

Mathematical Classification of the Modes of Tumour Evolution

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Declaration

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Abstract

In this work, the City University's Senate Regulation 25 (?) has been followed in order to obtain a L^AT_EX template providing the adequate format for a City University PhD dissertation.

Chapter 1

Introduction

Cancer remains one of the most formidable challenges in the realm of health and medicine, causing a quarter of all deaths in the UK (?). Despite advances in cancer research, the survival rates for many cancers remain low, with the disease being an increasing burden on healthcare systems (*Financial Burden of Cancer Care | Cancer Trends Progress Report* n.d.). The disease's heterogeneity, both within and between patients, is a major obstacle to effective treatment. Understanding the underlying evolutionary processes driving this heterogeneity is crucial to developing new treatments and improving patient outcomes. While having a comprehensive mathematical theory of cancer evolution may not be feasible, concrete mathematical models can provide valuable insights into the disease's dynamics. To this end, I consider different approaches to modelling cancer evolution, which includes the use of phylogenetic trees and agent-based models. Further, I employ methylation data to verify the accuracy of the models using Approximate Bayesian Computation (ABC).

Trees as a mathematical object have found use in a variety of fields, of which biology is my main focus. However, I have found interesting links to methods in computer science via information theory. In chapter 2, I expand upon three points. First, I further establish J^1 as a universal index of tree balance through connections with data structures in computer science. Second, I derive upper bounds on the error of the expected value approximations for the Yule process and the uniform model. Finally, I investigate the minimal values of J^1 in important special cases, with special emphasis on the large tree limit.

In chapter 3, I employ the index J^1 , along with two other tree shape indices, to test to what degree one can differentiate between different evolutionary regimes

in cancer by only relying tree shape indices. These results are compared to a new, more comprehensive system of tree shape indices (Noble & Verity n.d.) which further generalised the concepts of diversity, evenness and richness. These results lay the groundwork for future analysis of cancer tree data.

In chapter 4, I introduce a tailor-made model for simulating a specific type of molecular data, methylation arrays, obtained from multi-site sequencing of colorectal cancer. I show that the model is able to recapitulate the patterns observed in the data and that it can be used to infer the evolutionary history of the tumour. I further explore how the model can be expanded for more general use due to its modular design. I also demonstrate an approximate Bayesian computation workflow for inferring the parameters of the model from data, and discuss the choice of summary statistics and the performance of the ABC algorithm.

In chapter 5, I verify the utility of the model on the example of colorectal cancer methylation data. I discuss the results of the inference and compare the inferred gland divergence trees to ones generated by the model. I further discuss the implications of the results for the understanding of colorectal cancer evolution and the potential for future research.

1.1 Mathematical oncology

1.1.1 Introduction

Cancer emergence and progression is an evolutionary process (Nowell n.d., Merlo et al. n.d.). This statement is now widely accepted, and the applications of quantitative methods found in evolutionary biology in cancer research are numerous (Rockne et al. n.d., Yin et al. n.d., Kourou et al. n.d.). The, now well established, area of mathematical oncology is informed by clinicians, computer scientists, mathematicians of all flavours, and biologists alike (Bull & Byrne n.d.), which has led to a rapid development of more specific avenues of research spanning from the initiation of the disease (Paterson et al. n.d.) to the optimisation of therapy protocols (West, Adler, Gallaher, Strobl, Brady-Nicholls, Brown, Roberson-Tessi, Kim, Noble, Viossat, Basanta & Anderson n.d.). This is a perfect reflection of the complexity of the disease itself, as its rapid evolution, heterogeneity and constraints on how much information one can obtain from a patient take the combined efforts of thousands of scientists. Mathematics plays its own role in this effort, providing a common language through

rigour and methods development, and frameworks for the interpretation of data.

1.1.2 Mathematical models of tumour evolution

The specifics of tumour evolution are complex as, while deterministic equations may capture the evolutionary dynamics of a cohort of tumours, the individual tumour's evolutionary history is stochastic (Werner et al. n.d.). This only adds to the issue of how the surrounding tissue (West, Schenck, Gatenbee, Robertson-Tessi & Anderson n.d.) and the tumour's own spatial organisation will affect its progression (Noble, Burri, Le Sueur, Lemant, Viossat, Kather & Beerenwinkel n.d.). Therefore, existing models of tumour evolution have had to incorporate both general, large-scale processes and sometimes molecular level events to be able to claim progress towards personalised cancer care informed by quantitative models (Yin et al. n.d.).

As mentioned earlier, the applications of mathematics in oncology are diverse. Thus, my focus over the course of this PhD has been on modelling tumour evolution and progression from its early stages up to and excluding treatment. This makes the problem more of an exercise in population dynamics than strict oncology, as underlying assumptions of such models tend to focus less on the microenvironment impact and more on how mutations accumulate and spread in the tumour. A good example of one such model is the Big Bang model of tumour growth (Sottoriva et al. n.d.). Informed by multi-site sequencing, the authors' hypothesis was that colorectal cancer evolves neutrally after an initial period of rapid expansion and selection. Much like cosmic microwave background radiation is unevenly distributed across the observable universe, they observed an asymmetrical distribution of mutations across the tumour spheroid. This inspired a spatial branching process model based on gland fission, with each tumour gland approximated to rapid fixation in the event of a driver mutation, which showed good agreement with the data. A follow-up paper (Williams et al. n.d.) ignited a debate on neutral evolution in exponentially growing tumours within the community (Tarabichi et al. n.d., McDonald et al. n.d., Heide et al. n.d., Bozic et al. n.d.). However, theoretical considerations of the two-level model compared to the neutral model did, in fact, show that it is possible to distinguish the two based on mutation frequency spectra (Tung & Durrett n.d.).

One would be remiss, however, to only focus on models explicitly designed for cancer. The abstract nature of mathematical modelling has allowed for the transfer of knowledge between fields, with models developed for other purposes being

applicable in cancer. General models which are more easily tested on, for example, bacterial populations (Fusco et al. n.d., ?) can be adapted to cancer, as the underlying principles of evolution are the same. But digging even deeper, the underlying model of boundary growth dates back to the Eden model of crystal growth (Eden n.d.). Among similar examples are uses of the Fisher-Kolmogorov-Petrovsky-Piscounov equation in ecology and its modifications for the study of the spread of mutations in populations with a constant size (Houchmandzadeh & Vallade n.d.) as well as growing populations (Wodarz & Komarova n.d.). Further, the use of phylogenetic trees and methods in cancer is an emerging field introduced in the following section and expanded upon in chapters 2 and 3.

1.2 Trees and their applications

1.2.1 Introduction

In the most general sense, a tree is a connected graph with no cycles. In this thesis, when a tree is mentioned, I refer to a rooted tree, as formally defined in section ???. Trees have found use in a variety of fields, including computer science, biology, and linguistics. In computer science, trees are used to represent hierarchical data structures, such as file systems (Nievergelt n.d.) or the structure of a program's syntax (Knuth n.d.*b*), an approach that computer scientists share with linguists (Chomsky n.d.). The concept of search trees, dating back to the mid 20th century, revolutionised the field of computer science with applications in information retrieval in the form of binary search trees and self-balancing trees (Nievergelt & Reingold n.d., Knuth n.d.*a*). In evolutionary biology, one of the earliest appearances of trees dates back to the 19th century, when Charles Darwin used them to represent the evolutionary relationships between species. Phylogenetic trees have over time become a key tool in analysing the lineages of species, viral mutations, and cancer evolution. By investigating quantitative summaries of different properties of tree shapes, one can gain insight into the underlying processes driving the evolution of species (Mooers & Heard n.d.) or cancer (Scott et al. n.d., Noble, Burri, Le Sueur, Lemant, Viossat, Kather & Beerenwinkel n.d.). However, most of the inference work so far has been performed using methods which are not necessarily rooted in sound mathematical theory, but are rather based on heuristics (O'Meara n.d.). Specifically, measures of tree balance suffer from a lack of a common framework, with at least 19 different

metrics available in literature (Fischer et al. n.d.), and few of them being directly comparable. Also, due to the divergent terminology and interest in the use of trees as a tool, there is scarce literature on the transfer of knowledge between the fields of computer science and biology, with certain results being rediscovered nigh on half a century later, as discussed in section ??.

1.2.2 Quantifying tree balance

In a recent paper (Lemant et al. n.d.), Lemant and Noble proposed a new robust, universal index, J^1 , for quantifying the balance of rooted trees with arbitrary node degree and size distributions. This index is based on Shannon entropy and favours even distributions of node sizes. By generating large numbers of random trees using the alpha-gamma model, I showed that J^1 is robust, in the sense that it is insensitive to small changes in node sizes and to the removal of small nodes (figure 1.1B, C). Noble and I further showed that this index unites and generalises two of the most popular prior approaches to quantifying tree balance in biology, the Colless index and the Sackin index. Applied to evolutionary trees, J^1 outperforms conventional tree balance indices as a summary statistic for comparing model output to empirical data (Noble, Burri, Le Sueur, Lemant, Viossat, Kather & Beerenwinkel n.d.).

Given any tree shape index, an important task is to obtain its expected and extreme values under standard tree-generating processes, which can then be used as null-model reference points. In (Lemant et al. n.d.), Noble and I obtained analytical approximations to the expected values of J^1 under the Yule process and the uniform model, and I tested their accuracy numerically for trees with up to 128 leaves (figure 1.1A). In the same study, Noble and I proved that caterpillar trees minimise J^1 among bifurcating trees but not when larger outdegrees are permitted.

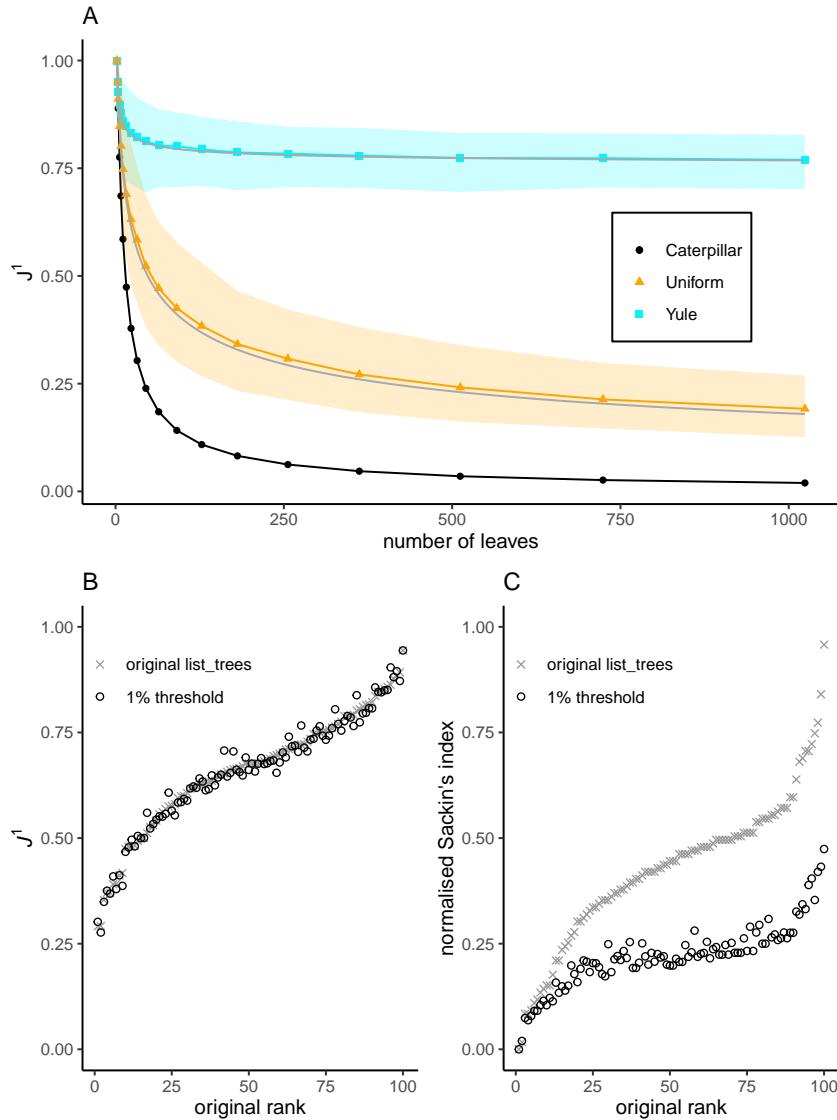


Figure 1.1: **A** J^1 values for trees generated under the Yule process and the uniform model. Solid grey curves represent the approximate expected values, and the dashed lines the 5th and 95th percentiles.

B J^1 values for 100 random trees on 16 leaves using the alpha-gamma model, with $\alpha \sim \text{Unif}(0, 1)$ and $\gamma \sim \text{Unif}(0, \alpha)$. The values were calculated before and after applying a 1% population threshold, i.e. removing all leaves with sizes smaller than 0.01 times the total population.

C Normalised Sackin index values for the same trees as in **B**.

1.3 Agent-based modelling in oncology

1.3.1 Introduction

Agent-based models (ABMs) are a class of computational models that simulate the actions and interactions of individual agents within a system. These agents can represent anything from cells in a tissue to animals in an ecosystem. ABMs are particularly useful in cancer research, as they can capture the complex interactions happening on the microscale in cancer. Spatial agent-based models (SABMs) are a subclass of ABMs that incorporate spatial information into the simulations. This is particularly useful for modelling solid tumours as it allows for the simulation of things like the spatial heterogeneity of the tumour microenvironment and the effects of spatial constraints on tumour growth. A strength of ABMs is that they can be as simple or as complex as the researcher needs them to be (Colyer et al. n.d.). However, therein lies their weakness, as oversimplification of a model can lead to rapid loss of its utility in capturing the behaviour of a complex system such as cancer. On the other hand, a model that is too complex, and attempts to include everything from epigenetic mutations to the effects of the immune system on the tumour, is likely too computationally expensive to be useful for modelling a tumour of reasonable size. This is an organic demonstration of many a researcher's favourite saying *all models are wrong, but some are useful, and some are more useful than others*. In parsing through the literature and developing a new model of my own, I have also been influenced by an alternative wording of this, that is *the best model is its own worst enemy*, by mathematical biologist Philip Maini (?). My interpretation is that a good model should address the questions it was designed to answer, but also open up new ones which require further investigation, improvements, and research. For example, one can use the **demon-warlock** framework (Bak et al. n.d.) to simulate the evolution of a tumour in space and draw conclusions on how spatial organisation will impact intratumour heterogeneity or patient outcomes (Noble, Burley, Le Sueur & Hochberg n.d., Noble, Burri, Le Sueur, Lemant, Viossat, Kather & Beerenswinkel n.d.). However, the model does not address the impact of the immune system, spatial heterogeneity in the microenvironment, or the effects of therapy without further modifications. Alternatively, one may want to include diffusion of nutrients and waste products in the model, or the effects of hypoxia on the tumour cells. Tools that would be appropriate for such tasks are, for example, HAL (Bravo et al. n.d.)

or PhysiCell (Ghaffarizadeh et al. n.d.), but they are not ideal either as simulating a realistically-sized tumour with these models is prohibitively expensive in terms of computational resources. Thus, my preferred approach is to develop a purpose-made model which is informed by the literature and the data, and which has ample room for future expansion and improvement.

1.3.2 The demon-warlock framework

In a recent paper (Bak et al. n.d.), a new agent-based model for simulating the evolution of a tumour in space was introduced. The model is designed to be versatile and able to simulate a wide range of spatial configurations and evolutionary properties of cancer. Spatially, the model is based on a 2D grid, where each grid cell represents a deme, that is a spatially homogeneous population of cells. Each cell in the model belongs to a genotype, a unique identifier based on the cell's mutations, and a driver genotype, which differentiates itself from the genotype by not taking into account passenger mutations. Cell migrations in the tumour have multiple modes, including invasion of tissue and other demes, and deme fission. The latter allows for the simulation of tumours with a glandular structure, such as colorectal cancer. Events in the model are scheduled according to the Gillespie algorithm, with the event hierarchy shown in figure 1.2. As the model was written predominantly in plain C, it is highly efficient considering the complexity of the simulations it can run. An accompanying R package, `demonanalysis`, is available for the analysis and visualisation of the model's output, e.g. figure 1.3.

Despite the model's versatility, it is not without its limitations. In its current form, it is not feasible to simulate tumours larger than a few million cells. This leaves out the possibility of simulating realistically-sized glandular tumours which can contain a few million glands containing thousands of cells each at the time of diagnosis. Furthermore, as the main limitation of the model's scalability is tied to the inherent inefficiency of generating random numbers, it is not well-suited to simulating neutral stochastic markers, such as fluctuating methylation clocks (Gabbutt, Schenck, Weisenberger, Kimberley, Berner, Househam, Lakatos, Robertson-Tessi, Martin, Patel, Clark, Latchford, Barnes, Leedham, Anderson, Graham & Shibata n.d.). This is further discussed in section 4.1.2.

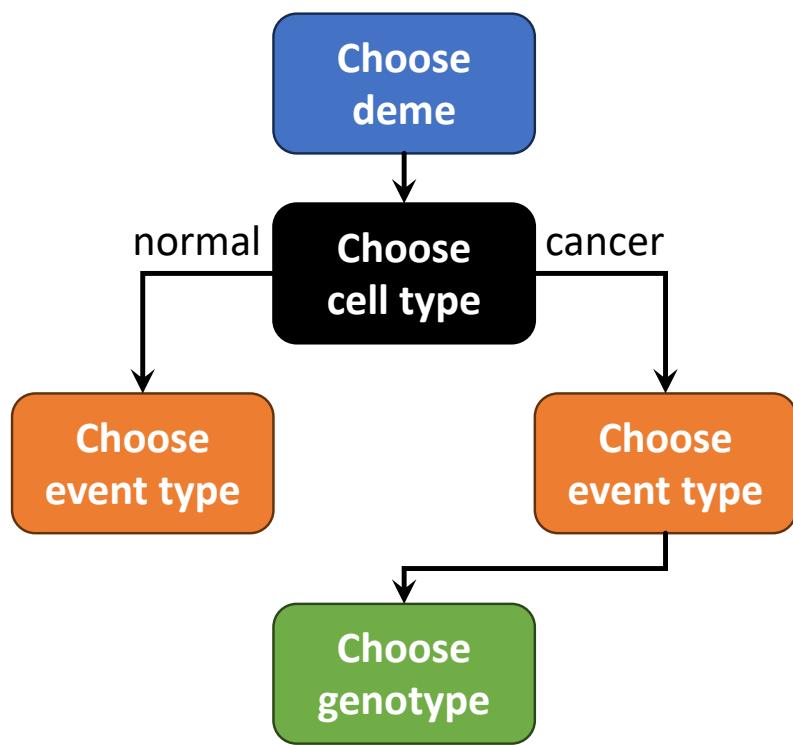


Figure 1.2: Event hierarchy in the `demon-warlock` framework. Figure reproduced from (Bak et al. n.d.) with the authors' permission.

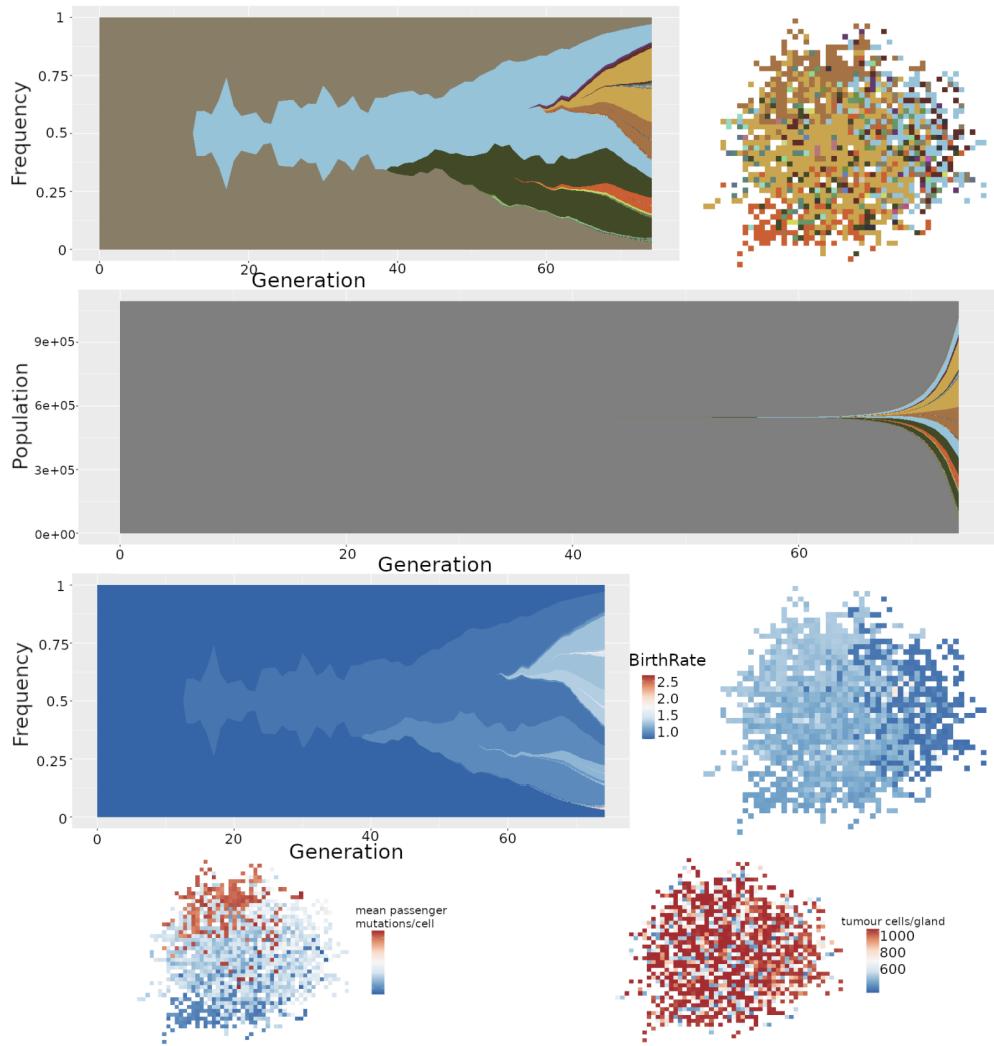


Figure 1.3: Example output from `demon`, visualised using the `demonanalysis` package. **a** Muller plot of clonal dynamics over time. Each colour represents a clone with a distinct combination of driver mutations. **b** Final proportions and spatial plot of clones. **c** Fish plot of clone populations over time using the same colours as in **a**. **d** Muller plot showing evolution of tumour cell division rate. **e** Final spatial distribution of cell division rates. **f** Final spatial distribution of the mean numbers of passenger mutations per cell. **g** Final spatial distribution of the number of tumour cells per gland.

1.4 Likelihood-free inference

1.4.1 Introduction

To verify whether a model predicts behaviour of the observed system, a common approach is comparing its output to measurements. The way this is done depends on the complexity of the model and the data. Here we discuss the general framework of likelihood-free inference, and more specifically the use of Approximate Bayesian Computation (ABC).

Models based on differential equations can be compared to data using likelihood-based methods. In the frequentist tradition, the likelihood function is used to estimate the parameters of the model under the assumption that there is a correct, or “true” value of those parameters. An alternative approach is Bayesian statistics, which uses random variables (θ) to represent the uncertainty in the parameters. The distribution of these random variables before observing the data is called the prior distribution ($P(\theta)$). After performing measurements in the system and obtaining data (D) which has an associated likelihood function ($P(D|\theta)$), the prior distribution is updated to the posterior distribution ($P(\theta|D)$) using Bayes’ theorem (Bayes & Price n.d.):

$$P(\theta|D) = \frac{P(D|\theta)P(\theta)}{P(D)} \quad (1.1)$$

The likelihood function is thus a key component of Bayesian statistics, quantifying the probability of observing the data given the parameters of the model. However, its greatest asset is also its greatest weakness. Depending on the complexity of the model and the data, the likelihood function can be difficult or impossible to calculate analytically, or can be too computationally expensive to calculate numerically. This is especially true for stochastic models, such as agent-based models, where the likelihood function is often intractable.

In the case of intractable likelihoods, a common approach is to use likelihood-free inference methods, designed to approximate the posterior distribution without the need to calculate the likelihood function. These methods rely on the generation of simulated data from the model, and the comparison of these simulations to the observed data. Instead of calculating the likelihood function, these methods often involve a process of simulation and rejection. A common drawback of likelihood-free inference methods is that they can be computationally expensive, as they often

require a large number of simulations to obtain a good approximation of the posterior distribution. However, as the computational power of modern computers increases, these methods are becoming more and more feasible for a wide range of models and data.

1.4.2 Approximate Bayesian Computation

Approximate Bayesian Computation (ABC) is a likelihood-free inference method that has gained popularity in the last three decades (Tavare et al. n.d., Sottoriva & Tavaré n.d., Jagiella et al. n.d.). The basic idea behind ABC is to approximate the posterior distribution of the parameters of a model by comparing simulated data to observed data.

In the most general form of ABC, the algorithm proceeds as follows:

1. Sample a set of parameters from the prior distribution, $\hat{\theta}$.
2. Simulate data from the model using the parameters.
3. Compare the simulated data (\hat{D}) to the observed data (D) using a distance function, $d(\hat{D}, D)$.
4. If the distance between the simulated and observed data is less than a certain threshold ϵ , accept the parameters. Otherwise, reject them.
5. Repeat steps 1-4 until a sufficient number of accepted parameters have been obtained.

The distance threshold must be strictly positive, and is often chosen to be a small value. Alternatively, in the case of high-dimensional data, the distance function can be replaced by a summary statistic, S , which is a function of the data, i.e. $d'(S(\hat{D}), S(D))$.

ABC does not come without its own set of challenges. As it relies on comparing relevant features of the simulated data to the observed data, the choice of summary statistic or distance metric is crucial, as it determines how the data is reduced before the comparison. Fortunately, there are methods for reducing dimensionality of the data which narrows in on its informative aspects (Blum et al. n.d.). The choice of the distance threshold is also important, as it determines the acceptance rate of the algorithm. Setting the threshold too high or too low can lead to biased or

inefficient estimates of the posterior distribution. However, this can be mitigated by using a dynamic threshold, which is adapted during the course of the algorithm (Prangle n.d.). Finally, as the algorithm relies on repeated simulations of potentially complex models. This can require a large amount of computational resources, raising questions about the method’s scalability and practicality. An obvious way to circumvent this is to use a model which is as lightweight as possible. Even in the case of infinite compute available, one must be mindful of the fact that the more complex the model, the more complex the inference problem, and the more complex the inference problem, the more complex the model. This is a feedback loop which can render both the model and subsequent analysis uninterpretable. Therefore, I believe that the best practice as a mathematician and applied scientist is to abstract the model enough to be able to draw conclusions from it, but not so much that it becomes uninformative.

In chapter 4, I introduce a simplified model of colorectal cancer gland fission and the accompanying ABC workflow. I discuss the choice of summary statistics and the performance of the ABC algorithm, and subsequently demonstrate the model’s utility in inferring the evolutionary history of a tumour from methylation data in chapter 5.

1.5 Fluctuating methylation clocks

The concept of the molecular clock is commonplace in molecular evolutionary biology. It is based on the idea that the rate of evolution of a particular gene or set of genes is constant over time, and can be used to estimate the time of divergence between species or the time of a particular event in the evolutionary history of a species. The most famous example of a molecular clock is the mitochondrial DNA clock, which is used to estimate the time of divergence between species (Hasegawa et al. n.d.). The key principle behind molecular clocks is that closely related species or individual will have more similar sequences than distantly related ones. This also translates to individual cells in cancer. The issue with using molecular clocks in cancer is that “slowly ticking” molecular clocks, i.e. ones with a low mutation rate, are not informative enough on the timescale of cancer evolution, limiting their utility to cell lineages which diverged too far in the past, with recent events remaining undetectable. On the other hand, “fast ticking” molecular clocks can reveal

recent evolutionary events but also have their own limitations, such as independent mutations in the same site (Kuipers et al. n.d.).

