sets, K in Ks, order in order_nos, rep
839         ↳ in 1:replicates
840             push!(job_ids, Chain_ID(obs_id, K, order, rep))
841     end
842
843     no_input_hmms, chains, input_hmms, output_hmms =
844         ↳ setup_EM_jobs!(job_ids, training_sets)
845     @test no_input_hmms ==
846         ↳ replicates*length(Ks)*length(order_nos)*length(training_sets)
847
848     while isready(input_hmms)
849         jobid, start_iterate, hmm, last_norm, observations =
850             ↳ take!(input_hmms)
851         @test last_norm == linear_likelihood(observations, hmm)
852         obs_lengths = [findfirst(iszero, observations[o,:])-1 for o in
853             ↳ 1:size(observations)[1]]
854         #make sure input HMMs are valid and try to mle_step them and
855             ↳ ensure their 1-step children are valid
856         @test assert_hmm(hmm.a, hmm.A, hmm.B)
857         new_hmm, prob = linear_step(hmm, observations, obs_lengths)
858         @test assert_hmm(hmm.a, hmm.A, hmm.B)
859         @test prob < 0
860     end
861
862     genome_reader = open(FASTA.Reader, genome, index=index)
863
864         ↳ seq=LongSequence{DNAAlphabet{2}}(FASTA.sequence(genome_reader["CM002885.2.1"]))
865     seqdict = Dict("test"⇒[seq for i in 1:3])
866     seqdict["blacklist"] = seqdict["test"]
867
868     job_ids=Vector{Chain_ID}()
869     push!(job_ids, Chain_ID("blacklist", 1, 0, 1))
870     push!(job_ids, Chain_ID("blacklist", 1, 0, 2))

```

```

864 Ks=[1,2]; order_nos=[0,1]
865 for K in Ks, order in order_nos, replicate in 1:2
866     push!(job_ids, Chain_ID("test", K, order, replicate))
867 end
868
869 wkpool=addprocs(2, topology=:master_worker)
870 @everywhere using BioBackgroundModels
871
872 load_dict=Dict{Int64,LoadConfig}()
873 for (n,wk) in enumerate(wkpool)
874     n==1 &&
875         (load_dict[wk]=LoadConfig(1:2,0:1,blacklist=[Chain_ID("blacklist",1,0,1)])
876     n==2 && (load_dict[wk]=LoadConfig(1:2,0:1))
877 end
878 #test work resumption, load configs
879
880 em_jobset=setup_EM_jobs!(job_ids, seqdict, delta_thresh=10000.)
881 execute_EM_jobs!(wkpool, em_jobset..., "testchains",
882     → delta_thresh=10000., verbose=true, load_dict=load_dict)
883
884 for (chain_id,chain) in em_jobset[2]
885     @test chain[end].converged = true
886     @test chain[end].delta ≤ 10000
887     @test typeof(chain[end].hmm)=BHMM{Float64}
888     @test 1 ≤ chain[end].iterate ≤ 5000
889 end
890
891 wkpool=addprocs(2, topology=:master_worker)
892 @everywhere using BioBackgroundModels
893
894 em_jobset=setup_EM_jobs!(job_ids, seqdict,
895     → chains=deserialize("testchains"), delta_thresh=.1)
896 execute_EM_jobs!(wkpool, em_jobset..., "testchains", delta_thresh=.1,
897     → verbose=true)
898
899 for (chain_id,chain) in em_jobset[2]
900     @test chain[end].converged = true
901     @test chain[end].delta ≤ .1
902     @test typeof(chain[end].hmm)=BHMM{Float64}
903     @test 1 ≤ chain[end].iterate ≤ 5000
904 end
905
906 #test for already converged warning

```

```

903     wkpool=addprocs(2, topology=:master_worker)
904     @everywhere using BioBackgroundModels
905     @test_throws ArgumentError execute_EM_jobs!(wkpool, em_jobset..., 
906         → "testchains", delta_thresh=.01, verbose=true)
907
908     rm("testchains")
909
910     @test_throws ArgumentError
911         → generate_reports(Dict{Chain_ID,Vector{EM_step}}(),seqdict)
912     @test_throws ArgumentError
913         → generate_reports(em_jobset[2],Dict{String,Vector{LongSequence{DNAAlphabet{2}}}}
914
915     @test_throws ArgumentError
916         → BioBackgroundModels.report_chains(em_jobset[2],Dict{String,Vector{LongSequence{DNAAlphabet{2}}}})
917
918     report_folders=generate_reports(em_jobset[2],seqdict)
919     folder=report_folders["test"]
920     @test "test"==folder.partition_id
921     show(folder.partition_report)
922     show(folder.replicate_report)
923     for id in folder.partition_report.best_repset
924         show(folder.chain_reports[id])
925     end
926
927     end
928
929     rm(genome)
930     rm(index)
931     rm(gff)
932     rm(posfasta)

```

16.6.27 /test/synthetic_sequence_gen.jl

```

1 function print_synthetic_fasta(path::String,line_length::Integer=80)
2     header=>CM002885.2.1 BioBackgroundModels Synthetic chromosome
3         → for tests\n"
4     write(path, header,
5         → format_lines(generate_synthetic_seq(),line_length))
6 end
7
8 function print_synthetic_index(path::String)
9     write(path,
10        → "CM002885.2.1          1000          5          80          81\n")

```

```

8 end
9
10 function print_synthetic_position(path::String)
11     write(path, ">1 start 501 end 641 smt_pos 111 smt_value 205.11821
12     ↳ fuzziness_score
13     ↳ 43.53698\n", generate_synthetic_seq()[501:641], "\n")
14 end
15
16
17
18
19
20
21
22 1 Ensembl chromosome 1 1000 .
23 ###
24 1 ensembl_havana gene 501 1000 . - .
25 ↳ gene
26 ↳ [Source:FAKESRC];gene_id=ENSDARG00000000001;logic_name=ensembl_havana_gene;version=1
27 1 ensembl_havana mRNA 501 1000 .
28 1 ensembl_havana three_prime_UTR 501 510 .
29 1 ensembl_havana exon 501 570 .
30 1 ensembl_havana CDS 511 570 .
31 1 ensembl_havana exon 601 660 .
32 1 ensembl_havana CDS 601 660 .
33 1 ensembl_havana exon 701 760 .
34 1 ensembl_havana CDS 701 760 .
35 1 ensembl_havana exon 801 860 .
36 1 ensembl_havana CDS 801 860 .
37 1 ensembl_havana five_prime_UTR 901 975 .
38 ###
39     write(path,gff_txt)
40 end
41

```

```

42 function generate_synthetic_seq(gene_start::Integer=501,
43     ↵ UTR3L::Integer=10, exon_length::Integer=60,
44     ↵ intron_length::Integer=40, no_exons::Integer=5, UTR5L::Integer=25,
45     ↵ OAL::Integer=1000,
46     ↵ (iseq,pseq,eseq)::Tuple{String,String,String}=("AT","CG","CAT");
47     ↵ verbose::Bool=false)
48     ↵ @assert length(iseq) == 2
49     ↵ @assert length(pseq) == 2
50     ↵ @assert length(eseq) == 3
51     ↵ @assert mod(gene_start-1,2) == 0
52     ↵ @assert mod(exon_length,3) == 0
53     ↵ @assert mod(intron_length,2) == 0
54     ↵ gene_length=gene_start+UTR3L+(no_exons*exon_length)+((no_exons-1)*intron_length)
55     ↵ @assert gene_length ≤ OAL
56     ↵ seq=""
57     ↵ for i in 1:((gene_start-1) / 2)
58         ↵     seq *= iseq
59     ↵ end
60     ↵ verbose && @info "Intergenic length $(length(seq))"
61     ↵ for i in 1:UTR3L / 2
62         ↵     seq *= pseq
63     ↵ end
64     ↵ verbose && @info "Added 3'UTR... $(length(seq))"
65     ↵ for ex in 1:no_exons-1
66         ↵     for i in 1:(exon_length/3)
67             ↵         seq*=eseq
68         ↵     end
69         ↵     ex == 1 ? ilength = 30 : ilength = 40
70         ↵     for i in 1:(ilength/2)
71             ↵         seq*=pseq
72         ↵     end
73     ↵ end
74     ↵ for i in 1:(exon_length/3)
75         ↵     seq*=eseq
76     ↵ end
77     ↵ verbose && @info "Added exons and introns... $(length(seq))"
78     ↵ for i in 1:UTR5L / 2
79         ↵     seq *= pseq
80     ↵ end
81     ↵ verbose && @info "Added 5'UTR... $(length(seq))"
82
83     ↵ for i in 1:((OAL-length(seq))/2)
84         ↵     seq*=iseq

```

```

80         end
81
82     verbose && @info "Generated synthetic genome sequence of length
83     ↪  $(length(seq))"
84
85     return seq
86
87     function format_lines(seq::String, line_length::Integer)
88         a=join((SubString(seq,i,min(i+line_length-1,length(seq))) for
89         ↪  i=1:line_length:length(seq)), '\n')
90         return(a)
91     end

```

16.7 BioMotifInference

16.7.1 /src/BioMotifInference.jl

```

1 module BioMotifInference
2     using BioBackgroundModels, BioSequences, Distributed, Distributions,
2     ↪  Serialization, UnicodePlots
3     import DataFrames:DataFrame
4     import ProgressMeter: AbstractProgress, Progress, @showprogress,
4     ↪  next!, move_cursor_up_while_clearing_lines, printover,
4     ↪  durationstring
5     import Printf: @sprintf
6     import StatsFuns: logaddexp, logsumexp #both are needed as logsumexp
6     ↪  for two terms is deprecated
7     import Random: rand, seed!, shuffle!
8     import Distances: euclidean
9
10    #CONSTANTS AND PERMUTE FUNCTION ARGUMENT DEFAULTS GIVING RISE TO
10    ↪  IMPLEMENTATION-SPECIFIC SAMPLING EFFECTS
11    global MOTIF_EXPECT=1. #motif expectation- this value to be divided
11    ↪  by obs lengths to obtain penalty factor for scoring
12
13    global REVCOMP=true #calculate scores on both strands?
14
15    global TUNING_MEMORY=20 #coefficient multiplied by Permute_Instruct
15    ↪  function call limit to give total number of calls remembered by
15    ↪  tuner per function

```

```

16   global CONVERGENCE_MEMORY=500 #number of iterates to display for
   ↳ convergence interval history
17
18   global SRC_PERM_FREQ=.5 #frequency with which random_decorrelate will
   ↳ permute a source
19
20   global PWM_SHIFT_DIST=Weibull(.5,.1) #distribution of weight matrix
   ↳ permutation magnitudes
21   global PWM_SHIFT_FREQ=.2 #proportion of positions in source to
   ↳ permute weight matrix
22   global PWM_LENGTHPERM_FREQ=.2 #proportion of sources to permute
   ↳ length
23   global LENGTHPERM_RANGE=1:3
24
25   global PRIOR_WT=3. #estimate prior dirichlets from product of this
   ↳ constant and sample "mle" wm
26   global PRIOR_LENGTH_MASS=.8
27
28   global EROSION_INFO_THRESH=1.
29
30   global CONSOLIDATE_THRESH=.035
31
32   include("IPM/ICA_PWM_Model.jl")
33   export Model_Record
34   export ICA_PWM_Model
35   include("IPM/IPM_likelihood.jl")
36   include("IPM/IPM_prior_utilities.jl")
37   assemble_source_priors
38   include("ensemble/IPM_Engine.jl")
39   export IPM_Engine, assemble_IPMs
40   include("permutation/permute_utilities.jl")
41   include("permutation/orthogonality_helper.jl")
42   include("permutation/permute_functions.jl")
43   export full_perm_funcvec
44   include("permutation/permute_control.jl")
45   export Permute_Instruct
46   include("permutation/Permute_Tuner.jl")
47   include("ensemble/ensemble_utilities.jl")
48   export ensemble_history, reset_ensemble!, move_ensemble!,
   ↳ copy_ensemble!, rewind_ensemble, complete_evidence, get_model,
   ↳ show_models
49   include("utilities/model_display.jl")
50   include("utilities/worker_diagnostics.jl")

```

```

51 include("utilities/ns_progressmeter.jl")
52 include("utilities/synthetic_genome.jl")
53 include("utilities/worker_sequencer.jl")
54 export synthetic_sample
55 include("nested_sampler/nested_step.jl")
56 include("nested_sampler/converge_ensemble.jl")
57 export converge_ensemble!
58
59 end # module

```

16.7.2 /src/IPM/ICA_PWM_Model.jl

```

1 struct Model_Record #record struct to associate a log_Li with a saved,
2   ↳ calculated model
3   path::String
4   log_Li::AbstractFloat
5 end
6
6 struct ICA_PWM_Model #Independent component analysis position weight
7   ↳ matrix model
8   name::String #designator for saving model to posterior
9   origin::String #functions instantiating IPMs should give an
10    ↳ informative desc of the means by which the model was generated
11   sources::Vector{Tuple{<:AbstractMatrix{<:AbstractFloat},<:Integer}}
12    ↳ #vector of PWM signal sources (LOG PROBABILITY!!!) tupled with an
13    ↳ index denoting the position of the first PWM base on the prior
14    ↳ matrix- allows us to permute length and redraw from the
15    ↳ appropriate prior position
16   source_length_limits::UnitRange{<:Integer} #min/max source lengths
17    ↳ for init and permutation
18   mix_matrix::BitMatrix # obs x sources bool matrix
19   log_Li::AbstractFloat
20   permute_blacklist::Vector{Function} #blacklist of functions that
21    ↳ ought not be used to permute this model (eg. because to do so
22    ↳ would not generate a different model for IPMs produced from
23    ↳ fitting the mix matrix)
24   function ICA_PWM_Model(name, origin, sources, source_length_limits,
25     ↳ mix_matrix, log_Li, permute_blacklist=Vector{Function}())
26     verify_srcs(name, origin, sources)
27     new(name, origin, sources, source_length_limits, mix_matrix,
28       ↳ log_Li, permute_blacklist)
29   end

```

```

18 end
19
20 #ICA_PWM_Model FUNCTIONS
21 ICA_PWM_Model(name::String,
22   ↳ source_priors::AbstractVector{<:Union{<:AbstractVector{<:Dirichlet{<:AbstractFloat},<:BitMatrix},<:AbstractFloat}}, 
23   ↳ mix_prior::Tuple{BitMatrix,<:AbstractFloat},
24   ↳ bg_scores::AbstractArray{<:AbstractFloat},
25   ↳ observations::AbstractArray{<:Integer},
26   ↳ source_length_limits::UnitRange{<:Integer}) = init_IPM(name,
27   ↳ source_priors,mix_prior,bg_scores,observations,source_length_limits)
28
29 function verify_srcs(name, ori,
30   ↳ sources::Vector{<:Tuple{<:AbstractMatrix{<:AbstractFloat},<:Integer}})
31   for (n,(pwm,pi)) in enumerate(sources)
32     !all(isprobvec(exp.(pwm[pos,:])) for pos in 1:size(pwm,1)) &&
33     ↳ throw(DomainError("Bad probvec in model $name, source $n,
34     ↳ origin $(ori):$(exp.(pwm)))")
35   end
36 end
37
38
39 #MODEL INIT
40 function init_IPM(name::String,
41   ↳ source_priors::AbstractVector{<:Union{<:AbstractVector{<:Dirichlet{<:AbstractFloat},<:BitMatrix},<:AbstractFloat}}, 
42   ↳ mix_prior::Tuple{BitMatrix,<:AbstractFloat},
43   ↳ bg_scores::AbstractArray{<:AbstractFloat},
44   ↳ observations::AbstractArray{<:Integer},
45   ↳ source_length_limits::UnitRange{<:Integer})
46   assert_obs_bg_compatibility(observations,bg_scores)
47   T,O = size(observations)
48   S=length(source_priors)
49   obs_lengths=[findfirst(iszero,observations[:,o])-1 for o in
50     ↳ 1:size(observations)[2]]
51   sources=init_logPWM_sources(source_priors, source_length_limits)
52   mix=init_mix_matrix(mix_prior,O,S)
53   log_lh = IPM_likelihood(sources, observations, obs_lengths,
54     ↳ bg_scores, mix)
55
56
57   return ICA_PWM_Model(name, "init_IPM", sources, source_length_limits,
58     ↳ mix, log_lh)
59 end
60
61           #init_IPM SUBFUNCS
62           function assert_obs_bg_compatibility(obs, bg_scores)
63             T,O=size(obs)

```

```

44
45     t,o=size(bg_scores)
46     O!=o && throw(DomainError("Background scores and
47         observations must have same number of observation
48         columns!"))
49     T!=t+1 && throw(DomainError("Background score array
50         must have the same observation lengths as
51         observations!"))
52 end
53
54 function
55     → init_logPWM_sources(prior_vector::AbstractVector{<:Union{<:Abstract
56     → source_length_limits::UnitRange{<:Integer}})
57     srcvec = Vector{Tuple{Matrix{Float64},Int64}}()
58     for prior in prior_vector
59         if
60             → typeof(prior)<:AbstractVector{<:Dirichlet{<:AbstractF
61             if length(prior) <
62                 → source_length_limits[1]
63                 length_dist=DiscreteNonParametric(
64                     [source_length_limits ... ],
65
66                         → [PRIOR_LENGTH_MASS,ones(length(source_len
67                         PWM_length=rand(length_dist)
68                         elseif source_length_limits[1] <
69                             length(prior) <
70                             source_length_limits[end]
71                             length_dist=DiscreteNonParametric(
72                                 [source_length_limits ... ],
73
74                                     [
75
76                                         → (ones(length(prior))-source_length_lim
77
78                                         → (ones(source_length_limits[end]-lengt
79
80                                         ]
81
82                                         )
83                                         PWM_length=rand(length_dist)
84                                         else
85                                         PWM_length=rand(source_length_limits)
86                                         end
87
88                                         if PWM_length>length(prior)
89
90                                             → prior_coord=rand(-(PWM_length-length(prior)):
```

```

73         else
74             ↪ prior_coord=rand(1:length(prior)-PWM_length+1)
75         end
76
77         PWM = zeros(PWM_length,4)
78
79         curr_pos=prior_coord
80         for pos in 1:PWM_length
81             curr_pos < 1 || curr_pos >
82                 ↪ length(prior) ?
83                 ↪ dirichlet=Dirichlet(ones(4)/4) :
84                 ↪ dirichlet=prior[curr_pos]
85             PWM[pos, :] = rand(dirichlet)
86         end
87         push!(srcvec, (log.(PWM), prior_coord))
88             ↪ #push the source PWM to the source
89             ↪ vector with the prior coord idx to
90             ↪ allow drawing from the appropriate
91             ↪ prior dirichlets on permuting source
92             ↪ length
93     elseif prior==false
94         PWM_length=rand(source_length_limits)
95         PWM=zeros(PWM_length,4)
96         dirichlet=Dirichlet(ones(4)/4)
97         for pos in 1:PWM_length
98             PWM[pos,:]=rand(dirichlet)
99         end
100        push!(srcvec, (log.(PWM), 1))
101    else
102        throw(ArgumentError("Bad prior supplied
103                         ↪ for ICA_PWM_Model!"))
104    end
105  end
106  return srcvec
107 end
108
109 function
110     ↪ init_mix_matrix(mix_prior:: Tuple{BitMatrix,<:AbstractFloat},
111     ↪ no_observations:: Integer, no_sources:: Integer)
112     inform, uninform=mix_prior
113     if size(inform,2) > 0

```

```

104     @assert size(inform,1)==no_observations &&
105         → size(inform,2)≤ no_sources "Bad informative
106         → mix prior dimensions!"
107     end
108     @assert 0.0 ≤ uninform ≤1.0 "Uninformative mix
109         → prior not between 0.0 and 1.0!"
110     mix_matrix = falses(no_observations, no_sources)
111     if size(inform,2)>0
112         mix_matrix[:,1:size(inform,2)]=inform
113     end
114     for index in
115         → CartesianIndices((1:no_observations,size(inform,2)+1:no_sources))
116         rand() ≤ uninform && (mix_matrix[index] = true)
117     end
118     return mix_matrix
119 end
120
121
122 function Base.show(io::IO, m::ICA_PWM_Model;
123     → nsrc::Integer=length(m.sources), progress=false)
124     nsrc = 0 && (nsrc=length(m.sources))
125     nsrc>length(m.sources) && (nsrc=length(m.sources))
126     nsrc=length(m.sources) ? (srcstr="All") : (srcstr="Top $nsrc")
127
128     printidxs,printsrcs,printfreqs=sort_sources(m,nsrc)
129
130     printstyled(io, "ICA PWM Model $(m.name) w/ logLi $(m.log_Li)\n",
131     → bold=true)
132     println(io, srcstr*" sources:")
133     for src in 1:nsrc
134         print(io, "S$(printidxs[src]), $(printfreqs[src]*100)%: ")
135         pwmstr_to_io(io, printsrcs[src])
136         println(io)
137     end
138     println(m.origin)
139
140     progress && return(nsrc+4)
141 end
142
143 function sort_sources(m, nsrc)
144     printidxs=Vector{Integer}__()
145     printsrcs=Vector{Matrix{Float64}}__()
146     printfreqs=Vector{Float64}__()

```

```

141     freqs=vec(sum(m.mix_matrix,dims=1)); total=size(m.mix_matrix,1)
142     sortfreqs=sort(freqs,rev=true); sortidxs=sortperm(freqs,rev=true)
143     for srcidx in 1:nsrc
144         push!(printidxs, sortidxs[srcidx])
145         push!(printsracs, m.sources[sortidxs[srcidx]][1])
146         push!(printfreqs, sortfreqs[srcidx]/total)
147     end
148     return printidxs, printsracs, printfreqs
149 end

```

16.7.3 /src/IPM/IPM_likelihood.jl

```

1 #LIKELIHOOD SCORING FUNCS
2 function
3     ↳ IPM_likelihood(sources :: AbstractVector{<: Tuple{<: AbstractMatrix{<: AbstractFloat},,
4         ↳ observations :: AbstractMatrix{<: Integer},,
5         ↳ obs_lengths :: AbstractVector{<: Integer},,
6         ↳ bg_scores :: AbstractArray{<: AbstractFloat}, mix :: BitMatrix,
7         ↳ revcomp :: Bool = REVCOMP, returncache :: Bool = false,
8         ↳ cache :: AbstractVector{<: AbstractFloat} = zeros(0),
9         ↳ clean :: AbstractVector{<: Bool} = Vector(false(size(observations)[2])))
10        ↳ source_wmls = [size(source[1])[1] for source in sources]
11        ↳ 0 = size(bg_scores)[2]
12        ↳ source_stops = [obs_l - wml + 1 for wml in source_wmls, obs_l in obs_lengths]
13            ↳ #stop scanning th source across the observation as the source
14            ↳ reaches the end
15        ↳ L = maximum(obs_lengths) + 1
16
17
18        ↳ obs_src_ids = mix_pull_ids(mix) #get vectors of sources emitting in
19            ↳ each obs
20
21
22        ↳ revcomp ? (srcs = cat(source[1], revcomp_pwm(source[1]), dims=3) for
23            ↳ source in sources; motif_expectations = [( (MOTIF_EXPECT/2)/obs_l)
24            ↳ for obs_l in obs_lengths; mat_dim=2) : (srcs = [source[1] for
25            ↳ source in sources]; ; motif_expectations = [(MOTIF_EXPECT/obs_l)
26            ↳ for obs_l in obs_lengths; mat_dim=1) #setup appropriate reverse
27            ↳ complemented sources if necessary and set
28            ↳ log_motif_expectation-nMica has 0.5 per base for including the
29            ↳ reverse complement, 1 otherwise
30
31        ↳ lme_vec = zeros(length(sources))
32
33

```



```

46             penalty_sum > 1. && (penalty_sum=1.)
47             cardinality_penalty=log(1.0-penalty_sum)
48         else
49             cardinality_penalty=0.0
50         end
51
52         revcomp ? (obs_lhs[t][i]=weave_scores_ds!(weavevec,
53             ↳ lh_vec, obsl, view(bg_scores,:,:,o), score_matrices,
54             ↳ oidxs, mixwmls, log(motif_expectations[o]),
55             ↳ cardinality_penalty, osi_emitting)) :
56             (obs_lhs[t][i]=weave_scores_ss!(lh_vec, obsl,
57                 ↳ view(bg_scores,:,:,o), score_matrices, oidxs,
58                 ↳ mixwmls, log(motif_expectations[o]),
59                 ↳ cardinality_penalty, osi_emitting))
60
61             empty!(osi_emitting)
62
63         end
64     end
65     end
66
67     returncache ? (return lps([lps(obs_lhs[t]) for t in 1:nt]),
68         ↳ vcat(obs_lhs ...)) : (return lps([lps(obs_lhs[t]) for t in 1:nt]))
69 end
70
71 @inline function mix_pull_idxs(A::AbstractArray{Bool})
72     n=count(A)
73     S=[Vector{Int64}() for o in 1:size(A,1)]
74     cnt=1
75     for (i,a) in pairs(A)
76         if a
77             push!(S[i[1]],i[2])
78             cnt+=1
79         end
80     end
81     return S
82 end
83
84
85 @inline function
86     ↳ revcomp_pwm(pwm::AbstractMatrix{<:AbstractFloat}) #in
87     ↳ order to find a motif on the reverse strand, we scan
88     ↳ the forward strand with the reverse complement of the
89     ↳ pwm, reordered 3' to 5', so that eg. an PWM for an
90     ↳ ATG motif would become one for a CAT motif

```

```

77         return pwm[end:-1:1,end:-1:1]
78     end
79
80     @inline function score_sources_ds!(score_mat,
81         ← score_matrices::AbstractVector{<:AbstractMatrix{<:AbstractFloat}}}
82         ← sources, observation::AbstractVector{<:Integer},
83         ← source_stops)
84         for (s,source) in enumerate(sources)
85             for t in 1:source_stops[s]
86                 for position in 1:size(source,1)
87                     score_loc = t+position-1 #score_loc is
88                     ← the position of the obs to be scored
89                     ← by PWM
90                     score_mat[t,1] +=
91                     ← source[position,observation[score_loc],1]
92                     ← #add the appropriate log PWM value
93                     ← from the source to the score
94                     score_mat[t,2] +=
95                     ← source[position,observation[score_loc],2]
96                     ← #add the appropriate log PWM value
97                     ← from the source to the score
98
99             end
100         end
101         score_matrices[s]=score_mat[1:source_stops[s],:]
102         ← #copy score matrix to vector
103         score_mat.=0. #reset score matrix
104     end
105
106     @inline function score_sources_ss!(score_mat,
107         ← score_matrices::AbstractVector{<:AbstractArray{<:AbstractFloat}},}
108         ← sources, observation::AbstractVector{<:Integer},
109         ← source_stops)
110         for (s,source) in enumerate(sources)
111             for t in 1:source_stops[s]
112                 for position in 1:size(source,1)
113                     score_loc = t+position-1 #score_loc is
114                     ← the position of the obs to be scored
115                     ← by PWM

```

```

100                         score_mat[t] +=
101                         ↵ source[position,observation[score_loc]]
102                         ↵ #add the appropriate log PWM value
103                         ↵ from the source to the score
104
105                     end
106                 end
107             end
108
109             @inline function weave_scores_ds!(weavevec, lh_vec,
110                         ↵ obsl::Integer, bg_scores::SubArray,
111                         ↵ score_mat::AbstractVector{<:AbstractMatrix{<:AbstractFloat}},
112                         ↵ obs_source_indices::AbstractVector{<:Integer},
113                         ↵ source_wmls::AbstractVector{<:Integer},
114                         ↵ log_motif_expectation::AbstractFloat,
115                         ↵ cardinality_penalty::AbstractFloat, osi_emitting)
116             for i in 2:obsl+1 #i=1 is ithe lh_vec initializing 0,
117                         ↵ i=2 is the score of the first background position
118                         ↵ (ie t=1)
119                         t=i-1
120                         score = lps(lh_vec[i-1], bg_scores[t],
121                         ↵ cardinality_penalty)
122
123                         #logic: all observations are scored from t=wml to
124                         ↵ the end of the obs-therefore check at each
125                         ↵ position for new sources to add (indexed by
126                         ↵ vector position to retrieve source wml and
127                         ↵ score matrix)
128             if
129                         ↵ length(osi_emitting)<length(obs_source_indices)
130             for n in 1:length(obs_source_indices)
131                         if !(n in osi_emitting)
132                             t ≥ source_wmls[n] &&
133                             ↵ (push!(osi_emitting,n))
134                         end
135                     end
136             end
137
138             for n in osi_emitting

```

```

124             wml = source_wmls[n]
125             from_score = lh_vec[i-wml+1] #score at the
126             ↪ first position of the PWM
127             score_array = score_mat[n] #get the source
128             ↪ score matrix
129             score_idx = t - wml + 1 #translate t to
130             ↪ score_array idx for emission score
131             f_emit_score = score_array[score_idx,1]
132             ↪ #emission score at the last position of
133             ↪ the PWM
134             r_emit_score = score_array[score_idx,2]
135
136             weavevec *= score, lps(from_score,
137             ↪ f_emit_score, log_motif_expectation),
138             ↪ lps(from_score, r_emit_score,
139             ↪ log_motif_expectation)
140
141             score=logsumexp(weavevec)
142         end
143         lh_vec[i] = score
144     end
145     return lh_vec[obs1+1]
146 end
147
148 @inline function weave_scores_ss!(lh_vec, obs1::Integer,
149   ↪ bg_scores::SubArray,
150   ↪ score_mat::AbstractVector{<:AbstractVector{<:AbstractFloat}},
151   ↪ obs_source_indices::AbstractVector{<:Integer},
152   ↪ source_wmls::AbstractVector{<:Integer},
153   ↪ log_motif_expectation::AbstractFloat,
154   ↪ cardinality_penalty::AbstractFloat, osi_emitting)
155   for i in 2:obs1+1 #i=1 is ithe lh_vec initializing 0,
156   ↪ i=2 is the score of the first background position
157   ↪ (ie t=1)
158     t=i-1
159     score = lps(lh_vec[i-1], bg_scores[t],
160     ↪ cardinality_penalty)
161     #logic: all observations are scored from t=wml to
162     ↪ the end of the obs-therefore check at each
163     ↪ position for new sources to add (indexed by
164     ↪ vector position to retrieve source wml and
165     ↪ score matrix)

```

```

145             if
146                 → length(osi_emitting)<length(obs_source_indices)
147                     for n in 1:length(obs_source_indices)
148                         if !(n in osi_emitting)
149                             t ≥ source_wmls[n] &&
150                             → (push!(osi_emitting,n))
151                         end
152                     end
153             end
154
155             for n in osi_emitting
156                 wml = source_wmls[n]
157                 from_score = lh_vec[i-wml+1] #score at the
158                 → first position of the PWM
159                 score_array = score_mat[n] #get the source
160                 → score matrix
161                 score_idx = t - wml + 1 #translate t to
162                 → score_array idx for emission score
163                 f_emit_score = score_array[score_idx]
164                 → #emission score at the last position of
165                 → the PWM
166
167                 score=logaddexp(score, lps(from_score,
168                 → f_emit_score, log_motif_expectation))
169             end
170             lh_vec[i] = score
171         end
172     return lh_vec[obsL+1]
173 end

```

16.7.4 /src/IPM/IPM_prior_utilities.jl

```

1 function read_fa_wms_tr(path::String)
2     wms=Vector{Matrix{Float64}}}()
3     wm=zeros(1,4)
4     f=open(path)
5     for line in eachline(f)
6         prefix=line[1:2]
7         prefix = "01" && (wm=transpose([parse(Float64,i) for i in
8             → split(line)[2:end]]))

```

```

8     prefix != "01" && prefix != "NA" && prefix != "PO" && prefix !=
9         "://" && (wm=vcat(wm, transpose([parse(Float64,i) for i in
10        split(line)[2:end]])))
11    prefix = "://" && push!(wms, wm)
12 end
13
14 #wm_samples are in decimal probability space, not log space
15 function assemble_source_priors(no_sources::Integer,
16     →   wm_samples::AbstractVector{<:AbstractMatrix{<:AbstractFloat}},
17     →   prior_wt::AbstractFloat=PRIOR_WT) #estimate a dirichlet prior on
18     →   wm_samples inputs; if the number of samples is lower than the number
19     →   of sources, return a false bool for init and permutation functions
20     source_priors = Vector{Union{Vector{Dirichlet{Float64}}, Bool}}()
21     for source in 1:no_sources
22         if source ≤ length(wm_samples)
23             push!(source_priors,
24                 →   estimate_dirichlet_prior_on_wm(wm_samples[source],
25                 →   prior_wt))
26         else
27             push!(source_priors, false)
28         end
29     end
30     return source_priors
31 end
32
33     function
34         →   estimate_dirichlet_prior_on_wm(wm::AbstractMatrix{<:AbstractFloat},
35         →   wt::AbstractFloat=PRIOR_WT)
36         for i in 1:size(wm)[1]
37             !(isprobvec(wm[i,:])) && throw(DomainError("Bad
38                 →   weight vec supplied to
39                 →   estimate_dirichlet_prior_on_wm! $(wm[i,:])"))
40         end
41         prior = Vector{Dirichlet{Float64}}()
42         for position in 1:size(wm)[1]
43             normvec=wm[position,:]
44             zero_idxs=findall(isequal(0.),wm[position,:])
45             normvec[zero_idxs].+=10^-99
46             push!(prior, Dirichlet(normvec.*wt))
47         end
48         return prior

```

```

39         end
40
41 function cluster_mix_prior!(df::DataFrame,
42     ↪ wms::AbstractVector{<:AbstractMatrix{<:AbstractFloat}})
43     mix=falses(size(df,1),length(wms))
44     for (o, row) in enumerate(eachrow(df))
45         row.cluster ≠ 0 && (mix[o, row.cluster]=true)
46     end
47
48     represented_sources=unique(df.cluster)
49     return mix[:,represented_sources]
50 end
51
52 function infocenter_wms_trim(wm::AbstractMatrix{<:AbstractFloat},
53     ↪ trimsize::Integer)
54     !(size(wm,2)==4) && throw(DomainError("Bad wm! 2nd dimension should
55     ↪ be size 4"))
56     infovec=get_pwm_info(wm, logsw=false)
57     maxval, maxidx=findmax(infovec)
58     upstream_extension=Int(floor((trimsize-1)/2))
59     downstream_extension=Int(ceil((trimsize-1)/2))
60     1+upstream_extension+downstream_extension > size(wm,1) &&
61     ↪ throw(DomainError("Src too short for trim! $upstream_extension
62     ↪ $downstream_extension"))
63     return
64     ↪ wm[max(1,maxidx-upstream_extension):min(maxidx+downstream_extension,size(wm,1))]
65 end
66
67 function filter_priors(target_src_no::Integer, target_src_size::Integer,
68     ↪ prior_wms::AbstractVector{<:AbstractMatrix{<:AbstractFloat}},
69     ↪ prior_mix::BitMatrix)
70     wms=Vector{Matrix{Float64}}(undef, target_src_no)
71     freqsort_ids=sortperm([sum(prior_mix[:,s]) for s in
72         ↪ 1:length(prior_wms)])
73     for i in 1:target_src_no
74         target_src_idx=freqsort_ids[i]
75         wms[i]=infocenter_wms_trim(prior_wms[target_src_idx],
76             ↪ target_src_size)
77     end
78     return wms
79 end
80
81 end

```

```

71 function combine_filter_priors(target_src_no::Integer,
72     → target_src_size::Integer,
73     → prior_wms::Tuple{<:AbstractVector{<:AbstractMatrix{<:AbstractFloat}},AbstractVect
74     → prior_mix::Tuple{BitMatrix, BitMatrix})
75     wms=Vector{Matrix{Float64}}(undef, target_src_no)
76     cat_wms=vcat(prior_wms[1],prior_wms[2])
77     first_freq=[sum(prior_mix[1][:,s]) for s in 1:length(prior_wms[1])]
78     second_freq=[sum(prior_mix[2][:,s]) for s in 1:length(prior_wms[2])]
79     freqsort_idxes=sortperm(vcat(first_freq,second_freq))
80     for i in 1:target_src_no
81         target_src_idx=freqsort_idxes[i]
82         wms[i]=infocenter_wms_trim(cat_wms[target_src_idx],
83             → target_src_size)
84     end
85     return wms
86 end

```

16.7.5 /src/ensemble/IPM_Engine.jl

```

1 mutable struct IPM_Engine
2     path::String #ensemble models and popped-out posterior samples
3     → serialised here
4     models::Vector{Model_Record} #ensemble keeps paths to serialised
5     → models and their likelihood tuples rather than keeping the
6     → models in memory
7
8     contour::AbstractFloat#current log_Li[end]
9     log_Li::Vector{AbstractFloat} #likelihood of lowest-ranked model
10    → at iterate i
11    log_Xi::Vector{AbstractFloat} #amt of prior mass included in
12    → ensemble contour at Li
13    log_wi::Vector{AbstractFloat} #width of prior mass covered in
14    → this iterate
15    log_Liwi::Vector{AbstractFloat} #evidentiary weight of the
16    → iterate
17    log_Zi::Vector{AbstractFloat} #ensemble evidence
18    Hi::Vector{AbstractFloat} #ensemble information
19
20    obs_array::Matrix{Integer} #observations Txo
21    obs_lengths::Vector{Integer}

```



```

48     IPM_likelihood(init_logPWM_sources(source_priors,
49                     ↵   source_length_limits), obs, [findfirst(iszero,obs[:,o])-1 for
50                     ↵   o in 1:size(obs)[2]], bg_scores,
51                     ↵   falses(size(obs)[2],length(source_priors))))
52
53 IPM_Ensemble(worker_pool::AbstractVector{<:Integer}, path::String,
54   ↵   no_models::Integer,
55   ↵   source_priors::AbstractVector{<:Union{<:AbstractVector{<:Dirichlet{<:AbstractFlo
56   ↵   mix_prior::Tuple{BitMatrix,<:AbstractFloat},
57   ↵   bg_scores::AbstractMatrix{<:AbstractFloat}, obs::Array{<:Integer},
58   ↵   source_length_limits; posterior_switch::Bool=false} =
59 IPM_Ensemble(
60   ↵   path,
61   ↵   distributed_IPM_assembly(worker_pool, path, no_models,
62     ↵   source_priors, mix_prior, bg_scores, obs,
63     ↵   source_length_limits)... ,
64   ↵   [-Inf], #L0 = 0
65   ↵   [0], #ie exp(0) = all of the prior is covered
66   ↵   [-Inf], #w0 = 0
67   ↵   [-Inf], #Liwi0 = 0
68   ↵   [-1e300], #Z0 = 0
69   ↵   [0], #H0 = 0,
70   ↵   obs,
71   ↵   [findfirst(iszero,obs[:,o])-1 for o in 1:size(obs)[2]],
72   ↵   source_priors,
73   ↵   mix_prior,
74   ↵   bg_scores, #precalculated background score
75   ↵   posterior_switch,
76   ↵   Vector{String}(),
77   ↵   no_models+1,
78   ↵   IPM_likelihood(init_logPWM_sources(source_priors,
79                     ↵   source_length_limits), obs, [findfirst(iszero,obs[:,o])-1 for
80                     ↵   o in 1:size(obs)[2]], bg_scores,
81                     ↵   falses(size(obs)[2],length(source_priors))))
82
83 function assemble_IPMs(path::String, no_models::Integer,
84   ↵   source_priors::AbstractVector{<:Union{<:AbstractVector{<:Dirichlet{<:AbstractFlo
85   ↵   mix_prior::Tuple{BitMatrix,<:AbstractFloat},
86   ↵   bg_scores::AbstractArray{<:AbstractFloat},
87   ↵   obs::AbstractArray{<:Integer},
88   ↵   source_length_limits::UnitRange{<:Integer})
89   ↵   ensemble_records = Vector{Model_Record}()
90   ↵   !isdir(path) && mkpath(path)

```

```

73
74     @assert size(obs)[2]==size(bg_scores)[2]
75
76     @showprogress 1 "Assembling IPM ensemble ... " for model_no in
77         1:no_models
78         model_path = string(path,'/',model_no)
79         if !isfile(model_path)
80             model = ICA_PWM_Model(string(model_no),
81             source_priors, mix_prior, bg_scores, obs,
82             source_length_limits)
83             serialize(model_path, model) #save the model to
84             the ensemble directory
85             push!(ensemble_records,
86                 Model_Record(model_path,model.log_Li))
87         else #interrupted assembly pick up from where we left off
88             model = deserialize(model_path)
89             push!(ensemble_records,
90                 Model_Record(model_path,model.log_Li))
91     end
92
93     return ensemble_records, minimum([record.log_Li for record in
94         ensemble_records])
95 end
96
97 function distributed_IPM_assembly(worker_pool::Vector{Int64},
98     path::String, no_models::Integer,
99     source_priors::AbstractVector{<:Union{<:AbstractVector{<:Dirichlet{<:AbstractFloat}},<:BitMatrix,<:AbstractFloat}},
100    mix_prior::Tuple{BitMatrix,<:AbstractFloat},
101    bg_scores::AbstractArray{<:AbstractFloat},
102    obs ::AbstractArray{<:Integer},
103    source_length_limits ::UnitRange{<:Integer})
104     ensemble_records = Vector{Model_Record}()
105     !isdir(path) && mkpath(path)
106
107     @assert size(obs)[2]==size(bg_scores)[2]
108
109     model_chan=
110         RemoteChannel(()→Channel{ICA_PWM_Model}(length(worker_pool)))
111     job_chan = RemoteChannel(()→Channel{Union{Tuple,Nothing}}(1))
112     put!(job_chan,(source_priors, mix_prior, bg_scores, obs,
113         source_length_limits))

```

```

101     sequence_workers(worker_pool, worker_assemble, job_chan,
102                     ↵ model_chan)
103
104     assembly_progress=Progress(no_models, desc="Assembling IPM
105                                ↵ ensemble ... ")
106
107     model_counter=check_assembly!(ensemble_records, path, no_models,
108                                ↵ assembly_progress)
109
110     while model_counter ≤ no_models
111         wait(model_chan)
112         candidate=take!(model_chan)
113         model = ICA_PWM_Model(string(model_counter),
114                               ↵ candidate.origin, candidate.sources,
115                               ↵ candidate.source_length_limits, candidate.mix_matrix,
116                               ↵ candidate.log_Li)
117         model_path=string(path,'/',model_counter)
118         serialize(model_path,model)
119         push!(ensemble_records,
120               ↵ Model_Record(model_path,model.log_Li))
121         model_counter+=1
122         next!(assembly_progress)
123     end
124
125     take!(job_chan),put!(job_chan,nothing)
126
127     return ensemble_records, minimum([record.log_Li for record in
128                                         ↵ ensemble_records])
129
130     end
131
132     function
133         ↵ check_assembly!(ensemble_records::AbstractVector{
134             ↵ path::String, no_models::Integer,
135             ↵ assembly_progress::Progress)
136             counter=1
137             while counter ≤ no_models
138                 model_path=string(path,'/',counter)
139                 if isfile(model_path)
140                     model=deserialize(model_path)
141                     push!(ensemble_records,
142                           ↵ Model_Record(model_path, model.log_Li))
143                     counter+=1
144                     next!(assembly_progress)
145                 else
146                 end
147             end
148         end
149     end
150 
```

```

132                                     return counter
133                                         end
134                                         end
135                                         return counter
136                                         end
137
138                                         function
139                                         → worker_assemble(job_chan::RemoteChannel,
140                                         → models_chan::RemoteChannel,
141                                         → comms_chan::RemoteChannel)
142                                         put!(comms_chan,myid())
143                                         wait(job_chan)
144                                         params=fetch(job_chan)
145                                         while !(fetch(job_chan) ===
146                                         → nothing)
147                                         → model=ICA_PWM_Model(string(myid()),pa
148                                         → put!(models_chan,model)
149                                         end
150                                         end
151
152                                         function Base.show(io::IO, e::IPM_E
153                                         livec=[model.log_Li for model in e.models]
154                                         maxLH=maximum(livec)
155                                         printstyled(io, "ICA PWM Model Ensemble @ $(e.path)\n",
156                                         → bold=true)
157                                         msg = @sprintf "Contour: %3.6e MaxLH:%3.3e Max/Naive:%3.3e log
158                                         → Evidence:%3.6e" e.contour maxLH (maxLH-e.naive_lh)
159                                         → e.log_Zi[end]
160                                         println(io, msg)
161                                         hist=UnicodePlots.histogram(livec, title="Ense
162                                         → mbles Likelihood
163                                         → Distribution")
164                                         show(io, hist)
165                                         println()
166                                         progress && return(nrows(hist.graphics)+6)
167
168                                         end

```

16.7.6 /src/ensemble/ensemble_utilities.jl

```

1 function ensemble_history(e::IPM_E
2   !e.sample_posterior && throw(ArgumentError("This ensemble has no
→ posterior samples to show a history for!"))

```

```

3    livec=vcat([model.log_Li for model in e.models],[model.log_Li for
4      ↵ model in e.posterior_samples])
5    show(histogram(livec, nbins=bins))
6
7 function e_backup(e::IPM_Ensemble, tuner::Permute_Tuner)
8     serialize(string(e.path,'/',"ens"), e)
9     serialize(string(e.path,'/',"tuner"), tuner)
10 end
11
12 function clean_ensemble_dir(e::IPM_Ensemble, model_pad::Integer;
13   ↵ ignore_warn=false)
14   !ignore_warn && e.sample_posterior && throw(ArgumentError("Ensemble
15     ↵ is set to retain posterior samples and its directory should not
16     ↵ be cleaned!"))
17   for file in readdir(e.path)
18     !(file in vcat([basename(model.path) for model in
19       ↵ e.models],"ens",[string(number) for number in
20         ↵ e.model_counter-length(e.models)-model_pad:e.model_counter-1]))
21     && rm(e.path*'*file)
22   end
23 end
24
25 function complete_evidence(e::IPM_Ensemble)
26   return final_logZ = logaddexp(e.log_Zi[end], (logsumexp([model.log_Li
27     ↵ for model in e.models]) + e.log_Xi[length(e.log_Li)] -
28     ↵ log(length(e.models))))
29 end
30
31 function reset_ensemble!(e::IPM_Ensemble)
32   new_e=deepcopy(e)
33   for i in 1:length(e.models)
34     if string(i) in [basename(record.path) for record in e.models]
35       new_e.models[i]=e.models[findfirst(isequal(string(i)),
36         ↵ [basename(record.path) for record in e.models])]
37     else
38
39       ↵ new_e.models[i]=e.posterior_samples[findfirst(isequal(string(i)),
40         ↵ [basename(record.path) for record in
41           ↵ e.posterior_samples])]
42     end
43   end
44 end

```

```

33     new_e.contour=minimum([record.log_Li for record in new_e.models])
34
35     new_e.log_Li=[new_e.log_Li[1]]
36     new_e.log_Xi=[new_e.log_Xi[1]]
37     new_e.log_wi=[new_e.log_wi[1]]
38     new_e.log_Liwi=[new_e.log_Liwi[1]]
39     new_e.log_Zi=[new_e.log_Zi[1]]
40     new_e.Hi=[new_e.Hi[1]]
41
42     new_e.posterior_samples=Vector{Model_Record}()
43
44     new_e.model_counter=length(new_e.models)+1
45
46     clean_ensemble_dir(new_e, 0; ignore_warn=true)
47     isfile(e.path*"tuner") && rm(e.path*"tuner")
48     serialize(e.path*"ens", new_e)
49
50     return new_e
51 end
52
53 function move_ensemble!(e::IPM_Ensemble,path::String)
54     !isdir(path) && mkdir(path)
55     for file in readdir(e.path)
56         mv(e.path*'*file,path*'*file)
57     end
58
59     for (n,model) in enumerate(e.models)
60         e.models[n]=Model_Record(path*'*basename(model.path),
61             ↳ model.log_Li)
62     end
63     if e.sample_posterior
64         for (n,model) in enumerate(e.posterior_samples)
65             ↳ e.posterior_samples[n]=Model_Record(path*'*basename(model.path),
66                 ↳ model.log_Li)
67         end
68     end
69     rm(e.path)
70     e.path=path
71     serialize(e.path*"ens",e)
72     return e
73 end

```

```

73
74 function copy_ensemble!(e::IPM_Ensemble,path::String)
75     new_e=deepcopy(e)
76     !isdir(path) && mkdir(path)
77     for file in readdir(e.path)
78         cp(e.path*'*file,path*'*file, force=true)
79     end
80
81     for (n,model) in enumerate(e.models)
82         new_e.models[n]=Model_Record(path*'*basename(model.path),
83                                     ↪ model.log_Li)
84     end
85     if e.sample_posterior
86         for (n,model) in enumerate(e.posterior_samples)
87             ↪ new_e.posterior_samples[n]=Model_Record(path*'*basename(model.path)
88                                         ↪ model.log_Li)
89         end
90     end
91     new_e.path=path
92     serialize(new_e.path*"/ens",new_e)
93     return new_e
94 end
95
96 function rewind_ensemble(e::IPM_Ensemble,rewind_idx)
97     !e.sample_posterior && throw(ArgumentError("An ensemble not retaining
98                                     ↪ posterior samples cannot be rewound!"))
99     rewind_idx ≥ length(e.log_Li) && throw(ArgumentError("rewind_idx
100                                         ↪ must be less than the current iterate!"))
101
102     n=length(e.models)
103     max_model_no=length(e.log_Li)+length(e.models)-1
104     rewind_model_no=rewind_idx+length(e.models)-1
105     new_e = deepcopy(e)
106
107     rm_models=[string(name) for name in rewind_model_no+1:max_model_no]
108
109     filter!(model→!(basename(model.path) in rm_models),new_e.models)
110     filter!(model→!(basename(model.path) in
111                 ↪ rm_models),new_e.posterior_samples)
112
113     while length(new_e.models) < n
114         new_e.models[1]=Model_Record(path*'*basename(e.models[1].path),
115                                     ↪ e.models[1].log_Li)
116     end
117
118     new_e.path=path
119     serialize(new_e.path*"/ens",new_e)
120     return new_e
121 end

```

```

110     push!(new_e.models, pop!(new_e.posterior_samples))
111   end
112   new_e.contour=new_e.log_Li[rewind_idx]
113   new_e.log_Li=new_e.log_Li[1:rewind_idx]
114   new_e.log_Xi=new_e.log_Xi[1:rewind_idx]
115   new_e.log_wi=new_e.log_wi[1:rewind_idx]
116   new_e.log_Liwi=new_e.log_Liwi[1:rewind_idx]
117   new_e.log_Zi=new_e.log_Zi[1:rewind_idx]
118   new_e.Hi=new_e.Hi[1:rewind_idx]
119
120   new_e.model_counter=length(new_e.models)+rewind_idx
121
122   return new_e
123 end
124
125 function show_models(e::IPM_Ensemble, idxs)
126   liperm=sortperm([model.log_Li for model in e.models], rev=true)
127   for idx in idxs
128     m=deserialize(e.models[liperm[idx]].path)
129     show(m)
130   end
131 end
132
133 function get_model(e::IPM_Ensemble, no)
134   return deserialize(e.path*"/"*string(no))
135 end

```

16.7.7 /src/nested_sampler/converge_ensemble.jl

```

1 function converge_ensemble!(e::IPM_Ensemble,
2   instruction::Permute_Instruct; max_iterates=typemax(Int64),
2   backup::Tuple{Bool, Integer}=(false, 0),
2   clean::Tuple{Bool, Integer, Integer}=(false, 0, 0), verbose::Bool=false,
2   converge_criterion::String="standard",
2   converge_factor::AbstractFloat=.001, progargs ... )
2   N = length(e.models); curr_it=length(e.log_Li)
2
3   curr_it>1 && isfile(e.path*"/tuner") ?
4     (tuner=deserialize(e.path*"/tuner")) : (tuner =
4     Permute_Tuner(instruction)) #restore tuner from saved if any
5   wk_mon = Worker_Monitor([1])

```

```

6     meter = ProgressNS(e, wk_mon, tuner, 0.; start_it=curr_it,
7                           ↵ progargs ... )
8
9     converge_check = get_convfunc(converge_criterion)
10    while !converge_check(e, converge_factor) && (curr_it ≤
11        ↵ max_iterates)
12        warn,step_report = nested_step!(e, instruction) #step the
13        ↵ ensemble
14        warn == 1 && "#" "1" passed for warn code means no workers persist;
15        ↵ all have hit the permute limit
16        (@error "Failed to find new models, aborting at current
17        ↵ iterate."; return e) #if there is a warning, just
18        ↵ return the ensemble and print info
19        curr_it += 1
20
21        tune_weights!(tuner, step_report)
22        instruction = tuner.inst
23
24        backup[1] && curr_it%backup[2] == 0 && e_backup(e,tuner) #every
25        ↵ backup interval, serialise the ensemble and instruction
26        clean[1] && !e.sample_posterior && curr_it%clean[2] == 0 &&
27        ↵ clean_ensemble_dir(e,clean[3]) #every clean interval, remove
28        ↵ old discarded models
29
30        update!(meter, converge_check(e,converge_factor,vals=true) ... )
31    end
32
33    if converge_check(e,converge_factor)
34        final_logZ = complete_evidence(e)
35        @info "Job done, sampled to convergence. Final logZ $final_logZ"
36
37        e_backup(e,tuner)
38        clean[1] && !e.sample_posterior && clean_ensemble_dir(e,0) #final
39        ↵ clean
40        return final_logZ
41    elseif curr_it==max_iterates
42        @info "Job done, sampled to maximum iterate $max_iterates.
43        ↵ Convergence criterion not obtained."
44
45        e_backup(e,tuner)
46        clean[1] && !e.sample_posterior && clean_ensemble_dir(e,0) #final
47        ↵ clean
48        return e.log_Zi[end]

```

```

37     end
38 end
39
40 function converge_ensemble!(e::IPM_Engsemble,
41     instruction::Permute_Instruct, wk_pool::Vector{Int64};
42     max_iterates=typemax(Int64), backup::Tuple{Bool, Integer}=(false, 0),
43     clean::Tuple{Bool, Integer, Integer}=(false, 0, 0), verbose::Bool=false,
44     converge_criterion::String="standard",
45     converge_factor::AbstractFloat=.001, progargs ... )
46     N = length(e.models)
47
48     converge_check = get_convfunc(converge_criterion)
49     model_chan=
50         RemoteChannel(()→Channel{Tuple{Union{ICA_PWM_Model, String}, Integer,
51             AbstractVector{<: Tuple}}}(10*length(wk_pool))) #channel to take
52         ← EM iterates off of
53     job_chan =
54         RemoteChannel(()→Channel{Tuple{<: AbstractVector{<: Model_Record},
55             Float64, Union{Permute_Instruct, String}}}(1))
56     put!(job_chan, (e.models, e.contour, instruction))
57
58     if !converge_check(e, converge_factor) #sequence workers only if not
59         ← already converged
60         @async sequence_workers(wk_pool, permute_IPM, e, job_chan,
61             ← model_chan)
62     end
63
64     curr_it=length(e.log_Li)
65     wk_mon=Worker_Monitor(wk_pool)
66     curr_it>1 && isfile(e.path*"/tuner") ?
67         (tuner=deserialize(e.path*"/tuner")) : (tuner =
68             Permute_Tuner(instruction)) #restore tuner from saved if any
69     meter = ProgressNS(e, wk_mon, tuner, 0.; start_it=curr_it,
70         ← progargs ... )
71
72     while !converge_check(e, converge_factor) && (curr_it ≤
73         ← max_iterates)
74         warn, step_report = nested_step!(e, model_chan, wk_mon) #step the
75             ← ensemble
76         warn = 1 && "#"1" passed for warn code means no workers persist;
77             ← all have hit the permute limit

```

