EVOLUTION AND EVOLVABILITY IN CHANGING ENVIRONMENTS

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ABSTRACT

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rework abstract

This thesis is dedicated to Kendall Koning.

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Your acknowledgements here acknowledge

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CHAPTER 1

INTRODUCTION - CHANGE, ADAPTATION AND THE EVOLUTION OF EVOLVABILITY

1.1 Evolvability and Evolutionary Potential - Why Study It

The evolutionary potential of a genome is a controversial and nuanced topic, ultimately measurable only in retrospect once the evolutionary success of its descendants is known. Questions relating to evolutionary potential, however, are some of the biggest in Evolutionary Biology: What selective pressures drive organisms to become more evolvable? What aspects of genetic architecture influence evolutionary potential? How do we go about predicting longer-term evolutionary success? And how do features of the environment, such as complexity, change, and periodicity drive and constrain movement across mutational landscapes?

The evolution of sex, multi-cellularity, and other major transitions are characterized by significant changes in genetic architecture that appear to have facilitated the transitions[1]. The adaptive radiations that accompanied Metazoan evolution were also accompanied by changes in genetic architecture that were carried along as species diversified and colonized new ecological niches[2]. The vast diversity of species and their complex ecological interplay depends fundamentally on the ability of populations to not only adapt to their environment, but also create new niches and rapidly explore and exploit their environment as it changes around them. Evolvability has many subtle forms that are produced in different types of changing environment.

Within evolutionary computation, evolvability is also fundamental. The "representation problem", which influences every aspect of evolutionary search, can be characterized as a problem of how to design the underlying genetic encoding such that genomes can not only express complex solutions, but can also be mutated in meaningful ways[3]. In particular,

good designs for genetic representations often involve increasing the probability that a recombination between potential solutions can produce a result that is not only viable, but more fit than either parent. The entire goal of the representation problem is to improve evolvability so that better solutions can be found. By definition, systems that exhibit good characteristics in evolvability produce good solutions more quickly, while avoiding premature convergence[4] so adaptive evolution continues to as high a level as possible. Beyond the representation problem, many of the barriers to complexity are actually barriers to evolvability.

1.2 What is Evolvability (and why is it so hard to pin down)

In its most abstract sense, evolvability appears to be a simple concept: the ability of genetic systems to produce adaptive variation. However, the devil is in the details. How, exactly do genetic systems generate adaptive variation? How do we measure this potential? Should all forms of variation count as evolvability? At what time-scales does evolvability act? And finally, how did it evolve in the first place? That is, are evolvable features under some form of direct selection, or are they by-products of other processes?

Evolvability, in its details, must mean different things at different evolutionary scopes and timescales. Depending on your perspective, evolvability can describe the response to selection at the population level[5, 6], the ability of populations to adapt to changing conditions[7], larger phenomena such as variability generation[8], exploration of neutral spaces and robustness[9, 10], generation of novel features[11, 12], or even the potential to generate the larger clade-level innovations[2] and major transitions[1]. Beyond that, there is a lot of confusion and controversy about the definitions and components of evolvability even within any one of these scopes[13].

Finally, it is unclear whether evolvability is acted upon by direct selection, or whether it is a byproduct of other traits that are selected upon, or some combination of the two. At the individual level, its possible that some traits that support evolvability, such as robustness of

developmental or cell processes[2] could have been selected for directly in response to adverse environmental conditions. However, at the population level, traits like neutral variation generation are more likely to have hitchhiked on the genomes of the adaptive variants that they produced. Finally, at the clade level, genetic structures that produced populations of adaptive variants with robust and flexible genetic architectures would have been more successful at adaptive radiations[3], and thus go on to found whole branches of life with those traits[2].

Of course, we must be careful when invoking selection at higher levels than the individual. While there is some evidence to support clade-level selection in the evolution of evolvability [14], caution should be applied when attributing evolutionary outcomes to higher levels of selection when random chance or lower levels of selection are adequately explanatory. Specifically, we need to be careful to avoid falling into the trap of adaptationism[15] by assuming that evolvability is an end in itself. Selection can only act on organisms and populations as they exist, and it is an error to assume that patterns identified in hind-sight are predictive of future evolution.

1.3 Changing Environments and Evolvability

Sustained directional selection adjusts the composition of phenotypes and genotypes in a population [16], typically moving that population across the mutational landscape to local regions of higher fitness. When populations find a fitness peak, they tend to cluster there, and exploration of that region of the landscape slows dramatically.

revise this

In changing environments, however, the direction of selection is not fixed and peaks are not stable. Instead, as the environment changes, populations are driven to explore new regions of the mutational landscape [17, 18]. As they proceed, populations accumulate and carry with the genetic material acquired in prior explorations and adaptations, and use this history as raw material for new adaptation [19]. Indeed, earlier work has shown that changing environments promote evolvability in many contexts, without compromising

robustness [20, 21]. Strength of selection is also an important component of this exploration, since the harshness of the environment drives the speed with which organisms adapt to new conditions [22].