Recently, a new type of molecular clock has been proposed, the fluctuating methylation clock, based on the observation that the methylation status of certain CpG sites in the genome is heritable but fluctuates stochastically over time (Gabbutt, Schenck, Weisenberger, Kimberley, Berner, Househam, Lakatos, Robertson-Tessi, Martin, Patel, Clark, Latchford, Barnes, Leedham, Anderson, Graham & Shibata n.d., Gabbutt, Duran-Ferrer, Grant, Mallo, Nadeu, Househam, Villamor, Krali, Nordlund, Zenz, Campo, Lopez-Guillermo, Fitzgibbon, Barnes, Shibata, Martin-Subero & Graham n.d.). A CpG site is a cytosine nucleotide followed by a guanine nucleotide in the linear sequence of bases along its 5' → 3' direction, and is a common site of methylation in the genome. As citosine and guanine are complementary, each CpG site in the 5' → 3' direction has a corresponding pair in the 3' → 5' direction. This means that each CpG pair can be in one of three states: both methylated (homozgously methylated), both unmethylated (homozgously unmethylated), or one methylated and the other unmethylated (heterozygously methylated). Depending on tissue type, the fluctuating CpG (fCpG) sites can number somewhere between 1000 and 2000, which means each cell has a potentially unique barcode in its fCpG array. As the array is not constant, with methylations and demethylations of fCpG sites happening over the course of cell divisions, the authors of the two papers covering fCpGs so far have been able to reconstruct the evolutionary history of healthy colonic crypts and lymphoid malignancies with high accuracy. This is a promising development, as sequencing methylation arrays is a cheaper method than genome sequencing, but may offer finer temporal resolution. In chapter 5, I investigate whether multi-site methylation array sequencing can be used to reconstruct clonal dynamics of colorectal cancer.

1.6 Aims

1.6.1 Hypotheses

1. The J^1 index can be used, in conjunction with other tree shape indices, to differentiate between evolutionary modes in cancer.
2. SABMs recapitulate molecular data observed in solid tumours (or sth like that)

3. These methods are useful for inferring the evolutionary history of colorectal cancer based on multi-site methylation array sequencing.

1.6.2 Aims

1. Calculate or approximate important properties of the J^1 index, such as its expected value under standard tree-generating processes.
 - (a) Contextualise J^1 within the broader field of tree shape indices in biology and computer science.
 - (b) Investigate extreme and expected values of J^1 under standard tree-generating processes.
2. Determine the utility of sets of tree shape indices for differentiating between evolutionary modes in cancer.
 - (a) Recapitulate the classification of evolutionary modes in cancer using a set of three tree shape indices.
 - (b) Extend the discussion to a more interpretable and general system of tree shape indices.
3. Extend the fluctuating methylation clock model to multi-site sequencing of solid tumours on the example of colorectal cancer.
 - (a) Develop an agent-based model for simulating the evolutionary dynamics of colorectal adenocarcinoma.
 - (b) Develop an ABC workflow for inferring the evolutionary parameters of the model, specifically the gland fission rate, methylation and demethylation rates, driver mutation rate, selective advantage, and the effective number of lineages per tumour gland.
 - (c) Apply the model to colorectal cancer data and compare the inferred phylogenies to the trees generated by the model.

Chapter 2

Expected and extreme values of universal tree balance index J^1

2.1 Introduction

Broadly speaking, the balance of a tree is the extent to which its terminal nodes (leaves) are evenly distributed among its branches. Despite the abundance of metrics of tree balance (Fischer et al. n.d.), universal indices are hard to come by. This limits practical applications of tree balance indices.

Following the J^1 index paper (Lemant et al. n.d.), where a universal index was proposed, shown to be robust to the removal of small nodes and to outperform conventional tree balance indices as a summary statistic for comparing model output to empirical data, I examined several important properties of J^1 . Given any new tree shape index, the expected value under standard tree-generating processes and the extreme values need to be known for the index to be useful in practice. In figure 1.1A, I showed the sample mean of J^1 up to 128 leaves under the Yule and uniform models, which appears to be close to the inverse Sackin index expression derived by Noble in (Lemant et al. n.d.). Additionally, as a consequence of this relationship, the caterpillar trees minimises J^1 for bifurcating trees. However, I showed in (Lemant et al. n.d.) that the caterpillar topology does not minimise J^1 for multifurcating trees by providing a counterexample on 6 leaves.

In this chapter, I will further show the universality of J^1 by identifying fundamental connections to classical results in computer science, related to Huffman coding and self-balancing tree data structures. I will also derive upper bounds on

the error of the expected value approximations for the Yule process and uniform model. For the Yule process, I show that the approximation rapidly converges to the true expected value in the large tree limit. Finally, I will investigate the minimal values of J^1 in important special cases, obtaining a counter-intuitive result in the large tree limit.

2.2 Prerequisites

2.2.1 Preliminary definitions from systematic biology

Definition 2.2.1 (Rooted tree). A **rooted tree** T is a connected acyclic graph with node set $V(T)$ and edge (or branch) set $E(T)$, in which one node is designated the root. Parent-child and ancestor-descendant relationships in a rooted tree are assigned along paths directed away from the root.

Definition 2.2.2 (Node size and tree magnitude, Lemant et al. (n.d.)). We assign to every node a non-negative size. The **magnitude** of a tree T , denoted $S(T)$, is then the sum of its node sizes.

Definition 2.2.3. (Leafy tree, Lemant et al. (n.d.)) A **leafy tree** is one with only zero-sized internal nodes.

Definition 2.2.4. (Node depth) As we will consider only trees with uniform edge lengths, we define the **depth** of a node as the number of edges in the shortest path from that node to the root.

Definition 2.2.5 (Sackin index, Sackin (n.d.)). The **Sackin index** of rooted tree T is the sum of its leaf depths:

$$I_S(T) = \sum_{l \in L(T)} \nu(l), \quad (2.1)$$

where $L(T)$ is the set of all leaves (terminal nodes) of T , and $\nu(l)$ is the depth of leaf l .

Definition 2.2.6 (Generalised Sackin index, Lemant et al. (n.d.)). The Sackin index can be generalised to account for arbitrary node sizes:

$$I_{S,\text{gen}}(T) = \sum_{i \in V(T)} S_i^*, \quad (2.2)$$

where $V(T)$ is the set of all internal nodes (non-leaves), and S_i^* is the magnitude of the subtree rooted at node i , excluding i . If T is a leafy tree in which all leaves have unit size then $I_{S,\text{gen}}(T) = I_S(T)$.

Definition 2.2.7 (Robust balance index, Lemant et al. (n.d.)). The robust balance index J^1 of tree T is

$$J^1(T) = \frac{1}{I_{S,\text{gen}}(T)} \sum_{i \in \tilde{V}(T)} S_i^* \sum_{j \in C(i)} W_{ij}^1, \quad (2.3)$$

where $\tilde{V}(T)$ the set of all internal nodes whose descendants are not all of zero size, $C(i)$ is the set of children of node i , and W_{ij}^1 is the node balance score, defined as the normalised Shannon entropy of the daughter subtree magnitudes:

$$W_{ij}^1 = \begin{cases} -\frac{S_j}{S_i^*} \log_{d^+(i)} \frac{S_j}{S_i^*}, & \text{for } d^+(i) > 1 \\ 0, & \text{otherwise,} \end{cases} \quad (2.4)$$

where S_i is the magnitude of the subtree rooted at node i , including i , and $d^+(i)$ is the outdegree of i .

Definition 2.2.8 (Binary tree and bifurcating tree). A **binary tree** is a rooted tree in which no node has more than 2 children. A **bifurcating tree** (or full binary tree) is a rooted tree in which each internal node has exactly 2 children.

Definition 2.2.9 (Cherry). A tree consisting of only a root and two leaves is a **cherry**.

Definition 2.2.10 (Caterpillar tree). A **caterpillar tree** is a bifurcating tree in which every internal node except one has exactly one child leaf.

Definition 2.2.11 (Fully symmetric tree). If, for every internal node i , the subtrees rooted at the children of i all contain the same number of leaves then the tree is **fully symmetric**.

2.2.2 Preliminary definitions from computer science

Definition 2.2.12 (Root balance and tree balance scores, Nievergelt et al. (n.d.)). The **root balance score** of a bifurcating leafy subtree T_i rooted at i and containing

at least three nodes is

$$\rho(T_i) = \frac{\min(S_{i_1}, S_{i_2})}{S_i} \in [0, \frac{1}{2}], \quad (2.5)$$

where S_i is the magnitude of T_i , and S_{i_1} and S_{i_2} are the magnitudes of the subtrees rooted at the children of i . The balance score of a bifurcating leafy tree T is then defined as

$$\beta(T) = \min(\rho(T_i)_{i \in V(T)}). \quad (2.6)$$

For any given leaf count, the balance score is minimal for the caterpillar tree and maximal for the fully symmetric bifurcating tree.

Definition 2.2.13 (Total and weighted path lengths, Nievergelt et al. (n.d.)). In computer science, the Sackin index is better known as the **total path length**. Let T be a rooted tree in which each node i is assigned a weight (or access frequency) w_i . Then the **weighted path length** of T is

$$|T| = \sum_{i \in V(T)} w_i \nu(i). \quad (2.7)$$

2.3 Results

2.3.1 J^1 unites and generalises prior notions of tree balance

In computer science, tree balance is effectively a binary property: a tree is considered balanced if its weighted path length is sufficiently small, given its leaf count (Nievergelt & Reingold n.d.). In biology, where comparisons between trees are more relevant, researchers instead use a normalised form of the Sackin index or various other indices to assign balance values on a continuum (??Mir, Rosselló & Rotger n.d., Mir, Rotger & Rosselló n.d., Fischer et al. n.d.). I will show that J^1 uniquely connects these two historically separate notions of tree balance. Let us note first that the weighted path length is equivalent to the generalised Sackin index:

$$|T| = \sum_{i \in V(T)} \alpha_i \nu(i) = \sum_{i \in V(T)} S_i^* = I_{S,gen}(T). \quad (2.8)$$

Consider then the following proposition.

Proposition 2.3.1 (Lemant et al. (n.d.)). *Let T be a leafy tree with $d^+(i) = m > 1$*

for all internal nodes i . Then

$$J^1(T) = \frac{H_m(T)S(T)}{I_{S,gen}(T)}, \quad (2.9)$$

where $H_m(T)$ is the Shannon entropy (base m) of the proportional leaf sizes.

Corollary 2.3.1. We can rewrite equation (2.9) for bifurcating trees as

$$J^1(T) = \frac{H_2(T)S(T)}{|T|}. \quad (2.10)$$

Hence, for any given set of leaf sizes, minimising the weighted path length is equivalent to maximising J^1 .

Theorem 2.3.1 (Section 5 of Nievergelt et al. (n.d.)). *Let T be a bifurcating leafy tree with balance score β_T . Then the total path length $|T|$ satisfies the inequality*

$$|T| \leq \frac{S(T) \log_2 S(T) + H_2(T)}{H_2(\beta_T)}. \quad (2.11)$$

If the node sizes sum to unity then this simplifies to

$$|T| \leq \frac{H_2(T)}{H_2(\beta_T)}. \quad (2.12)$$

A special case of this theorem is considered as Theorem 2 in Wong & Nievergelt (n.d.): If the tree has n leaves, all of size 1 then

$$|T| \leq \frac{n \log n}{H(\beta_T)}. \quad (2.13)$$

The proof of this theorem defines the *average entropy* of a general tree T , corrected for typo in original paper, as

$$\bar{H}(T) = \frac{1}{|T|} \sum_{k \in \tilde{V}(T)} \sum_{j \in C(k)} n_j \log_2 \frac{n_k}{n_j}, \quad (2.14)$$

which is identical to the definition of J^1 , equation (2.3), up to the base of the logarithm in the expression for the entropy of internal node k .

Remark 2.3.1. We can trivially expand the definition of the balance score to m -furcating trees, by considering all m descendants of internal nodes in the root balance score. The root balance score of subtree T_j rooted in node j of m -furcating leafy

tree T can be defined as

$$\rho_m(T_j) = \frac{\min(S_{j_1}, \dots, S_{j_m})}{S_j}, \quad (2.15)$$

where $j_1, \dots, j_m \in C(i)$ are the children of node j . By extension, we define

$$\beta_m(T) = \min(\rho_m(T_i)_{i \in V(T)}), \quad (2.16)$$

the balance score of m -furcating leafy tree T .

Corollary 2.3.2. There is a lower bound on J^1 for an m -furcating leafy tree T on n leaves, with balance score β_T , and it equals

$$J_{\text{lower}}^1 = \frac{H_m(T)S(T)}{|T|_{\text{upper}}} = \frac{n \log_m n}{(H_m(\vec{\beta}_T))^{-1}n \log_m n} = H_m(\vec{\beta}_T). \quad (2.17)$$

where $\vec{\beta}_T = (S_{1,\min}, \dots, S_{m,\min})$ is the vector of magnitudes of subtrees rooted in the children of the node with the smallest root balance score.

The connections between J^1 and measures of tree balance and entropy in computer science show that these properties are universally important. However, the similarities may well end at this point, as evolutionary biologists and computer scientists use these measures for different purposes and take their research in opposite directions directions, for example inferring evolutionary processes which produced the tree shape (Mooers & Heard n.d.) versus shaping the tree to optimise data storage and retrieval (Nagaraj n.d.).

2.3.2 J^1 is maximised by Huffman coding

Definition 2.3.1 (Binary search tree). A **binary search tree** T_n over n entries (w.l.g. numbers) x_1, \dots, x_n is a labelled binary tree, each of whose nodes have been labelled with a distinct number chosen from x_1, \dots, x_n such that for each node N , all nodes in the left subtree of N have a smaller x_i as their label than x_N , and all nodes in the right subtree of N have a larger number as their label than node N (e.g. figure 2.1).

Remark 2.3.2. Each node i in a binary search tree can have an associated non-negative number called access frequency (or weight, size, probability) w_i .

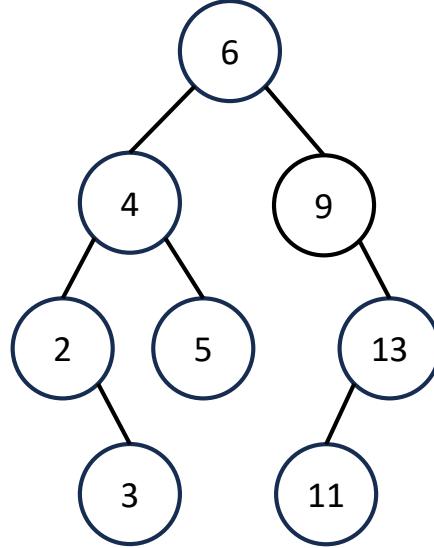


Figure 2.1: A simple example of a binary search tree over the set of labels $S = \{2, 3, 4, 5, 6, 9, 11, 13\}$.

To construct an optimal binary search tree, that is one with a minimal weighted path length, we can use Huffman coding.

Definition 2.3.2 (Huffman coding, Huffman (n.d.)). Let $A = (\alpha_1, \dots, \alpha_n)$ be a tuple of non-negative numbers. To construct an optimal binary tree on n leaves with sizes given by A we choose the two smallest ones, w.l.g. α_1 and α_2 , and join them in a cherry, so that their parent node has size $\alpha_1 + \alpha_2$. We now have $A' = (\alpha_1 + \alpha_2, \alpha_3, \dots, \alpha_n)$ as our set of $n - 1$ nodes. By repeating this procedure until we have only one node left, an optimal binary search tree is obtained.

Proposition 2.3.2. *The Huffman method maximises J^1 on bifurcating trees for a given set of node sizes.*

Proof. By corollary 2.3.2, the Huffman method maximises J^1 as it minimises the weighted path length. \square

As Huffman coding is an optimisation algorithm, J^1 can be used to measure how close a tree constructed using a given set of node sizes is to the maximally balanced binary tree on the same set. This means we can quantify how close an alternative algorithm which runs in a faster time complexity, such as arithmetic coding (Pasco n.d.), gets to the optimal solution.

2.3.3 Expected value of J^1 under simple evolutionary processes

For applications in evolutionary biology, an important property of balance indices is their expected value under an evolutionary process. This quantity helps us compare the trees generated under a null model to the observed data, and is a valuable part of inferring the underlying evolutionary properties. Two of the simplest, and most widely studied, processes of tree generation are the uniform model (Rosen n.d.) and the Yule model (Yule n.d.), which generate bifurcating trees and are useful null models in evolutionary biology. The Yule model, also known as the pure birth or coalescent model, is used when considering speciation rates and patterns (Aldous n.d., Steel & McKenzie n.d.). The uniform model is used as a null model for comparing neutral evolutionary patterns against ones which include more complex biological mechanisms (Mooers & Heard n.d., McKenzie & Steel n.d.). While in section 2.2.2 I discussed the static calculation of a balance index for a given tree, I am also interested in how balanced binary search trees generated under some stochastic process are. The Yule model is also useful for these considerations as it is connected to the BST martingale, a statistical tool used to analyze and predict the behavior of binary search trees, via L_1 convergence (Chauvin & Rouault n.d.). From here, one can extend the discussion to AVL and red-black trees in a similar way to more complex evolutionary processes as self-balancing trees will by definition have higher expected values of J^1 than those generated under the Yule process.

The expected value of a few indices, and even some higher moments in certain cases, are known for both Yule and uniform models (Mir, Rosselló & Rotger n.d., M. Coronado et al. n.d., Goh et al. n.d.). Among these is the Sackin index, which is particularly useful for our purposes.

Definition 2.3.3 (Yule model, Yule (n.d.)). Consider a bifurcating tree T on n leaves. To obtain the probability of generating T under the Yule model, start with a single node and replace it with a cherry. Then, at each step, choose one leaf uniformly at random and replace it with a cherry, until the tree has n leaves (figure 2.2A). The sum of probabilities of generating T in all possible ways is the probability of generating T under the Yule model.

Definition 2.3.4 (Uniform model, Rosen (n.d.)). Under the **uniform model** of tree generation, every bifurcating tree on n leaves has an equal probability of being generated, which is equal to $n \binom{2n-2}{n-1}^{-1}$ (figure 2.2B).

Remark 2.3.3. We only consider leafy trees with equal leaf sizes generated by the processes in definitions 2.3.3 and 2.3.4.

Remark 2.3.4. We calculated the exact values of the expectation of J^1 under both the Yule and uniform models semi-manually by creating all possible $(n + 1)$ -leaf trees given a set of n -leaf trees, eliminating duplicates and assigning appropriate probabilities in the Yule case, and thus computing the exact value of the expectation. The process is inefficient for large trees, and we limited our search to $n \leq 11$, the exact and approximate expected values for which are found in table 2.1.

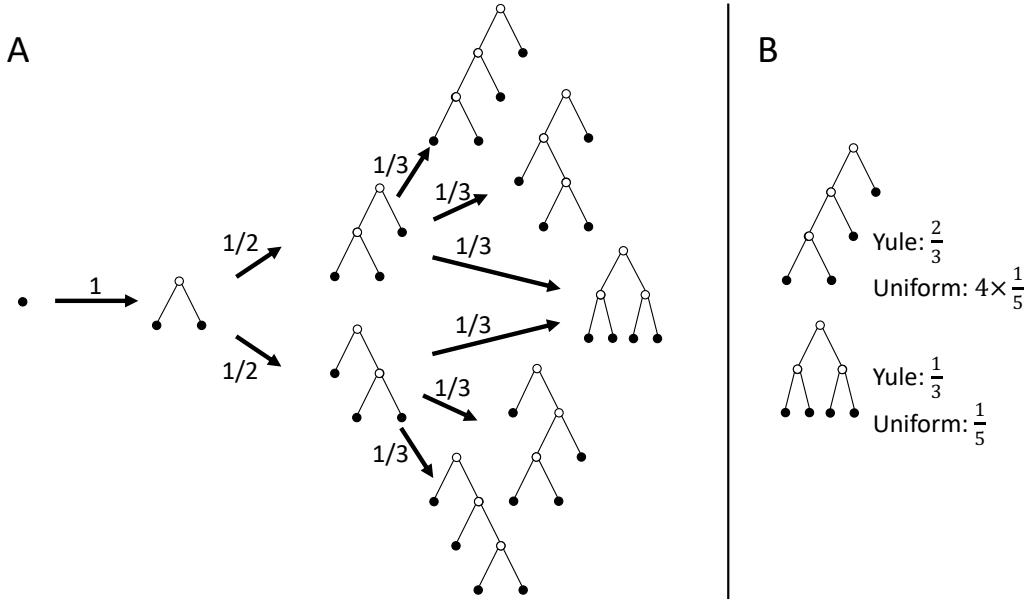


Figure 2.2: Comparison of probabilities for generation of trees on 4 leaves under the Yule and uniform models. **A:** Arrows show generation under the Yule model. Each of the trees shown on 4 leaves has the same probability under the uniform model. **B:** Comparison of probabilities of tree topologies on 4 leaves under the Yule and uniform models.

Under the Yule model, the expected value of the Sackin index for trees on n leaves is

$$\mathbb{E}_Y^n(I_S) = 2n \sum_{i=2}^n \frac{1}{i}, \quad (2.18)$$

as shown in Kirkpatrick & Slatkin (n.d.). Equation (2.9) implies then that the expected value of J^1 for a tree on n leaves is

$$\mathbb{E}_Y^n(J^1) = \mathbb{E}_Y^n\left(\frac{n \log_2 n}{I_S}\right) = n \log_2 n \mathbb{E}_Y^n(1/I_S), \quad (2.19)$$

where $\mathbb{E}_Y^n(1/I_S)$ is the harmonic mean of the Sackin index. The harmonic mean under the Yule process is not a standard result in literature, nor have I been able to obtain a closed-form solution for this problem so far. It is possible, however, to

compare the harmonic and arithmetic means of I_S by considering the Jensen gap

$$\mathcal{J}(f, X) = \mathbb{E}[f(X)] - f(\mathbb{E}[X]), \quad (2.20)$$

with $f(x) = 1/x$.

Theorem 2.3.2 (Liao & Berg (n.d.)). *Let X be a one-dimensional random variable with mean μ , and $P(X \in (a, b)) = 1$, where $\infty \leq a < b \leq \infty$. If $f(x)$ is a twice differentiable function on (a, b) , and*

$$h(x; \nu) = \frac{f(x) - f(\nu)}{(x - \nu)^2} - \frac{f'(\nu)}{x - \nu}, \quad (2.21)$$

then

$$\inf_{x \in (a, b)} \{h(x; \mu)\} \text{Var}(X) \leq \mathbb{E}[f(X)] - f(\mathbb{E}[X]) \leq \sup_{x \in (a, b)} \{h(x; \mu)\} \text{Var}(X). \quad (2.22)$$

Proposition 2.3.3. *Let $\mathbb{E}_Y(J^1)$ and $\mathbb{E}_U(J^1)$ be expectation values of J^1 under the Yule and uniform models respectively. Then:*

$$(i) \quad \mathbb{E}_Y(J^1) \rightarrow \frac{n \log_2 n}{\mathbb{E}_Y(I_S)},$$

$$(ii) \quad \mathbb{E}_U(J^1) - \frac{n \log_2 n}{\mathbb{E}_U(I_S)} \text{ is bounded from both sides,}$$

as $n \rightarrow \infty$.

Proof. (i) Let μ_Y be the expected value of the Sackin index under the Yule process for trees on n leaves, and $f(x) = \frac{1}{x}$. By theorem 2.3.2

$$h(x; \mu_Y) = \frac{f(x) - f(\mu_Y)}{(x - \mu_Y)^2} - \frac{f'(\mu_Y)}{x - \mu_Y} = \frac{1}{x \mu_Y^2}. \quad (2.23)$$

We can then substitute this into the inequality given in the theorem

$$\frac{n \log_2 n}{\frac{(n-1)(n+2)}{2} \mu_Y^2} \text{Var}_Y(I_S) \leq \mathbb{E}[J^1] - \frac{n \log_2 n}{\mathbb{E}[I_S]} \leq \frac{n \log_2 n}{\mu_Y^2 n \log_2 n} \text{Var}_Y(I_S), \quad (2.24)$$

where the supremum and infimum of $h(x, \mu)$ are substituted with extremal values of the Sackin index on bifurcating trees (Fischer n.d.). The expectation of the Sackin index under the Yule process is given in equation (2.28), and its variance is calculated

as (Cardona et al. n.d.)

$$\text{Var}_Y(I_S) = 7n^2 - 4n^2 \sum_{i=1}^n \frac{1}{i^2} - 2n \sum_{i=1}^n \frac{1}{i} - n. \quad (2.25)$$

Substituting these expressions into equation (2.24), we find limits

$$\begin{aligned} \frac{n \log_2 n}{\frac{(n-1)(n+2)}{2} \mu_Y^2} \text{Var}_Y(I_S) &\xrightarrow{n \rightarrow \infty} \frac{\log n (7n^2 - 4n^2 \sum_{i=2}^n \frac{1}{i^2} - 2n \sum_{i=2}^n \frac{1}{i} - n)}{4n^3 (\sum_{i=2}^n \frac{1}{i})^2} \\ &\sim \frac{\log n}{n} \rightarrow 0 \end{aligned}$$

for the lower bound on the gap, and

$$\begin{aligned} \frac{n \log_2 n}{\mu_Y^2 n \log_2 n} \text{Var}_Y(I_S) &= \frac{7n^2 - 4n^2 \sum_{i=2}^n \frac{1}{i^2} - 2n \sum_{i=2}^n \frac{1}{i} - n}{4n^2 (\sum_{i=2}^n \frac{1}{i})^2} \\ &\xrightarrow{n \rightarrow \infty} \frac{1}{(\sum_{i=2}^n \frac{1}{i})^2} \rightarrow 0 \end{aligned}$$

for the upper bound on the gap. The upper bound reaches a maximum at $n = 13$ and is approximately 0.008, while the lower bound reaches a maximum at $n = 8$ and is approximately 0.005.