```

60             (@error "All workers failed to find new models, aborting
61             ↳ at current iterate."; return e) #if there is a
62             ↳ warning, just return the ensemble and print info
63         curr_it += 1
64         tune_weights!(tuner, step_report)
65         instruction = tuner.inst
66         take!(job_chan); put!(job_chan,(e.models,e.contour,instruction))
67         backup[1] && curr_it%backup[2] == 0 && e_backup(e,tuner) #every
68             ↳ backup interval, serialise the ensemble and instruction
69         clean[1] && !e.sample_posterior && curr_it%clean[2] == 0 &&
70             ↳ clean_ensemble_dir(e,clean[3]) #every clean interval, remove
71             ↳ old discarded models
72
73         update!(meter, converge_check(e,converge_factor,vals=true) ... )
74     end
75
76     take!(job_chan); put!(job_chan, (e.models, e.contour, "stop")) #stop
77         ↳ instruction terminates worker functions
78
79     if converge_check(e,converge_factor)
80         final_logZ = complete_evidence(e)
81         @info "Job done, sampled to convergence. Final logZ $final_logZ"
82
83         e_backup(e,tuner)
84         clean[1] && !e.sample_posterior && clean_ensemble_dir(e,0) #final
85             ↳ clean
86         return final_logZ
87     elseif curr_it==max_iterates
88         @info "Job done, sampled to maximum iterate $max_iterates.
89             ↳ Convergence criterion not obtained."
90
91         e_backup(e,tuner)
92         clean[1] && !e.sample_posterior && clean_ensemble_dir(e,0) #final
93             ↳ clean
94         return e.log_Zi[end]
95     end
96 end
97
98
99     function evidence_converge(e, evidence_fraction;
100         ↳ vals=false)
101         val=lps(findmax([model.log_Li for model in
102             ↳ e.models])[1], e.log_Xi[end])
103         thresh=lps(log(evidence_fraction),e.log_Zi[end])
104     end
105 end

```

```

92             vals ? (return val, thresh) : (return val


---



```

16.7.8 /src/nested_sampler/nested_step.jl

```

1 ##### IMPLEMENTATION OF JEFF SKILLINGS' NESTED SAMPLING ALGORITHM #####
2 function nested_step!(e::IPM_Ensemble, instruction::Permute_Instruct)
3     N = length(e.models) #number of sample models/particles on the
4         ← posterior surface
5     i = length(e.log_Li) #iterate number, index for last values
6     j = i+1 #index for newly pushed values
7
8     e.contour, least_likely_idx = findmin([model.log_Li for model in
9         ← e.models])
10    Li_model = e.models[least_likely_idx]
11
12    #SELECT NEW MODEL, SAVE TO ENSEMBLE DIRECTORY, CREATE RECORD AND PUSH
13        ← TO ENSEMBLE
14    model_selected=false; step_report=0
15    while !model_selected
16        candidate,step_report=permute_IPM(e, instruction)
17        if !(candidate==nothing)

```

```

15         if !(candidate.log_Li in [m.log_Li for m in e.models])
16             model_selected=true
17
18             new_model_record =
19                 → Model_Record(string(e.path,'/'),e.model_counter),
20                 → candidate.log_Li);
21             e.sample_posterior && push!(e.posterior_samples,
22                 → Li_model)#if sampling posterior, push the model
23                 → record to the ensemble's posterior samples vector
24             deleteat!(e.models, least_likely_idx)
25             push!(e.models, new_model_record);
26
27             final_model=ICA_PWM_Model(string(e.model_counter),
28                 → candidate.origin, candidate.sources,
29                 → candidate.source_length_limits, candidate.mix_matrix,
30                 → candidate.log_Li, candidate.permute_blacklist)
31             serialize(new_model_record.path, final_model)
32
33             e.model_counter +=1
34         end
35     else
36         push!(e.models, Li_model)
37         return 1, step_report
38     end
39 end
40
41 #UPDATE ENSEMBLE QUANTITIES
42 push!(e.log_Li, minimum([model.log_Li for model in e.models])) #log
43             → likelihood of the least likely model - the current ensemble ll
44             → contour at Xi
45 push!(e.log_Xi, -i/N) #log Xi - crude estimate of the iterate's
46             → enclosed prior mass
47 push!(e.log_wi, lps(e.log_Xi[i], -((j+1)/N)-log(2))) #log width of
48             → prior mass spanned by the last step-trapezoidal approx
49 push!(e.log_Liwi, lps(e.log_Li[j],e.log_wi[j])) #log likelihood + log
50             → width = increment of evidence spanned by iterate
51 push!(e.log_Zi, logaddexp(e.log_Zi[i],e.log_Liwi[j]))      #log
52             → evidence
53 #information- dimensionless quantity
54 push!(e.Hi, lps(
55             (exp(lps(e.log_Liwi[j],-e.log_Zi[j])) * e.log_Li[j]), #term1
56             (exp(lps(e.log_Zi[i],-e.log_Zi[j])) *
57                 → lps(e.Hi[i],e.log_Zi[i])), #term2

```

```

44         -e.log_Zi[j])) #term3
45
46     return 0, step_report
47 end
48
49 function nested_step!(e::IPM_Ensemble, model_chan::RemoteChannel,
50   ↳ wk_mon::Worker_Monitor)
51   N = length(e.models)+1 #number of sample models/particles on the
52   ↳ posterior surface- +1 as one has been removed in the distributed
53   ↳ dispatch for converge_ensemble
54   i = length(e.log_Li) #iterate number, index for last values
55   j = i+1 #index for newly pushed values
56
57   e.contour, least_likely_idx = findmin([model.log_Li for model in
58   ↳ e.models])
59   Li_model = e.models[least_likely_idx]
60
61   #SELECT NEW MODEL, SAVE TO ENSEMBLE DIRECTORY, CREATE RECORD AND
62   ↳ REPLACE LEAST LIKELY MODEL
63   model_selected=false;wk=0;step_report=0
64   while !model_selected
65     @async wait(model_chan)
66     candidate,wk,step_report = take!(model_chan)
67     if !(candidate=="quit")
68       if (candidate.log_Li > e.contour) && !(candidate.log_Li in
69         ↳ [m.log_Li for m in e.models])
70         model_selected=true
71
72         new_model_record =
73           Model_Record(string(e.path,'/',e.model_counter),
74             ↳ candidate.log_Li);
75         e.sample_posterior && push!(e.posterior_samples,
76           ↳ Li_model)#if sampling posterior, push the model
77           ↳ record to the ensemble's posterior samples vector
78         deleteat!(e.models, least_likely_idx)
79         push!(e.models, new_model_record);
80
81         final_model=ICA_PWM_Model(string(e.model_counter),
82           ↳ candidate.origin, candidate.sources,
83           ↳ candidate.source_length_limits, candidate.mix_matrix,
84           ↳ candidate.log_Li, candidate.permute_blacklist)
85         serialize(new_model_record.path, final_model)
86
87
88
89
90
91
92
93

```

```

74         e.model_counter +=1
75     end
76     update_worker_monitor!(wk_mon,wk,true)
77   else
78     update_worker_monitor!(wk_mon,wk,false)
79     !any(wk_mon.persist) && (return 1,step_report)
80   end
81 end
82
83 #UPDATE ENSEMBLE QUANTITIES
84 push!(e.log_Li, minimum([model.log_Li for model in e.models])) #log
  ↳ likelihood of the least likely model - the current ensemble ll
  ↳ contour at Xi
85 push!(e.log_Xi, -i/N) #log Xi - crude estimate of the iterate's
  ↳ enclosed prior mass
86 push!(e.log_wi, lps(e.log_Xi[i], -((j+1)/N)-log(2))) #log width of
  ↳ prior mass spanned by the last step-trapezoidal approx
87 push!(e.log_Liwi, lps(e.log_Li[j],e.log_wi[j])) #log likelihood + log
  ↳ width = increment of evidence spanned by iterate
88 push!(e.log_Zi, logaddexp(e.log_Zi[i],e.log_Liwi[j]))    #log
  ↳ evidence
89
90 #information- dimensionless quantity
91 push!(e.Hi, lps(
  ↳ (exp(lps(e.log_Liwi[j],-e.log_Zi[j])) * e.log_Li[j]), #term1
  ↳ (exp(lps(e.log_Zi[i],-e.log_Zi[j])) *
  ↳ lps(e.Hi[i],e.log_Zi[i])), #term2
  ↳ -e.log_Zi[j])) #term3
92
93 return 0, step_report
94 end

```

16.7.9 /src/permuation/Permute_Tuner.jl

```

1 mutable struct Permute_Tuner
2   inst::Permute_Instruct
3   velocities::Matrix{Float64}
4   successes::BitMatrix #(memoryxfunc)
5   tabular_display::DataFrame
6   time_history::Vector{Float64}
7   override::Bool
8 end

```

```

9
10 """
11     Permute_Tuner(instruction)
12
13 Generate a Permute_Tuner for the given instruction.
14 """
15 function Permute_Tuner(instruction::Permute_Instruct)
16     nfuncs=length(instruction.funcs)
17     funcnames=Vector{String}()
18     vels=ones(TUNING_MEMORY*instruction.func_limit,nfuncs)
19     succs=true.(TUNING_MEMORY*instruction.func_limit,nfuncs)
20     for (idx,func) in enumerate(instruction.funcs)
21         length(instruction.args[idx])>0 ?
22             (kwstr="(+$(length(instruction.args[idx])))kwa)") : (kwstr="")
23         push!(funcnames, string(nameof(func),kwstr))
24     end
25
26     tabular_display=DataFrame("Function"⇒funcnames,
27     → "Succeed"⇒zeros(Int64,nfuncs), "Fail"⇒zeros(Int64,
28     → nfuncs), "Velocity"⇒ones(nfuncs), "Weights"⇒instruction.weights)
29
30     instruction.override_time>0. ? (override=true) : (override=false)
31     return
32         → Permute_Tuner(instruction,vels,succs,tabular_display,zeros(CONVERGENCE_MEMORY))
33 end
34
35 """
36     tune_weights!(tuner, call_report)
37
38 Given a call_report from permute_IPM(), adjust tuner's Permute_Instruct
39     → weights for function success rate and likelihood surface velocity.
40 """
41
42 function tune_weights!(tuner::Permute_Tuner,
43     → call_report::Vector{Tuple{Int64,Float64,Float64}})
44     for call in call_report
45         funcidx,time,distance=call
46         distance!==-Inf &&
47             (tuner.velocities[:,funcidx]=update_velocity!(tuner.velocities[:,funcidx])
48             → #do not push velocity to array if it has -Inf probability
49             → (usu no new model found))
50         if call==call_report[end]
51             → tuner.successes[:,funcidx]=update_sucvec!(tuner.successes[:,funcidx],)

```

```

42     tuner.tabular_display[funcidx, "Succeed"]+=1
43   else
44
45     → tuner.successes[:,funcidx]=update_sucvec!(tuner.successes[:,funcidx],
46       tuner.tabular_display[funcidx, "Fail"]+=1
47   end
48 end
49 tuner.override && mean(tuner.time_history)>tuner.inst.override_time ?
50   → (tuner.inst.weights=tuner.inst.override_weights;
51   → tuner.tabular_display[!, "Weights"]=tuner.inst.override_weights) :
52     update_weights!(tuner)
53 end
54
55 function update_velocity!(velvec,time,distance)
56   popfirst!(velvec) #remove first value
57   vel = distance - log(time)
58   push!(velvec,distance-log(time))
59 end
60
61 function update_sucvec!(sucvec, bool)
62   popfirst!(sucvec)
63   push!(sucvec, bool)
64 end
65
66 function update_weights!(t::Permute_Tuner)
67   mvels=[mean(t.velocities[:,n]) for n in 1:length(t.inst.funcs)]
68   t.tabular_display[!, "Velocity"]=copy(mvels)
69
70   any(i→i<0,mvels) && (mvels.=minimum(mvels)+1.) #to calculate
71     → weights, scale negative values into >1.
72   pvec=[mvels[n]*(sum(t.successes[:,n])/length(t.successes[:,n])) for n
73     → in 1:length(t.inst.funcs)]
74   pvec./=sum(pvec)
75   (any(pvec.<t.inst.min_clmps) || any(pvec.>t.inst.max_clmps)) &&
76     → clamp_pvec!(pvec,t.inst.min_clmps,t.inst.max_clmps)
77
78   @assert isprobvec(pvec)
79   t.inst.weights=pvec; t.tabular_display[!, "Weights"]=pvec
80 end
81
82 """
83   clamp_pvec(pvec, tuner)
84 """

```

```

79 Clamp the values of a probability vector between the minimums and
  ↳ maximums provided by a Permute_Tuner.
80 """
81     function clamp_pvec!(pvec, min_clmps, max_clmps)
82         #logic- first accumulate on low values, then distribute
83         ↳ excess from high values
84         any(pvec.<min_clmps) ? (low_clamped=false) :
85             ↳ (low_clamped=true)
86         while !low_clamped
87             vals_to_accumulate=pvec.<min_clmps
88             vals_to_deplete=pvec.>min_clmps
89
90             ↳ depletion=sum(min_clmps[vals_to_accumulate]--pvec[vals_to_accumulate])
91             ↳ pvec[vals_to_accumulate].=min_clmps[vals_to_accumulate]
92             ↳ pvec[vals_to_deplete].-=depletion/sum(vals_to_deplete)
93
94             !any(pvec.<min_clmps)&&(low_clamped=true)
95         end
96
97         any(pvec.>max_clmps) ? (high_clamped=false) :
98             ↳ (high_clamped=true)
99         while !high_clamped
100            vals_to_deplete=pvec.>max_clmps
101            vals_to_accumulate=pvec.<max_clmps
102
103            ↳ depletion=sum(pvec[vals_to_deplete]--max_clmps[vals_to_deplete])
104            pvec[vals_to_deplete].=max_clmps[vals_to_deplete]
105
106            ↳ pvec[vals_to_accumulate].+=depletion/sum(vals_to_accumulate)
107
108        !any(pvec.>max_clmps)&&(high_clamped=true)
109    end
110
111 end

```

16.7.10 /src/permuation/orthogonality_helper.jl

```

1 ##ORTHOGONALITY_HELPER
2 function consolidate_srcs(con_idxs::Dict{Integer,Vector{Integer}}|,
3     ↳ m::ICA_PWM_Model, obs_array::AbstractMatrix{<:Integer},
4     ↳ obs_lengths::AbstractVector{<:Integer},
5     ↳ bg_scores::AbstractMatrix{<:AbstractFloat}, contour::AbstractFloat,
6     ↳ models::AbstractVector{<:Model_Record};
7     ↳ iterates::Integer=length(m.sources)*2, remote=false)
8     new_log_Li=-Inf; iterate = 1
9     T,0 = size(obs_array); T=T-1; S = length(m.sources)
10    new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
11    a, cache = IPM_likelihood(new_sources, obs_array, obs_lengths,
12        ↳ bg_scores, new_mix, true, true)
13
14    while new_log_Li ≤ contour && iterate ≤ iterates #until we produce
15        ↳ a model more likely than the lh contour or exceed iterates
16        new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
17        clean=Vector{Bool}(trues(0))
18
19        for host_src in filter(!in(vcat(values(con_idxs) ... )),
20            ↳ keys(con_idxs)) #copy mix information to the source to be
21            ↳ consolidated on as host
22            for cons_src in con_idxs[host_src]
23                new_mix[:,host_src]=[new_mix[o,host_src] ||
24                    ↳ new_mix[o,cons_src] for o in 1:size(new_mix,1)]
25            end
26        end
27
28        remote ? (merger_m = deserialize(rand(models).path)) : (merger_m
29            ↳ = remotecall_fetch(deserialize, 1, rand(models).path))
30            ↳ #randomly select a model to merge
31        used_m_srcs=Vector{Int64}()
32
33        for src in unique(vcat(values(con_idxs) ... )) #replace all
34            ↳ non-host sources with sources from a merger model unlike the
35            ↳ one being removed
36            distvec=[pwm_distance(m.sources[src][1],m_src[1]) for m_src
37                ↳ in merger_m.sources]
38            m_src=findmax(distvec)[2]
39            while m_src in used_m_srcs
40                distvec[m_src]=0.; m_src=findmax(distvec)[2]

```

```

26         length(used_m_srcs)==length(merger_m.sources) &&
27             → break; break
28     end
29
30     clean[new_mix[:,src]]*=false #mark dirty any obs that start
31         → with the source
32     new_sources[src]=merger_m.sources[m_src]
33     new_mix[:,src]=merger_m.mix_matrix[:,m_src]
34     clean[new_mix[:,src]]*=false #mark dirty any obs that end
35         → with the source
36     push!(used_m_srcs, m_src)
37 end
38
39 if consolidate_check(new_sources)[1] #if the new sources pass the
40     → consolidate check
41     new_log_Li, cache = IPM_likelihood(new_sources, obs_array,
42         → obs_lengths, bg_scores, new_mix, true, true, cache,
43         → clean) #assess likelihood
44 end
45
46 iterate += 1
47 end
48
49 return ICA_PWM_Model("candidate", "consolidated $(m.origin)",
50     → new_sources, m.source_length_limits, new_mix, new_log_Li)
51 end
52
53 function
54     → consolidate_check(sources :: AbstractVector{<: Tuple{<: AbstractMatrix{<: AbstractFloat}}}
55     → thresh=CONSOLIDATE_THRESH, revcomp=REVCOMP)
56     pass=true
57     lengthδmat=[size(src1[1],1) - size(src2[1],1) for src1 in sources,
58         → src2 in sources]
59     cons_idxs=Dict{Integer, Vector{Integer}}()
60     for src1 in 1:length(sources), src2 in src1+1:length(sources)
61         if lengthδmat[src1,src2]==0
62             revcomp ? (info_condition =
63                 → (pwm_distance(sources[src1][1],sources[src2][1]) < thresh
64                 ||
65                 → pwm_distance(sources[src1][1],revcomp_pwm(sources[src2][1]))
66                 < thresh)) : (info_condition =
67                 → (pwm_distance(sources[src1][1],sources[src2][1]) <
68                 thresh))

```

```

53         if info_condition
54             if !in(src1,keys(cons_idxs))
55                 cons_idxs[src1]=[src2]; pass=false
56             else
57                 push!(cons_idxs[src1], src2)
58             end
59         end
60     end
61
62     return pass, cons_idxs
63 end
64
65
66     function pwm_distance(pwm1,pwm2)
67         minwml=min(size(pwm1,1),size(pwm2,1))
68         return sum([euclidean(exp.(pwm1[pos,:]),
69             → exp.(pwm2[pos,:])) for pos in 1:minwml])/minwml
70     end

```

16.7.11 /src/permuation/permute_control.jl

```

1 mutable struct Permute_Instruct
2     funcs :: AbstractVector{<:Function}
3     weights :: AbstractVector{<:AbstractFloat}
4     args :: AbstractVector{<:AbstractVector{<:Tuple{<:Symbol,<:Any}}}
5     model_limit :: Integer
6     func_limit :: Integer
7     min_clmps :: AbstractVector{<:AbstractFloat}
8     max_clmps :: AbstractVector{<:AbstractFloat}
9     override_time :: AbstractFloat
10    override_weights :: AbstractVector{<:AbstractFloat}
11    Permute_Instruct(funcs,
12                      weights,
13                      model_limit,
14                      func_limit;
15                      min_clmps=fill(.01,length(funcs)),
16                      max_clmps=fill(1.,length(funcs)),
17                      override_time=0.,
18                      override_weights=zeros(length(funcs)),

```



```

45         funcidx=rand(filtered_weights)
46         permute_func=filtered_funcs[funcidx]
47
48             ↳ pos_args,kw_args=get_permfunc_args(permute_func,e,m,filtered_args[fun
49             new_m=permute_func(pos_args ... ;kw_args ... )
50
51             ↳ push!(call_report,(filteridxs[funcidx],time()-start,new_m.log_Li
52             ↳ - e.contour))
53
54     if length(new_m.permute_blacklist) > 0 && new_m.log_Li ==
55         ↳ -Inf #in this case the blacklisted function will not work
56         ↳ on this model at all; it should be removed from the
57         ↳ functions to use
58         vcat(model_blacklist,new_m.permute_blacklist)
59         filteridxs, filtered_funcs, filtered_weights,
60         ↳ filtered_args = filter_permutes(instruction,
61         ↳ model_blacklist)
62     end
63
64
65         dupecheck(new_m,m) && new_m.log_Li > e.contour &&
66             ↳ return new_m, call_report
67         end
68     end
69     return nothing, call_report
70 end
71
72 function permute_IPM(e::IPM_Ensemble, job_chan::RemoteChannel,
73     ↳ models_chan::RemoteChannel, comms_chan::RemoteChannel)
74     ↳ #ensemble.models is partially updated on the worker to populate
75     ↳ arguments for permute funcs
76         persist=true
77         id=myid()
78         put!(comms_chan,id)
79         while persist
80             wait(job_chan)
81             start=time()
82             e.models, e.contour, instruction = fetch(job_chan)
83             instruction = "stop" && (persist=false; break)
84
85             call_report=Vector{Tuple{Int64,Float64,Float64}}()
86             for model=1:instruction.model_limit
87                 found::Bool=false
88                 m_record = rand(e.models)

```

```

76
77     remotecall_fetch(isfile,1,m_record.path) ? m =
    →   (remotecall_fetch(deserialize,1,m_record.path)) : (break)
78
79     model_blacklist=copy(m.permute_blacklist)
80     filteridxs, filtered_funcs, filtered_weights, filtered_args =
    →   filter_permutes(instruction, model_blacklist)
81
82     for call in 1:instruction.func_limit
83         start=time()
84         funcidx=rand(filtered_weights)
85         permute_func=filtered_funcs[funcidx]
86
    →   pos_args,kw_args=get_permfunc_args(permute_func,e,m,filtered_args)
87         new_m=permute_func(pos_args ... ;kw_args ... )
88
    →   push!(call_report,(filteridxs[funcidx],time()-start,new_m.log_Li
    →   - e.contour))
89
90     if length(new_m.permute_blacklist) > 0 && new_m.log_Li ==
    →   -Inf #in this case the blacklisted function will not
    →   work on this model at all; it should be removed from
    →   the functions to use
91         vcat(model_blacklist,new_m.permute_blacklist)
92         filteridxs, filtered_funcs, filtered_weights,
    →   filtered_args = filter_permutes(instruction,
    →   model_blacklist)
93     end
94
95     dupecheck(new_m,m) && new_m.log_Li > e.contour &&
    →   ((put!(models_chan, (new_m ,id, call_report)));
    →   found=true; break)
96
97     end
98     found=true && break;
99     wait(job_chan)
100    fetch(job_chan)≠e.models && (break) #if the ensemble has
    →   changed during the search, update it
101    model=instruction.model_limit &&
    →   (put!(models_chan, ("quit", id,
    →   call_report));persist=false)#worker to put
    →   "quit" on channel if it fails to find a model
    →   more likely than contour

```



```

136         end
137     end
138
139     kw_args = NamedTuple()
140     if length(args) > 0 #if there are any keyword
141         ← arguments to pass
142         sym=Vector{Symbol}(){}
143         val=Vector{Any}(){}
144         for arg in args
145             push!(sym, arg[1]); push!(val, arg[2])
146         end
147         kw_args = (;zip(sym,val) ... )
148     end
149
150     return pos_args, kw_args
151
152     function dupecheck(new_model, model)
153         (new_model.sources==model.sources &&
154         ← new_model.mix_matrix==model.mix_matrix) ? (return
155         ← false) : (return true)
156     end

```

16.7.12 /src/permuation/permute_functions.jl

```

1 #DECORRELATION SEARCH PATTERNS
2 function permute_source(m::ICA_PWM_Model, models::Vector{Model_Record},
3     ← obs_array :: AbstractMatrix{<:Integer},
3     ← obs_lengths :: AbstractVector{<:Integer},
3     ← bg_scores :: AbstractMatrix{<:AbstractFloat}, contour :: AbstractFloat,
3     ← source_priors :: AbstractVector{<:Union{<:AbstractVector{<:Dirichlet{<:AbstractFloat}},<:UnitRange{<:Integer}}}},
3     iterate :: Integer=length(m.sources)*2,
3     ← weight_shift_freq :: AbstractFloat=PWM_SHIFT_FREQ,
3     ← weight_shift_dist :: Distributions.ContinuousUnivariateDistribution=PWM_SHIFT_DIST,
3     ← length_change_freq :: AbstractFloat=PWM_LENGTHPERM_FREQ,
3     ← length_perm_range :: UnitRange{<:Integer}=LENGTHPERM_RANGE,
3     ← remote=false)
3 #weight_shift_dist is given in decimal probability values- converted to
3     ← log space in permute_source_lengths!
4     new_log_Li=-Inf; iterate = 1
5     O = size(obs_array,2);S = length(m.sources)
6     new_sources=deepcopy(m.sources);

```



```

31     a, cache = IPM_likelihood(m.sources, obs_array, obs_lengths,
32                               ↵ bg_scores, m.mix_matrix, true, true)
33     dirty=false
34
35     while (new_log_Li <= contour || !dirty) && iterate <= iterates #until
36       ↵ we produce a model more likely than the lh contour or exceed
37       ↵ iterates
38       mix_moves=rand(mix_move_range)
39       mix_moves > length(m.mix_matrix) && (mix_moves =
40         ↵ length(m.mix_matrix))
41
42       new_mix, clean = mix_matrix_decorrelate(m.mix_matrix, mix_moves)
43         ↵ #generate a decorrelated candidate mix
44       c_log_li, c_cache = IPM_likelihood(m.sources, obs_array,
45         ↵ obs_lengths, bg_scores, new_mix, true, true, cache, clean)
46         ↵ #calculate the model with the candidate mix
47       positive_indices=c_cache.>(cache) #obtain any obs indices that
48         ↵ have greater probability than we started with
49       clean=Vector{Bool}(trues(0)-positive_indices)
50       if any(positive_indices) #if there are any such indices
51         new_log_Li, cache = IPM_likelihood(m.sources, obs_array,
52           ↵ obs_lengths, bg_scores, new_mix, true, true, cache,
53           ↵ clean) #calculate the new model
54         dirty=true
55     end
56     iterate += 1
57   end
58
59   return ICA_PWM_Model("candidate","PM from $(m.name)", m.sources,
60     ↵ m.source_length_limits, new_mix, new_log_Li, Vector{Function}())
61     ↵ #no consolidate check is necessary as sources havent changed
62 end
63
64 function perm_src_fit_mix(m::ICA_PWM_Model,
65   ↵ models::Vector{Model_Record}, obs_array::AbstractMatrix{<:Integer},
66   ↵ obs_lengths::AbstractVector{<:Integer},
67   ↵ bg_scores::AbstractMatrix{<:AbstractFloat}, contour::AbstractFloat,
68   ↵ source_priors::AbstractVector{<:Union{<:AbstractVector{<:Dirichlet{<:AbstractFloat}},  

69   ↵ iterates::Integer=length(m.sources)*2,
70   ↵ weight_shift_freq::AbstractFloat=PWM_SHIFT_FREQ,
71   ↵ length_change_freq::AbstractFloat=PWM_LENGTHPERM_FREQ,
72   ↵ length_perm_range::UnitRange{<:Integer}=LENGTHPERM_RANGE, weight_shift_dist::Distr
73   ↵ remote=false)
74 end

```

```

53     new_log_Li=-Inf; iterate = 1
54     O = size(obs_array,2); S = length(m.sources)
55     new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
56     fit_mix in m.permute_blacklist ? (new_bl=m.permute_blacklist) :
57       (new_bl=Vector{Function}())
58
58     while new_log_Li <= contour && iterate <= iterates #until we produce
59       a model more likely than the lh contour or exceed iterates
60       new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix);
61         tm_one=deepcopy(m.mix_matrix);
62       clean=Vector{Bool}(trues(0))
63       s = rand(1:S);
64       clean[new_mix[:,s]]*=false #all obs starting with source are
65         dirty
66
66     new_mix[:,s]*=false;tm_one[:,s]*=true
67
67     weight_shift_freq > 0 &&
68       (new_sources[s]=permute_source_weights(new_sources[s],
69         weight_shift_freq, weight_shift_dist))
70     rand() < length_change_freq &&
71       (new_sources[s]=permute_source_length(new_sources[s],
72         source_priors[s], m.source_length_limits, length_perm_range))
73
73     l,zero_cache=IPM_likelihood(new_sources, obs_array, obs_lengths,
74       bg_scores, new_mix, true, true)
75     l,one_cache=IPM_likelihood(new_sources, obs_array, obs_lengths,
76       bg_scores, tm_one, true, true)
77     fit_mix=one_cache. ≥ zero_cache
78
78     if REVCOMP #if we're looking at reverse strands, we want to see
79       if sources fit better on the reverse strand, and if so switch
80       over to the revcomp source and use its fitted mix
81       revsrc=(revcomp_pwm(new_sources[s][1]),new_sources[s][2])
82       revsrcs=deepcopy(new_sources)
83       revsrcs[s]=revsrc
84       l, revsrc_cache=IPM_likelihood(revsrcs, obs_array,
85         obs_lengths, bg_scores, tm_one, true, true)
86       rfit_mix=revsrc_cache. ≥ zero_cache
87       lps(revsrc_cache[rfit_mix])>lps(one_cache[fit_mix]) &&
88         (new_sources=revsrcs;fit_mix=rfit_mix) #if the total
89         likelihood contribution for the revsource fit is greater
90         than the source fit, take the revsource

```

```

80     end
81
82     new_mix[:,s]=fit_mix
83
84     clean[fit_mix] *= false #all obs ending with source are dirty
85
86     new_log_Li = IPM_likelihood(new_sources, obs_array, obs_lengths,
87         ↪ bg_scores, new_mix, true, false, zero_cache, clean)
87     iterate += 1
88
89     end
90
91     cons_check, cons_idxs = consolidate_check(new_sources)
92     cons_check ? (return ICA_PWM_Model("candidate","PSFM from
93         ↪ $(m.name)",new_sources, m.source_length_limits, new_mix,
94         ↪ new_log_Li, new_bl)) : (return consolidate_srcs(cons_idxs,
95         ↪ ICA_PWM_Model("candidate","PSFM from $(m.name)",new_sources,
96         ↪ m.source_length_limits, new_mix, new_log_Li, new_bl), obs_array,
97         ↪ obs_lengths, bg_scores, contour, models; remote=remote))
98   end
99
100
101 function fit_mix(m::ICA_PWM_Model, models::Vector{Model_Record},
102     ↪ obs_array::AbstractMatrix{<:Integer},
103     ↪ obs_lengths::AbstractVector{<:Integer},
104     ↪ bg_scores::AbstractMatrix{<:AbstractFloat}; remote=false)
105     new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix);
106     ↪ test_mix=falses(size(m.mix_matrix))
107     new_bl=[fit_mix]
108
109
110     l,zero_cache=IPM_likelihood(m.sources, obs_array, obs_lengths,
111         ↪ bg_scores, test_mix, true, true)
112     for (s,source) in enumerate(m.sources)
113         test_mix=falses(size(m.mix_matrix))
114         test_mix[:,s]=true
115         l,src_cache=IPM_likelihood(m.sources, obs_array, obs_lengths,
116             ↪ bg_scores, test_mix, true, true)
117         fit_mix=src_cache. ≥ zero_cache
118
119
120         if REVCOMP #if we're looking at reverse strands, we want to see
121             ↪ if sources fit better on the reverse strand, and if so switch
122             ↪ over to the revcomp source and use its fitted mix
123             revsrc=(revcomp_pwm(source[1]),source[2])
124             revsrcs=deepcopy(new_sources)
125             revsrcs[s]=revsrc

```

```

109     l, revsrc_cache=IPM_likelihood(revsrcts, obs_array,
110         ↵ obs_lengths, bg_scores, test_mix, true, true)
111     rfit_mix=revsrc_cache.≥zero_cache
112     lps(revsrc_cache[rfit_mix])>lps(src_cache[fit_mix]) &&
113         ↵ (new_sources=revsrcts;fit_mix=rfit_mix) #if the total
114         ↵ likelihood contribution for the revsource fit is greater
115         ↵ than the source fit, take the revsource
116     end
117
118     new_mix[:,s]=fit_mix
119 end
120 new_mix=m.mix_matrix ? (new_log_Li=-Inf) : (new_log_Li =
121     ↵ IPM_likelihood(m.sources, obs_array, obs_lengths, bg_scores,
122     ↵ new_mix, true)) #bail if the mix matrix doesnt change
123 new_log_Li in [model.log_Li for model in models] && (new_log_Li=-Inf)
124     ↵ #an existing identical log_Li is almost certainly a fitted model
125     ↵ from another origin with the same sources, in this case abort to
126     ↵ prevent dupes
127
128 return ICA_PWM_Model("candidate","FM from $(m.name)",new_sources,
129     ↵ m.source_length_limits, new_mix, new_log_Li, new_bl) #no
130     ↵ consolidate check necessary as no change to sources
131 end
132
133 function random_decorrelate(m::ICA_PWM_Model,
134     ↵ models::Vector{Model_Record}, obs_array::AbstractMatrix{<:Integer},
135     ↵ obs_lengths::AbstractVector{<:Integer},
136     ↵ bg_scores::AbstractMatrix{<:AbstractFloat}, contour::AbstractFloat,
137     ↵ source_priors::AbstractVector{<:Union{<:AbstractVector{<:Dirichlet{<:AbstractFlo
138     ↵ iterates::Integer=length(m.sources)*2,
139     ↵ source_permute_freq::AbstractFloat=SRC_PERM_FREQ,
140     ↵ weight_shift_freq::AbstractFloat=PWM_SHIFT_FREQ,
141     ↵ length_change_freq::AbstractFloat=PWM_LENGTHPERM_FREQ,
142     ↵ weight_shift_dist::Distributions.ContinuousUnivariateDistribution=PWM_SHIFT_DIST,
143     ↵ mix_move_range::UnitRange=1:size(m.mix_matrix,1), remote=false)
144     ↵ new_log_Li=-Inf; iterate = 1
145     ↵ O = size(obs_array,2); S = length(m.sources)
146     ↵ new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
147
148 a, cache = IPM_likelihood(new_sources, obs_array, obs_lengths,
149     ↵ bg_scores, new_mix, true, true)

```

```

129   while new_log_Li ≤ contour && iterate ≤ iterates #until we produce
130     ↵ a model more likely than the lh contour or exceed iterates
131     new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
132     clean=Vector{Bool}(trues(0))
133     s = rand(1:S)
134     clean[new_mix[:,s]]*=false #all obs starting with source are
135     ↵ dirty
136     rand() < source_permute_freq &&
137       ↵ (new_sources[s]=permute_source_weights(new_sources[s],
138         ↵ weight_shift_freq, weight_shift_dist))
139     rand() < length_change_freq &&
140       ↵ (new_sources[s]=permute_source_length(new_sources[s],
141         ↵ source_priors[s], m.source_length_limits))
142
143     ↵ new_mix[:,s]=mixvec_decorrelate(new_mix[:,s],rand(mix_move_range))
144     clean[new_mix[:,s]]*=false #all obs ending with source are dirty
145     new_log_Li, cache = IPM_likelihood(new_sources, obs_array,
146       ↵ obs_lengths, bg_scores, new_mix, true, true, cache, clean)
147     iterate += 1
148
149 end
150
151
152 function shuffle_sources(m::ICA_PWM_Model,
153   ↵ models :: AbstractVector{<:Model_Record},
154   ↵ obs_array :: AbstractMatrix{<:Integer},
155   ↵ obs_lengths :: AbstractVector{<:Integer},
156   ↵ bg_scores :: AbstractMatrix{<:AbstractFloat}, contour::AbstractFloat;
157   ↵ remote=false)
158   new_log_Li=-Inf; 0 = size(obs_array,2); S = length(m.sources)
159   new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
160
161   a, cache = IPM_likelihood(m.sources, obs_array, obs_lengths,
162     ↵ bg_scores, m.mix_matrix, true, true)

```

```

152     remote ? (merger_m = deserialize(rand(models).path)) : (merger_m =
153         ← remotecall_fetch(deserialize, 1, rand(models).path))#randomly
154         ← select a model to merge
155
156     svec=[1:S ... ]
157
158     while new_log_Li ≤ contour && length(svec)>0 #until we produce a
159         ← model more likely than the lh contour or no more sources to
160         ← attempt merger
161         new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
162         clean=Vector{Bool}(trues(0))
163
164         s = popat!(svec,rand(1:length(svec))) #randomly select a source
165             ← to merge
166
167         new_sources[s]=merger_m.sources[s];
168             ← new_mix[:,s] .= merger_m.mix_matrix[:,s] #shuffle in the merger
169             ← model source from this index
170
171         clean[m.mix_matrix[:,s]] .= false #mark dirty any obs that have the
172             ← source
173         new_log_Li, cache = IPM_likelihood(new_sources, obs_array,
174             ← obs_lengths, bg_scores, m.mix_matrix, true, true, cache,
175             ← clean) #assess likelihood
176     end
177
178     cons_check, cons_idxs = consolidate_check(new_sources)
179     cons_check ? (return ICA_PWM_Model("candidate","SS from
180         ← $(m.name)",new_sources, m.source_length_limits, new_mix,
181         ← new_log_Li,Vector{Function}())) : (return
182         ← consolidate_srcs(cons_idxs, ICA_PWM_Model("candidate","SS from
183         ← $(m.name)",new_sources, m.source_length_limits, new_mix,
184         ← new_log_Li), obs_array, obs_lengths, bg_scores, contour, models;
185         ← remote=remote))
186 end
187
188 function accumulate_mix(m::ICA_PWM_Model,
189     ← models::AbstractVector{<:Model_Record},
190     ← obs_array::AbstractMatrix{<:Integer},
191     ← obs_lengths::AbstractVector{<:Integer},
192     ← bg_scores::AbstractMatrix{<:AbstractFloat}, contour::AbstractFloat;
193     ← remote=false)
194     new_log_Li=-Inf; 0 = size(obs_array,2); S = length(m.sources)

```

```

174     new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
175     new_bl=Vector{Function}()
176
177     a, cache = IPM_likelihood(m.sources, obs_array, obs_lengths,
178     ↪ bg_scores, m.mix_matrix, true, true)
179
180     remote ? (merger_m = deserialize(rand(models).path)) : (merger_m =
181     ↪ remotecall_fetch(deserialize, 1, rand(models).path))#randomly
182     ↪ select a model to merge
183
184     svec=[1:S ... ]
185
186     while new_log_Li ≤ contour && length(svec)>0 #until we produce a
187     ↪ model more likely than the lh contour or no more sources to
188     ↪ attempt merger
189     new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
190     clean=Vector{Bool}(trues(0))
191
192     s = popat!(svec,rand(1:length(svec))) #randomly select a source
193     ↪ to merge
194     distvec=[pwm_distance(src[1],m_src[1]) for src in m.sources,
195     ↪ m_src in merger_m.sources]
196     S > 1 ? merge_s=findmin(distvec)[2][2] :
197     ↪ merge_s=findmin(distvec)[2]
198
199     new_mix[:,s]=[new_mix[o,s] || merger_m.mix_matrix[o,merge_s] for
200     ↪ o in 1:size(new_mix,1)] #accumulate the mix vector for this
201     ↪ source
202
203     clean[m.mix_matrix[:,s]]*=false #mark dirty any obs that have the
204     ↪ source
205     new_log_Li, cache = IPM_likelihood(new_sources, obs_array,
206     ↪ obs_lengths, bg_scores, m.mix_matrix, true, true, cache,
207     ↪ clean) #assess likelihood
208
209     if new_log_Li ≤ contour #if accumulating on the host source
210     ↪ doesnt work, try copying over the merger source
211     new_sources[s]=merger_m.sources[merge_s]
212     new_log_Li, cache = IPM_likelihood(new_sources, obs_array,
213     ↪ obs_lengths, bg_scores, m.mix_matrix, true, true, cache,
214     ↪ clean) #assess likelihood
215   end
216 end

```

```

201
202     (new_log_Li == m.log_Li || new_log_Li in [model.log_Li for model in
203     models]) && (new_log_Li != -Inf) #accumulate can sometimes duplicate
204     models if source mix is identical
205
206     cons_check, cons_idxs = consolidate_check(new_sources)
207     cons_check ? (return ICA_PWM_Model("candidate", "AM from
208     $(m.name)", new_sources, m.source_length_limits, new_mix,
209     new_log_Li)) : (return consolidate_srcs(cons_idxs,
210     ICA_PWM_Model("candidate", "AM from $(m.name)", new_sources,
211     m.source_length_limits, new_mix, new_log_Li), obs_array,
212     obs_lengths, bg_scores, contour, models; remote=remote))
213   end
214
215
216   function distance_merge(m::ICA_PWM_Model,
217     models::AbstractVector{<:Model_Record},
218     obs_array::AbstractMatrix{<:Integer},
219     obs_lengths::AbstractVector{<:Integer},
220     bg_scores::AbstractMatrix{<:AbstractFloat}, contour::AbstractFloat;
221     remote=false)
222     new_log_Li = -Inf; O = size(obs_array, 2); S = length(m.sources)
223     new_sources = deepcopy(m.sources); new_mix = deepcopy(m.mix_matrix)
224
225     a, cache = IPM_likelihood(new_sources, obs_array, obs_lengths,
226     bg_scores, new_mix, true, true)
227
228     remote ? (merger_m = deserialize(rand(models).path)) : (merger_m =
229     remotecall_fetch(deserialize, 1, rand(models).path)) #randomly
230     select a model to merge
231
232     svec = [1:S ... ]
233
234     while new_log_Li <= contour && length(svec) > 0 #until we produce a
235       model more likely than the lh contour or no more sources to
236       attempt merger
237       new_sources = deepcopy(m.sources); new_mix = deepcopy(m.mix_matrix)
238       clean = Vector{Bool}(trues(O))
239
240       s = popat!(svec, rand(1:length(svec))) #randomly select a source
241       to merge
242       merge_s = most_dissimilar(new_mix[:, s], merger_m.mix_matrix) #find
243       the source in the merger model whose mixvec is most
244       dissimilar to the one selected

```

```

224
225     clean[new_mix[:,s]] = false #mark dirty any obs that start with
226     ↪   the source
227     new_sources[s] = merger_m.sources[merge_s] #copy the source
228     new_mix[:,s] = merger_m.mix_matrix[:,merge_s] #copy the mixvector
229     ↪   (without which the source will likely be highly improbable)
230     clean[new_mix[:,s]] = false #mark dirty any obs that end with the
231     ↪   source
232
233     new_log_Li, cache = IPM_likelihood(new_sources, obs_array,
234     ↪   obs_lengths, bg_scores, new_mix, true, true, cache, clean)
235     ↪   #assess likelihood
236   end
237
238   cons_check, cons_idxs = consolidate_check(new_sources)
239   cons_check ? (return ICA_PWM_Model("candidate", "DM from
240   ↪   $(m.name)", new_sources, m.source_length_limits, new_mix,
241   ↪   new_log_Li, Vector{Function}())) : (return
242   ↪   consolidate_srcs(cons_idxs, ICA_PWM_Model("candidate", "DM from
243   ↪   $(m.name)", new_sources, m.source_length_limits, new_mix,
244   ↪   new_log_Li), obs_array, obs_lengths, bg_scores, contour, models;
245   ↪   remote=remote))
246 end
247
248 function similarity_merge(m::ICA_PWM_Model,
249   ↪   models::AbstractVector{<:Model_Record},
250   ↪   obs_array::AbstractMatrix{<:Integer},
251   ↪   obs_lengths::AbstractVector{<:Integer},
252   ↪   bg_scores::AbstractMatrix{<:AbstractFloat}, contour::AbstractFloat;
253   ↪   remote=false)
254   new_log_Li=-Inf; O = size(obs_array,2); S = length(m.sources)
255   new_sources=deepcopy(m.sources)
256
257   a, cache = IPM_likelihood(m.sources, obs_array, obs_lengths,
258   ↪   bg_scores, m.mix_matrix, true, true)
259
260   remote ? (merger_m = deserialize(rand(models).path)) : (merger_m =
261   ↪   remotecall_fetch(deserialize, 1, rand(models).path))#randomly
262   ↪   select a model to merge
263
264   svec=[1:S ... ]
265
266

```

```

247   while new_log_Li ≤ contour && length(svec)>0 #until we produce a
248     ← model more likely than the lh contour or no more sources to
249     ← attempt merger
250
251     new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
252     clean=Vector{Bool}(trues(0))
253
254     s = popat!(svec,rand(1:length(svec))) #randomly select a source
255     ← to merge
256     merge_s=most_similar(m.mix_matrix[:,s],merger_m.mix_matrix)
257     ← #obtain the source in the merger model whose mixvec is most
258     ← similar to the one in the original
259
260     clean[m.mix_matrix[:,s]]*=false #mark dirty any obs that have the
261     ← source
262     new_sources[s] = merger_m.sources[merge_s] #copy the source, but
263     ← dont copy the mixvector
264     new_log_Li, cache = IPM_likelihood(new_sources, obs_array,
265     ← obs_lengths, bg_scores, m.mix_matrix, true, true, cache,
266     ← clean) #assess likelihood
267   end
268
269   cons_check, cons_idxs = consolidate_check(new_sources)
270   cons_check ? (return ICA_PWM_Model("candidate","SM from
271     ← $(m.name)",new_sources, m.source_length_limits, m.mix_matrix,
272     ← new_log_Li,Vector{Function}())) : (return
273     ← consolidate_srcs(cons_idxs, ICA_PWM_Model("candidate","SM from
274     ← $(m.name)",new_sources, m.source_length_limits, m.mix_matrix,
275     ← new_log_Li), obs_array, obs_lengths, bg_scores, contour, models;
276     ← remote=remote))
277 end
278
279 function reinit_src(m::ICA_PWM_Model,
280   ← models::AbstractVector{<:Model_Record},
281   ← obs_array::AbstractMatrix{<:Integer},
282   ← obs_lengths::AbstractVector{<:Integer},
283   ← bg_scores::AbstractMatrix{<:AbstractFloat},contour::AbstractFloat,
284   ← source_priors::AbstractVector{<:Union{<:AbstractVector{<:Dirichlet{<:AbstractFlo
285   ← iterates::Integer=length(m.sources)*2, remote=false}
286   new_log_Li=-Inf; iterate = 1
287   O = size(obs_array,2); S = length(m.sources)
288   new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix)
289
290

```

```

268   while new_log_Li ≤ contour && iterate ≤ iterates #until we produce
269     ↵ a model more likely than the lh contour or exceed iterates
270   new_sources=deepcopy(m.sources); new_mix=deepcopy(m.mix_matrix);
271   ↵ tm_one=deepcopy(m.mix_matrix);
272   clean=Vector{Bool}(trues(0))
273   s = rand(1:S);
274   clean[new_mix[:,s]]*=false #all obs starting with source are
275   ↵ dirty
276
277   new_mix[:,s]*=false;tm_one[:,s]*=true
278
279   new_sources[s] = init_logPWM_sources([source_priors[s]],
280   ↵ m.source_length_limits)[1] #reinitialize the source
281
282   l,zero_cache=IPM_likelihood(new_sources, obs_array, obs_lengths,
283   ↵ bg_scores, new_mix, true, true)
284   l,one_cache=IPM_likelihood(new_sources, obs_array, obs_lengths,
285   ↵ bg_scores, tm_one, true, true)
286
287   fit_mix=one_cache.≥ zero_cache
288
289   new_mix[:,s]=fit_mix
290
291   clean[fit_mix]*=false #all obs ending with source are dirty
292
293   new_log_Li = IPM_likelihood(new_sources, obs_array, obs_lengths,
294   ↵ bg_scores, new_mix, true, false, zero_cache, clean)
295   iterate += 1
296
297 end
298
299 cons_check, cons_idxs = consolidate_check(new_sources)
300 cons_check ? (return ICA_PWM_Model("candidate","RS from $(m.name)",
301   ↵ new_sources, m.source_length_limits, new_mix,
302   ↵ new_log_Li,Vector{Function}())) : (return
303   ↵ consolidate_srcs(cons_idxs, ICA_PWM_Model("candidate","RS from
304   ↵ $(m.name)",new_sources, m.source_length_limits, new_mix,
305   ↵ new_log_Li), obs_array, obs_lengths, bg_scores, contour, models;
306   ↵ remote=remote))
307 end
308
309

```

```

295 function erode_model(m::ICA_PWM_Model,
296   → models :: AbstractVector{<:Model_Record},
297   → obs_array :: AbstractMatrix{<:Integer},
298   → obs_lengths :: AbstractVector{<:Integer},
299   → bg_scores :: AbstractMatrix{<:AbstractFloat}, contour::AbstractFloat;
300   → info_thresh::AbstractFloat=EROSION_INFO_THRESH, remote=false)
301   new_log_Li=-Inf; 0 = size(obs_array,2);
302   new_sources=deepcopy(m.sources)

303
304   erosion_sources=Vector{Integer}()
305   for (s,src) in enumerate(m.sources)
306     pwm,pi=src
307     if size(pwm,1)>m.source_length_limits[1] #do not consider
308       → eroding srcs at min length limit
309       infovec=get_pwm_info(pwm)
310       any(info-><(info, info_thresh),infovec) &&
311       → push!(erosion_sources,s)
312   end
313 end

314
315
316
317
318
319
320

```

length(erosion_sources)==0 && return ICA_PWM_Model("candidate","EM
 → from \$(m.name)",new_sources, m.source_length_limits,
 → m.mix_matrix, new_log_Li,[erode_model])#if we got a model we cant
 → erode bail out with a model marked -Inf lh and with EM
 → blacklisted

a, cache = IPM_likelihood(m.sources, obs_array, obs_lengths,
 → bg_scores, m.mix_matrix, true, true)

while new_log_Li ≤ contour && length(erosion_sources) > 0 #until we
 → produce a model more likely than the lh contour or there are no
 → more sources to erode
 clean=Vector{Bool}(trues(0))
 s=popat!(erosion_sources,rand(1:length(erosion_sources)))

new_sources[s]=erode_source(new_sources[s],
 → m.source_length_limits, info_thresh)
 clean[m.mix_matrix[:,s]]*=false

new_log_Li, cache = IPM_likelihood(new_sources, obs_array,
 → obs_lengths, bg_scores, m.mix_matrix, true, true, cache,
 → clean) #assess likelihood

end

```

321 new_log_Li <= contour ? (blacklist=[erode_model]) :
322   ↵ (blacklist=Vector{Function}())
323
324 cons_check, cons_idxs = consolidate_check(new_sources)
325 cons_check ? (return ICA_PWM_Model("candidate", "EM from
326   ↵ $(m.name)", new_sources, m.source_length_limits, m.mix_matrix,
327   ↵ new_log_Li, blacklist)) : (return consolidate_srcs(cons_idxs,
328   ↵ ICA_PWM_Model("candidate", "EM from $(m.name)", new_sources,
329   ↵ m.source_length_limits, m.mix_matrix, new_log_Li, blacklist),
330   ↵ obs_array, obs_lengths, bg_scores, contour, models;
331   ↵ remote=remote))
332 end
333
334 function info_fill(m::ICA_PWM_Model,
335   ↵ models::AbstractVector{<:Model_Record},
336   ↵ obs_array::AbstractMatrix{<:Integer},
337   ↵ obs_lengths::AbstractVector{<:Integer},
338   ↵ bg_scores::AbstractMatrix{<:AbstractFloat}, contour::AbstractFloat;
339   ↵ remote=false)
340   new_log_Li=-Inf; O = size(obs_array,2); S = length(m.sources);
341   ↵ iterate=1
342   new_sources=deepcopy(m.sources)
343
344   a, cache = IPM_likelihood(m.sources, obs_array, obs_lengths,
345     ↵ bg_scores, m.mix_matrix, true, true)
346
347   svec=[1:S ... ]
348
349   while new_log_Li <= contour && length(svec)>0 #until we produce a
350     ↵ model more likely than the lh contour or no more sources to
351     ↵ attempt infofill
352       new_sources=deepcopy(m.sources)
353       clean=Vector{Bool}(trues(O))
354       s = popat!(svec,rand(1:length(svec)))
355       pwm=new_sources[s][1]
356       fill_idx=findmin(get_pwm_info(pwm))[2]
357       fill_bases=[1,2,3,4]
358       fill_candidate=findmax(pwm[fill_idx,:])[2]
359       deleteat!(fill_bases, findfirst(b→b==fill_candidate,fill_bases))
360       new_sources[s][1][fill_idx,:]=-Inf;
361       ↵ new_sources[s][1][fill_idx,fill_candidate]=0.
362       clean[m.mix_matrix[:,s]]-=false

```

```

347     new_log_Li, cache = IPM_likelihood(new_sources, obs_array,
348         ↵ obs_lengths, bg_scores, m.mix_matrix, true, true, cache,
349         ↵ clean) #assess likelihood

350     while new_log_Li <= contour && length(fill_bases)>0
351         fill_candidate=popat!(fill_bases,rand(1:length(fill_bases)))
352         new_sources[s][1][fill_idx,:]==-Inf;
353             ↵ new_sources[s][1][fill_idx,fill_candidate]=0.
354         new_log_Li, cache = IPM_likelihood(new_sources, obs_array,
355             ↵ obs_lengths, bg_scores, m.mix_matrix, true, true, cache,
356             ↵ clean) #assess likelihood
357     end
358 end
359
360 new_log_Li <= contour ? (blacklist=[info_fill]) :
361     ↵ (blacklist=Vector{Function}())
362
363 cons_check, cons_idxs = consolidate_check(new_sources)
364 cons_check ? (return ICA_PWM_Model("candidate","IF from
365     ↵ $(m.name)",new_sources, m.source_length_limits, m.mix_matrix,
366     ↵ new_log_Li, blacklist)) : (return consolidate_srcs(cons_idxs,
367     ↵ ICA_PWM_Model("candidate","IF from $(m.name)",new_sources,
368     ↵ m.source_length_limits, m.mix_matrix, new_log_Li, blacklist),
369     ↵ obs_array, obs_lengths, bg_scores, contour, models;
370     ↵ remote=remote))
371
372 full_perm_funcvec=[permute_source, permute_mix, perm_src_fit_mix,
373     ↵ fit_mix, random_decorrelate, shuffle_sources, accumulate_mix,
374     ↵ distance_merge, similarity_merge, reinit_src, erode_model, info_fill]

```

16.7.13 /src/permuation/permute_utilities.jl