MORE HERE about other things CE can or can't do

1.4 Historical Conceptions of Evolvability

Evolvability is described at many different scopes and levels in the literature, each with varying amounts of detail and predictive power. As such, it may be best to avoid attempting to unify the concept, and rather acknowledge that evolvability is not a singular idea, but rather an overlapping and interrelated set of concepts relating to adaptation and evolutionary potential. In order to synthesize the large field of evolvability and understand how the distinct scopes and ideas connect together, a historical narrative is clearly useful.

1.4.1 Modern Synthesis

The evolution of evolvability as a formalized theory originated with Dawkins[3] and Albrech[11], though the underlying concept (as the response to selection, measured by heritability) existed much earlier, in the work of Fisher[5] and Wright[16]. Fishers fundamental theorem of the response of a population to selection identified narrow-sense heritability (h^2) as a measure for how evolvable populations were. Evolvability as heritability (h^2) is a measure of the portion of the phenotypic variation in a population that can be accounted for by additive genetic effects. h^2 therefore is the component that directly relates to a populations response to selection[6].

$$h^2 = \frac{Var_A}{Var_P} \tag{1.1}$$

In contrast to narrow-sense heritability (h^2), broad-sense heritability (H^2) refers to the entire genetic contribution to a populations variance, including dominance and epistasis. Because of these other contributors, it is unsuitable for isolating the response to selection.

As a measure of evolvability, narrow-sense heritability (h^2) was also used as a term in the breeders equation, in order to estimate the response of a population to artificial selection.

$$R = h^2 S \tag{1.2}$$

Heritability, however, is not an ideal predictor for the response to selection because it fails to integrate factors such as the population distribution of variability in a trait[6]. Heritability, being scaled by total population variation in a trait, would predict the same response to selection regardless of whether the standard deviation of variance of that trait was large or small, or where the mean of that trait lay.

Houle advocated for an alternative genetic variability measure that suffered from fewer of these problems: the Additive Genetic Coefficient of Genetic Variation (CV_A).

$$CV_A = 100\sqrt{\frac{V_A}{\bar{X}}} \tag{1.3}$$

Using CV_A as the measure of genetic variability is superior to narrow-sense heritability because it scales additive genetic variance by the trait mean, rather than by total population variation. Thus, the additive variation component isnt overwhelmed by large population trait variance[23]. Since life-history (fitness-related) traits tend to have large population variances, h^2 predicts that life-history traits have low heritability and thus low response to selection[24]. CV_A, however, being scaled by trait mean, predicts much higher response to selection for life-history traits[25, 6].

CV_A still suffers from significant drawbacks as predictors for adaptation and evolvability in a larger sense [25]. Both h^2 and CV_A measures predict the response to selection based on the expressed trait variation in a population, under the current environmental conditions. They say nothing of the potential for cryptic variation that may be revealed in different genetic background, nor do they address differences in genetic architecture that may promote faster adaptation. Ultimately, CV_A is best when examining the short-term response to

selection in artificially-selected populations, in static environments, with low mutational load[6].

Clearly, such short-term, population-based measures are unsuitable for measuring larger patterns of the evolution of evolvability, especially over the long term.

1.4.2 Evolvability as a Distinct Concept

Dawkins, in his foundational paper on evolvability and evolutionary constraint [3], re-framed the problem of evolvability in the context of computational evolution and development. Dawkins described a generative genetic system based on a few alleles, and rules that governed development based on the traits encoded in the alleles. Each allele would govern the execution of a generative rule, and the rules would interact with each other as they produced the phenotype. As he added new kinds of rules (constraints) into the generative process, he showed that the system produced more and more complexity.

Dawkins used this example to draw parallels to biological generative developmental systems and how evolutionary constraints in development allow for more complex and robust phenotypes. Dawkins identified a few key themes that underlay the more powerful features of developmental systems. These systems would be organized in such a way as to facilitate cumulative effects. That is, innovations in constraints can build upon each other and are cumulative in evolutionarily interesting ways[3].

Dawkins hypothesized that these kinds of generative developmental systems, or embryologies were the basis for evolvability, and that they must have evolved as a result of their intrinsic power to produce adaptive variation. Dawkins further suggested that the genetic systems that persisted were those that facilitated adaptive radiations into new or otherwise empty ecological niches.

Alberch followed up Dawkins ideas with a more thorough accounting of how, exactly, these kinds of evolvable traits translate into an analyzable phenotype space[11]. Alberch dismantled the concept of a simplistic, hierarchical genotype-to-phenotype mapping function

and emphasized that developmental and cell metabolic systems are strongly dynamical, nonlinear systems, for which genes are just one part of the regulatory cycle. Because of the dynamic nature of cell processes, it was clear that the gene-centric, population genetics view was inadequate to fully describe the complexity of the processes involved, and how they translated complex parameters into phenotypes. To that end, a new framework for analysis was required.