(ii) Let μ_U be the expected value of the Sackin's index under the uniform model for trees on n leaves, and $f(x) = \frac{1}{x}$. By theorem 2.3.2

$$h(x; \mu_U) = \frac{f(x) - f(\mu_U)}{(x - \mu_U)^2} - \frac{f'(\mu_U)}{x - \mu_U} = \frac{1}{x \mu_U^2}. \quad (2.26)$$

We can then substitute this into the inequality given in the theorem as in

$$\frac{n \log_2 n}{\frac{(n-1)(n+2)}{2} \mu_U^2} \text{Var}_U(I_S) \leq \mathbb{E}[J^1] - \frac{n \log_2 n}{\mathbb{E}[I_S]} \leq \frac{n \log_2 n}{\mu_U^2 n \log_2 n} \text{Var}_U(I_S), \quad (2.27)$$

analogously to (2.24). The expectation of Sackin's index under the uniform model is given by (Cardona et al. n.d.)

$$\mathbb{E}_U(I_S) = \frac{4^{n-1} n! (n-1)!}{(2n-2)!} - n, \quad (2.28)$$

and its variance is

$$\text{Var}_U(I_S) = n \frac{10n^2 - 3n - 1}{3} - \frac{(n+1)(n+2)}{2} \frac{(2n-2)!!}{(2n-3)!!} - n^2 \left(\frac{(2n-2)!!}{(2n-3)!!} \right)^2. \quad (2.29)$$

For the limit $n \rightarrow \infty$ we can use Stirling's approximation

$$n! \xrightarrow{n \rightarrow \infty} \sqrt{2\pi n} \left(\frac{n}{e}\right)^n \quad (2.30)$$

$$n! \xrightarrow{n \rightarrow \infty} \begin{cases} \sqrt{\pi n} \left(\frac{n}{e}\right)^{n/2}, & n \text{ even,} \\ \sqrt{2n} \left(\frac{n}{e}\right)^{n/2}, & n \text{ odd,} \end{cases} \quad (2.31)$$

to obtain asymptotic behaviour of the expected value and variance of I_S under the uniform model. The expectation reduces to

$$\begin{aligned} \mathbb{E}_U(I_S) &\xrightarrow{n \rightarrow \infty} \frac{4^{n-1} \sqrt{2\pi n} \left(\frac{n}{e}\right)^n \sqrt{2\pi(n-1)} \left(\frac{n-1}{e}\right)^{n-1}}{\sqrt{2\pi(2n-2)} \left(\frac{2n-2}{e}\right)^{2n-2}} - n \\ &\sim \frac{\sqrt{\pi n} n^n}{e(n-1)^{n-1}} - n \\ &\sim \sqrt{\pi} \exp \left[\left(n + \frac{1}{2} \right) \log n - (n-1) \log(n-1) \right] - n \\ &\sim \sqrt{\pi} n^{\frac{3}{2}} - n \end{aligned}$$

and the variance

$$\begin{aligned} \text{Var}_U(I_S) &\xrightarrow{n \rightarrow \infty} \frac{10}{3} n^3 - n^2 - \frac{1}{3} n - \frac{n^2 + 3n + 2}{2} \frac{\sqrt{\pi(2n-2)} \left(\frac{2n-2}{e}^{n-1}\right)}{\sqrt{2(2n-3)} \left(\frac{2n-3}{e}^{n-1/2}\right)} \\ &\quad - n^2 \left(\frac{\sqrt{\pi(2n-2)} \left(\frac{2n-2}{e}^{n-1}\right)}{\sqrt{2(2n-3)} \left(\frac{2n-3}{e}^{n-1/2}\right)} \right)^2 \\ &\sim \frac{10}{3} n^3 - n^2 - \frac{1}{3} n - \frac{n^2 + 3n + 2}{2} \sqrt{\frac{e\pi}{2}} \exp \left[(n-1) \log \frac{2n-2}{2n-3} + \frac{1}{2} \log(2n-3) \right] \\ &\quad - n^2 \exp[\log(2n-3)] \\ &\sim \frac{4}{3} n^3 + 2n^2 - \frac{1}{3} n - \frac{n^{\frac{5}{2}} + 3n^{\frac{3}{2}} + 2n^{\frac{1}{2}}}{2} \sqrt{e\pi}. \end{aligned}$$

□

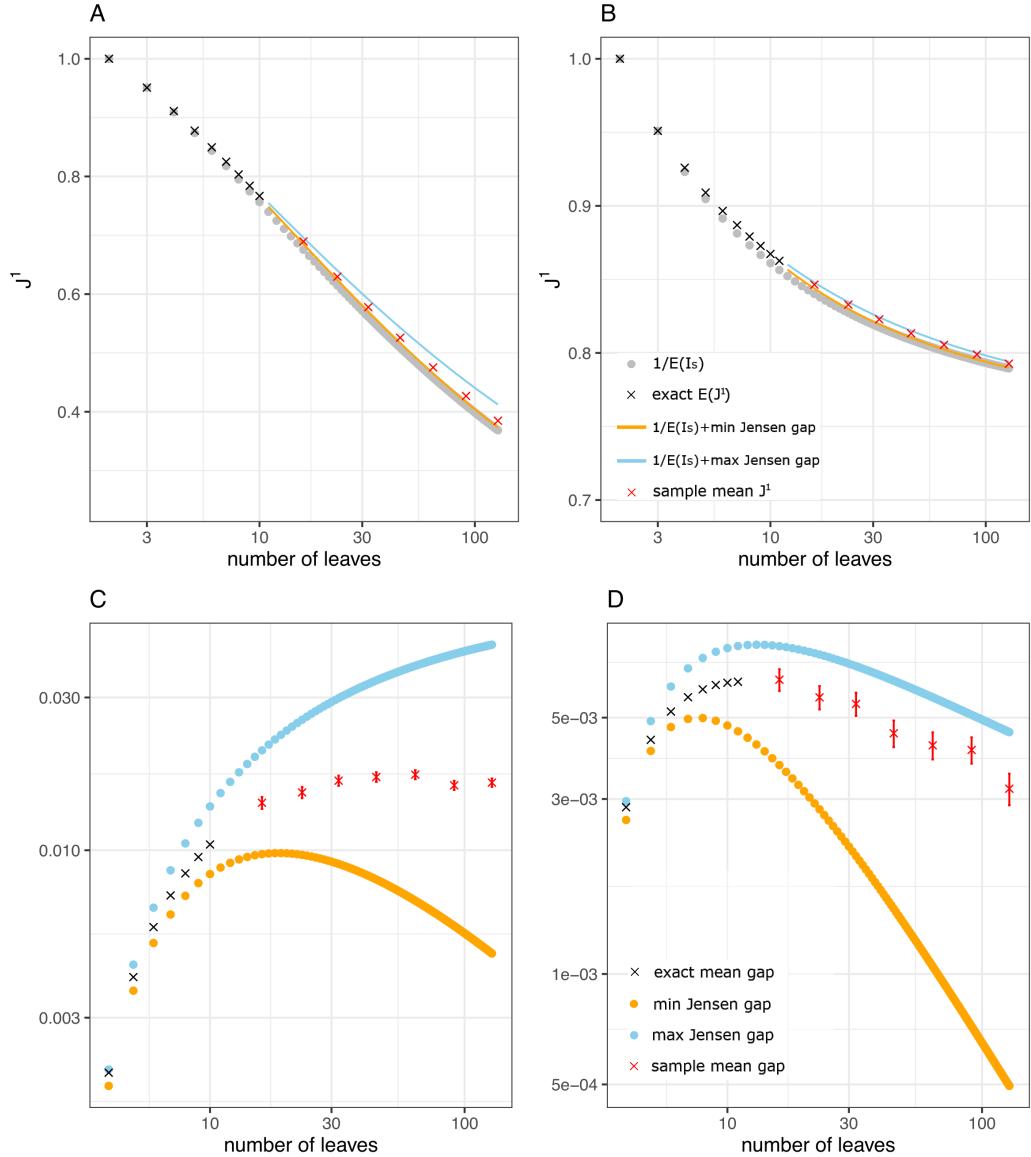


Figure 2.3: Top row: True values of $E(J^1)$ for up to 10 leaves were calculated manually, and the approximations up to 128 leaves were calculated as $n \log_2 n / E(I_S)$. **A** — uniform model, **B** — Yule model.

Bottom row: The Jensen gap of $E(J^1)$ calculated for trees up to 128 leaves under the uniform model (**C**), and the Yule model (**D**). The size of the gap is calculated as the difference between the true and approximate expected value, with the gaps for 2 and 3 leaves equal to zero as there is only one possible bifurcating tree shape for each of those values. Refer to table 2.1 for numerical values of the gap size for the first several values of n . The red crosses in **A** and **B** represent sample mean J^1 values for 100000 trees generated under the uniform model and Yule process, and the difference between the approximate gap size and the sample mean, with standard error represented by error bars, in **C** and **D**.

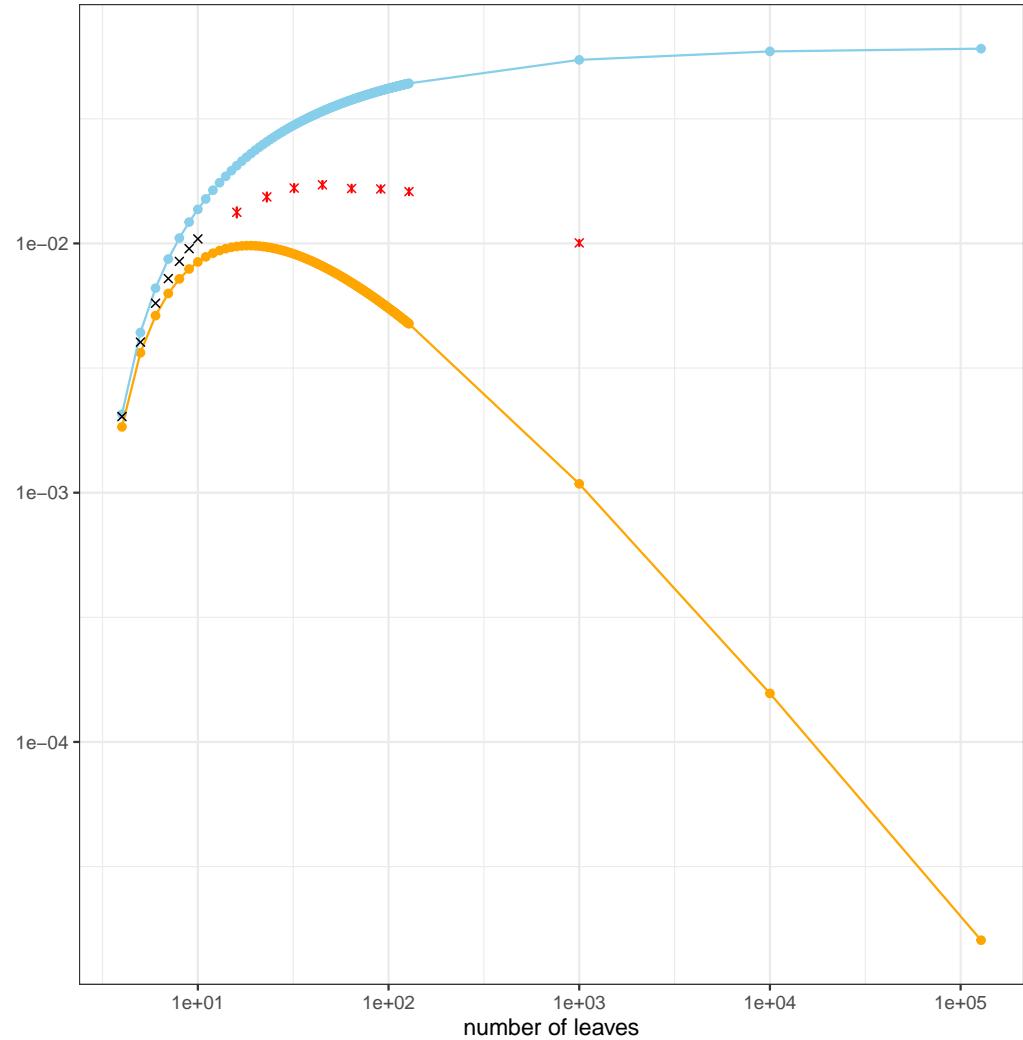


Figure 2.4: The convergence of the upper bound to $4/3\pi$ is much slower than the convergence of the lower bound to 0, and the maximum it reaches over the plotted range is 0.0604 for $n = 128000$. The red crosses, as in figure 2.3, suggest convergence of the gap size.

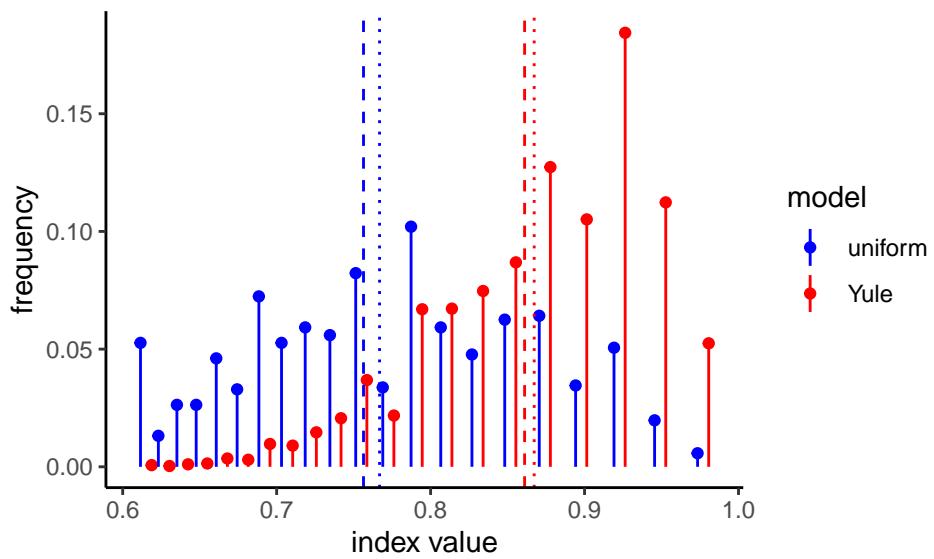


Figure 2.5: Higher variance in the uniform model leads to a non-zero upper bound on the Jensen gap. Shown are frequencies of J^1 values on 10-leaf trees generated under the Yule and uniform models. The dashed lines represent the true expected value of J^1 , and the dotted lines the approximate value calculated as $\frac{n \log_2 n}{\mathbb{E}(I_S)}$

The lower bound of $\mathbb{E}_U(J^1) - \frac{n \log_2 n}{\mathbb{E}_U(I_S)}$ goes to 0 as $\frac{\log n}{n}$, while the upper bound tends to $\frac{4}{3\pi}$ for $n \rightarrow \infty$. This is a consequence of high variance in the uniform model (figure 2.5), as each tree on n leaves is selected with equal probability while the number of trees on n grows exponentially with n , the number of leaves. While I cannot prove analytically that the size of the Jensen gap in this case tends to 0, I can generate random trees using the uniform model and compare the sample mean to the approximation using the expected value of the Sackin index. In figure 2.3, I show behaviour of the Jensen gap and its bounds for J^1 under the Yule and uniform models. The red crosses in figures 2.3C and 2.4 indicate that the gap size does converge for $n \rightarrow \infty$. Therefore, I propose the following conjecture:

Conjecture 2.3.1. *For trees generated under the uniform model on $n \rightarrow \infty$ leaves, the following holds*

$$\mathbb{E}_U(J^1) \rightarrow \frac{n \log_2 n}{\mathbb{E}_U(I_S)}. \quad (2.32)$$

n	$n \log_2 n / \mathbb{E}_Y(J^1)$	$\mathbb{E}_Y(I_S)$	$n \log_2 n / \mathbb{E}_U(J^1)$	$\mathbb{E}_U(I_S)$
2	2	2	2	2
3	5	5	5	5
4	216/25	26/3	360/41	44/5
5	728/57	77/6	3822/289	99/7
6	1162800/67217	87/5	18.25643	386/21
7	199806750/9017743	223/10	23.81979	793/33
8	27.29901	962/35	29.87282	12952/492
9	32.68993	4609/140	36.38201	26333/715
10	38.30246	4861/126	43.31989	106762/2431
11	44.11464	55991/1260	n/a	n/a

Table 2.1: Comparison of exact and approximate expected values of J^1 and I_S under the Yule and uniform models.

2.3.4 Analytic properties of the J^1 index

The index J^1 is normalised in a way which makes comparison of its values on trees of different sizes valid (Lemant et al. n.d.). As J^1 was defined to take into account node sizes, it can take any value between 0 and 1 for any given tree topology (figure 2.6). Furthermore, since J^1 is defined to be 0 on linear trees, finding its minimal value on a given number of nodes is trivial. In this section I investigate extremal values on trees where I impose restrictions to both topology and node size distributions, i.e. consider only leafy trees with out-degree of each internal node greater than 1.

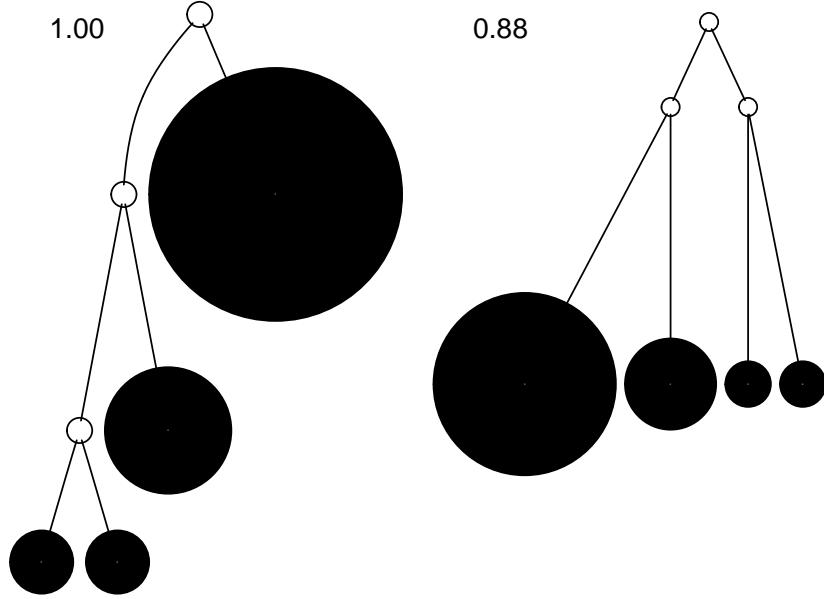


Figure 2.6: By including the node-balance function W^1 in J^1 , we allow for the possibility of perfectly balanced caterpillars (left) and less balanced fully symmetric trees (right) based on the node size distribution in the tree. The leaf sizes in these two trees are identical, with a ratio 4 : 2 : 1 : 1 from largest to smallest.

2.3.5 Properties of J^1 on different tree families

For most balance indices in use in evolutionary biology, the least balanced tree for a given number of leaves n is the binary caterpillar tree. I have previously derived a general expression for leafy trees of this topology (Lemant et al. n.d.)

$$J^1(T_C) = \frac{2n \log_2 n}{(n-1)(n+2)}. \quad (2.33)$$

Most balance indices in literature define the caterpillar topology as the least balanced one (Fischer et al. n.d.). Intuitively, this makes sense as balance is often associated with symmetry, and the caterpillar is the most asymmetric bifurcating tree. However, in the context of the J^1 index, tree topology is just one of a few factors which contribute to the balance score of a tree, especially since the index does not limit the space of trees to bifurcating ones. Also important to consider are node sizes and, more specifically, how the population is split across different subtrees in the tree of interest. Let us consider a slightly altered caterpillar topology.

Definition 2.3.5. Let T_B be a leafy tree on n leaves. Let every internal node of T_B except for the most distant one from the root have out-degree 2 such that one of its

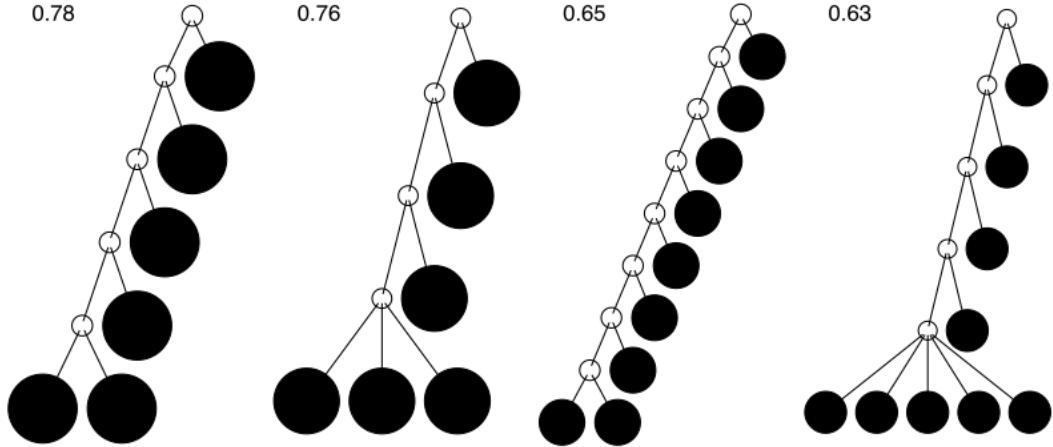


Figure 2.7: If we limit our search to leafy trees with equal leaf sizes, the least balanced tree on a given number of leaves is not necessarily the caterpillar. Pictured are the caterpillar trees on 6 and 9 leaves, as well as minimally balanced brooms for 6 and 9 leaves, with corresponding J^1 values.

descendants is a leaf, and the other an internal node. Further, let the internal node most distant from the root have out-degree k . Then we call tree T_B a **broom tree**. We call the leaves attached to the internal node with the highest out-degree the **broom head**, and the remaining leaves are attached to the **handle**.

A general expression of J^1 for this family of trees is then derived.

Proposition 2.3.4. *The value of J^1 for a broom tree T_B on n leaves, of which k in the broom head is*

$$J^1(T_B) = \frac{2(n \log_2 n - k \log_2 k + k)}{(n+k)(n-k+1)}. \quad (2.34)$$

Proof.

$$\begin{aligned}
J^1(T_B) &= \frac{1}{\sum_{l=k}^n l} \sum_{i \in \tilde{V}} S_i^* \sum_{j \in C(i)} W_{ij}^1 \\
&= \frac{-2}{(n+k)(n-k+1)} \sum_{i \in \tilde{V}} S_i^* \sum_{j \in C(i)} \frac{S_j}{S_i^*} \log_{d^+(i)} \frac{S_j}{S_i^*} \\
&= \frac{-2}{(n+k)(n-k+1)} \left(\sum_{\substack{i \in \tilde{V} \\ d^+(i)=2}} S_i^* \sum_{j \in C(i)} \frac{S_j}{S_i^*} \log_2 \frac{S_j}{S_i^*} + k \cdot k \cdot \frac{1}{k} \log_k \frac{1}{k} \right) \\
&= \frac{-2}{(n+k)(n-k+1)} \left(\sum_{\substack{i \in \tilde{V} \\ d^+(i)=2}} S_i \left(\frac{S_i-1}{S_i} \log_2 \frac{S_i-1}{S_i} + \frac{1}{S_i} \log_2 \frac{1}{S_i} \right) - k \right) \\
&= \frac{2}{(n+k)(n-k+1)} \left(\sum_{i=k+1}^n i \left(\frac{i-1}{i} \log_2 \frac{i}{i-1} + \frac{1}{i} \log_2 i \right) + k \right) \\
&= \frac{2}{(n+k)(n-k+1)} \left(\sum_{i=k+1}^n \left((i-1) \log_2 \frac{i}{i-1} + \log_2 i \right) + k \right) \\
&= \frac{2}{(n+k)(n-k+1)} \left(\log_2 \frac{n^n k!}{k^k n!} + \log_2 \frac{n!}{k!} + k \right) \\
&= \frac{2}{(n+k)(n-k+1)} \left(\log_2 \frac{n^n}{k^k} + k \right) \\
&= \frac{2}{(n+k)(n-k+1)} (n \log_2 n - k \log_2 k + k)
\end{aligned}$$

□

The result of proposition 2.3.4 is directly generalisable in the following way.

Proposition 2.3.5. *For a broom tree T_{Bq} on n leaves, of which k in the broom head, such that the sizes of leaves in the head sum to $q \in \mathbb{R}$, and the leaves in the handle all of equal size 1, the value of J^1 is*

$$\begin{aligned}
J^1(T_{Bq}) &= \frac{1}{(n-k+1)(q+(n-k)/2)} \\
&\times \left(q \log_k q - \left(\sum_{i=1}^k l_i \log_k l_i \right) + (q+n-k) \log_2 (q+n-k) - q \log_2 q \right),
\end{aligned} \tag{2.35}$$

where l_1, \dots, l_k are the leaf sizes which add up to q .

In figure 2.7 I show that the caterpillar is not the minimally balanced leafy tree

for a few tree sizes. To take it a step further, consider the following proposition.

Proposition 2.3.6. *For leafy trees on n leaves and no linear parts, the caterpillar minimises J^1 iff $n < 5$.*

Proof. Let $J_B^1(n, k)$ denote the value of J^1 on a broom tree with n leaves, of which k in the broom head. Then

$$J_B^1(n, 2) = \frac{2n \log_2 n}{(n+2)(n-1)}, \quad (2.36)$$

$$J_B^1(n, 3) = \frac{2}{(n+3)(n-2)}(n \log_2 n - 3 \log_2 3 + 3). \quad (2.37)$$

Consider the case when $J_B^1(n, 2) < J_B^1(n, 3)$. Plugging in equations (2.36) and (2.37), we can rearrange the inequality to find

$$8n \log_2 n - 6(n^2 + n - 2) \log_2 3 + 6(n^2 + n - 2) > 0, \quad (2.38)$$

which changes sign at 0, 0.667, 1 and 4.168. Setting the first derivative of this expression to zero

$$8 \log_2 n + \frac{8}{\log 2} - (12n + 6) \log_2 3 + 12n + 6 = 0$$

we find solutions around $n = 0.822$ and $n = 2.888$, the latter of which signifies a local maximum. Therefore, as n can only take positive integer values, valid solutions for which the caterpillar is less balanced than the broom with 3 leaves in the broom head according to the index J^1 are 3 and 4, with the $k = 3$ broom being less balanced otherwise. \square

This proposition gives us a threshold for the number of leaves at which the caterpillar is no longer the minimally balanced tree for the given number of leaves, which sets J^1 apart from conventional balance indices (figure 2.8A). However, I am yet to prove the following statement.

Conjecture 2.3.2. *For leafy trees on n leaves and no linear parts, the tree that minimises J^1 belongs to the broom family.*

2.3.6 Behaviour as $n \rightarrow \infty$

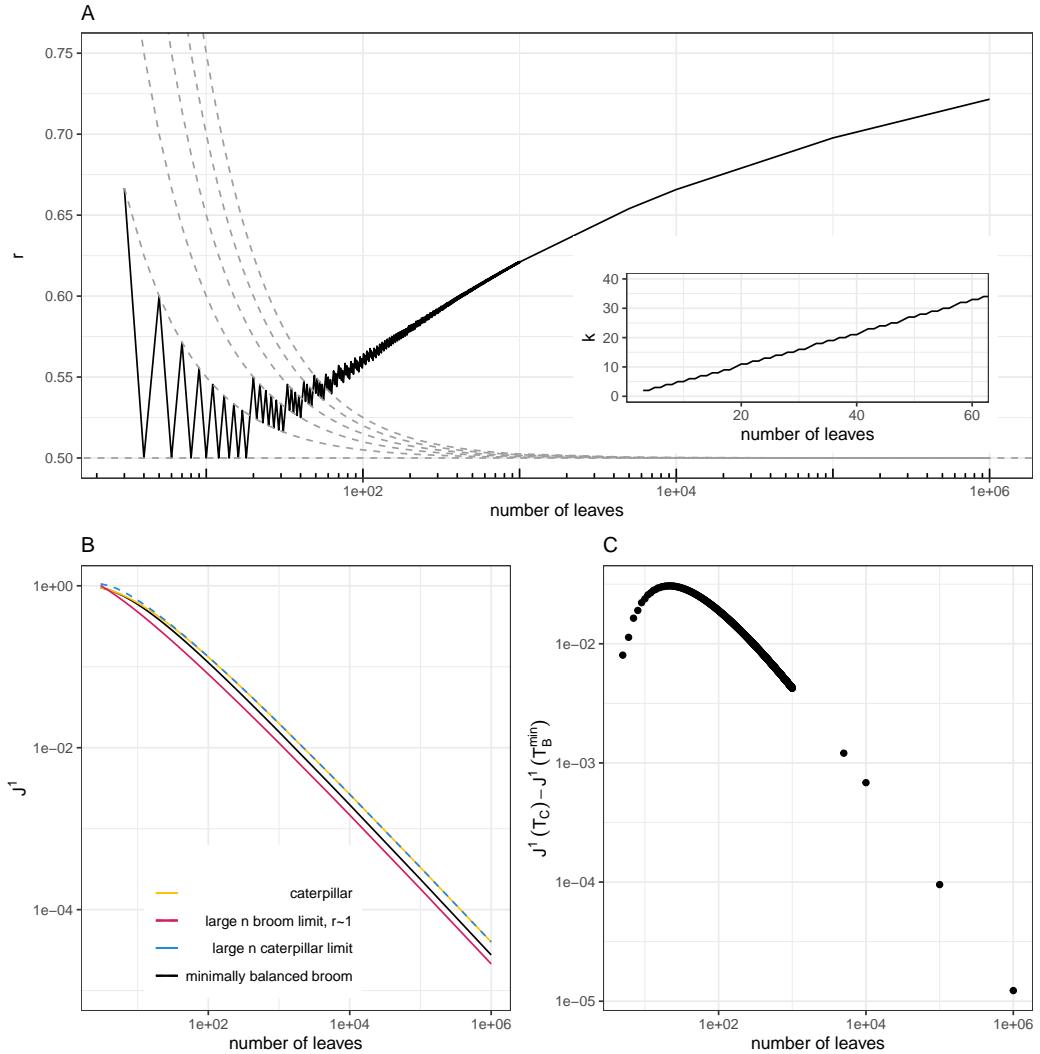


Figure 2.8: The labels used in the figures are as above - n for number of leaves, k for number of leaves in the broom head, $r = n/k$. **A:** Value of r for which the minimum value of J^1 is obtained on leafy trees. Trees on n leaves which satisfy $r = \frac{n+a}{2n}$, for $a = 0, 1, 2, \dots$ lie on the dashed grey lines. The inset plot shows $k = rn$, the number of leaves attached at the broom head. **B:** Comparison of true and approximate values of J^1 for the caterpillar and minimally balanced broom trees as a function of n . **C:** The difference between values of J^1 of the minimally balanced broom and the caterpillar trees.

I have derived general behaviour of J^1 on broom and caterpillar trees for a given number of leaves n , showing that caterpillar trees are not necessarily minimally balanced for a given number of leaves. If we let $n \rightarrow \infty$, the value of J^1 for the caterpillar from equation (2.33) will behave like

$$\lim_{n \rightarrow \infty} J^1(T_C) = \frac{2 \log_2 n}{n}. \quad (2.39)$$

As J^1 is not limited to trees with equal leaf sizes, there is a threshold we can impose on the broom tree beyond which the caterpillar is less balanced.

Proposition 2.3.7. *Let $T_B(n)$ be a broom tree on n leaves such that the leaves on the handle and head have sizes f and fp respectively, and $T_C(n)$ be a caterpillar tree on n leaves of equal sizes f . Then*

$$J^1(T_B) > J^1(T_C) \quad \text{iff} \quad p < \frac{1}{2}, \quad (2.40)$$

as $n \rightarrow \infty$.