```
1 ##BASIC UTILITY FUNCTIONS
2 #SOURCE PERMUTATION
3 function
4     ↳ permute_source_weights(source::Tuple{<:AbstractMatrix{<:AbstractFloat},<:Integer})
5     ↳ shift_freq::AbstractFloat,
6     ↳ PWM_shift_dist::Distribution{Univariate,Continuous})
7     dirty=false; source_length=size(source[1],1)
8     new_source=deepcopy(source)
9
10
```

```

7   for source_pos in 1:source_length
8     if rand() ≤ shift_freq
9       pos_WM = exp.(source[1][source_pos,:]) #leaving logspace, get
10      ↳ the wm at that position
11      new_source[1][source_pos,:] = log.(wm_shift(pos_WM,
12        ↳ PWM_shift_dist)) #accumulate probility at a randomly
13        ↳ selected base, reassign in logspace and carry on
14        !dirty && (dirty=true)
15      end
16    end
17
18    if !dirty #if no positions were shifted, pick one and shift
19      rand_pos=rand(1:source_length)
20      pos_WM = exp.(source[1][rand_pos,:])
21      new_source[1][rand_pos,:]=log.(wm_shift(pos_WM, PWM_shift_dist))
22    end
23
24    return new_source
25  end
26
27  function
28    ↳ wm_shift(pos_WM::AbstractVector{<:AbstractFloat},
29    ↳ PWM_shift_dist::Distribution{Univariate,Continuous})
30    base_to_shift = rand(1:4) #pick a base to accumulate
31    ↳ probability
32    permute_sign = rand(-1:2:1)
33    shift_size = rand(PWM_shift_dist)
34    new_wm=zeros(4)
35
36    for base in 1:4 #ACGT
37      if base == base_to_shift
38        new_wm[base] =
39        clamp(0, #no lower than 0 prob
40          (pos_WM[base] #selected PWM posn
41           + permute_sign * shift_size), #randomly
42           ↳ permuted by size param
43           1) #no higher than prob 1
44      else
45        size_frac = shift_size / 3 #other bases
46        ↳ shifted in the opposite direction by 1/3
47        ↳ the shift accumulated at the base to
48        ↳ permute
49        new_wm[base] =
50      end
51    end
52  end
53
```

```

40             clamp(0,
41             (pos_WM[base]
42             - permute_sign * size_frac),
43             1)
44         end
45     end
46     new_wm = new_wm ./ sum(new_wm) #renormalise to sum 1
47     ↪ - necessary in case of clamping at 0 or 1
48     !isprobvec(new_wm) && throw(DomainError(new_wm, "Bad
49     ↪ weight vector generated in wm_shift!")) #throw
50     ↪ assertion exception if the position WM is invalid
51
52     return new_wm
53 end
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72

```

function

- permute_source_length(source::Tuple{<:AbstractMatrix{<:AbstractFloat},<:Integer},
- prior::Union{<:AbstractVector{<:Dirichlet{<:AbstractFloat}},<:Bool},
- length_limits::UnitRange{<:Integer},
- permute_range::UnitRange{<:Integer}=LENGTHPERM_RANGE,
- uninformative::Dirichlet=Dirichlet([.25,.25,.25,.25]))

source_PWM, prior_idx = source
source_length = size(source_PWM,1)

permute_sign, permute_length = get_length_params(source_length,
length_limits, permute_range)

permute_sign==1 ? permute_pos = rand(1:source_length+1) :
permute_pos=rand(1:source_length-permute_length)

if permute_sign == 1 #if we're to add positions to the PWM
ins_WM=zeros(permute_length,4)
if prior=false
for pos in 1:permute_length
ins_WM[pos,:]= log.(transpose(rand(uninformative)))
end
else
for pos in 1:permute_length
prior_position=permute_pos+prior_idx-1
prior_position<1 || prior_position>length(prior) ?
ins_WM[pos,:]= log.(transpose(rand(uninformative)))
↪ :

```

73         ins_WM[pos,:] =
    ↳ log.(transpose(rand(prior[prior_position])))
74     end
75 end
76 upstream_source=source_PWM[1:permute_pos-1,:]
77 downstream_source=source_PWM[permute_pos:end,:]
78 source_PWM=vcat(upstream_source,ins_WM,downstream_source)
79 permute_pos==1 && (prior_idx-=permute_length)
80 else #if we're to remove positions
81     upstream_source=source_PWM[1:permute_pos-1,:]
82     downstream_source=source_PWM[permute_pos+permute_length:end,:]
83     source_PWM=vcat(upstream_source,downstream_source)
84     permute_pos==1 && (prior_idx+=permute_length)
85 end
86
87 return (source_PWM, prior_idx) #return a new source
88 end
89
90 function get_length_params(source_length::Integer,
91     ↳ length_limits::UnitRange{<:Integer},
92     ↳ permute_range::UnitRange{<:Integer})
93     extendable = length_limits[end]-source_length
94     contractable = source_length-length_limits[1]
95
96     if extendable == 0 && contractable > 0
97         permute_sign=-1
98     elseif contractable == 0 && extendable > 0
99         permute_sign=1
100    else
101        permute_sign = rand(-1:2:1)
102    end
103
104    permute_sign==1 && extendable<permute_range[end] &&
    ↳ (permute_range=permute_range[1]:extendable)
105    permute_sign== -1 && contractable<permute_range[end]
    ↳ && (permute_range=permute_range[1]:contractable)
106    permute_length = rand(permute_range)
107
108    return permute_sign, permute_length
109 end
110
111 function
    ↳ erode_source(source :: Tuple{<:AbstractMatrix{<:AbstractFloat},<:Integer},length_li

```

```

110     pwm,prior_idx=source
111     infovec=get_pwm_info(pwm)
112     start_idx,end_idx=get_erosion_idxs(infovec, info_thresh,
113                                         ↪ length_limits)
114
115     return new_source=(pwm[start_idx:end_idx,:], prior_idx+start_idx-1)
116 end
117
118 function get_pwm_info(pwm::AbstractMatrix{<:AbstractFloat};
119                         ↪ logsw::Bool=true)
120     wml=size(pwm,1)
121     infovec=zeros(wml)
122     for pos in 1:wml
123         logsw ? wvec=deepcopy(exp.(pwm[pos,:])) :
124             ↪ wvec=deepcopy(pwm[pos,:])
125         !isprobvec(wvec) && throw(DomainError(wvec, "Bad wvec in
126                                         ↪ get_pwm_info -Original sources must be in logspace!!"))
127         wvec.=10^-99
128         infscore = (2.0 + sum([x*log(2,x) for x in wvec]))
129         infovec[pos]=infscore
130     end
131     return infovec
132 end
133
134 function get_erosion_idxs(infovec::AbstractVector{<:AbstractFloat},
135                           ↪ info_thresh::AbstractFloat, length_limits::UnitRange{<:Integer})
136     srcl=length(infovec)
137     contractable = srcl-length_limits[1]
138     contractable ≤ 0 && throw(DomainError(contractable, "erode_source
139                                         ↪ passed a source at its lower length limit!"))
140     centeridx=findmax(infovec)[2]
141
142     start_idx=findprev(info→<(info,info_thresh),infovec,centeridx)
143     start_idx==nothing ? (start_idx=1) : (start_idx+=1)
144     end_idx=findnext(info→<(info, info_thresh),infovec,centeridx)
145     end_idx==nothing ? (end_idx=srcl) : (end_idx-=1)
146
147     pos_to_eroде=srcl-(end_idx-start_idx)
148     if pos_to_eroде > contractable
149         pos_to_restore = pos_to_eroде-contractable
150         while pos_to_restore>0
151             end_die=rand()
152             if end_die ≤ .5

```

```

147             start_idx>1 && (pos_to_restore-=1; start_idx-=1)
148         else
149             end_idx<srcl && (pos_to_restore-=1; end_idx+=1)
150         end
151     end
152 end
153
154     return start_idx, end_idx
155 end
156
157 #MIX MATRIX FUNCTIONS
158 function mixvec_decorrelate(mix::BitVector, moves::Integer)
159     new_mix=deepcopy(mix)
160     idxs_to_flip=rand(1:length(mix), moves)
161     new_mix[idxs_to_flip] *= .!mix[idxs_to_flip]
162     return new_mix
163 end
164
165 function mix_matrix_decorrelate(mix::BitMatrix, moves::Integer)
166     clean=Vector{Bool}(trues(size(mix,1)))
167     new_mix=deepcopy(mix)
168     indices_to_flip = rand(CartesianIndices(mix), moves)
169     new_mix[indices_to_flip] *= .!mix[indices_to_flip]
170     clean[unique([idx[1] for idx in indices_to_flip])] *= false #mark all
171     → obs that had flipped indices dirty
172     return new_mix, clean
173 end
174
175 # function most_dissimilar(mix1, mix2)
176 #     S1=size(mix1,2);S2=size(mix2,2)
177 #     dist_mat=zeros(S1,S2)
178 #     for s1 in 1:S1, s2 in 1:S2
179 #         dist_mat[s1,s2]=sum(mix1[:,s1].==mix2[:,s2])
180 #     end
181 #     scores=vec(sum(dist_mat,dims=1))
182 #     return findmin(scores)[2]
183 # end
184
185
186 function most_dissimilar(src_mixvec, target_mixmat)

```

```

187     src_sim = [sum(src_mixvec.=target_mixmat[:,s]) for s in
188                 1:size(target_mixmat,2)] #compose array of elementwise equality
189                 comparisons between mixvectors and sum to score
190
191     merge_s=findmin(src_sim)[2] #source from merger model will be the one
192                 with the highest equality comparison score
193
194 end
195
196
197 function most_similar(src_mixvec, target_mixmat)
198     src_sim = [sum(src_mixvec.=target_mixmat[:,s]) for s in
199                 1:size(target_mixmat,2)] #compose array of elementwise equality
200                 comparisons between mixvectors and sum to score
201     merge_s=findmax(src_sim)[2] #source from merger model will be the one
202                 with the highest equality comparison score
203
204 end

```

16.7.14 /src/utilities/model_display.jl

```

1 #THIS CODE BASED ON N. REDDY'S THICWEED.JL DISPLAY SCRIPT
2
3 #CONSTANTS
4 # each tuple specifies the x, y, fontsize, xscale to print the character
5 # in a 100×100 box at position 100,100.
6 charvals_dict = Dict{Char,Tuple}('A'⇒(88.5,199.,135.,1.09),
7                                     'C'⇒(79.,196.,129.,1.19),
8                                     'G'⇒(83.,196.,129.,1.14),
9                                     'T'⇒(80.,199.,135.,1.24))
10
11 colour_dict = Dict{Char,String}('A'⇒"(0,128,0)", #green
12                                 'C'⇒"(0,0,128)", #blue
13                                 'G'⇒"(255,69,0)", #yellow-brown
14                                 'T'⇒"(150,0,0)") #red
15
16 #char output params for string display of PWMs in nested sampler
16     ↪ instrumentation
17 lwcs_vec=['a','c','g','t']
18 upcs_vec=['A','C','G','T']
19 cs_vec=[:green,:blue,:yellow,:red]
20 thresh_dict=Dict([(0.,'_'),(.25,'.'),(.5,"lwcs"),(1,"upcs")])
21
22 source_left_x = 10
23 xlen = 20
24 yscale = 250

```

```

25 scale_factor = 0.9
26 ypixels_per_source = 250
27 ypad = 10
28 xpad = 20
29 xpixels_per_position = 40
30 fontsize1=60
31 fontsize2=40
32
33 #function to convert ICA_PWM_Model sources lists to PWM sequence logo
34   ↵  diagrams
34 function
35   ↵  logo_from_model(model::ICA_PWM_Model,svg_output::String;freq_sort::Bool=false)
35     source_tups = Vector{Tuple{AbstractFloat, Integer,
36       ↵  Matrix{AbstractFloat}}}()

36       ↵  #(%of sequences w/ source, prior index,
37       ↵  weight matrix)
37     mix = model.mix_matrix #o x s
38     for (prior, source) in enumerate(model.sources)
39       push!(source_tups,
40         ↵  (sum(model.mix_matrix[:,prior])/size(model.mix_matrix)[1],
41         ↵  prior, exp.(source[1])))
41     end
42
43     freq_sort && sort(source_tups)
44
45     file = open(svg_output, "w")
46     write(file,
47       ↵  svg_header(xpad+xpixels_per_position*maximum([length(source[1])
48       ↵  for source in
49       ↵  model.sources]),ypixels_per_source*length(model.sources)+ypad))
50
51     curry = 0
52     for (frequency, index, source) in source_tups
53       curry += yscale
54       font1y = curry-190
55       ndig = ndigits(index+1)
56       write(file,
57         ↵  pwm_to_logo(source,source_left_x,curry,xlen,yscale*scale_factor))
58       write(file, "<text x=\"10\" y=\"$font1y\""
59         ↵  font-family=\"Helvetica\" font-size=\"$fontsize1\""
60         ↵  font-weight=\"bold\" >$index</text>")

```

```

53     write(file, "<text x=\"$((20+fontsize1*0.6*ndig))\""
54     ↪   y=\"$((font1y-fontsize1+1.5*fontsize2))\""
55     ↪   font-family=\"Helvetica\" font-size=\"$fontsize2\""
56     ↪   font-weight=\"bold\" >$((frequency*100)) % of
57     ↪   sequences</text>\n")
58 end
59
60
61 function svg_header(canvas_size_x, canvas_size_y)
62     return """
63     <?xml version="1.0" standalone="no"?>
64     <!DOCTYPE svg PUBLIC "-//W3C//DTD SVG 1.1//EN"
65     "http://www.w3.org/Graphics/SVG/1.1/DTD/svg11.dtd">
66     <svg width=\"$canvas_size_x\" height=\"$canvas_size_y"
67     version="1.1" xmlns="http://www.w3.org/2000/svg">
68     """
69 end
70
71 function pwm_to_logo(source,xpos,ypos,xlen,yscale)
72     outstr = ""
73     for n in 1:size(source,1)
74         outstr *=
75             → print_weightvec_at_x_y(source[n,:],xpos+xlen*n,ypos,xlen,yscale)
76     end
77     outstr *= "\n"
78     return outstr
79 end
80
81 function pwmstr_to_io(io,source;log=true)
82     log && (source=exp.(source))
83     for pos in 1:size(source,1)
84         char,color=uni_wvec_params(source[pos,:])
85         printstyled(io, char; color=color)
86     end
87 end
88 function uni_wvec_params(wvec) #assign a single unicode char and color
89     ↪ symbol for the most informational position in a position weight
90     ↪ vector

```

```

89     wvec.+=10^-99
90     infoscore=(2.0 + sum([x*log(2,x) for x in wvec]))
91     infovec = [x*infoscore for x in wvec]
92     val,idx=findmax(infovec)
93     return char,color=get_char_by_thresh(idx,val)
94 end
95     function get_char_by_thresh(idx,val)
96         char = '?'; seen_thresh=0.
97         for (thresh,threshchar) in thresh_dict
98             val ≥ thresh && thresh ≥ seen_thresh &&
99                 (char=threshchar; seen_thresh=thresh)
100            end
101            char=='?' && println(val)
102            char=="lwcs" && (char=lwcs_vec[idx])
103            char=="upcs" && (char=upcs_vec[idx])
104            color=cs_vec[idx]
105
106        return char,color
107    end
108
109
110
111
112 function print_weightvec_at_x_y(wvec, xpos, ypos, xlen, yscale)
113     # xlen is the length occupied by that column
114     # yscale is the total height for 2 information bits i.e. the
115     # maximum height available for a "perfect" nucleotide
116     outstr = ""
117     basestr = "ACGT"
118     wvec.+=10^-99 #prevent log(0) = -Inf
119     wvec = [x/sum(wvec) for x in wvec] #renorm
120     infscore = (2.0 + sum([x*log(2,x) for x in wvec]))
121     if infscore==0.0
122         return ""
123     end
124     wvec = [x*infscore*yscale/2.0 for x in wvec]
125     # at this point, the sum of all wvec is a maximum of yscale
126     wveclist = [(wvec[n],basestr[n]) for n in 1:4]
127     wveclist = sort(wveclist)
128     curr_ypos = ypos
129     for n in 1:4
130         curr_ypos -= wveclist[n][1]

```

```

131         outstr *=
132             ↪ print_char_in_rect(wveclist[n][2],xpos,curr_ypos,xlen,wveclist[n][1])
133     end
134   return outstr
135 end
136 blo = 1
137
138 function print_char_in_rect(c,x,y,width,height)
139     raw_x, raw_y, raw_fontsize, raw_xsclae = charvals_dict[c]
140     raw_x = (raw_x*raw_xsclae-100)/raw_xsclae
141     raw_y = raw_y-100
142
143     xscale = width/100.0 * raw_xsclae
144     yscale = height/100.0
145
146     scaled_x = x/xscale + raw_x
147     scaled_y = y/yscale + raw_y
148
149     return "<text x=\"$scaled_x\" y=\"$scaled_y\""
150         ↪ font-size=\"$raw_fontsize\" font-weight=\"bold\""
151         ↪ font-family=\"Helvetica\" fill=\"$rgb*colour_dict[c]*\""
152         ↪ transform=\"scale($xscale,$yscale)\">$c</text>\n"
153 end
154
155 function svg_footer()
156     return "</svg>"
157 end

```

16.7.15 /src/utilities/ns_progressmeter.jl

```

1 #UTILITY REPORTS WORKER NUMBER AND CURRENT ITERATE
2 mutable struct ProgressNS{T<:Real} <: AbstractProgress
3     interval::T
4     dt::AbstractFloat
5     start_it::Integer
6     counter::Integer
7     triggered::Bool
8     tfirst::AbstractFloat
9     tlast::AbstractFloat
10    tstop::AbstractFloat

```

```

1    printed::Bool          # true if we have issued at least one status
2    ↳ update
3
4    desc::AbstractString # prefix to the percentage, e.g. "Computing ... "
5    color::Symbol         # default to green
6    output::IO             # output stream into which the progress is
7    ↳ written
8    numprintedvalues::Integer # num values printed below progress in
9    ↳ last iteration
10   offset::Integer        # position offset of progress bar
11   ↳ (default is 0)
12
13
14
15
16
17
18   e::IPM_Ensemble
19   wm::Worker_Monitor
20   tuner::Permute_Tuner
21   top_m::ICA_PWM_Model
22
23   mean_stp_time::AbstractFloat
24
25   wk_disp::Bool
26   tuning_disp::Bool
27   conv_plot::Bool
28   ens_disp::Bool
29   lh_disp::Bool
30   liwi_disp::Bool
31   src_disp::Bool
32   no_displayed_srcs::Integer
33
34   disp_rotate_inst::Vector{Any}
35
36   convergence_history::Vector{Float64}
37
38   function ProgressNS{T}(
39       e::IPM_Ensemble,
40       top_m::ICA_PWM_Model,
41       wm::Worker_Monitor,
42       tuner::Permute_Tuner,
43       interval::T;
44       dt::Real=0.1,
45       desc::AbstractString="Nested Sampling::",
46       color::Symbol=:green,
47       output::IO=stdout,
48       offset::Int=0,
49       start_it::Int=1,
50       wk_disp::Bool=false,
51       )
52       ...
53   end

```

```

50          tuning_disp::Bool=false,
51          conv_plot::Bool=true,
52          ens_disp::Bool=false,
53          lh_disp::Bool=false,
54          liwi_disp::Bool=false,
55          src_disp::Bool=true,
56          nsrcts::Integer=0,
57
58          ↳ disp_rotate_inst=[false,0,0,Vector{Vector{Symbol}}]
59          ↳ where T
60
61      tfirst = tlast = time()
62      printed = false
63      new{T}(interval,
64          dt,
65          start_it,
66          start_it,
67          false,
68          tfirst,
69          tlast,
70          0.,
71          printed,
72          desc,
73          color,
74          output,
75          0,
76          offset,
77          e,
78          wM,
79          tuner,
80          top_m,
81          0.,
82          wk_disp,
83          tuning_disp,
84          conv_plot,
85          ens_disp,
86          lh_disp,
87          liwi_disp,
88          src_disp,
89          nsrcts,
90          disp_rotate_inst,
91          zeros(CONVERGENCE_MEMORY))
92
93      end
94  end

```

```

91
92 function ProgressNS(e::IPM_E ensemble, wm::Worker_Monitor,
93   tuner::Permute_Tuner, interval::Real; dt::Real=0.1,
94   desc::AbstractString="Nested Sampling:: ", color::Symbol=:green,
95   output::IO=stderr, offset::Integer=0, start_it::Integer=1,
96   wk_disp::Bool=false, tuning_disp::Bool=false, conv_plot::Bool=false,
97   lh_disp::Bool=false, liwi_disp::Bool=false, ens_disp::Bool=false,
98   src_disp::Bool=false, nsrcts=0,
99   disp_rotate_inst::Vector{Any}=[false,0,0,Vector{Vector{Symbol}}()])
100 top_m = deserialize(e.models[findmax([model.log_Li for model in
101   e.models])[2]].path)
102
103 return ProgressNS{typeof(interval)}(e, top_m, wm, tuner, interval,
104   dt=dt, desc=desc, color=color, output=output, offset=offset,
105   start_it=start_it, wk_disp=wk_disp, tuning_disp=tuning_disp,
106   conv_plot=conv_plot, lh_disp=lh_disp, liwi_disp=liwi_disp,
107   ens_disp=ens_disp, src_disp=src_disp, nsrcts=nsrcts,
108   disp_rotate_inst=disp_rotate_inst)
109 end
110
111
112
113
114
115 function update!(p::ProgressNS, val, thresh; options ... )
116   p.counter += 1
117
118   p.tstp=time()-p.tlast
119   popfirst!(p.tuner.time_history)
120   push!(p.tuner.time_history, p.tstp)
121
122   p.interval = val - thresh
123   popfirst!(p.convergence_history)
124   push!(p.convergence_history, p.interval)
125
126   p.top_m.name != basename(p.e.models[findmax([model.log_Li for model
127     in p.e.models])[2]].path) &&
128   (p.top_m=deserialize(p.e.models[findmax([model.log_Li for model
129     in p.e.models])[2]].path))
130
131   updateProgress!(p; options ... )
132
133 end
134
135 function updateProgress!(p::ProgressNS; offset::Integer = p.offset, keep
136   = (offset == 0))
137   p.offset = offset

```

```

117     t = time()
118     p.disp_rotate_inst[1] && p.counter%p.disp_rotate_inst[2] == 0 &&
119         → rotate_displays(p)
120
121     if p.interval ≤ 0 && !p.triggered
122         p.triggered = true
123         if p.printed
124             p.triggered = true
125             dur = durationstring(t-p.tfirst)
126             msg = @sprintf "%s Converged. Time: %s (%d iterations). logZ:
127             → %s\n" p.desc dur p.counter p.e.log_Zi[end]
128
129             print(p.output, "\n" ^ (p.offset + p.numprintedvalues))
130             move_cursor_up_while_clearing_lines(p.output,
131                 → p.numprintedvalues)
132             upper_lines=display_upper_dash(p)
133             printover(p.output, msg, :magenta)
134             lower_lines=display_lower_dash(p)
135
136             p.numprintedvalues=upper_lines + lower_lines + 1
137
138             if keep
139                 println(p.output)
140             else
141                 print(p.output, "\r\u1b[A" ^ (p.offset +
142                 → p.numprintedvalues))
143             end
144         end
145         return
146     end
147
148     if t > p.tlast+p.dt && !p.triggered
149         p.counter < CONVERGENCE_MEMORY ?
150             mean_step_time=mean(p.tuner.time_history[end-(p.counter-1):end])
151             :
152
153             → mean_step_time=mean(p.tuner.time_hist
154
155             msg = @sprintf "%s Iterate: %s Recent step time μ: %s Convergence
156             → Interval: %g\n" p.desc p.counter hmss(mean_step_time)
157             → p.interval
158
159             print(p.output, "\n" ^ (p.offset + p.numprintedvalues))
160             move_cursor_up_while_clearing_lines(p.output, p.numprintedvalues)

```

```

151     upper_lines=display_upper_dash(p)
152     printover(p.output, msg, p.color)
153     lower_lines=display_lower_dash(p)
154
155     p.numprintedvalues=upper_lines + lower_lines + 1
156     print(p.output, "\r\u1b[A" ^ (p.offset + p.numprintedvalues))
157
158     # Compensate for any overhead of printing. This can be
159     # especially important if you're running over a slow network
160     # connection.
161     p.tlast = t + 2*(time()-t)
162     p.printed = true
163   end
164 end
165
166 function rotate_displays(p)
167   curr_inst=p.disp_rotate_inst[3]
168   curr_inst+1>length(p.disp_rotate_inst[4]) ?
169     ↪ next_inst=1 : next_inst=curr_inst+1
170   next_disps=p.disp_rotate_inst[4][next_inst]
171   for disp in
172     ↪ [:wk_disp,:tuning_disp,:conv_plot,:ens_disp,:lh_disp,:liwi_di
173       disp in next_disps ? setproperty!(p, disp, true)
174         ↪ : setproperty!(p, disp, false)
175   end
176   p.disp_rotate_inst[3]=next_inst
177 end
178
179 function hmss(dt)
180   dt<0 ? (dt=-dt; prfx="-") : (prfx="")
181   isnan(dt) && return "NaN"
182   (h,r) = divrem(dt,60*60)
183   (m,r) = divrem(r, 60)
184   (isnan(h)||isnan(m)||isnan(r)) && return "NaN"
185   string(prfx,Int(h),":",Int(m),":",Int(ceil(r)))
186 end
187
188 function display_upper_dash(p::ProgressNS)
189   wklines = tunelines = cilines = 0
190   p.wk_disp && (wklines=show(p.output, p.wm,
191     ↪ progress=true);println())
192   p.tuning_disp && (tunelines=show(p.output, p.tuner,
193     ↪ progress=true);println())
194   if p.conv_plot

```

```

189          ↵ ciplot=lineplot([p.counter-(length(p.convergence_history)
190          ↵ p.convergence_history, title="Convergence
191          ↵ Interval Recent History",
192          ↵ xlabel="Iterate",ylabel="CI", color=:yellow)
193          cilines=nrows(ciplot.graphics)+5
194          show(p.output, ciplot); println()
195      end
196      return wklines + tunelines + cilines
197  end
198
199  function display_lower_dash(p::ProgressNS)
200      lhlines = liwilines = ensemblelines = srclines = 0
201      if p.lh_disp
202          lhplot=lineplot(p.e.log_Li[2:end], title="Contour
203          ↵ History", xlabel="Iterate", color=:magenta,
204          ↵ name="Ensemble logLH")
205          lineplot!(lhplot, [p.e.naive_lh for it in
206          ↵ 1:length(p.e.log_Li[2:end])], name="Naive
207          ↵ logLH")
208          lhlines=nrows(lhplot.graphics)+5
209          show(p.output, lhplot); println()
210      end
211      if p.liwi_disp && p.counter>2
212          ↵ liwiplot=lineplot([max(2,p.counter-(CONVERGENCE_MEMORY-1)
213          ↵ title="Recent iterate evidentiary weight",
214          ↵ xlabel="Iterate", name="Ensemble log Liwi",
215          ↵ color=:cyan)
216          liwilines=nrows(liwiplot.graphics)+5
217          show(p.output, liwiplot); println()
218      end
219      p.ens_disp && (ensemblelines=show(p.output, p.e,
220          ↵ progress=true))
221      p.src_disp && (println("MLE Model
222          ↵ Sources:");srclines=show(p.output, p.top_m,
223          ↵ nsr=p.no_displayed_srcs, progress=true))
224      return lhlines + liwilines + ensemblelines + srclines
225  end

```

16.7.16 /src/utilities/synthetic_genome.jl

```

1  function synthetic_sample(no_obs::Integer, obsl,
2    ↳ bhmm_vec::AbstractVector{<:BHMM}, bhmm_dist::Categorical,
3    ↳ spikes::AbstractVector{<:AbstractMatrix{<:AbstractFloat}}, 
4    ↳ spike_instruct::AbstractVector{<:Tuple{<:Bool, <:Tuple}})
5      !(typeof(obsl) <:UnitRange || typeof(obsl) <:Integer) &&
6        ↳ throw(ArgumentError("obsl must be Integer or UnitRange"))
7      length(spike_instruct)≠length(spikes) &&
8        ↳ throw(ArgumentError("spike_instruct must be as long as spikes"))
9
10     obs, hmm_truth=obs_array_from_bhmms(no_obs, obsl, bhmm_vec, bhmm_dist)
11
12   spike_truth = spike_obs!(obs, spikes, spike_instruct)
13
14   bg_scores = score_synthetic(obs, bhmm_vec, hmm_truth)
15
16   return obs, bg_scores, hmm_truth, spike_truth
17 end
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

```

```

36 function spike_struc!(obs, spike, frac_obs, periodicity)
37     truth=false(size(obs,2))
38     for o in 1:size(obs,2)
39         if rand() < frac_obs
40             truth[o]=true
41             rand()<.5 ? (source=revcomp_pwm(spike)) : (source=spike)
42             pos=rand(1:periodicity)
43             oidx=findfirst(iszero,obs[:,o])
44             while pos<oidx
45                 pos_ctr=pos
46                 pwm_ctr=1
47                 while pos_ctr<oidx&&pwm_ctr≤size(source,1)
48                     obs[pos_ctr,o]=rand(Categorical(source[pwm_ctr,:]))
49                     pos_ctr+=1
50                     pwm_ctr+=1
51                 end
52                 pos+=periodicity+pwm_ctr
53             end
54         end
55     end
56     return truth
57 end
58
59 function spike_irreg!(obs, spike, frac_obs, recur)
60     truth=false(size(obs,2))
61     for o in 1:size(obs,2)
62         oidx=findfirst(iszero,obs[:,o])
63         if rand()<frac_obs
64             truth[o]=true
65             for r in 1:rand(recur)
66                 rand()<.5 ? (source=revcomp_pwm(spike)) : source=spike
67                 pos=rand(1:oidx-1)
68                 pwm_ctr=1
69                 while pos<oidx && pwm_ctr≤size(source,1)
70                     obs[pos,o]=rand(Categorical(source[pwm_ctr,:]))
71                     pos+=1
72                     pwm_ctr+=1
73                 end
74             end
75         end
76     end
77     return truth
78 end

```

```

79
80 function score_synthetic(obs, bhmm_vec, hmm_truth)
81     lh_mat=zeros(size(obs,1)-1, size(obs,2))
82     for o in 1:size(obs,2)
83         oidx=findfirst(iszero,obs[:,o])
84
85         → lh_mat[1:oidx-1,o]=BioBackgroundModels.get_BGHMM_symbol_lh(transpose(obs[1:oidx-1,:]))
86     end
87     return lh_mat
88 end

```

16.7.17 /src/utilities/worker_diagnostics.jl

```

1 struct Worker_Monitor
2     idx::Dict{Integer, Integer}
3     persist::BitMatrix
4     last_seen::Matrix{Float64}
5 end
6
7 function Worker_Monitor(wk_pool::Vector{<:Integer})
8     idx=Dict{Integer, Integer}()
9     for (n,wk) in enumerate(wk_pool)
10        idx[wk]=n
11    end
12    persist=true.(1:length(wk_pool))
13    last_seen=[time() for x in 1:1, y in 1:length(wk_pool)]
14    return Worker_Monitor(idx, persist, last_seen)
15 end
16
17 function update_worker_monitor!(mon,wk,persist)
18     mon.persist[1,mon.idx[wk]]=persist
19     mon.last_seen[1,mon.idx[wk]]=time()
20 end
21
22 function Base.show(io::IO, mon::Worker_Monitor; progress=false)
23     printstyled("Worker Diagnostics\n", bold=true)
24     pers=heatmap(float.(mon.persist), colormap=persistcolor,
25                 → title="Persistence", labels=false)
26     ls=heatmap([time()-ls for ls in mon.last_seen], title="Last
27                 → Seen", labels=false)
28     show(pers)
29     println()

```

```

28     show(ls)
29     progress && return nrows(pers.graphics)+nrows(ls.graphics)+7
30 end
31
32     function persistcolor(z, zmin, zmax)
33         z=1. && return 154
34         z=0. && return 160
35     end

```

16.7.18 /src/utilities/worker_sequencer.jl

```

1 #waits for one worker to begin before calling the next so that network is
2   ↳ not slammed trying to send huge arrays to many workers simultaneously
3
4 function sequence_workers(wk_pool, func, args ... )
5     comms_chan = RemoteChannel(()→Channel{Integer}(length(wk_pool)))
6
7     for worker in wk_pool
8         remote_do(func, worker, args ... , comms_chan)
9         wait(comms_chan); report=take!(comms_chan)
10        @assert worker==report
11    end
11 end

```

16.7.19 /test/consolidate_unit_tests.jl

```

1 @testset "Orthogonality helper" begin
2     bg_scores = log.(fill(.25, (17,3)))
3     obs=[BioSequences.LongSequence{DNAAlphabet{2}}("ATGATTACGATGATGCA")
4           BioSequences.LongSequence{DNAAlphabet{2}}("TCAGTTACGATGATCAG")
5           BioSequences.LongSequence{DNAAlphabet{2}}("TTACGCACAGATGTTAC")]
6     order_seqs = BioBackgroundModels.get_order_n_seqs(obs, 0)
7     coded_seqs = BioBackgroundModels.code_seqs(order_seqs)
8     obs=Array(transpose(coded_seqs))
9     obsl=[findfirst(iszero,obs[:,o])-1 for o in 1:size(obs)[2]]
10
11     src_ATG = [.7 .1 .1 .1
12                 .1 .1 .1 .7
13                 .1 .1 .7 .1]
14
15     src_CAG = [.1 .7 .1 .1

```

```

16     .7 .1 .1 .1
17     .1 .1 .7 .1]

18
19     src_TTAC = [.1 .1 .1 .7
20     .1 .1 .1 .7
21     .7 .1 .1 .1
22     .1 .7 .1 .1]

23
24     src_GCA = [.1 .1 .7 .1
25     .1 .7 .1 .1
26     .7 .1 .1 .1]

27
28     consolidate_one =
29     ↳ [(log.(src_ATG),0),(log.(src_TTAC),0),(log.(src_ATG),0)]
30     cons_one_mix = BitMatrix([true true false
31                               true false true
32                               false true true])

33     consolidate_two =
34     ↳ [(log.(src_ATG),0),(log.(src_ATG),0),(log.(src_ATG),0)]
35     cons_two_mix = BitMatrix([true false false
36                               false true false
37                               false false true])

38     distance_model =
39     ↳ [(log.(src_TTAC),0),(log.(src_CAG),0),(log.(src_GCA),0)]

40     c1model = ICA_PWM_Model("c1", "c1", consolidate_one, 3:4,
41     ↳ cons_one_mix, IPM_likelihood(consolidate_one, obs, obsl,
42     ↳ bg_scores, cons_one_mix))
43     c2model = ICA_PWM_Model("c2", "c2", consolidate_two, 3:4,
44     ↳ cons_two_mix, IPM_likelihood(consolidate_two, obs, obsl,
45     ↳ bg_scores, cons_two_mix))
46     dmodel = ICA_PWM_Model("d", "d", distance_model, 3:4, trues(3,3),
47     ↳ IPM_likelihood(distance_model, obs, obsl, bg_scores, trues(3,3)))

48     dpath=randstring()
49     serialize(dpath, dmodel)
50     drec=Model_Record(dpath,dmodel.log_Li)

#check distance calculation
51     pwmtest_1=zeros(1,4);pwmtest_1[1]=1.
52     pwmtest_2=zeros(1,4);pwmtest_2[2]=1.

```

```

51  @test pwm_distance(log.(pwmtest_1),log.(pwmtest_2)) =
52    → euclidean(pwmtest_1,pwmtest_2) = 1.4142135623730951
53
54  #test consolidate check
55  @test consolidate_check(distance_model) =
56    → (true,Dict{Integer,Vector{Integer}}())
57  @test consolidate_check(consolidate_one) = (false,
58    → Dict{Integer,Vector{Integer}}(1⇒[3]))
59  @test consolidate_check(consolidate_two) = (false,
60    → Dict{Integer,Vector{Integer}}(1⇒[2,3], 2⇒[3]))
61
62  #test overall consolidate function
63  _,con_idxs=consolidate_check(consolidate_one)
64  c1consmod=consolidate_srcs(con_idxs, c1model, obs, obsl, bg_scores,
65    → drec.log_Li, [drec])
66
67  @test consolidate_check(c1consmod.sources)[1]
68  @test c1consmod.log_Li > dmodel.log_Li
69  @test all(c1consmod.mix_matrix[:,1])
70  @test c1consmod.sources[1][1]==log.(src_ATG)
71  @test c1consmod.sources[3][1]≠log.(src_ATG)
72  @test c1consmod.origin=="consolidated c1"
73
74  _,con_idxs=consolidate_check(consolidate_two)
75  c2consmod=consolidate_srcs(con_idxs, c2model, obs, obsl, bg_scores,
76    → drec.log_Li, [drec])
77
78  @test consolidate_check(c2consmod.sources)[1]
79  @test c2consmod.log_Li > dmodel.log_Li
80  @test all(c2consmod.mix_matrix[:,1])
81  @test c2consmod.sources[1][1]==log.(src_ATG)
82  @test c2consmod.sources[2][1]≠log.(src_ATG)
83  @test c2consmod.sources[3][1]≠log.(src_ATG)
84  @test c2consmod.origin=="consolidated c2"
85
86  rm(dpath)
87 end

```

16.7.20 /test/ensemble_tests.jl

```

1 @testset "Ensemble assembly and nested sampling functions" begin
2     ensambledir = randstring()

```

```

3 spensemblendir = randstring()
4 distdir = randstring()
5
6 source_pwm = [.7 .1 .1 .1
7 .1 .1 .1 .7
8 .1 .1 .7 .1]
9
10 source_pwm_2 = [.6 .1 .1 .2
11 .2 .1 .1 .6
12 .1 .2 .6 .1]
13
14 src_length_limits=2:12
15 no_sources=3
16
17 source_priors = assemble_source_priors(no_sources, [source_pwm,
18   ↵ source_pwm_2])
mix_prior=.5
19
20 bg_scores = log.(fill(.1, (30,4)))
21
22   ↵ obs=[BioSequences.LongSequence{DNAAlphabet{2}}("CCGTTGACGATGTGATGAATAATGAAAGA")
23
24   ↵ BioSequences.LongSequence{DNAAlphabet{2}}("CCCCGATGATGACCGTTGACCAGATGGATG")
25
26   ↵ BioSequences.LongSequence{DNAAlphabet{2}}("CCCCGATGATGACCCGATTTGAAAAAAA")
27
28   ↵ BioSequences.LongSequence{DNAAlphabet{2}}("TCATCATGCTGATGATGAATCAGATGAAAG")
29 ]
30
31 order_seqs = BioBackgroundModels.get_order_n_seqs(obs, 0)
32 coded_seqs = BioBackgroundModels.code_seqs(order_seqs)
33 obs=Array(transpose(coded_seqs))
34
35 ensemble = IPM_Engine(ensembedir, 150, source_priors,
36   ↵ (falses(0,0),mix_prior), bg_scores, obs, src_length_limits)
37 ensemble = IPM_Engine(ensembedir, 200, source_priors,
38   ↵ (falses(0,0),mix_prior), bg_scores, obs, src_length_limits) #test
39   ↵ resumption
40
41 sp_ensemble = IPM_Engine(spensemblendir, 200, source_priors,
42   ↵ (falses(0,0),mix_prior), bg_scores, obs, src_length_limits,
43   ↵ posterior_switch=true)
44
45

```

```

36     @test length(ensemble.models) == 200
37     for model in ensemble.models
38         @test -350 < model.log_Li < -150
39     end
40
41     @test length(sp_ensemble.models) == 200
42     for model in sp_ensemble.models
43         @test -350 < model.log_Li < -150
44     end
45
46     assembler=addprocs(1)
47
48     @everywhere using BioMotifInference
49
50     dist_ensemble=IPM_Engsemble(assembler, distdir, 150, source_priors,
51     ↪ (falses(0,0),mix_prior), bg_scores, obs, src_length_limits)
52     dist_ensemble=IPM_Engsemble(assembler, distdir, 200, source_priors,
53     ↪ (falses(0,0),mix_prior), bg_scores, obs, src_length_limits) #test
54     ↪ resumption
55
56     @test length(dist_ensemble.models) == 200
57     for model in ensemble.models
58         @test -350 < model.log_Li < -150
59     end
60
61     rmprocs(assembler)
62     rm(distdir, recursive=true)
63
64     models_to_permute = 600
65     funclimit=200
66     funcvec=full_perm_funcvec
67
68     instruct = Permute_Instruct(funcvec,
69     ↪ ones(length(funcvec))./length(funcvec),models_to_permute,200,
70     ↪ min_clmps=fill(.02,length(funcvec)))
71
72     @info "Testing convergence displays..."
73     sp_logZ = converge_ensemble!(sp_ensemble, instruct,
74     ↪ converge_factor=500., wk_disp=true, tuning_disp=true,
75     ↪ ens_disp=true, conv_plot=true, src_disp=true, lh_disp=true,
76     ↪ liwi_disp=true, max_iterates=50)
77
78     sp_ensemble=reset_ensemble!(sp_ensemble)

```

```

71
72     @info "Testing threaded convergence ... "
73     sp_logZ = converge_ensemble!(sp_ensemble, instruct,
74         → converge_factor=500., wk_disp=false, tuning_disp=false,
75         → ens_disp=false, conv_plot=false, src_disp=false, lh_disp=false,
76         → liwi_disp=false, backup=(true, 150), max_iterates=300)
77
78     @info "Testing resumption ... "
79     sp_logZ = converge_ensemble!(sp_ensemble, instruct,
80         → converge_factor=500., wk_disp=false, tuning_disp=false,
81         → ens_disp=false, conv_plot=false, src_disp=false, lh_disp=false,
82         → liwi_disp=false, backup=(true, 150))
83     @test length(sp_ensemble.models) == 200
84     @test length(sp_ensemble.log_Li) == length(sp_ensemble.log_Xi) ==
85         → length(sp_ensemble.log_wi) == length(sp_ensemble.log_Liwi) ==
86         → length(sp_ensemble.log_Zi) == length(sp_ensemble.Hi) ==
87         → sp_ensemble.model_counter-200
88     for i in 1:length(sp_ensemble.log_Li)-1
89         @test sp_ensemble.log_Li[i] ≤ sp_ensemble.log_Li[i+1]
90     end
91     for i in 1:length(sp_ensemble.log_Zi)-1
92         @test sp_ensemble.log_Zi[i] ≤ sp_ensemble.log_Zi[i+1]
93     end
94     @test sp_logZ > -1500.0
95
96
97     @info "Testing multiprocess convergence ... "
98     @info "Spawning worker pool..."
99     worker_pool=addprocs(2, topology=:master_worker)
100    @everywhere using BioMotifInference
101
102    #####CONVERGE#####
103    final_logZ = converge_ensemble!(ensemble, instruct, worker_pool,
104        → converge_factor=500., wk_disp=false, tuning_disp=false,
105        → ens_disp=false, conv_plot=false, src_disp=false, lh_disp=false,
106        → liwi_disp=false, backup=(true,500), clean=(true, 500, 1000))
107
108    convits=length(ensemble.log_Li)
109
110    #test converging already converged, wih different converge criterion

```

```

98     final_logZ = converge_ensemble!(ensemble, instruct, worker_pool,
99         ← converge_factor=150., converge_criterion="compression",
100        ← wk_disp=false, tuning_disp=false, ens_disp=false,
101        ← conv_plot=false, src_disp=false, lh_disp=false, liwi_disp=false,
102        ← backup=(true,500), clean=(true, 500, 1000))
103
104     length(ensemble.log_Li)==convits
105
106     rmprocs(worker_pool)
107
108
109     @test length(ensemble.models) == 200
110     @test length(ensemble.log_Li) == length(ensemble.log_Xi) ==
111         ← length(ensemble.log_wi) == length(ensemble.log_Liwi) ==
112         ← length(ensemble.log_Zi) == length(ensemble.Hi) ==
113         ← ensemble.model_counter-200
114     for i in 1:length(ensemble.log_Li)-1
115         @test ensemble.log_Li[i] ≤ ensemble.log_Li[i+1]
116     end
117     for i in 1:length(ensemble.log_Zi)-1
118         @test ensemble.log_Zi[i] ≤ ensemble.log_Zi[i+1]
119     end
120     @test typeof(final_logZ) == Float64
121     @test final_logZ > -1500.0
122
123     rm(ensembedir, recursive=true)
124     rm(spensembedir, recursive=true)
125
126 end

```

16.7.21 /test/lh_perf.jl

```

1 using BenchmarkTools, BioMotifInference, BioBackgroundModels,
2     ← BioSequences
3 println(Threads.nthreads())
4 source_pwm = [.7 .1 .1 .1
4   .1 .1 .1 .7
5   .1 .1 .7 .1]
6 source_pwm_2 = [.6 .1 .1 .2
7   .2 .1 .1 .6
8   .1 .2 .6 .1]
9 sources = [(log.(source_pwm), 1),(log.(source_pwm_2), 1)]
10
11 bg_scores = log.(fill(.1, (150,10000)))

```

```

12
13 obs=zeros(UInt8,151,10000)
14 obs[1:150,1:end]=1
15 obsl=[findfirst(iszero,obs[:,o])-1 for o in 1:size(obs)[2]]
16 mix=trues(10000,2)

17
18 println(median(@benchmark (BioMotifInference.IPM_likelihood($sources,
    → $obs, $obsl, $bg_scores, $mix)) samples=5 evals=20))
19
20 println(median(@benchmark (BioMotifInference.dev_likelihood($sources,
    → $obs, $obsl, $bg_scores, $BitMatrix(transpose(mix)))) samples=5
    → evals=20))

```

16.7.22 /test/likelihood_unit_tests.jl

```

1 @testset "Model scoring and likelihood functions" begin
2     #test a trivial scoring example
3     obs=[BioSequences.LongSequence{DNAAlphabet{2}}("AAAAAA")]
4     order_seqs = BioBackgroundModels.get_order_n_seqs(obs, 0)
5     coded_seqs = BioBackgroundModels.code_seqs(order_seqs)
6     obs=Array(transpose(coded_seqs))

7
8     source_pwm = zeros(1,4)
9     source_pwm[1,1] = 1
10    log_pwm = log.(source_pwm)

11
12    source_stop=5

13
14    #score preallocates
15    ss_srcs = [revcomp_pwm(log_pwm)]
16    ds_srcs=[cat(log_pwm,revcomp_pwm(log_pwm),dims=3)]
17    score_mat_ds=zeros(source_stop,2)
18    score_mat_ss=zeros(source_stop,1)
19    score_matrices_ds=Vector{Matrix{Float64}}(undef, 2)
20    score_matrices_ss=Vector{Vector{Float64}}(undef, 2)

21
22    score_sources_ds!(score_mat_ds, score_matrices_ds, ds_srcs, obs[:,1],
    → source_stop)
23    @test score_matrices_ds[1] = [0.0 -Inf; 0.0 -Inf; 0.0 -Inf; 0.0
    → -Inf; 0.0 -Inf]

```

24

```

25 score_sources_ss!(score_mat_ss, score_matrices_ss, ss_srcs, obs[:,1],
26   ↵ source_stop)
27
28
29 #test a more complicated scoring example with two sources
30 obs=[BioSequences.LongSequence{DNAAlphabet{2}}]("ATGATGATGATG")
31 order_seqs = BioBackgroundModels.get_order_n_seqs(obs, 0)
32 coded_seqs = BioBackgroundModels.code_seqs(order_seqs)
33 obs=Array(transpose(coded_seqs))
34
35 source_pwm = [.7 .1 .1 .1
36               .1 .1 .1 .7
37               .1 .1 .7 .1]
38
39 source_pwm_2 = [.6 .1 .1 .2
40                 .2 .1 .1 .6
41                 .1 .2 .6 .1]
42
43 target_s1 = [.7^3 .1^3; .1^3 .1^3; .1^3 (.1*.7^2); .7^3 .1^3; .1^3
44   ↵ .1^3; .1^3 (.1*.7^2); .7^3 .1^3; .1^3 .1^3; .1^3 (.1*.7^2); .7^3
45   ↵ .1^3]
46 target_s2 = [.6^3 (.2*.1^2); (.2*.1^2) (.2*.1^2); (.2*.1^2)
47   ↵ (.2*.6^2); .6^3 (.2*.1^2); (.2*.1^2) (.2*.1^2); (.2*.1^2)
48   ↵ (.2*.6^2); .6^3 (.2*.1^2); (.2*.1^2) (.2*.1^2); (.2*.1^2)
49   ↵ (.2*.6^2); .6^3 (.2*.1^2)]
50 log_pwms = [log.(source_pwm), log.(source_pwm_2)]
51
52 source_start = 1
53 source_stop = 10
54
55 #score preallocates
56 ss_srcs = log_pwms
57 ds_srcs=[cat(pwm,revcomp_pwm(pwm),dims=3) for pwm in log_pwms]
58 score_mat_ds=zeros(source_stop,2)
59 score_mat_ss=zeros(source_stop,1)
60
61 score_sources_ds!(score_mat_ds, score_matrices_ds, ds_srcs, obs[:,1],
62   ↵ [source_stop, source_stop])
63 @test isapprox(score_matrices_ds[1], log.(target_s1))
64 @test isapprox(score_matrices_ds[2], log.(target_s2))

```

```

60     score_sources_ss!(score_mat_ss, score_matrices_ss, ss_srcs, obs[:,1],
61     ↳ [source_stop, source_stop])
62     @test isapprox(score_matrices_ss[1], log.(target_s1[:,1]))
63     @test isapprox(score_matrices_ss[2], log.(target_s2[:,1]))
64
65     #test score weaving and IPM likelihood calculations
66     #trivial example using fake cardinality_penalty
67     target=logaddexp(log(.25), 0.)
68     lh_vec=zeros(2)
69     obsl=1
70     bg_scores=view([log(.25)],:,:)
71     score_mat=[[0.]]
72     osi=[1]
73     source_wmls=[1]
74     lme=0.
75     cardinality_penalty=0. #not correct but for test purposes
76     osi_emit=Vector{Int64}()
77
78     @test weave_scores_ss!(lh_vec, obsl, bg_scores, score_mat, osi,
79     ↳ source_wmls, lme, cardinality_penalty, osi_emit) = target
80
81     target2=logsumexp([log(.25), log(.5), log(.5)])
82     lh_vec=zeros(2)
83     weavevec=zeros(3)
84     score_mat=[zeros(1,2)]
85     empty!(osi_emit)
86     lme=log(.5)
87
88     @test weave_scores_ds!(weavevec, lh_vec, obsl, bg_scores, score_mat,
89     ↳ osi, source_wmls, lme, cardinality_penalty, osi_emit) = target2
90
91     #test more complicated weaves
92     obs=[BioSequences.LongSequence{DNAAlphabet{2}}("ATGATGATGATG")
93           BioSequences.LongSequence{DNAAlphabet{2}}("TGATGATGATGA")]
94     order_seqs = BioBackgroundModels.get_order_n_seqs(obs, 0)
95     coded_seqs = BioBackgroundModels.code_seqs(order_seqs)
96     obs=Array(transpose(coded_seqs))
97
98     sources = [(log.(source_pwm), 1),(log.(source_pwm_2), 1)]
99     dbl1_sources=[(log.(source_pwm), 1),(log.(source_pwm), 1)]
100
101    ds_srcs=[cat(pwm[1],revcomp_pwm(pwm[1])),dims=3) for pwm in sources]
```



```

134     o1_lh = weave_scores_ds!(weavevec, lh_vec, 12, view(bg_scores,:,:1),
135                               ↳ o1_mats, osi, source_wmls, log_motif_expectation,
136                               ↳ cardinality_penalty, osi_emit)
137     @test isapprox(lh_target,o1_lh)

138
139     empty!(osi_emit)

140
141     o2_lh = weave_scores_ds!(weavevec, lh_vec, 12, view(bg_scores,:,:2),
142                               ↳ o2_mats, osi, source_wmls, log_motif_expectation,
143                               ↳ cardinality_penalty, osi_emit)

144     lh,cache = IPM_likelihood(sources, obs, [12,12], bg_scores,
145                               ↳ trues(2,2), true,true)
146     @test isapprox(o1_lh,cache[1])
147     @test isapprox(o2_lh,cache[2])
148     @test isapprox(lps(o1_lh,o2_lh),lh)

149     #test source penalization
150     score_sources_ds!(score_mat_ds, score_matrices_ds, dbl1_srcs,
151                       ↳ obs[:,1], source_stops)

152
153     dbl_score_mat=copy(score_matrices_ds)

154
155     empty!(osi_emit)

156
157     naive=weave_scores_ds!(weavevec, lh_vec, 12,
158                               ↳ view(bg_scores,:,:1),Vector{Matrix{Float64}}(), Vector{Int64}(),
159                               ↳ Vector{Int64}(), log_motif_expectation, cardinality_penalty,
160                               ↳ osi_emit)

161
162     empty!(osi_emit)

163     single=weave_scores_ds!(weavevec, lh_vec, 12, view(bg_scores,:,:1),
164                               ↳ [dbl_score_mat[1]], [1], [3], log_motif_expectation,
165                               ↳ cardinality_penalty, osi_emit)

166
167     empty!(osi_emit)

168
169     double=weave_scores_ds!(weavevec, lh_vec, 12, view(bg_scores,:,:1),
170                               ↳ dbl_score_mat, [1,2], [3,3], log_motif_expectation,
171                               ↳ cardinality_penalty, osi_emit)

172
173     empty!(osi_emit)

```

```

164
165     triple=weave_scores_ds!(weavevec, lh_vec, 12, view(bg_scores,:,:1),
166     ↳ [dbl_score_mat[1] for i in 1:3], [1,2,3], [3,3,3],
167     ↳ log_motif_expectation, cardinality_penalty, osi_emit)
168
169     @test (single-naive) > (double-single) > (triple-double)
170
171     naive_target=-16.635532333438686
172     naive = IPM_likelihood(sources, obs, [findfirst(iszero,obs[:,o])-1
173     ↳ for o in 1:size(obs)[2]],
174     ↳ bg_scores,falses(size(obs)[2],length(sources)))
175     @test naive==naive_target
176
177     #test IPM_likelihood clean vector and cache calculations
178     mix_matrix=trueones(2,2)
179     baselh,basecache = IPM_likelihood(sources, obs, [12,12], bg_scores,
180     ↳ mix_matrix, true,true)
181
182     clean=[true,false]
183
184     unchangedlh,unchangedcache=IPM_likelihood(sources, obs, [12,12],
185     ↳ bg_scores, mix_matrix, true, true, basecache, clean)
186
187     changed_lh,changedcache=IPM_likelihood(sources, obs, [12,12],
188     ↳ bg_scores, BitMatrix([true true; false false]), true, true,
189     ↳ basecache, clean)
190
191     indep_lh, indepcache=IPM_likelihood(sources, obs,[12,12], bg_scores,
192     ↳ BitMatrix([true true; false false]), true, true)
193
194     @test baselh==unchangedlh!=changed_lh==indep_lh
195     @test basecache==unchangedcache!=changedcache==indepcache
196
197     #check that IPM_likelihood works in single stranded operation
198     IPM_likelihood(sources, obs,[12,12], bg_scores, BitMatrix([true true;
199     ↳ false false]), false)
200 end

```

16.7.23 /test/mix_matrix_unit_tests.jl