Alberch introduced the concept of parameter spaces to describe the variation in genotypic parameters that results in distinct phenotypes, while addressing the lack of one-to-one correlation between alleles (parameters) and phenotype. Parameter spaces are multidimensional spaces, divided by parameter thresholds (bifurcation boundaries) that form borders between phenotypes. The domains bounded by these thresholds include all of the parameter combinations that produce a given phenotype. Larger domains can be described as more stable than smaller domains, because there are larger ranges of neutral variation available before organisms tip into a different phenotype. Populations with distinct phenotypes and varying parameters can thus be visualized as blobs occupying areas in parameter space.

Alberch contended that the evolvability potential of a dynamical system is encapsulated by the properties of the parameter space. Specifically, the topology of the bifurcation boundaries govern the ease with which the systems can produce both neutral and adaptive variation. Alberch asserted that the generative systems must have undergone selection that favors those systems that provide a good balance between exploration and stability, but provided no mechanism for that selection.

Dawkins and Alberch laid out a compelling case for the role generative developmental systems in facilitating evolvability, but their theoretical frameworks were far from complete.

1.4.3 Theoretical Frameworks for the Evolution of Evolvability

The Wagner and Altenberg paper on the evolution of evolvability significantly expanded the theoretical framework behind the evolution of the genotype-phenotype map[8]. The authors

draw on knowledge from computational evolution to inform their perspective on evolvability, since the problem of evolvability is central to the representation problem in evolutionary computer science.

Initially, Wagner and Altenberg emphasized a distinction between variation and variability. *Variation* is the realized diversity in a population, which is a concept that lies firmly within population genetics and the gene-centric modern synthesis. *Variability*, on the other hand, is a concept that they introduced to describe the ability to generate new phenotypes in response to mutation or environmental change. Variability is a metric associated with a local neighborhood in a genotype to phenotype map, and depends on features of that map, including pleiotropy and modularity, and robustness and flexibility of biological processes.

Wagner and Altenbergs paper led to a vast proliferation of new work exploring the evolution of evolvability. Of particular note is the Kirschner and Gerhart 1998 paper[2], which explored metazoan evolution for examples of traits that, in combination, acted to increase evolvability. The authors found numerous examples of new, evolvable features coinciding with adaptive radiations. The authors also develop a case for a combination of direct selection upon the individual for evolvability-enhancing features, and those traits persisting as by-products as a result of adaptive radiations, setting the stage for the evolution of more and more complex evolvable features.

1.5 So, what do I mean by Evolvability?

As I described above, evolvability is a series of distinct, but overlapping concepts that are generally concerned with adaptation, variation, and/or novelty generation. For the purposes of my research, I am using the Wagner/Altenberg conception of evolvability, which focuses on variability (i.e., the generation of adaptive variation in response to mutation). Variability depends primarily on the organization and interrelation of the components of the genome; that is, the genetic architecture, and the resulting genotype-to-phenotype map.

The major features that influence this metric for evolvability appear to be modularity

remove "proposal", revise this

of functional components and phenotypic robustness to mutation and environmental perturbation. While there are other architectural features that are also likely to contribute to evolvability, they will not be the focus of this proposal.

1.5.1 What is Modularity?

Modularity is the degree to which traits are both self-contained and decoupled from each other. Modular organization can appear at different scales, from the reduction of overlap between unrelated gene regions (spatial modularity[26]), to the decoupling the mutational effects on distinct traits (functional modularity[8]), to the composition of groups of related trait complexes (variational modularity [27, 28]).

Features such as evolvability and robustness are thought to rely heavily on modularity[8]. For example, traits with high functional modularity will have low pleiotropy and therefore should be able to evolve independently—a critical feature if individual traits need to quickly respond to changes in selection. Additionally, modular traits may be more easily re-purposed or co-opted by other traits to add new function[28]. Conversely, spatially modular genomic regions, because they are more self-contained, tend to better resist disruption from recombination, thus increasing robustness[26].

The relationship between modularity and pleiotropy is complex. At small scales, spatial modularity acts to directly reduce pleiotropy by reducing the number of traits affected by a single locus[26]. However, at higher scales, modularity may rely on pleiotropic links within groups of related trait complexes to enable those groups to evolve and optimize in concert[27].

Despite the benefits described above, modularity, like many other variational trait complexes, may not be an unmitigated boon for evolvability. High levels of functional modularity may reduce the overall evolvability of a genotype by reducing the incidence of mutations of large effect and reducing the size of mutational targets[29]. Reducing the incidence of large changes reduces the likelyhood of the development of entirely new traits as a result of relatively few mutations. Thus, the evolvability benefit of modularity may be mediated by the

scale and degree to which it occurs.

1.5.1.1 Measuring Modularity

At the phenotypic level, modularity is assessed based on the functional independence of traits and trait complexes. Spatial modularity is correlated with functional modularity, though it is possible to have spatially modular genomes that are not functionally modular and vice-versa[30, 31].

For the purposes of this research, I will focus on spatial modularity. Spatial modularity may be measured by calculating the proportion of traits that are affected by a given site in the genome, normalized by the number of sites that code for a trait [26]. Trivially, this can be measured by performing knock-out experiments to identify the sites that contribute to particular function.