Proof of proposition 2.3.7. Let $n \rightarrow \infty$. The the value of J^1 for the caterpillar tree tends to

$$J^1(T_C) = \frac{2 \log_2 n}{n},$$

and for the broom tree with equally sized leaves of size p in the broom head

$$J^1(T_{B,p}) = \frac{2}{n(1-r)(1+r(2p-1))} [(r(p-1)+1) \log_2 n(r(p-1)+1) - rp \log_2 nrp].$$

We can evaluate the difference between these expressions:

$$\begin{aligned} J^1(T_C) - J^1(T_{B,p}) &\sim (1-r)(1+r(2p-1)) \log_2 n + rp \log_2 nrp \\ &\quad - (r(p-1)+1) \log_2 n(r(p-1)+1) \\ &\sim ((1-r)(1+r(2p-1)) - (r(p-1)+1) + rp) \log_2 n + o(\log_2 n). \end{aligned}$$

The difference is dominated by the term containing $\log_2 n$ which is always positive. The term in the brackets preceding it can be negative, however:

$$(1-r)(1+r(2p-1)) - r(p-1) + 1 + rp = r(1-r)(2p-1).$$

As $r = k/n$, with k the number of leaves in the broom head, it is always positive. Thus, the expression is negative only when $2p - 1 < 0$ or $p < \frac{1}{2}$ \square

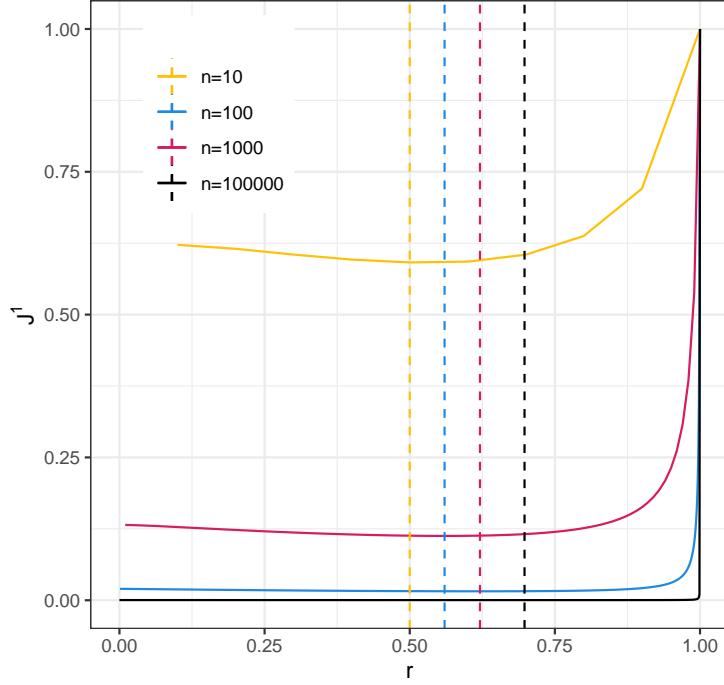


Figure 2.9: Values of J^1 on trees of different sizes calculated using equation (2.34) for different values of $r = k/n$. The dashed lines are at values of r which minimise J^1 .

For broom trees, the behaviour is a little more complicated and, perhaps, counter-intuitive (figure 2.9). Consider the following.

Proposition 2.3.8. *Let $\mathcal{T}_B(n)$ be the set of all leafy broom trees with equal leaf sizes on n leaves, $r = \frac{k}{n}$ where k is the number of leaves in the broom head for a given tree, and r_{opt} the value of r which minimises J^1 for a given n . Then $r_{opt} \rightarrow 1$ as $n \rightarrow \infty$.*

Proof. Let $r = k/n$ and $J_B^1(n, r)$ the value of J^1 for a broom tree on n leaves, of which k in the head. Then

$$J^1 \xrightarrow{n \rightarrow \infty} \frac{2}{n(1-r)(r+1)}((r+1)\log_2 n(r+1) - r\log_2 nr) \quad (2.41)$$

which is minimised for $r \rightarrow 1$. \square

The proposition says that most leaves on a minimally balanced broom tree will be concentrated in the head, with comparatively few on the handle, resembling a

star tree more closely than a caterpillar tree. However, one must take into account how imbalanced the nodes above the broom head are, since one of their subtrees contains most of the tree’s leaves, whereas the other is a single leaf. For practical purposes, the difference between the J^1 values of the minimally balanced broom and the caterpillar for the number of leaves $n \rightarrow \infty$ is small and decreases rapidly as n grows (figure 2.8B, 2.8C).

Finding the true value of k which minimises $J^1(T_B)$ analytically is difficult. The derivative with respect to k of equation (2.34) yields a transcendental equation which is not analytically solvable. I also cannot analytically determine whether broom trees minimise J^1 for a given number of leaves. However, I have exhaustively checked whether the broom minimises J^1 up to 12 leaves — which it does. Beyond that, the number of possible trees grows too rapidly for a similar verification to be computationally feasible without an efficient tree generating algorithm for trees with arbitrary node degree distributions.

2.4 Discussion

The aim of this chapter was to explore deeper analytic properties of the universal balance index J^1 and carve its place in the broader context of tree balance by extending past results and uncovering new connections.

In the chapter I focussed on trees with uniform branch lengths, as J^1 was not defined with them under consideration. A further generalisation of metric describing tree properties is therefore the logical next step (Noble & Verity n.d.).

I calculated an approximate expectation of J^1 under the most common null models used in evolutionary biology. Having a good approximation for the expected value of J^1 is a crucial result in the development of this index, as it allows us to employ it in the analysis of evolutionary processes on phylogenetic trees. The next step in this direction would be to obtain a closed-form solution for the expectation of J^1 , as well as its variance.

Finally, I only touched upon directly obtainable relationships without considering different real-world use cases of the index and the implications of equation (2.9). This is another avenue of future research as there may exist a relationship between the way indices vary with time and the underlying evolutionary process growing the associated tree.

Chapter 3

Tracking cancer evolution *in silico* via evolutionary indices

3.1 Introduction

A trajectory is a path described by any object (or indeed point) in some space according to some parameter, usually time. Intuitively then, an evolutionary trajectory refers to the changes that a lineage or population undergoes over time — the series of genetic, morphological, and behavioral transformations that occur as organisms evolve and diversify. We are interested in the evolutionary trajectory of cancers but reliably obtaining time-series data is, at the time of writing, not feasible at a larger scale. This stems from multiple issues. Firstly, at time of diagnosis, solid tumours have likely already been growing for long enough to reach a size visible in standard medical imaging (Patrone et al. n.d.). This means that even initial data obtained in the clinic represents a relatively late stage in the cancer’s evolutionary history most of the time. Secondly, solid tumours are just that — clumps of cells organised in some way in space — meaning that taking a sample from one point in the tumour is not necessarily representative of the rest of the cell population. Finally, a biopsy is an invasive procedure which can cause considerable discomfort to patients, depending on where the tumour is situated. Therefore, having a reasonable estimate of a tumours evolutionary trajectory based on the data that is available at time of sequencing would allow for a more informed treatment strategy. This begs the question — how can we distinguish between different ways tumours evolve? Is it necessary to wade through sequences of genetic data to do so or is it

possible to abstract the key properties of a tumour’s evolutionary trajectory into a few numerical summaries?

In this chapter, we will examine the utility of two different sets of evolutionary indices for tracking the evolution of tumours on the example of agent-based simulations.

3.2 Preliminaries

3.2.1 Modes of evolution

Over the years, there have been a number of different definitions of what a mode of evolution is. Initially, it was introduced as the term which covers the way or manner in which a species evolves (Eiseley n.d.). Depending on the piece of literature, it could also refer to the model used in the study of a population’s evolutionary trajectory (Yotoko et al. n.d.), or the mechanism which drives the evolution such as genetic drift (Glassman et al. n.d., Wolf & Koonin n.d.). This ambiguity of terminology is present in cancer research as well. In this thesis, I will use the term mode of evolution as originally defined by (Eiseley n.d.), and used by (Davis et al. n.d., Noble, Burri, Le Sueur, Lemant, Viossat, Kather & Beerenwinkel n.d.). That is, the way in which a tumour evolves (figure 3.1).

3.2.2 Why even bother with indices?

Before introducing the sets of indices used to analyse properties of trees, let us consider a simpler question — can we map the set of all possible trees to the set of real numbers? For this purpose we should decide how to define the set of trees. The number of nodes in a tree is a natural number, $n \in \mathbb{N}$, as is the number of possible tree topologies for a given n . We denote with $T(n)$ the set of enumerated tree topologies (Nakano n.d.). Each node then has a corresponding size, giving us an n -tuple of real numbers $(\alpha_1, \dots, \alpha_n) \in \mathbb{R}^n$, and each edge (branch) has a corresponding length or $(l_i, \dots, l_{(n-1)}) \in \mathbb{R}^{(n-1)}$. This means we would need a family of maps

$$f_n : A(n) \times \mathbb{R}^n \times \mathbb{R}^{n-1} \rightarrow R. \quad (3.1)$$

It would be easy to construct a mapping which would allow us to “enumerate” each possible tree with a real number. The problem with this approach, however,

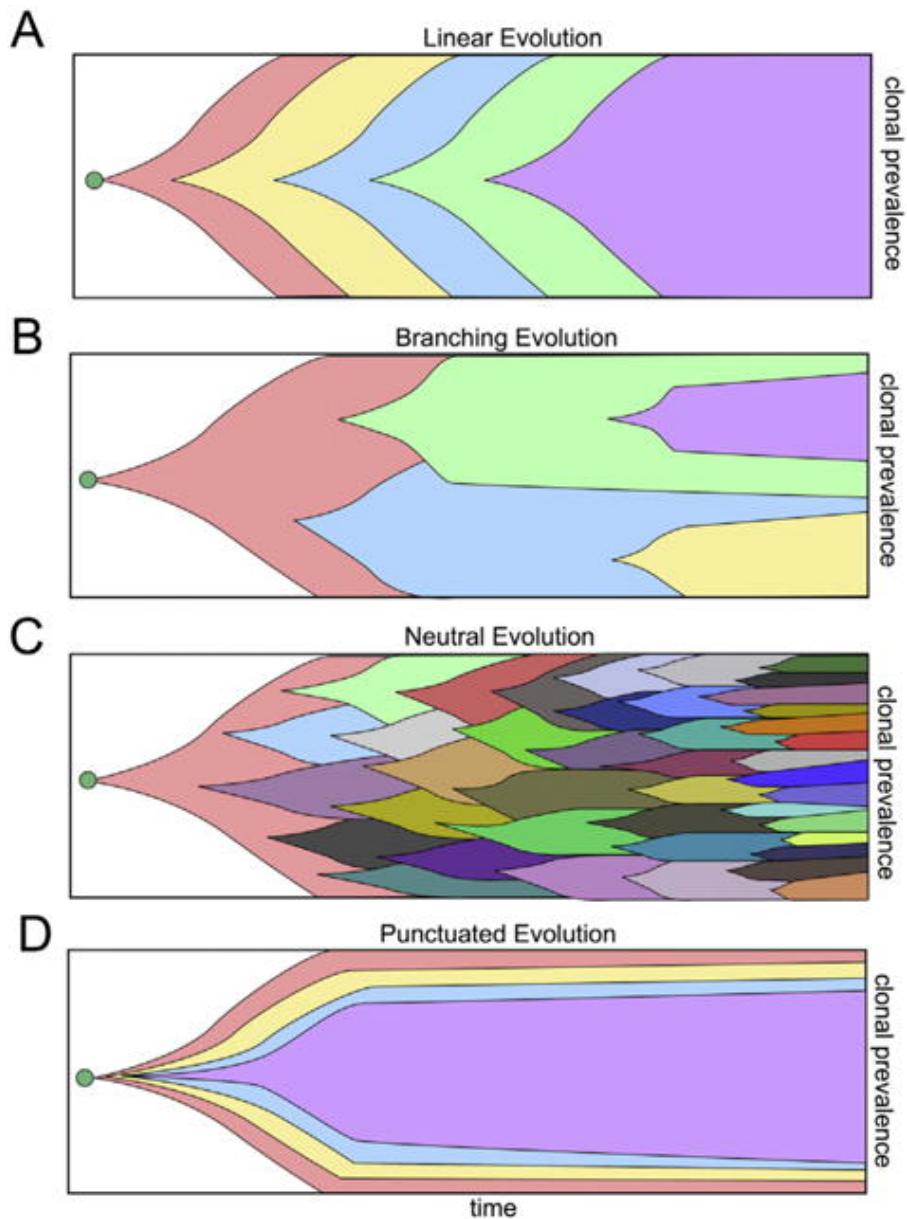


Figure 3.1: Four different modes of tumour evolution.
 Picture adapted from (Davis et al. n.d.) under a CC BY 4.0 license.

is that it would not be very informative. The real numbers are not ordered in any way that would allow us to meaningfully compare trees. The lack of interpretability would render any application of such a mapping useless. This is where tree shape indices come in as a way to summarise key properties of a tree in a way that is both interpretable and mathematically sound.

3.2.3 A 3-dimensional index space — trees with uniform branch lengths

Shannon diversity

Shannon entropy is a fundamental concept in information theory, that quantifies the uncertainty or randomness of a system (Shannon n.d.). By considering a system where diversity represents the variety of elements, such as intra-tumour heterogeneity, we can define the Shannon diversity as the exponential of the Shannon entropy,

$$^1D = \exp [{}^1H] = \exp \left[- \sum_{i=1}^N p_i \log p_i \right], \quad (3.2)$$

where N is the total number of categories (or elements, species, etc.), and p_i the frequency of category i . The Shannon diversity was chosen because of the nice property that it is maximised and equal to the number of categories when all categories are equally represented, and minimised when only one category is present. Previous work on a similar topic (Noble, Burri, Le Sueur, Lemant, Viossat, Kather & Beerenwinkel n.d.) was based on the Simpson index (Simpson n.d.). However, the Shannon index was chosen for this work because it is more sensitive to changes in the frequencies of subclones, better interpretability, and the fact that the J^1 index is based on the Shannon entropy.

Mean number of drivers per cell — distance from the root

Each speciation event in phylogenetics or driver mutation in cancer evolution is associated with a change in the corresponding tree's topology. To capture the average number of these events, we use the mean number of drivers per cell. This is defined as the average of distances from all nodes to the root (with the root distance from itself defined as 1) weighted by the frequencies of the subclones,

$$n = \sum_{i=1}^N p_i \nu(i), \quad (3.3)$$

where $\nu(i)$ is the root distance of node i .

Balance index

As discussed in chapter 2, the balance index J^1 is a weighted average of the evenness of the population distribution within a tree. We use it as the third index in this

space.

3.2.4 A general set of indices — any rooted tree

Expanding upon the 3-dimensional space defined above, a new comprehensive set of interpretable robust indices based on Hill numbers was introduced recently (Noble & Verity n.d.). The authors expanded and improved upon the existing quantifiers of tree shape properties by deriving methods for trees with arbitrary node size, node degree, and branch length distributions. The methods for calculating all of the indices are included as part of an R package (kimverity n.d.).

Each generalised index has three components, depending on which part of the tree it is applied — the longitudinal mean, node-wise mean, star mean.

Richness — 0D

Richness in the context of phylogenetics is simply the number of extant species, i.e. the number of tips in a phylogenetic tree. The generalised richness's three components are:

1. 0D_L — the average number of branches across the tree;
2. 0D_N — the average effective outdegree, ignoring branch sizes;
3. 0D_S — the effective number of non-root nodes.

Diversity — qD , $q > 0$

The generalised diversity index represents an extension of the Shannon diversity index. Its three components are:

1. qD_L — the effective number of maximally distant nodes (leaves);
2. qD_N — the average effective outdegree, accounting for branch sizes, i.e. bushiness;
3. qD_S — the effective numbering of branches, accounting for branch sizes.

Evenness — qJ , $q > 0$

Finally, the extension of the robust universal balance index J^1 , this set of indices generalises tree balance in the following way:

1. ${}^q J_L$ — evenness of branch sizes across the tree, or tree symmetry for leafy and ultrametric trees;
2. ${}^q J_N$ — tree balance, or evenness of the node size distribution;
3. ${}^q J_S$ — evenness of all branch sizes.

3.3 Tree resolution

I rejected the idea of simply mapping trees to real numbers due to the lack of interpretability. Tree shape indices are nominally better, as they summarise properties of a tree, but they may have limitations in the form of a lack of resolution for certain types of trees.

Starting simple, we examine leafy trees with all leaves of equal size in the 3-dimensional index space. The first thing to note is that the Shannon diversity will simply equal the number of leaves in the tree. This already takes away a degree of freedom. The next thing to consider is the value of J^1 . If we limit our search, for now, to perfectly balanced trees, we are left with symmetric trees on a fixed number of leaves N . To make the final index equal between two trees, they need to have equal average depths of their leaves. As we are only looking at perfectly symmetric trees, that means that the average depth will be exactly equal to the individual leaf depths. We can then show the following

Proposition 3.3.1. *Let T be a symmetric leafy tree on N leaves with equal leaf sizes. If the canonical factorisation of N is*

$$N = \prod_{i=1}^k \alpha_i^{l_i}, \quad (3.4)$$

then there are

$$\frac{\left(\sum_{i=1}^k l_i\right)!}{\prod_{i=1}^k l_i!} \quad (3.5)$$

distinct trees with the same values of J^1 , ${}^1 D$, and n , including T .

Proof. First, the values of indices J^1 and D for a symmetric leafy tree with N

equally-sized leaves are

$$J^1 = 1,$$

$$D = N,$$

$$n = 1 + \sum_{i=1}^k l_i.$$

The result is then a simple combinatorial problem of placing $n - 1$ balls (α_i 's) into $n - 1$ bins, with each α_i repeated l_i times. Therefore, the number of distinct trees is

$$\frac{\left(\sum_{i=1}^k l_i\right)!}{\prod_{i=1}^k l_i!}. \quad (3.6)$$

□

This case may be interesting mathematically, but is not too relevant for practical purposes as it is highly unlikely that sequencing data would yield a perfectly symmetric leafy tree. As the space of trees is so large, there is little point in performing a grid search, especially when arbitrary node sizes are considered. In testing, there have been no cases of trees with the same values of indices, but different topologies. This is a good sign, as it means that the indices are able to distinguish between different trees. However, the question remains whether there is a set of indices which can differentiate between any two trees.

3.4 Computational methods

3.4.1 Agent-based modelling framework - *warlock/demon*

There is no shortage of agent-based models of tumour evolution (Colyer et al. n.d.), and the can range from purpose-built complex frameworks to more stripped-down and abstract ones. Since each model should be “as simple as possible but no simpler”, the appropriate framework for our purposes must satisfy certain requirements — flexibility, efficiency, and reproducibility. The first requirement is deceptively specific. As the main inspiration behind this work stems from cancer evolution, we want our simulations to have parameters for controlling aspects of the cell population’s physical properties which would in turn imply a different way in which it evolves. This would, for example, include spatial arrangement of cells, mutation

rates, migration rates, and selective advantage. Furthermore, while the goal is to simulate large populations of cells, we also need a large number of simulations over which we can infer more general deterministic properties. Stochastic effects could make vastly different evolutionary modes look more similar than expected in theory. Finally, reproducibility allows us to share parameters of our models for verification by peers, and possible further investigation.

The agent-based modelling framework we decided to use is `warlock` (Bak et al. n.d.), a `snakemake` wrapper written for `demon` (Noble n.d.). It satisfies the requirements above, with a few associated comments. Firstly, it is a flexible agent-based model of tumour evolution as it does have parameters which control for spatial arrangement, mutation rates and selective advantage, as well as migration. While it is able to simulate spatial structure, `demon` covers at most two spatial dimensions. This is not an issue since we approximate the cell population to undergo stochastic isotropic growth, that is the tumour has equal probability of expanding in all directions in space. This implies approximate spherical symmetry of simulated solid tumours, which allows us to effectively consider the two-dimensional simulation as a cross-section of a tumour spheroid. In terms of efficiency, `demon` was written mainly in C++, and conceptualised so that instead of tracking individual cells, it simulates unique cell genotypes on a two-dimensional grid comprised of demes, or well-mixed patches of cells. The procedure for simulation cell events is based on the Gillespie algorithm (Gillespie n.d.), and follows the steps of selecting a deme, then cell type, event type, and finally cell genotype. This approach sacrifices micro-scale interactions between cells to benefit efficiency and the feasibility of mathematical analysis of the model using, for example, diffusion approximations. Finally, all associated code is free and open source (cite github repos once finished), which allows reproducibility using identical parameters and random seeds. Parameter values for different batches can be found in the appendix (ref).

3.5 Results

3.5.1 Sensitivity of evolutionary mode to parameter values

- there is clear variance in trajectories within a spatial config but less than one might expect for parameters within an order of magnitude of each other

Average temporal plots for boundary growth

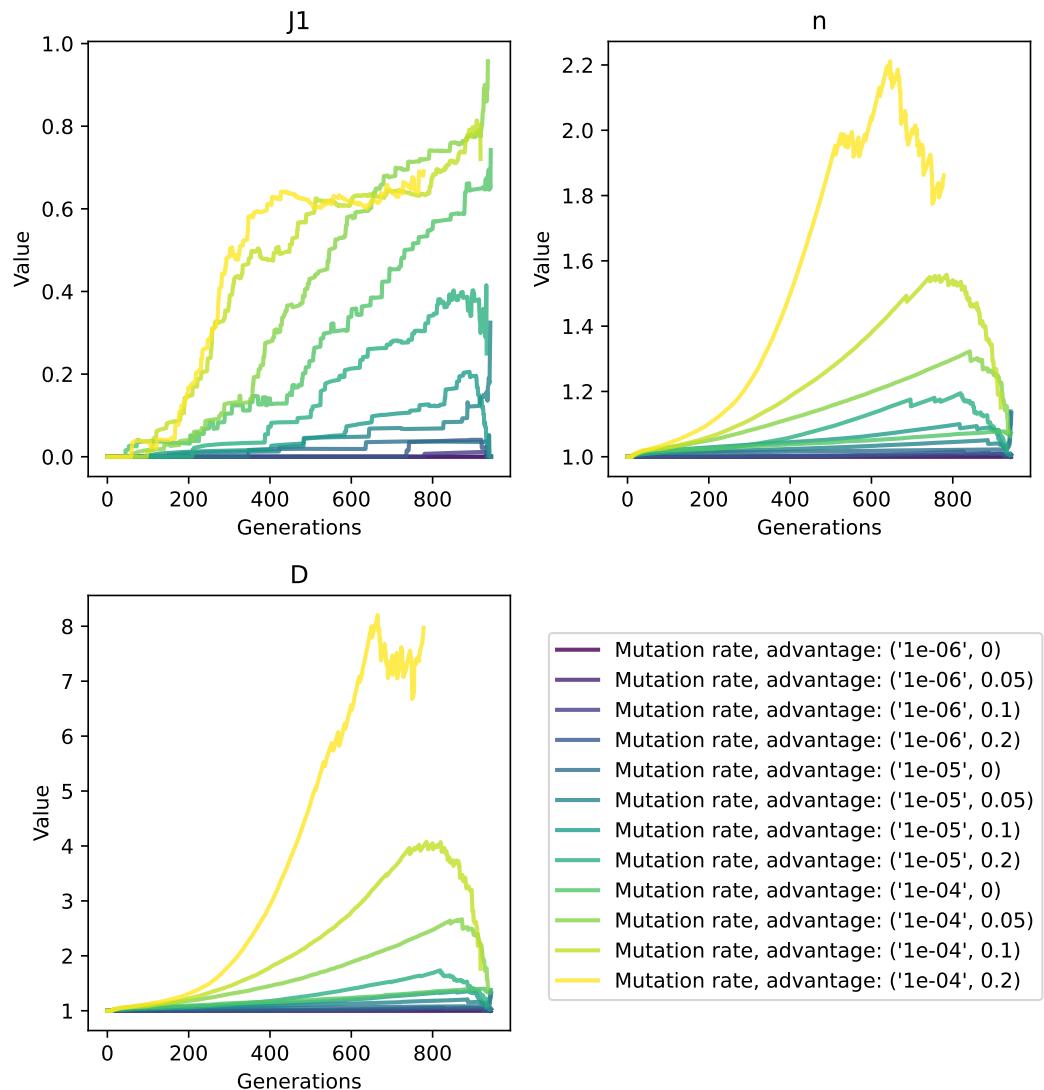


Figure 3.2: Average trajectories of the three indices for different values of driver mutation rate and selective advantage for tumours progressing via boundary growth.

- all things but spatial config being equal, the trajectories seem to be distinct in later stages of evolution
- should formalise somehow??

TO DO: add figures for index space, add figures to appendix, add expanded index set figures (both temporal and index space)

Average temporal plots for non-spatial tumours

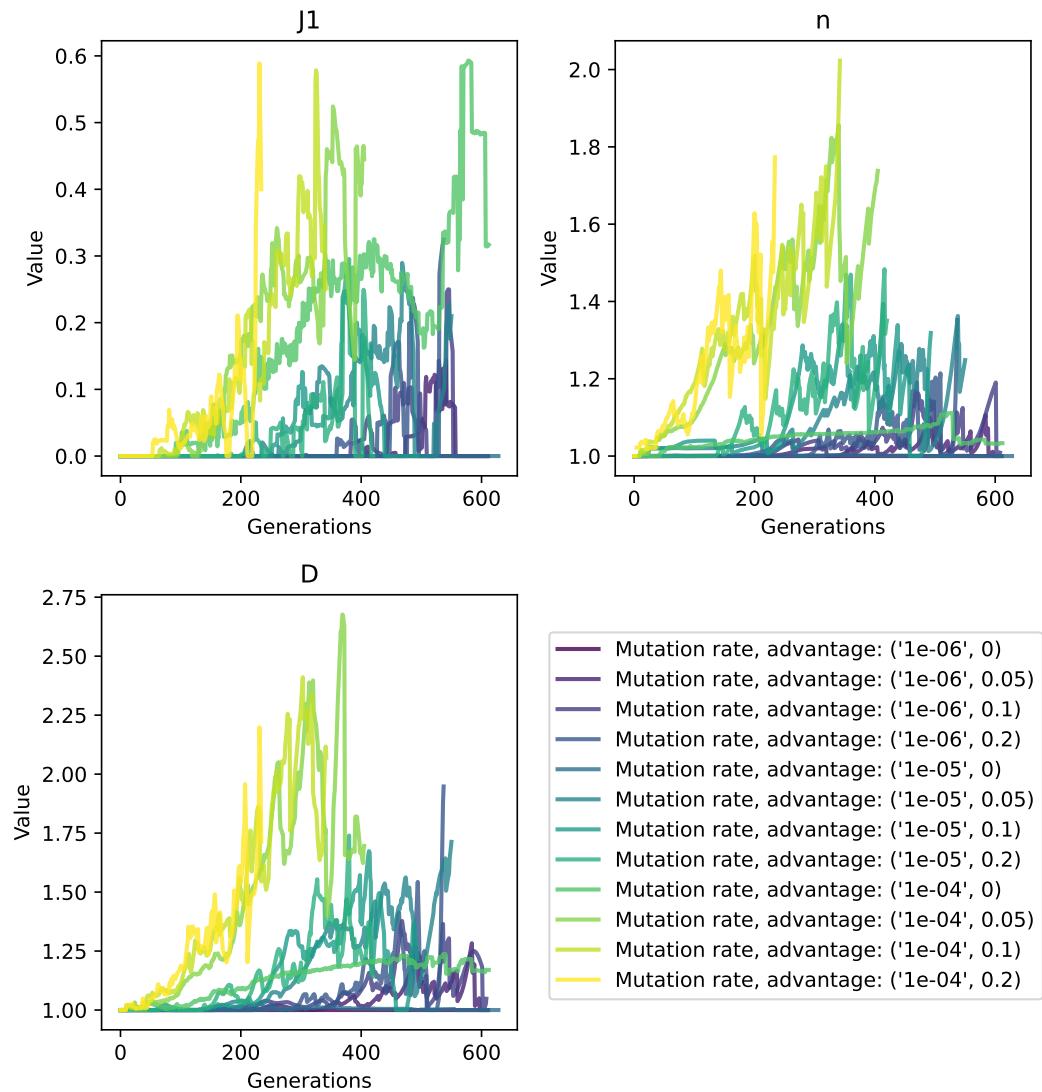


Figure 3.3: Average trajectories of the three indices for different values of driver mutation rate and selective advantage for well-mixed cancer cell populations.