```

1 @testset "Mix matrix initialisation and manipulation functions" begin
2     #test mix matrix init

```

```

3   prior_mix_test=init_mix_matrix((trues(2,10),0.0),2, 20)
4   @test all(prior_mix_test[:,1:10])
5   @test !any(prior_mix_test[:,11:20])
6
7   @test sum(init_mix_matrix((falses(0,0),1.0), 0, S)) = 0*S
8   @test sum(init_mix_matrix((falses(0,0),0.0), 0, S)) = 0
9   @test 0 < sum(init_mix_matrix((falses(0,0),0.5), 0, S)) < 0*S
10
11  #test mix matrix decorrelation
12  empty_mixvec=falses(0)
13  one_mix=mixvec_decorrelate(empty_mixvec,1)
14  @test sum(one_mix)==1
15
16  empty_mix = falses(0,S)
17  new_mix,clean=mix_matrix_decorrelate(empty_mix, 500)
18  @test 0 < sum(new_mix) ≤ 500
19  @test !all(clean)
20
21  full_mix = trues(0,S)
22  less_full_mix,clean=mix_matrix_decorrelate(full_mix, 500)
23
24  @test 0*S-sum(less_full_mix) ≤ 500
25  @test !all(clean)
26
27  #test matrix similarity and dissimilarity functions
28  test_mix=falses(0,S)
29  test_mix[1:Int(floor(0/2)),:]=true
30  test_idx=3
31  compare_mix=deepcopy(test_mix)
32  compare_mix[:,test_idx] *= .!compare_mix[:,test_idx]
33  @test most_dissimilar(test_mix,compare_mix)=test_idx
34
35  src_mixvec=falses(0)
36  src_mixvec[Int(ceil(0/2)):end]=true
37  @test most_similar(src_mixvec,compare_mix)=test_idx
38 end

```

16.7.24 /test/permute_func_tests.jl

```

1 @testset "Model permutation functions" begin
2     source_pwm = [.7 .1 .1 .1
3                   .1 .1 .1 .7

```

```

4     .1 .1 .7 .1]
5
6     source_pwm_2 = [.6 .1 .1 .2
7     .2 .1 .1 .6
8     .1 .2 .6 .1]
9
10    pwm_to_erode = [.25 .25 .25 .25
11          .97 .01 .01 .01
12          .01 .01 .01 .97
13          .01 .01 .97 .01
14          .25 .25 .25 .25]
15
16    src_length_limits=2:5
17
18    source_priors = assemble_source_priors(3, [source_pwm, source_pwm_2])
19    mix_prior=.5
20
21    bg_scores = log.(fill(.25, (12,2)))
22    obs=[BioSequences.LongSequence{DNAAlphabet{2}}("ATGATGATGATG")
23        BioSequences.LongSequence{DNAAlphabet{2}}("TGATGATGATGA")]
24    order_seqs = BioBackgroundModels.get_order_n_seqs(obs, 0)
25    coded_seqs = BioBackgroundModels.code_seqs(order_seqs)
26    obs=Array(transpose(coded_seqs))
27    obsl=[findfirst(iszero,obs[:,o])-1 for o in 1:size(obs)[2]]
28
29    test_model = ICA_PWM_Model("test", source_priors,
30        ~ (falses(0,0),mix_prior), bg_scores, obs, src_length_limits)
31
32    ps_model= permute_source(test_model, Vector{Model_Record}(), obs,
33        ~ obsl, bg_scores, test_model.log_Li, source_priors,
34        ~ iterates=1000, weight_shift_freq=.2,length_change_freq=.2)
35    @test ps_model.log_Li > test_model.log_Li
36    @test ps_model.sources ≠ test_model.sources
37    @test ps_model.mix_matrix = test_model.mix_matrix
38    @test ps_model.origin = "PS from test"
39
40    pm_model= permute_mix(test_model, obs, obsl, bg_scores,
41        ~ test_model.log_Li, iterates=1000)
42    @test pm_model.log_Li > test_model.log_Li
43    @test pm_model.sources = test_model.sources
44    @test pm_model.mix_matrix ≠ test_model.mix_matrix
45    @test "PM from test" = pm_model.origin

```

```

43 psfm_model=perm_src_fit_mix(test_model, Vector{Model_Record}(),obs,
44   ↪ obsl, bg_scores, test_model.log_Li, source_priors,
45   ↪ iterates=1000)
46 @test psfm_model.log_Li > test_model.log_Li
47 @test psfm_model.sources ≠ test_model.sources
48 @test psfm_model.mix_matrix ≠ test_model.mix_matrix
49 @test "PSFM from test" = psfm_model.origin

50 fm_model=fit_mix(test_model, Vector{Model_Record}(),
51   ↪ obs,obsl, bg_scores)
52 @test fm_model.log_Li > test_model.log_Li
53 @test fm_model.sources = test_model.sources
54 @test fm_model.mix_matrix ≠ test_model.mix_matrix
55 @test "FM from test" = fm_model.origin
56 @test fit_mix in fm_model.permute_blacklist

57 post_fm_psfm=perm_src_fit_mix(fm_model, Vector{Model_Record}(),obs,
58   ↪ obsl, bg_scores, test_model.log_Li, source_priors, iterates=1000)
59 @test "PSFM from candidate" = post_fm_psfm.origin
60 @test fit_mix in post_fm_psfm.permute_blacklist

61 rd_model=random_decorrelate(test_model, Vector{Model_Record}(),obs,
62   ↪ obsl, bg_scores, test_model.log_Li, source_priors, iterates=1000)
63 @test rd_model.log_Li > test_model.log_Li
64 @test rd_model.sources ≠ test_model.sources
65 @test rd_model.mix_matrix ≠ test_model.mix_matrix
66 @test "RD from test" = rd_model.origin

67 rs_model=reinit_src(test_model, Vector{Model_Record}(),obs, obsl,
68   ↪ bg_scores, test_model.log_Li, source_priors, iterates=1000)
69 @test rs_model.log_Li > test_model.log_Li
70 @test rs_model.sources ≠ test_model.sources
71 @test rs_model.mix_matrix ≠ test_model.mix_matrix
72 @test "RS from test" = rs_model.origin

73
74 → erosion_sources=[(log.(source_pwm),1),(log.(source_pwm_2),1),(log.(pwm_to_ero
75
76 eroded_mix=true(2,3)

77 erosion_lh=IPM_likelihood(erosion_sources,obs,obsl, bg_scores,
    ↪ eroded_mix)

```

```

78     erosion_model=ICA_PWM_Model("erode", "", erosion_sources,
    ↳ test_model.source_length_limits, eroded_mix, erosion_lh)

79
80     eroded_model=erode_model(erosion_model, Vector{Model_Record}(), obs,
    ↳ obsl, bg_scores, erosion_model.log_Li)
81     @test eroded_model.log_Li > erosion_model.log_Li
82     @test eroded_model.sources ≠ erosion_model.sources
83     @test eroded_model.mix_matrix = erosion_model.mix_matrix
84     @test "EM from erode" = eroded_model.origin
85     @test eroded_model.sources[1]=erosion_model.sources[1]
86     @test eroded_model.sources[2]=erosion_model.sources[2]
87     @test eroded_model.sources[3]≠erosion_model.sources[3]
88     @test eroded_model.sources[3][1]=erosion_model.sources[3][1][2:4,:]

89
90     dbl_eroded=erode_model(eroded_model, Vector{Model_Record}(), obs,
    ↳ obsl, bg_scores, eroded_model.log_Li)
91     @test dbl_eroded.permute_blacklist = [erode_model]

92
93
94     merger_srcs=  [(log.([.1 .7 .1 .1
95         .1 .7 .1 .1
96         .15 .35 .35 .15]),
97         1
98     ),
99     (log.([.1 .7 .1 .1
100        .1 .65 .15 .1
101        .1 .7 .1 .1]),
102        1
103     ),
104     (log.([.1 .7 .1 .1
105        .1 .7 .1 .1
106        .1 .7 .1 .1
107        .7 .1 .1 .1]),
108        1
109     )]
110
111     accurate_srcs=[ (log.([0.90 0.06 0.02 0.02; 0.02 0.02 0.02 0.94; 0.02
    ↳ 0.02 0.94 0.02]), 1),
112     (log.([0.94 0.02 0.02 0.02; 0.02 0.07 0.07 0.84; 0.02 0.02 0.94
    ↳ 0.02]), 1),
113     (log.([0.95 0.01 0.02 0.02; 0.02 0.02 0.02 0.94; 0.02 0.02 0.94
    ↳ 0.02]), 1)]

```

```

115     merger_mix = BitMatrix([true false false
116                             true true false])
117
118     accurate_mix=BitMatrix([true false false
119                             true true false])
120
121     merger_base=ICA_PWM_Model("merge", "", merger_srcs,src_length_limits,
122                               → merger_mix, IPM_likelihood(merger_srcs,obs,obsl, bg_scores,
123                               → merger_mix))
124
125     merger_target=ICA_PWM_Model("target", "", accurate_srcs,
126                               → src_length_limits, accurate_mix,
127                               → IPM_likelihood(accurate_srcs,obs,obsl, bg_scores, accurate_mix))
128
129     path=randstring()
130     test_record = Model_Record(path, merger_target.log_Li)
131     serialize(path, merger_target)
132
133     dm_model=distance_merge(merger_base, [test_record], obs, obsl,
134                               → bg_scores, merger_base.log_Li)
135     @test dm_model.log_Li > merger_base.log_Li
136     @test dm_model.sources ≠ merger_base.sources
137     distance_dict=Dict(1⇒3,2⇒1,3⇒1)
138     @test all([src=merger_base.sources[n] ||
139                → src=merger_target.sources[distance_dict[n]] for (n,src) in
140                → enumerate(dm_model.sources)])
141     @test "DM from merge" == dm_model.origin
142
143     sm_model=similarity_merge(merger_base, [test_record], obs, obsl,
144                               → bg_scores, merger_base.log_Li)
145     @test sm_model.log_Li > merger_base.log_Li
146     @test sm_model.sources ≠ merger_base.sources
147     @test all([src=merger_base.sources[n] ||
148                → src=merger_target.sources[n] for (n,src) in
149                → enumerate(sm_model.sources)])
150     @test "SM from merge" == sm_model.origin
151
152     shuffled_model=shuffle_sources(merger_base, [test_record], obs, obsl,
153                                   → bg_scores, merger_base.log_Li)
154     @test
155         → sum(shuffled_model.sources.=merger_base.sources)=length(shuffled_model.sources)
156     for (s,src) in enumerate(shuffled_model.sources)

```

```

145     @test src==merger_base.sources[s] ||
146         → src==merger_target.sources[s]
147     if src == merger_base.sources[s]
148         @test
149             → shuffled_model.mix_matrix[:,s]==merger_base.mix_matrix[:,s]
150     else
151         @test
152             → shuffled_model.mix_matrix[:,s]==merger_target.mix_matrix[:,s]
153     end
154 end
155
156 acc_base_mix=false(2,1);acc_base_mix[1,1]=true
157 acc_merge_mix=false(2,1);acc_merge_mix[2,1]=true
158
159 acc_base=ICA_PWM_Model("accbase", "",
160     → [merger_srcs[1]],src_length_limits, acc_base_mix,
161     → IPM_likelihood([merger_srcs[1]],obs,obsl, bg_scores,
162     → acc_base_mix))
163 acc_merge=ICA_PWM_Model("accmerge", "",
164     → [accurate_srcs[1]],src_length_limits, acc_merge_mix,
165     → IPM_likelihood([accurate_srcs[1]],obs,obsl, bg_scores,
166     → acc_merge_mix))
167
168 accpath=randstring()
169 acc_record = Model_Record(accpath, acc_merge.log_Li)
170 serialize(accpath, acc_merge)
171
172 acc_model=accumulate_mix(acc_base, [acc_record], obs, obsl,
173     → bg_scores, acc_merge.log_Li)
174 @test acc_model.mix_matrix=true(2,1)
175 @test acc_model.sources == acc_merge.sources
176 @test "AM from accbase" == acc_model.origin
177
178 testwk=addprocs(1)[1]
179 @everywhere import BioMotifInference
180
181 ddm_model=remotecall_fetch(distance_merge, testwk, merger_base,
182     → [test_record], obs, obsl, bg_scores, merger_base.log_Li,
183     → remote=true)
184 @test ddm_model.log_Li > merger_base.log_Li
185 @test ddm_model.sources ≠ merger_base.sources

```

```

175  @test all([src=merger_base.sources[n] ||
176    ↵ src=merger_target.sources[distance_dict[n]] for (n,src) in
177    ↵ enumerate(ddm_model.sources)])
178  @test "DM from merge" == ddm_model.origin
179
180
181  dsm_model=remotecall_fetch(similarity_merge, testwk, merger_base,
182    ↵ [test_record], obs, obsl, bg_scores, merger_base.log_Li,
183    ↵ remote=true)
184  @test dsm_model.log_Li > merger_base.log_Li
185  @test dsm_model.sources != merger_base.sources
186  @test all([src=merger_base.sources[n] ||
187    ↵ src=merger_target.sources[n] for (n,src) in
188    ↵ enumerate(dsm_model.sources)])
189  @test "SM from merge" == dsm_model.origin
190
191
192  dshuffled_model=remotecall_fetch(shuffle_sources, testwk,
193    ↵ merger_base, [test_record], obs, obsl, bg_scores,
194    ↵ merger_base.log_Li, remote=true)
195  @test
196    ↵ sum(dshuffled_model.sources.=merger_base.sources)=length(dshuffled_model.sou
197  for (s,src) in enumerate(dshuffled_model.sources)
198    @test src=merger_base.sources[s] ||
199      ↵ src=merger_target.sources[s]
200    if src == merger_base.sources[s]
201      @test
202        ↵ dshuffled_model.mix_matrix[:,s]=merger_base.mix_matrix[:,s]
203    else
204      @test
205        ↵ dshuffled_model.mix_matrix[:,s]=merger_target.mix_matrix[:,s]
206    end
207  end
208  @test "SS from merge" == dshuffled_model.origin
209
210
211  dacc_model=remotecall_fetch(accumulate_mix, testwk, acc_base,
212    ↵ [acc_record], obs, obsl, bg_scores, acc_merge.log_Li)
213  @test dacc_model.mix_matrix=trues(2,1)
214  @test dacc_model.sources == acc_merge.sources
215  @test "AM from accbase" == dacc_model.origin
216
217
218  info_base=ICA_PWM_Model("info_base", "", [accurate_srcs[1]],
219    ↵ src_length_limits, accurate_mix[:,1:1],
220    ↵ IPM_likelihood([accurate_srcs[1]],obs,obsl,bg_scores,accurate_mix[:,1:1]))

```

```

202     → info_model=info_fill(info_base,Vector{Model_Record}(),obs,obsl,bg_scores,info)
203     @test info_model.sources[1][1][:] == [0., -Inf, -Inf, -Inf]
204     @test info_model.log_Li > info_base.log_Li
205     @test "IF from info_base" == info_model.origin
206
207     rmprocs(testwk)
208     rm(path)
209     rm(accpath)
210 end

```

16.7.25 /test/permute_tuner_tests.jl

```

1 @testset "Permute Tuner" begin
2     funcvec=[random_decorrelate,fit_mix]
3     instruct=Permute_Instruct(funcvec, [.5,.5],100,100)
4     tuner=Permute_Tuner(instruct)
5     #need to test update_weights functionality
6     #want to supply some fake data to induce a .8 .2 categorical
7     tuner.successes[:,1]=falses(TUNING_MEMORY*instruct.func_limit)
8     tuner.successes[:,2]=falses(TUNING_MEMORY*instruct.func_limit)
9
10    → tuner.successes[1:Int(floor(TUNING_MEMORY*instruct.func_limit*.8)),1]==true
11
12    → tuner.successes[1:Int(floor(TUNING_MEMORY*instruct.func_limit*.2)),2]==true
13    update_weights!(tuner)
14    @test tuner.inst.weights==[.8,.2]      #check clamping
15    tuner.successes[:,1]=falses(TUNING_MEMORY*instruct.func_limit)
16    @test tuner.inst.min_clmps==[.01,.01]
17    @test tuner.inst.max_clmps==[1.,1.]
18    update_weights!(tuner)
19    @test tuner.inst.weights==[.01,1-.01]
20
21    #test override
22    instruct=Permute_Instruct(funcvec,
23        → [.5,.5],100,100,override_time=2.,override_weights=[.75,.25])
24    tuner=Permute_Tuner(instruct)
25    tune_weights!(tuner, [(1,1.,1.)])
26    @test tuner.inst.weights==[.5,.5] #no override
27    tuner.time_history=fill(3.,CONVERGENCE_MEMORY)
28    tune_weights!(tuner, [(1,1.,1.)])
29    @test tuner.inst.weights==[.75,.25]

```

```

27
28     #more clamping tests
29     testvec=[0.02693244970132842, 0.031512823560878485,
30             ↪ 0.050111382705776294, 0.6893314065796903, 0.04206537140157707,
31             ↪ 0.02013161919125878, 0.026535815205008566, 0.051758654139053166,
32             ↪ 0.03497967993105292, 0.013999262917669668, 0.01264153466670629]
33     target=[0.02527900179828276, 0.029859375657832827,
34             ↪ 0.04845793480273063, 0.6876779586766446, 0.040411923498531406,
35             ↪ 0.02, 0.02488236730196291, 0.050105206236007505,
36             ↪ 0.03332623202800726, 0.02, 0.02]
37
38
39         → clamp_pvec!(testvec,fill(.02,length(testvec)),fill(1.,length(testvec)))
40     @test isprobvec(testvec)
41     @test testvec==target
42
43
44     #high clamp
45     testvec=[.9,.08,.02]
46     clamp_pvec!(testvec, fill(.05,length(testvec)), [.45,.45,.45])
47     @test testvec==[.45, .2825, .2675]
48 end

```

16.7.26 /test/pwm_unit_tests.jl

```

1 @testset "PWM source prior setup, PWM source initialisation and
2     manipulation functions" begin
3     #test dirichlet prior estimation from wm inputs
4     wm_input = [.0 .2 .3 .5; .0 .2 .3 .5]
5     est_dirichlet_vec = estimate_dirichlet_prior_on_wm(wm_input)
6     @test typeof(est_dirichlet_vec) == Vector{Dirichlet{Float64}}
7     for pos in 1:length(est_dirichlet_vec)
8         @test isapprox(est_dirichlet_vec[pos].alpha,
9             ↪ wm_input[pos,:].*PRIOR_WT)
10    end
11
12    bad_input = wm_input .* 2
13    @test_throws DomainError estimate_dirichlet_prior_on_wm(bad_input)
14
15    wm_input = [.1 .2 .3 .4; .1 .2 .3 .4]
16    est_dirichlet_vec = estimate_dirichlet_prior_on_wm(wm_input)
17    @test typeof(est_dirichlet_vec) == Vector{Dirichlet{Float64}}
18    for pos in 1:length(est_dirichlet_vec)

```

```

17         @test est_dirichlet_vec[pos].alpha == wm_input[pos,:].*PRIOR_WT
18     end
19
20     length_range = 2:2
21
22     #test informative/uninformative source prior vector assembly
23     test_priors = assemble_source_priors(2, [wm_input])
24     @test length(test_priors) == 2
25     for pos in 1:length(test_priors[1])
26         @test test_priors[1][pos].alpha == wm_input[pos,:].*PRIOR_WT
27     end
28     @test test_priors[2] == false
29
30     #test source wm initialisation from priors
31     test_sources = init_logPWM_sources(test_priors, length_range)
32     for source in test_sources
33         for pos in 1:size(source[1])[1]
34             @test isprobvec(exp.(source[1][pos,:]))
35         end
36     end
37
38     #test that wm_shift is returning good shifted probvecs
39     rando_dist=Dirichlet([.25,.25,.25,.25])
40     for i in 1:1000
41         wm=rand(rando_dist)
42         new_wm=wm_shift(wm,Weibull(1.5,.1))
43         @test isprobvec(new_wm)
44         @test wm!=new_wm
45     end
46
47     #test that legal new sources are generated by permute_source_weights
48     permuted_weight_sources=deepcopy(test_sources)
49
50     → permuted_weight_sources[1]=permute_source_weights(permuted_weight_sources[1],
51     → permuted_weight_sources[2]=permute_source_weights(permuted_weight_sources[2],
52     @test permuted_weight_sources ≠ test_sources
53     for (s,source) in enumerate(permuted_weight_sources)
54         for pos in 1:size(source[1],1)
55             @test isprobvec(exp.(source[1][pos,:]))
56             @test source[1][pos,:] ≠ test_sources[s][1][pos,:]
57         end
58     end
59 
```



```

96          .25 .25 .25 .25]),1)
97
98     infovec=get_pwm_info(erosion_test_source[1])
99     start_idx, end_idx = get_erosion_idxs(infovec, .25, 2:8)
100    @test start_idx==3
101    @test end_idx==5
102
103    eroded_pwm,eroded_prior_idx=erode_source(erosion_test_source,2:8,.25)
104    for pos in 1:size(eroded_pwm,1)
105        @test isprobvec(exp.(eroded_pwm[pos,:]))
106    end
107    @test eroded_prior_idx==3
108    @test isapprox(exp.(eroded_pwm), [.7 .1 .1 .1
109      .06 .06 .06 .82
110      .7 .1 .1 .1])
111
112
113    #make sure revcomp_pwm is reversing pwms across both dimensions
114    revcomp_test_pwm = zeros(2,4)
115    revcomp_test_pwm[1,1] = 1
116    revcomp_test_pwm[2,3] = 1
117    log_revcomp_test_pwm = log.(revcomp_test_pwm)
118    @test revcomp_pwm(log_revcomp_test_pwm) = [-Inf 0. -Inf -Inf
119                                -Inf -Inf -Inf
120                                → 0.]
121
122 end

```

16.7.27 /test/runtests.jl

```

1 @info "Loading test packages ... "
2
3 using BioMotifInference, BioBackgroundModels, BioSequences,
4   ↳ Distributions, Distributed, Random, Serialization, Test
4 import StatsFuns: logsumexp, logaddexp

```

```

5 import BioMotifInference:estimate_dirichlet_prior_on_wm,
→ assemble_source_priors, init_logPWM_sources, wm_shift,
→ permute_source_weights, get_length_params, permute_source_length,
→ get_pwm_info, get_erosion_idxs, erode_source, init_mix_matrix,
→ mixvec_decorrelate, mix_matrix_decorrelate, most_dissimilar,
→ most_similar, revcomp_pwm, score_sources_ds!, score_sources_ss!,
→ weave_scores_ss!, weave_scores_ds!, IPM_likelihood,
→ consolidate_check, consolidate_srcs, pwm_distance, permute_source,
→ permute_mix, perm_src_fit_mix, fit_mix, random_decorrelate,
→ reinit_src, erode_model, reinit_src, shuffle_sources, accumulate_mix,
→ distance_merge, similarity_merge, info_fill, converge_ensemble!,
→ reset_ensemble!, Permute_Tuner, PRIOR_WT, TUNING_MEMORY,
→ CONVERGENCE_MEMORY, tune_weights!, update_weights!, clamp_pvec!
6 import Distances: euclidean
7
8 @info "Beginning tests ... "
9 using Random
10 Random.seed!(786)
11 O=1000;S=50
12
13 include("pwm_unit_tests.jl")
14 include("mix_matrix_unit_tests.jl")
15 include("likelihood_unit_tests.jl")
16 include("consolidate_unit_tests.jl")
17 include("permute_func_tests.jl")
18 include("permute_tuner_tests.jl")
19 include("ensemble_tests.jl")
20 @info "Tests complete!"

```

16.7.28 /test/spike_recovery.jl

```

1 #Testbed for synthetic spike recovery from example background
2
3 using nnlearn, BGHMM, HMMBase, Distributions, Random, Serialization,
→   Distributions
4
5 Random.seed!(786)
6
7 #CONSTANTS
8 no_obs=1000
9 obsl=140
10

```

```

11 hmm=HMM{Univariate,Float64}([0.4016518533961019, 0.2724399569450827,
→ 0.3138638675018568, 0.012044322156962559], [3.016523036789942e-9
→ 2.860631288328858e-6 0.2299906524188302 0.7700064839333549;
→ 3.0102278323431375e-11 0.7494895424906354 0.23378615437778671
→ 0.016724303101477486; 8.665894321573098e-17 0.2789950381410553
→ 0.7141355461949104 0.006869415664033568; 0.006872526597796038
→ 0.016052425322133648 0.017255179541768192 0.9598198685383041],
→ [Categorical([0.1582723599684065, 0.031949729618356536,
→ 0.653113286526948, 0.15666462388628763]),
→ Categorical([0.4610499748798372, 0.2613013005680122,
→ 0.15161801872560146, 0.12603070582654768]),
→ Categorical([0.08601960130086236, 0.13869192872427524,
→ 0.26945182329686523, 0.5058366466779973]),
→ Categorical([0.3787366527613563, 0.11034604356978756,
→ 0.1119586976058099, 0.3989586060630392])))

12
13 struc_sig=[.1 .7 .1 .1
14           .1 .1 .1 .7
15           .1 .7 .1 .1]
16 periodicity=8
17 struc_frac_obs=.35
18
19 tata_box=[.05 .05 .05 .85
20           .85 .05 .05 .05
21           .05 .05 .05 .85
22           .85 .05 .05 .05
23           .425 .075 .075 .425
24           .85 .05 .05 .05
25           .425 .075 .075 .425]
26 tata_frac_obs=.7
27 tata_recur_range=1:4
28
29 combined_ensemble = "/bench/PhD/NGS_binaries/nnlearn/combined_ensemble"
30
31 #JOB CONSTANTS
32 const position_size = 141
33 const ensemble_size = 100
34 const no_sources = 3
35 const source_min_bases = 3
36 const source_max_bases = 12
37 @assert source_min_bases < source_max_bases
38 const source_length_range= source_min_bases:source_max_bases
39 const mixing_prior = .3

```

```

40 @assert mixing_prior ≥ 0 & mixing_prior ≤ 1
41 const models_to_permute = ensemble_size * 3
42
43 job_sets=[[
44 ([[
45     ("PS", (no_sources)),
46     ("PM", (no_sources)),
47     ("PSFM", (no_sources)),
48     ("PSFM", (no_sources*10, .8, 1.)),
49     ("FM", (())),
50     ("DM", (no_sources)),
51     ("SM", (no_sources)),
52     ("RD", (no_sources)),
53     ("RI", (no_sources)),
54     ("EM", (no_sources))
55 ], [.025, .025, .025, .025, .775, .025, .025, .025, .025, .025]),,
56 ([[
57     ("PS", (no_sources)),
58     ("PM", (no_sources)),
59     ("PSFM", (no_sources)),
60     ("PSFM", (no_sources, 0., 1.)),
61     ("PSFM", (no_sources, 0.8, .0)),
62     ("FM", (())),
63     ("DM", (no_sources)),
64     ("SM", (no_sources)),
65     ("RD", (no_sources)),
66     ("RI", (no_sources*10)),
67     ("EM", (no_sources))
68 ], [.05, .15, .15, .10, .05, .20, 0.0, 0.05, 0.15, 0.10, .0]),,
69 ]
70 job_limit=4
71
72 const prior_wt=1.1
73
74 #FUNCTIONS
75 function setup_obs(hmm, no_obs, obsl)
76     obs=vec(Int64.(rand(hmm,obsl)[2]))
77     for o in 2:no_obs
78         obs=hcat(obs, vec(Int64.(rand(hmm,obsl)[2])))
79     end
80     obs=vcat(obs,zeros(Int64,1,no_obs))
81     return obs
82 end

```

```

83
84 function spike_irreg!(obs, source, frac_obs, recur)
85     truth=Vector{Int64}()
86     for o in 1:size(obs,2)
87         if rand()<frac_obs
88             push!(truth, o)
89             for r in rand(recur)
90                 rand()<.5 && (source=nnlearn.revcomp_pwm(source))
91                 pos=rand(1:size(obs,1)-1)
92                 pwm_ctr=1
93                 while pos ≤ size(obs,1)-1&& pwm_ctr ≤ size(source,1)
94                     obs[pos,o]=rand(Categorical(source[pwm_ctr,:]))
95                     pos+=1
96                     pwm_ctr+=1
97                 end
98             end
99         end
100    return truth
101 end
102
103
104 function spike_struc!(obs, source, frac_obs, periodicity)
105     truth=Vector{Int64}()
106     truthpos=Vector{Vector{Int64}}()
107     for o in 1:size(obs,2)
108         if rand()<frac_obs
109             push!(truth, o)
110             rand()<.5 && (source=nnlearn.revcomp_pwm(source))
111             pos=rand(1:periodicity)
112             posvec=Vector{Int64}()
113             while pos ≤ size(obs,1)
114                 pos_ctrl=pos
115                 pwm_ctrl=1
116                 while pos_ctrl ≤ size(obs,1)-1&&pwm_ctrl ≤ size(source,1)
117                     push!(posvec,pos_ctrl)
118                     base=rand(Categorical(source[pwm_ctrl,:]))
119                     obs[pos_ctrl,o]=base
120                     pos_ctrl+=1
121                     pwm_ctrl+=1
122                 end
123                 pos+=periodicity+pwm_ctrl
124             end
125             push!(truthpos,posvec)

```

```

126     end
127   end
128   return truth,truthpos
129 end
130
131 function get_BGHMM_lhs(obs,hmm)
132   lh_mat=zeros(size(obs,1)-1,size(obs,2))
133   for o in 1:size(obs,2)
134     obso=zeros(Int64,size(obs,1),1)
135     obso[1:end,1] = obs[:,o]
136
137     → lh_mat[:,o]=BGHMM.get_BGHMM_symbol_lh(Matrix(transpose(obso)),hmm)
138   end
139   return lh_mat
140 end
141 @info "Setting up synthetic observation set ... "
142 obs=setup_obs(hmm, no_obs, obsl)
143 struc_truth,struc_postruth=spike_struc!(obs, struc_sig, struc_frac_obs,
144   → periodicity)
145 irreg_truth=spike_irreg!(obs, tata_box, tata_frac_obs, tata_recur_range)
146
147 @info "Calculating background likelihood matrix ... "
148 bg_lhs=get_BGHMM_lhs(obs,hmm)
149
150 @info "Assembling source priors ... "
151 #prior_array= [struc_sig, tata_box]
152 prior_array= Vector{Matrix{Float64}}(){}
153 source_priors = nnlearn.assemble_source_priors(no_sources, prior_array,
154   → prior_wt, source_length_range)
155
156 @info "Assembling ensemble ... "
157 path=randstring()
158 if isfile(string(path,'/',"ens"))
159   ens = deserialize(string(path,'/',"ens"))
160   job_set_thresh=[-Inf,ens.naive_lh]
161   param_set=(job_sets,job_set_thresh,job_limit)
162
163 @info "Converging ensemble ... "

```

```

164 nnlearn.ns_converge!(ens, param_set, models_to_permute, .0001,
    ↳ model_display=no_sources, backup=(true,5))
165
166 rm(path,recursive=true)
167
168 #811973

```

16.8 AWSWrangler

16.8.1 /src/AWSWrangler.jl

```

1 module AWSWrangler
2
3 using AWSSDK, Dates, Sockets
4
5 function spot_wrangle(no_instances::Integer, spot_price::AbstractFloat,
    ↳ sgrp_name::String, sgrp_desc::String, skeys::String, zone::String,
    ↳ ami::String, instance_type::String)
6     #ssh permissions
7     WANip=chomp(read(pipeline(`dig +short myip.opendns.com
    ↳ @resolver1.opendns.com`), String))
8     permissions = [
9         [
10            "FromPort" => 22,
11            "IpProtocol" => "tcp",
12            "IpRanges" => [
13                [
14                    "CidrIp" => WANip*"/32",
15                    "Description" => "SSH access"
16                ]
17            ],
18            "ToPort" => 22
19        ]
20    ]
21
22     @info "Configuring AWS credentials ... "
23     aws=aws_config()
24
25     @info "Getting security group info ... "
26     sgrp=Dict()
27
28     try

```

```

29      ↵ sgrp=AWSSDK.EC2.create_security_group(aws,GroupDescription=sgrp_desc,Grou
30      inret=AWSSDK.EC2.authorize_security_group_ingress(aws,
31          ↵ GroupId=sgrp["groupId"], IpPermissions=permissions)
32      outret=AWSSDK.EC2.authorize_security_group_egress(aws,
33          ↵ GroupId=sgrp["groupId"], IpPermissions=permissions)
34  catch error
35      if error.code == "InvalidGroup.Duplicate"
36
37      ↵ sgrp=AWSSDK.EC2.describe_security_groups(aws,GroupName=sgrp_name)[ "se
38  end
39
40  #Launch specs
41 LaunchSpec = [
42      "ImageId" => ami,
43      "InstanceType" => instance_type,
44      "SecurityGroupId" => [
45          sgrp["groupId"]
46      ],
47      "Placement" => [
48          "AvailabilityZone" => zone
49      ],
50      "KeyName" => skeys
51  ]
52
53  @info "Requesting spot instances ... "
54
55  spot_reqs=AWSSDK.EC2.request_spot_instances(aws,
56      ↵ LaunchSpecification=LaunchSpec, InstanceCount=no_instances,
57      ↵ SpotPrice=spot_price, Type="one-time")
58
59  instance_public_ips=Vector()
60
61  @info "Waiting for instances to become active ... "
62
63  if no_instances==1
64
65      ↵ spot_req_Id=spot_reqs["spotInstanceRequestSet"]["item"]["spotInstanceReq
66      active=false
67      while !active

```

```

65         AWSSDK.EC2.describe_spot_instance_requests(aws,
66             → SpotInstanceRequestId=spot_req_Id)[ "spotInstanceRequestSet" ][ "item" ][
67                 → = "active" ? active=true : sleep(10)
68     end
69     instance_Id=AWSSDK.EC2.describe_spot_instance_requests(aws,
70         → SpotInstanceRequestId=spot_req_Id)[ "spotInstanceRequestSet" ][ "item" ][ "ins
71     address=AWSSDK.EC2.describe_instances(aws,
72         → InstanceId=instance_Id)[ "reservationSet" ][ "item" ][ "instancesSet" ][ "item"
73     push!(instance_public_ips,address)
74   else
75     for spot_req in spot_reqs[ "spotInstanceRequestSet" ][ "item" ]
76       spot_req_Id = spot_req[ "spotInstanceRequestId" ]
77       active=false
78       while !active
79         AWSSDK.EC2.describe_spot_instance_requests(aws,
80             → SpotInstanceRequestId=spot_req_Id)[ "spotInstanceRequestSet" ][ "item" ][
81                 → = "active" ? active=true : sleep(10)
82     end
83     instance_Id = AWSSDK.EC2.describe_spot_instance_requests(aws,
84         → SpotInstanceRequestId=spot_req_Id)[ "spotInstanceRequestSet" ][ "item" ][
85     address=AWSSDK.EC2.describe_instances(aws,
86         → InstanceId=instance_Id)[ "reservationSet" ][ "item" ][ "instancesSet" ][ "item"
87     push!(instance_public_ips,address)
88   end
89 end
90
91     return instance_public_ips
92 end
93
94 function get_cheapest_zone(instance_type::String)
95   arguments=[  

96     "ProductDescription"⇒["Linux/UNIX"]  

97     "InstanceType"⇒[instance_type]  

98     "StartTime"⇒Dates.now(Dates.UTC)  

99   ]
100   history=AWSSDK.EC2.describe_spot_price_history(aws_config(),
101     → arguments)
102
103   price_dict=Dict{String,Float64}()  

104   for record in history[ "spotPriceHistorySet" ][ "item" ]
105     price_dict[record[ "availabilityZone" ]]=parse(Float64,
106           → record[ "spotPrice" ])
107 end

```

```

98
99     return sort(collect(price_dict), by=x→x[2])[1]
100 end
101
102 export spot_wrangle,get_cheapest_zone
103
104 end # module

```

16.9 Analyses

16.9.1 /cmz/a10correlation.jl

```

1 using
   ↳ BayesianLinearRegression,Csv,DataFrames,Distributions,NGRefTools,StatsBase,Plots,
   ↳ Measurements
2 import Measurements:value,uncertainty
3
4 default(legendfont = (8), guidefont = (10,), tickfont = (8), guide = "x")
5
6 a10pth="/bench/PhD/datasets/A10 measurements 2018update.csv"
7
8 a10df=DataFrame(Csv.read(a10pth))
9 a10df.BSR=[string(row.Block,',',row.Slide,',',row.Row) for row in
   ↳ eachrow(a10df)]
10
11 X=Vector{Float64}()
12 measure_dict=Dict{String,Vector{Vector{Float64}}}()
13
14 measure_dict["PopEst"]=Vector{Vector{Float64}}()
15 measure_dict["VolEst"]=Vector{Vector{Float64}}()
16
17 #EXTRACT ALL MEASUREMENTS FROM DF TO VECTORS
18 for t_df in groupby(a10df, "Time point (d)")
19     push!(X,Float64(unique(t_df."Time point (d)")[1]))
20     pevec,tvec,dvec,vvec,vevec, rtvec, rplvec, rptvec, ldvec, ondvec,
   ↳ onlvec, oplvec, inlvec, iplvec, gclvec = [zeros(0) for i in 1:15]
21
22     for i_df in groupby(t_df,"BSR")
23         length([skipmissing(i_df."Lens diameter") ... ])>0 &&
   ↳ push!(ldvec,mean(skipmissing(i_df."Lens diameter"))))
24     end
25

```

```

26   for i_df in groupby(t_df, "BSR")
27     if length([skipmissing(i_df."CMZ Sum") ... ])>0
28       push!(tvec,mean(skipmissing(i_df."CMZ Sum")))
29       if length([skipmissing(i_df."Lens diameter") ... ])>0
30         push!(pevec,mean(skipmissing(i_df."CMZ
31           → Sum"))*mean(skipmissing(i_df."Lens diameter"))/14.)
32       else
33         push!(pevec,mean(skipmissing(i_df."CMZ
34           → Sum"))*mean(ldvec)/14.)
35       end
36     end
37     if length([skipmissing(i_df."Retina thickness") ... ])>0 &&
38       length([skipmissing(i_df."IPL") ... ])>0 &&
39       length([skipmissing(i_df."OPL") ... ])>0 &&
40       length([skipmissing(i_df."RPE length") ... ])>0
41       rthi=mean(skipmissing(i_df."Retina
42         → thickness"))-mean(skipmissing(i_df."OPL"))-mean(skipmissing(i_df."
43       rpel=mean(skipmissing(i_df."RPE length"))
44       if length([skipmissing(i_df."Lens diameter") ... ])>0
45         rcirc=rpel+mean(skipmissing(i_df."Lens diameter"))
46       else
47         rcirc=rpel+mean(ldvec)
48       end
49
50
51       or=rcirc/2π
52       ir=or-.5*rthi
53
54       ov=(4/3)*π*(or^3)
55       iv=(4/3)*π*(ir^3)
56
57       push!(vevec,(ov-iv)*(4/5))
58     end
59   end
60 end
61
62 x=measure_dict["PopEst"][1]
63 uncorrX3d=ones(length(x),1)
64 corrX3d=hcat(ones(length(x)),x)
65 y=measure_dict["VolEst"][1]

```

```

66 uncorr=BayesianLinearRegression.fit!(BayesianLinReg(uncorrX3d,y))
67 corr=BayesianLinearRegression.fit!(BayesianLinReg(corrX3d,y))
68
69 function blr_plt(x,y,Xs,blrs,colors,labels,q=.975)
70
71     plt=scatter(x,y,marker=:diamond,markersize=8,markerstrokestyle=:bold,markerco
72     retinal volume",xlabel="Estimated CMZ Annulus
73     Population",legend=:bottomleft)
74     for (X,blr,color,label) in zip(Xs, blrs,colors,labels)
75         line=zeros(size(X,1))
76         ribbon=zeros(size(X,1))
77         predictions=BayesianLinearRegression.predict(blr,X)
78         for (n,p) in enumerate(predictions)
79             normal=Normal(value(p),uncertainty(p))
80             ribbon[n]=quantile(normal,q)-mean(normal)
81             line[n]=mean((value(p)))
82         end
83         plot!(plt,x,line,ribbon=ribbon,color=color,label=label)
84     end
85     return plt
86 end
87 p=blr_plt(x,y,[corrX3d,uncorrX3d],[corr,uncorr],[:darkmagenta,:green],["Correlated
88     Model", "Uncorrelated Model"])
89
90 savefig(p, "/bench/PhD/Thesis/images/cmz/3dcorr.png")
91 @info "Saved fig."
92
93 println("\begin{tabular}{l|l|l|l}")
94 println("\\hline")
95 println("{\bf Age (dpf)} & {\bf Uncorrelated logZ} & {\bf Correlated
96     logZ} & {\bf logZR}\hline")
97
98 for (x,xs,ys) in zip(X,measure_dict["PopEst"],measure_dict["VolEst"])
99     uncorrX=ones(length(xs),1)
100    corrX=hcat(ones(length(xs)),xs)
101    uncorr=BayesianLinearRegression.fit!(BayesianLinReg(uncorrX,ys))
102    corr=BayesianLinearRegression.fit!(BayesianLinReg(corrX,ys))
103    println("$x & {\bf $(round(logEvidence(uncorr),digits=3))} &
104        $(round(logEvidence(corr),digits=3)) &
105        $(round(logEvidence(uncorr)-logEvidence(corr),digits=3))\hline")
106
107 end

```

```

101
102 println("\end{tabular}")
103
104
105

```

16.9.2 /cmz/a10dvratio.jl

```

1 using BayesianLinearRegression, CSV, DataFrames, Distributions, GMC_NS,
  ↵   Serialization, Plots, NGRefTools
2 a10pth="/bench/PhD/datasets/A10 measurements 2018update.csv"
3
4 a10df=DataFrame(CSV.read(a10pth))
5 a10df.BSR=[string(row.Block,',',row.Slide,',',row.Row) for row in
  ↵   eachrow(a10df)]
6
7 X=Vector{Float64}()
8 measure_dict=Dict{String,Vector{Vector{Float64}}}()
9
10 measure_dict["Dorsal CMZ (#)"]=Vector{Vector{Float64}}()
11 measure_dict["Ventral CMZ (#)"]=Vector{Vector{Float64}}()
12 measure_dict["Ratio"]=Vector{Vector{Float64}}()
13
14 #EXTRACT ALL MEASUREMENTS FROM DF TO VECTORS
15 for t_df in groupby(a10df, "Time point (d)")
16     push!(X,Float64(unique(t_df."Time point (d)")[1]))
17     rvec,dvec,vvec = [zeros(0) for i in 1:3]
18
19     for i_df in groupby(t_df,"BSR")
20         length(skipmissing(i_df."Dorsal CMZ (#)")...)]>0 &&
21             mean(skipmissing(i_df."Dorsal CMZ (#)")) >0. &&
22             push!(dvec,mean(skipmissing(i_df."Dorsal CMZ (#)")))
23         length(skipmissing(i_df."Ventral CMZ (#)")...)]>0 &&
24             mean(skipmissing(i_df."Ventral CMZ (#)")) >0. &&
25             push!(vvec,mean(skipmissing(i_df."Ventral CMZ (#)")))
26         length(skipmissing(i_df."Dorsal CMZ (#)")...)]>0 &&
27             mean(skipmissing(i_df."Dorsal CMZ (#)")) >0. &&
28             length(skipmissing(i_df."Ventral CMZ (#)")...)]>0 &&
29             mean(skipmissing(i_df."Ventral CMZ (#)")) >0. &&
30             push!(rvec,mean(skipmissing(i_df."Dorsal CMZ
  ↵   (#)"))/mean(skipmissing(i_df."Ventral CMZ (#)")))
31     end

```

```

24
25     push!(measure_dict["Ratio"],rvec)
26     push!(measure_dict["Dorsal CMZ (#)"],dvec)
27     push!(measure_dict["Ventral CMZ (#)"],vvec)
28 end
29
30 d_logn_mts=[fit(MarginalTDist,log.(measure_dict["Dorsal CMZ (#)"])[n]))
31   →  for n in 1:length(X)]
32 d_mean=[exp(mean(mt)) for mt in d_logn_mts]
33 d_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in d_logn_mts]
34 d_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in d_logn_mts]
35
36 v_logn_mts=[fit(MarginalTDist,log.(measure_dict["Ventral CMZ (#)"])[n)))
37   →  for n in 1:length(X)]
38 v_mean=[exp(mean(mt)) for mt in v_logn_mts]
39 v_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in v_logn_mts]
40 v_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in v_logn_mts]
41
42 r_n_mts=[fit(MarginalTDist,measure_dict["Ratio"][n]) for n in
43   →  1:length(X)]
44 r_mean=[mean(mt) for mt in r_n_mts]
45 r_lower=[mean(mt)-quantile(mt,.025) for mt in r_n_mts]
46 r_upper=[quantile(mt,.975)-mean(mt) for mt in r_n_mts]
47
48 plotticks=[30*i for i in 1:12]
49
50 dv_chart=Plots.plot(X, d_mean, ribbon=(d_lower,d_upper), color=:green,
51   →  label="Dorsal mean", ylabel="CMZ sectional population size",
52   →  showaxis=:y, xticks=plotticks, xformatter=_→"")
53 plot!(X, v_mean, ribbon=(v_lower,v_upper), color=:darkmagenta,
54   →  label="Ventral mean")
55 scatter!(vcat([[X[n] for i in 1:length(measure_dict["Dorsal CMZ
56   →  (#)"])[n]]) for n in 1:length(X)] ... ),vcat(measure_dict["Dorsal CMZ
57   →  (#)"] ... ), marker=:utriangle, color=:green, markersize=3,
58   →  markerstrokecolor=:green, label="Dorsal data")
59 scatter!(vcat([[X[n] for i in 1:length(measure_dict["Ventral CMZ
60   →  (#)"])[n]]) for n in 1:length(X)] ... ),vcat(measure_dict["Ventral CMZ
61   →  (#)"] ... ), marker=:dtriangle, color=:darkmagenta, markersize=3,
62   →  markerstrokecolor=:darkmagenta, label="Ventral data")
63 annotate!([(1.5,125,Plots.text("A",18))])
64 lens!([2, 31], [10, 150], inset = (1, bbox(0.3, 0.1, 0.45, 0.6)))

```

```

54 ratio_chart=scatter(vcat([[X[n] for i in
→ 1:length(measure_dict["Ratio"])[n]]) for n in
→ 1:length(X)] ... ), vcat(measure_dict["Ratio"] ... ), marker=:cross,
→ color=:black, markersize=3, markerstrokecolor=:black, label="Ratio
→ data", ylabel="Individual asymmetry ratio", xlabel="Age (dpf)",
→ xticks=plotticks)
55 plot!(X, r_mean, ribbon=(r_lower,r_upper), color=:green, label="Ratio
→ mean")
56 plot!(X, [1. for i in 1:length(X)], style=:dot, color=:black, label="Even
→ ratio")
57 annotate!([(1.5,.5,Plots.text("B",18))])
58
59
60 l=@layout [a{.6h}
61             b]
62
63 combined=Plots.plot(dv_chart,ratio_chart,layout=l, link=:x,
→ size=(900,600))
64
65 savefig(combined, "/bench/PhD/Thesis/images/cmz/DVontology.png")

```

16.9.3 /cmz/a10dvslice.jl

```

1 using
→ BayesianLinearRegression, CSV, DataFrames, Distributions, CMZNicheSims, GMC_NS,
→ Serialization, Plots,
→ KernelDensityEstimate, KernelDensityEstimatePlotting, Gadfly
2 a10pth="/bench/PhD/datasets/A10 measurements 2018update.csv"
3
4 edpath="/bench/PhD/NGS_binaries/CNS/A10/ed"
5 evpath="/bench/PhD/NGS_binaries/CNS/A10/ev"
6 edvpath="/bench/PhD/NGS_binaries/CNS/A10/edv"
7 paths=[edpath, evpath, edvpath]
8
9 default(legendfont = (10), guidefont = (12), tickfont = (10))
10
11 a10df=DataFrame(CSV.read(a10pth))
12 a10df.BSR=[string(row.Block,',',row.Slide,',',row.Row) for row in
→ eachrow(a10df)]
13
14 X=Vector{Float64}(){}
15 measure_dict=Dict{String,Vector{Vector{Float64}}}(){}

```

```

16
17 measure_dict["CMZ Sum"] = Vector{Vector{Float64}}()
18 measure_dict["Dorsal CMZ (#)"] = Vector{Vector{Float64}}()
19 measure_dict["Ventral CMZ (#)"] = Vector{Vector{Float64}}()
20 measure_dict["Lens circumference"] = Vector{Vector{Float64}}()

21
22 #EXTRACT ALL MEASUREMENTS FROM DF TO VECTORS
23 for t_df in groupby(a10df, "Time point (d)")
24     if !(Float64(unique(t_df."Time point (d)")[1])) in [180., 360.])