To measure Spatial Modularity, m_S:

1. count the total number of traits expressed in a genome: T
2. identify the number of sites that code for any trait: set K
3. count the number of items in set K: k
4. count the number of traits coded for by each site within set K: t_k;
5. calculate the inverse of the average number of traits coded for per site to reflect the level of spatial modularity (m_S) of coding regions of a genome

$$m_S = \frac{1}{\frac{1}{k}\sum_{i=1}^{k} \frac{t_k}{T}} \tag{1.4}$$

1.5.2 What is Robustness?

Much like evolvability, robustness is a set of overlapping concepts concerned with the ability of a genotype to maintain a given phenotype despite an unexpected disruption[10, 32]. Most

commonly, robustness is studied in regard to either perturbations in the environment or else mutational disruptions. In the first case, the evolution of robustness to environmental disturbances depends heavily on the flexibility and decoupling of gene regulatory or signaling pathways[2]. For example, a gene-regulatory or signaling pathway that is loosely coupled may make use of signaling from multiple incoming paths, rather than depending on a single, rigid precursor. This type of arrangement is more likely to continue to function even if some part of the signaling path is disrupted. An example of this kind of robust arrangement is nerve conduction in vertebrates where axons connect several cells, thus routing signals in parallel, and avoiding single points of failure[2].

For the purposes of my research, I will focus on the second case: mutational robustness. Distinct from robustness to environmental perturbation, robustness against mutation depends largely on degeneracy, redundancy, and regulatory decoupling[10]. Degeneracy refers to a many-to-one relationship between an encoding and a product, such that several codes can produce a single output. Thus, there is a chance that mutations in the code will not alter the product. One example of this feature is codon degeneracy in biological organisms, where, depending on the hydropathy of the amino-acid, single, or even double mutations in some positions of the encoding do not affect the binding of the encoded amino-acid[33].

Similarly, redundancy refers to the duplication of function in multiple places in the genome, such that mutations altering function in one copy of a gene do not alter function in the other copy. Redundancy may also refer to redundancy of function within genes, such that if a mutation occurs in one portion of a gene, other neighboring portions of the protein will compensate, and the protein will retain its structure and function.[34]

Finally, regulatory decoupling allows for more than one kind regulatory precursor to provide inputs for a process[34]. Thus, if mutation were to damage one set of precursors, others can take their place and preserve function. An example of this kind of architecture is in the production of the acetate precursor for the Krebs cycle, which produces ATP in all aerobic organisms[35]. Acetate can be derived from either carbohydrates, lipids, or proteins,

thus if any of those pathways are damaged by mutation, acetate can still be produced from other sources, and ATP production can continue.

It is worth noting that many of the architectural features that confer robustness to processes and genomes are based on arrangements of modular structures[36, 37]. In this way, much of robustness is facilitated by the evolution of modularity.

1.5.2.1 Measuring Robustness to Mutation

Robustness to mutation can be assessed in multiple ways, either from the perspective of a specific phenotype, a specific genotype, or combinations of the two. From the perspective of an individual genotype, you can assess its robustness by calculating the proportion of mutations that produce a phenotype that is different from the one expressed by the target genotype[38]. In most cases it is easiest to focus on single-step mutations (to cover the 1-neighborhood in the fitness landscape), but sampling from the full distribution of mutation combinations that occur naturally will produce a more exact results.

To measure Genotypic Robustness, r_G, of a genotype G:

1. count the number of loci in the genome: n
2. count the number of possible alleles at a given site: D
3. enumerate all possible single-step mutants that may arise from the given genotype (or sample from a more realistic distribution): $n(D-1)$;
4. count those mutants that prove to be neutral phenotypic variants: R_G;
5. calculate the proportion of neutral phenotypic variants to reflect the probability of a neutral variant being produced by this genotype in response to mutation.

$$r_G = \frac{R_G}{n(D-1)} \tag{1.5}$$

Genotypic robustness is trivially negatively correlated with genotypic evolvability, because each neutral variant in the 1-neighborhood of a genotype is, by definition, not of a different phenotype. However, the inverse is not necessarily the case, because each non-neutral neighbor phenotype may not be unique. Therefore, a non-robust genotype may not necessarily have high evolvability if its neighborhood is dominated by a single or few distinct phenotypes [38].

From the perspective of the phenotype, robustness may be assessed by to taking the average genotypic robustness across the phenotype.

To measure Phenotypic robustness, r_P:

1. count the number of distinct neutral genetic variants that produce a given phenotypic trait in a population, $(SetK : k)$;
2. calculate the proportion of neutral variants produced by single-step mutations r_G, averaged over all of the neutral genetic variants to reflect the probability of a neutral genotype currently in the population producing another neutral genotype in response to mutation.

$$r_P = \frac{1}{k} \sum_{i=1}^{k} r_G \tag{1.6}$$

Unlike genotypic robustness, higher phenotypic robustness has been shown to correlate with phenotypic evolvability in cases where the possible number of neutral variants in a phenotype (the frequency of the phenotype) is high[38]. With increasing numbers of neutral variants, the number of potential unique phenotypes in the 1-neighborhood of the phenotype increases.