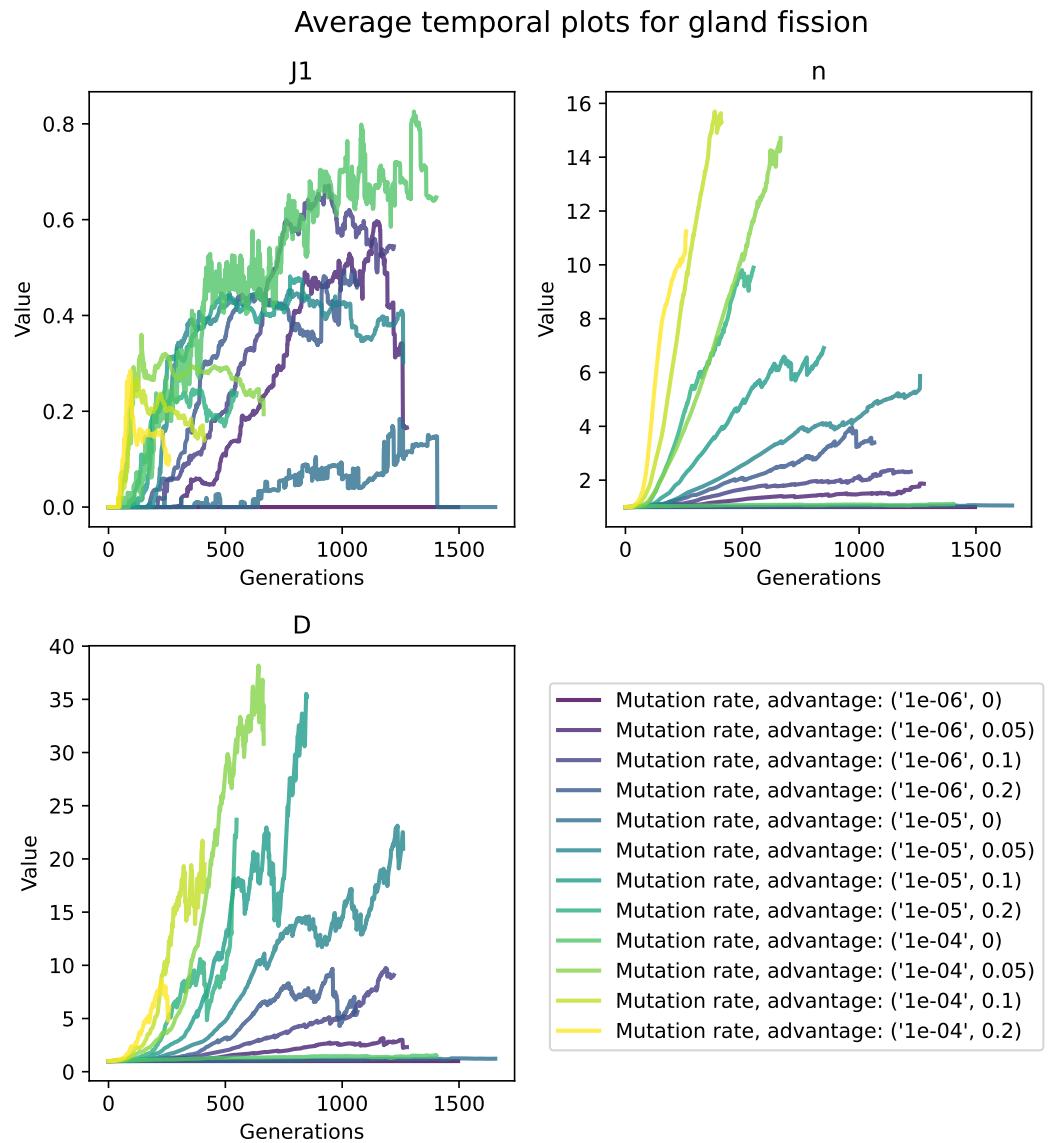


Figure 3.4: Average trajectories of the three indices for different values of driver mutation rate and selective advantage for gland fission.

Average temporal plots for invasive glandular tumours

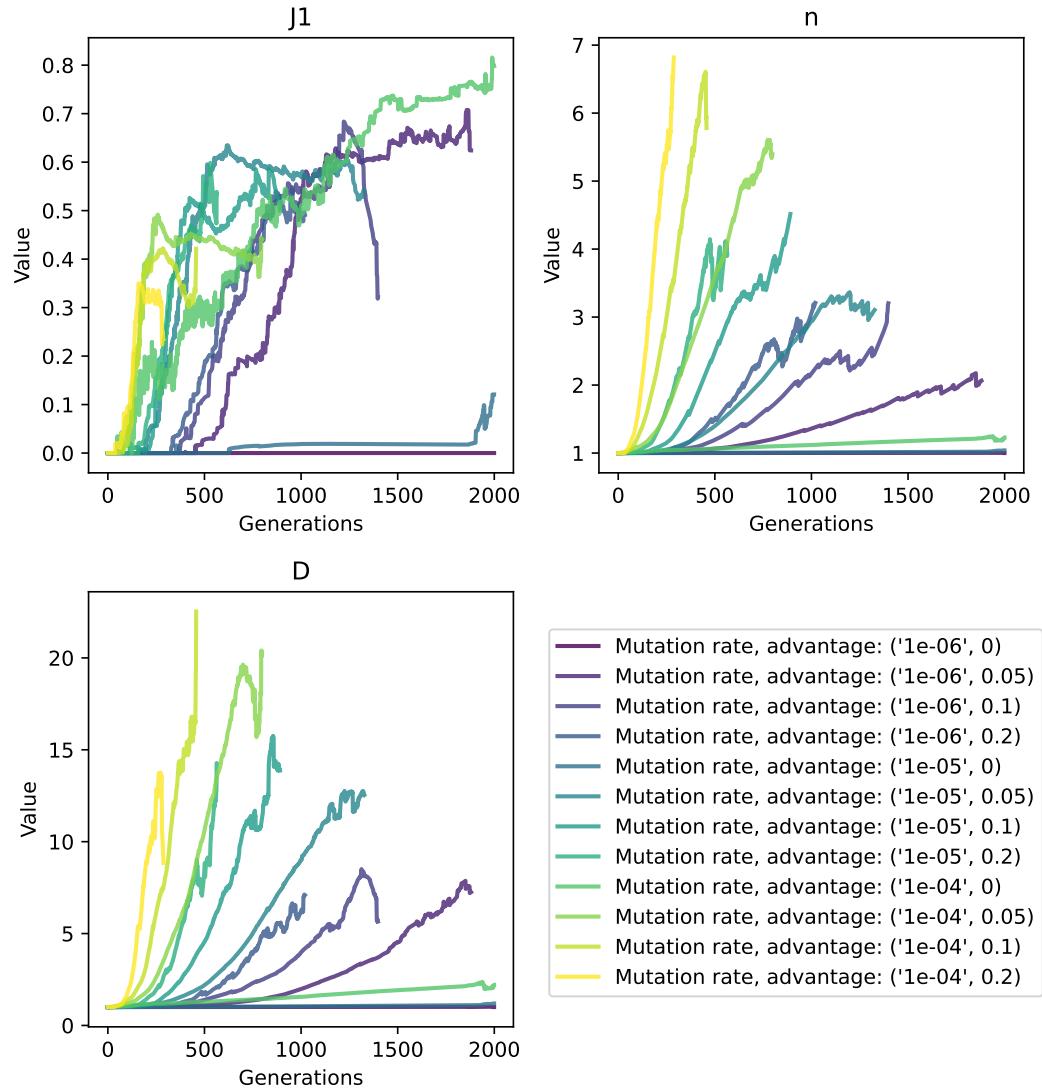


Figure 3.5: Average trajectories of the three indices for different values of driver mutation rate and selective advantage for invasive glandular tumours.

Average trajectories in index space for boundary growth

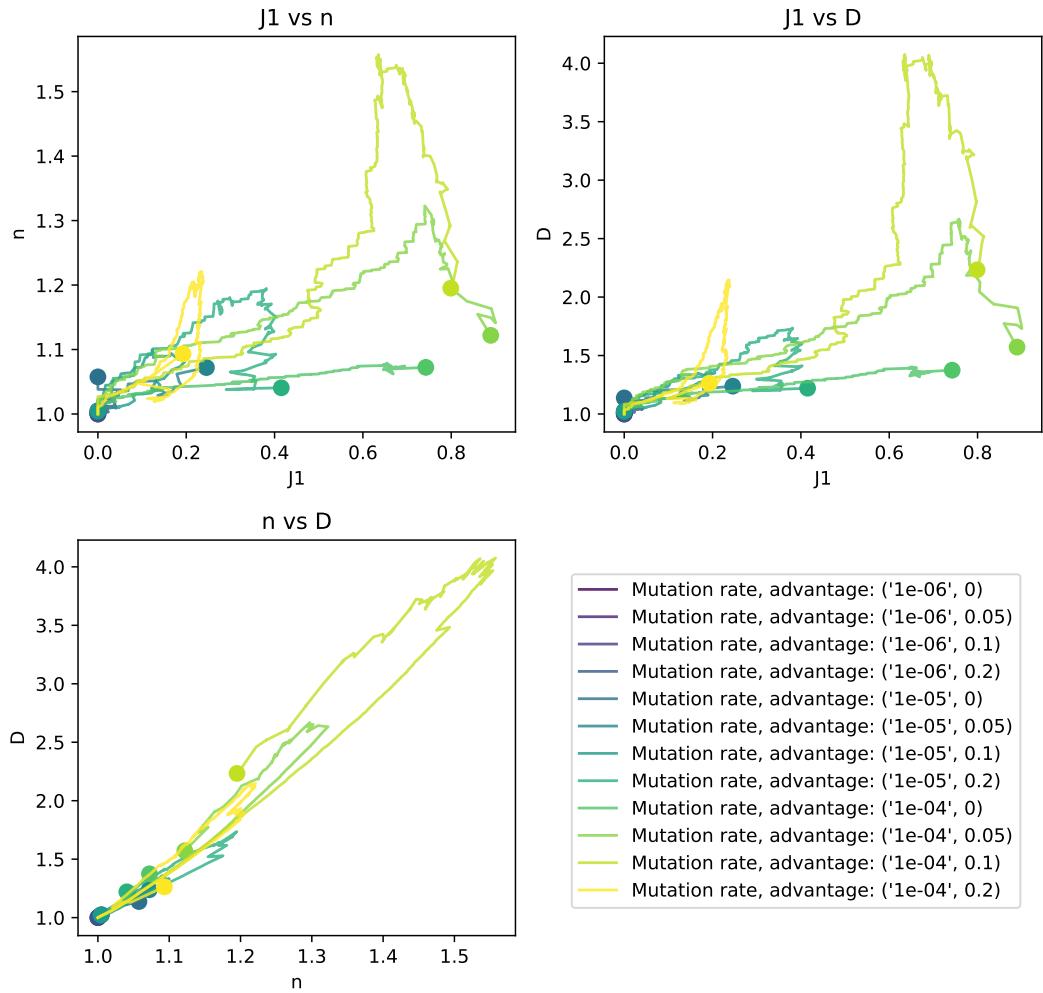


Figure 3.6: Average trajectories in index space for tumours progressing via boundary growth.

Average trajectories in index space for non-spatial tumours

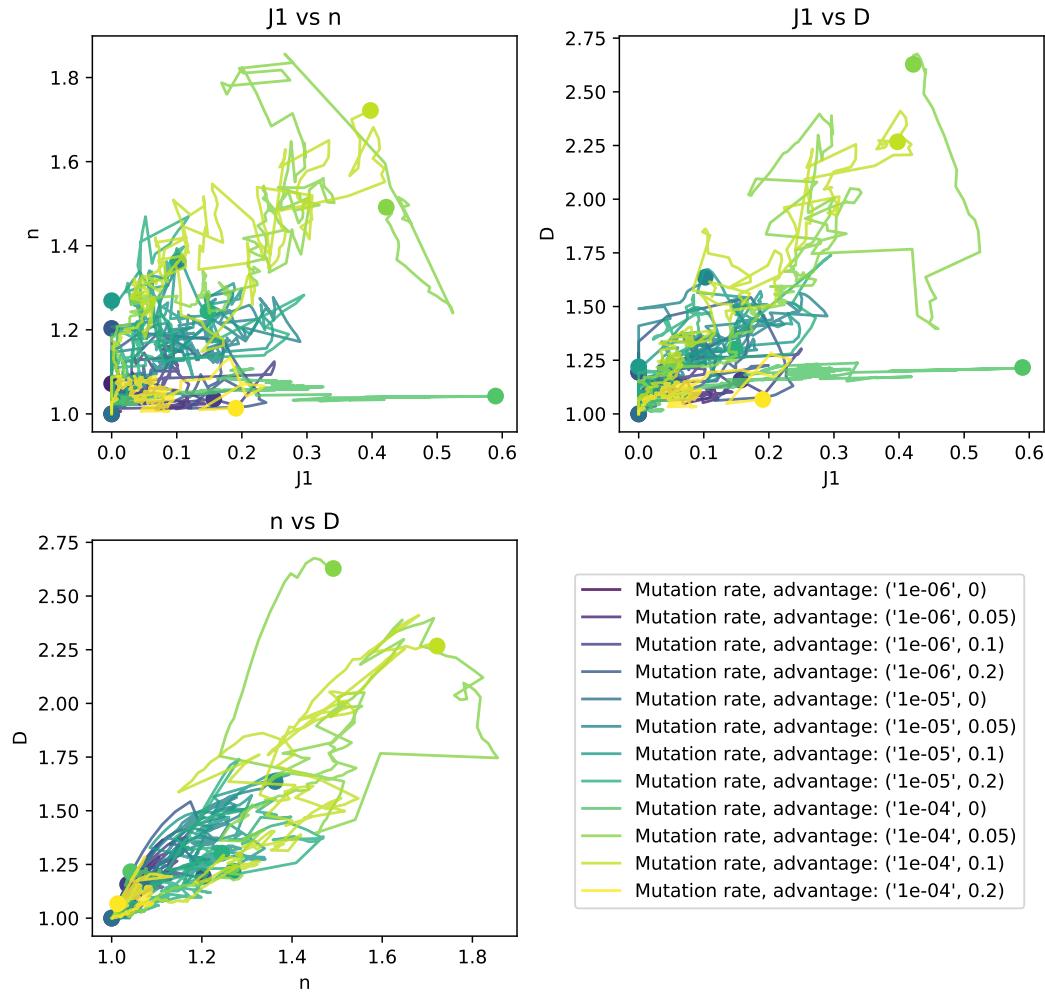


Figure 3.7: Average trajectories in index space for well-mixed cancer cell populations.

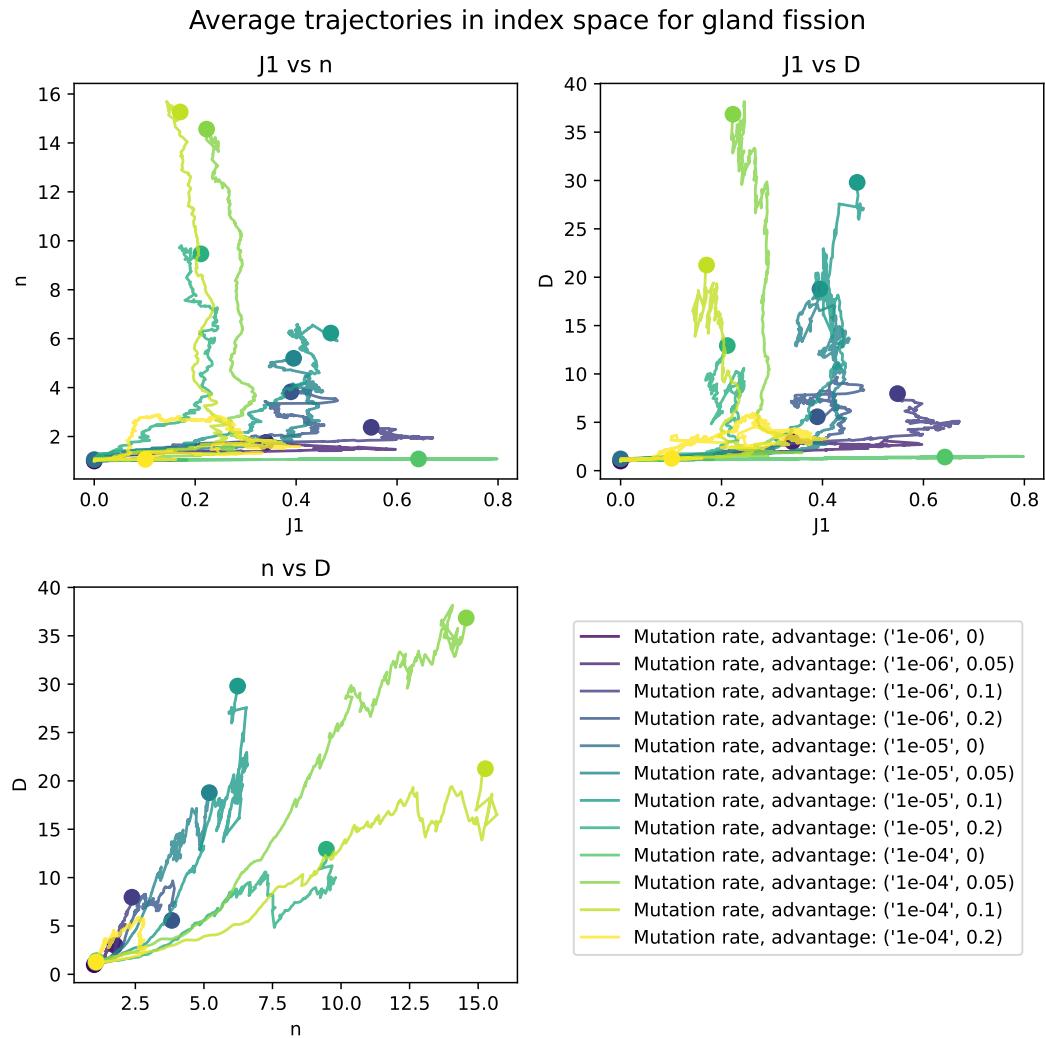


Figure 3.8: Average trajectories in index space for tumours progressing via gland fission.

Average trajectories in index space for invasive glandular tumours

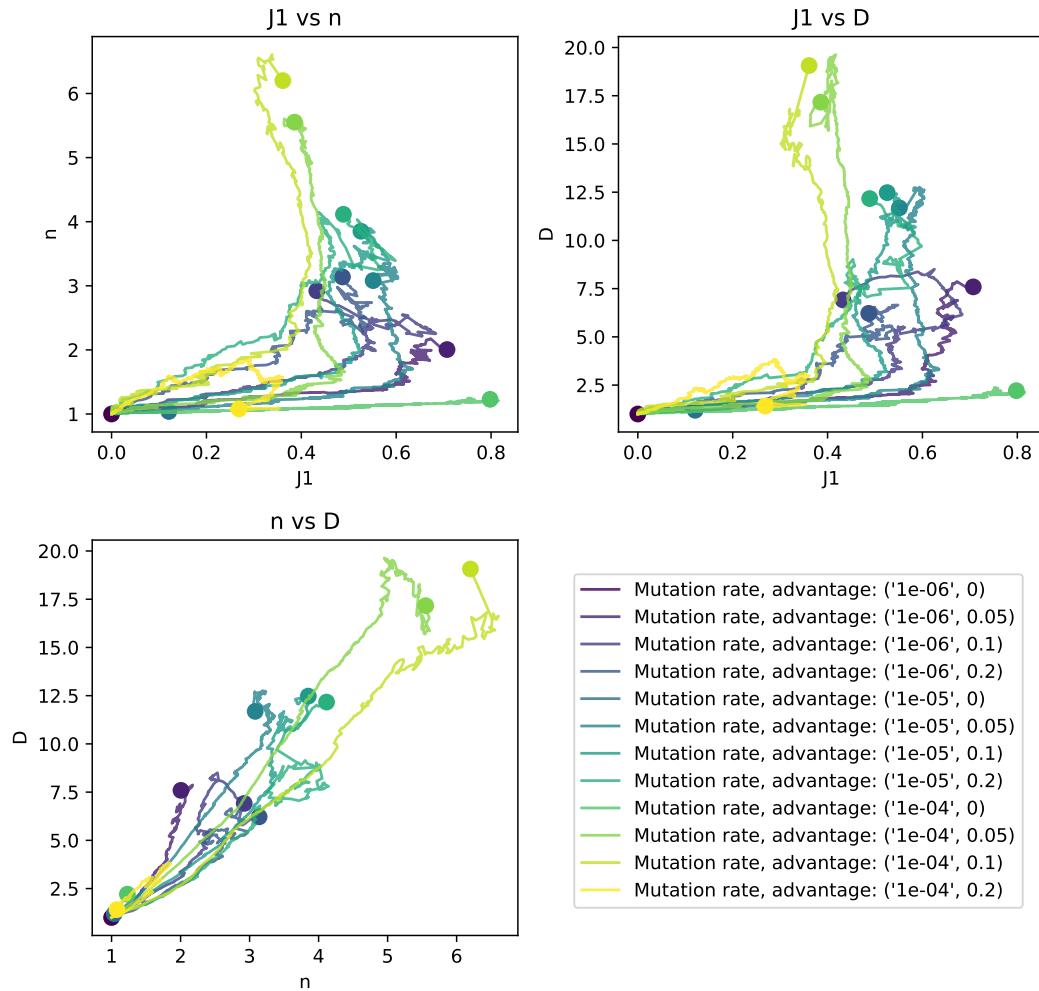


Figure 3.9: Average trajectories in index space for invasive glandular tumours.

3.6 Discussion

- clear differences between different tumour trajectories, but also decent amount of variance depending on parameters — which ones are realistic? (need to be inferred from real data)
- what are the limitations of the approach? — clear starting point is data availability, but also general inter-patient variation of tumour progression
- next steps — further refining of the methods, sourcing and applying to more data (Kim's work in progress)

Chapter 4

Agent-based model of fluctuating methylation arrays in growing fragmented cancer cell populations

4.1 Introduction

In chapter 3, I used a general spatial agent-based model to investigate broad evolutionary patterns as related to spatial organisation. While the model was capable of simulating the dynamics of tumour growth, its utility is limited by the computational cost of simulating a large number of cells. This means that using the model's outputs in comparison to or to draw inference from real data is not feasible.

There are a few ways to address this issue. For example, rather than simulating all clones in a tumour, one could take the approach of (Sottoriva et al. n.d.) and use demes (tumour glands) as the principal agent of our simulation. This would allow for a realistically-sized tumour to be generated as the number of glands would be around the right order of magnitude. A problem with this approach is that it loses resolution since a gland's population is assumed to be clonal, undergoing rapid fixation in the case of an emerging mutant. If one wanted to study evolutionary dynamics on a finer scale it would be necessary to at least simulate the dynamics of cell lineages, if not individual cells, as performed earlier.

In (Gabbutt, Duran-Ferrer, Grant, Mallo, Nadeu, Househam, Villamor, Krali,

Nordlund, Zenz, Campo, Lopez-Guillermo, Fitzgibbon, Barnes, Shibata, Martin-Subero & Graham n.d.), the authors employ a stochastic model for an expanding cell population to model the behaviour of fluctuating CpG sites in blood cancers. The model is capable of simulating the dynamics and the corresponding fluctuating methylation arrays of lymphoid malignancies at scale. However, this model is not spatially explicit, which is a feature that has to be distinguished between different glands in a solid tumour. In this chapter, I present a purpose-written agent-based model, `methdemon`, which reduces the computational cost of simulating a tumour’s growth and models the fluctuating methylation arrays in colorectal cancer.

4.1.1 Fluctuating methylation arrays

As mathematicians new to biology quickly learn, perfectly clean data containing detailed information about the population structure of a tumour is non-existent. In fact, most data is noisy and at best measures a decent proxy for the properties which can be described by a mathematical model. Therefore, one learns very quickly to adapt their thinking when working with biological data. Specifically, when it comes to cancer, a compromise has to be made between resolution and scale. Where single-cell data can provide a detailed view of the mutations accumulated in the genome, it is not feasible to obtain it for a whole tumour. On the other hand, bulk data gives a high-level view of the tumour’s population structure, but a lot of the details get lost in the process.

However, DNA sequencing is not the only way to obtain information about a tumour’s population structure. Early work with methylation arrays in colorectal cancer has shown potential for inferring the ancestry and age of a tumour (Hong et al. n.d., Siegmund, Marjoram, Tavaré & Shibata n.d.). In a way the genome shows more mutations in older populations, methylation arrays will also be more diverse as time goes on. Current techniques allow for the sequencing of some 850, 000 CpG sites which, while a small fraction of the genome, is still enough to provide valuable insight into the underlying dynamics of the cell population. Initial studies on methylation as a tracker of evolution made use of the whole array (Siegmund, Marjoram & Shibata n.d., ?). However, more recent work has shown that just a small subset of CpG sites is enough to infer the evolutionary dynamics of a cell population (Gabbutt, Schenck, Weisenberger, Kimberley, Berner, Househam, Lakatos, Robertson-Tessi, Martin, Patel, Clark, Latchford, Barnes, Leedham, Anderson, Graham & Shibata

n.d., Gabbutt, Duran-Ferrer, Grant, Mallo, Nadeu, Househam, Villamor, Krali, Nordlund, Zenz, Campo, Lopez-Guillermo, Fitzgibbon, Barnes, Shibata, Martin-Subero & Graham n.d.). This is the set of fluctuating CpG (fCpG) loci, which is also the topics of chapter 5.

As fCpG loci seem to be a neutral marker of cell population dynamics, I will define the following set of assumptions for modelling their behaviour:

- (i') **Each cell has a corresponding fCpG array inherited from its parent cell.**
- (ii') **Upon cell division, each methylated fCpG site has an independent and equal probability of being demethylated, and vice-versa.**
- (iii') **The rates of methylation and demethylation do not change over time.**

These assumptions are based on the findings of (Gabbutt, Schenck, Weisenberger, Kimberley, Berner, Househam, Lakatos, Robertson-Tessi, Martin, Patel, Clark, Latchford, Barnes, Leedham, Anderson, Graham & Shibata n.d., Gabbutt, Duran-Ferrer, Grant, Mallo, Nadeu, Househam, Villamor, Krali, Nordlund, Zenz, Campo, Lopez-Guillermo, Fitzgibbon, Barnes, Shibata, Martin-Subero & Graham n.d.).

4.1.2 A comment on using existing models

As discussed in section 1.3, it is preferable to use established frameworks and models for simulating tumour growth and evolution. Therefore, with the assumptions outlined in the previous section, my initial approach was to employ a general agent-based model with small modifications, to simulate the behaviour of fCpG loci in cancer. The first model I considered was `demon`, with which I had already worked in chapter 3, as its light weight and reasonable wall times (total execution time) had shown promise. A naive approach to simulating methylation arrays is to use the model's passenger mutations as a proxy for epigenetic changes. This way, the model could be run as usual with modified passenger mutation rates, and methylation arrays could be assigned to the cells post-hoc. The main issue with this approach is memory management, as the output files tend to be large and thus difficult to handle in the post-processing steps. This meant that applying any sort of inference workflow would take a long time, making the approach impractical. This stands for

other SABMs as well, due to the amount of data produced when simulating a whole tumour's growth.

4.2 An ABM of fluctuating methylation arrays in cancer

To tackle the issue of models generating too much unused data, I wrote a new ABM, `methdemon`, capable of simulating a growing cell population and their corresponding fCpG arrays. The model can be run as a well-mixed population expansion, or in a 1D spatial setting, with the latter being relevant in the case of multi-site sequencing of a tumour spheroid. The model is written in C++, with an emphasis on execution time and dynamic memory management.