25         push!(X,Float64(unique(t_df."Time point (d)")[1]))
26         tvec,dvec,vvec,lcirc = [zeros(0) for i in 1:4]

28
29         for i_df in groupby(t_df,"BSR")
30             length([skipmissing(i_df."Lens diameter") ... ]) > 0 &&
31                 → push!(lcirc,mean(skipmissing(i_df."Lens diameter"))*π)
32         end

33         for i_df in groupby(t_df,"BSR")
34             length([skipmissing(i_df."CMZ Sum") ... ]) > 0 &&
35                 → mean(skipmissing(i_df."CMZ Sum")) > 0. &&
36                     → push!(tvec,mean(skipmissing(i_df."CMZ Sum")))
37             length([skipmissing(i_df."Dorsal CMZ (#)") ... ]) > 0 &&
38                 → mean(skipmissing(i_df."Dorsal CMZ (#)")) > 0. &&
39                     → push!(dvec,mean(skipmissing(i_df."Dorsal CMZ (#)")))
40             length([skipmissing(i_df."Ventral CMZ (#)") ... ]) > 0 &&
41                 → mean(skipmissing(i_df."Ventral CMZ (#)")) > 0. &&
42                     → push!(vvec,mean(skipmissing(i_df."Ventral CMZ (#)")))
43         end

44     end

45
46 obsset=[measure_dict["Dorsal CMZ (#)"],measure_dict["Ventral CMZ
47     (#)"],measure_dict["CMZ Sum"]]

48 dpopdist=fit(LogNormal,measure_dict["Dorsal CMZ (#)"][1])
49 vpopdist=fit(LogNormal,measure_dict["Ventral CMZ (#)"][1])
50 dvpopdist=fit(LogNormal,measure_dict["CMZ Sum"][1])

```

```

51
52 logLC=vcat([log.(measure_dict["Lens circumference"])[n]) for n in
   ↪ 1:length(X) ... )
53 logX=vcat([[log.(X[n]) for ms in measure_dict["Lens circumference"]][n]
   ↪ for n in 1:length(X)] ... )
54 logX=hcat(ones(length(logX)),logX)
55
56 lm=BayesianLinearRegression.fit!(BayesianLinReg(logX,logLC))
57 w1,w2=posteriorWeights(lm)
58 factor=10^w1.val
59 power=w2.val
60
61 lm1=Lens_Model(factor,power,14.)
62 lm2=Lens_Model(factor,power,28.)
63
64 mc_its=Int64(1e6)
65 base_scale=[144.,5.]
66 base_min=[10.,0.]
67 time_scale=90
68 time_min=4.
69
70 cycle_prior=[LogNormal(log(20),log(2)),LogNormal(log(.9),log(1.6))]
71 end_prior=[Uniform(4,90)]
72
73 function compose_priors(phases)
74     prs=Vector{Vector{Distribution}} }()
75     bxes=Vector{Matrix{Float64}} }()
76     for p in phases
77         pr=[repeat(cycle_prior,p) ... ,repeat(end_prior,p-1) ... ]
78         push!(prs,pr)
79         bx=hcat([repeat(base_min,p) ... ,fill(time_min,p-1) ... ],
80                 [repeat(base_scale,p) ... ,fill(time_scale,p-1) ... ])
81         push!(bxes,GMC_NS.to_unit_ball.(bx,pr))
82     end
83     return prs,bxes
84 end
85
86 prior,box=compose_priors(2)
87
88 d_constants=[X,dpopdist,lm1,mc_its,2]
89 v_constants=[X,vpopdist,lm1,mc_its,2]
90 dv_constants=[X,dvpopdist,lm2,mc_its,2]
91 constants=[d_constants,v_constants,dv_constants]

```

```

92
93 uds=Vector{Vector{Function}}([[],[liwi_display],[convergence_display]])
94 lds=Vector{Vector{Function}}([[model_obs_display],[ensemble_display],[ensemble_displa
95
96 gmc_settings=GMC_DEFAULTS
97 gmc_settings[end]=25000
98
99 for (pth,constants,obs) in zip(paths,constants,obsset)
100     if isfile(pth*"/ens")
101         e=deserialize(pth*"/ens")
102     else
103         e=Slice_Ensemble(pth,3000,obs, prior..., constants, box..., 
104                         ↪ gmc_settings)
105     end
106
107     converge_ensemble!(e,backup=true,50),upper_displays=uds,
108     ↪ lower_displays=lds, disp_rot_its=100, mc_noise=.3,
109     ↪ converge_criterion="compression", converge_factor=1.)
110 end
111
112 ed=deserialize(edpath*"/ens")
113 ev=deserialize(epath*"/ens")
114 edv=deserialize(edvpath*"/ens")
115
116 edev=measure_evidence(ed)
117 evev=measure_evidence(ev)
118 edvev=measure_evidence(edv)
119
120 evidence_ratio=edvev-(edev+evev)
121
122 println("\begin{tabular}{l|l|l|l|l}")
123 println("\hline")
124 println("{\bf Total logZ} & {\bf Dorsal logZ} & {\bf Ventral logZ} &
125     & {\bf logZR} & {\bf $\sigma$ Significance}\hline")
126 println("\textbf{\$(round(edvev,digits=3))} & \$(round(edev,digits=3)) &
127     & \$(round(evev,digits=3)) & \$(round(evidence_ratio,digits=3)) &
128     & \$(round(evidence_ratio.val/evidence_ratio.err,digits=3))\hline")
129 println("\end{tabular}")
130
131 mapd=deserialize(ed.models[findmax([m.log_Li for m in
132                             ↪ ed.models])[2]].path)

```

```

126 mapv=deserialize(ev.models[findmax([m.log_Li for m in
    ↵ ev.models])[2]].path)
127 mapdv=deserialize(edv.models[findmax([m.log_Li for m in
    ↵ edv.models])[2]].path)
128
129 println("\\begin{tabular}{l|l|l|l}")
130 println("\\hline")
131 println("{\\bf Parameter} & {\\bf Total MAP} & {\\bf Dorsal MAP} & {\\bf
    ↵ Ventral MAP}\\\"\\hline")
132 println("Phase 1 \$CT\$ (h) & $(round(mapdv.θ[1], digits=1)) &
    ↵ $(round(mapd.θ[1], digits=1)) & $(round(mapv.θ[1], digits=1))\\\\\\
    ↵ \\\"\\hline")
133 println("Phase 1 \$\\epsilon\$ & $(round(mapdv.θ[2], digits=2)) &
    ↵ $(round(mapd.θ[2], digits=2)) & $(round(mapv.θ[2], digits=2))\\\\\\
    ↵ \\\"\\hline")
134 println("Phase 2 \$CT\$ (h) & $(round(mapdv.θ[3], digits=1)) &
    ↵ $(round(mapd.θ[3], digits=1)) & $(round(mapv.θ[3], digits=1))\\\\\\
    ↵ \\\"\\hline")
135 println("Phase 2 \$\\epsilon\$ & $(round(mapdv.θ[4], digits=2)) &
    ↵ $(round(mapd.θ[4], digits=2)) & $(round(mapv.θ[4], digits=2))\\\\\\
    ↵ \\\"\\hline")
136 println("Transition age & $(round(mapdv.θ[5], digits=1)) &
    ↵ $(round(mapd.θ[5], digits=1)) & $(round(mapv.θ[5], digits=1))\\\\\\
    ↵ \\\"\\hline")
137 println("\\end{tabular}")
138
139 X=ed.constants[1]
140
141 plotticks=[10*i for i in 1:9]
142
143 catdobs=vcat([ed.obs[t] for t in 1:length(X)] ...)
144 catvobs=vcat([ev.obs[t] for t in 1:length(X)] ...)
145 catdvobs=vcat([edv.obs[t] for t in 1:length(X)] ...)
146
147 dXs=vcat([[X[n] for i in 1:length(ed.obs[n])] for n in 1:length(X)] ...)
148 vXs=vcat([[X[n] for i in 1:length(ev.obs[n])] for n in 1:length(X)] ...)
149 dvXs=vcat([[X[n] for i in 1:length(edv.obs[n])] for n in 1:length(X)] ...)
150
151 d_mean=mapd.disp_mat[:,2]
152 d_upper=mapd.disp_mat[:,3]-mapd.disp_mat[:,2]
153 d_lower=mapd.disp_mat[:,2]-mapd.disp_mat[:,1]
154
155 v_mean=mapv.disp_mat[:,2]

```

```

156 v_upper=mapv.disp_mat[:,3]--mapv.disp_mat[:,2]
157 v_lower=mapv.disp_mat[:,2]--mapv.disp_mat[:,1]
158
159 dv_mean=mapdv.disp_mat[:,2]
160 dv_upper=mapdv.disp_mat[:,3]--mapdv.disp_mat[:,2]
161 dv_lower=mapdv.disp_mat[:,2]--mapdv.disp_mat[:,1]
162
163 mapd_plt=scatter(dXs,catdobs, marker=:cross, color=:black, markersize=3,
    ↵ label="Dorsal CMZ population data", showaxis=:y, xticks=plotticks,
    ↵ xformatter=_→ "", ylabel="Population")
164 plot!(mapd_plt, X, d_mean, ribbon=(d_lower,d_upper), color=:green,
    ↵ label="Dorsal model population")
165 annotate!([(8,150,Plots.text("B",18))])
166
167 mapv_plt=scatter(vXs,catvobs, marker=:cross, color=:black, markersize=3,
    ↵ label="Ventral CMZ population data", xlabel="Age (dpf)",
    ↵ ylabel="Population", xticks=plotticks)
168 plot!(mapv_plt, X, v_mean, ribbon=(v_lower,v_upper), color=:darkmagenta,
    ↵ label="Ventral model population")
169 annotate!([(8,175,Plots.text("C",18))])
170
171 mapdv_plt=scatter(dvXs,catdvobs, marker=:cross, color=:black,
    ↵ markersize=3, label="Total CMZ population data", showaxis=:y,
    ↵ xticks=plotticks, xformatter=_→ "", ylabel="Population")
172 plot!(mapdv_plt, X, dv_mean, ribbon=(dv_lower,dv_upper),
    ↵ color=:darkorange, label="Total model population")
173 annotate!([(8,300,Plots.text("A",18))])
174
175 combined_map=Plots.plot(mapdv_plt, mapd_plt, mapv_plt, layout=grid(3,1),
    ↵ size=(800,900), link=:x)
176
177 savefig(combined_map, "/bench/PhD/Thesis/images/cmz/a10dvMAP.png")
178
179 kde=posterior_kde(edv)
180
181 ph1marg=marginal(kde,[1;2])
182 ph2marg=marginal(kde,[3;4])
183 ph12marg=marginal(kde,[1;3])
184 transmarg=marginal(kde,[5])
185
186 ph1mplt=KernelDensityEstimatePlotting.plot(ph1marg;dimLbls=[ "Phase 1 CT
    ↵ (hr)", "Phase 1 ε rate"], axis=[0. 144.; 0. 4.])

```

```

187 ph2mplt=KernelDensityEstimatePlotting.plot(ph2marg;dimLbls=[ "Phase 2 CT
    ↵ (hr)", "Phase 2 e rate"], axis=[0. 144.; 0. 4.])
188 ph12mplt=KernelDensityEstimatePlotting.plot(ph12marg; dimLbls=[ "Phase 1
    ↵ CT (hr)", "Phase 2 CT (hr)"],axis=[0. 144.; 0. 144.])
189 tmplt=KernelDensityEstimatePlotting.plotKDE(transmarg,xlbl="Phase
    ↵ transition age (dpf)", points=false, c=[ "green"])
190
191 combined_marg=Gadfly.gridstack([ph1mplt ph12mplt; ph2mplt tmplt])
192 img=SVG( "/bench/PhD/Thesis/images/cmz/a10dvmarginals.svg",24cm,24cm)
193 draw(img,combined_marg)

```

16.9.4 /cmz/a10nvln.jl

```

1 using
    ↵ CSV,DataFrames,Distributions,NGRefTools,StatsBase,Plots,GMC_NS,Serialization,
    ↵ Measurements
2
3 a10pth="/bench/PhD/datasets/A10 measurements 2018update.csv"
4 pne_pth="/bench/PhD/NGS_binaries/GMC_NS/A10/pop_norm"
5 plne_pth="/bench/PhD/NGS_binaries/GMC_NS/A10/pop_lognorm"
6 vne_pth="/bench/PhD/NGS_binaries/GMC_NS/A10/vol_norm"
7 vlne_pth="/bench/PhD/NGS_binaries/GMC_NS/A10/vol_lognorm"
8
9 a10df=DataFrame(CSV.read(a10pth))
10 a10df.BSR=[string(row.Block,',',row.Slide,',',row.Row) for row in
    ↵ eachrow(a10df)]
11
12 X=Vector{Float64}()
13 measure_dict=Dict{String,Vector{Vector{Float64}}}()
14
15 measure_dict["PopEst"]=Vector{Vector{Float64}}()
16 measure_dict["VolEst"]=Vector{Vector{Float64}}()
17
18 #EXTRACT ALL MEASUREMENTS FROM DF TO VECTORS
19 for t_df in groupby(a10df, "Time point (d)")
20     push!(X,Float64(unique(t_df."Time point (d)")[1]))
21     pevec,ldvec,spvec = [zeros(0) for i in 1:3]
22
23     for i_df in groupby(t_df,"BSR")
24         length([skipmissing(i_df."Lens diameter") ... ])>0 &&
            ↵ push!(ldvec,mean(skipmissing(i_df."Lens diameter")))
25     end

```

```

26
27     for i_df in groupby(t_df, "BSR")
28         if length([skipmissing(i_df."CMZ Sum") ... ])>0 &&
29             → mean(skipmissing(i_df."CMZ Sum")) > 0
30             if length([skipmissing(i_df."Lens diameter") ... ])>0
31                 push!(pevec,mean(skipmissing(i_df."CMZ
32                     → Sum"))*mean(skipmissing(i_df."Lens diameter"))/14.)
33             else
34                 push!(pevec,mean(skipmissing(i_df."CMZ
35                     → Sum"))*mean(ldvec)/14.)
36             end
37         end
38         if length([skipmissing(i_df."Retina thickness") ... ])>0 &&
39             length([skipmissing(i_df."IPL") ... ])>0 &&
40             length([skipmissing(i_df."OPL") ... ])>0 &&
41             length([skipmissing(i_df."RPE length") ... ])>0
42
43             rthi=mean(skipmissing(i_df."Retina
44                 → thickness"))-mean(skipmissing(i_df."OPL"))-mean(skipmissing(i_df."IPL"))
45             rpel=mean(skipmissing(i_df."RPE length"))
46             if length([skipmissing(i_df."Lens diameter") ... ])>0
47                 rcirc=rpel+mean(skipmissing(i_df."Lens diameter"))
48             else
49                 rcirc=rpel+mean(ldvec)
50             end
51
52             or=rcirc/2π
53             ir=or-.5*rthi
54
55             ov=(4/3)*π*(or^3)
56             iv=(4/3)*π*(ir^3)
57
58             push!(spvec,(ov-iv)*(4/5))
59         end
60     end
61
62     push!(measure_dict["PopEst"],pevec)
63     push!(measure_dict["VolEst"],spvec)
64 end
65
66 gmc=GMC_DEFAULTS
67 gmc[1]=5
68 gmc[2]=1.e-15

```

```

65 gmc[end]=2e4
66
67 uds=Vector{Vector{Function}}([[convergence_display],[evidence_display],[info_display]]
68
69 lds=Vector{Vector{Function}}([[model_obs_display],[ensemble_display]])
70
71 evdict=Dict{String,Measurement}()
72
73 pmax_μ=2e5
74 pmin_μ=1
75 pmax_λ=1e-3
76 pmin_λ=1e-10
77 vmax_μ=1e10
78 vmin_μ=100
79 vmax_λ=1e-10
80 vmin_λ=1e-20
81
82 pn_priors=[Uniform(pmin_μ,pmax_μ),Uniform(pmin_λ,pmax_λ)]
83 pn_box=[pmin_μ pmax_μ;pmin_λ pmax_λ]
84 vn_priors=[Uniform(vmin_μ,vmax_μ),Uniform(vmin_λ,vmax_λ)]
85 vn_box=[vmin_μ vmax_μ;vmin_λ vmax_λ]
86
87 n_models=50
88
89 for (pth,prior,box,eststring) in zip([pne_pth, vne_pth],[pn_priors,
    ↪ vn_priors],[pn_box,vn_box],["PopEst","VolEst"])
90     for (nx,x) in enumerate(X)
91         enspth=pth*"/$x"
92         if isfile(enspth*"/ens")
93             e=deserialize(enspth*"/ens")
94         else
95             e=Normal_Ensemble(enspth,n_models,filter(i→!iszero(i),
    ↪ measure_dict[eststring][nx]), prior, box, gmc ... )
96     end
97
98     pth in keys(evdict) ? (evdict[pth] +=
    ↪ converge_ensemble!(e,backup=true,10000),upper_displays=uds,
    ↪ lower_displays=lds, disp_rot_its=10000,
    ↪ converge_factor=1e-6)) : (evdict[pth] =
    ↪ converge_ensemble!(e,backup=true,10000),upper_displays=uds,
    ↪ lower_displays=lds, disp_rot_its=10000,
    ↪ converge_factor=1e-6))
99 end

```

```

100 end

101
102 ln_pmax_mu=log(pmax_mu^2/sqrt(pmax_mu^2 + inv(pmax_lambda)))
103 ln_pmin_mu=log(pmin_mu^2/sqrt(pmin_mu^2 + inv(pmin_lambda)))
104 ln_pmax_lambda=1/log(1+(inv(pmax_lambda)/pmax_mu^2))
105 ln_pmin_lambda=1/log(1+(inv(pmin_lambda)/pmin_mu^2))

106
107 ln_vmax_mu=log(vmax_mu^2/sqrt(vmax_mu^2 + inv(vmax_lambda)))
108 ln_vmin_mu=log(vmin_mu^2/sqrt(vmin_mu^2 + inv(vmin_lambda)))
109 ln_vmax_lambda=1/log(1+(inv(vmax_lambda)/vmax_mu^2))
110 ln_vmin_lambda=1/log(1+(inv(vmin_lambda)/vmin_mu^2))

111
112 pln_priors=[Uniform(ln_pmin_mu,ln_pmax_mu),Uniform(ln_pmin_lambda,ln_pmax_lambda)]
113 pln_box=[ln_pmin_mu ln_pmax_mu;ln_pmin_lambda ln_pmax_lambda]
114 vln_priors=[Uniform(ln_vmin_mu,ln_vmax_mu),Uniform(ln_vmin_lambda,ln_vmax_lambda)]
115 vln_box=[ln_vmin_mu ln_vmax_mu;ln_vmin_lambda ln_vmax_lambda]

116
117 for (pth,prior,box,eststring) in zip([plne_pth, vlne_pth],[pn_priors,
    ↪ vn_priors],[pn_box,vn_box],["PopEst","VolEst"])
    for (nx,x) in enumerate(X)
        enspth=pth*"/$x"
        if isfile(enspth*"/ens")
            e=deserialize(enspth*"/ens")
        else
            ↪ e=LogNormal_Ensemble(enspth,n_models,filter(i→i>0,measure_dict[estst
                ↪ prior, box, gmc ... )
        end
    end
    pth in keys(evdict) ? (evdict[pth] +=
        ↪ converge_ensemble!(e,backup=true,10000),upper_displays=uds,
        ↪ lower_displays=lds, disp_rot_its=10000,
        ↪ converge_factor=1e-6)) : (evdict[pth] =
        ↪ converge_ensemble!(e,backup=true,10000),upper_displays=uds,
        ↪ lower_displays=lds, disp_rot_its=10000,
        ↪ converge_factor=1e-6)
end
end

pop_evidence_ratio=evdict[plne_pth]-evdict[pne_pth]
vol_evidence_ratio=evdict[vlne_pth]-evdict[vne_pth]

```

```

133 println("CMZ Population & $(round(evdict[pne_pth],digits=3)) &
→   $(round(evdict[plne_pth],digits=3)) &
→   $(round(pop_evidence_ratio,digits=3)) &
→   $(pop_evidence_ratio.val/pop_evidence_ratio.err)\\\\\\ \\hline")
134 println("Estimated Retinal Volume & $(round(evdict[vne_pth],digits=3)) &
→   $(round(evdict[vlne_pth],digits=3)) &
→   $(round(vol_evidence_ratio,digits=3)) &
→   $(vol_evidence_ratio.val/vol_evidence_ratio.err)\\\\\\ \\hline")

```

16.9.5 /cmz/a10periodisation.jl

```

1 using CSV,DataFrames,Distributions,StatsBase,GMC_NS,Serialization,
→   CMZNicheSims, Random, KernelDensityEstimate,
→   KernelDensityEstimatePlotting, Plots, Gadfly
2 gr()
3 default(legendfont = (10), guidefont = (12), tickfont = (10))
4
5 Random.seed!(786)
6
7 a10pth="/bench/PhD/datasets/A10 measurements 2018update.csv"
8 e2ph="/bench/PhD/NGS_binaries/CNS/A10/e2ph"
9 e3ph="/bench/PhD/NGS_binaries/CNS/A10/e3ph"
10
11 paths=[e2ph, e3ph]
12
13 a10df=DataFrame(CSV.read(a10pth))
14 a10df.BSR=[string(row.Block,',',row.Slide,',',row.Row) for row in
→   eachrow(a10df)]
15
16 X=Vector{Float64}()
17 measure_dict=Dict{String,Vector{Vector{Float64}}}()
18
19 measure_dict["PopEst"]=Vector{Vector{Float64}}()
20 measure_dict["VolEst"]=Vector{Vector{Float64}}()
21 measure_dict["SphEst"]=Vector{Vector{Float64}}()
22 measure_dict["CircEst"]=Vector{Vector{Float64}}()
23 measure_dict["ThiEst"]=Vector{Vector{Float64}}()
24
25 #EXTRACT ALL MEASUREMENTS FROM DF TO VECTORS
26 for t_df in groupby(a10df, "Time point (d)")
27     push!(X,Float64(unique(t_df."Time point (d)")[1]))
28     pevec,vevec,tvec,ldvec,spvec,cvec,thivec = [zeros(0) for i in 1:7]

```

```

29
30     for i_df in groupby(t_df, "BSR")
31         length([skipmissing(i_df."Lens diameter") ... ])>0 &&
32             → push!(ldvec,mean(skipmissing(i_df."Lens diameter")))
33     end
34
35     for i_df in groupby(t_df, "BSR")
36         if length([skipmissing(i_df."CMZ Sum") ... ])>0 &&
37             → mean(skipmissing(i_df."CMZ Sum")) > 0
38             push!(tvec,mean(skipmissing(i_df."CMZ Sum")))
39             if length([skipmissing(i_df."Lens diameter") ... ])>0
40                 push!(pevec,mean(skipmissing(i_df."CMZ
41                     → Sum"))*mean(skipmissing(i_df."Lens diameter"))/14.)
42             else
43                 push!(pevec,mean(skipmissing(i_df."CMZ
44                     → Sum"))*mean(ldvec)/14.)
45             end
46     end
47     if length([skipmissing(i_df."Retina thickness") ... ])>0 &&
48         length([skipmissing(i_df."IPL") ... ])>0 &&
49         length([skipmissing(i_df."OPL") ... ])>0 &&
50         length([skipmissing(i_df."RPE length") ... ])>0
51
52         rthi=mean(skipmissing(i_df."Retina
53             → thickness"))-mean(skipmissing(i_df."OPL"))-mean(skipmissing(i_df."IPL"))
54         push!(thivec,rthi)
55         rpel=mean(skipmissing(i_df."RPE length"))
56         if length([skipmissing(i_df."Lens diameter") ... ])>0
57             rcirc=rpel+mean(skipmissing(i_df."Lens diameter"))
58         else
59             rcirc=rpel+mean(ldvec)
60         end
61
62         push!(cvec,rcirc)
63
64         or=rcirc/2π
65         ir=or-rthi
66
67         ov=(4/3)*π*(or^3)
68         iv=(4/3)*π*(ir^3)
69
70         push!(spvec,(ov-iv)*(4/5))
71     end

```

```

67     end
68
69     push!(measure_dict["PopEst"],pevec)
70     push!(measure_dict["SphEst"],spvec)
71     push!(measure_dict["CircEst"],cvec)
72     push!(measure_dict["ThiEst"],thivec)
73 end
74
75 obs=[(measure_dict["PopEst"][t],measure_dict["SphEst"][t]) for t in
76      1:length(X)]
77
78 nucpop3d=2075.6 #from a38 sib_measure_dict NucPop mean
79 or=mean(measure_dict["CircEst"][[1]])/2π
80 ir=or-.5*mean(measure_dict["ThiEst"][[1]])
81 sxvol_3d=((π*or^2)-(π*ir^2))*14)*(4/5)
82 vol_const=sxvol_3d/nucpop3d
83 mc_its=Int64(1.e6)
84
85 popdist=fit(LogNormal,measure_dict["PopEst"][[1]])
86 voldist=fit(LogNormal,measure_dict["SphEst"][[1]])
87
88 ph2_constants=[X, popdist, voldist, vol_const, mc_its, 2]
89 ph3_constants=[X, popdist, voldist, vol_const, mc_its, 3]
90 constants=[ph2_constants, ph3_constants]
91
92 cycle_prior=[Uniform(10.,144.), LogNormal(log(.9),log(2))]
93 end_prior=[Uniform(4.,359.)]
94
95 phase_max=[144.,5.]
96 phase_min=[10.,0.]
97 time_max=359
98 time_min=4.
99
100 function compose_priors(phases)
101     prs=Vector{Vector{Distribution}}()
102     bxes=Vector{Matrix{Float64}}()
103     for p in phases
104         pr=[repeat(cycle_prior,p) ... ,repeat(end_prior,p-1) ... ]
105         push!(prs,pr)
106         bx=hcat([repeat(phase_min,p) ... ,fill(time_min,p-1) ... ],
107                  [repeat(phase_max,p) ... ,fill(time_max,p-1) ... ])
108         push!(bxes,GMC_NS.to_unit_ball.(bx,pr))
109     end

```

```

109     return prs, bxes
110 end

111
112 phases=[2,3]
113 priors,boxes=compose_priors(phases)

114
115 uds=Vector{Vector{Function}}([[[],[liwi_display],[convergence_display]])}
116 lds=Vector{Vector{Function}}([[model_obs_display],[ensemble_display],[ensemble_displa

117
118 gmc_settings=GMC_DEFAULTS
119 gmc_settings[end]=1000

120
121
122 for (pth,prior,constants,box) in zip(paths,priors,constants,boxes)
123     if isfile(pth*"/ens")
124         e=deserialize(pth*"/ens")
125     else
126         e=CMZ_Ensemble(pth,3000,obs, prior, constants, box, gmc_settings)
127     end
128
129     converge_ensemble!(e,backup=(true,50),upper_displays=uds,
130                         lower_displays=lds, disp_rot_its=100, mc_noise=.3,
131                         converge_criterion="compression", converge_factor=1.)
132 end

133 e2=deserialize(e2ph*"/ens")
134 e3=deserialize(e3ph*"/ens")

135 e2ev=measure_evidence(e2)
136 e3ev=measure_evidence(e3)

137
138 evidence_ratio=e2ev-e3ev

139
140 println("\\begin{tabular}{l|l|l|l|l}")
141 println("\\hline")
142 println("${\\bf 2-phase logZ} & ${\\bf 3-phase logZ} & ${\\bf logZR} & ${\\bf
143     \\$\\sigma\$ Significance}\\ \\hline")
144 println("${\\textbf{(round(e2ev,digits=3))}} & ${\\textbf{(round(e3ev,digits=3))}} &
145     ${\\textbf{(round(evidence_ratio,digits=3))}} &
146     ${\\textbf{(round(evidence_ratio.val/evidence_ratio.err,digits=3))}}\\\\\\
147     \\hline")
148
149 println("\\end{tabular}")

```

```

146 map2=deserialize(e2.models[findmax([m.log_Li for m in
    ↵ e2.models])[2]].path)
147 map3=deserialize(e3.models[findmax([m.log_Li for m in
    ↵ e3.models])[2]].path)
148
149 println("\\begin{tabular}{l|l|l|}")
150 println("\\hline")
151 println("{\\bf Parameter} & {\\bf 2-phase MAP} & {\\bf 3-phase MAP}\\\
    ↵ \\hline")
152 println("Phase 1 \$CT\$ (h) & $(round(map2.θ[1], digits=1)) &
    ↵ $(round(map3.θ[1], digits=1))\\\\\\hline")
153 println("Phase 1 \$\\epsilon\$ & $(round(map2.θ[2], digits=2)) &
    ↵ $(round(map3.θ[2], digits=2))\\\\\\hline")
154 println("Phase 2 \$CT\$ (h) & $(round(map2.θ[3], digits=1)) &
    ↵ $(round(map3.θ[3], digits=1))\\\\\\hline")
155 println("Phase 2 \$\\epsilon\$ & $(round(map2.θ[4], digits=2)) &
    ↵ $(round(map3.θ[4], digits=2))\\\\\\hline")
156 println("Phase 3 \$CT\$ (h) & NA & $(round(map3.θ[5], digits=1))\\\\\
    ↵ \\hline")
157 println("Phase 3 \$\\epsilon\$ & NA & $(round(map3.θ[6], digits=2))\\\\\
    ↵ \\hline")
158 println("Transition 1 age & $(round(map2.θ[5], digits=1)) &
    ↵ $(round(map3.θ[7], digits=1))\\\\\\hline")
159 println("Transition 2 age & NA & $(round(map3.θ[8], digits=1))\\\\\
    ↵ \\hline")
160 println("\\end{tabular}")

161
162 X=e2.constants[1]
163 plotticks=[30*i for i in 1:12]

164
165 catpobs=vcat([e2.obs[t][1] for t in 1:length(X)]...)
166 catvobs=vcat([e2.obs[t][2] for t in 1:length(X)]...)
167 Xs=vcat([[X[n] for i in 1:length(e2.obs[n][1])] for n in 1:length(X)]...)

168
169 p2_mean=map2.disp_mat[:,2,1]
170 p2_upper=map2.disp_mat[:,1,1]-map2.disp_mat[:,2,1]
171 p2_lower=map2.disp_mat[:,2,1]-map2.disp_mat[:,3,1]

172
173 map2_popplt=scatter(Xs,catpobs, marker=:cross, color=:black,
    ↵ markersize=3, label="CMZ population estimate", ylabel="Population",
    ↵ showaxis=:y, xticks=plotticks, xformatter=_→'', legend=:top,
    ↵ background_color_legend=nothing)

```

```

174 plot!(map2_popplt, X, p2_mean, ribbon=(p2_lower,p2_upper),
    ↳ color=:darkmagenta, label="2-phase model population")
175 lens!([2, 32], [300, 5700], inset = (1, bbox(0.6, 0.0, 0.38, 0.7)))
176 annotate!([(30,1.25e4,Plots.text("A1",18))])

177
178 v2_mean=map2.disp_mat[:,2,2]
179 v2_upper=map2.disp_mat[:,1,2]--map2.disp_mat[:,2,2]
180 v2_lower=map2.disp_mat[:,2,2]--map2.disp_mat[:,3,2]

181
182 map2_volplt=scatter(Xs,catvobs, marker=:cross, color=:black,
    ↳ markersize=3, label="Retinal volume estimate", ylabel="Volume
    ↳ ( $\mu\text{m}^3$ )", xlabel="Age (dpf)", legend=:topleft, showaxis=:y,
    ↳ xticks=plotticks, xformatter=_ $\rightarrow$  "", background_color_legend=nothing)
183 plot!(map2_volplt, X, v2_mean, ribbon=(v2_lower,v2_upper), color=:green,
    ↳ label="2-phase model volume")
184 lens!([2, 32], [2e6, 3e7], inset = (1, bbox(0.6, 0.0, 0.34, 0.5)))
185 annotate!([(30,3.5e8,Plots.text("A2",18))])

186
187
188 p3_mean=map3.disp_mat[:,2,1]
189 p3_upper=map3.disp_mat[:,1,1]--map3.disp_mat[:,2,1]
190 p3_lower=map3.disp_mat[:,2,1]--map3.disp_mat[:,3,1]

191
192 map3_popplt=scatter(Xs,catpobs, marker=:cross, color=:black,
    ↳ markersize=3, label="CMZ population estimate", ylabel="Population",
    ↳ showaxis=:y, xticks=plotticks, xformatter=_ $\rightarrow$  "", legend=:top,
    ↳ background_color_legend=nothing)
193 plot!(map3_popplt, X, p3_mean, ribbon=(p3_lower,p3_upper),
    ↳ color=:darkmagenta, label="3-phase model population")
194 lens!([2, 32], [300, 5700], inset = (1, bbox(0.6, 0.0, 0.38, 0.7)))
195 annotate!([(30,1.5e4,Plots.text("B1",18))])

196
197 v3_mean=map3.disp_mat[:,2,2]
198 v3_upper=map3.disp_mat[:,1,2]--map3.disp_mat[:,2,2]
199 v3_lower=map3.disp_mat[:,2,2]--map3.disp_mat[:,3,2]

200
201 map3_volplt=scatter(Xs,catvobs, marker=:cross, color=:black,
    ↳ markersize=3, label="Retinal volume estimate", ylabel="Volume
    ↳ ( $\mu\text{m}^3$ )", xlabel="Age (dpf)", legend=:topleft, xticks=plotticks,
    ↳ background_color_legend=nothing)
202 plot!(map3_volplt, X, v3_mean, ribbon=(v3_lower,v3_upper), color=:green,
    ↳ label="3-phase model volume")
203 lens!([2, 32], [2e6, 3e7], inset = (1, bbox(0.6, 0.0, 0.34, 0.5)))

```

```

204 annotate!([(30,3.5e8,Plots.text("B2",18))])
205
206 combined_map=Plots.plot(map2_popplt,map2_volplt,map3_popplt,map3_volplt,layout=grid(4
    ↵   size=(1200,1200), link=:x)
207
208 savefig(combined_map,"/bench/PhD/Thesis/images/cmz/a10pMAP.png")
209
210 kde2=posterior_kde(e2)
211
212 ph1marg=marginal(kde2,[1;2])
213 ph2marg=marginal(kde2,[3;4])
214 ph12marg=marginal(kde2,[1;3])
215 transmarg=marginal(kde2,[5])
216
217 ph1mplt=KernelDensityEstimatePlotting.plot(ph1marg;dimLbls=[ "Phase 1 CT
    ↵   (hr)", "Phase 1 e rate"], axis=[0. 144.; 0. 4.])
218 ph2mplt=KernelDensityEstimatePlotting.plot(ph2marg;dimLbls=[ "Phase 2 CT
    ↵   (hr)", "Phase 2 e rate"], axis=[0. 144.; 0. 4.])
219 ph12mplt=KernelDensityEstimatePlotting.plot(ph12marg; dimLbls=[ "Phase 1
    ↵   CT (hr)", "Phase 2 CT (hr)"],axis=[0. 144.; 0. 144.])
220 tmplt=KernelDensityEstimatePlotting.plotKDE(transmarg,xlbl="Phase
    ↵   transition age (dpf)", points=false, c=["green"])
221
222 combined_marg=Gadfly.gridstack([ph1mplt ph12mplt; ph2mplt tmplt])
223 img=SVG("/bench/PhD/Thesis/images/cmz/a10pmarginals.svg",24cm,24cm)
224 draw(img,combined_marg)

```

16.9.6 /cmz/a10popsurvey.jl

```

1 using CSV,DataFrames,Distributions,NGRefTools,StatsBase,Plots
2 gr()
3 default(legendfont = (10), guidefont = (11), tickfont = (10))
4
5 a10pth="/bench/PhD/datasets/A10 measurements 2018update.csv"
6
7 a10df=DataFrame(CSV.read(a10pth))
8 a10df.BSR=[string(row.Block,',',row.Slide,',',row.Row) for row in
    ↵ eachrow(a10df)]
9
10 X=Vector{Float64}()
11 measure_dict=Dict{String,Vector{Vector{Float64}}}()
12

```

```

13 measure_dict["PopEst"] = Vector{Vector{Float64}}}()
14 measure_dict["CMZ Sum"] = Vector{Vector{Float64}} }()
15 measure_dict["Dorsal CMZ (#)"] = Vector{Vector{Float64}} }()
16 measure_dict["Ventral CMZ (#)"] = Vector{Vector{Float64}} }()
17 measure_dict["VolEst"] = Vector{Vector{Float64}} }()
18 measure_dict["Retina thickness"] = Vector{Vector{Float64}} }()
19 measure_dict["RPE length"] = Vector{Vector{Float64}} }()
20 measure_dict["Lens diameter"] = Vector{Vector{Float64}} }()
21 measure_dict["ON diameter"] = Vector{Vector{Float64}} }()
22 measure_dict["ONL"] = Vector{Vector{Float64}} }()
23 measure_dict["OPL"] = Vector{Vector{Float64}} }()
24 measure_dict["INL"] = Vector{Vector{Float64}} }()
25 measure_dict["IPL"] = Vector{Vector{Float64}} }()
26 measure_dict["GCL"] = Vector{Vector{Float64}} }()
27 measure_dict["Pop/VolEst"] = Vector{Vector{Float64}}}()

28
29 #EXTRACT ALL MEASUREMENTS FROM DF TO VECTORS
30 for t_df in groupby(a10df, "Time point (d)")
31     push!(X, Float64(unique(t_df."Time point (d)")[1]))
32     pevec, tvec, dvec, vvec, vevec, rtvec, rplvec, rptvec, ldvec, ondvec,
33     → onlvec, oplvec, inlvec, iplvec, gclvec, pvest = [zeros(0) for i
34     → in 1:16]

35
36     for i_df in groupby(t_df, "BSR")
37         if length([skipmissing(i_df."Lens diameter") ... ]) > 0 &&
38             → push!(ldvec, mean(skipmissing(i_df."Lens diameter")))
39     end

40
41     for i_df in groupby(t_df, "BSR")
42         ipop=false
43         ivol=false
44         if length([skipmissing(i_df."CMZ Sum") ... ]) > 0
45             push!(tvec, mean(skipmissing(i_df."CMZ Sum")))
46             if length([skipmissing(i_df."Lens diameter") ... ]) > 0
47                 push!(pevec, mean(skipmissing(i_df."CMZ
48                 → Sum")) * mean(skipmissing(i_df."Lens diameter")) / 14.)
49                 ipop=true
50             else
51                 push!(pevec, mean(skipmissing(i_df."CMZ
52                 → Sum")) * mean(ldvec) / 14.)
53                 ipop=true
54             end
55         end
56     end

```

```

51
52     length([skipmissing(i_df."Dorsal CMZ (#)") ... ])>0 &&
53         ↳ push!(dvec,mean(skipmissing(i_df."Dorsal CMZ (#)")))
54     length([skipmissing(i_df."Ventral CMZ (#)") ... ])>0 &&
55         ↳ push!(vvec,mean(skipmissing(i_df."Ventral CMZ (#)")))
56     if length([skipmissing(i_df."Retina thickness") ... ])>0 &&
57         length([skipmissing(i_df."IPL") ... ])>0 &&
58         length([skipmissing(i_df."OPL") ... ])>0 &&
59         length([skipmissing(i_df."RPE length") ... ])>0
60
61         rthi=mean(skipmissing(i_df."Retina
62             ↳ thickness"))-mean(skipmissing(i_df."OPL"))-mean(skipmissing(i_df."IPL"))
63         rpel=mean(skipmissing(i_df."RPE length"))
64         if length([skipmissing(i_df."Lens diameter") ... ])>0
65             rcirc=rpel+mean(skipmissing(i_df."Lens diameter"))
66         else
67             rcirc=rpel+mean(ldvec)
68         end
69
70         or=rcirc/2π
71         ir=or-.5*rthi
72
73         ov=(4/3)*π*(or^3)
74         iv=(4/3)*π*(ir^3)
75
76         push!(vevec,(ov-iv)*(4/5))
77     end
78     length([skipmissing(i_df."Retina thickness") ... ])>0 &&
79         ↳ push!(rtvec,mean(skipmissing(i_df."Retina thickness")))
80     length([skipmissing(i_df."RPE length") ... ])>0 &&
81         ↳ push!(rptvec,mean(skipmissing(i_df."RPE length")))
82     length([skipmissing(i_df."ON diameter") ... ])>0 &&
83         ↳ push!(ondvec,mean(skipmissing(i_df."ON diameter")))
84     length([skipmissing(i_df.ONL) ... ])>0 &&
85         ↳ push!(onlvec,mean(skipmissing(i_df.ONL)))
86     length([skipmissing(i_df.OPL) ... ])>0 &&
87         ↳ push!(oplvec,mean(skipmissing(i_df.OPL)))
88     length([skipmissing(i_df.INL) ... ])>0 &&
89         ↳ push!(inlvec,mean(skipmissing(i_df.INL)))
90     length([skipmissing(i_df.IPL) ... ])>0 &&
91         ↳ push!(iplvec,mean(skipmissing(i_df.IPL)))
92     length([skipmissing(i_df.GCL) ... ])>0 &&
93         ↳ push!(gclvec,mean(skipmissing(i_df.GCL)))

```

```

83     ivol && ipop &&
84     push!(pvest,
85     mean(skipmissing(i_df."CMZ Sum"))/
86     ((mean(skipmissing(i_df."Retina
87     → thickness"))-mean(skipmissing(i_df."OPL"))-mean(skipmissing(i_df."IPL
88     *mean(skipmissing(i_df."RPE length"))*(π/4)))
89   end
90
91   push!(measure_dict["PopEst"],pevec)
92   push!(measure_dict["CMZ Sum"],tvec)
93   push!(measure_dict["Dorsal CMZ (#)"],dvec)
94   push!(measure_dict["Ventral CMZ (#)"],vvec)
95   push!(measure_dict["VolEst"],vevec)
96   push!(measure_dict["Retina thickness"],rtvec)
97   push!(measure_dict["RPE length"],rptvec)
98   push!(measure_dict["Lens diameter"],ldvec)
99   push!(measure_dict["ON diameter"],ondvec)
100  push!(measure_dict["ONL"],onlvec)
101  push!(measure_dict["OPL"],oplvec)
102  push!(measure_dict["INL"],inlvec)
103  push!(measure_dict["IPL"],iplvec)
104  push!(measure_dict["GCL"],gclvec)
105  push!(measure_dict["Pop/VolEst"],pvest)
106 end
107 #TEST IF DATA IS BETTER MODELLED NORMALLY OR LOGNORMALLY
108 for ms in ["CMZ Sum", "Lens diameter", "PopEst", "RPE length", "Retina
109   → thickness", "VolEst"]
110   normal_mts=[fit(Normal,measure_dict[ms][n]) for n in 1:length(X)]
111   logn_mts=[fit(LogNormal,filter!(i→!iszero(i),measure_dict[ms][n]))
112   → for n in 1:length(X)]
113
113   normal_lh=sum([sum(logpdf(normal_mts[n],measure_dict[ms][n])) for n
114   → in 1:length(X)])
115   logn_lh=sum([sum(logpdf(logn_mts[n],measure_dict[ms][n])) for n in
116   → 1:length(X)])
117
117   println("$ms & $(round(normal_lh,digits=3)) &
118   → $(round(logn_lh,digits=3)) &
119   → $(round(logn_lh-normal_lh,digits=3))\\\\ \\hline")
116 end
117
118

```

```

119 pe_n_mts=[fit(MarginalTDist,measure_dict["PopEst"][[n]) for n in
    ↵ 1:length(X)]
120 pen_lower=[mean(mt)-quantile(mt,.025) for mt in pe_n_mts]
121 pen_upper=[quantile(mt,.975)-mean(mt) for mt in pe_n_mts]
122
123 pe_logn_mts=[fit(MarginalTDist,log.(filter!(i→!iszero(i),measure_dict["PopEst"][[n])))
    ↵ for n in 1:length(X)]
124 pe_mean=[exp(mean(mt)) for mt in pe_logn_mts]
125 pe_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in pe_logn_mts]
126 pe_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in pe_logn_mts]
127
128 plotticks=[30*i for i in 1:12]
129
130 ann_chart=scatter(vcat([[X[n] for i in
    ↵ 1:length(measure_dict["PopEst"][[n])]] for n in
    ↵ 1:length(X)] ... ),vcat(measure_dict["PopEst"] ... ), marker=:cross,
    ↵ color=:black, markersize=3, label="Pop. data", ylabel="Est. CMZ
    ↵ annulus population", showaxis=:y, xticks=plotticks, xformatter=_→"")
131 plot!(ann_chart, X, pe_mean, ribbon=(pe_lower,pe_upper),
    ↵ color=:darkmagenta, label="Pop. mean")
132 lens!([2, 20], [500, 2500], inset = (1, bbox(0.3, 0.1, 0.45, 0.45)))
133 annotate!([(8,4500,Plots.text("A",18))])
134
135 poprate_mean,poprate_lower,poprate_upper=[Vector{Float64}() for i in 1:3]
136 for (nx, x) in enumerate(X)
137     if nx > 1
138         d=X[nx]-X[nx-1]
139         l,m,u=MTDist_MC_func((a,b)→((exp(a)-exp(b))/d),
            ↵ [log.(filter!(i→!iszero(i),measure_dict["PopEst"][[nx])),),
            ↵ log.(filter!(i→!iszero(i),measure_dict["PopEst"][[nx-1]])),,
            ↵ summary=true)
140         push!(poprate_mean,m);push!(poprate_lower,m-l);push!(poprate_upper,u-m)
141     end
142 end
143
144 poprate_chart=plot(X[2:end],poprate_mean,ribbon=(poprate_lower,
    ↵ poprate_upper), color=:grey, ylabel="Est. CMZ pop. rate of change
    ↵ (cells/d)", label="Simulated mean rate", legend=:topright,
    ↵ showaxis=:y, xticks=plotticks, xformatter=_→"")
145 annotate!([(8,400,Plots.text("B",18))])
146
147

```

```

148 l3,m3,u3=MTDist_MC_func((a,b)→(exp(a)-exp(b))/5.,
  ↵ [filter!(isinf,log.(measure_dict["PopEst"])[2])),
  ↵ filter!(isinf,log.(measure_dict["PopEst"])[1])), summary=true)

149
150 ratedist23=MTDist_MC_func((a,b)→(exp(a)-exp(b))/6.,
  ↵ [filter!(isinf,log.(measure_dict["PopEst"])[6])),
  ↵ filter!(isinf,log.(measure_dict["PopEst"])[5])), summary=false)

151
152 println("3dpf poprate mean: $(m3) 23dpf poprate mean
  ↵ $(mean(ratedist23))poprate 23dpf quantile :
  ↵ $(quantile(ratedist23,.025)))")

153
154 ve_logn_mts=[fit(MarginalTDist,log.(filter!(i→!iszero(i),measure_dict["VolEst"])[n]))
  ↵ for n in 1:length(X)]
155 ve_mean=[exp(mean(mt)) for mt in ve_logn_mts]
156 ve_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in ve_logn_mts]
157 ve_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in ve_logn_mts]

158
159 vol_chart=scatter(vcat([[X[n] for i in
  ↵ 1:length(measure_dict["VolEst"])[n]]) for n in
  ↵ 1:length(X)] ... ),vcat(measure_dict["VolEst"] ... ), marker=:cross,
  ↵ color=:black, markersize=3, label="Vol. data", ylabel="Est. retinal
  ↵ volume ( $\mu\text{m}^3$ )", showaxis=:y, xticks=plotticks, xformatter=_→"",
  ↵ legend=:bottomright)
160 plot!(vol_chart, X, ve_mean, ribbon=(ve_lower,ve_upper), color=:blue,
  ↵ label="Vol. mean")
161 lens!([2, 20], [2.5e6, 1e7], inset = (1, bbox(0.12, 0., 0.39, 0.40)))
162 annotate!([(8,4.5e8,Plots.text("C",18))])

163
164 println($"(ccdf(ve_logn_mts[2],log(ve_mean[1]))) % of marginal posterior
  ↵ mass of 5dpf volume estimate is greater than the 3dpf estimated
  ↵ mean")

165
166 volrate_mean,volrate_lower,volrate_upper=[Vector{Float64}() for i in 1:3]
167 for (nx, x) in enumerate(X)
  if nx > 1
    d=X[nx]-X[nx-1]
168    l,m,u=MTDist_MC_func((a,b)→(exp(a)-exp(b))/d,
      ↵ [filter!(isinf,log.(measure_dict["VolEst"])[nx])),
      ↵ filter!(isinf,log.(measure_dict["VolEst"])[nx-1])), summary=true)
169
170    push!(volrate_mean,m);push!(volrate_lower,l);push!(volrate_upper,u)

```

```

172     end
173 end
174 volrate_lower=volrate_mean--volrate_lower
175 volrate_upper=volrate_upper--volrate_mean
176
177 volrate_chart=plot(X[2:end],volrate_mean,ribbon=(volrate_lower,
    ↵  volrate_upper), color=:grey, label="Simulated mean rate",
    ↵  legend=:topright, xlabel="Age(dpf)", ylabel="Est. volume rate
    ↵  ( $\mu\text{m}^3\text{d}^{-1}$ )", xticks=plotticks)
178 annotate!([(8,4.5e6,Plots.text("D",18))])
179
180 l=@layout [a{0.3h}
181             b{0.2h}
182             c{0.3h}
183             d]
184 combined=plot(ann_chart,poprate_chart,vol_chart,volrate_chart,layout=(4,1),size=(1200
185 savefig(combined, "/bench/PhD/Thesis/images/cmz/CMZoverall.png")
186
187 l30,m30,u30=MTDist_MC_func((a,b)→(exp(a)-exp(b))/7.,
    ↵  [filter{!isinf,log.(measure_dict["VolEst"][[7]])},
    ↵  filter{!isinf,log.(measure_dict["VolEst"][[6]])}], summary=true)
188
189 ratedist60=MTDist_MC_func((a,b)→(exp(a)-exp(b))/30.,
    ↵  [filter{!isinf,log.(measure_dict["VolEst"][[8]])},
    ↵  filter{!isinf,log.(measure_dict["VolEst"][[7]])}], summary=false)
190
191 println("6*30dpf volrate mean: $(6*m30) volrate 60dpf quantile :
    ↵  $(quantile(ratedist60,.01))")
```

16.9.7 /cmz/a19lineagetrace.jl

```

1 using CSV,DataFrames,Distributions,NGRefTools,StatsBase,Plots,
    ↵  Measurements,GMC_NS,Serialization
2 gr()
3
4 a19_1pth="/bench/PhD/datasets/A19GR1.csv"
5 a19_2pth="/bench/PhD/datasets/A19GR2.csv"
6 a19_3pth="/bench/PhD/datasets/A19GR3.csv"
7
8 gr1df=DataFrame(CSV.read(a19_1pth))
9 gr2df=DataFrame(CSV.read(a19_2pth))
10 gr3df=DataFrame(CSV.read(a19_3pth))
```

```

11
12 X=sort(unique(gr1df."Pulse age (dpf)"))
13 t_measure_dict=Dict{String,Vector{Vector{Float64}}}()
14 d_measure_dict=Dict{String,Vector{Vector{Float64}}}()
15 v_measure_dict=Dict{String,Vector{Vector{Float64}}}()
16
17 mds=[t_measure_dict,d_measure_dict,v_measure_dict]
18
19 for md in mds
20     md["ONL"]=[zeros(0) for i in 1:length(X)]
21     md["INL"]=[zeros(0) for i in 1:length(X)]
22     md["GCL"]=[zeros(0) for i in 1:length(X)]
23     md["Isl"]=[zeros(0) for i in 1:length(X)]
24     md["Pax6"]=[zeros(0) for i in 1:length(X)]
25     md["Isl_Pax6"]=[zeros(0) for i in 1:length(X)]
26     md["INL_Pax6"]=[zeros(0) for i in 1:length(X)]
27     md["PKCB"]=[zeros(0) for i in 1:length(X)]
28     md["GS"]=[zeros(0) for i in 1:length(X)]
29     md["HM"]=[zeros(0) for i in 1:length(X)]
30     md["Zpr1"]=[zeros(0) for i in 1:length(X)]
31 end
32
33 function check_layerfrac(row)
34     !ismissing(row.#GCL) && !ismissing(row.#INL) &&
35         !ismissing(row.#PRL) && !ismissing(row.Total) ? (return true)
36         : (return false)
37 end
38
39 function gr1_dfcheck(subdf)
40     pass=false(size(subdf,1))
41     for (n,row) in enumerate(eachrow(subdf))
42         !ismissing(row.GCL - Isl) && !ismissing(row.GCL - Pax6) &&
43             !ismissing(row.GCL - Isl/Pax6) && !ismissing(row.INL -
44                 Pax6) ? (pass[n]=true) : (pass[n]=false)
45     end
46     all(pass) ? (return true) : (return false)
47 end
48
49 for df in [gr1df,gr2df,gr3df]
50     for agedf in groupby(df, "Pulse age (dpf)")
51         xidx=findfirst(i→i==unique(agedf."Pulse age (dpf)")[1],X)
52         for inddf in groupby(agedf, "Individual")
53             if df ≡ gr1df

```

```

50         if gr1_dfcheck(inddf)
51             clean=false(2)
52             for row in eachrow(inddf)
53                 if row."D/V" == "D"
54                     push!(d_measure_dict["Isl"][xidx],row."GCL -
55                         →  Isl"/row.#GCL")
56                     push!(d_measure_dict["Pax6"][xidx],row."GCL -
57                         →  Pax6"/row.#GCL")
58
59                     →  push!(d_measure_dict["Isl_Pax6"][xidx],row."GCL
60                         →  - Isl/Pax6"/row.#GCL")
61
62                     if check_layerfrac(row)
63                         →  push!(d_measure_dict["GCL"][xidx],row.#GCL"/row.
64
65                         →  push!(d_measure_dict["INL"][xidx],row.#INL"/row.
66
67                         →  push!(d_measure_dict["ONL"][xidx],row.#PRL"/row.
68                         clean[1]=true
69                     end
70             else
71                 push!(v_measure_dict["Isl"][xidx],row."GCL -
72                     →  Isl"/row.#GCL")
73                 push!(v_measure_dict["Pax6"][xidx],row."GCL -
74                     →  Pax6"/row.#GCL")
75
76                     →  push!(v_measure_dict["Isl_Pax6"][xidx],row."GCL
77                         →  - Isl/Pax6"/row.#GCL")
78
79                     →  push!(v_measure_dict["INL_Pax6"][xidx],row."INL
80                         →  - Pax6"/row.#INL")
81             if check_layerfrac(row)
82
83                 →  push!(v_measure_dict["GCL"][xidx],row.#GCL"/row.
84
85                 →  push!(v_measure_dict["INL"][xidx],row.#INL"/row.
86
87                 →  push!(v_measure_dict["ONL"][xidx],row.#PRL"/row.
88                 clean[2]=true
89             end

```

```

75         end
76     end
77     push!(t_measure_dict["Isl"][xidx],sum(inddf."GCL -
78         ↪ Isl")/sum(inddf.#GCL"))
79     push!(t_measure_dict["Pax6"][xidx],sum(inddf."GCL -
80         ↪ Pax6")/sum(inddf.#GCL"))
81     push!(t_measure_dict["Isl_Pax6"][xidx],sum(inddf."GCL
82         ↪ - Isl/Pax6")/sum(inddf.#GCL"))
83     push!(t_measure_dict["INL_Pax6"][xidx],sum(inddf."INL
84         ↪ - Pax6")/sum(inddf.#INL"))
85     if all(clean)
86
87         → push!(t_measure_dict["GCL"][xidx],sum(inddf.#GCL)/sum(i
88
89         → push!(t_measure_dict["INL"][xidx],sum(inddf.#INL)/sum(i
90
91         → push!(t_measure_dict["ONL"][xidx],sum(inddf.#PRL)/sum(i
92     end
93
94     elseif df === gr2df
95         for row in eachrow(inddf)
96             if row."D/V" == "D"
97
98                 → push!(d_measure_dict["PKCB"][xidx],row."INL-PKCB"/row.#I
99
100                → push!(d_measure_dict["GS"][xidx],row."INL-GS"/row.#INL)
101
102                → push!(d_measure_dict["HM"][xidx],row."INL-HM"/row.#INL)
103
104                → push!(d_measure_dict["GCL"][xidx],row.#GCL/row.Total)
105
106                → push!(d_measure_dict["INL"][xidx],row.#INL/row.Total)
107
108                → push!(d_measure_dict["ONL"][xidx],row.#PRL/row.Total)
109
110            else
111
112                → push!(v_measure_dict["PKCB"][xidx],row."INL-PKCB"/row.#I
113
114                → push!(v_measure_dict["GS"][xidx],row."INL-GS"/row.#INL)
115
116                → push!(v_measure_dict["HM"][xidx],row."INL-HM"/row.#INL)
117
118                → push!(v_measure_dict["GCL"][xidx],row.#GCL/row.Total)

```

```

101          ↳ push!(v_measure_dict["INL"][xidx],row.">#INL"/row."Total")
102          ↳ push!(v_measure_dict["ONL"][xidx],row.">#PRL"/row."Total")
103      end
104  end
105
106          ↳ push!(t_measure_dict["PKCB"][xidx],sum(inddf."INL-PKCB")/sum(inddf.
107          ↳ push!(t_measure_dict["GS"][xidx],sum(inddf."INL-GS")/sum(inddf."#
108          ↳ push!(t_measure_dict["HM"][xidx],sum(inddf."INL-HM")/sum(inddf."#
109          ↳ push!(t_measure_dict["GCL"][xidx],sum(inddf."#GCL")/sum(inddf."To
110          ↳ push!(t_measure_dict["INL"][xidx],sum(inddf."#INL")/sum(inddf."To
111  elseif df ≡ gr3df
112      for row in eachrow(inddf)
113          if row."D/V" == "D"
114              push!(d_measure_dict["Zpr1"][xidx],row."PRL -
115                  ↳ Zpr1"/row."#PRL")
116              ↳ push!(d_measure_dict["GCL"][xidx],row.">#GCL"/row."Total")
117              ↳ push!(d_measure_dict["INL"][xidx],row.">#INL"/row."Total")
118              ↳ push!(d_measure_dict["ONL"][xidx],row.">#PRL"/row."Total")
119  else
120      push!(v_measure_dict["Zpr1"][xidx],row."PRL -
121          ↳ Zpr1"/row."#PRL")
122      ↳ push!(v_measure_dict["GCL"][xidx],row.">#GCL"/row."Total")
123      ↳ push!(v_measure_dict["INL"][xidx],row.">#INL"/row."Total")
124      ↳ push!(v_measure_dict["ONL"][xidx],row.">#PRL"/row."Total")
125  end
end
push!(t_measure_dict["Zpr1"][xidx],sum(inddf."PRL -
→ Zpr1")/sum(inddf."#PRL"))

```

```

126      ↳ push!(t_measure_dict["GCL"][xidx],sum(inddf."#GCL")/sum(inddf."To
127      ↳ push!(t_measure_dict["INL"][xidx],sum(inddf."#INL")/sum(inddf."To
128      ↳ push!(t_measure_dict["ONL"][xidx],sum(inddf."#PRL")/sum(inddf."To
129    end
130  end
131 end
132 end
133
134 for d in mds
135   for (k,v) in d
136     for ovec in v
137       if any(i→i==0.,ovec)
138         ovec[findall(i→i==0.,ovec)]*=1e-3
139       end
140     end
141     d[k]=v
142   end
143 end
144
145 onl_n_mts=[fit(MarginalTDist,t_measure_dict["ONL"][n]) for n in
146   ↳ 1:length(X)]
146 onl_mean=[mean(mt) for mt in onl_n_mts]
147 onl_lower=[mean(mt)-quantile(mt,.025) for mt in onl_n_mts]
148 onl_upper=[quantile(mt,.975)-mean(mt) for mt in onl_n_mts]
149
150 inl_n_mts=[fit(MarginalTDist,t_measure_dict["INL"][n]) for n in
151   ↳ 1:length(X)]
151 inl_mean=[mean(mt) for mt in inl_n_mts]
152 inl_lower=[mean(mt)-quantile(mt,.025) for mt in inl_n_mts]
153 inl_upper=[quantile(mt,.975)-mean(mt) for mt in inl_n_mts]
154
155 gcl_ln_mts=[fit(MarginalTDist,log.(t_measure_dict["GCL"][n])) for n in
156   ↳ 1:length(X)]
156 gcl_mean=[exp(mean(mt)) for mt in gcl_ln_mts]
157 gcl_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in gcl_ln_mts]
158 gcl_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in gcl_ln_mts]
159

```