These measures of robustness are each limited in that they do not address realized population composition, the shape of the mutational landscape, nor the expected frequency of the target phenotype. In particular, the correlation of phenotypic robustness with evolvability depends on the expected phenotypic frequency [38]. Thus, if the frequency is unknown, phenotypic robustness may not predict evolvability.

Further, different populations may have vastly different numbers of realized neutral variants for a given phenotype. Factors such as gene-flow, bottle-necking, linkage dis-equilibrium, founder effects, and sexual selection may strongly affect overall diversity in populations, including the neutral diversity for a particular phenotypic trait that we are concerned with[11].

For this reason, while population level metrics may cause a phenotype to appear to be non-robust, this apparent value may be the result of the amount and type of realized diversity present in a given population, rather than the robustness of that phenotype as predicted by its potential neutral network[11].

1.5.3 Predicting Short-Term Evolvability with Landscape Metrics

As indicated above, the features that confer robustness may also promote evolvability by allowing for greater neutral genetic diversity within a given phenotype. The larger the number of distinct genotypes with the same phenotype in a connected region of the fitness landscape, the more exploration of the genotype space that can be done without decreasing organismal fitness. As a population diffuses through such a neutral region, more potential phenotypes become available in few mutational steps[9].

Historically, predicting this robust-yet-evolvable quality has been challenging. Previously-used measures for robustness that focus on counting the proportion of unique genotypes that compose a phenotype (Phenotypic Robustness [38]) are limited in their ability to predict the evolvability of a population, especially where phenotypic frequency is unknown.

In contrast, we will use Genomic Diffusion Rate, which is the probability that an offspring will be different from its parent, while expressing a neutral or positive fitness effect. This metric may be used to characterize overall population evolvability as it approximates the overall rate in which entirely new genotypes are encountered[39].

To calculate the **Genomic Diffusion Rate** (D_g) in the local neighborhood of a genotype, first calculate its *Fidelity* (F), or the probability of an offspring sharing this genotype with its parent, by measuring the probability that a single locus is not mutated ($1-\mu$) and raising

it to the power of the genome length (l). Next, measure the proportion of 1-step mutants that are neutral or beneficial when compared to the parent (p_ν) as well as those that are detrimental or lethal (p_d), which must sum to one ($p_\nu + p_d = 1$). The *Neutral Fidelity* (F_ν) of a genotype is thus the probability that no harmful mutations occur, assuming no epistasis. Finally, subtracting Fidelity from Neutral Fidelity will yield the overall probability of producing an neutral offspring with a different genotype, yet neutral or better fitness (D_g).

$$F = (1 - \mu)^l \tag{1.7}$$

$$F_\nu = (1 - \mu p_d)^l \tag{1.8}$$

$$D_g = F_\nu - F \tag{1.9}$$

Measures of neutral exploration, however, only show part of the picture. While some form of neutrality is necessary for exploring a fitness landscape, new phenotypes must be discovered to achieve higher local evolvability. In order to assess evolvability more specifically, we introduce a related measure, the **Phenotypic Diffusion Rate** (D_p), which represents the probability that an offspring will be fitness-neutral, but also express a different phenotype than its parent. To do so, we must first measure the proportion of one-step mutants that are *phenotypically* neutral as compared to their parent ($p_{p\nu}$) and follow a similar procedure as above, first calculating the probability that a phenotype-changing mutation will occur (μ_{pheno}), then the phenotypic-level fidelity ($F_{p\nu}$).

$$\mu_{pheno} = \mu(1 - p_{p\nu}) \tag{1.10}$$

$$F_{p\nu} = (1 - \mu_{pheno})^l \tag{1.11}$$

$$D_p = F_\nu - F_{p\nu} \tag{1.12}$$

The difference between the overall neutral fidelity and the phenotype-preserving neutral fidelity ($F_\nu - F_{p\nu}$) yields the phenotypic diffusion rate.

1.5.4 Expected Value of Fitness Landscapes

In the context of changing environments, the expected fitness value ($E(w)$), and thus the neutrality, of a mutant in the mutational landscape will vary depending on the environmental context. So, in one environment, a mutant may be highly fit, but the same allele may be highly deleterious in a different environment. In order to address this variation, all metrics must be normalized by the probability that a particular environment will occur (P_i). That is, the nearby mutational landscape must be evaluated in each possible environment, yielding a traditional fitness landscape. Then, the set of fitnesses of each mutant (w_i) in each environment must be aggregated according to the probability of that environment occurring.

$$E(w) = \sum_{i=1}^{e} w_i P_i \tag{1.13}$$

1.6 Digital Evolution

Digital Evolution uses self-replicating computer programs as model organisms to study evolutionary dynamics [40]. Unlike theoretical simulations, digital organisms have a fully functional genome that direct them to self-replicate, mutate, and compete with their peers for resources and space in which to reproduce. Because digital organisms undergo genetic mutations (i.e., variation) that are passed on to their offspring (inheritance), and their survival is based on the actions they take (differential selection), they undergo evolution by natural selection.