4.2.1 Model structure

Inspired by the `demon` model, event scheduling happens according to the Gillespie algorithm, with a deme being chosen first, followed by a cell within that deme. Events are then chosen based on the sum of rates in the tumour, between cell birth, cell death, and deme fission. Upon cell division, both the parent and daughter cells have the same probability of acquiring a driver mutation, and each cell's fCpG array is updated according to the rules outlined in the previous section. A list of relevant model parameters is given in table 4.1, with source code and examples available in the model's github repository (?). Consider a tumour consisting of N demes at the end of growth. Each deme, during growth, has a probability p of undergoing fission, and the final mean number of fissions per deme is $\log_2 N$. Further, the expected number of descendant demes of deme i is given by

$$\frac{N_i}{N} = \frac{(2pt)^i}{i!} e^{-2pt}, \quad (4.1)$$

i.e. the Poisson distribution with mean $2pt$, as derived in (Kharlamov n.d.). This condition governs how fissions are handled in the model. As a real tumour can consist of millions of glands, the model narrows the focus to the subset of demes sequenced at the end of growth. Fissions which produce untracked demes, untracked fissions from here, are the main way of accumulating fission events in a deme. Each untracked fission simply discards half of the deme's population, stochastically rounded. Tracked

Parameter	Description	Units
<code>deme_carrying_capacity</code>	The maximum number of cells in a gland	cell
<code>init_migration_rate</code>	The probability of a gland undergoing fission	$\text{cell}^{-1}(\text{cell division})^{-1}$
<code>mu_driver_birth</code>	The probability of a cell acquiring a driver mutation	$\text{cell}^{-1}(\text{cell division})^{-1}$
<code>s_driver_birth</code>	The selective advantage of a mutant cell	n/a
<code>meth_rate</code>	The probability of an unmethylated fCpG site changing state	$(\text{cell div})^{-1}(\text{fCpG site})^{-1}$
<code>demeth_rate</code>	The probability of a methylated fCpG site changing state	$(\text{cell div})^{-1}(\text{fCpG site})^{-1}$

Table 4.1: Parameters used in the `methdemon` model.

fissions are implemented by assigning a probability to each fission event of being tracked (figure 4.1), equal to

$$\phi = \frac{p}{\mathbb{E}[\text{fissions per deme}]/2}. \quad (4.2)$$

By using this discrete uniform distribution, I ensure that the expected number of fissions before a tracked fission event in a deme is about half of the mean total number of fissions. Were the individual probabilities equal to $1/\mathbb{E}[\text{fissions per deme}]$, the expected number of fission events before a tracked fission would be equal to $\mathbb{E}[\text{fissions per deme}]$. This would, on average, lead to a lot of simulations with the mean number of fissions considerably above the target value. Ignoring untracked fissions, this is equivalent to having a fission rate of $p \times \phi$. Untracked fissions are important, however, not just for the purpose of tracking the mean number of fission events, but also for population dynamics of the demes. Depending on the deme carrying capacity, that is the maximum number of cells in a deme, and mutation and epimutation rates, a fission can impact the population structure of a deme in different ways. Let K be the carrying capacity of a deme, μ the driver mutation rate, γ the epimutation rate, and L the number of fCpG sites per cell (assuming equal methylation and demethylation rates for simplicity). Upon fission, the population of the deme is divided into two, and there need to be $K/2$ cell division events before the deme is back to its carrying capacity. The expected number of mutations is then

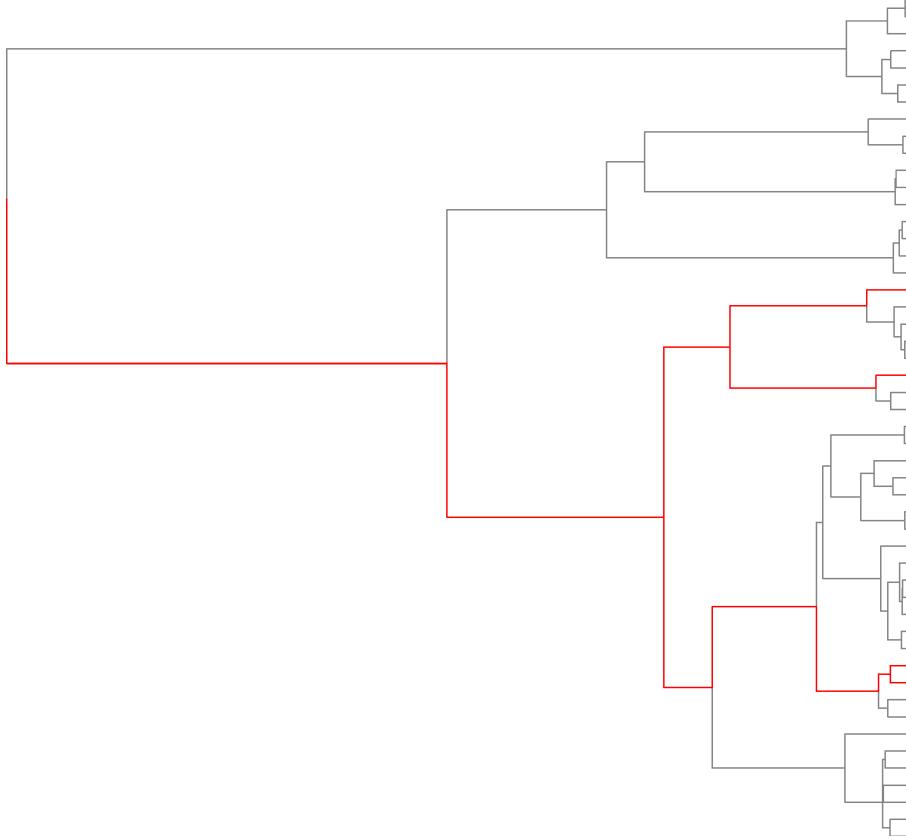


Figure 4.1: A toy example of how fissions are handled in the model. The red branches represent tracked fissions, and the grey branches are hypothetical untracked fissions occurring under a regular branching process.

simply $K/2 \times \mu$, and the expected number of epimutations is $K/2 \times \gamma \times L$. Depending on the rates, these numbers can be quite different. Realistically, the number of driver mutations during repopulation of a deme is likely negligible. While epimutations are more probable, fCpG arrays are inherited with a high degree of fidelity, and the fCpG distribution in a deme post-fission will resemble the state just before. When a deme is at carrying capacity, its dynamics are equivalent to a Moran process, meaning the probability of neutral fixation for a mutant population of m in a deme of carrying capacity K is equal to m/K , ignoring fissions. However, as cells grow into empty space post-fission until carrying capacity is reached, fissions increase the probability of neutral fixation at smaller deme sizes as they will offset some of the effects of genetic drift which is more pronounced at smaller population sizes.

4.2.2 Stopping conditions

When simulating the whole tumour, imposing a stopping condition based on the total cell population is one of the most straightforward ways to end the simulation.

However, as this model is focussed on a subset of relevant cells, I decided on a few different options for stopping conditions. If the model is run in the non-spatial setting, the simulation can be terminated after a maximum number of cells is reached. In the spatial setting, more concretely in the case of deme fission, the main indicator of a tumour’s growth is the mean number of fissions per deme. This condition is based on the assumption that a tumour’s progression by fission is equivalent to a birth-only branching process, similar to the model of (Sottoriva et al. n.d.). The model also has the option of simulating steady-state turnover of cells in the simulated demes after the initial growth phase. This is an approximation of a saturation growth regime, as real tumours are not expected to grow indefinitely.

4.2.3 Sensitivity analysis

To check whether the model’s behaviour is consistent with the expectations, I wrote a `snakemake` workflow to test many combinations of the parameters from table 4.1. The code is available at the github repository for the workflow, `walter` (?). A summary of the results is shown in figures 4.2, and 4.3, 4.4, and 4.5. Further details are included in the appendix.

Carrying capacity

By carrying capacity, I mean the maximum number of cells with proliferative potential in a gland. This is effectively equivalent to the maximum number of lineages allowed in the gland. A more likely situation is that some of the simulated cells are closely related. These could be considered as cancer stem, i.e. cells with infinite proliferative potential which maintain the population of a cancer gland. I considered three different carrying capacities: 10, 100, and 1000. A real tumour gland contains about 10^5 cells, but the current understanding of how colorectal cancer evolves suggests that not all of them are able to divide indefinitely, rather following a similar hierarchical structure to real colonic crypts (Cernat et al. n.d.). The results follow intuition: higher carrying capacity leads to less likelihood of neutral fixation but also more diversity in the presence of selection.

Fission rate

The fission rate in this model is tracked per cell, with a gland’s fission rate being the sum of the fission rates of all cells in the gland. In testing, I considered per cell fission

rates of 10^{-5} , 10^{-4} , and 10^{-3} . As a consequence, the per deme fission rates ranged from 10^{-4} to 1. This led to a complication in the form of unfinished simulations, as with too low of a fission rate, the first deme never splits and the simulation never ends. This is important to note when choosing priors in the ABC workflow. As we only consider tumours which have grown in a reasonable time, the prior distribution of fission rates will be chosen with the carrying capacity taken into account. The results, as expected, show that tumours with a higher fission rate grow faster than those with a lower fission rate. Further, fCpG array diversity depends on the fission rate, epigenetic switching rates and the carrying capacity, as a high fission rate for smaller demes can still produce a diverse array with a high epimutation rate, where slower fissions still lead to a diverse array with a relatively low epimutation rate.

Driver mutation rate and selective advantage

The driver mutation rate is the probability of a cell acquiring a driver mutation upon division. The selective advantage is the proliferative advantage of cells carrying a driver mutation. In testing, I considered driver mutation rates of 10^{-5} , 10^{-4} , and 10^{-3} , and selective advantages of 0, 0.1, and 1. For larger deme sizes, the presence of selection seems to lead to more desynchronised arrays than the neutral case, as neutral fixation is less likely. However, at very strong selection, array diversity again decreases as the mutant quickly fixes in the population. Higher mutation rates lead to more diverse arrays, but an overly high mutation rate leads to the emergence of many mutants, effectively voiding the effect of selection in some cases due to clonal interference.

Epimutation rates

The epimutation rates are the probabilities of a fCpG site changing state upon cell division. In testing, I considered epimutation rates of 10^{-5} , 10^{-4} , and 10^{-3} . The results are consistent with the findings of (Gabbutt, Schenck, Weisenberger, Kimberley, Berner, Househam, Lakatos, Robertson-Tessi, Martin, Patel, Clark, Latchford, Barnes, Leedham, Anderson, Graham & Shibata n.d.), where too slow switching means less diversity, too fast means complete desynchronisation and regression to a normal distribution around 0.5.

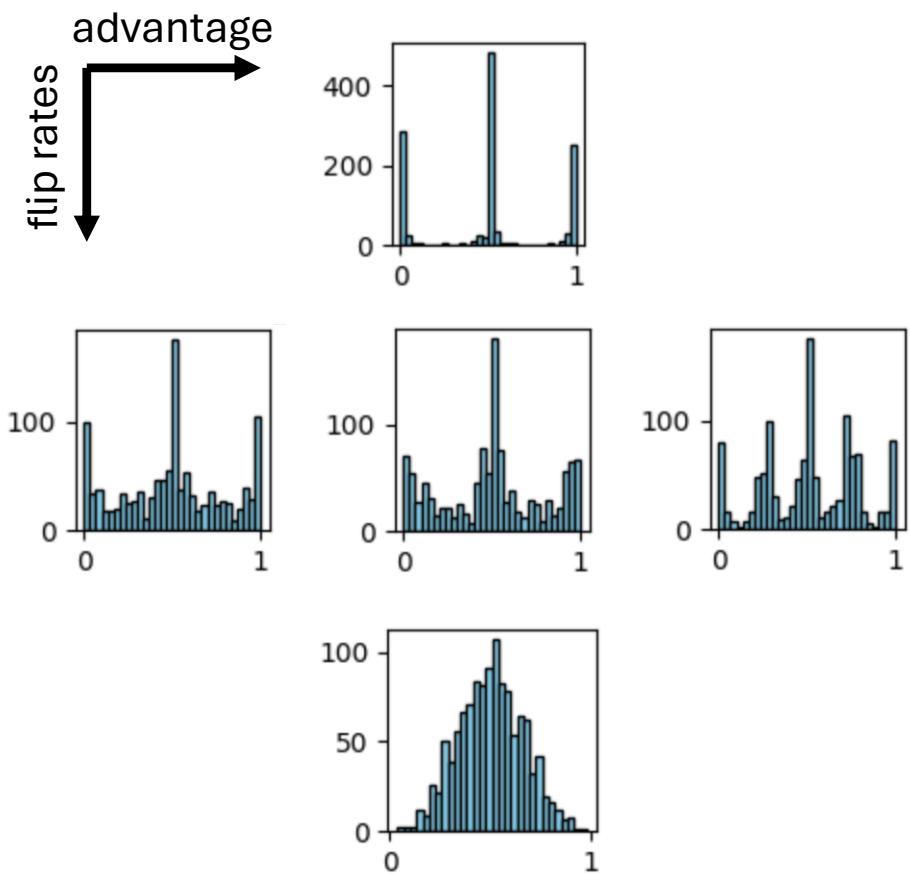


Figure 4.2: Epigenetic mutation rates and strength of selection impact the fCpG distribution within a gland. **x-axis:** selective advantage of driver mutations from neutral to weak ($s = 0.1$) to strong ($s = 0.5$). Strong selection leads to clonal interference and fewer dominant lineages, reflected in the peaks between 0 and 0.5, and 0.5 and 1. Neutral and weak selection have similar signatures in the simulations, with small intermediate peaks emerging occasionally due to the stochastic nature of the model and the probability of neutral fixation. **y-axis:** epimutation rates from slowest (10^{-4}) to medium (10^{-3}) to fastest (10^{-2}). Slower switching shows very little deviation from the progenitor cell's fCpG array, while too fast switching makes the fCpG distribution tend to a Gaussian around 0.5.

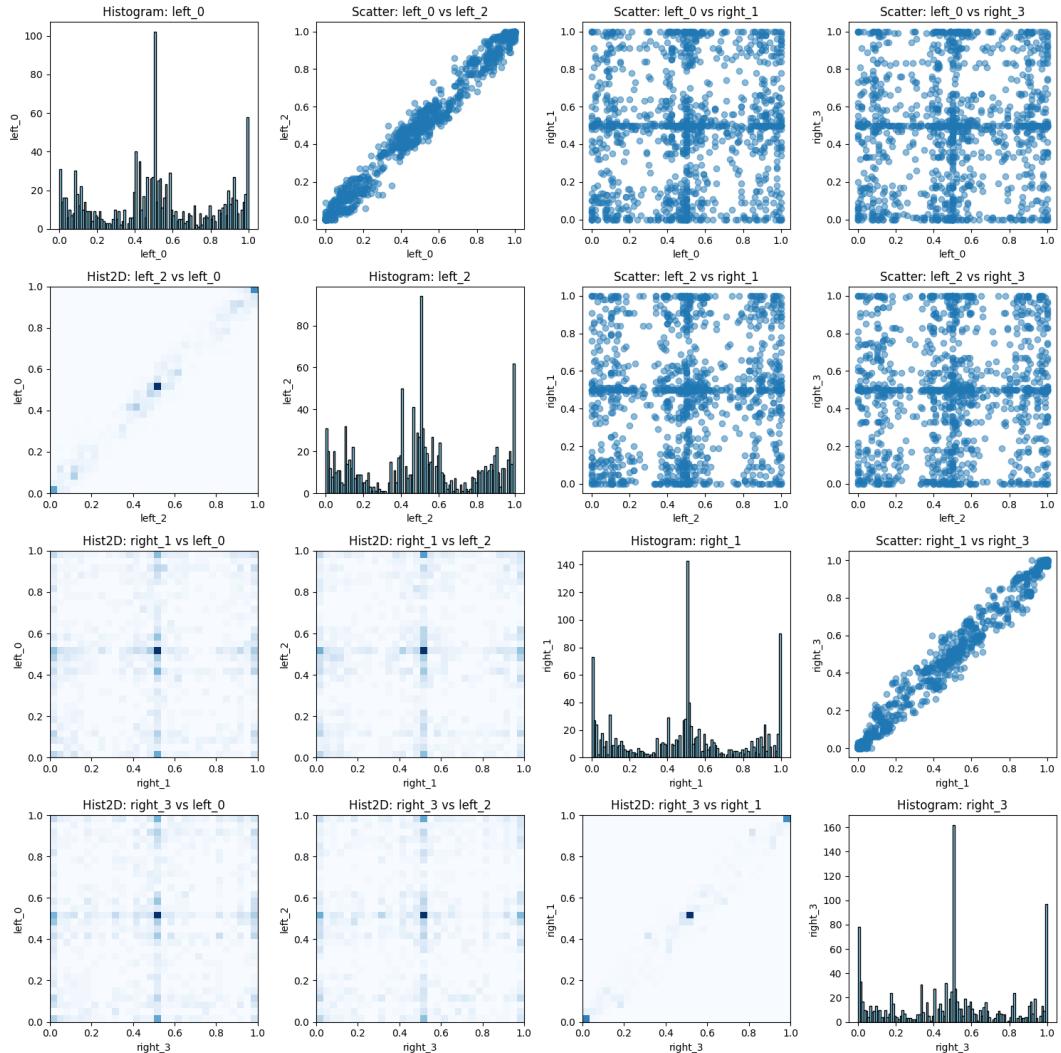


Figure 4.3: Slower fission rates lead to more different fCpG arrays across the sides of the simulated tumour. **diagonal:** Histograms of each gland's fCpG array at the end of the simulation. **above diagonal:** Pairwise scatter plots of the glands' fCpG arrays. **below diagonal:** Pairwise 2D histograms of the scatter plots showing the density of points.

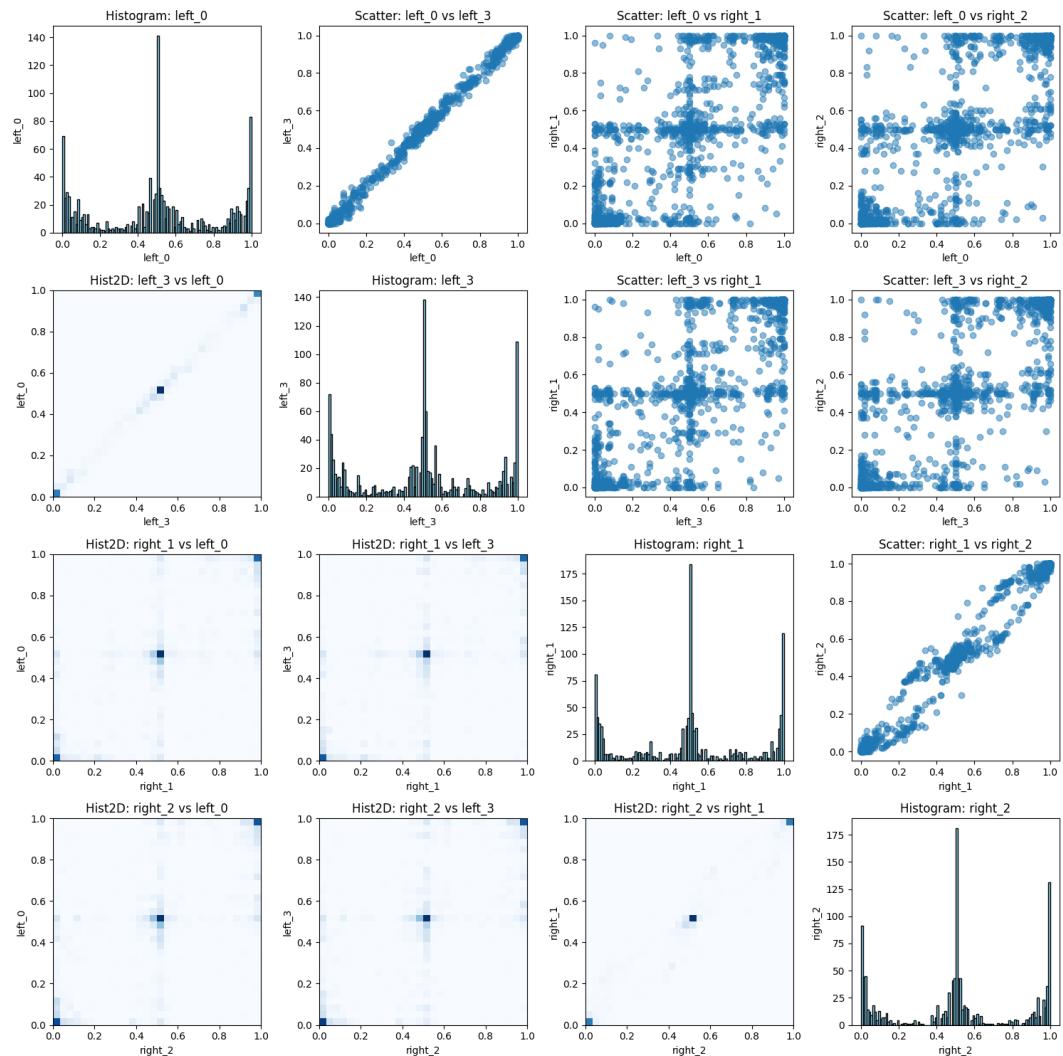


Figure 4.4: Increasing the fission rate leads to more closely related fCpG arrays.

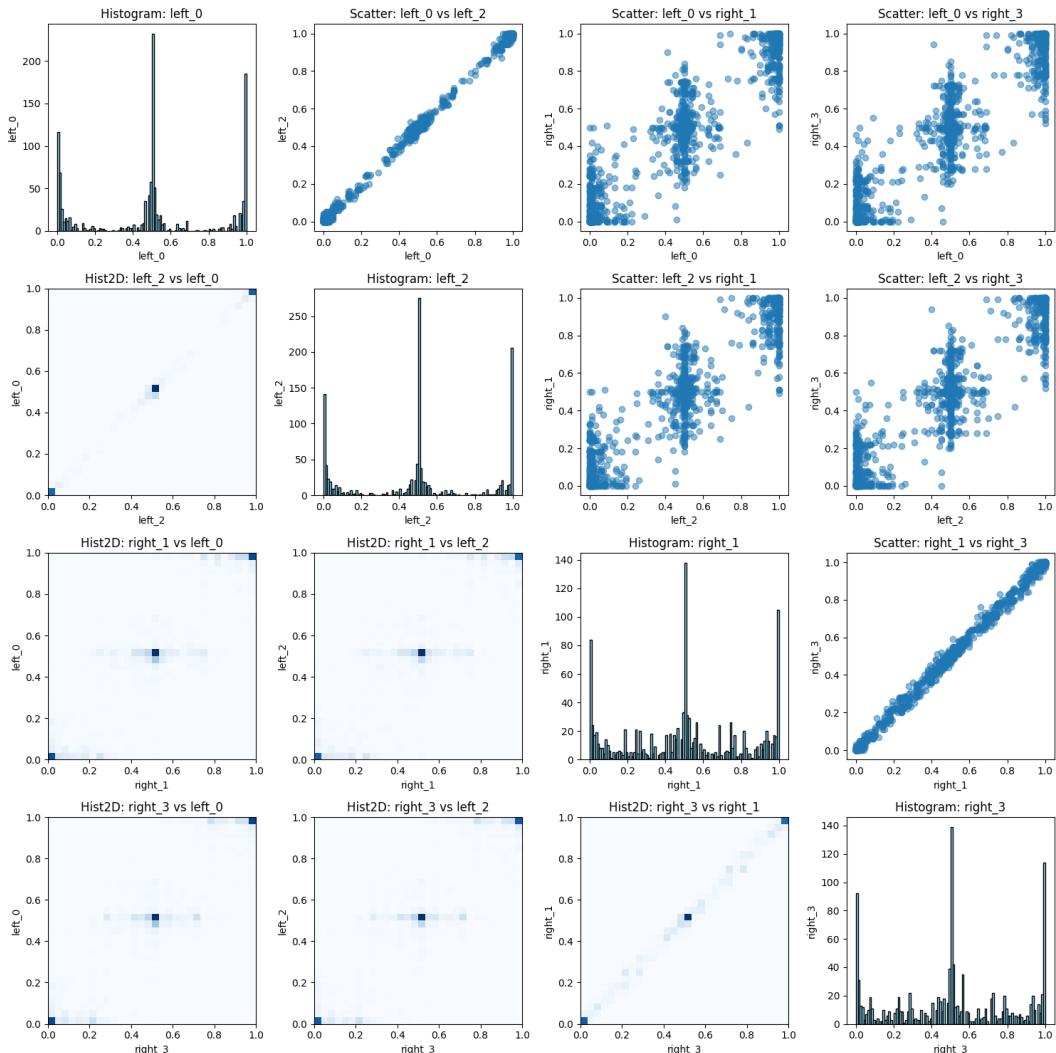


Figure 4.5: Too high a fission rate leads to much less time spent in independent turnover, and thus the most closely related fCpG arrays.

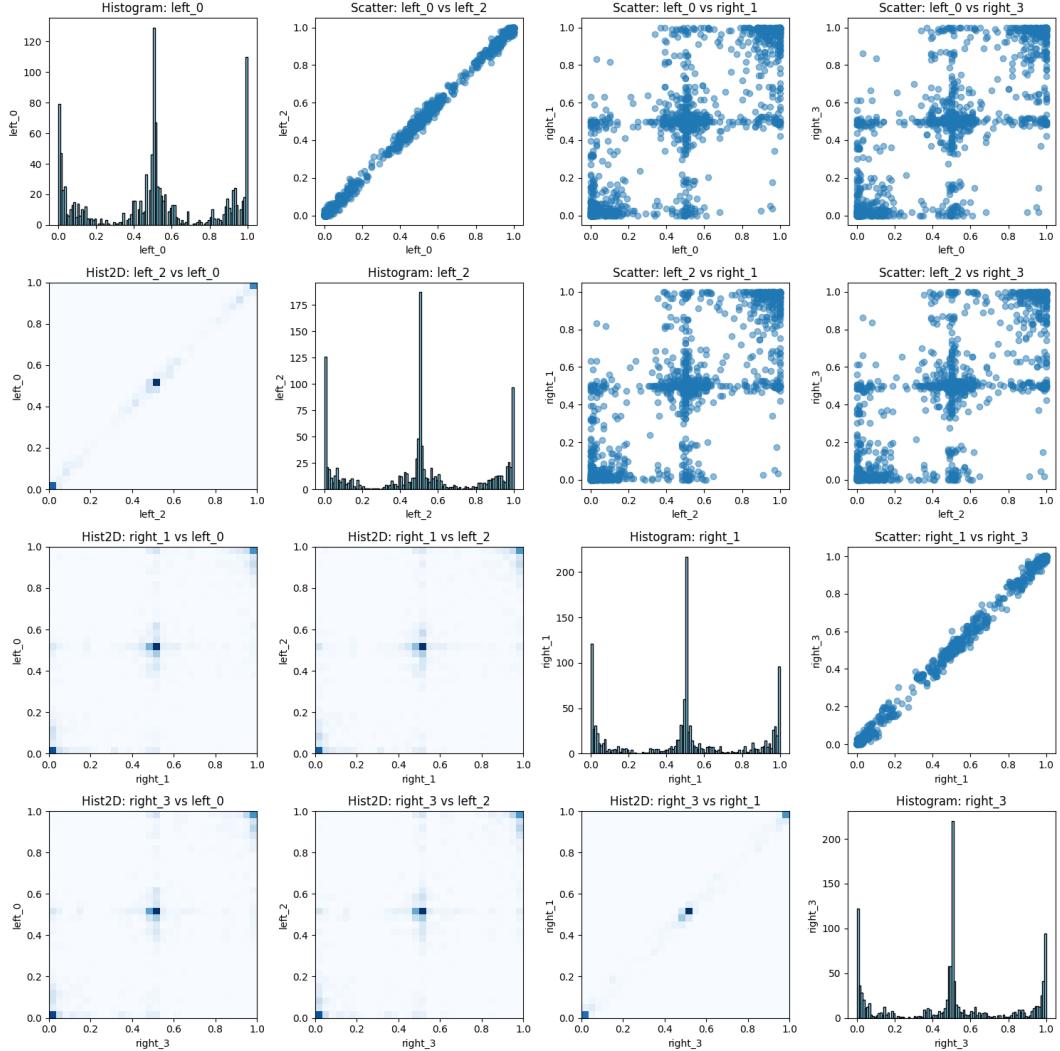


Figure 4.6: High driver mutation rate and strong selection can “compensate” for fast fission rates, leading to slightly more diverse fCpG arrays. While not necessarily a realistic scenario in a real tumour, this example shows how different parts of parameter space can lead to similar results.

4.2.4 Efficiency and memory requirements

Apart from standard C++ libraries, the model makes use of the `boost` library for random number generation and reading in parameters from a config file.

Time complexity

The model's time complexity is dominated by the fCpG array updates, both in individual calls and demes. Each fCpG site has an independent probability of flipping upon division. This means that, if a cell has L fCpG loci, each time a cell division occurs $2L$ random numbers have to be generated. Consider a tumour consisting of N demes, each with a carrying capacity of K , and with a target of F mean fissions per deme. If the fission rate per cell is ϕ , the expected number of cell births before fission is $1/\phi$. The time complexity of the model is then

$$O(F \times N \times K \times L \times \phi^{-1}). \quad (4.3)$$

Memory usage

The bulk of the program's memory usage comes from the tracking of each cell's and deme's fCpG array, with other memory usage being negligible in comparison. A cell's fCpG array is a $1 \times 2L$ vector of integers, where L is the number of fCpG loci per cell. A deme's fCpG array is a $1 \times L$ vector of floats, calculated as the average of the fCpG arrays of all cells in the deme. For a tumour of N demes, each with a carrying capacity of K , the total memory used by the program is approximately $2NKL \times 4\text{bytes} + NL \times 4\text{bytes}$, for a total memory complexity of

$$\theta(NKL). \quad (4.4)$$

Output files

The model writes to at least one csv file, and at most two. The compulsory file contains essential information about the simulated demes and is written at the end of the simulation or every 10 generations. This file consists of the columns:

- Generation - time of writing the row
- Deme - unique identifier of the deme

- `Parent` - unique identifier of the parent deme
- `Population` - number of cells in the deme at time of writing
- `OriginTime` - time of the deme's birth
- `AverageArray` - the average fCpG array of the deme at time of writing

The other file contains information about all cells in the simulation and is written every 10 generations. This file can be quite large, depending on deme size, and is not necessary for the inference workflow described in the next section.