```

160 layers_chart=scatter(vcat([[X[n] for i in
→ 1:length(t_measure_dict["GCL"])[n]])] for n in
→ 1:length(X) ... ), vcat(t_measure_dict["GCL"] ... ), marker=:utriangle,
→ color=:darkmagenta, markersize=3, markerstrokecolor=:darkmagenta,
→ label="GCL data", ylabel="Fraction of cohort in layer", xlabel="Age
→ (dpf)", xticks=X, foreground_color_legend=nothing,
→ background_color_legend=nothing, legend=:inside)
161 plot!(X, gcl_mean, ribbon=(gcl_lower,gcl_upper), color=:darkmagenta,
→ label="GCL mean")
162 scatter!(vcat([[X[n] for i in 1:length(t_measure_dict["INL"])[n]])] for n
→ in 1:length(X) ... ), vcat(t_measure_dict["INL"] ... ), marker=:cross,
→ color=:blue, markersize=3, label="INL data")
163 plot!(X, inl_mean,ribbon=(inl_lower,inl_upper), color=:blue, label="INL
→ mean")
164 scatter!(vcat([[X[n] for i in 1:length(t_measure_dict["ONL"])[n]])] for n
→ in 1:length(X) ... ), vcat(t_measure_dict["ONL"] ... ),
→ marker=:dtriangle, color=:green, markersize=3,
→ markerstrokecolor=:green, label="ONL data")
165 plot!(X, onl_mean,ribbon=(onl_lower,onl_upper), color=:green, label="ONL
→ mean")
166 annotate!([(15,.7,Plots.text("A",18))])
167
168 isl_ln_mts=[fit(MarginalTDist,log.(t_measure_dict["Isl"][n])) for n in
→ 1:length(X)]
169 isl_mean=[exp(mean(mt)) for mt in isl_ln_mts]
170 isl_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in isl_ln_mts]
171 isl_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in isl_ln_mts]
172
173 islchart=scatter(vcat([[X[n] for i in 1:length(t_measure_dict["Isl"])[n]])]
→ for n in 1:length(X) ... ), vcat(t_measure_dict["Isl"] ... ),
→ marker=:square, color=:darkmagenta, markerstrokecolor=:darkmagenta,
→ markersize=3, label="Isl+ data", xticks=X,
→ foreground_color_legend=nothing, background_color_legend=nothing,
→ legend=:bottom, xformatter=_→"", showaxis=:y)
174 plot!(X, isl_mean,ribbon=(isl_lower,isl_upper), color=:darkmagenta,
→ label="Isl+ mean", ylims=[0,.5])
175 annotate!([(15,.05,Plots.text("B",18))])
176
177 pax6_ln_mts=[fit(MarginalTDist,log.(t_measure_dict["Pax6"][n])) for n in
→ 1:length(X)]
178 pax6_mean=[exp(mean(mt)) for mt in pax6_ln_mts]
179 pax6_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in pax6_ln_mts]
180 pax6_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in pax6_ln_mts]

```

```

181
182 pax6chart=scatter(vcat([[X[n] for i in
    ↳ 1:length(t_measure_dict["Pax6"])[n]])] for n in
    ↳ 1:length(X)] ... ), vcat(t_measure_dict["Pax6"] ... ), marker=:circle,
    ↳ color=:darkmagenta, markersize=3, markerstrokecolor=:darkmagenta,
    ↳ label="Pax6+ data", xticks=X, foreground_color_legend=nothing,
    ↳ background_color_legend=nothing, legend=:top, xformatter=_→"",
    ↳ showaxis=:y, ylabel="Fraction of GCL cohort labelled")
183 plot!(X, pax6_mean, ribbon=(pax6_lower,pax6_upper), color=:darkmagenta,
    ↳ label="Pax6+ mean", ylims=[0,.6])
184 annotate!([(15,.45,Plots.text("C",18))])

185
186 islp_ln_mts=[fit(MarginalTDist,log.(t_measure_dict["Isl_Pax6"])[n])) for n
    ↳ in 1:length(X)]
187 islp_mean=[exp(mean(mt)) for mt in islp_ln_mts]
188 islp_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in islp_ln_mts]
189 islp_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in islp_ln_mts]

190
191 islpchart=scatter(vcat([[X[n] for i in
    ↳ 1:length(t_measure_dict["Isl_Pax6"])[n]])] for n in
    ↳ 1:length(X)] ... ), vcat(t_measure_dict["Isl_Pax6"] ... ),
    ↳ marker=:triangle, color=:darkmagenta, markersize=3,
    ↳ markerstrokecolor=:darkmagenta, label="Isl/Pax6+ data", xticks=X,
    ↳ foreground_color_legend=nothing, background_color_legend=nothing,
    ↳ legend=:bottom, xlabel="Age (dpf)")
192 plot!(X, islp_mean, ribbon=(islp_lower,islp_upper), color=:darkmagenta,
    ↳ label="Isl/Pax6+ mean")
193 annotate!([(15,.05,Plots.text("D(+)",18))])

194
195 gcl_subplot=plot(islchart, pax6chart, islpchart, layout=grid(3,1),
    ↳ link=:x)

196
197 pax6i_n_mts=[fit(MarginalTDist,t_measure_dict["INL_Pax6"])[n]) for n in
    ↳ 1:length(X)]
198 pax6i_mean=[mean(mt) for mt in pax6i_n_mts]
199 pax6i_lower=[mean(mt)-quantile(mt,.025) for mt in pax6i_n_mts]
200 pax6i_upper=[quantile(mt,.975)-mean(mt) for mt in pax6i_n_mts]

201

```

```

202 inlpax6chart=scatter(vcat([[X[n] for i in
→ 1:length(t_measure_dict["INL_Pax6"])[n]]) for n in
→ 1:length(X)] ... ),vcat(t_measure_dict["INL_Pax6"] ... ), marker=:square,
→ color=:blue, markersize=3, markerstrokecolor=:blue, label="Pax6+
→ data", xticks=X, foreground_color_legend=nothing,
→ background_color_legend=nothing, legend=:bottom, xformatter=_→"",
→ showaxis=:y)
203 plot!(X, pax6i_mean,ribbon=(pax6i_lower,pax6i_upper), color=:blue,
→ label="Pax6+ mean")
204 annotate!([(15,.075,Plots.text("E(+)"),18))])
205
206 pkc_ln_mts=[fit(MarginalTDist,log.(t_measure_dict["PKCB"])[n])) for n in
→ 1:length(X)]
207 pkc_mean=[exp(mean(mt)) for mt in pkc_ln_mts]
208 pkc_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in pkc_ln_mts]
209 pkc_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in pkc_ln_mts]
210
211 pkcchart=scatter(vcat([[X[n] for i in
→ 1:length(t_measure_dict["PKCB"])[n]]) for n in
→ 1:length(X)] ... ),vcat(t_measure_dict["PKCB"] ... ), marker=:circle,
→ color=:blue, markersize=3, markerstrokecolor=:blue, label="PKCβ+
→ data", xticks=X, foreground_color_legend=nothing,
→ background_color_legend=nothing, legend=:top, xformatter=_→"",
→ showaxis=:y, ylabel="Fraction of INL cohort labelled")
212 plot!(X, pkc_mean,ribbon=(pkc_lower,pkc_upper), color=:blue, label="PKCβ+
→ mean", ylims=[0,.2])
213 annotate!([(15,.18,Plots.text("F(+)"),18))])
214
215 gs_ln_mts=[fit(MarginalTDist,log.(t_measure_dict["GS"])[n])) for n in
→ 1:length(X)]
216 gs_mean=[exp(mean(mt)) for mt in gs_ln_mts]
217 gs_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in gs_ln_mts]
218 gs_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in gs_ln_mts]
219
220 gschart=scatter(vcat([[X[n] for i in 1:length(t_measure_dict["GS"])[n]])
→ for n in 1:length(X)] ... ),vcat(t_measure_dict["GS"] ... ),
→ marker=:diamond, color=:blue, markersize=3, markerstrokecolor=:blue,
→ label="GS+ data", xticks=X, foreground_color_legend=nothing,
→ background_color_legend=nothing, legend=:topright, xformatter=_→"",
→ showaxis=:y)
221 plot!(X, gs_mean,ribbon=(gs_lower,gs_upper), color=:blue, label="GS+
→ mean")
222 annotate!([(15,.125,Plots.text("G(+)"),18))])

```

```

223
224 hm_ln_mts=[fit(MarginalTDist,log.(t_measure_dict["HM"][n])) for n in
  ↪ 1:length(X)]
225 hm_mean=[exp(mean(mt)) for mt in hm_ln_mts]
226 hm_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in hm_ln_mts]
227 hm_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in hm_ln_mts]
228
229 hmchart=scatter(vcat([[X[n] for i in 1:length(t_measure_dict["HM"][n])])
  ↪ for n in 1:length(X)] ... ),vcat(t_measure_dict["HM"] ... ),
  ↪ marker=:cross, color=:blue, markersize=3, markerstrokecolor=:blue,
  ↪ label="HM+ data", xticks=X, foreground_color_legend=nothing,
  ↪ background_color_legend=nothing, legend=:topright, xlabel="Age
  ↪ (dpf)")
230 plot!(X, hm_mean,ribbon=(hm_lower,hm_upper), color=:blue, label="HM+
  ↪ mean")
231 annotate!([(15,.13,Plots.text("H",18))])
232
233 inl_subplot=plot(inlpax6chart, pkcchart, gschart, hmchart,
  ↪ layout=grid(4,1), link=:x)
234
235 z_n_mts=[fit(MarginalTDist,t_measure_dict["Zpr1"][n]) for n in
  ↪ 1:length(X)]
236 z_mean=[mean(mt) for mt in z_n_mts]
237 z_lower=[mean(mt)-quantile(mt,.025) for mt in z_n_mts]
238 z_upper=[quantile(mt,.975)-mean(mt) for mt in z_n_mts]
239
240 zchart=scatter(vcat([[X[n] for i in 1:length(t_measure_dict["Zpr1"][n])])
  ↪ for n in 1:length(X)] ... ),vcat(t_measure_dict["Zpr1"] ... ),
  ↪ marker=:square, color=:green, markerstrokecolor=:green, markersize=3,
  ↪ label="Zpr1+ data", xticks=X, foreground_color_legend=nothing,
  ↪ background_color_legend=nothing, legend=:bottom, xlabel="Age (dpf)",
  ↪ ylabel="Frac. OPL labelled")
241 plot!(X, z_mean,ribbon=(z_lower,z_upper), color=:green, label="HM+ mean",
  ↪ ylims=[0,.55])
242 annotate!([(15,.15,Plots.text("I(Nt)",18))])
243
244 l=@layout [[layers{0.5h};gcl] [inl{0.8h};opl]]
245 combined=plot(layers_chart,gcl_subplot, inl_subplot,zchart,
  ↪ size=(1200,1200),layout=l)
246 savefig(combined, "/bench/PhD/Thesis/images/cmz/layercontributions.png")
247
248 for ms in keys(t_measure_dict)

```

```

249     normal_mts=[fit(Normal,filter!(i→!isnan(i),t_measure_dict[ms][n]))
250     ↪   for n in 1:length(X)]
250     logn_mts=[fit(LogNormal,filter!(i→!isnan(i),t_measure_dict[ms][n]))
251     ↪   for n in 1:length(X)]
251
252     normal_lh=sum([sum(logpdf(normal_mts[n],t_measure_dict[ms][n])) for n
253     ↪   in 1:length(X)])
253     logn_lh=sum([sum(logpdf(logn_mts[n],t_measure_dict[ms][n])) for n in
254     ↪   1:length(X)])
254
255     println("$ms & $(round(normal_lh,digits=3)) &
256     ↪   $(round(logn_lh,digits=3)) &
257     ↪   $(round(logn_lh-normal_lh,digits=3))\\\\ \\hline")
258 end
259
260 gmc=GMC_DEFAULTS
261 gmc[1]=5
262 gmc[2]=1.e-15
263
264
265 n_models=50
266
267 evdict=Dict{String,Measurement}()
268
269 max_μ=1.
270 min_μ=0.
271 max_λ=4000.
272 min_λ=1.
273
274 prior=[Uniform(min_μ,max_μ),Uniform(min_λ,max_λ)]
275 box=[min_μ max_μ;min_λ max_λ]
276
277 for ms in keys(t_measure_dict)
278     for (n,ovec) in enumerate(t_measure_dict[ms])
279         e_basepth="/bench/PhD/NGS_binaries/GMC_NS/A19/n_*${ms}"
280         x=X[n]
281         enspth=e_basepth*"/$x"
282         if isfile(enspth*"/ens")
283             e=deserialize(enspth*"/ens")
284         else

```

```

285         e=Normal_Ensemble(enspth,n_models,filter(i→i>0,ovec), prior,
286                         ← box, gmc ... )
287     end
288
289     "n_"*ms in keys(evdict) ? (evdict["n_"*ms] +=
290         ← converge_ensemble!(e,backup=(true,10000),upper_displays=uds,
291         ← lower_displays=lds, disp_rot_its=10000,
292         ← converge_factor=1e-6)) : (evdict["n_"*ms] =
293         ← converge_ensemble!(e,backup=(true,10000),upper_displays=uds,
294         ← lower_displays=lds, disp_rot_its=10000,
295         ← converge_factor=1e-6))
296   end
297
298   for (n,ovec) in enumerate(t_measure_dict[ms])
299     e_basepth="/bench/PhD/NGS_binaries/GMC_NS/A19/ln_"*ms
300     x=X[n]
301     enspth=e_basepth*"/$x"
302     if isfile(enspth*"/ens")
303       e=deserialize(enspth*"/ens")
304     else
305       e=LogNormal_Ensemble(enspth,n_models,filter(i→i>0,ovec),
306                             ← prior, box, gmc ... )
307     end
308
309     "ln_"*ms in keys(evdict) ? (evdict["ln_"*ms] +=
310         ← converge_ensemble!(e,backup=(true,10000),upper_displays=uds,
311         ← lower_displays=lds, disp_rot_its=10000,
312         ← converge_factor=1e-6)) : (evdict["ln_"*ms] =
313         ← converge_ensemble!(e,backup=(true,10000),upper_displays=uds,
314         ← lower_displays=lds, disp_rot_its=10000,
315         ← converge_factor=1e-6))
316   end
317 end
318
319 for ms in keys(t_measure_dict)
320   nev=evdict["n_"*ms]
321   lnev=evdict["ln_"*ms]
322   ratio=lnev-nev
323   println("$ms & $(round(nev,digits=3)) & $(round(lnev,digits=3)) &
324           ← $(round(ratio,digits=3)) & $(round(ratio.val/ratio.err,
325           ← digits=1))\\\\\\ \\hline")
326 end
327
328
329
330
331

```

```

312 for ms in keys(t_measure_dict)
313     if ms in ["INL_Pax6", "INL", "Isl", "Isl_Pax6", "ONL", "Zpr1"]
314         efunc=Normal_Ensemble
315         pf="cn_"
316     else
317         efunc=LogNormal_Ensemble
318         pf="cln_"
319     end
320
321     ovec=vcat(t_measure_dict[ms] ... )
322     e_basepth="/bench/PhD/NGS_binaries/GMC_NS/A19/"*pf*ms
323
324     if isfile(e_basepth*"/ens")
325         e=deserialize(e_basepth*"/ens")
326     else
327         e=efunc(e_basepth,n_models,filter(i→i>0,ovec), prior, box,
328             ↪ gmc ... )
329     end
330
331     evdict[pf*ms] =
332         → converge_ensemble!(e,backup=(true,10000),upper_displays=uds,
333         → lower_displays=lds, disp_rot_its=10000, converge_factor=1e-6)
334 end
335
336 for ms in keys(t_measure_dict)
337     if ms in ["INL_Pax6", "INL", "Isl", "Isl_Pax6", "ONL", "Zpr1"]
338         pf="n_"
339     else
340         pf="ln_"
341     end
342
343     cev=evdict["c"*pf*ms]
344     sev=evdict[pf*ms]
345     ratio=cev-sev
346     println("$ms & $(round(cev,digits=3)) & $(round(sev,digits=3)) &
347             → $(round(ratio,digits=3)) &
348             → $(round(ratio.val/ratio.err,digits=1))\\\\ \\hline")
349 end
350
351 dv_evdict=Dict{String,Measurement}()
352
353 for ms in keys(d_measure_dict)
354     if ms in ["INL_Pax6", "INL", "Isl", "Isl_Pax6", "ONL", "Zpr1"]

```

```

350         efunc=Normal_Ensemble
351         pf="d_cn_"
352     else
353         efunc=LogNormal_Ensemble
354         pf="d_cln_"
355     end
356
357     ovec=vcat(d_measure_dict[ms] ... )
358     e_basepth="/bench/PhD/NGS_binaries/GMC_NS/A19/*pf*ms
359
360     if isfile(e_basepth*"/ens")
361         e=deserialize(e_basepth*"/ens")
362     else
363         e=efunc(e_basepth,n_models,filter(i→!isnan(i),ovec), prior, box,
364             → gmc ... )
365     end
366
367     dv_evdict[pf*ms] =
368         → converge_ensemble!(e,backup=(true,10000),upper_displays=uds,
369         → lower_displays=lds, disp_rot_its=10000, converge_factor=1e-6)
370 end
371
372 for ms in keys(v_measure_dict)
373     if ms in ["INL_Pax6","INL","Isl","Isl_Pax6","ONL","Zpr1"]
374         efunc=Normal_Ensemble
375         pf="v_cn_"
376     else
377         efunc=LogNormal_Ensemble
378         pf="v_cln_"
379     end
380
381     ovec=vcat(d_measure_dict[ms] ... )
382     e_basepth="/bench/PhD/NGS_binaries/GMC_NS/A19/*pf*ms
383
384     if isfile(e_basepth*"/ens")
385         e=deserialize(e_basepth*"/ens")
386     else
387         e=efunc(e_basepth,n_models,filter(i→!isnan(i),ovec), prior, box,
388             → gmc ... )
389     end
390
391     dv_evdict[pf*ms] =
392         → converge_ensemble!(e,backup=(true,10000),upper_displays=uds,
393         → lower_displays=lds, disp_rot_its=10000, converge_factor=1e-6)
394 end

```

```

387     dv_evdict[pf*ms] =
388         ← converge_ensemble!(e, backup=(true, 10000), upper_displays=uds,
389         ← lower_displays=lds, disp_rot_its=10000, converge_factor=1e-6)
390 end
391
392
393
394 for ms in keys(t_measure_dict)
395     if ms in ["INL_Pax6", "INL", "Isl", "Isl_Pax6", "ONL", "Zpr1"]
396         pf="n_"
397     else
398         pf="ln_"
399     end
400
401     cev=evdict["c"*pf*ms]
402     dev=dv_evdict["d_c"*pf*ms]
403     vev=dv_evdict["v_c"*pf*ms]
404     sev=dev+vev
405     ratio=cev-sev
406     println("$ms & $(round(cev,digits=3)) & $(round(sev,digits=3)) &
407         ← $(round(ratio,digits=3)) &
408         ← $(round(ratio.val/ratio.err,digits=1))\\\\ \\hline")
409 end

```

16.9.8 /cmz/a25GMC_NS.jl

```

1 using GMC_NS, CMZNicheSims, ConjugatePriors, CSV, DataFrames,
2     ← Distributed, Distributions, NGRefTools, ProgressMeter, Serialization
3
4 a10path="/bench/PhD/datasets/A10 measurements 2018update.csv"
5 a10df=DataFrame(CSV.File(a10path))
6
7 df3=a10df[a10df."Time point (d)" .== 3, :]
8
9 inddict=Dict{Tuple,Vector}()
10 for row in eachrow(df3)
11     ind=(row.Block, row.Slide, row.Row)
12     if !(ind in keys(inddict))
13         inddict[ind]=[(row["Dorsal CMZ (#)"]+row["Ventral CMZ (#)"])]
14     else
15         push!(inddict[ind],((row["Dorsal CMZ (#)"]+row["Ventral CMZ
16             ← (#)"])))
17     end

```

```

16 end
17
18 popvec=Vector{Float64}()
19 for (ind, pops) in inddict
20     push!(popvec,mean(pops))
21 end
22
23 a25path="/bench/PhD/datasets/a25.csv"
24 a25df=DataFrame(CSV.File(a25path))
25
26 X=Vector{Float64}()
27 y=Vector{Vector{Int64}}()
28
29 for timedf in groupby(a25df,"Time")
30     push!(X,timedf[1,"Time"])
31     dict=Dict{Tuple,Vector{Int64}}()
32     for row in eachrow(timedf)
33         ind=(row.Block,row.Slide,row.Row)
34         if !(ind in keys(dict))
35             dict[ind]=[row.PCNA,row.EdU]
36         else
37             dict[ind][1]+=row.PCNA
38             dict[ind][2]+=row.EdU
39         end
40     end
41
42     tcounts=Vector{Int64}()
43     for (ind,counts) in dict
44         global popvec=vcat(popvec, counts[1])
45         push!(tcounts, counts[2])
46     end
47     push!(y,tcounts)
48 end
49
50 y=y[sortperm(X)]
51 sort!(X)
52
53 sp_max_mean,sp_max_std=get_lognormal_desc(fit(LogNormal,popvec))
54 sp_max_var=sp_max_std^2
55
56 pop_mu_prior=Uniform(1.,sp_max_mean)
57 pop_std_prior=Uniform(1.,sp_max_var)
58 r_prior=Normal(5,1.5)

```

```

59 tc_prior=NormalInverseGamma(3.,1.,5.,2.)
60 s_prior=NormalInverseGamma(6.,2.5,1.,1.)
61 sister_prior=Beta(8.,75.)
62
63 priors_1_pop=[pop_mu_prior, pop_std_prior, r_prior,
    ↪ marginals(tc_prior) ... , marginals(s_prior) ... , sister_prior]
64 priors_2_pop=vcat(priors_1_pop,priors_1_pop)
65
66 p1_box=[1. sp_max_mean
    1. sp_max_var
    1. 10.
    1. 4.5
    eps() .5
    eps() .5]
67
68
69
70
71
72 p2_box=vcat(p1_box,p1_box)
73
74
75 boxes=[p1_box,p2_box]
76
77 prior_sets=[priors_1_pop,priors_2_pop]
78 ensemble_paths=[ "/bench/PhD/NGS_binaries/CNS/A25/1pop",
    ↪ "/bench/PhD/NGS_binaries/CNS/A25/2pop"]
79 const pulse_time=10.5
80 const mc_its=Int64(5e5)
81 const end_time=10.5
82 const retain_run=false
83 constants=[X, pulse_time, mc_its, end_time, retain_run]
84
85
86 ensembles=Vector{Thymidine_Ensemble}()
87 for (ps, ep, box) in zip(prior_sets,ensemble_paths,boxes)
88     if isfile(ep*"/ens")
89         push!(ensembles,deserialize(ep*"/ens"))
90     else
91         @info "Assembling ensemble at $ep"
92         push!(ensembles,Thymidine_Ensemble(ep, 50, y, ps, constants, box,
93             ↪ GMC_DEFAULTS))
94     end
95 end
96 #uds=Vector{Vector{Function}}([[tuning_display],[convergence_display],[tuning_display
97 uds=Vector{Vector{Function}}([[tuning_display]])
98 lds=Vector{Vector{Function}}([[model_obs_display],[ensemble_display]]))
```

```

99
100 evidence=zeros(length(ensembles))
101 for (ne,e) in enumerate(ensembles)
102     evidence[ne]=converge_ensemble!(e, τ0=.03,
103         ↳ backup=true,1),upper_displays=uds, lower_displays=lds,
104         ↳ disp_rot_its=5, mc_noise=.139)
105 end

```

16.9.9 /cmz/a25dvlinreg.jl

```

1 using Distributions, Serialization, CSV, BayesianLinearRegression, Plots,
2     ↳ DataFrames, Measurements
2 import Measurements:value,uncertainty
3
4
5 a25path="/bench/PhD/datasets/a25.csv"
6 a25df=DataFrame(CSV.File(a25path))
7
8 X=Vector{Float64}()
9 yD=Vector{Vector{Float64}}()
10 yV=Vector{Vector{Float64}}()
11
12 for timedf in groupby(a25df,"Time")
13     push!(X,timedf[1,"Time"])
14     dvec=Vector{Float64}()
15     vvec=Vector{Float64}()
16     for row in eachrow(timedf)
17         dv=row."D/V"
18         if dv == "D"
19             push!(dvec, row.EdU/row.PCNA)
20         else
21             push!(vvec, row.EdU/row.PCNA)
22         end
23     end
24
25     push!(yD,dvec)
26     push!(yV,vvec)
27 end
28
29 sp=sortperm(X)
30 yD=yD[sp]; yV=yV[sp]
31 sort!(X)

```

```

32 xYD=zeros(0); xYV=zeros(0)
33 YD=zeros(0); YV=zeros(0)
34
35
36 for (n,vec) in enumerate(yD)
37     global xYD=vcat(xYD,[X[n] for i in 1:length(vec)])
38     global YD=vcat(YD,vec)
39 end
40
41 for (n,vec) in enumerate(yV)
42     global xYV=vcat(xYV,[X[n] for i in 1:length(vec)])
43     global YV=vcat(YV,vec)
44 end
45
46 xYD=hcat(ones(length(xYD)),xYD)
47 xYV=hcat(ones(length(xYV)),xYV)
48
49 xY=vcat(xYD,xYV);Y=vcat(YD,YV)
50
51 dm=BayesianLinearRegression.fit!(BayesianLinReg(xYD,YD))
52 vm=BayesianLinearRegression.fit!(BayesianLinReg(xYV,YV))
53 tm=BayesianLinearRegression.fit!(BayesianLinReg(xY,Y))
54
55 println("Dorsal model logZ: $(logEvidence(dm))")
56 println("Ventral model logZ: $(logEvidence(vm))")
57 println("Joint D/V logZ: $(logEvidence(dm) + logEvidence(vm)) ")
58 println("Combined model logZ: $(logEvidence(tm))")
59 println("Combined/Joint ratio : $(logEvidence(tm)-(logEvidence(dm) +
→ logEvidence(vm)))")
60
61 function blr_plt(x,y,Xs,blrs,colors,labels,q=.975)
62
63     ← plt=scatter(x,y,marker=:cross,markersize=3,markercolor=:black,label="Data",yl
64     ← labelled fraction", xlabel="Pulse time (hr)", legend=:bottomright,
65     ← ylims=(-0.1,1.))
66     for (X,blr,color,label) in zip(Xs, blrs,colors,labels)
67         line=zeros(size(X,1))
68         ribbon=zeros(size(X,1))
69         predictions=BayesianLinearRegression.predict(blr,X)
70         for (n,p) in enumerate(predictions)
71             normal=Normal(value(p),uncertainty(p))
72             ribbon[n]=quantile(normal,q)-mean(normal)
73             line[n]=mean((value(p)))
74         end
75         plot!(plt,X,blr,color=color,ribbon=ribbon)
76     end
77     return plt
78 end

```

```

71         end
72         plot!(plt,x,line,ribbon=ribbon,color=color,label=label)
73     end
74     return plt
75 end
76
77 dplt=blr_plt(xYD[:,2],YD,[xYD],[dm],[:green],["Dorsal model"])
78 annotate!([(1,.9,Plots.text("A",12))])
79 vplt=blr_plt(xYV[:,2],YV,[xYV],[vm],[:magenta],["Ventral model"])
80 annotate!([(1,.9,Plots.text("B",12))])
81 tplt=blr_plt(xY[:,2],Y,[xY],[tm],[:black],["Combined model"])
82 annotate!([(1,.9,Plots.text("C",12))])
83
84 combined=plt(dplt,vplt,tplt,layout=(3,1), size=(600,900))
85
86 savefig(combined, "/bench/PhD/Thesis/images/cmz/3ddvlinreg.png")
87 @info "Saved fig."
88
89
90 println("\begin{tabular}{l|l|l|l}")
91 println("\hline")
92 println("{\bf Model} & {\bf Implied } $T_c$  & {\bf Implied } $T_s$  &
93   {\bf logZ}\hline")
94 for (name,model) in zip(["Dorsal","Ventral","Combined"],[dm,vm,tm])
95     local intercept,slope=posteriorWeights(model)
96     println("$name & $(1/slope) & $(intercept*1/slope) &
97       $(round(logEvidence(model),digits=3))\hline")
98 end
99 println("\end{tabular}")

```

16.9.10 /cmz/a25thymidinesim.jl

```

1 using GMC_NS, CMZNicheSims, ConjugatePriors, CSV, DataFrames,
2   Distributions, Serialization, NGRefTools
3
4 a10path="/bench/PhD/datasets/A10 measurements 2018update.csv"
5 a10df=DataFrame(CSV.File(a10path))
6 df3=a10df[a10df."Time point (d)" .== 3, :]
7

```

```

8 inddict=Dict{Tuple,Vector}(){}
9 for row in eachrow(df3)
10    ind=(row.Block, row.Slide, row.Row)
11    if !(ind in keys(inddict))
12        inddict[ind]=[(row["Dorsal CMZ (#)"]+row["Ventral CMZ (#)"])]
13    else
14        push!(inddict[ind],((row["Dorsal CMZ (#)"]+row["Ventral CMZ
15           (#)"])))
16    end
17 end
18 popvec=Vector{Float64}(){}
19 for (ind, pops) in inddict
20     push!(popvec,mean(pops))
21 end
22
23 a25path="/bench/PhD/datasets/a25.csv"
24 a25df=DataFrame(CSV.File(a25path))
25
26 X=Vector{Float64}(){}
27 y=Vector{Vector{Int64}}(){}
28
29 for timedf in groupby(a25df,"Time")
30     push!(X,timedf[1,"Time"])
31     dict=Dict{Tuple,Vector{Int64}}(){}
32     for row in eachrow(timedf)
33         ind=(row.Block, row.Slide, row.Row)
34         if !(ind in keys(dict))
35             dict[ind]=[row.PCNA, row.EdU]
36         else
37             dict[ind][1]+=row.PCNA
38             dict[ind][2]+=row.EdU
39         end
40     end
41
42     tcounts=Vector{Int64}(){}
43     for (ind,counts) in dict
44         global popvec=vcat(popvec, counts[1])
45         push!(tcounts, counts[2])
46     end
47     push!(y,tcounts)
48 end
49

```

```

50 y=y[sortperm(X)]
51 sort!(X)
52
53 pop_prior=fit(NormalInverseGamma,log.(popvec))
54
55 g1_prior=Normal(4.,2.)
56 tc_prior=NormalInverseGamma(3.5,1.,5.,2.)
57 s_prior=NormalInverseGamma(4.,2.,1.,1.)
58 sister_prior=Beta(8.,75.)
59
60 priors_1_pop=[marginals(pop_prior) ... , g1_prior, marginals(tc_prior) ... ,
   ↳ marginals(s_prior) ... , sister_prior]
61
62 p1_box=[3.3 5.8
63           .07 .26
64           1. 10.
65           .1 10.
66           eps() 2.7
67           eps() 20.
68           .1 200.
69           eps() .5]
70
71 p1_box=GMC_NS.to_unit_ball.(p1_box,priors_1_pop)
72
73 ep="/bench/PhD/NGS_binaries/CNS/A25/1pop"
74 const pulse_time=10.5
75 const mc_its=Int64(1.5e5)
76 const end_time=10.5
77 constants=[X, pulse_time, mc_its, end_time]
78
79 if isfile(ep*"/ens")
80     e=deserialize(ep*"/ens")
81 else
82     @info "Assembling ensemble at $ep"
83     gmcd=GMC_DEFAULTS
84     gmcd[1]=5
85     e=Thymidine_Ensemble(ep, 100, y, priors_1_pop, constants, p1_box,
   ↳ gmcd)
86 end
87
88 uds=Vector{Vector{Function}}([[liwi_display],[convergence_display],[evidence_display]]
89 lds=Vector{Vector{Function}}([[model_obs_display],[ensemble_display],[ensemble_displa
90

```

```

91 converge_ensemble!(e,backup=(true,1),upper_displays=uds,
→   lower_displays=lds, disp_rot_its=5, mc_noise=.14,
→   converge_factor=1e-3)

```

16.9.11 /cmz/a27GMC_NS.jl

```

1 using Distributions, Serialization, CSV, BayesianLinearRegression, Plots,
→   DataFrames, Measurements, GMC_NS
2 import Measurements:value,uncertainty
3
4 a27path="/bench/PhD/datasets/a27_r.csv"
5 a27df=DataFrame(CSV.File(a27path))
6
7 X=unique(a27df[ "Age "]).*30.
8 CR=Dict{Float64,Vector{Float64}}(){}
9 mo1=Dict{Float64,Vector{Float64}}{}
10
11 for x in X
12     CR[x]=Vector{Float64}(){}
13     x<90. && (mo1[x]=Vector{Float64}{}())
14 end
15
16 for row in eachrow(a27df)
17     x=row. "Age "*30.
18     !ismissing(row. "LR") && push!(CR[x],row. "LR")
19     !ismissing(row. "D/N1") && !ismissing(row. "V/T1") &&
→       push!(mo1[x],sum([row. "D/N1",row. "V/T1"]))
20 end
21
22
23 gmc=GMC_DEFAULTS
24 gmc[1]=5
25 gmc[2]=1.e-15
26
27 uds=Vector{Vector{Function}}([[convergence_display],[evidence_display],[info_display]])
28 lds=Vector{Vector{Function}}([[model_obs_display],[ensemble_display]])
29
30 n_models=50
31
32 evdict=Dict{String,Measurement}(){}
33
34 max_mu=2000.

```

```

35 min_μ=0.
36 max_λ=500.
37 min_λ=1e-6
38
39 prior=[Uniform(min_μ,max_μ),Uniform(min_λ,max_λ)]
40 box=[min_μ max_μ;min_λ max_λ]
41
42 cobs=vcat([v for v in values(CR)] ... )
43 mobs=vcat([v for v in values(mo1)] ... )
44
45 for (md, pstring) in zip([CR,mo1],["CR","mo1"])
46     for (k,v) in md
47         pfx=pstring*string(k)
48         enspth="/bench/PhD/NGS_binaries/GMC_NS/A27/*pfx
49         if isfile(enspth*/ens")
50             e=deserialize(enspth*/ens")
51         else
52             e=LogNormal_Ensemble(enspth,n_models,v, prior, box, gmc ... )
53         end
54
55         pstring in keys(evdict) ?
56             (evdict[pstring]+=converge_ensemble!(e,backup=(true,10000),upper_displays=
57             lower_displays=lds, disp_rot_its=10000,
58             converge_factor=1e-6)) :
59             (evdict[pstring]=converge_ensemble!(e,backup=(true,10000),upper_displays=
60             lower_displays=lds, disp_rot_its=10000,
61             converge_factor=1e-6))
62         end
63     end
64
65     for (obs,pstring) in zip([cobs,mobs],["cCR","cmo1"])
66         enspth="/bench/PhD/NGS_binaries/GMC_NS/A27/*pstring
67         if isfile(enspth*/ens")
68             e=deserialize(enspth*/ens")
69         else
70             e=LogNormal_Ensemble(enspth,n_models,obs, prior, box, gmc ... )
71         end
72
73         evdict[pstring]=converge_ensemble!(e,backup=(true,10000),upper_displays=uds,
74         lower_displays=lds, disp_rot_its=10000, converge_factor=1e-6)
75     end
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
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15
```

```

70 for ms in ["CR", "mo1"]
71     cev=evdict["c"*ms]
72     sev=evdict[ms]
73     ratio=cev-sev
74     println("$ms & $(round(cev,digits=3)) & $(round(sev,digits=3)) &
75             ↳ $(round(ratio,digits=3)) &
76             ↳ $(round(ratio.val/ratio.err,digits=1))\\\\ \\hline")
77 end

```

16.9.12 /cmz/a27linreg.jl

```

1 using Distributions, Serialization, CSV, BayesianLinearRegression, Plots,
2   ↳ DataFrames, Measurements
3 import Measurements:value,uncertainty
4
5 a27path="/bench/PhD/datasets/a27_r.csv"
6 a27df=DataFrame(CSV.File(a27path))
7
8 X=unique(a27df["Age"]).*30.
9 CR=Dict{Float64,Vector{Float64}}(){}
10 mo1=Dict{Float64,Vector{Float64}}(){}
11
12 for x in X
13     CR[x]=Vector{Float64}(){}
14     x<90. && (mo1[x]=Vector{Float64}())
15 end
16
17 for row in eachrow(a27df)
18     x=row["Age"]*30.
19     !ismissing(row["LR"]) && push!(CR[x],row["LR"])
20     !ismissing(row["D/N1"]) && !ismissing(row["V/T1"]) &&
21         ↳ push!(mo1[x],sum([row["D/N1"],row["V/T1"]]))
22 end
23
24 xCR=zeros(0); xmo1=zeros(0)
25 yCR=zeros(0); ymo1=zeros(0)
26
27 for (age,vec) in CR
28     println([age for i in 1:length(vec)])
29     global xCR=vcat(xCR,[age for i in 1:length(vec)])
30     global yCR=vcat(yCR,vec)
31 end

```

```

31 for (time,vec) in mo1
32     global xmo1=vcat(xmo1,[time for i in 1:length(vec)])
33     global ymo1=vcat(ymo1,vec)
34 end
35
36 xCR=hcat(ones(length(xCR)),xCR)
37 xmo1=hcat(ones(length(xmo1)),xmo1)
38
39 uncorrCR2=BayesianLinearRegression.fit!(BayesianLinReg(ones(length(yCR),1),yCR))
40 corrCR2=BayesianLinearRegression.fit!(BayesianLinReg(xCR,yCR))
41 uncorrCRX=ones(length(X),1)
42 corrCRX=hcat(ones(length(X)),X)
43
44 uncorrmo1=BayesianLinearRegression.fit!(BayesianLinReg(ones(length(ymo1),1),ymo1))
45 corrmo1=BayesianLinearRegression.fit!(BayesianLinReg(xmo1,ymo1))
46 uncorrmo1X=ones(length(X[1:2]),1)
47 corrmo1X=hcat(ones(length(X[1:2])),X[1:2])
48
49 function blr_plt(i,x,y,Xs,blrs,colors,labels,q=.975)
50
51     ← plt=scatter(x,y,marker=:diamond,markersize=8,markerstrokestyle=:bold,markerco
52     ← size", xlabel="Age
53     ← (dpf)", legend=:topright, ylims=[0,maximum(y)+25])
54     for (X,blr,color,label) in zip(Xs, blrs,colors,labels)
55         line=zeros(size(X,1))
56         ribbon=zeros(size(X,1))
57         predictions=BayesianLinearRegression.predict(blr,X)
58         for (n,p) in enumerate(predictions)
59             normal=Normal(value(p),uncertainty(p))
60             ribbon[n]=quantile(normal,q)-mean(normal)
61             line[n]=mean((value(p)))
62         end
63         plot!(plt,i,line,ribbon=ribbon,color=color,label=label)
64     end
65     return plt
66 end
67
68 pCR=blr_plt(X,xCR[:,2],yCR,[corrCRX,uncorrCRX],[corrCR2,uncorrCR2],[{:magenta,:green},
69     ← Model - CR", "Uncorrelated Model - CR"])
70 annotate!([(35,250,Plots.text("A",18))])

```

```

68 pmo1=blr_plt(X[1:2],xmo1[:,2],ymo1,[corrmo1X,uncorrmo1X],[corrmo1,uncorrmo1],[::magenta])
   ↳  Model - 1mo", "Uncorrelated Model - 1mo CMZ"])
69 annotate!([(32,350,Plots.text("B",18))])

70
71 combined=plot(pCR,pmo1,layout=grid(2,1),size=(600,800))
72 savefig(combined,"/bench/PhD/Thesis/images/cmz/a27linreg.png")

73
74 println("\\"begin{tabular}{l|l|l|l}")
75 println("\\"hline")
76 println("{\\bf Measurement} & {\\bf Uncorrelated logZ} & {\\bf Correlated
   ↳  logZ} & {\\bf logZR}\\"\\\"\\\"hline")
77 println("1dpf Central Remnant & {\\"bf
   ↳  $(round(logEvidence(uncorrCR2),digits=3))} &
   ↳  $(round(logEvidence(corrCR2),digits=3)) &
   ↳  $(round(logEvidence(uncorrCR2)-logEvidence(corrCR2),digits=3))\\\"\\\"\\\"hline")
78 println("30dpf Cohort & {\\"bf $(round(logEvidence(uncorrmo1),digits=3))} &
   ↳  & $(round(logEvidence(corrmo1),digits=3)) &
   ↳  $(round(logEvidence(uncorrmo1)-logEvidence(corrmo1),digits=3))\\\"\\\"\\\"hline")
79 println("\\"end{tabular}")

```

16.9.13 /rys/a37.jl

```

1 using CSV,DataFrames,Distributions,Measurements,GMC_NS, NGRefTools,
   ↳  Plots,Measures, Serialization
2 gr()
3 default(fontsize = 10, guidefont = (12,:bold), tickfont = 10, guide =
   ↳  "x")
4
5
6 pth="/bench/PhD/Thesis/datasets/a37.csv"
7
8 a37df=DataFrame(CSV.read(pth))
9
10 atg_measure_dict=Dict{String,Vector{Float64}}}()
11 spl_measure_dict=Dict{String,Vector{Float64}}}()
12 ctl_measure_dict=Dict{String,Vector{Float64}}}()
13 uninj_measure_dict=Dict{String,Vector{Float64}}}()
14
15 msd=[atg_measure_dict,spl_measure_dict,ctl_measure_dict,uninj_measure_dict]
16

```

```

17 for dict in msd
18     dict[ "DCMZ" ]=Vector{Float64}()
19     dict[ "VCMZ" ]=Vector{Float64}()
20     dict[ "TCMZ" ]=Vector{Float64}()
21     dict[ "NucPop" ]=Vector{Float64}()
22     dict[ "Sphericity" ]=Vector{Float64}()
23     dict[ "Volume" ]=Vector{Float64}()
24 end
25
26 #EXTRACT ALL MEASUREMENTS FROM DF TO VECTORS
27 for (gdf,dict) in zip(groupby(a37df, "Group"),msd)
28     for row in eachrow(gdf)
29         if row."D/V"=="D"
30             push!(dict[ "DCMZ" ],row."PCNA +ve")
31             !ismissing(row."Total PCNA") && push!(dict[ "TCMZ" ],row."Total
32             ← PCNA")
33             !ismissing(row."Total nuclei") &&
34             ← push!(dict[ "NucPop" ],row."Total nuclei")
35             push!(dict[ "Sphericity" ],row."Sphericity")
36             push!(dict[ "Volume" ],row."Volume")
37         else
38             push!(dict[ "VCMZ" ],row."PCNA +ve")
39             ← dict[ "Sphericity" ][end]+=row."Sphericity";dict[ "Sphericity" ][end]/=
40             dict[ "Volume" ][end]+=row."Volume";dict[ "Volume" ][end]/=
41         end
42     end
43 end
44 for md in msd
45     md[ "NucPer" ]=md[ "NucPop" ]./md[ "TCMZ" ]
46 end
47
48 gmc=GMC_DEFAULTS
49 gmc[1]=5
50 gmc[2]=1.e-15
51 gmcdir="/bench/PhD/NGS_binaries/BSS/A37"
52
53
54 uds=Vector{Vector{Function}}([[convergence_display],[evidence_display],[info_display]]
55 lds=Vector{Vector{Function}}([[model_obs_display],[ensemble_display]]))
56

```

```

57 n_models=50
58
59 max_mu=300.;min_mu=0.
60 max_lambda=1.;min_lambda=1e-5
61 prior=[Uniform(min_mu,max_mu),Uniform(min_lambda,max_lambda)]
62 box=[min_mu max_mu;min_lambda max_lambda]
63
64 np_prior=[Uniform(min_mu,5000),Uniform(min_lambda,max_lambda)]
65 np_box=[min_mu 5000;min_lambda max_lambda]
66
67 s_max_mu=1.;s_min_mu=0.
68 s_max_lambda=5e5;s_min_lambda=1e3
69 s_prior=[Uniform(s_min_mu,s_max_mu),Uniform(s_min_lambda,s_max_lambda)]
70 s_box=[s_min_mu s_max_mu;s_min_lambda s_max_lambda]
71
72 totalmsvec=[ "TCMZ", "NucPop", "NucPer", "DCMZ", "VCMZ", "Volume", "Sphericity"]
73 popmsvec=[ "TCMZ", "NucPop", "NucPer", "DCMZ", "VCMZ"]
74 normmsvec=[ "Volume", "Sphericity"]
75
76 for (md,morph) in zip(atg_measure_dict,spl_measure_dict,[ "ATG", "Spl"])
77     evdict=Dict{String,Measurement}()
78
79     for ms in totalmsvec
80         evdict[ms*"_Combined"] = measurement(0,0)
81         evdict[ms*"_Separate"] = measurement(0,0)
82     end
83
84
85     for ms in popmsvec
86         ms=="NucPop" ? (pr=np_prior; bx=np_box) : (pr=prior; bx=box)
87
88         c_ens=gmcdir*/" * morph * "_c_*ms
89         c_obs=vcat(md[ms],ctl_measure_dict[ms])
90         if isfile(c_ens*"/ens")
91             e=deserialize(c_ens*"/ens")
92         else
93             e=LogNormal_Ensemble(c_ens,n_models, c_obs, pr, bx, gmc ... )
94         end
95     end
96

```

```

97
    ↵ evdict[ms*"_Combined"]+=converge_ensemble!(e,backup=(true,10000),upper_di
    ↵ lower_displays=lds, disp_rot_its=10000,
    ↵ converge_criterion="compression", converge_factor=1e-6)

98
for (msd,p) in zip([md,ctl_measure_dict],[/*morph*, "/ctl_"])
100    ens=gmcdir*p*ms
101    obs=msd[ms]
102    if isfile(ens*/ens")
103        e=deserialize(ens*/ens")
104    else
105        e=LogNormal_Ensemble(ens,n_models, obs, pr, bx, gmc ... )
106    end
107
    ↵ evdict[ms*"_Separate"]+=converge_ensemble!(e,backup=(true,10000),upper_di
    ↵ lower_displays=lds, disp_rot_its=10000,
    ↵ converge_criterion="compression", converge_factor=1e-6)
108 end
109 end
110
111 for ms in normmsvec
112     c_ens=gmcdir/* * morph *_c_*ms
113     c_obs=vcat(md[ms],ctl_measure_dict[ms])
114     if isfile(c_ens*/ens")
115         e=deserialize(c_ens*/ens")
116     else
117         if ms=="Sphericity"
118             e=Normal_Ensemble(c_ens,n_models, c_obs, s_prior, s_box,
119             ↵ gmc ... )
120         else
121             e=Normal_Ensemble(c_ens,n_models, c_obs, prior, box,
122             ↵ gmc ... )
123         end
124     end
125
    ↵ evdict[ms*"_Combined"]+=converge_ensemble!(e,backup=(true,10000),upper_di
    ↵ lower_displays=lds, disp_rot_its=10000,
    ↵ converge_criterion="compression", converge_factor=1e-6)

126 for (msd,p) in zip([md,ctl_measure_dict],[/*morph*, "/ctl_"])
127     ens=gmcdir*p*ms
128     obs=msd[ms]

```

```

129         if isfile(ens*"/ens")
130             e=deserialize(ens*"/ens")
131         else
132             if ms=="Sphericity"
133                 e=Normal_Ensemble(ens,n_models, obs, s_prior, s_box,
134                             ↪ gmc ... )
135             else
136                 e=Normal_Ensemble(ens,n_models, obs, prior, box,
137                             ↪ gmc ... )
138             end
139         end
140
141     end
142
143     for ms in totalmsvec
144         sev=evdict[ms*"_Separate"]
145         cev=evdict[ms*"_Combined"]
146         ratio=sev-cev
147         sig=ratio.val/ratio.err
148
149         println("$morph $ms & $(round(sev,digits=3)) &
150             ↪ $(round(cev,digits=3)) & $(round(ratio,digits=3)) &
151             ↪ $(round(sig,digits=1)) \\\\" \\\hline")
152     end
153 end
154
155 colvec=[:darkmagenta,:darkorange,:green]
156
157 np_plt=plot_logn_MTDist([atg_measure_dict["NucPer"],spl_measure_dict["NucPer"],ctl_me
158                         ↪ Mo.,"Spl Mo.,"Ctrl Mo."],"Mean Sectional Population per CMZ
159                         ↪ Cell","Posterior mean lh.")
160
161 savefig(np_plt, "/bench/PhD/Thesis/images/rys/morphnuclei.png")

```

16.9.14 /rys/a38.jl

```

1 using CSV,DataFrames,Distributions,NGRefTools,StatsBase,StatsPlots
2 gr()
3 default(legendfont = 10, guidefont = (12,:bold), tickfont = 10, guide =
4   ↪ "x")
5
6 pth="/bench/PhD/datasets/A38.csv"
7
8 a38df=DataFrame(CSV.read(pth))
9 a38df.BSR=[string(row.Block,',',row.Slide,',',row.Row) for row in
10   ↪ eachrow(a38df)]
11 a38df.Group[1:10].="sib"
12 a38df.Group[11:22].="rys"
13
14 sib_measure_dict=Dict{String,Vector{Float64}}(){}
15 rys_measure_dict=Dict{String,Vector{Float64}}(){}
16
17 for dict in [sib_measure_dict,rys_measure_dict]
18   dict["DCMZ"]=Vector{Float64}(){}
19   dict["VCMZ"]=Vector{Float64}(){}
20   dict["TCMZ"]=Vector{Float64}(){}
21   dict["NucPop"]=Vector{Float64}(){}
22   dict["Sphericity"]=Vector{Float64}(){}
23   dict["Volume"]=Vector{Float64}(){}
24 end
25
26 #EXTRACT ALL MEASUREMENTS FROM DF TO VECTORS
27 for (gdf,dict) in zip(groupby(a38df,
28   ↪ "Group"),[sib_measure_dict,rys_measure_dict])
29   for row in eachrow(gdf)
30     if row."D/V"=="D"
31       push!(dict["DCMZ"],row."PCNA +ve")
32       push!(dict["TCMZ"],row."Total PCNA")
33       push!(dict["NucPop"],row."Total nuclei")
34       push!(dict["Sphericity"],row."Sphericity")
35       push!(dict["Volume"],row."Volume")
36     else
37       push!(dict["VCMZ"],row."PCNA +ve")
38
39       ↪ dict["Sphericity"][[end]]+=row."Sphericity";dict["Sphericity"][[end]]/=2
40       dict["Volume"][[end]]+=row."Volume";dict["Volume"][[end]]/=2

```

```

38         end
39     end
40 end
41
42 #TEST IF DATA IS BETTER MODELLED NORMALLY OR LOGNORMALLY
43 for ms in ["DCMZ", "VCMZ", "NucPop", "Sphericity", "Volume"]
44     n_ms=[fit(Normal,dict[ms]) for dict in [sib_measure_dict,
45         ↪ rys_measure_dict]]
46     logn_ms=[fit(LogNormal,dict[ms]) for dict in [sib_measure_dict,
47         ↪ rys_measure_dict]]
48
49     ↪ normal_lh=sum(logpdf(n_ms[1],sib_measure_dict[ms]))+sum(logpdf(n_ms[2],rys_me
50     ↪ logn_lh=sum(logpdf(logn_ms[1],sib_measure_dict[ms]))+sum(logpdf(logn_ms[2],ry
51     println("$ms & $(round(normal_lh,digits=3)) &
52         ↪ $(round(logn_lh,digits=3)) &
53         ↪ $(round(logn_lh-normal_lh,digits=3))\\\\ \\hline")
54 end
55
56
57 TCMZ_plt=plot_logn_MTDist([sib_measure_dict["TCMZ"],rys_measure_dict["TCMZ"]],[:green
58     ↪ CMZ Population","Posterior mean lh."]
59 annotate!([(55,3.8,Plots.text("A",18))])
60
61 PopRatio_plt=plot_logn_MTDist([sib_measure_dict["NucPop"]./sib_measure_dict["TCMZ"],r
62     ↪ Sectional Population per CMZ Cell","Posterior mean lh."]
63 annotate!([(7.5,3.,Plots.text("B",18))])
64
65 DCMZ_plt=plot_logn_MTDist([sib_measure_dict["DCMZ"],rys_measure_dict["DCMZ"]],[:green
66     ↪ CMZ Population","Posterior mean lh."]
67 annotate!([(35,4.5,Plots.text("C",18))])
68
69 log_mean_mass_comparator(rys_measure_dict["DCMZ"],sib_measure_dict["DCMZ"],labels=[ "r
70     ↪ 5dpf sectional DCMZ population","sib 5dpf sectional DCMZ
71     ↪ population"])
72 log_mean_mass_comparator(rys_measure_dict["VCMZ"],sib_measure_dict["VCMZ"],labels=[ "r
73     ↪ 5dpf sectional VCMZ population","sib 5dpf sectional VCMZ
74     ↪ population"])
75
76 VCMZ_plt=plot_logn_MTDist([sib_measure_dict["VCMZ"],rys_measure_dict["VCMZ"]],[:green
77     ↪ CMZ Population","Posterior mean lh."]

```

```

67 annotate!([(15,2.25,Plots.text("D",18))])
68
69
70 Vol_plt=plot_n_MTDist([sib_measure_dict["Volume"],rys_measure_dict["Volume"]],[:green
    ↪ "nuclear volume","Posterior mean lh."])
71 annotate!([(2.1,105,Plots.text("E",18))])
72
73 Sph_plt=plot_n_MTDist([sib_measure_dict["Sphericity"],rys_measure_dict["Sphericity"]]
    ↪ "nuclear sphericity","Posterior mean lh.")
74 annotate!([(2.1,325,Plots.text("F",18))])
75 plot!(legend=:top)
76
77 combined=plot(TCMZ_plt,PopRatio_plt,DCMZ_plt,VCMZ_plt,Vol_plt,Sph_plt,layout=grid(3,2
    ↪ size=(900,900)))
78
79 savefig(combined,"/bench/PhD/Thesis/images/rys/nuclearstudy.png")

```

16.9.15 /rys/a38GMC_NS.jl

```

1 using CSV,DataFrames,Distributions,GMC_NS,Measurements,Serialization
2
3 pth="/bench/PhD/datasets/A38.csv"
4
5 a38df=DataFrame(CSV.read(pth))
6 a38df.BSR=[string(row.Block,',',row.Slide,',',row.Row) for row in
    ↪ eachrow(a38df)]
7 a38df.Group[1:10].="sib"
8 a38df.Group[11:22].="rys"
9
10 sib_measure_dict=Dict{String,Vector{Float64}} }()
11 rys_measure_dict=Dict{String,Vector{Float64}} }()
12
13 for dict in [sib_measure_dict,rys_measure_dict]
14     dict["DCMZ"]=Vector{Float64}()
15     dict["VCMZ"]=Vector{Float64}()
16     dict["TCMZ"]=Vector{Float64}()
17     dict["NucPop"]=Vector{Float64}()
18     dict["Sphericity"]=Vector{Float64}()
19     dict["Volume"]=Vector{Float64}()
20 end
21
22 #EXTRACT ALL MEASUREMENTS FROM DF TO VECTORS

```