Digital organisms do not suffer from many of the drawbacks of experimentation on natural organisms. Three of the advantages of digital organisms are particularly relevant for our study. First, the rates of reproduction in digital systems are much faster than in even the most rapidly-reproducing physical organisms; we can process generations of organisms in seconds, rather than the hours required for the fastest biological organisms under sustained conditions [41, 42], or the weeks to years needed for more complex multicellular organisms [43, 44].

Second, using digital organisms allows us to tightly control and verify experimental conditions. For example, in physical organisms, factors such as mutation rate can generally be measured only after the fact, or coarsely altered through mutagens. In digital organisms, however, we can not only control mutation rates with fine-grained precision, but also types and probabilities of different types mutations (e.g., substitutions vs. insertions vs. deletions). Furthermore, we are also able to track and replay the evolutionary history of every organism at any point in time to verify that unusual or unexpected results do not represent measurement error. This ability to exactly replicate evolutionary results at an individual organism level is firmly out of reach for experiments with physical organisms.

Finally, we can precisely and perfectly map the mutational landscape around the genome of a digital organism, and identify the role of every site in its genome[39]; such exhaustive techniques are not feasible in even the simplest physical organisms. All of these factors make digital organisms ideal for studying the effects of changing environments on the mutational landscape.

1.6.1 Avida

Throughout the rest of this thesis, I use the Avida digital evolution platform to explore the effects of changing environments on the evolvability of populations of digital organisms. Avida is a software platform for performing evolution experiments with digital organisms in a virtual world.

what goals? (see following sentence)

An Avida organism is composed of a circular genome of assembly-like computer instructions that are executed in a virtual CPU (Fig 1.1). Populations of these organisms are placed in a toroidal world in individual cells where they are allowed to execute, reproduce, compete for space, mutate, and evolve.

Organisms in Avida are self-replicating, and experience mutation. The genomes of the initial default organisms contain all of the instructions necessary for reproduction. However, the instructions are not copied into an offspring with perfect fidelity. By default, the

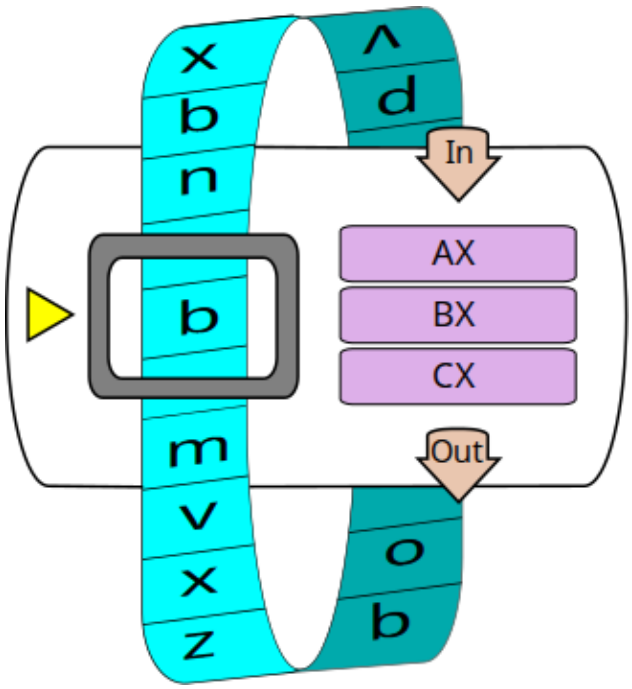

Figure 1.1: **An example virtual CPU from Avida**, with a circular genome (blue), three registers (purple), input and output handlers (tan), and an instruction pointer (yellow) indicating the next instruction to be executed.

reproductive copy instruction is faulty, meaning that it will probabilistically introduce errors (mutations) into the offspring genomes. These offspring organisms execute their own genomes even when different from their parent, and in turn pass on their inherited mutations, along with new mutations, to their own offspring (i.e., variation in the systems is heritable).

Avida worlds can be space- or resource-constrained. Avida allows the experimenter to configure many aspects of the environment, thus subjecting the organisms to various kinds of selective pressures. In many cases, these environments will include resources that can be metabolized by performing specific functions or activities, resulting in a boost to execution speed that gives the organisms a competitive advantage. However, even without explicit external pressures, organisms still experience an implicit pressure to execute more quickly and efficiently. The organisms that run fastest are typically able to also reproduce fastest, and thus out-compete their peers for space.

Avida is available for download without cost from `http://avida.devosoft.org/`,

and specific versions along with data-files to reproduce the experiments described in this thesis may be found at https://github.com/voidptr/avida and https://github.com/voidptr/dissertation.

CHAPTER 2

CHANGING ENVIRONMENTS PROMOTE RAPID ADAPTATION IN DIGITAL ORGANISMS

2.1 Background

The interaction between an environment and possible genomes can be mathematically expressed by a fitness landscape. Fitness landscapes are a mathematical tool to map genetic sequences to reproductive fitness. Many studies have examined the important role that different types of fitness landscapes play on evolutionary dynamics and outcomes, both in biological populations [45, 46, 47, 48] and in evolutionary computation settings [49, 50, 51]. However, real-world fitness landscapes are far more complex and varied than the limited or idealized models that are used in most of these studies. Neighboring regions of real landscapes can have starkly different properties from each other based on the effects of and interactions among mutations; as such, a local region of a fitness landscape around a genotype is commonly referred to as its mutational landscape.