4.3 ABC workflow for inferring methdemon parameters

4.3.1 Overview

For black-box simulations, likelihood-free inference is the most popular method of parameter estimation. Of these, ABC is preferred by most judging by its representation in the literature (Tavare et al. n.d., ?, Sottoriva et al. n.d., Wang et al. n.d., Bondi et al. n.d.). In its most basic form, ABC is a rejection algorithm which draws parameter values from a prior distribution, simulates data using a given model, and compares the simulated data to the observed data. If the distance between the two is less than a given threshold, the parameter values are accepted. This is repeated until a sufficient number of accepted parameter values is obtained. The main issue with this approach is that a completely random search of the parameter space is not efficient, and the number of simulations required to obtain a sufficient number of accepted parameter values blows up as the dimension of the parameter space increases. To address this, the `pyabc` package was developed (Klinger et al. n.d., Schälte et al. n.d.). The package uses a sequential Monte Carlo algorithm to sample the parameter space, with the option of using dynamic sampling, thresholds and particle population sizes for improved efficiency. I decided to use this package for the inference workflow because of its robust implementation and intuitive communication with high-performance infrastructure, such as the City, University of London's cluster, Hyperion.

While ABC is a powerful tool for complex models, it is limited by the way data is compared to simulation outputs. This includes using a summary statistic to reduce the dimensionality of the data and summarise the most important features of the

output. This also minimises the computational cost of the comparison step of the workflow. Further, the choice of summary statistic can introduce a bias, as does the non-zero tolerance threshold. The bias can be reduced with a smaller threshold, but this increases the overall complexity of the workflow as more simulations are required to obtain a sufficient number of accepted parameter values. Additionally, it is difficult to say whether any bias observed during the inference is due to the inference method or the model itself.

4.3.2 Distance functions

In the case of the `methdemon` model, the relevant output data is a set of average fCpG arrays, one for each deme, at the end of the simulation. As the fCpG sites fluctuate independently and stochastically, considering the absolute value of individual fCpG loci is not meaningful. Instead, my focus is on the way arrays differ from each other at the end of growth. There are two parts to this approach.

Inter-gland distance matrix

To reduce the dimensionality of a single tumour's output, I have defined the inter-gland distance matrix as a pairwise distance matrix of the average fCpG arrays of the demes.

Definition 4.3.1. Let a simulated tumour consist of N demes, with the array of deme i given by \mathbf{a}_i . The inter-gland distance matrix is then defined as

$$D_{ij} = \frac{1}{L} \sum_{k=1}^L (\mathbf{a}_i^k - \mathbf{a}_j^k)^2, \quad (4.5)$$

where L is the number of fCpG sites in the array.

The idea behind this distance matrix is to emphasise larger differences between sites, and reduce the impact of small differences. This is done to mitigate the impact of the noise when comparing the arrays. To compare two distance matrices, I use the Frobenius norm of their difference.

Definition 4.3.2. The distance between two inter-gland distance matrices is defined as

$$\delta(D_1, D_2) = \|D_1 - D_2\|_F / \sqrt{2} = \left(\frac{1}{2} \sum_{i=1}^N \sum_{j=1}^N a_{ij}^2 \right)^{\frac{1}{2}}, \quad (4.6)$$

where a_{ij} is the element of the difference matrix $D_1 - D_2$ at the i -th row and j -th column. The factor of $\sqrt{2}$ is included to account for the fact that the matrix is symmetric, and therefore each pairwise distance is counted twice.

Distance of fCpG distributions

While the inter-gland distance matrix is an overall measure of the differences between two tumours, having independent methylation and demethylation rates means that the fCpG distribution in a deme can be skewed in different ways. To make sure that the model is capable of capturing the right relationship between methylation and demethylation rates, I also compare individual demes' fCpG distributions using the Wasserstein distance (or the Kantorovich-Rubinstein metric) (Kantorovich n.d.):

Definition 4.3.3. The Kantorovich-Rubinstein or Wasserstein distance between two probability distributions P and Q is defined as

$$W(P, Q) = \inf_{\gamma \in \Pi(P, Q)} \int_{\mathbb{R}^2} d(x, y) \gamma(x, y) dx dy, \quad (4.7)$$

where $\Pi(P, Q)$ is the set of all joint distributions with marginals P and Q , and $d(x, y)$ is the distance between x and y .

Intuitively, the Wasserstein distance is the minimum cost of transforming one distribution into another.

4.3.3 Example inference

To demonstrate the utility of the `methdemon` model and the ABC workflow, I have performed an example inference using synthetic data generated from the model. The simulated tumour consists of 8 demes, each with a carrying capacity of 100. The ground truth parameter values and the prior and posterior distribution of the parameters are shown in table 4.2. The inference was performed over 3 generations

Parameter	Ground truth	Prior	Posterior
Demethylation rate	0.0018	$U[0, 0.5]$	0.0091 ± 0.0046
Methylation rate	0.0022	$U[0, 0.5]$	0.010 ± 0.005
Fission rate per cell	0.009	$U[0.001, 0.1]$	0.056 ± 0.02
Driver mutation rate	0.0001	$U[0, 0.01]$	0.0044 ± 0.0027
Selective advantage	0.1	$U[0, 0.5]$	0.31 ± 0.12

Table 4.2: Broad priors lead to acceptance of multiple parts of parameter space, resulting in broad posterior distributions.

using 200 particles per generation, with a dynamic tolerance threshold calculated using the `SilkOptimalEpsilon` method in the `pyabc` package. The prior distribution of the parameters was chosen to be uniform and broad. Visualisation of the synthetic data is included in the appendix (figure ??). The results of the inference are shown in figure 4.7.

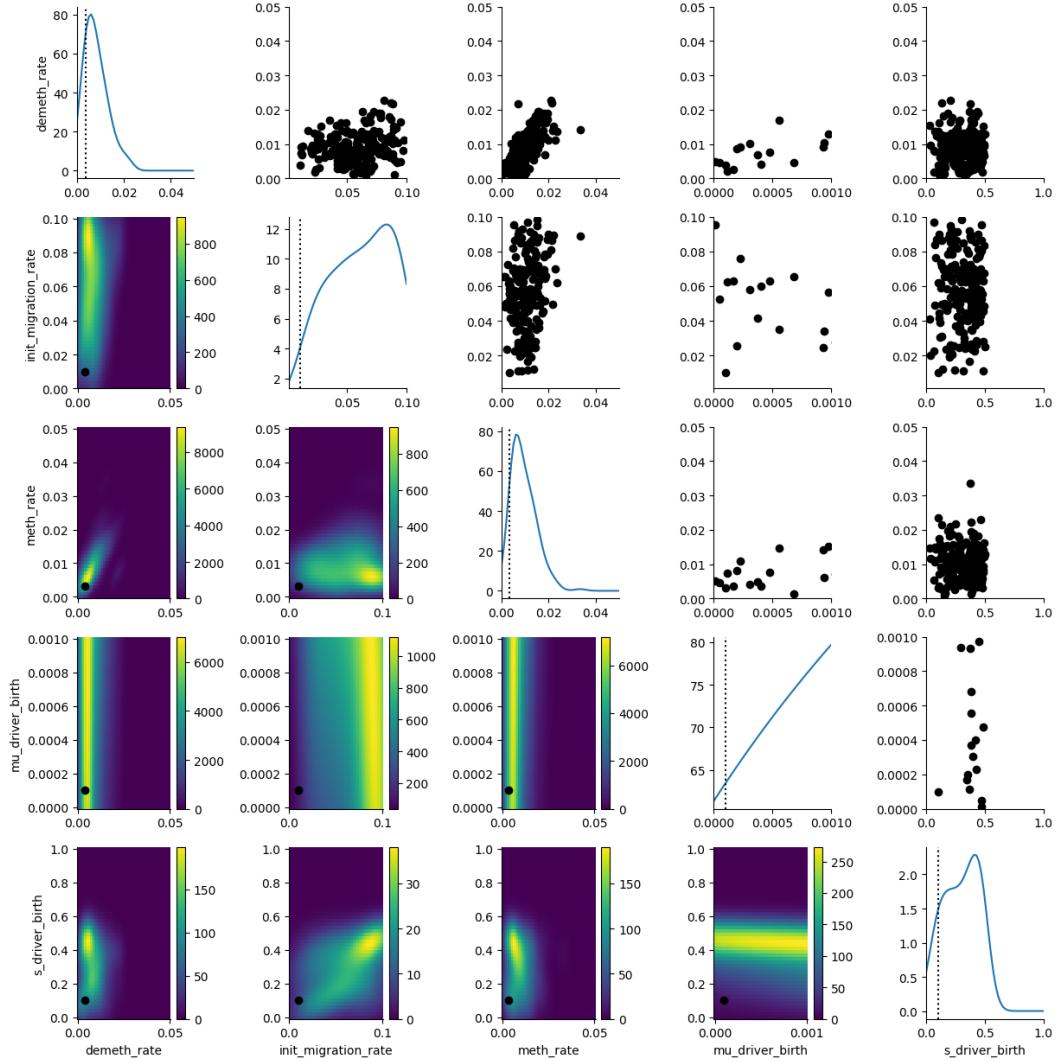


Figure 4.7: Results of the example inference of the `methdemon` model. The ground truth parameter values are shown as dotted vertical lines in the plots on the diagonal.

The results show a few interesting things. Firstly, the posterior distribution of the epimutation rates has narrowed down to a small range, close to the ground truth values. This is likely due to the way in which epimutation rates are expressed in the fCpG array - too fast and the distribution becomes Gaussian, too slow and few changes occur. The Wasserstein distance favours simulations with a similar bias to the observed data, meaning that the ratio of the two is likely to stay preserved. Additionally, the magnitude of the epimutation rates is encoded in the inter-gland distance matrices in a similar way to the individual deme fCpG distributions, with the difference between glands being more pronounced close to the sweet-spot of the rates.

The posterior distributions of the other three parameters are less informative, as they have remained broad. This could be the case for a few reasons. Firstly, the data itself could be uninformative, meaning that the model is not capable of capturing the relationship between the input parameters and the output data. Despite prior testing showing that the outputs are sensitive to the input parameters, this is a complex model and it is possible that the relationship between the parameters and the output data is not straightforward. For example, a slowly growing tumour (lower fission rate) with no to weak selection and a moderate mutation rate could produce similar outputs to a fast-growing tumour with strong selection and a high mutation rate. This is not a problem with the model, but a feature of the tumour's growth dynamics. The priors in this test were chosen to be broad, covering both realistic and unrealistic behaviour of cancer. According to mathematical models, weak selection or effectively neutral dynamics fit the growth of many tumours (Williams et al. n.d.). Secondly, the distance between observed and simulated data could be uninformative. The choice of summary statistics was a natural one, but it is possible that weaker signatures in the data are not captured by the distance metrics. This is a common problem in ABC, and is usually addressed by refining the rejection step, in addition to a more informative prior. Finally, having multiple possible parameter combinations which produce similar outputs could lead to a broad posterior distribution. This is an issue commonly debated in the literature, as selection is difficult to quantify in real tumour data. None the less, the results of the example inference are useful in that they show the ability of ABC to narrow down at least parts of the parameter space even in the case of broad priors in a complex model.

4.4 Discussion

In this chapter, I have presented a new agent-based model, `methdemon`, which is capable of simulating the growth of a tumour and the corresponding fluctuating methylation arrays. The model is developed with efficiency and dynamic memory management in mind, and is based on well-established models of tumour growth and evolution. The model is capable of simulating the growth of a tumour in a reasonable time, and the outputs are sensitive to the input parameters to varying degrees. I have also presented and tested an ABC workflow for inferring the parameters of the model from observed data. The workflow is capable of narrowing down the parameter space, and the results of an example inference show important features which need to be addressed when using the workflow on real data.

Chapter 5

Modelling colorectal cancer methylation data with `methdemon`

5.1 Introduction

A mathematical model is only as good as its ability to describe its system of interest. Therefore, in this chapter, I will test how well the `methdemon` model describes methylation data sampled from colorectal cancer (CRC). CRC is the third most common cancer worldwide, with over 40000 new cases diagnosed in the UK each year on average (?). The disease is characterised by the accumulation of genetic and epigenetic mutations in colonic cells (Fleming et al. n.d.). The most common type of CRC is adenocarcinoma, which arises from the epithelial cells lining the colon, covering the majority of cases. The tumour forms hierarchical cell structures similar to those of normal tissue, organising into crypt-like glands (Ponz de Leon & Di Gregorio n.d.). The tumour spreads by the process of gland fission (Preston et al. n.d.), which is similar to the branching processes seen in normal crypts (Almet et al. n.d.).

5.2 Data collection

The data used in this study were provided by Dr Darryl Shibata from the Keck School of Medicine at the University of Southern California. The data consist of DNA methylation arrays sequenced from multiple glands within colorectal tumours post-surgery. All samples are anonymised. The arrays were obtained from bulk samples of tumour glands, which means that the data are nominally not single-

cell resolved. Each tumour sample consists of 8 glands, with each gland’s array containing some 850000 CpG sites. The arrays were obtained using the Illumina Infinium MethylationEPIC BeadChip array. The data were pre-processed by Dr Shibata to remove low-quality samples and normalise the arrays. The sample purity was high, with the vast majority of cells in the samples being tumour cells.

5.3 Results

5.3.1 Identification of fCpG loci in colorectal cancer

The first step in the analysis was to identify the fCpG loci in the data. As multiple samples come from the same tumour, a larger cohort of samples is needed to reliably identify fCpG loci using the methods described by (Gabbutt, Duran-Ferrer, Grant, Mallo, Nadeu, Househam, Villamor, Krali, Nordlund, Zenz, Campo, Lopez-Guillermo, Fitzgibbon, Barnes, Shibata, Martin-Subero & Graham n.d.), i.e. isolating a set of CpG loci which are the least informative about the methylation state across the cohort. Dr Gabbutt ran the analysis on colorectal cancer data from the Cancer Genome Atlas (TCGA) and identified 1258 fCpG loci. For comparison, I ran a similar analysis only on the data provided by Dr Shibata and identified a set of some 950 loci. Of these, only 120 were common to both sets. The discrepancy is likely due to the small sample size of the data provided by Dr Shibata. The fCpG loci identified in one of the samples are shown in figure 5.1, with additional figures in appendix ??.

A notable feature of the data is that some samples show a clear bias towards either hyper- or hypomethylation. This was also the case for fCpG arrays filtered using only the samples provided by Dr Shibata. This suggests that the progenitor cell’s methylation state is not necessarily random, but could be affected by internal or external factors. Furthermore, I developed the model under the assumption that methylation and demethylation rates are constant and equal across all cells and fCpG loci. While this may be reasonable on average, there might be mutations which affect these rates, leading to the observed distributions of fCpG loci states.

5.3.2 Spatial proximity predicts similarity between fCpG arrays

With the assumed hierarchical structure of the tumour in mind, it should make sense intuitively that glands which are spatially close to each other likely diverged

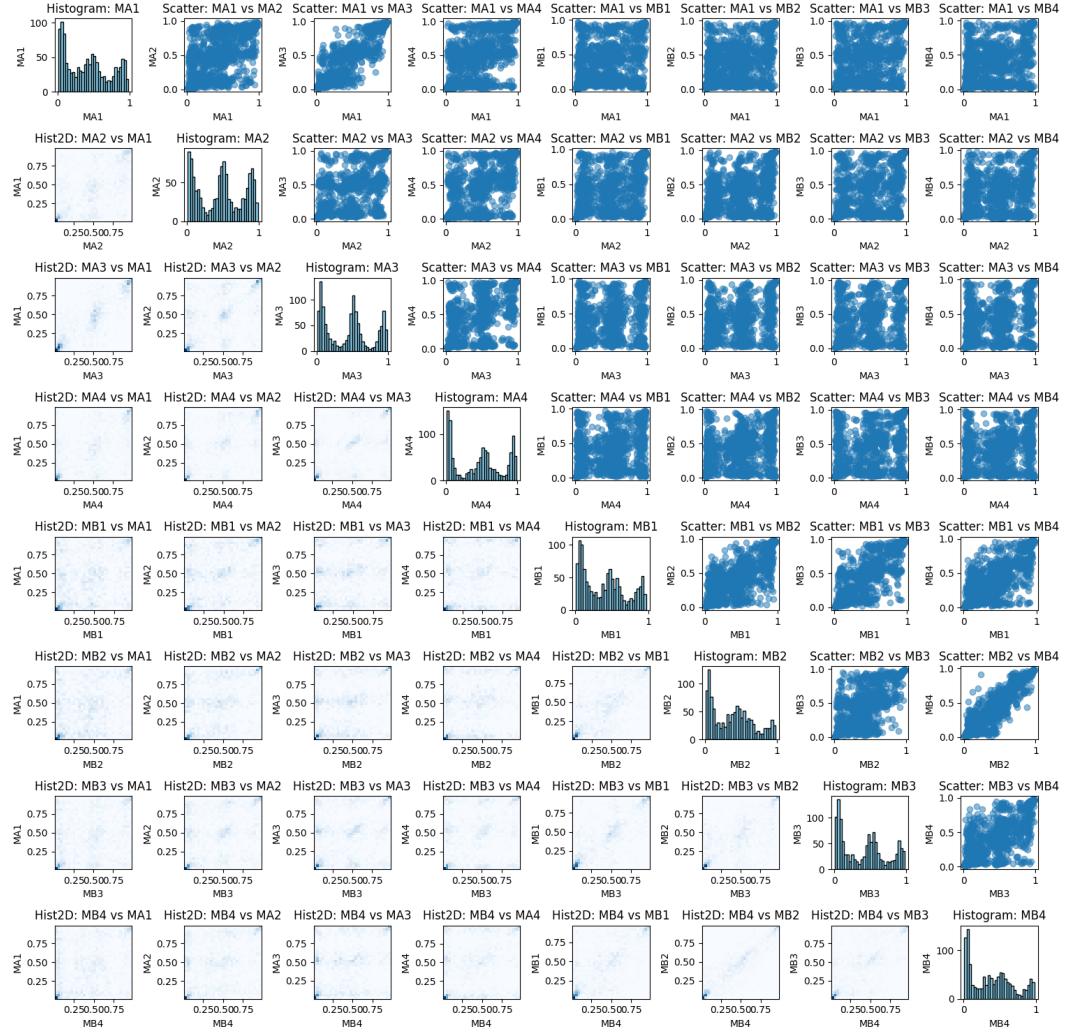


Figure 5.1: Glands from the same side (A, B) of the tumour have more similar fCpG arrays than glands from different sides. **diagonal** — histograms of fCpG arrays for each gland; **above diagonal** — scatter plots of correlations between glands; **below diagonal** — 2D histograms of the above-diagonal plots.

more recently than glands which are further apart. As a result, they have spent less time evolving independently and should have more similar fCpG arrays. To test this hypothesis, I calculated the inter-gland distance matrix for each tumour sample. The resulting matrices show a clear correlation between side and distance values. The distance matrix for one of the samples is shown in figure 5.2 for tumour M, and in appendix ?? for the other samples.

5.3.3 Development of the `methdemon` model

The `methdemon` model was developed for the purpose of simulating the data provided by Dr Shibata. The model's assumptions are based on the general understanding of colorectal cancer evolution and, translated into the language of an agent-based

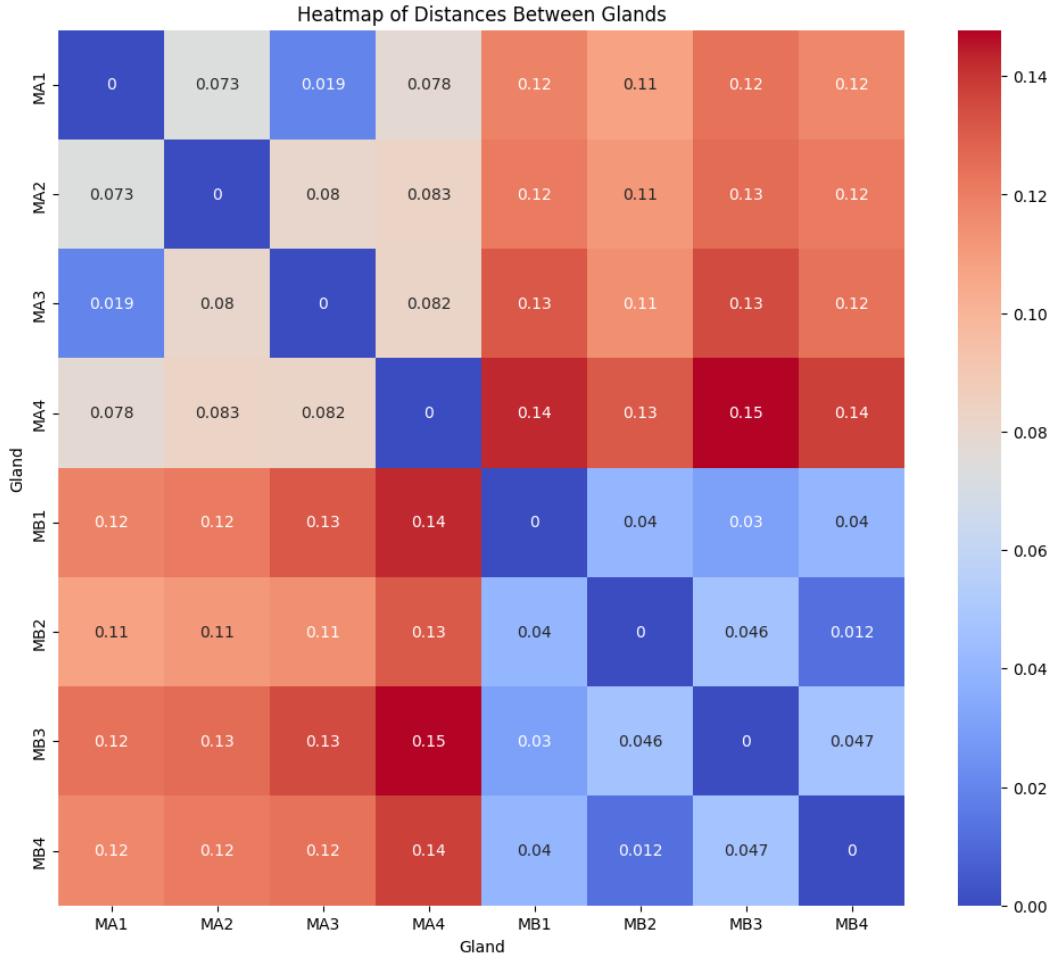


Figure 5.2: The inter-gland distance matrix for tumour M shows that spatial proximity is correlated to similarity between fCpG arrays.

model, are as follows:

- (i) **A single cell forms the first gland and initiates tumour growth.** This assumption skips over the process of tumorigenesis, during which a cell accumulates mutations and becomes malignant (Tariq & Ghias n.d.). This is a simplification to be sure, but a reasonable one, given that the focus of this work is on the evolutionary dynamics of the tumour rather than its initiation.
- (ii) **The rate of driver mutations is Poisson distributed and identical for all cells.** This assumption is consistent with most models of tumour evolution (Metzcar et al. n.d., Niida et al. n.d.).
- (iii) **The cell population within a gland grows exponentially and is well-mixed.** While not necessarily consistent with the biology of a solid tumour, this assumption allows for more efficiency in the simulation as opposed to

a multi-level spatial model. Further, as the data discussed in chapter 5 is obtained from bulk samples of tumour glands, this assumption is not unreasonable.

- (iv) **Once a gland reaches a certain size, which we call the carrying capacity, the population undergoes steady-state turnover according to the Moran process.**
- (v) **At carrying capacity, a gland has a certain probability of undergoing fission, which splits the gland's population randomly into two.** As a consequence of assumption (iii), fissions do not take into account a gland's spatial organisation.
- (vi) **Gland fission occurs as a neutral spatial branching process.** The previous two assumptions and this one together form the basis of the model's spatial dynamics. While there are other mechanisms of colorectal adenocarcinoma progression, gland fission is the principal way in which the tumour grows (Preston et al. n.d.). The assumption of neutrality in the spatial branching process is consistent with the findings of (Sottoriva et al. n.d.). Additionally, this assumption is based on the fact that the data used in this study only contains information about whether a gland was sample from side A or B, without any further spatial information other than the approximate size of the full tumour.

5.3.4 Higher deme carrying capacity requires stronger selection to recapitulate the data

To begin the analysis of cancer data using the `methdemon` model, I tested the ranges of parameters based on the assumption that each cancer cell has infinite proliferative potential. This would mean setting the carrying capacity of a gland to about 10000 cells, which is consistent with the size of the glands in the literature (Sottoriva et al. n.d.) and our data. Due to the glandular structure of the tumour, this is an effectively neutral model, as selection acts within glands but not between them, leading to progressive diversification of the population, as discussed in chapter 3 and (Noble, Burri, Le Sueur, Lemant, Viossat, Kather & Beerenwinkel n.d.).