```

23 for (gdf,dict) in zip(groupby(a38df,
24   → "Group"),[sib_measure_dict,rys_measure_dict])
25   for row in eachrow(gdf)
26     if row."D/V"=="D"
27       push!(dict["DCMZ"],row."PCNA +ve")
28       push!(dict["TCMZ"],row."Total PCNA")
29       push!(dict["NucPop"],row."Total nuclei")
30       push!(dict["Sphericity"],row."Sphericity")
31       push!(dict["Volume"],row."Volume")
32     else
33       push!(dict["VCMZ"],row."PCNA +ve")
34
35       → dict["Sphericity"][[end]]+=row."Sphericity";dict["Sphericity"][[end]]/=2
36       dict["Volume"][[end]]+=row."Volume";dict["Volume"][[end]]/=2
37     end
38   end
39 end
40
41 for md in [sib_measure_dict,rys_measure_dict]
42   md["NucPer"]=md["NucPop"]./md["TCMZ"]
43 end
44
45 gmc=GMC_DEFAULTS
46 gmc[1]=5
47 gmc[2]=1.e-15
48 gmc[[end]]=2e4
49 gmmdir="/bench/PhD/NGS_binaries/GMC_NS/A38"
50
51 uds=Vector{Vector{Function}}([[convergence_display],[evidence_display],[info_display]])
52 lds=Vector{Vector{Function}}([[model_obs_display],[ensemble_display]])
53
54 n_models=50
55
56 evdict=Dict{String,Measurement}()
57
58 totalmsvec=[ "TCMZ", "NucPer", "DCMZ", "VCMZ", "Volume", "Sphericity" ]
59 popmsvec=[ "TCMZ", "NucPer", "DCMZ", "VCMZ" ]
60 normmsvec=[ "Volume", "Sphericity" ]
61
62 for ms in totalmsvec
63   evdict[ms*"_Combined"] = measurement(0,0)

```

```

64     evdict[ms*"Separate"]=measurement(0,0)
65 end
66
67 max_mu=300.;min_mu=0.
68 max_lambda=.3;min_lambda=0.
69 prior=[Uniform(min_mu,max_mu),Uniform(min_lambda,max_lambda)]
70 box=[min_mu max_mu;min_lambda max_lambda]
71
72 s_max_mu=1.;s_min_mu=0.
73 s_max_lambda=5e5;s_min_lambda=0.
74 s_prior=[Uniform(s_min_mu,s_max_mu),Uniform(s_min_lambda,s_max_lambda)]
75 s_box=[s_min_mu s_max_mu;s_min_lambda s_max_lambda]
76
77 for ms in popmsvec
78     c_ens=gmmdir*/c_*ms
79     c_obs=vcat(sib_measure_dict[ms],rys_measure_dict[ms])
80     if isfile(c_ens*/ens")
81         e=deserialize(c_ens*/ens")
82     else
83         e=LogNormal_Ensemble(c_ens,n_models, c_obs, prior, box, gmc ... )
84     end
85
86     → evdict[ms*"Combined"]+=converge_ensemble!(e,backup=(true,10000),upper_displa
     → lower_displays=lds, disp_rot_its=10000, converge_factor=1e-6)
87
88     for (msd,p) in zip([sib_measure_dict,rys_measure_dict],["/s_","/r_"])
89         ens=gmmdir*p*ms
90         obs=msd[ms]
91         if isfile(ens*/ens")
92             e=deserialize(ens*/ens")
93         else
94             e=LogNormal_Ensemble(ens,n_models, obs, prior, box, gmc ... )
95         end
96
97         → evdict[ms*"Separate"]+=converge_ensemble!(e,backup=(true,10000),upper_di
         → lower_displays=lds, disp_rot_its=10000, converge_factor=1e-6)
98     end
99
100    for ms in normmsvec
101        c_ens=gmmdir*/c_*ms
102        c_obs=vcat(sib_measure_dict[ms],rys_measure_dict[ms])

```

```

103     if isfile(c_ens*"/ens")
104         e=deserialize(c_ens*"/ens")
105     else
106         if ms=="Sphericity"
107             e=Normal_Ensemble(c_ens,n_models, c_obs, s_prior, s_box,
108                                → gmc ... )
109         else
110             e=Normal_Ensemble(c_ens,n_models, c_obs, prior, box, gmc ... )
111         end
112     end
113
114     → evdict[ms*"_Combined"]+=converge_ensemble!(e,backup=(true,10000),upper_displa
115     → lower_displays=lds, disp_rot_its=10000, converge_factor=1e-6)
116
117     for (msd,p) in zip([sib_measure_dict,rys_measure_dict],[ "/s_","/r_"])
118         ens=gmcdir*p*ms
119         obs=msd[ms]
120         if isfile(ens*"/ens")
121             e=deserialize(ens*"/ens")
122         else
123             if ms=="Sphericity"
124                 e=Normal_Ensemble(ens,n_models, obs, s_prior, s_box,
125                                    → gmc ... )
126             else
127                 e=Normal_Ensemble(ens,n_models, obs, prior, box, gmc ... )
128             end
129         end
130     end
131
132     for ms in totalmsvec
133         sev=evdict[ms*"_Separate"]
134         cev=evdict[ms*"_Combined"]
135         ratio=sev-cev
136         sig=ratio.val/ratio.err
137
138         println("$ms & $(round(sev,digits=3)) & $(round(cev,digits=3)) &
139             → $(round(ratio,digits=3)) & $(round(sig,digits=1)) \\\\" \\\\"")

```

 139 end

16.9.16 /rys/caspase.jl

```

1 using CSV, DataFrames, Plots, Distributions, Measures
2 import Plots:Plot
3
4 gr()
5
6 caspase_pth="/bench/PhD/Thesis/datasets/rys_caspase.csv"
7 caspasedf=DataFrame(CSV.read(caspase_pth))
8
9 sib_ms_dict=Dict{String,Vector{Vector{Float64}}}()
10 rys_ms_dict=Dict{String,Vector{Vector{Float64}}}()
11
12 X=[4.,6.]
13
14 for md in [sib_ms_dict,rys_ms_dict]
15     md["CMZ"]=[zeros(0) for i in 1:2]
16     md["Border"]=[zeros(0) for i in 1:2]
17     md["Central"]=[zeros(0) for i in 1:2]
18     md["Total"]=[zeros(0) for i in 1:2]
19 end
20
21 for row in eachrow(caspasedf)
22     row.dpf==4 ? (xidx=1) : (xidx=2)
23     row.genotype=="sib" ? (md=sib_ms_dict) : (md=rys_ms_dict)
24     push!(md["CMZ"][xidx],row.CMZ)
25     push!(md["Border"][xidx],row."CMZ/central border region")
26     push!(md["Central"][xidx],row."central retina")
27     push!(md["Total"][xidx],row.CMZ + row."CMZ/central border region" +
28           ↳ row."central retina")
29 end
30 plot_halves=Vector{Plot}()
31 for (n, age) in enumerate(X)
32     subplots=Vector{Plot}()
33     for (m,ms) in enumerate([ "CMZ", "Border", "Central", "Total"])
34         s_data=sib_ms_dict[ms][n]
35         sl=length(s_data)
36         r_data=rys_ms_dict[ms][n]
37         rl=length(r_data)

```

```

38     if m == 1
39
40         → subplot=bar(1:1:sl,s_data,barwidths=1,color=:green,xticks=(1:1:sl+rl,v
41             → for i in 1:sl],["R" for i in 1:rl])),legend=:none,
42             → xlabel=ms, margin=0mm,ylabel="Caspase-3+ cells")
43     else
44
45         → subplot=bar(1:1:sl,s_data,barwidths=1,color=:green,xticks=(1:1:sl+rl,v
46             → for i in 1:sl],["R" for i in 1:rl])),legend=:none,
47             → xlabel=ms, leftmargin=-7.5mm, yformatter=_→"")
48     end
49     bar!(sl+1:1:sl+rl,r_data,color=:darkmagenta, barwidths=1.)
50     push!(subplots,subplot)
51
52     end
53
54     title = plot(title = "$(Int64(age)) dpf", grid = false, showaxis =
55         → nothing, bottom_margin = -35Plots.px)
56     l=@layout [t{.02h};[a{.25w} b{.25w} c{.25w} d{.25w}]]
57     half=plot(title, subplots ...,layout=l,link=:y)
58     push!(plot_halves,half)
59
60 end
61
62 combined=plot(plot_halves ...,layout=grid(1,2), size=(900,450))
63
64 savefig(combined,"/bench/PhD/Thesis/images/rys/caspase.png")
65
66 println("Rys CMZ caspase 4dpf: $(mean(rys_ms_dict["CMZ"][[1]])) ±
67     → $(std(rys_ms_dict["CMZ"][[1]]))")
68 println("Rys CMZ caspase 6dpf: $(mean(rys_ms_dict["CMZ"][[2]])) ±
69     → $(std(rys_ms_dict["CMZ"][[2]]))")
70
71 println("Rys central caspase 4dpf: $(mean(rys_ms_dict["Central"][[1]])) ±
72     → $(std(rys_ms_dict["Central"][[1]]))")
73 println("Rys central caspase 6dpf: $(mean(rys_ms_dict["Central"][[2]])) ±
74     → $(std(rys_ms_dict["Central"][[2]]))")
75 println("Sib central caspase 4dpf: $(mean(sib_ms_dict["Central"][[1]])) ±
76     → $(std(sib_ms_dict["Central"][[1]]))")
77 println("Sib central caspase 6dpf: $(mean(sib_ms_dict["Central"][[2]])) ±
78     → $(std(sib_ms_dict["Central"][[2]]))")

```

16.9.17 /rys/em.jl

```

1 using Plots, Images, FileIO, Plots.PlotMeasures
2
3 sib36=load("/bench/PhD/Thesis/images/rys/C2 cell 1/Rys sib C2 36k cell
4   ↵ 1.jpg")
5 sib36=sib36[1:2465,:]
6 sib110=load("/bench/PhD/Thesis/images/rys/C2 cell 1/Rys sib C2 110k cell
7   ↵ 1.jpg")
8 sib110=sib110[1:2465,:]
9
10 sib210=load("/bench/PhD/Thesis/images/rys/C2 cell 1/Rys sib C2 210k cell
11   ↵ 1.jpg")
12 sib210=sib210[1:2465,:]
13
14 rys36=load("/bench/PhD/Thesis/images/rys/D1 cell 1/Rys mut D1 CMZ
15   ↵ 36k.jpg")
16 rys36=rys36[1:2465,:]
17 rys110=load("/bench/PhD/Thesis/images/rys/D1 cell 1/Rys mut D1 CMZ
18   ↵ 110k.jpg")
19 rys110=rys110[1:2465,:]
20 rys210=load("/bench/PhD/Thesis/images/rys/D1 cell 1/Rys mut D1 CMZ
21   ↵ 210k.jpg")
22 rys210=rys210[1:2465,:]
23
24 s36p=plot(sib36, axis=nothing, border=:none, margin=0mm)
25 annotate!([(200,105,Plots.text("SIB",25,:white,:bold))])
26 s110p=plot(sib110, axis=nothing, border=:none, margin=0mm)
27 annotate!([(200,105,Plots.text("SIB",25,:white,:bold))])
28 s210p=plot(sib210, axis=nothing, border=:none, margin=0mm)
29 annotate!([(200,105,Plots.text("SIB",25,:white,:bold))])
30 r36p=plot(rys36, axis=nothing, border=:none, margin=0mm)
31 annotate!([(200,105,Plots.text("RYS",25,:white,:bold))])
32 r110p=plot(rys110, axis=nothing, border=:none, margin=0mm)
33 annotate!([(200,105,Plots.text("RYS",25,:white,:bold))])
34
35 combined=plot(s36p, r36p, s110p, r110p, s210p, r210p,
36   ↵ layout=grid(3,2),size=(1200,2400))
37
38 savefig(combined, "/bench/PhD/Thesis/images/emtogiimp.png")

```

16.9.18 /rys/occupancy.py

```
 1 import os
 2 import numpy as np
 3 import pandas as pd
 4 from Bio import SeqIO
 5 import matplotlib.pyplot as plt
 6 from matplotlib.gridspec import GridSpec
 7 from matplotlib import cm
 8 from matplotlib import colors
 9
10 from PIL import Image
11 from io import BytesIO
12
13 def get_chr_props(genome):
14     genome_dict = SeqIO.index(genome, "fasta")
15     length_dict = {}
16     prop_dict = {}
17
18     total_length = 0
19     nonchr_length = 0
20     for entry in genome_dict:
21         length=len(genome_dict[entry].seq)
22         if entry.isdigit():
23             length_dict[entry] = length
24             total_length += length
25         else:
26             nonchr_length += length
27             total_length += length
28     length_dict['nonchr']=nonchr_length
29
30     for entry in length_dict:
31         prop_dict[entry] = length_dict[entry]/total_length
32
33     return prop_dict
34
35 def build_position_dict(df):
36     observed_dict={}
37     observed_nonchr_positions = 0
38
39     for chr, chrgp_data in df.groupby('chr'):
40         length=len(chrgp_data)
41         if chr.isdigit():
```

```

42         observed_dict[chr]=length
43     else:
44         observed_nonchr_positions+=length
45     observed_dict['nonchr']=observed_nonchr_positions
46
47     return observed_dict
48
49 def build_signal_dict(df):
50     observed_dict={}
51     observed_nonchr_signal = 0
52
53     for chr, chrgp_data in df.groupby('chr'):
54         signal=chrgp_data['smt_value'].sum()
55         if chr.isdigit():
56             observed_dict[chr]=signal
57         else:
58             observed_nonchr_signal+=signal
59     observed_dict['nonchr']=observed_nonchr_signal
60
61     return observed_dict
62
63 #relevant file locations
64 reference_genome = flow_variables['reference_genome_input']
65 sib_positions =
66     ↳ os.path.join(input_table_1['PathToDanposResults'][0], 'pooled', input_table_2['Danp
66 rys_positions =
67     ↳ os.path.join(input_table_1['PathToDanposResults'][0], 'pooled', input_table_2['Danp
68
68 #read in dataframes
69 sib_pos_df = pd.read_table(sib_positions, dtype={'chr':'category',
70     ↳ 'start':'uint32', 'end':'uint32', 'smt_pos':'uint32',
71     ↳ 'smt_value':'float32', 'fuzziness_score':'float32'})
70 rys_pos_df = pd.read_table(rys_positions, dtype={'chr':'category',
71     ↳ 'start':'uint32', 'end':'uint32', 'smt_pos':'uint32',
72     ↳ 'smt_value':'float32', 'fuzziness_score':'float32'})
71
72 #get proportional lengths of extrachromosomal material from reference
73     ↳ genome
73 prop_dict=get_chr_props(reference_genome)
74
75 #get total positions and signals from sib and rys dfs
76 sib_total_positions=len(sib_pos_df)
77 sib_total_signal=sib_pos_df['smt_value'].sum()

```

```

78 rys_total_positions=len(rys_pos_df)
79 rys_total_signal=rys_pos_df['smt_value'].sum()
80
81 #build dict of expected position numbers and signal sums for sibs based
     ↪ on percentage of genome comprised by relevant chr
82 sib_expected_pos_d={}
83 sib_expected_sig_d={}
84 for entry in prop_dict:
85     sib_expected_pos_d[entry]=sib_total_positions * prop_dict[entry]
86     sib_expected_sig_d[entry]=sib_total_signal * prop_dict[entry]
87
88 #build dicts of observed position numbers and signal summit sums by
     ↪ chromosome
89 sib_posn_d=build_position_dict(sib_pos_df)
90 sib_sig_d=build_signal_dict(sib_pos_df)
91 rys_posn_d=build_position_dict(rys_pos_df)
92 rys_sig_d=build_signal_dict(rys_pos_df)
93
94 #build arrays of observed positions, signals, and proportional
     ↪ differences
95 sib_observed_positions=[]
96 sib_observed_signal=[]
97 rys_observed_positions=[]
98 rys_observed_signal=[]
99
100 rys_expected_posn_from_sib=[]
101 rys_expected_sig_from_sib=[]
102
103 sib_posn_dif_from_expected=[]
104 sib_sig_dif_from_expected=[]
105 rys_posn_dif_from_sib=[]
106 rys_sig_dif_from_sib=[]
107
108 #for chr1-25
109 for i in range (1,26):
110     #build observed position & signal arrays
111     sib_observed_positions.append(sib_posn_d[str(i)])
112     sib_observed_signal.append(sib_sig_d[str(i)])
113     rys_observed_positions.append(rys_posn_d[str(i)])
114     rys_observed_signal.append(rys_sig_d[str(i)])
115
116     #build rys expected arrays

```

```

117     ↳ rys_expected_posn_from_sib.append(rys_total_positions*(sib_posn_d[str(i)]/sib_
118     ↳ rys_expected_sig_from_sib.append(rys_total_signal*(sib_sig_d[str(i)]/sib_total_
119
120 #build proportional difference arrays
121
122     ↳ sib_posn_dif_from_expected.append((sib_posn_d[str(i)]-sib_expected_posn_d[str(i)])
123     ↳ sib_sig_dif_from_expected.append((sib_sig_d[str(i)]-sib_expected_sig_d[str(i)])
124     ↳ rys_posn_dif_from_sib.append((rys_posn_d[str(i)]-rys_expected_posn_from_sib[i]))
125     ↳ rys_sig_dif_from_sib.append((rys_sig_d[str(i)]-rys_expected_sig_from_sib[i-1])
126
127 #lastly append nonchromosomal material to all arrays
128 sib_observed_positions.append(sib_posn_d['nonchr'])
129 sib_observed_signal.append(sib_sig_d['nonchr'])
130 rys_observed_positions.append(rys_posn_d['nonchr'])
131 rys_observed_signal.append(rys_sig_d['nonchr'])
132
133 rys_expected_posn_from_sib.append(rys_total_positions*(sib_posn_d['nonchr']/sib_total_
134
135 sib_posn_dif_from_expected.append((sib_posn_d['nonchr']-sib_expected_posn_d['nonchr']))
136 sib_sig_dif_from_expected.append((sib_sig_d['nonchr']-sib_expected_sig_d['nonchr'])/sib_
137 rys_posn_dif_from_sib.append((rys_posn_d['nonchr']-rys_expected_posn_from_sib[25])/rys_
138 rys_sig_dif_from_sib.append((rys_sig_d['nonchr']-rys_expected_sig_from_sib[25])/rys_e_
139
140 #!!sib nonchromosomal signal is blown out relative to other scaffolds.
141     ↳ store value and annotate, color separately
142 NC_sig_dif = sib_sig_dif_from_expected[25]
143 sib_sig_dif_from_expected[25] = 0
144
145 #build proportional difference color arrays
146 sib_norm =
147     ↳ colors.Normalize(vmin=-abs(max(sib_posn_dif_from_expected+sib_sig_dif_from_expect_
148     ↳ key=abs)),
149     ↳ vmax=abs(max(sib_posn_dif_from_expected+sib_sig_dif_from_expected,
150     ↳ key=abs)))

```

```

146 rys_norm =
147   ↳ colors.Normalize(vmin=-abs(max(rys_posn_dif_from_sib+rys_sig_dif_from_sib,
148   ↳ key=abs)), vmax=abs(max(rys_posn_dif_from_sib+rys_sig_dif_from_sib,
149   ↳ key=abs)))
150
151 sib_pos_colors = cm.PiYG(sib_norm(sib_posn_dif_from_expected))
152 sib_sig_colors = cm.PiYG(sib_norm(sib_sig_dif_from_expected))
153 sib_sig_colors[25] = [0, .1, 1, 1]
154 rys_pos_colors = cm.PRGn(rys_norm(rys_posn_dif_from_sib))
155 rys_sig_colors = cm.PRGn(rys_norm(rys_sig_dif_from_sib))
156
157 #build figure
158 fig = plt.figure(figsize=(6,6.5))
159
160 gs1 = GridSpec (2,2, wspace=.1, hspace=.05, left=.05, right=.95, top=.95,
161   ↳ bottom=.15)
162 pos_sib = plt.subplot(gs1[0, 0])
163 pos_rys = plt.subplot(gs1[0, 1])
164 sig_sib = plt.subplot(gs1[1, 0])
165 sig_rys = plt.subplot(gs1[1, 1])
166
167 pos_sib.set_ylabel('Positions', fontsize=12, weight='bold')
168 sig_sib.set_ylabel('Occupancy', fontsize=12, weight='bold')
169
170 pos_sib.text(.01,.95, 'A', transform=pos_sib.transAxes,
171   ↳ fontweight='bold', va='top')
172 pos_rys.text(.01,.95, 'B', transform=pos_rys.transAxes,
173   ↳ fontweight='bold', va='top')
174 sig_sib.text(.01,.95, 'C', transform=sig_sib.transAxes,
175   ↳ fontweight='bold', va='top')
176 sig_rys.text(.01,.95, 'D', transform=sig_rys.transAxes,
177   ↳ fontweight='bold', va='top')
178
179 gs2 = GridSpec(4, 2, wspace=.1, hspace=.05, left=.05, right=.95, top=.15,
180   ↳ bottom=.05)
181 sib_cbax = plt.subplot(gs2[3, 0])
182 rys_cbax = plt.subplot(gs2[3, 1])
183
184 #colorbar stuff
185 ssm = cm.ScalarMappable(cmap=cm.PiYG, norm=sib_norm)
186 ssm._A =[]
187

```

```

178 sib_cb=plt.colorbar(ssm, cax=sib_cbax, orientation='horizontal',
→   extend='max',
→   ticks=[-abs(max(sib_posn_dif_from_expected+sib_sig_dif_from_expected,
→   key=abs)), -.3, -.2, -.1, 0, .1, .2, .3])
179 sib_cb.ax.set_xticklabels(['{:0:.2f}'.format(-abs(max(sib_posn_dif_from_expected+sib_s
→   key=abs))), '-0.3', '-0.2', '-0.1', '0.0', '0.1', '0.2', '0.3'])
180 sib_cbax.set_xlabel('Fold difference from per-kb uniformity')
181 sib_cbax.xaxis.set_label_position('top')

182
183 rsm = cm.ScalarMappable(cmap=cm.PRGn, norm=rys_norm)
184 rsm._A =[]
185 rsm_cb=plt.colorbar(rsm, cax=rys_cbax, orientation='horizontal',
→   ticks=[-abs(max(rys_posn_dif_from_sib+rys_sig_dif_from_sib,
→   key=abs)), -.05, -.025, 0, .025, .05,
→   abs(max(rys_posn_dif_from_sib+rys_sig_dif_from_sib, key=abs))])
186 rsm_cb.ax.set_xticklabels(['{:0:.2f}'.format(-abs(max(rys_posn_dif_from_sib+rys_sig_di
→   key=abs))), '-0.05', '-0.025', '0.0', '0.025', '0.05', '{0:.2f}'.format(abs(max(rys_pos
→   key=abs)))])
187 rys_cbax.set_xlabel('Fold difference from sib proportions')
188 rys_cbax.xaxis.set_label_position('top')

189
190 #labels for chr wedges
191 labels=list(range(1,26))
192 labels.append('NC')

193
194 #make the donut plots
195 pos_sib.pie(sib_observed_positions, colors=sib_pos_colors, radius=1,
→   startangle=90, counterclock=False,
→   wedgeprops=dict(width=.7,edgecolor='w'), labels=labels,
→   textprops=dict(fontsize=6))
196 pos_sib.text(0.5, 0.5, 'sib', horizontalalignment='center',
→   verticalalignment='center', transform=pos_sib.transAxes,
→   weight='bold', fontsize='16')
197 pos_rys.pie(rys_observed_positions, colors=rys_pos_colors, radius=1,
→   startangle=90, counterclock=False,
→   wedgeprops=dict(width=.7,edgecolor='w'), labels=labels,
→   textprops=dict(fontsize=6))
198 pos_rys.text(0.5, 0.5, 'rys', horizontalalignment='center',
→   verticalalignment='center', transform=pos_rys.transAxes,
→   weight='bold', fontsize='16')
199

```

```

200 wedges, texts = sig_sib.pie(sig_observed_signal, colors=sib_sig_colors,
201     ↵ radius=1, startangle=90, counterclock=False,
202     ↵ wedgeprops=dict(width=.7, edgecolor='w'), labels=labels,
203     ↵ textprops=dict(fontsize=6))
204
205 sig_sib.text(0.5, 0.5, 'sib', horizontalalignment='center',
206     ↵ verticalalignment='center', transform=sig_sib.transAxes,
207     ↵ weight='bold', fontsize='16')
208
209 sig_rys.pie(rys_observed_signal, colors=rys_sig_colors, radius=1,
210     ↵ startangle=90, counterclock=False,
211     ↵ wedgeprops=dict(width=.7, edgecolor='w'), labels=labels,
212     ↵ textprops=dict(fontsize=6))
213 sig_rys.text(0.5, 0.5, 'rys', horizontalalignment='center',
214     ↵ verticalalignment='center', transform=sig_rys.transAxes,
215     ↵ weight='bold', fontsize='16')
216
217
218 fig.savefig(os.path.join(input_table_1['PathToDanposResults'][0], 'proportional_chromo'
219 png_memory = BytesIO()
220 fig.savefig(png_memory, format='png', dpi=600)
221 PILpng = Image.open(png_memory)
222 PILpng.save(os.path.join(input_table_1['PathToDanposResults'][0], 'proportional_chromo'
223 png_memory.close()
224
225 # Copy input to output
226 output_table_1 = input_table_1.copy()
227 output_table_2 = input_table_2.copy()

```

16.9.19 /rys/qPCR.jl

```

1 using CSV, DataFrames, Measurements, Plots, Distributions
2 import Plots:Plot
3
4 rand6pth="/bench/PhD/Thesis/datasets/6dpfRys-Histones random primers
5   ↪ qPCR.csv"
6 rand8pth="/bench/PhD/Thesis/datasets/8dpfRys-Histones random primers
7   ↪ qPCR.csv"
8 dt6pth="/bench/PhD/Thesis/datasets/6dpfRys-Histones oligo dT qPCR.csv"
9 dt8pth="/bench/PhD/Thesis/datasets/8dpfRys-Histones oligo dT qPCR.csv"
10
11 rand6=DataFrame(CSV.read(rand6pth))
12 rand8=DataFrame(CSV.read(rand8pth))
13 dt6=DataFrame(CSV.read(dt6pth))
14 dt8=DataFrame(CSV.read(dt8pth))
15
16 randdfs=[rand6,rand8]
17 dtdfs=[dt6,dt8]
18 samples=Dict{Tuple{String,Int64},Vector{String}}(){}
19
20 for (df,d) in zip(randdfs,[6,8])
21   samples[("rand",d)]=unique(df.Sample)
22   for row in eachrow(df)
23     str=("rand",d,row.Plate,row.Sample,row.Detector)
24     if !ismissing(row.Ct)
25       str in keys(measure_dict) ? push!(measure_dict[str],row.Ct) :
26         → measure_dict[str]=[row.Ct]
27     end
28   end
29 end
30
31 for (df,d) in zip(dtdfs,[6,8])
32   samples[("dt",d)]=unique(df."Sample Name")
33   for row in eachrow(df)
34     str=("dt",d,row.Plate,row."Sample Name",row."Target Name")
35     if !ismissing(row.CT)
36       str in keys(measure_dict) ? push!(measure_dict[str],row.CT) :
37         → measure_dict[str]=[row.CT]
38     end
39   end
40 end

```

```

38 end
39
40 X=[6.,8.]
41 rand_sib=Dict{String,Vector{Vector{Measurement}}}()
42 rand_rys=Dict{String,Vector{Vector{Measurement}}}()
43 dt_sib=Dict{String,Vector{Vector{Measurement}}}()
44 dt_rys=Dict{String,Vector{Vector{Measurement}}}()

45
46
47 for ms in ["H2A","H2B","H3","H4"]
48     for d in [6,8]
49         xidx=findfirst(isequal(d),X)
50         for plate in [1,2,3]
51             wt_hk=measure_dict(["rand",d,plate,"WT cDNA 10x","ActinB"])
52             length(wt_hk)>1 ? wt_hk_m=measurement(mean(wt_hk),std(wt_hk))
53             ← : wt_hk_m=measurement(mean(wt_hk),0.)
54             wt_trg=measure_dict(["rand",d,plate,"WT cDNA 10x",ms])
55             length(wt_hk)>1 ?
56                 ← wt_trg_m=measurement(mean(wt_trg),std(wt_trg)) :
57                 ← wt_trg_m=measurement(mean(wt_trg),0.)
58             wt_ΔCt=wt_trg_m-wt_hk_m
59
60             for (smpl, msdict) in zip(["Sib cDNA 10x","Rys cDNA
61             ← 10x"],[rand_sib,rand_rys])
62                 smpl_hk=measure_dict(["rand",d,plate,smpl,"ActinB"])
63                 length(smpl_hk)>1 ?
64                     ← smpl_hk_m=measurement(mean(smpl_hk),std(smpl_hk)) :
65                     ← smpl_hk_m=measurement(mean(smpl_hk),0.)
66                 smpl_trg=measure_dict(["rand",d,plate,smpl,ms])
67                 length(wt_hk)>1 ?
68                     ← smpl_trg_m=measurement(mean(smpl_trg),std(smpl_trg))
69                     ← : smpl_trg_m=measurement(mean(smpl_trg),0.)
70                 smpl_ΔCt=smpl_trg_m-smpl_hk_m
71                 ΔΔCt=smpl_ΔCt-wt_ΔCt
72                 fold_change=2^(-ΔΔCt)
73                 ms in keys(msdict) ? push!(msdict[ms][xidx],fold_change)
74                 ← : msdict[ms]=[[fold_change],Vector{Float64}()]
75             end
76         end
77     end
78 end
79
80 msvec=["H2A","H2B","H3","H4"]

```

```

72
73 for ms in msvec
74     for d in [6,8]
75         xidx=findfirst(isequal(d),X)
76         for plate in [1,2,3]
77             wt_hk=measure_dict[("dt",d,plate,"WT cDNA","ActinB")]
78             length(wt_hk)>1 ? wt_hk_m=measurement(mean(wt_hk),std(wt_hk))
79             → : wt_hk_m=measurement(mean(wt_hk),0.)
80             wt_trg=measure_dict[("dt",d,plate,"WT cDNA",ms)]
81             length(wt_trg)>1 ?
82                 → wt_trg_m=measurement(mean(wt_trg),std(wt_trg)) :
83                 → wt_trg_m=measurement(mean(wt_trg),0.)
84             wt_ΔCt=wt_trg_m-wt_hk_m
85
86
87         for (smpl, msdict) in zip(["Sib cDNA", "Rys
88             → cDNA"],[dt_sib,dt_rsys])
89             smpl_hk=measure_dict[("dt",d,plate,smpl,"ActinB")]
90             length(smpl_hk)>1 ?
91                 → smpl_hk_m=measurement(mean(smpl_hk),std(smpl_hk)) :
92                 → smpl_hk_m=measurement(mean(smpl_hk),0.)
93             smpl_trg=measure_dict[("dt",d,plate,smpl,ms)]
94             length(smpl_trg)>1 ?
95                 → smpl_trg_m=measurement(mean(smpl_trg),std(smpl_trg))
96                 → : smpl_trg_m=measurement(mean(smpl_trg),0.)
97             smpl_ΔCt=smpl_trg_m-smpl_hk_m
98             ΔΔCt=smpl_ΔCt-wt_ΔCt
99             fold_change=2^(-ΔΔCt)
100            ms in keys(msdict) ? push!(msdict[ms][xidx],fold_change)
101            → : msdict[ms]=[[fold_change],Vector{Float64}()]
102
103        end
104    end
105 end
106
107 plot_halves=Vector{Plot}()
108 for ((sib_dict, rys_dict),assay_name) in
109     → zip([(rand_sib,rand_rsys),(dt_sib,dt_rsys)],["Total transcript",
110     → "Polyadenylated Transcript"])
111     subplots=Vector{Plot}()
112     for (n,ms) in enumerate(msvec)
113         plt=0.
114         mx=0.
115         mnn=Inf

```

```

104     for (dict,color,symbol,leg) in zip([sib_dict,
105       ↪ rys_dict],[:green,:darkmagenta],[::circle,:diamond],["Sib","rys"])
106       means=[mean(valvec) for valvec in dict[ms]]
107       stdevs=[std(valvec) for valvec in dict[ms]]
108       upper=Vector{Float64}()
109       mns=[mn.val for mn in means]
110       stds=[sd.val for sd in stdevs]
111       lower=Vector{Float64}()
112
113       for (mn,sd) in zip(mns,stds)
114         dist=Normal(mn,sd)
115         push!(upper,quantile(dist,.975)-mn)
116         push!(lower,mn-quantile(dist,.025))
117       end
118
119       xs=vcat([[X[n] for i in 1:length(dict[ms][n])] for n in
120         ↪ 1:length(X)] ... )
121       ys=vcat([[m.val for m in dict[ms][n]] for n in
122         ↪ 1:length(X)] ... )
123
124       mx=max(mx,maximum(vcat(upper,ys)))
125       mnn=min(mnn,minimum(vcat(lower,ys)))
126
127       if dict==sib_dict
128         if n!=4 && n!=1
129           plt=scatter(xs,ys, marker=symbol, color=color,
130             ↪ markerstrokecolor=color, markersize=3,
131             ↪ label=leg*" data", showaxis=:y, xticks=X,
132             ↪ xformatter=_→"", ylabel="Fold
133             ↪ change", xlims=[5.5,8.5],legend=:none)
134         elseif n==1
135           plt=scatter(xs,ys, marker=symbol, color=color,
136             ↪ markerstrokecolor=color, markersize=3,
137             ↪ label=leg*" data", showaxis=:y, xticks=X,
138             ↪ xformatter=_→"", ylabel="Fold
139             ↪ change", xlims=[5.5,8.5],legend=:topleft,
140             ↪ title=assay_name)
141         else
142           plt=scatter(xs,ys, marker=symbol, color=color,
143             ↪ markerstrokecolor=color, markersize=3,
144             ↪ label=leg*" data", xticks=X, ylabel="Fold
145             ↪ change", xlabel="Age (dpf)",
146             ↪ xlims=[5.5,8.5],legend=:none)
147
148       end
149
150   end

```



```

167         wtdifs=[measurement(mean(valvec).val,std(valvec).val) for
168             → valvec in dict[ms]]-1.
169         stds=[v.val/v.err for v in wtdifs]
170         stdmat[n,assay_idx,2,:]=stds
171     end
172   end
173 end
174
175 for (a,assay) in enumerate(["Total","polyA"])
176   for (m,ms) in enumerate(msvec)
177     for (d,age) in enumerate([6,8])
178       println("$assay & $ms & $age &
179           → $(round(stdmat[m,a,1,d],digits=2)) &
180           → $(round(stdmat[m,a,2,d],digits=2))\\ \\ \\ \\hline")
181   end
182 end
183 rr_h2a=[Normal(m,s) for (m,s) in
184   → zip(mean.(rand_rys["H2A"]),std.(rand_rys["H2A"]))]
185 rs_h2a=[Normal(m,s) for (m,s) in
186   → zip(mean.(rand_sib["H2A"]),std.(rand_sib["H2A"]))]
187 rand_h2a_joint=exp(sum(logccdf.(rr_h2a,[d.μ for d in rs_h2a])))
188
189 rr_h2b=[Normal(m,s) for (m,s) in
190   → zip(mean.(rand_rys["H2B"]),std.(rand_rys["H2B"]))]
191 rs_h2b=[Normal(m,s) for (m,s) in
192   → zip(mean.(rand_sib["H2B"]),std.(rand_sib["H2B"]))]
193 rand_h2b_joint=exp(sum(logccdf.(rr_h2b,[d.μ for d in rs_h2b])))
194
195 dtr_h2a=[Normal(m,s) for (m,s) in
196   → zip(mean.(dt_rys["H2A"]),std.(dt_rys["H2A"]))]
197 dts_h2a=[Normal(m,s) for (m,s) in
198   → zip(mean.(dt_sib["H2A"]),std.(dt_sib["H2A"]))]
199 dt_h2a_joint=exp(sum(logccdf.(dtr_h2a,[d.μ for d in dts_h2a])))
200
201 dtr_h2b=[Normal(m,s) for (m,s) in
202   → zip(mean.(dt_rys["H2B"]),std.(dt_rys["H2B"]))]
203 dts_h2b=[Normal(m,s) for (m,s) in
204   → zip(mean.(dt_sib["H2B"]),std.(dt_sib["H2B"]))]
205 dt_h2b_joint=exp(sum(logccdf.(dtr_h2b,[d.μ for d in dts_h2b])))

```

```
199 all_joint=rand_h2b_joint*dt_h2a_joint*dt_h2b_joint
```

16.9.20 /rys/rysp_GMC_NS.jl

```

1 using CSV,DataFrames,Distributions,GMC_NS,Measurements,Serialization
2
3 a38pth="/bench/PhD/datasets/A38.csv"
4 a49pth="/bench/PhD/datasets/A49.csv"
5
6 a38df=DataFrame(CSV.read(a38pth))
7 a38df.Group[1:10]::="sib"
8 a38df.Group[11:22]::="rysp"
9 a49df=DataFrame(CSV.read(a49pth))
10 a49df=a49df[2:end,:]
11
12 a38df.BSR=[string(row.Block,',',row.Slide,',',row.Row) for row in
13   eachrow(a38df)]
14
15 sib_measure_dict=Dict{String,Vector{Vector{Float64}}}()
16 rys_measure_dict=Dict{String,Vector{Vector{Float64}}}()
17 XCMZ=Vector{Float64}()
18 XEdU=Vector{Float64}()
19
20 for dict in [sib_measure_dict,rysp_measure_dict]
21   dict["DCMZ"]=Vector{Float64}()
22   dict["VCMZ"]=Vector{Float64}()
23   dict["TCMZ"]=Vector{Float64}()
24   dict["EdU"]=Vector{Float64}()
25 end
26
27 for tdf in groupby(a49df,"Age")
28   age=unique(tdf."Age")[1]
29   push!(XCMZ,age);push!(XEdU,age)
30   xidx=length(XCMZ)
31   for sdf in groupby(tdf, "Rys/Sib")
32     sr=unique(sdf."Rys/Sib")[1]
33     for mdf in groupby(sdf, "D/V/P/WE")
34       if unique(mdf."D/V/P/WE")[1] == "D"
35         if sr=="sib"
36           push!(sib_measure_dict["DCMZ"],mdf."PCNA +ve")
37           push!(sib_measure_dict["TCMZ"],mdf."PCNA +ve")

```

```

38         push!(sib_measure_dict["EdU"], mdf."EdU +ve, PCNA
39             ↪ +ve")
40     else
41         push!(rys_measure_dict["DCMZ"], mdf."PCNA +ve")
42         push!(rys_measure_dict["TCMZ"], mdf."PCNA +ve")
43         push!(rys_measure_dict["EdU"], mdf."EdU +ve, PCNA
44             ↪ +ve")
45     end
46 elseif unique(mdf."D/V/P/WE")[1] == "V"
47     if sr=="sib"
48         push!(sib_measure_dict["VCMZ"], mdf."PCNA +ve")
49         sib_measure_dict["TCMZ"][xidx].+=mdf."PCNA +ve"
50         sib_measure_dict["EdU"][xidx].+=mdf."EdU +ve, PCNA
51             ↪ +ve"
52     else
53         push!(rys_measure_dict["VCMZ"], mdf."PCNA +ve")
54         rys_measure_dict["TCMZ"][xidx].+=mdf."PCNA +ve"
55         rys_measure_dict["EdU"][xidx].+=mdf."EdU +ve, PCNA
56             ↪ +ve"
57     end
58 end
59 end
60
61 push!(XCMZ, 5.)
62 for sdf in groupby(a38df, "Group")
63     xidx=length(XCMZ)
64     if unique(sdf."Group")[1]=="sib"
65         for mdf in groupby(sdf, "D/V")
66             if unique(mdf."D/V")[1] == "D"
67                 push!(sib_measure_dict["DCMZ"], mdf."PCNA +ve")
68                 push!(sib_measure_dict["TCMZ"], mdf."PCNA +ve")
69             else
70                 push!(sib_measure_dict["VCMZ"], mdf."PCNA +ve")
71                 sib_measure_dict["TCMZ"][xidx].+=mdf."PCNA +ve"
72             end
73         end
74     else
75         for mdf in groupby(sdf, "D/V")
76             if unique(mdf."D/V")[1] == "D"
77                 push!(rys_measure_dict["DCMZ"], mdf."PCNA +ve")
78                 push!(rys_measure_dict["TCMZ"], mdf."PCNA +ve")

```

```

77         else
78             push!(rys_measure_dict["VCMZ"],mdf."PCNA +ve")
79             rys_measure_dict["TCMZ"][:idx].+=mdf."PCNA +ve"
80         end
81     end
82 end
83
84
85 perm=sortperm(XCMZ)
86 for ms in ["DCMZ","VCMZ","TCMZ"]
87     sib_measure_dict[ms]=sib_measure_dict[ms][perm]
88     rys_measure_dict[ms]=rys_measure_dict[ms][perm]
89 end
90 XCMZ=[4.,10.]
91
92 for msd in [sib_measure_dict,rys_measure_dict]
93     for (k,v) in msd
94         for vec in v
95             if any(i→i==0,vec)
96                 vec[findall(i→i==0,vec)]-=1e-3
97             end
98         end
99     end
100 end
101
102 gmc=GMC_DEFAULTS
103 gmc[1]=5
104 gmc[2]=1.e-15
105 gmc[end]=2e4
106
107 uds=Vector{Vector{Function}}([[convergence_display],[evidence_display],[info_display]]
108 lds=Vector{Vector{Function}}([[model_obs_display],[ensemble_display]])
109
110 n_models=50
111
112 evdict=Dict{String,Measurement}()
113
114 for ms in ["TCMZ","EdU"]
115     evdict[ms*"Combined"]=measurement(0,0)
116     evdict[ms*"Separate"]=measurement(0,0)
117 end
118
119 max_μ=2000.

```

```

120 min_μ=0.
121 max_λ=500.
122 min_λ=1e-6
123
124 prior=[Uniform(min_μ,max_μ),Uniform(min_λ,max_λ)]
125 box=[min_μ max_μ;min_λ max_λ]
126
127 gmmdir="/bench/PhD/NGS_binaries/GMC_NS/rysp/"
128
129 for ms in ["TCMZ","EdU"]
130     for (n,x) in enumerate(XCMZ)
131         c_ens=gmmdir*/c_*ms*string(Int64(x))
132         c_obs=vcat(sib_measure_dict[ms][n],rysp_measure_dict[ms][n])
133         if isfile(c_ens*/ens")
134             e=deserialize(c_ens*/ens")
135         else
136             e=LogNormal_Ensemble(c_ens,n_models, c_obs, prior, box,
137             → gmc ... )
138         end
139
140
141
142     for (msd,p) in
143         zip([sib_measure_dict,rysp_measure_dict],["/s_","/r_"])
144         ens=gmmdir*p*ms*string(Int64(x))
145         obs=msd[ms][n]
146         if isfile(ens*/ens")
147             e=deserialize(ens*/ens")
148         else
149             e=LogNormal_Ensemble(ens,n_models, obs, prior, box,
150             → gmc ... )
151         end
152
153     end
154 end

```

16.9.21 /rys/ryspont.jl

```

1 using CSV,DataFrames,Distributions,NGRefTools,StatsBase,StatsPlots
2 gr()
3 default(legendfont = (8), guidefont = (12,:bold), tickfont = (8), guide =
4   "x")
5
6 a38pth="/bench/PhD/datasets/A38.csv"
7 a49pth="/bench/PhD/datasets/A49.csv"
8
9 a38df=DataFrame(CSV.read(a38pth))
10 a38df.Group[1:10]::="sib"
11 a38df.Group[11:22]::="rys"
12 a49df=DataFrame(CSV.read(a49pth))
13 a49df=a49df[2:end,:]
14
15 a38df.BSR=[string(row.Block,',',row.Slide,',',row.Row) for row in
16   eachrow(a38df)]
17
18 sib_measure_dict=Dict{String,Vector{Vector{Float64}}}()
19 rys_measure_dict=Dict{String,Vector{Vector{Float64}}}()
20
21 XCMZ=Vector{Float64}()
22 XEdU=Vector{Float64}()
23
24 for dict in [sib_measure_dict,rys_measure_dict]
25   dict["DCMZ"]=Vector{Float64}()
26   dict["VCMZ"]=Vector{Float64}()
27   dict["TCMZ"]=Vector{Float64}()
28   dict["EdU"]=Vector{Float64}()
29 end
30
31 for tdf in groupby(a49df,"Age")
32   age=unique(tdf."Age")[1]
33   push!(XCMZ,age);push!(XEdU,age)
34   xidx=length(XCMZ)
35   for sdf in groupby(tdf, "Rys/Sib")
36     sr=unique(sdf."Rys/Sib")[1]
37     for mdf in groupby(sdf,"D/V/P/WE")
38       if unique(mdf."D/V/P/WE")[1] == "D"
39         if sr=="sib"
40           push!(sib_measure_dict["DCMZ"],mdf."PCNA +ve")

```

```

40         push!(sib_measure_dict["TCMZ"],mdf."PCNA +ve")
41         push!(sib_measure_dict["EdU"],mdf."EdU +ve, PCNA
42             ↵     +ve")
43     else
44         push!(rys_measure_dict["DCMZ"],mdf."PCNA +ve")
45         push!(rys_measure_dict["TCMZ"],mdf."PCNA +ve")
46         push!(rys_measure_dict["EdU"],mdf."EdU +ve, PCNA
47             ↵     +ve")
48     end
49 elseif unique(mdf."D/V/P/WE")[1] == "V"
50     if sr=="sib"
51         push!(sib_measure_dict["VCMZ"],mdf."PCNA +ve")
52         sib_measure_dict["TCMZ"][xidx].+=mdf."PCNA +ve"
53         sib_measure_dict["EdU"][xidx].+=mdf."EdU +ve, PCNA
54             ↵     +ve"
55     else
56         push!(rys_measure_dict["VCMZ"],mdf."PCNA +ve")
57         rys_measure_dict["TCMZ"][xidx].+=mdf."PCNA +ve"
58         rys_measure_dict["EdU"][xidx].+=mdf."EdU +ve, PCNA
59             ↵     +ve"
60     end
61 end
62 end
63
64 push!(XCMZ,5.)
65 for sdf in groupby(a38df, "Group")
66     xidx=length(XCMZ)
67     if unique(sdf."Group")[1]=="sib"
68         for mdf in groupby(sdf, "D/V")
69             if unique(mdf."D/V")[1] == "D"
70                 push!(sib_measure_dict["DCMZ"],mdf."PCNA +ve")
71                 push!(sib_measure_dict["TCMZ"],mdf."PCNA +ve")
72             else
73                 push!(sib_measure_dict["VCMZ"],mdf."PCNA +ve")
74                 sib_measure_dict["TCMZ"][xidx].+=mdf."PCNA +ve"
75             end
76         end
77     else
78         for mdf in groupby(sdf, "D/V")
79             if unique(mdf."D/V")[1] == "D"
80                 push!(rys_measure_dict["DCMZ"],mdf."PCNA +ve")
81             end
82         end
83     end
84 end

```

```

79     push!(rys_measure_dict["TCMZ"],mdf."PCNA +ve")
80   else
81     push!(rys_measure_dict["VCMZ"],mdf."PCNA +ve")
82     rys_measure_dict["TCMZ"][:xidx].+=mdf."PCNA +ve"
83   end
84 end
85 end
86 end
87
88 perm=sortperm(XCMZ)
89 for ms in ["DCMZ","VCMZ","TCMZ"]
90   sib_measure_dict[ms]=sib_measure_dict[ms][perm]
91   rys_measure_dict[ms]=rys_measure_dict[ms][perm]
92 end
93 XCMZ=XCMZ[perm]
94
95 sp_logn_mts=[fit(MarginalTDist,log.(sib_measure_dict["TCMZ"][:n])) for n
96   in 1:length(XCMZ)]
97 sp_mean=[exp(mean(mt)) for mt in sp_logn_mts]
98 sp_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in sp_logn_mts]
99 sp_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in sp_logn_mts]
100
101 rp_logn_mts=[fit(MarginalTDist,log.(rys_measure_dict["TCMZ"][:n])) for n
102   in 1:length(XCMZ)]
103 rp_mean=[exp(mean(mt)) for mt in rp_logn_mts]
104 rp_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in rp_logn_mts]
105 rp_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in rp_logn_mts]
106
107 log_mean_mass_comparator(rys_measure_dict["TCMZ"][:1],sib_measure_dict["TCMZ"][:1],labe
108   → $(XCMZ[1]) dpf sectional CMZ population","sib $(XCMZ[1]) dpf
109   → sectional CMZ population"])
110 log_mean_mass_comparator(rys_measure_dict["TCMZ"][:end],sib_measure_dict["TCMZ"][:end],labe
111   → $(XCMZ[:end]) dpf sectional CMZ population","sib $(XCMZ[:end]) dpf
112   → sectional CMZ population"])
113
114 println("At 4dpf,
115   → $(mean(sib_measure_dict["EdU"][:1] ./ sib_measure_dict["TCMZ"][:1])) ±
116   → $(std(sib_measure_dict["EdU"][:1] ./ sib_measure_dict["TCMZ"][:1])) of
117   → sib PCNA+ve are EdU positive")
118
119

```

```

110 println("At 4dpf,
→ $(mean(rys_measure_dict["EdU"][[1]]./rys_measure_dict["TCMZ"][[1]])) ±
→ $(std(rys_measure_dict["EdU"][[1]]./rys_measure_dict["TCMZ"][[1]])) of
→ rys PCNA+ve are EdU positive")

111
112 println("At 10dpf,
→ $(mean(sib_measure_dict["EdU"][[end]]./sib_measure_dict["TCMZ"][[end]]))
→ ± $(std(sib_measure_dict["EdU"][[end]]./sib_measure_dict["TCMZ"][[end]]))
→ of sib PCNA+ve are EdU positive")

113
114
115 println("At 10dpf,
→ $(mean(rys_measure_dict["EdU"][[end]]./rys_measure_dict["TCMZ"][[end]]))
→ ± $(std(rys_measure_dict["EdU"][[end]]./rys_measure_dict["TCMZ"][[end]]))
→ of rys PCNA+ve are EdU positive")

116
117 se_logn_mts=[fit(MarginalTDist,log.(sib_measure_dict["EdU"][[n]])) for n in
→ 1:length(XEdU)]
118 se_mean=[exp(mean(mt)) for mt in se_logn_mts]
119 se_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in se_logn_mts]
120 se_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in se_logn_mts]

121
122 re_logn_mts=[fit(MarginalTDist,log.(filter(val→!iszero(val),rys_measure_dict["EdU"])[
→ for n in 1:length(XEdU)])
123 re_mean=[exp(mean(mt)) for mt in re_logn_mts]
124 re_lower=[exp(mean(mt))-exp(quantile(mt,.025)) for mt in re_logn_mts]
125 re_upper=[exp(quantile(mt,.975))-exp(mean(mt)) for mt in re_logn_mts]

126
127
128 pcna_plt=plot(XCMZ,sp_mean,ribbon=(sp_lower,sp_upper),color=:green,
→ label="sib mean", xlabel="Age (dpf)", ylabel="PCNA+ve CMZ Cells")
129 plot!(pcna_plt,XCMZ, rp_mean,ribbon=(rp_lower, rp_upper),color=:darkmagenta,
→ label="rys mean", legend=:none)
130 scatter!(pcna_plt,vcat([[XCMZ[n] for i in
→ 1:length(sib_measure_dict["TCMZ"][[n]])] for n in
→ 1:length(XCMZ)] ... ),vcat(sib_measure_dict["TCMZ"] ... ),marker=:circle,markersize=:
→ data")
131 scatter!(pcna_plt,vcat([[XCMZ[n] for i in
→ 1:length(rys_measure_dict["TCMZ"][[n]])] for n in
→ 1:length(XCMZ)] ... ),vcat(rys_measure_dict["TCMZ"] ... ),marker=:dtriangle,markersize=:
→ data")
132 annotate!([(4.5,175,Plots.text("A",18))])
133

```

```

134 edu_plt=plot(XEdU,se_mean,ribbon=(se_lower,se_upper),color=:green,
    ↵ label="sib mean", xlabel="Age (dpf)", ylabel="EdU+ve CMZ Cells")
135 plot!(edu_plt,XEdU,re_mean,ribbon=(re_lower,re_upper),color=:darkmagenta,
    ↵ label="rys EdU")
136 scatter!(edu_plt,vcat([[XEdU[n] for i in
    ↵ 1:length(sib_measure_dict["EdU"])[n]]) for n in
    ↵ 1:length(XEdU)] ... ),vcat(sib_measure_dict["EdU"] ... ),marker=:circle,markersize=:3
    ↵ data")
137 scatter!(edu_plt,vcat([[XEdU[n] for i in
    ↵ 1:length(rys_measure_dict["EdU"])[n]]) for n in
    ↵ 1:length(XEdU)] ... ),vcat(rys_measure_dict["EdU"] ... ),marker=:dtriangle,markersize
    ↵ data")
138 annotate!([(4.5,150,Plots.text("B",18))])
139
140
141 combined=plt=plot(pcna_plt,edu_plt,layout=grid(1,2),size=(900,500))
142 savefig(combined=plt,"/bench/PhD/Thesis/images/rys/CMZontogeny.png")

```

16.9.22 /rys/bg_models/BBM_refinement_analysis.jl

```

1 using BioBackgroundModels, Serialization
2
3 hmm_output = "/bench/PhD/NGS_binaries/BBM/refined_chains"
4 sample_output = "/bench/PhD/NGS_binaries/BBM/refinement_samples"
5
6 survey_folders = "/bench/PhD/NGS_binaries/BBM/refined_folders"
7
8 chains=deserialize(hmm_output)
9 sample_dfs = deserialize(sample_output)
10 training_sets, test_sets = split_obs_sets(sample_dfs)
11
12 report_folders=generate_reports(chains, test_sets)
13 serialize(survey_folders, report_folders)

```

16.9.23 /rys/bg_models/BBM_refinement_prep.jl

```

1 #JOB FILEPATHS
2 #GFF3 feature database, FASTA genome and index paths
3 danio_gff_path = "/bench/PhD/seq/GRCz11/Danio rerio.GRCz11.94.gff3"
4 danio_genome_path =
    ↵ "/bench/PhD/seq/GRCz11/GCA_000002035.4_GRCz11_genomic.fna"

```

```

5 danio_gen_index_path =
   ↵ "/bench/PhD/seq/GRCz11/GCA_00002035.4_GRCz11_genomic.fna.fai"
6 #sample record and hmm serialisation output path
7 sample_output = "/bench/PhD/NGS_binaries/BBM/refinement_samples"
8 #non registered libs
9
10 #GENERAL SETUP
11 @info "Loading libraries ... "
12 using Distributed, Serialization
13
14 #JOB CONSTANTS
15 #CONSTANTS FOR GENOMIC SAMPLING
16 const sample_set_length = Int64(16e6)
17 const sample_window_min = 10
18 const sample_window_max = 3000
19 const perigenic_pad = 500
20 const partitions = 3 #exonic, periexonic, intragenic
21
22 #Setup sampling workers
23 @info "Spawning workers ... "
24 pool_size = partitions                                #number of workers to use
25 worker_pool = addprocs(pool_size) # add processes up to the worker pool
   ↵ size + 1 control process
26 @everywhere using BioBackgroundModels, Random
27 @everywhere Random.seed!(1)
28
29 #GET SEQUENCE OBSERVATIONS TO TRAIN AND TEST MODELS - PARTITIONING GENOME
   ↵ INTO EXONIC, PERIEXONIC, INTRAGENIC SEQUENCE
30 @info "Building observation db ... "
31 #generate the genomic sample dataframe
32 if isfile(sample_output) #if the sample DB has already been built for the
   ↵ current project, terminate
      @error "Existing sample dataframe at $sample_output"
33 else #otherwise, build it from scratch
      @info "Setting up sampling jobs ... "
34   #setup worker channels, input gets the genome partitions
35   channels = setup_sample_jobs(danio_genome_path, danio_gen_index_path,
36     ↵ danio_gff_path, sample_set_length, sample_window_min,
37     ↵ sample_window_max, perigenic_pad)
38
39   @info "Sampling genome ... "
40   sample_record_dfs=execute_sample_jobs(channels, worker_pool)
41

```