Examples of the type of properties that we are interested in include robustness, epistasis, and modularity, all of which are measurements of how information is organized inside of a genome and commonly categorized as components of an organism's "genetic architecture". Isolated pockets in a landscape can often be characteristically different from the landscape as a whole due to the amount and organization of genetic information. In fact, in most natural fitness landscapes, the vast majority of neighborhoods consist entirely of non-replicating genomes with zero fitness (and thus no genetic information), making life itself appear to be a rare exception [52].

Evolution on these convoluted landscapes is clearly limited to those regions that have non-zero fitness, with a selective pressure for fitness to increase. Beyond that, however, populations can evolve toward neighborhoods with specific local properties based on the

evolutionary forces acting upon the populations. For example, high mutation rates drive populations toward neighborhoods with a higher fraction of neutral mutations in an effect dubbed survival of the flattest [21]. Similarly, sexual populations tend toward regions of the fitness landscape with more modularity [26] and more negative epistasis [53] than otherwise equivalent asexual populations.

Understanding these dynamics is of broad interest. It is important to evolutionary computation, given the strong influence of local landscape properties on the quality of the final solutions that an evolving population is able to obtain. Its relevance to evolutionary biology is equally obvious – the local landscape that a population occupies will influence the selective forces at play in the population, creating a feedback cycle between these two important evolutionary factors [54, 55]. Disentangling such interactions is likely to provide further insights into fundamental evolutionary dynamics. Computational artificial life systems have the advantage of being able to bridge these two realms: they have unconstrained evolutionary dynamics similar to natural systems, while maintaining the ability to rapidly perform experiments and collect any data we need about populations or their local landscapes.

2.1.1 Evolvability and Genetic Architecture

As described in Chapter 1, evolvability refers to a series of distinct but overlapping concepts that are generally concerned with adaptation, variation, and/or novelty generation [13]. Depending on your perspective, evolvability can describe the response to selection at the population level[5, 6], the ability of populations to adapt to changing conditions[7], larger phenomena such as variability generation[8], exploration of neutral spaces and robustness[9, 10], generation of novel features[11, 12], or even the potential to generate clade-level innovations[2] and major transitions[1]. For the purposes this chapter, we will focus on evolvability as the capability of genomes to generate adaptive variation in response to mutation.

In the short-term, this kind of evolvability determines a population's response to selection. This kind of evolvability depends primarily on the organization and interrelation of

information in the genome; that is, the genetic architecture, and the resulting genotype-to-phenotype map [8]. An example of evolvable architecture can be found in some bacterial genomes that contain highly mutable genome regions, called contingency loci. Small sets of insertions or deletions to these regions create transcription frameshifts that alter the expression of nearby coding regions, thus allowing populations to easily switch phenotypes via minor mutations. Contingency loci are most often seen in the genomes of pathogens, which are subject to frequent environmental shifts caused by the host immune system [56]. Thus, these populations are able to produce large amounts of heritable variation despite the reduction in population diversity resulting from population bottlenecks.

2.1.1.1 Mutational Landscapes

Properties of genetic architectures such as evolvability and robustness are determined by the shape of the resulting mutational landscape (local fitness landscape around a genotype, accessible in a single mutation) [38]. Robust genetic architectures that can tolerate more mutations without altering their phenotype reside in mutational landscapes that connect to more neutral mutants. Similarly, architectures that more easily switch phenotypes in response to mutation without substantial reduction in fitness, reside in more evolvable regions of genotype-space.

It is worth noting that not all regions of the mutational landscape are equally accessible. Some genome regions may be more resistant to mutation than others [57], thereby altering the probabilities of mutations occurring that lead into certain regions of the mutational landscape. This kind of differential probability may therefore moderate a population's diffusion through the mutational landscape.

clarify

Further, response to selection is likely to be weaker in regions of the landscape where there are fewer available mutations that provide potentially adaptive traits, whereas response to selection will be stronger in regions where there are many adaptive variants available within a few mutational steps [11, 58]. This differential response to selection therefore constrains

the ability of populations to diffuse across a fitness landscape.

In order to assess the potential of different regions of the fitness landscape to promote or hinder evolvability, we will use both the **Genomic Diffusion Rate** (D_g)1.9 and the **Phenotypic Diffusion Rate** (D_p)1.12, as normalized across changing environments1.13.

2.2 Methods

2.2.1 Experimental Design

In order to examine the dynamics and mechanisms of evolving populations in changing environments, we performed two sets of experiments. We subjected populations of evolving digital organisms to a set of cyclic changing environments, and a set stochastic changing environments. The cyclic environments were designed to simulate predictable cycles of change, such as day/night or seasonal cycles, whereas the stochastic environments represent less predictable oscillations in environmental states, such as random weather patterns, or climactic changes. These experiments allow organisms to adapt to a predictable set of environments, and explores short-term evolvability dynamics. See Table 2.1

A diagram of how the environments are placed. stochastic as jumbled, cyclic as periodic. Harsh is red/blue, Benign is white/blue.