As a first test, I ran the model with weak selection, $s = 0.1$. The resulting outputs are shown in figures 5.3 and 5.4. There are a few notable features about the output

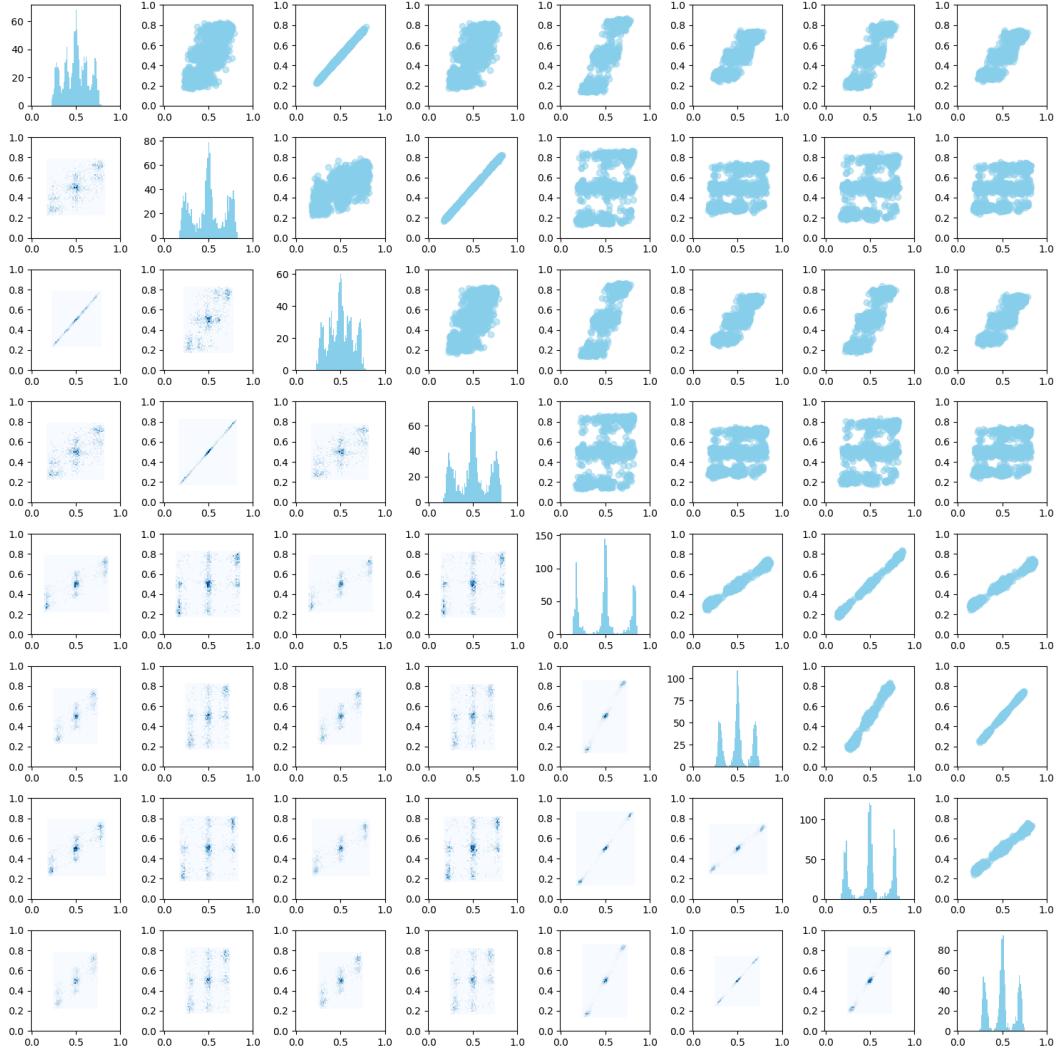


Figure 5.3

fCpG arrays and the distance matrix. The most obvious is that the peaks associated with homozygous methylation and demethylation states have moved towards the middle. This is to be expected, as we are treating all 10000 cells in a deme as being able to divide ad infinitum, leading to a lot of stochastic noise. This is a consequence of the time spent in turnover, and is adjusted by increasing the fission rate. However, the distance matrix shows that the glands are still very similar to each other, especially when compared to the data. This is likely due to the fact that there is only a small probability of a partial or full sweep of a lineage within a gland. The similarity is a consequence of the large deme carrying capacity, which allows for a lot of stochastic noise to accumulate over time but lowers the probability of fixation in the weak selection regime. Increasing the selection coefficient to $s = 0.3$ leads to more divergence between the glands, since emerging lineages are more likely to fully

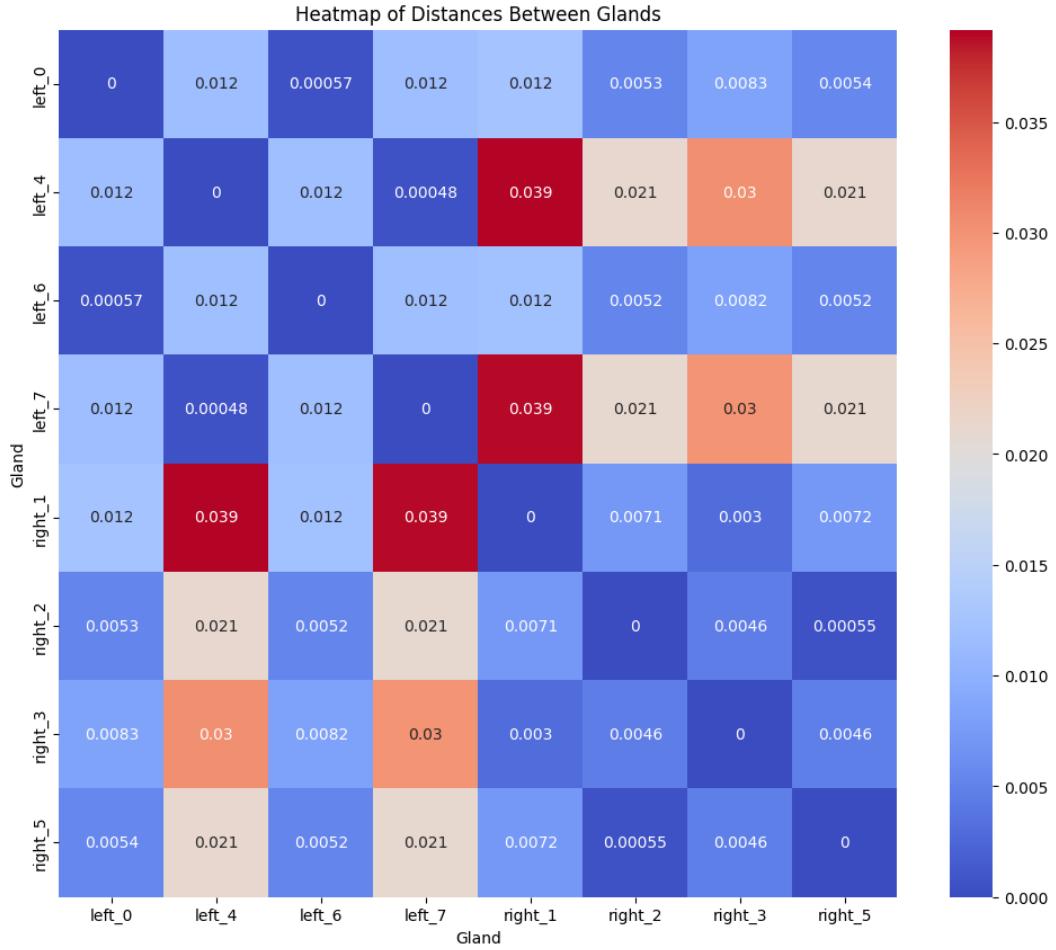


Figure 5.4: Inter-gland distances for the output fCpG arrays from the `methdemon` model with weak selection.

or partially sweep the gland’s population and establish more distinct fCpG arrays between glands. However, as discussed in chapter 4, strong selection can quickly become problematic in an ABM like this due to the accumulation of advantageous drivers. An example of strong selection at deme size 10000 is in appendix ??.

Considering that the number of stem cells in a normal crypt is on the order of 10 (Gehart & Clevers n.d., Gabbett, Schenck, Weisenberger, Kimberley, Berner, Househam, Lakatos, Robertson-Tessi, Martin, Patel, Clark, Latchford, Barnes, Leedham, Anderson, Graham & Shibata n.d.), with the total number of cells in a crypt being on the order of 1000, I next tested the model with a deme carrying capacity of 100 i.e. about 1% of the total cell population in the gland. The currently available data supports this percentage as a reasonable estimate of the proportion of cancer stem cells (CSCs) in colorectal cancer (O’Brien et al. n.d., Munro et al. n.d.). In this case, the model’s outputs are as expected, with the fCpG arrays diverging over time

even with no or weak selection. Examples are shown in figures 5.5 and 5.6.

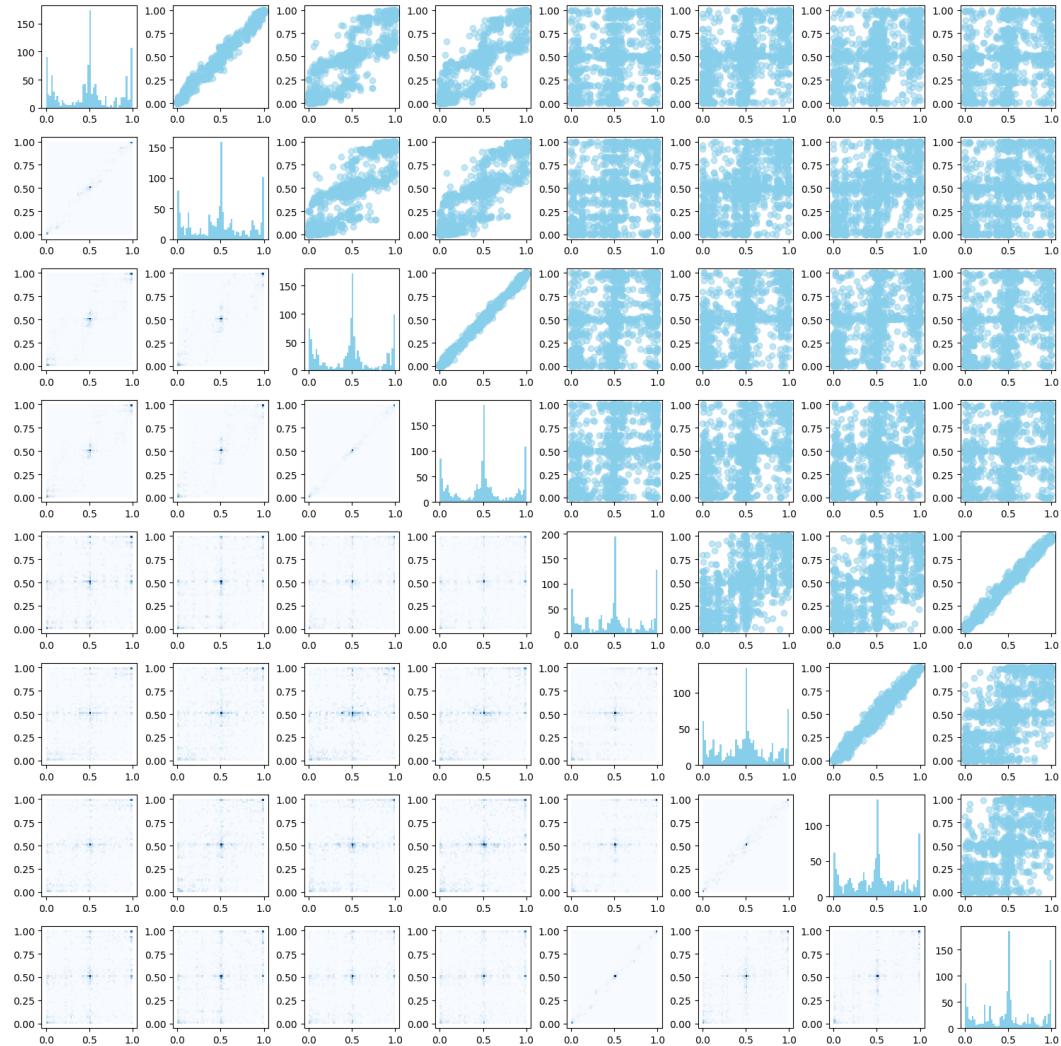


Figure 5.5: Output fCpG arrays from the `methdemon` model with weak selection and deme carrying capacity 100 reflect the data better than larger deme carrying capacity.

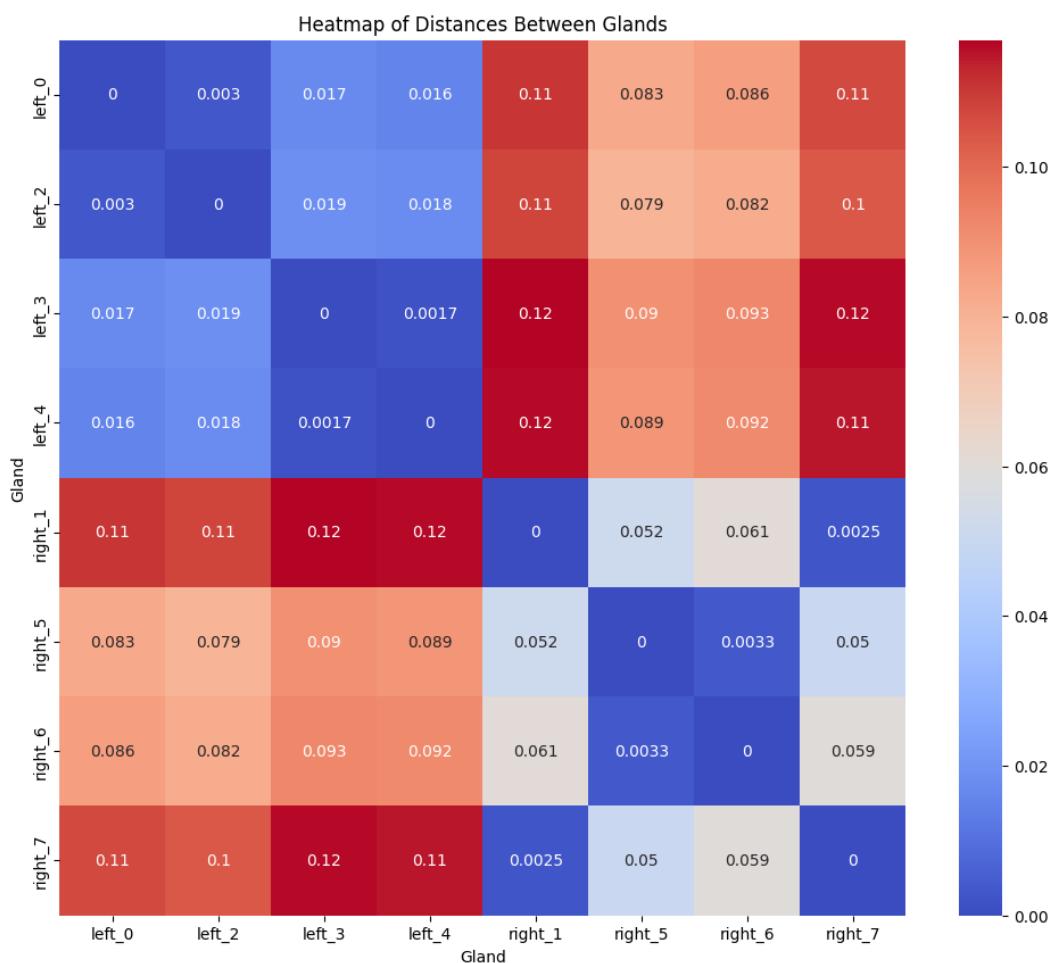


Figure 5.6: Inter-gland distance matrix corresponding to the run from figure 5.5.

5.3.5 Parameter inference from colorectal cancer data

Regular model

Having established `methdemon`'s ability to output data which resembles observations, I next attempted to fit the model to the colorectal cancer data. For this, I used the ABC workflow described in chapter 4. Considering the results of the previous section, I set the deme carrying capacity to 100 for all inference runs. I made this choice as there is no evidence to suggest different numbers of stem cells across tumours or even glands within a tumour. Furthermore, having the deme carrying capacity as a free parameter would slow down the inference process significantly. In the first instance, I ran the inference with parameter ranges given in table 5.1. The results of the inference are shown in figure 5.7, with more details in appendix ??.

Parameter	Prior
methylation rate	$U(0, 0.1)$
demethylation rate	$U(0, 0.1)$
fission rate	$U(10^{-4}, 10^{-2})$
driver mutation rate	$U(0, 10^{-2})$
selective advantage	$U(0, 0.2)$

Table 5.1: Parameter priors for the first inference run.

The first inference run did produce some results, but the posterior distributions of some parameters remained broad. There are a few possible reasons for this. Firstly, I intentionally used overly broad priors to see how well the ABC workflow would delineate the parameter space. This choice makes the inference process more difficult, as the prior distributions are not informative and the parameter space is large. However, it allows for future runs with more informative priors. The second reason may just be that the model is not able to recover all of the parameters in its current form. Selective advantage and driver mutation rate in particular seem to be difficult to infer for the model. This could be due to the signature of selection being too weak in the model or data (or both). It could also be down to the distance functions in the ABC rejection step not being able to capture the differences between the arrays well enough. Either way, the results prompted further testing with the important change of log-transforming the parameters. This change allows for more efficient exploration of the parameter space, as parameters are sampled on the same scale and steps between generations will cover more of the space.

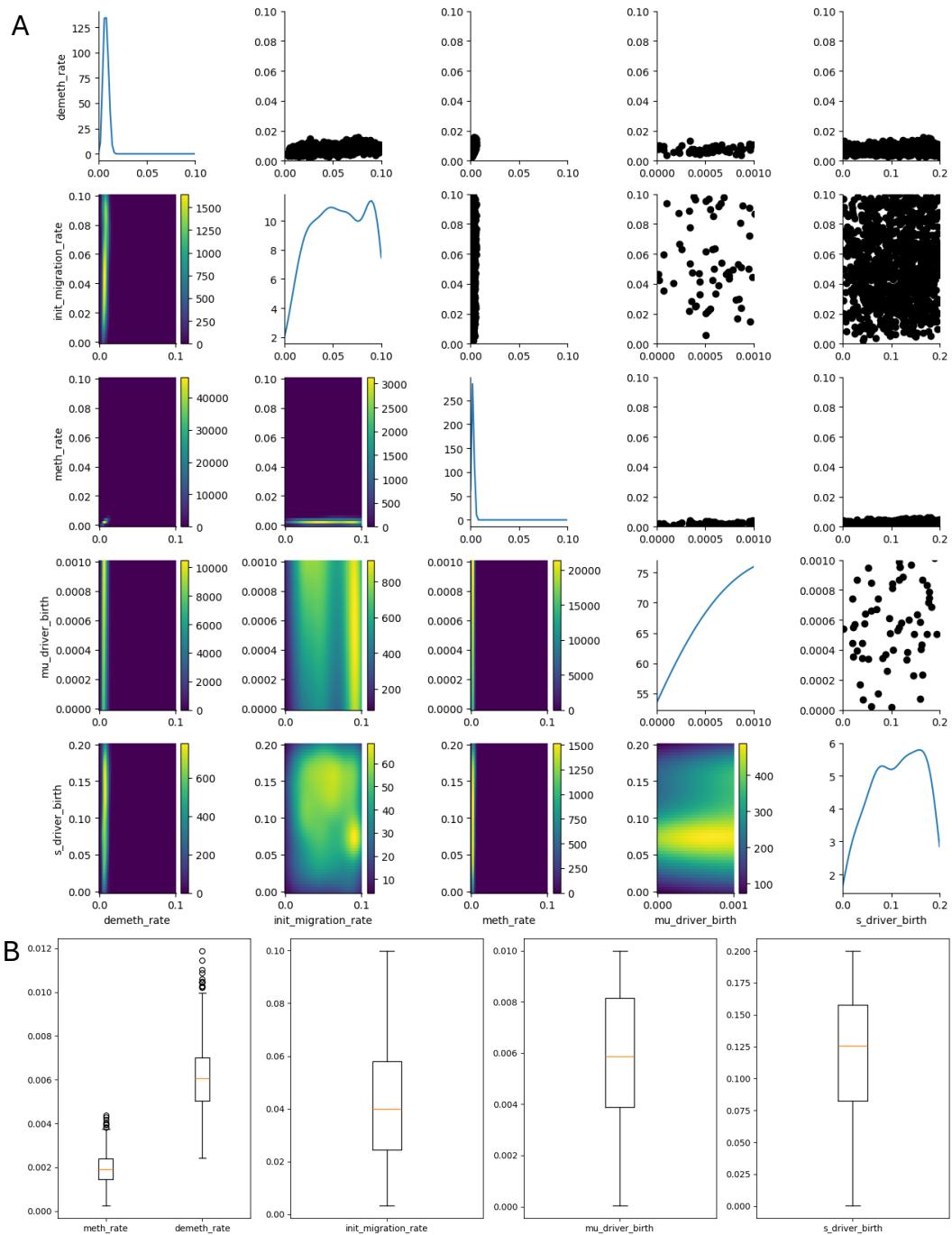


Figure 5.7: Using absolute parameter values in the inference process leads to less efficient inference. **A** — posterior distributions of fCpG fluctuation rates have narrowed down rapidly, but other parameters' posteriors remain broad. **B** — box plots of the posteriors show that the model is not able to resolve the effects of selection from the data, and leaves a lot of uncertainty in the fission rates.

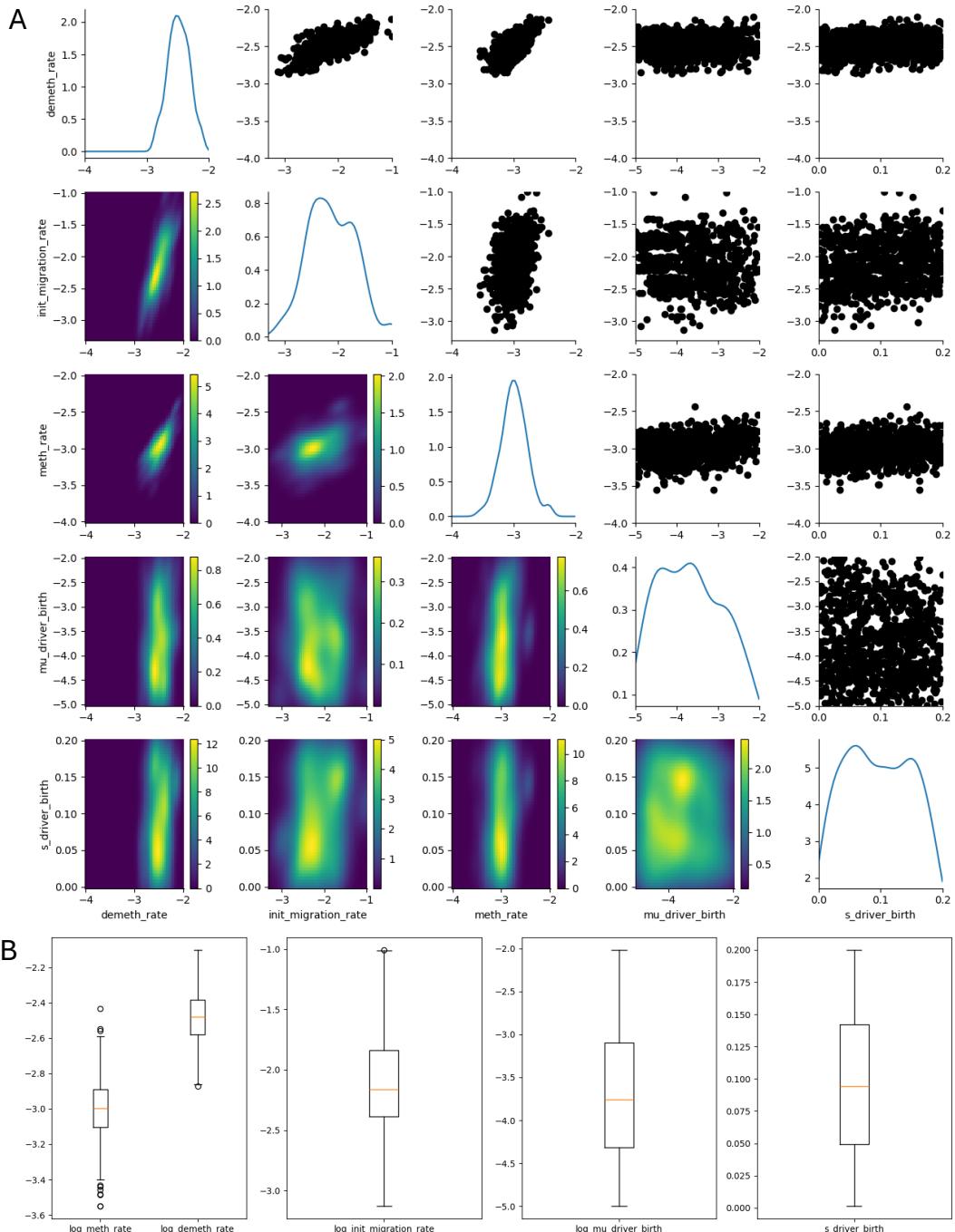
Log-transformed model

The second inference run was done with similar parameter ranges as the first. The log-transformed parameter ranges are give in table 5.2 and the results of the inference are shown in figure 5.8, with more details in appendix ???. All log transformations were done with base 10.

Parameter	Prior
log methylation rate	$U(-4, -2)$
log demethylation rate	$U(-4, -2)$
log fission rate	$U(-3.3, -1)$
log driver mutation rate	$U(-5, -2)$
selective advantage	$U(0, 0.2)$

Table 5.2: Log-transformed priors for the second inference run.

The results of the second inference run are more promising than the first, with the fission rate posterior distribution being considerably narrower. However, selection and driver mutation rate are still difficult to narrow down. This further supports the idea that the model is not able to detect weak selection at the gland level.



5.3.6 Fast- and slow-growing tumours

The data provided by Dr Shibata contains samples from different patients, with each tumour being potentially at a different stage of growth. From exploratory analysis, it seems that some tumours are growing faster than others, if we work under the assumption that gland fission is the only mechanism of growth. This hypothesis is supported by the inference results, as samples with higher values in the inter-gland distance matrix tend to grow slower, and ones where gland fCpG arrays are more similar have grown faster. The inferred median fission rates are shown in table 5.3.

Tumour	Inferred median fission rate [cell ⁻¹ cell div ⁻¹]	L_2 half-norm	Tumour size [cm]	Stage
I	0.017	0.176	3.6	III
J	0.003444	0.421	5	III
S	0.007	0.295	6	n/a

Table 5.3: Inferred median fission rates for different tumours and their sizes. **NOTE:** Still running a few more samples on the cluster, will update the table with more results when they are done.

As the inferred fission rates from table 5.3 are set per cell per cell division, the fission rate per gland would be 100 times higher, or roughly in the interval $(0.1, 1)$ — one order of magnitude less, or about the same as the stem cell division rate. In my model, the tumour grows as a pure birth branching process, meaning that the growth is exponential. Let ϕ be the fission rate and $N(t)$ the number of glands in the tumour at time t . Then

$$N(t) = e^{\phi t} N(0). \quad (5.1)$$

As the tumour grows from a single gland, the time τ to reach a certain size N_τ is

$$\tau = \frac{1}{\phi} \log N_\tau. \quad (5.2)$$

The literature does not provide clear estimates for cancer stem cell division rates, but I think a reasonable estimate would be from about 1 per month to 1 per week, or about 0.034 to 0.1 per day. This would mean that the time to reach a size of 10^7 glands would lie in the interval $(230, 7000)$ days, or about 8 months to 20 years. This range is broad, and is not meant as a precise estimate, but rather a sanity check of the model's outputs. Considering that the orders of magnitude are correct, the model seems to be able to describe the observed data well.

5.4 Discussion

In this chapter, I tested the `methdemon` model on colorectal cancer methylation data using approximate Bayesian computation. The model was able to recapitulate the patterns observed in the data, and the inferred parameters lay within reasonable ranges. However, the model struggled to infer the selection coefficient and the driver mutation rate. This could be due to the signature of selection being too weak in the model or data, or the distance functions in the ABC rejection step not being fine-grained enough to detect the differences between evolutionary modes. Having said that, the results do support an effectively neutral evolutionary mode of colorectal cancer growth, with the effects of selection constrained to within glands. Furthermore, it seems clear that multi-site sequencing of methylation arrays from solid tumours can be used to draw inferences about the evolutionary dynamics of the tumour, and warrants further investigation with a more sophisticated model.

Chapter 6

Discussion

6.1 Summary

6.2 Combining ABM with deterministic models

- ABM allows for study of cell-level dynamics, making it invaluable for examining the early stages of cancer evolution, but is not practical at whole-tumour scale
- cancer, broadly, follows certain deterministic growth laws, but the equations fall apart early on or even when just observing individual patients
- combining the two approaches could allow for a more accurate multi-scale model, such as `methdemon` which leverages coarse-grained approximations globally, and ABM locally

6.3 Hybrid inference approach

ABM is a powerful tool for studying the evolutionary dynamics of cancer, but the stochastic nature of small-scale events can make it difficult to obtain results which are closely aligned with the data, and `methdemon` is no exception. Prior work in fCpG modelling was done using a likelihood-based approach (Gabbatt, Schenck, Weisenberger, Kimberley, Berner, Househam, Lakatos, Robertson-Tessi, Martin, Patel, Clark, Latchford, Barnes, Leedham, Anderson, Graham & Shibata n.d., Gabbatt, Duran-Ferrer, Grant, Mallo, Nadeu, Househam, Villamor, Krali, Nordlund, Zenz, Campo, Lopez-Guillermo, Fitzgibbon, Barnes, Shibata, Martin-Subero & Graham

n.d.), and has shown promising results. Due to the complexity of colorectal cancer, an exclusively likelihood-based approach may not be feasible, but a hybrid model which leverages likelihood-based inference locally for detecting potentially small effects of selection, with ABC on the global scale could be a good compromise. The main hurdle in this approach is reconciling fissions with the steady-state turnover process.

6.4 Gland phylogenies

Another piece of the colon cancer fCpG puzzle is the reconstruction of gland phylogenetic trees. In (Gabbett, Duran-Ferrer, Grant, Mallo, Nadeu, Househam, Villamor, Krali, Nordlund, Zenz, Campo, Lopez-Guillermo, Fitzgibbon, Barnes, Shiba, Martin-Subero & Graham n.d.), the authors used a custom BEAST pipeline, a Bayesian phylogenetic inference tool (Bouckaert et al. n.d.), to infer clone phylogenies from blood cancer data. The main differences between the two data sets include the fact that the blood cancer data is non-spatial, and therefore possible for meaningful sequencing at multiple points in time. In the case of colorectal cancer, it is not possible to obtain multiple samples from the same gland over time. Further, the spatial nature of the data means that sequencing it in the first place may not be possible before the tumour has been removed. This means that the clock rate of the gland phylogenies is unknown. However, sequencing multiple glands does allow for accurate reconstruction of tree topologies, with the clock rate being a nuisance parameter. Having discussed the potential for tree shape indices being used in evolutionary mode inference, the next logical step would be testing whether they point to signs of global selective pressures in the data. Because of the way `methdemon` is written, phylogenies are easily constructed as a byproduct of the simulation, making it easy to test effectively neutral growth as a null model. Resources permitting, it would be interesting to use a larger-scale spatial model to test different ways of gland organisation in space, which could result in different modes of evolution, and thus tree shapes.

6.5 Conclusion

Appendix A

Title of the First Appendix

Two possibilities for the appendices are presented in this template. The Appendix A is included in the main matter of the thesis after the `\appendix` command. This produces that the appendix input in the table of contents is labelled with the corresponding capital letter (in this case 'A'). The text 'Appendix A' will appear on top of the first page of the appendix, above the appendix's title, in case you have given a title to it.

Appendix B

The Appendix B is included in the back matter of the thesis. No `\appendix` command is used. This produces that the appendix input in the table of contents is not labelled, neither with arabic numbers nor capital letters. The only text that will appear in the appendix title is that written in the `\chapter{}` command brackets. In this cases this is 'Appendix B'.

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