```

42     @info "Serializing sample dataframes ... "
43     serialize(sample_output, sample_record_dfs) #write the dataframes to
        ↳   file
44 end
45
46 rmprocs(worker_pool)
47
48 @info "Done sampling jobs!"

```

16.9.24 /rys/bg_models/BBM_sample_prep.jl

```

1 #JOB FILEPATHS
2 #GFF3 feature database, FASTA genome and index paths
3 danio_gff_path = "/bench/PhD/seq/GRCz11/Danio rerio.GRCz11.94.gff3"
4 danio_genome_path =
    ↳   "/bench/PhD/seq/GRCz11/GCA_00002035.4_GRCz11_genomic.fna"
5 danio_gen_index_path =
    ↳   "/bench/PhD/seq/GRCz11/GCA_00002035.4_GRCz11_genomic.fna.fai"
6 #sample record and hmm serialisation output path
7 sample_output = "/bench/PhD/NGS_binaries/BBM/survey_samples"
8 #non registered libs
9
10 #GENERAL SETUP
11 @info "Loading libraries ... "
12 using Distributed, Serialization
13
14 #JOB CONSTANTS
15 #CONSTANTS FOR GENOMIC SAMPLING
16 const sample_set_length = Int64(4e6)
17 const sample_window_min = 10
18 const sample_window_max = 3000
19 const perigenic_pad = 500
20 const partitions = 3 #exonic, periexonic, intragenic
21
22 #Setup sampling workers
23 @info "Spawning workers ... "
24 pool_size = partitions                                #number of workers to use
25 worker_pool = addprocs(pool_size) # add processes up to the worker pool
        ↳   size
26 @everywhere using BioBackgroundModels, Random
27 @everywhere Random.seed!(1)
28

```

```

29 #GET SEQUENCE OBSERVATIONS TO TRAIN AND TEST MODELS - PARTITIONING GENOME
   ↳ INTO EXONIC, PERIEXONIC, INTRAGENIC SEQUENCE
30 @info "Building observation db ... "
31 #generate the genomic sample dataframe
32 if isfile(sample_output) #if the sample DB has already been built for the
   ↳ current project, terminate
      @error "Existing sample dataframe at $sample_output"
33 else #otherwise, build it from scratch
      @info "Setting up sampling jobs ... "
34 #setup worker channels, input gets the genome partitions
35 channels = setup_sample_jobs(danio_genome_path, danio_gen_index_path,
   ↳ danio_gff_path, sample_set_length, sample_window_min,
   ↳ sample_window_max, perigenic_pad)
36
37
38
39 @info "Sampling genome ... "
40 sample_record_dfs=execute_sample_jobs(channels, worker_pool)
41
42 @info "Serializing sample dataframes ... "
43 serialize(sample_output, sample_record_dfs) #write the dataframes to
   ↳ file
44 end
45
46 rmprocs(worker_pool)
47
48 @info "Done sampling jobs!"

```

16.9.25 /rys/bg_models/BBM_survey.jl

```

1 #JOB FILEPATHS
2 #sample record and hmm serialisation output path
3 sample_output = "/bench/PhD/NGS_binaries/BBM/survey_samples"
4 hmm_output = "/bench/PhD/NGS_binaries/BBM/survey_chains"
5
6 #GENERAL SETUP
7 @info "Loading libraries ... "
8 using BioBackgroundModels, DataFrames, Distributed, Serialization
9 include("/srv/git/rys_nucleosomes/aws/aws_wrangler.jl")
10
11 #JOB CONSTANTS
12 const replicates = 3 #repeat optimisation from this many separately
   ↳ initialised samples from the prior
13 const Ks = [1,2,4,6] #mosaic class #s to test

```

```

14 const order_nos = [0,1,2] #DNA kmer order #s to test
15 const delta_thresh=1e-3 #stopping/convergence criterion (log probability
    ↪ difference btw subsequent EM iterates)
16 const max_iterates=15000
17
18 #LOAD SAMPLES
19 @info "Loading samples from $sample_output ..."
20 sample_dfs = deserialize(sample_output)
21
22 #BUILD TRAINING AND TEST SETS FROM SAMPLES
23 training_sets, test_sets = split_obs_sets(sample_dfs)
24
25 #PROGRAMATICALLY GENERATE Chain_ID Vector
26 job_ids=Vector{Chain_ID}()
27 for (obs_id, obs) in training_sets, K in Ks, order in order_nos, rep in
    ↪ 1:replicates
        push!(job_ids, Chain_ID(obs_id, K, order, rep))
28 end
29
30
31 #DISTRIBUTED CLUSTER CONSTANTS
32 load_dict=Dict{Int64,LoadConfig}()
33 local_config=LoadConfig(1:6,2:2)
34 remote_config=LoadConfig(1:6,0:1)
35 aws_instance_config=LoadConfig(1:6,0:2)
36 remote_machine = "10.0.0.3"
37 no_local_processes = 1
38 no_remote_processes = 1
39 #SETUP DISTRIBUTED BAUM WELCH LEARNERS
40 @info "Spawning local cluster workers ..."
41 worker_pool=addprocs(no_local_processes, topology=:master_worker)
42 for worker in worker_pool
43     load_dict[worker]=local_config
44 end
45
46 remote_pool=addprocs([(remote_machine,no_remote_processes)], tunnel=true,
    ↪ topology=:master_worker)
47
48 for worker in remote_pool
49     load_dict[worker]=remote_config
50 end
51
52 worker_pool=vcat(worker_pool, remote_pool)
53

```

```

54 #AWS PARAMS
55 @info "Setting up AWS wrangling ... "
56
57 security_group_name="calc1"
58 security_group_desc="calculation group"
59 ami="ami-02df7e1881a08f163"
60 skeys="AWS"
61 instance_type="c5.4xlarge"
62 zone,spot_price=get_cheapest_zone(instance_type)
63 no_instances=1
64 instance_workers=8
65 bid=spot_price+.01
66
67 @assert bid >= spot_price
68
69 @info "Wrangling AWS instances ... "
70 aws_ips = spot_wrangle(no_instances, bid, security_group_name,
    ↪ security_group_desc, skeys, zone, ami, instance_type)
71 @info "Giving instances 90s to boot ... "
72 sleep(90)
73
74 @info "Spawning AWS cluster workers ... "
75 for ip in aws_ips
76     instance_pool=addprocs([(ip, instance_workers)], tunnel=true,
    ↪ topology=:master_worker, sshflags="-o StrictHostKeyChecking=no")
77     for worker in instance_pool
78         load_dict[worker]=aws_instance_config
79     end
80     global worker_pool=vcat(worker_pool, instance_pool)
81 end
82
83 @info "Loading worker libraries everywhere ... "
84 @everywhere using BioBackgroundModels
85 #INITIALIZE HMMS
86 @info "Setting up HMMS ... "
87 if isfile(hmm_output) #if some results have already been collected, load
    ↪ them
88     @info "Loading incomplete results ... "
89     hmm_results_dict = deserialize(hmm_output)
90 else #otherwise, pass a new results dict
91     @info "Initialising new HMM results file at $hmm_output"
92     hmm_results_dict = Dict{Chain_ID,Vector{EM_step}}()
93 end

```

```

94
95 em_jobset = setup_EM_jobs!(job_ids, training_sets;
  ↵ chains=hmm_results_dict)
96 execute_EM_jobs!(worker_pool, em_jobset..., hmm_output;
  ↵ load_dict=load_dict, delta_thresh=delta_thresh,
  ↵ max_iterates=max_iterates)

```

16.9.26 /rys/bg_models/BBM_survey_analysis.jl

```

1 using BioBackgroundModels, Serialization, Plots, Measures
2 import Measures:mm
3 import Plots:Plot
4
5 hmm_output = "/bench/PhD/NGS_binaries/BBM/survey_chains"
6 sample_output = "/bench/PhD/NGS_binaries/BBM/survey_samples"
7
8 survey_folders = "/bench/PhD/NGS_binaries/BBM/survey_folders"
9
10 chains=deserialize(hmm_output)
11 sample_dfs = deserialize(sample_output)
12 training_sets, test_sets = split_obs_sets(sample_dfs)
13
14 report_folders=generate_reports(chains, test_sets)
15 serialize(survey_folders, report_folders)
16
17 partplts=Vector{Plot}()
18
19 for o in [0,1,2]
20     for p in ["exon","intergenic","periexonic"]
21         rf=report_folders[p]
22         pr=rf.partition_report
23         od=pr.orddict
24         or=od[o]
25
26         p=="exon" ? (ylabel="Order $o lh"; yfmt=y→y; lm=0mm) : (ylabel="";
27           ↵ yfmt=_→""; lm=-7mm)
28         o==2 ? (xlabel=p; xfmt=x→Int64(x); bm=0mm) : (xlabel="";
29           ↵ xfmt=_→""; bm=-7mm)

```

```

29     plt=scatter(or.converged_K,or.converged_lh, marker=:cross,
30                 ↵ markersize=15, markercolor=:black, label="Ord. $o model lhs",
31                 ↵ xlabel=xlbl, ylabel=ylbl, legend=nothing, xformatter=xfmt,
32                 ↵ yformatter=yfmt, xticks=[1,2,4,6],
33                 ↵ leftmargin=lm,bottommargin=bm)
34 plot!(unique(or.converged_K),[pr.naive_lh for l in
35             ↵ 1:length(unique(or.converged_K))], label="Naive lh",
36             ↵ color=:darkmagenta,linewidth=2.5)
37 push!(partplts,plt)
38 end
39 end
40 combined=plot(partplts ... ,layout=grid(3,3),link=:xy, size=(600,600))
41 savefig(combined,"/bench/PhD/Thesis/images/rys/bghmm.png")

```

16.9.27 /rys/bg_models/BBM_survey_refinement.jl

```

1 #JOB FILEPATHS
2 hmm_output = "/bench/PhD/NGS_binaries/BBM/survey_chains"
3 refined_path = "/bench/PhD/NGS_binaries/BBM/refined_chains"
4 sample_output = "/bench/PhD/NGS_binaries/BBM/refinement_samples"
5
6 survey_folders = "/bench/PhD/NGS_binaries/BBM/survey_folders"
7
8 #GENERAL SETUP
9 @info "Loading libraries ..."
10 using BioBackgroundModels, DataFrames, Distributed, Serialization
11
12 const delta_thresh=1e-4 #stopping/convergence criterion (log probability
13             ↵ difference btw subsequent EM iterates)
14 const max_iterates=15000
15
16 #LOAD SAMPLES
17 @info "Loading samples from $sample_output ..."
18 sample_dfs = deserialize(sample_output)
19
20 #BUILD TRAINING AND TEST SETS FROM SAMPLES
21 training_sets, test_sets = split_obs_sets(sample_dfs)
22
23 #GENERATE Chain_ID Vector AND CHAINS SUBSET
24 @info "Loading reports from $survey_folders"
25 report_folders=deserialize(survey_folders)
26 job_ids=Vector{Chain_ID}()

```

```

26 for (partition,folder) in report_folders
27     report=folder.partition_report
28     for id in report.best_repset
29         push!(job_ids,id)
30     end
31 end
32
33 if isfile(refined_path) #if some results have already been collected,
34     ↪ load them
35     @info "Loading incomplete results ..."
36     refined_chains=deserialize(refined_path)
37 else
38     @info "Initialising new HMM results file at $refined_path"
39     chains = deserialize(hmm_output)
40     refined_chains=Dict{Chain_ID,Vector{EM_step}}(){}
41     for job_id in job_ids
42         refined_chains[job_id]=[EM_step(1, chains[job_id][end].hmm, 0, 0,
43             ↪ false)]
44     end
45 end
46
47 #DISTRIBUTED CLUSTERS CONSTANTS
48 remote_machine = "10.0.0.3"
49 no_local_processes = 1
50 no_remote_processes = 1
51 load_dict=Dict{Int64,LoadConfig}()
52 local_config=LoadConfig(1:6,0:2)
53 remote_config=LoadConfig(1:6,0:2)
54
55 #SETUP DISTRIBUTED BAUM WELCH LEARNERS
56 @info "Spawning local cluster workers ..."
57 worker_pool=addprocs(no_local_processes, topology=:master_worker)
58 for worker in worker_pool
59     load_dict[worker]=local_config
60 end
61
62 remote_pool=addprocs([(remote_machine,no_remote_processes)], tunnel=true,
63     ↪ topology=:master_worker)
64 for worker in remote_pool
65     load_dict[worker]=remote_config
66 end
67
68 worker_pool=vcat(worker_pool, remote_pool)

```

```

66
67 @info "Loading worker libraries everywhere ... "
68 @everywhere using BioBackgroundModels
69
70 em_jobset = setup_EM_jobs!(job_ids, training_sets;
    ↳ delta_thresh=delta_thresh, chains=refined_chains)
71 execute_EM_jobs!(worker_pool, em_jobset..., refined_path;
    ↳ delta_thresh=delta_thresh, max_iterates=max_iterates,
    ↳ load_dict=load_dict)

```

16.9.28 /rys/nested_sampling/dif_pos_assembly.jl

```

1 @info "Setting up for job ... "
2 #JOB FILEPATHS
3 sib_wms_path =
4     ↳ "/bench/PhD/NGS_binaries/BMI/sib_nuc_position_sequences.fa_wms.tr"
5 rys_wms_path =
6     ↳ "/bench/PhD/NGS_binaries/BMI/rys_nuc_position_sequences.fa_wms.tr"
7
8 sib_df_binary = "/bench/PhD/NGS_binaries/BMI/sib_diff_positions"
9 rys_df_binary = "/bench/PhD/NGS_binaries/BMI/rys_diff_positions"
10 combined_df_binary =
11     ↳ "/bench/PhD/NGS_binaries/BMI/combined_diff_positions"
12
13 sib_code_binary = "/bench/PhD/NGS_binaries/BMI/sib_diff_codes"
14 rys_code_binary = "/bench/PhD/NGS_binaries/BMI/rys_diff_codes"
15 combined_code_binary = "/bench/PhD/NGS_binaries/BMI/combined_diff_codes"
16
17 sib_diff_bg = "/bench/PhD/NGS_binaries/BMI/sib_diff_bg"
18 rys_diff_bg = "/bench/PhD/NGS_binaries/BMI/rys_diff_bg"
19 combined_diff_bg = "/bench/PhD/NGS_binaries/BMI/combined_diff_bg"
20
21 #JOB CONSTANTS
22 const ensemble_size = 500
23 const no_sources = 8
24 const source_min_bases = 3
25 const source_max_bases = 10
26
27 @assert source_min_bases < source_max_bases

```

```

28 const source_length_range= source_min_bases:source_max_bases
29 const mixing_prior = .07
30 @assert mixing_prior ≥ 0 & mixing_prior ≤ 1
31
32 @info "Loading master libraries ... "
33 using Distributed, Distributions, Serialization
34
35 @info "Adding workers ... "
36 no_local_procs=2
37 worker_pool=addprocs(no_local_procs, topology=:master_worker)
38
39 @info "Loading libraries everywhere ... "
40 @everywhere using BioMotifInference, Random
41 Random.seed!(myid()*10000)
42
43 @info "Assembling uninformative source priors ... "
44 sib_ui_sp = BioMotifInference.assemble_source_priors(no_sources,
    ↳ Vector{Matrix{Float64}}())
45 rys_ui_sp = BioMotifInference.assemble_source_priors(no_sources,
    ↳ Vector{Matrix{Float64}}())
46 combined_ui_sp = BioMotifInference.assemble_source_priors(no_sources,
    ↳ Vector{Matrix{Float64}}())
47
48 @info "Loading BGHMM likelihood matrix binaries ... "
49 sib_matrix=deserialize(sib_diff_bg)
50 rys_matrix=deserialize(rys_diff_bg)
51 combined_matrix=deserialize(combined_diff_bg)
52
53 @info "Loading coded observation sets ... "
54 sib_obs = deserialize(sib_code_binary)
55 rys_obs = deserialize(rys_code_binary)
56 combined_obs = deserialize(combined_code_binary)
57
58 @info "Assembling sib IPM ensemble on uninformative priors ... "
59 isfile(string(sib_e_pth,'/','ens')) ? (sib_e =
    ↳ deserialize(string(sib_e_pth,'/','ens'))) :
60     (sib_e = BioMotifInference.IPM_Elensembles(worker_pool, sib_e_pth,
        ↳ ensemble_size, sib_ui_sp, (falses(0,0), mixing_prior),
        ↳ sib_matrix, sib_obs, source_length_range, posterior_switch=true);
        ↳ serialize(string(sib_e_pth,'/','ens'),sib_e))
61 sib_e=[]; Base.GC.gc();
62
63 @info "Assembling rys IPM ensemble on uninformative priors ... "

```

```

64 isfile(string(rys_e_pth,'/','ens')) ? (rys_e =
  ↳ deserialize(string(rys_e_pth,'/','ens'))) :
65   (rys_e = BioMotifInference.IPM_Engineer(worker_pool, rys_e_pth,
  ↳ ensemble_size, rys_ui_sp, (falses(0,0), mixing_prior),
  ↳ rys_matrix, rys_obs, source_length_range, posterior_switch=true);
  ↳ serialize(string(rys_e_pth,'/','ens'),rys_e))
66 rys_e=[]; Base.GC.gc();
67
68
69 @info "Assembling combined IPM ensemble on uninformative priors ... "
70 isfile(string(combined_e_pth,'/','ens')) ? (combined_e =
  ↳ deserialize(string(combined_e_pth,'/','ens'))) :
71   (combined_e = BioMotifInference.IPM_Engineer(worker_pool,
  ↳ combined_e_pth, ensemble_size, combined_ui_sp, (falses(0,0),
  ↳ mixing_prior), combined_matrix, combined_obs,
  ↳ source_length_range, posterior_switch=true);
  ↳ serialize(string(combined_e_pth,'/','ens'),combined_e))
72 combined_e=[]; Base.GC.gc();
73
74 rmprocs(worker_pool)
75
76 @info "Job done!"

```

16.9.29 /rys/nested_sampling/dif_pos_learner.jl

```

1 using Distributed, Distributions, Serialization
2 include("/srv/git/rys_nucleosomes/aws/aws_wrangler.jl")
3
4 sib_e_pth = "/bench/PhD/NGS_binaries/BMI/sib_e"
5 rys_e_pth = "/bench/PhD/NGS_binaries/BMI/rys_e"
6 combined_e_pth = "/bench/PhD/NGS_binaries/BMI/combined_e"
7
8 @info "Assembling worker pool ... "
9
10 #DISTRIBUTED CLUSTERS CONSTANTS
11 remote_machine = "10.0.0.3"
12 no_local_processes = 2
13 no_remote_processes = 6
14
15 @info "Spawning local cluster workers ... "
16 worker_pool=addprocs(no_local_processes, topology=:master_worker)

```

```

17 remote_pool=addprocs([(remote_machine,no_remote_processes)], tunnel=true,
   ↵ topology=:master_worker)

18
19 worker_pool=vcat(worker_pool, remote_pool)

20
21 #AWS PARAMS
22 @info "Setting up AWS wrangling ..."

23
24 security_group_name="calc1"
25 security_group_desc="calculation group"
26 ami="ami-0bb0f5609b995d39e"
27 skeys="AWS"
28 instance_type="c5a.24xlarge"
29 zone,spot_price=get_cheapest_zone(instance_type)
30 no_instances=5
31 instance_workers=48
32 bid=spot_price+.1

33
34 @assert bid ≥ spot_price

35
36 @info "Wrangling AWS instances ... "
37 aws_ips = spot_wrangle(no_instances, bid, security_group_name,
   ↵ security_group_desc, skeys, zone, ami, instance_type)
38 @info "Giving instances 150s to boot ... "
39 sleep(150)

40
41 # aws_ips=["18.223.214.57","18.224.4.81",
   ↵ "3.17.13.150","3.21.113.229","18.222.211.44"]

42
43
44 @info "Spawning AWS cluster workers ... "
45 for ip in aws_ips
46     instance_pool=addprocs([(ip, instance_workers)], tunnel=true,
   ↵ topology=:master_worker, sshflags="-o StrictHostKeyChecking=no")
47     global worker_pool=vcat(worker_pool, instance_pool)
48 end

49
50 @info "Loading worker libraries everywhere ... "
51 @everywhere using BioMotifInference,Random
52 @everywhere Random.seed!(myid()*100000)

53
54 #JOB CONSTANTS
55 funcvec=full_perm_funcvec

```

```

56 models_to_permute=10000
57 func_limit=25
58 push!(funcvec, BioMotifInference.perm_src_fit_mix)
59 push!(funcvec, BioMotifInference.random_decorrelate)
60
61 min_clamps=fill(.01,length(funcvec))
62 min_clamps[2:3] *= .1 #perm_src_fit_mix & permute_mix
63 min_clamps[8]=.1 #difference_merge
64 max_clamps=fill(.5,length(funcvec))
65 max_clamps[6:7] *= .15 #ss and am
66 max_clamps[9]=.15 #sim merge
67
68 initial_weights= ones(length(funcvec))./length(funcvec)
69 # override_weights=fill(.034375,length(funcvec))
70 # override_weights[6:9] *= .1;override_weights[13:14] *= .1625
71 # override_time=20.

72
73 args=[Vector{Tuple{Symbol,Any}}() for i in 1:length(funcvec)]
74 args[end-1]=[:weight_shift_freq,0.],(:length_change_freq,1.),(:length_perm_range,1:1)
75 args[end]=[:iterates,50],(:source_permute_freq,.3),(:mix_move_range,1:10)

76
77 instruct = Permute_Instruct(funcvec, initial_weights, models_to_permute,
    → func_limit;min_clmps=min_clamps, max_clmps=max_clamps, args=args)

78
79 display_rotation=[true,10,1,[[:tuning_disp,:lh_disp,:src_disp],[:conv_plot,:liwi_disp
80
81 @info "Beginning nested sampling for sib ensemble ... "
82 sib_e=deserialize(sib_e_pth*"/ens")
83 sib_logZ = converge_ensemble!(sib_e, instruct, worker_pool,
    → converge_criterion="compression", converge_factor=150.,
    → backup=(true,25), tuning_disp=true,lh_disp=true,src_disp=true,
    → disp_rotate_inst=display_rotation)
84 sib_e=[]; Base.GC.gc();

85
86 @info "Beginning nested sampling for rys ensemble ... "
87 rys_e=deserialize(rys_e_pth*"/ens")
88 rys_logZ = converge_ensemble!(rys_e, instruct, worker_pool,
    → converge_criterion="compression", converge_factor=150.,
    → backup=(true,25), tuning_disp=true,lh_disp=true,src_disp=true,
    → disp_rotate_inst=display_rotation)
89 rys_e=[]; Base.GC.gc();

90
91 @info "Beginning nested sampling for combined ensemble ... "

```

```

92 combined_e=deserialize(combined_e_pth*"/ens")
93 combined_logZ = converge_ensemble!(combined_e, instruct, worker_pool,
    ↪ converge_criterion="compression", converge_factor=150.,
    ↪ backup=(true,25),
    ↪ tuning_disp=true, lh_disp=true, src_disp=true, disp_rotate_inst=display_rotation)
94
95 joint_logZ=rys_logZ+sib_logZ
96 evratio=joint_logZ-combined_logZ
97 sig=evratio.val/evratio.err
98
99 println("""$(round(sib_logZ,digits=3)) & $(round(rys_logZ,digits=3)) &
    ↪ {\\bf $(round(joint_logZ,digits=3))} &
    ↪ $(round(combined_logZ,digits=3)) & $(round(evratio,digits=3)) &
    ↪ $(round(sig,digits=1)) \\\\ \\hline""")
100
101 rmprocs(worker_pool)
102
103 @info "Job done!"

```

16.9.30 /rys/nested_sampling/dif_pos_sample_prep.jl

```

1 using BioSequences, Distributed, DataFrames, BioBackgroundModels, CSV,
    ↪ Serialization
2 import StatsBase:sample
3
4 include("/bench/PhD/Thesis/Analyses/rys/position_analysis/position_overlap.jl")
5
6 #JOB PATHS AND CONSTANTS
7
8 sample_rows = 7092 #2 million bases
9
10 sib_pos =
    ↪ "/bench/PhD/danpos_results/pooled/sib.Fnor.smooth.positions.xls"
11 rys_pos =
    ↪ "/bench/PhD/danpos_results/pooled/rys.Fnor.smooth.positions.xls"
12
13 danio_genome_path =
    ↪ "/bench/PhD/seq/GRCz11/GCA_000002035.4_GRCz11_genomic.fna"
14 danio_gen_index_path =
    ↪ "/bench/PhD/seq/GRCz11/GCA_000002035.4_GRCz11_genomic.fna.fai"
15 danio_gff_path = "/bench/PhD/seq/GRCz11/Danio rerio.GRCz11.94.gff3"
16

```

```

17 refined_folders_path = "/bench/PhD/NGS_binaries/BBM/refined_folders"
18 selected_hmms = "/bench/PhD/NGS_binaries/BBM/selected_hmms"
19
20 sib_df_binary = "/bench/PhD/NGS_binaries/BMI/sib_diff_positions"
21 rys_df_binary = "/bench/PhD/NGS_binaries/BMI/rys_diff_positions"
22 combined_df_binary =
23   ↳ "/bench/PhD/NGS_binaries/BMI/combined_diff_positions"
24
25 sib_code_binary = "/bench/PhD/NGS_binaries/BMI/sib_diff_codes"
26 rys_code_binary = "/bench/PhD/NGS_binaries/BMI/rys_diff_codes"
27 combined_code_binary = "/bench/PhD/NGS_binaries/BMI/combined_diff_codes"
28
29 sib_diff_bg = "/bench/PhD/NGS_binaries/BMI/sib_diff_bg"
30 rys_diff_bg = "/bench/PhD/NGS_binaries/BMI/rys_diff_bg"
31 combined_diff_bg = "/bench/PhD/NGS_binaries/BMI/combined_diff_bg"
32
33 sib_fa = "/bench/PhD/thicweed_results/sib_nuc_position_sequences.fa"
34 sib_arch =
35   ↳ "/bench/PhD/thicweed_results/sib_nuc_position_sequences.fa_archs.txt"
36
37 rys_fa = "/bench/PhD/thicweed_results/rys_nuc_position_sequences.fa"
38 rys_arch =
39   ↳ "/bench/PhD/thicweed_results/rys_nuc_position_sequences.fa_archs.txt"
40
41 @info "Reading from position.xls ... "
42 sib_df=CSV.read(sib_pos)
43 rys_df=CSV.read(rys_pos)
44
45 @info "Mapping ... "
46 map_positions!(sib_df, rys_df)
47 map_positions!(rys_df, sib_df)
48
49 @info "Making differential position dataframes ... "
50 sib_diff_df = deepcopy(sib_df[findall(iszero,sib_df.mapped_pos),:])
51 sib_diff_df = sib_diff_df[findall(!isequal("MT"), sib_diff_df.chr),:]
52 rys_diff_df = deepcopy(rys_df[findall(iszero,rys_df.mapped_pos),:])
53 rys_diff_df = rys_diff_df[findall(!isequal("MT"), rys_diff_df.chr),:]
54
55 add_position_sequences!(sib_diff_df, danio_genome_path,
56   ↳ danio_gen_index_path)
57 add_position_sequences!(rys_diff_df, danio_genome_path,
58   ↳ danio_gen_index_path)

```

```

55 @info "Filtering ambiguous sequences ... "
56 deleterows!(sib_diff_df, [hasambiguity(seq) for seq in sib_diff_df.seq])
57 deleterows!(rys_diff_df, [hasambiguity(seq) for seq in rys_diff_df.seq])
58
59 @info "Sampling subsets of sequences ... "
60
61 sib_diff_df=sib_diff_df[sample(axes(sib_diff_df, 1), sample_rows;
62   → replace=false, ordered=true), :]
62 rys_diff_df=rys_diff_df[sample(axes(rys_diff_df, 1), sample_rows;
63   → replace=false, ordered=true), :]
64
64 @info "Masking positions by genome partition and strand ... "
65 BioBackgroundModels.add_partition_masks!(sib_diff_df, danio_gff_path,
66   → 500, (:chr,:seq,:start))
66 BioBackgroundModels.add_partition_masks!(rys_diff_df, danio_gff_path,
67   → 500, (:chr,:seq,:start))
68
68 @info "Obtaining cluster data ... "
69 get_cluster!(sib_diff_df, sib_fa, sib_arch)
70 get_cluster!(rys_diff_df, rys_fa, rys_arch)
71
72 @info "Serializing dataframes ... "
73 combined_diff_df = vcat(sib_diff_df, rys_diff_df)
74
75 serialize(sib_df_binary, sib_diff_df)
76 serialize(rys_df_binary, rys_diff_df)
77 serialize(combined_df_binary, combined_diff_df)
78
79 @info "Creating coded observation sets ... "
80 sib_codes = observation_setup(sib_diff_df, order=0, symbol=:seq)
81 rys_codes = observation_setup(rys_diff_df, order=0, symbol=:seq)
82 combined_codes = observation_setup(combined_diff_df, order=0,
83   → symbol=:seq)
84
84 @info "Serializing coded observation sets ... "
85 serialize(sib_code_binary, Matrix(transpose(sib_codes)))
86 serialize(rys_code_binary, Matrix(transpose(rys_codes)))
87 serialize(combined_code_binary, Matrix(transpose(combined_codes)))
88
89 @info "Setting up for BBM likelihood calculations ... "
90 BHMM_dict = Dict{String,BHMM}()
91 refined_folders=deserialize(refined_folders_path)
92 for (part, folder) in refined_folders

```

```

93     BHMM_dict[part]=folder.partition_report.best_model[2]
94 end
95
96 serialize(selected_hmms,BHMM_dict)
97
98 @info "Performing calculations ... "
99 sib_lh_matrix = BGHMM_likelihood_calc(sib_diff_df, BHMM_dict,
100   ↪ symbol=:seq)
100 rys_lh_matrix = BGHMM_likelihood_calc(rys_diff_df, BHMM_dict,
101   ↪ symbol=:seq)
101 combined_lh_matrix = hcat(sib_lh_matrix,rys_lh_matrix)
102
103 @info "Serializing background matrices ... "
104 serialize(sib_diff_bg, sib_lh_matrix)
105 serialize(rys_diff_bg, rys_lh_matrix)
106 serialize(combined_diff_bg, combined_lh_matrix)
107
108 @info "Job done!"

```

16.9.31 /rys/nested_sampling/local_test.jl

```

1 using Distributed, Distributions, Serialization
2
3 test_e_pth = "/bench/PhD/NGS_binaries/BMI/test_e"
4
5 @info "Assembling worker pool ... "
6
7 #DISTRIBUTED CLUSTERS CONSTANTS
8 remote_machine = "10.0.0.3"
9 no_local_processes = 1
10
11 @info "Spawning local cluster workers ... "
12 worker_pool=addprocs(no_local_processes, topology=:master_worker)
13
14 @info "Loading worker libraries everywhere ... "
15 @everywhere using BioMotifInference,Random
16 @everywhere Random.seed!(myid()*100000)
17
18 #JOB CONSTANTS
19 models_to_permute=5000
20 func_limit=30
21 clamp=.02

```

```

22 funcvec=full_perm_funcvec
23 push!(funcvec, BioMotifInference.permute_source)
24 push!(funcvec, BioMotifInference.permute_source)
25 args=[Vector{Tuple{Symbol,Any}}() for i in 1:length(funcvec)]
26 args[end-1]=[:weight_shift_freq,0.],(:length_change_freq,1.),(:length_perm_range,1:1)
27 args[end]=[:weight_shift_freq,.1],(:length_change_freq,0.),(:weight_shift_dist,Unifo
28
29 instruct = Permute_Instruct(funcvec,
30     ↳ ones(length(funcvec))./length(funcvec), models_to_permute,
31     ↳ func_limit, clamp; args=args)
32
33 @info "Beginning nested sampling for test ensemble ... "
34 test_e=deserialize(test_e_pth*"/ens")
35 test_logZ = converge_ensemble!(test_e, instruct, worker_pool, .001,
36     ↳ backup=true,1),tuning_disp=true, lh_disp=true,src_disp=true,
37     ↳ disp_rotate_inst=display_rotation)
38
39 rmpprocs(worker_pool)
40
41 @info "Job done!"

```

16.9.32 /rys/nested_sampling/prior_test.jl

```

1 @info "Setting up for job ... "
2 #JOB FILEPATHS
3 sib_wms_path =
4     ↳ "/bench/PhD/NGS_binaries/BMI/sib_nuc_position_sequences.fa_wms.tr"
4 rys_wms_path =
5     ↳ "/bench/PhD/NGS_binaries/BMI/rys_nuc_position_sequences.fa_wms.tr"
5
6 sib_df_binary = "/bench/PhD/NGS_binaries/BMI/sib_diff_positions"
7 rys_df_binary = "/bench/PhD/NGS_binaries/BMI/rys_diff_positions"
8 combined_df_binary =
9     ↳ "/bench/PhD/NGS_binaries/BMI/combined_diff_positions"
10
10 sib_code_binary = "/bench/PhD/NGS_binaries/BMI/sib_diff_codes"
11 rys_code_binary = "/bench/PhD/NGS_binaries/BMI/rys_diff_codes"
12 combined_code_binary = "/bench/PhD/NGS_binaries/BMI/combined_diff_codes"
13
14 sib_diff_bg = "/bench/PhD/NGS_binaries/BMI/sib_diff_bg"

```

```

15 rys_diff_bg = "/bench/PhD/NGS_binaries/BMI/rys_diff_bg"
16 combined_diff_bg = "/bench/PhD/NGS_binaries/BMI/combined_diff_bg"
17
18 sib_e_ui_pth = "/bench/PhD/NGS_binaries/BMI/sib_e_ui"
19 sib_e_inf_pth = "/bench/PhD/NGS_binaries/BMI/sib_e_inf"
20 rys_e_ui_pth = "/bench/PhD/NGS_binaries/BMI/rys_e_ui"
21 rys_e_inf_pth = "/bench/PhD/NGS_binaries/BMI/rys_e_inf"
22 combined_e_ui_pth = "/bench/PhD/NGS_binaries/BMI/combined_e_ui"
23 combined_e_inf_pth = "/bench/PhD/NGS_binaries/BMI/combined_e_inf"
24
25
26 #JOB CONSTANTS
27 const ensemble_size = 1000
28 const no_sources = 8
29 const source_min_bases = 3
30 const source_max_bases = 10
31 @assert source_min_bases < source_max_bases
32 const source_length_range= source_min_bases:source_max_bases
33 const mixing_prior = .07
34 @assert mixing_prior ≥ 0 & mixing_prior ≤ 1
35 const prior_wt=1.2
36
37 @info "Loading master libraries ... "
38 using Distributed, Distributions, Serialization
39
40 @info "Adding librarians and workers ... "
41 no_local_procs=2
42 no_remote_procs=6
43 remote_machine = "10.0.0.119"
44 remote_pool=addprocs([(remote_machine, no_remote_procs)], tunnel=true,
    ↳ topology=:master_worker)
45 local_pool=addprocs(no_local_procs, topology=:master_worker)
46 worker_pool=vcat(remote_pool,local_pool)
47
48 @info "Loading libraries everywhere ... "
49 @everywhere using BioMotifInference, Random
50 Random.seed!(myid()*10000)
51
52 @info "Loading informative source priors ... "
53 sib_wms = BioMotifInference.read_fa_wms_tr(sib_wms_path)
54 rys_wms = BioMotifInference.read_fa_wms_tr(rys_wms_path)

```

```

55 sib_mix_prior =
  ↵ BioMotifInference.cluster_mix_prior!(deserialize(sib_df_binary),
  ↵ sib_wms)
56 rys_mix_prior =
  ↵ BioMotifInference.cluster_mix_prior!(deserialize(rys_df_binary),
  ↵ rys_wms)

57
58 @info "Filtering informative priors ... "
59 sib_prior_wms = BioMotifInference.filter_priors(Int(floor(no_sources/2)),
  ↵ source_max_bases, sib_wms, sib_mix_prior)
60 rys_prior_wms = BioMotifInference.filter_priors(Int(floor(no_sources/2)),
  ↵ source_max_bases, rys_wms, rys_mix_prior)
61 combined_prior_wms =
  ↵ BioMotifInference.combine_filter_priors(Int(floor(no_sources/2)),
  ↵ source_max_bases, (sib_wms, rys_wms), (sib_mix_prior, rys_mix_prior))

62
63 @info "Assembling informative source priors ... "
64 sib_inf_sp = BioMotifInference.assemble_source_priors(no_sources,
  ↵ sib_prior_wms, prior_wt)
65 rys_inf_sp = BioMotifInference.assemble_source_priors(no_sources,
  ↵ rys_prior_wms, prior_wt)
66 combined_inf_sp = BioMotifInference.assemble_source_priors(no_sources,
  ↵ combined_prior_wms, prior_wt)

67
68 @info "Assembling uninformative source priors ... "
69 sib_ui_sp = BioMotifInference.assemble_source_priors(no_sources,
  ↵ Vector{Matrix{Float64}}())
70 rys_ui_sp = BioMotifInference.assemble_source_priors(no_sources,
  ↵ Vector{Matrix{Float64}}())
71 combined_ui_sp = BioMotifInference.assemble_source_priors(no_sources,
  ↵ Vector{Matrix{Float64}}())

72
73 @info "Loading BGHMM likelihood matrix binaries ... "
74 sib_matrix=deserialize(sib_diff_bg)
75 rys_matrix=deserialize(rys_diff_bg)
76 combined_matrix=deserialize(combined_diff_bg)

77
78 @info "Loading coded observation sets ... "
79 sib_obs = deserialize(sib_code_binary)
80 rys_obs = deserialize(rys_code_binary)
81 combined_obs = deserialize(combined_code_binary)

82
83 @info "Assembling sib IPM ensemble on uninformative priors ... "

```

```

84 isfile(string(sib_e_ui_pth,'/','ens')) ? (sib_e_ui =
85   → deserialize(string(sib_e_ui_pth,'/','ens'))) :
86   (sib_e_ui = BioMotifInference.IPM_Ensemble(worker_pool, sib_e_ui_pth,
87     → ensemble_size, sib_ui_sp, (falses(0,0), mixing_prior),
88     → sib_matrix, sib_obs, source_length_range);
89   → serialize(string(sib_e_ui_pth,'/','ens'),sib_e_ui))

90
91 @info "Assembling sib IPM ensemble on informative priors ... "
92 isfile(string(sib_e_inf_pth,'/','ens')) ? (sib_e_inf =
93   → deserialize(string(sib_e_inf_pth,'/','ens'))) :
94   (sib_e_inf = BioMotifInference.IPM_Ensemble(worker_pool,
95     → sib_e_inf_pth, ensemble_size, sib_inf_sp, (falses(0,0),
96     → mixing_prior), sib_matrix, sib_obs, source_length_range);
97   → serialize(string(sib_e_inf_pth,'/','ens'),sib_e_inf))

98
99 @info "Assembling rys IPM ensemble on uninformative priors ... "
100 isfile(string(rys_e_ui_pth,'/','ens')) ? (rys_e_ui =
101   → deserialize(string(rys_e_ui_pth,'/','ens'))) :
102   (rys_e_ui = BioMotifInference.IPM_Ensemble(worker_pool,
103     → rys_e_ui_pth, ensemble_size, rys_ui_sp, (falses(0,0),
104     → mixing_prior), rys_matrix, rys_obs, source_length_range);
105   → serialize(string(rys_e_ui_pth,'/','ens'),rys_e_ui))

106
107 @info "Assembling rys IPM ensemble on informative priors ... "
108 isfile(string(rys_e_inf_pth,'/','ens')) ? (rys_e_inf =
109   → deserialize(string(rys_e_inf_pth,'/','ens'))) :
110   (rys_e_inf = BioMotifInference.IPM_Ensemble(worker_pool,
111     → rys_e_inf_pth, ensemble_size, rys_inf_sp, (falses(0,0),
112     → mixing_prior), rys_matrix, rys_obs, source_length_range);
113   → serialize(string(rys_e_inf_pth,'/','ens'),rys_e_inf))

114
115 @info "Assembling combined IPM ensemble on uninformative priors ... "
116 isfile(string(combined_e_ui_pth,'/','ens')) ? (combined_e_ui =
117   → deserialize(string(combined_e_ui_pth,'/','ens'))) :
118   (combined_e_ui = BioMotifInference.IPM_Ensemble(worker_pool,
119     → combined_e_ui_pth, ensemble_size, combined_ui_sp, (falses(0,0),
120     → mixing_prior), combined_matrix, combined_obs,
121     → source_length_range);
122   → serialize(string(combined_e_ui_pth,'/','ens'),combined_e_ui))

123
124 @info "Assembling combined IPM ensemble on informative priors ... "
125 isfile(string(combined_e_inf_pth,'/','ens')) ? (combined_e_inf =
126   → deserialize(string(combined_e_inf_pth,'/','ens'))) :

```

```

105     (combined_e_inf = BioMotifInference.IPM_Elusive(worker_pool,
106      ↵ combined_e_inf_pth, ensemble_size, combined_inf_sp,
107      ↵ (falses(0,0), mixing_prior), combined_matrix, combined_obs,
108      ↵ source_length_range);
109      ↵ serialize(string(combined_e_inf_pth,'/',"ens"),combined_e_inf))
110
111 rmprocs(worker_pool)
112
113 @info "Fitting distributions ... "
114
115 sib_ui_dist=fit_mle(Normal,[model.log_Li for model in sib_e_ui.models])
116 sib_inf_dist=fit_mle(Normal,[model.log_Li for model in sib_e_inf.models])
117 @info "Is informative a better prior for sibs?"
118 println(sib_inf_dist.μ > sib_ui_dist.μ && quantile(sib_inf_dist,.5) >
119   ↵ sib_ui_dist.μ)
120
121 rys_ui_dist=fit_mle(Normal,[model.log_Li for model in rys_e_ui.models])
122 rys_inf_dist=fit_mle(Normal,[model.log_Li for model in rys_e_inf.models])
123 @info "Is informative a better prior for rys?"
124 println(rys_inf_dist.μ > rys_ui_dist.μ && quantile(rys_inf_dist,.5) >
125   ↵ rys_ui_dist.μ)
126
127 @info "Job done!"

```

16.9.33 /rys/nested_sampling/spike_recovery.jl

```

1 #Testbed for synthetic spike recovery from example background
2 using BioBackgroundModels, BioMotifInference, Random, Distributed,
2   ↵ Distributions, Serialization
3 Random.seed!(786)
4 #CONSTANTS
5 no_obs=500
6 obsl=100:200
7

```

```

8 const folder_path="/bench/PhD/NGS_binaries/BBM/refined_folders"
9
10 report_folders=deserialize(folder_path)
11
12 bhmm_vec=Vector{BHMM}()
13 push!(bhmm_vec,report_folders["intergenic"].partition_report.best_model[2])
14 push!(bhmm_vec,report_folders["periexonic"].partition_report.best_model[2])
15 push!(bhmm_vec,report_folders["exon"].partition_report.best_model[2])
16
17 bhmm_dist=Categorical([.6,.2,.2])
18
19 struc_sig_1=[.1 .7 .1 .1
20             .1 .1 .1 .7
21             .1 .7 .1 .1]
22 struc_sig_2=[.1 .1 .7 .1
23             .1 .1 .7 .1
24             .7 .1 .1 .1]
25 periodicity=8
26 struc_frac_obs=.75
27
28
29 tata_box=[.05 .05 .05 .85
30             .85 .05 .05 .05
31             .05 .05 .05 .85
32             .85 .05 .05 .05
33             .425 .075 .075 .425
34             .85 .05 .05 .05
35             .425 .075 .075 .425]
36 caat_box=[.15 .15 .55 .15
37             .15 .15 .55 .15
38             .05 .85 .05 .05
39             .05 .85 .05 .05
40             .85 .05 .05 .05
41             .85 .05 .05 .05
42             .05 .05 .05 .85
43             .15 .55 .15 .15
44             .15 .15 .15 .55]
45 motif_frac_obs=.7
46 motif_recur_range=1:4
47
48 @info "Constructing synthetic sample set 1..."
49 obs1, bg_scores1, hmm_truth1, spike_truth1 =
    → synthetic_sample(no_obs,obs1,bhmm_vec,bhmm_dist,[struc_sig_1,tata_box],[(true,(st

```

```

50
51 @info "Constructing synthetic sample set 2 ... "
52 obs2, bg_scores2, hmm_truth2, spike_truth2 =
    ↳ synthetic_sample(no_obs,obs1,bhmm_vec,bhmm_dist,[struc_sig_2,caat_box],[(true,(st
53
54 @info "Assembling combined sample set ... "
55 obs3=hcat(obs1,obs2)
56 bg_scores3=hcat(bg_scores1, bg_scores2)
57
58 @info "Assembling worker pool ... "
59
60 #DISTRIBUTED CLUSTERS CONSTANTS
61 remote_machine = "10.0.0.3"
62 no_local_processes = 2
63 no_remote_processes = 6
64
65 @info "Spawning local cluster workers ... "
66 worker_pool=addprocs(no_local_processes, topology=:master_worker)
67 remote_pool=addprocs([(remote_machine,no_remote_processes)], tunnel=true,
    ↳ topology=:master_worker)
68
69 worker_pool=vcat(worker_pool, remote_pool)
70
71 @info "Loading worker libraries everywhere ... "
72 @everywhere using BioMotifInference, Random
73 @everywhere Random.seed!(myid())
74
75 e1 = "/bench/PhD/NGS_binaries/BMI/e1"
76 e2 = "/bench/PhD/NGS_binaries/BMI/e2"
77 e3 = "/bench/PhD/NGS_binaries/BMI/e3"
78
79 #JOB CONSTANTS
80 const ensemble_size = 250
81 const no_sources = 2
82 const source_min_bases = 3
83 const source_max_bases = 12
84 const source_length_range= source_min_bases:source_max_bases
85 const mixing_prior = .5
86 const models_to_permute = ensemble_size * 3
87 funcvec=full_perm_funcvec
88 push!(funcvec, BioMotifInference.permute_source)
89 push!(funcvec, BioMotifInference.permute_source)
90 args=[Vector{Tuple{Symbol,Any}}() for i in 1:length(funcvec)]

```



```

119 @info "Converging ensemble 3 ... "
120
121 logZ3 = BioMotifInference.converge_ensemble!(ens3, instruct, worker_pool,
    ↵ .001, backup=(true,250), wk_disp=false, tuning_disp=true,
    ↵ ens_disp=false, conv_plot=true, src_disp=true, lh_disp=false,
    ↵ liwi_disp=false)
122
123 @info "Evidence ratio of joint 182 / 3:"
124 ratio=exp((logZ1+logZ2)-logZ3)
125 println(ratio)
126
127 rmprocs(worker_pool)

```

16.9.34 /rys/position_analysis/position_overlap.jl

```

1 using DataFrames, BioSequences, BioBackgroundModels, CSV, FASTX,
    ↵ Statistics
2
3 function add_position_sequences!(df::DataFrame, genome_path::String,
    ↵ genome_idx_path::String)
4     scaffold_seq_record_dict::Dict{String,BioSequences.LongSequence} =
        ↵ BioBackgroundModels.build_scaffold_seq_dict(genome_path,
        ↵ genome_idx_path)
5
6     seqs=[BioSequences.LongSequence{DNAAlphabet{4}}() for i in
        ↵ 1:size(df,1)]
7     df[!, :seq] *= seqs
8
9     Threads.@threads for entry in eachrow(df)
10         entry.seq=BioBackgroundModels.fetch_sequence(entry.chr,
11             ↵ scaffold_seq_record_dict ,entry.start, entry.end, '+')
12     end
13 end
14
15 function get_cluster!(df::DataFrame, fasta::String, arch::String)
16     df[!, :cluster] *= zeros(Int64, size(df,1))
17     art=CSV.read(arch, header=0)
18
19     position_vec=Vector{Vector{Int64}}()
20     reader=FASTA.Reader(open(fasta))
21     for (n, entry) in enumerate(reader)

```

```
22     scaffold = FASTA.identifier(entry)
23     desc_array = split(FASTA.description(entry))
24     pos_start = parse(Int64, desc_array[2])
25     pos_end = parse(Int64, desc_array[4])
26     seq = FASTA.sequence(entry)
27
28     idx = filter(in(findall(chr→chr=scaffold, df.chr)),
29                  ↳ findall(start→start=pos_start, df.start))
30
31     if length(idx)==1
32         match=df[idx[1],:]
33         @assert match.end == pos_end
34         @assert match.seq==seq
35         match.cluster=art.Column1[n]
36     end
37 end
38
39 function make_position_df(position_fasta::String)
40     position_reader = FASTA.Reader(open((position_fasta), "r"))
41     position_df = DataFrame(SeqID = String[], Start=Int64[], End=Int64[],
42                             ↳ Seq = LongSequence[])
43
44     for entry in position_reader
45         scaffold = FASTA.identifier(entry)
46
47         if scaffold != "MT"
48             desc_array = split(FASTA.description(entry))
49             pos_start = parse(Int64, desc_array[2])
50             pos_end = parse(Int64, desc_array[4])
51             seq = FASTA.sequence(entry)
52
53             if !hasambiguity(seq)
54                 push!(position_df, [scaffold, pos_start, pos_end, seq])
55             end
56         end
57     end
58
59     close(position_reader)
60     return position_df
61 end
```

```

62 function observation_setup(position_df :: DataFrame; order :: Int64=0,
63   → symbol :: Symbol=:Seq)
64   order_seqs =
65     → BioBackgroundModels.get_order_n_seqs(Vector{LongSequence{DNAAlphabet{2}}})(pos-
66       → symbol),order)
67   coded_seqs = BioBackgroundModels.code_seqs(order_seqs)
68
69   return coded_seqs
70 end
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94

```

`function observation_setup(position_df :: DataFrame; order :: Int64=0,
 symbol :: Symbol=:Seq)
 order_seqs =
 BioBackgroundModels.get_order_n_seqs(Vector{LongSequence{DNAAlphabet{2}}})(pos-
 symbol),order)
 coded_seqs = BioBackgroundModels.code_seqs(order_seqs)

 return coded_seqs
end

function map_positions!(base_df, map_df)
 df_rows = size(base_df,1)
 base_df[!, :mapped_pos] = zeros(Int64, df_rows)
 base_df[!, :overlap_bp] = zeros(Int64, df_rows)
 base_df[!, :rel_overlap] = zeros(df_rows)

 chrgroups=groupby(base_df, :chr)

 for chr_df in chrgroups #split the df by scaffold so base numbers are
 → local to scaffold
 base_chr = chr_df.chr[1]
 matched=false
 for map_chr_df in groupby(map_df, :chr)
 base_chr = map_chr_df.chr[1] && subDFmap!(chr_df,
 → map_chr_df)
 matched=true
 end
 matched=false &&
 → (chr_df.mapped_pos=zeros(Int64,length(chr_df.start));
 → chr_df.overlap_bp=zeros(Int64,length(chr_df.start));
 → chr_df.rel_overlap=zeros(length(chr_df.start)))
 end

 return base_df
end

function subDFmap!(chr_df :: SubDataFrame,
 → map_chr_df :: SubDataFrame)
 Threads.@threads for position in eachrow(chr_df)
 search_start = position.start;
 → search_end=position.end
 end
end`

```

95          ↵ start_idxs=findall(in(findall(ende→search_start ≤ ende,ma
96          ↵ findall(start→search_start ≥ start,map_chr_df.start))
97
98          ↵ end_idxs=findall(in(findall(ende→search_end ≤ ende,map_ch
99          ↵ findall(start→search_end ≥ start,map_chr_df.start))
100         mapped_idxs = unique(vcat(start_idxs, end_idxs))

101
102         overlaps=Vector{Int64}()
103         for idx in mapped_idxs
104             #println(idx)
105             mapped_start=map_chr_df.start[idx];
106             ↵ mapped_end=map_chr_df.end[idx]
107             if (mapped_start==search_start &&
108                 ↵ mapped_end==search_end) #complete
109                 ↵ position overlap (all positions are the
110                 ↵ same size)
111
112                 ↵ push!(overlaps,(mapped_end-mapped_start+1))
113                 #println("same")
114             elseif (mapped_start < search_start ≤
115                 ↵ mapped_end) #5' end of the searched
116                 ↵ position overlaps with the mapped
117                 ↵ position
118
119                 ↵ push!(overlaps,(mapped_end-search_start+1))
120                 #println("5' overlap")
121             elseif (mapped_start ≤ search_end <
122                 ↵ mapped_end) #3' end of searched position
123                 ↵ overlaps with the mapped position
124
125                 ↵ push!(overlaps,(search_end-mapped_start+1))
126                 #println("3' overlap")
127             end
128         end
129
130         if length(mapped_idxs)>0 #1 or more mapped
131             ↵ candidates: take the one with the most
132             ↵ overlap
133             overlap, oidx = findmax(overlaps)
134             map_idx = mapped_idxs[oidx]
135             position.mapped_pos=map_idx
136             position.overlap_bp=overlap

```

```

120             position.rel_overlap=overlap /
121             ↳ (search_end-search_start+1)
122         end
123     end
124
125 sib_pos =
126   ↳ "/bench/PhD/danpos_results/pooled/sib.Fnor.smooth.positions.xls"
127 rys_pos =
128   ↳ "/bench/PhD/danpos_results/pooled/rys.Fnor.smooth.positions.xls"
129 sib_refpos =
130   ↳ "/bench/PhD/danpos_results/pooled/sib.Fnor.smooth.positions.ref_adjust.xls"
131 rys_refpos =
132   ↳ "/bench/PhD/danpos_results/pooled/rys.Fnor.smooth.positions.ref_adjust.xls"
133
134
135 Threads.@threads for t in 1:4
136     t==1 && nucpos.map_positions!(sib_df, rys_df)
137     t==2 && nucpos.map_positions!(rys_df, sib_df)
138     t==3 && nucpos.map_positions!(sib_refdf, rys_refdf)
139     t==4 && nucpos.map_positions!(rys_refdf, sib_refdf)
140 end
141
142 println("Unmapped positions: sib
143   ↳ $(length(findall(iszero,sib_df.mapped_pos))), sib_ref
144   ↳ $(length(findall(iszero,sib_refdf.mapped_pos))))")
145 println("Unmapped positions: rys
146   ↳ $(length(findall(iszero,rys_df.mapped_pos))), rys_ref
147   ↳ $(length(findall(iszero,rys_refdf.mapped_pos))))")
148 println("Avg overlap: sib $(mean(sib_df.rel_overlap)), sib_ref
149   ↳ $(mean(sib_refdf.rel_overlap))")
150 println("Avg overlap: rys $(mean(rys_df.rel_overlap)), rys_ref
151   ↳ $(mean(rys_refdf.rel_overlap))")
```

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