2.2.1.1 Cyclic and Stochastic Changing Environments

For the cyclic environment, we subjected a total of 150 replicate populations of digital organisms to two different treatments of two-phase cyclically changing environments, plus a static control. The environment cycles between 500 updates of reward, and 500 updates of punishment; as such each full cycle is 1000 updates, or roughly 30 generations. We chose this cycle length after surveying a series of possible values in order to determine an optimal

Table 2.1: **Experimental Treatments**

Treatment	**Changing Environment**	**Rewarded Tasks**	
		XOR	EQU
Control	None (static)	constant 2^3	constant 2^5
CCE Benign	Cyclic	constant 2^3	benign fluctuating 0 or 2^5
CCE Harsh	Cyclic	constant 2^3	harsh fluctuating -2^5 or 2^5
SCE Benign	Stochastic	constant 2^3	benign fluctuating 0 or 2^5
SCE Harsh	Stochastic	constant 2^3	harsh fluctuating -2^5 or 2^5

Experimental treatments. Four types of changing environment, plus a static control. In the first two treatments, the environment switches in a predictable cycle, whereas in the second two, the environment switches at random intervals.

length of time. That is, long enough to allow adaptation to occur and spread through the population, but short enough to reduce the effects of drift destroying vestigial genetic information. For more details about this survey, please refer to Appendix A.

In the static control, there is no cycle. Rather, the rewards remain constant. The first phase of the experiment extends for 200 cycles, or 200,000 updates, approximately 6,000 generations.

The stochastic changing environment experiment is similar to the cyclic environment, except that rather than the environment toggling every 500 updates, the environmental switch happens randomly, with a 0.002 probability of changing on every update. This averages, in the long term, to approximately one switch every 500 updates, but in the short term, the environmental switches are unpredictable.

We set up the system to detect organisms that performed XOR or EQU, two challenging

bitwise logical tasks. In the static control, XOR is rewarded with a CPU speed (and thus fitness) multiple of 8, while EQU is rewarded with a CPU speed multiple of 32. In the harsh treatment, as the cycle progresses, the XOR reward remains constant, while the EQU reward cycles between a 32-fold bonus and a correspondingly harsh 32-fold penalty (i.e., CPU speed is divided by 32 when EQU is performed in the off cycle). The benign treatment is nearly identical to the harsh treatment, except that the reward merely goes away in the off-cycle as opposed to incurring a severe penalty.

In both environments, we identify EQU as the *Fluctuating Task*. XOR, because it is rewarded continuously, is the *Backbone Task*, and is used as a background for comparing the separation or intertwining of functional genetic components in the evolution of EQU. Further, the 4-fold difference in reward level between XOR and EQU encourages the evolution and maintenance of EQU when possible.

For all of the experiments described in this section, we held the individual genomes at a fixed length of 121[1] instructions, but tested the new genomes for mutations after each successful replication event at a substitution probability of 0.00075 per site. We configured the Avida world to have local interactions on a toroidal grid that is 60-by-60 cells (3600 cells in total), and we seeded the initial populations with an ancestor that was previously evolved to perform XOR and EQU under a static reward. The genetic architecture for performing XOR and EQU is tightly intertwined in this ancestral organism, as it was evolved with no selective pressure for modularity.

2.3 Results and Discussion

try to make the images intersperse with the text more. Not super sure how to do that

Our experiments demonstrate that digital organisms that were evolved in changing environments differ substantially from those that evolved in static environments in a number

[1]As part of our initial controls, we hand-wrote an organism with separated sections that performed XOR and EQU. This hand-written organism had 121 instructions and as such we used this genome length as a constraint for the evolve organisms as well.

of ways. These differences include the number of mutations that fix in the lineage from the ancestor (the "phylogenetic depth"), key metrics of their genetic architecture, and the presence of reservoirs of pseudogenes that change the nearby mutational landscape. These features represent adaptation to the larger regime of repeated environmental switching. We also show that while populations evolved in cyclic environments are slightly better adapted to change than those that evolved in stochastic environments, in most measures of adaptation and short-term evolvability, these differences are generally not significant. This result indicates that while regular periodicity may offer a slight advantage for adaptation, stochastic environments perform similarly in most respects.

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2.3.1 Cyclic Changing Environments

We will begin by examining the characteristics of populations evolved in cyclic changing environments.

2.3.1.1 Evolutionary History and Population Structure

Evolution in the harsh cyclic changing environment resulted in many more mutations fixing, and thus populations with substantially higher phylogenetic depth as compared to those evolved in static or benign environments. At each environmental shift, adaptive mutations rapidly swept and fixed in the populations. (Fig 2.1)

The populations that evolved in the control and benign environments displayed more genetic diversity as compared to those evolved in the harsh cyclic environment, which underwent a bottleneck at each cycle shift. Because a selective sweep reduces current diversity within a population, the smaller number of sweeps in the benign and control treatments led populations in them to have higher standing diversity for most of their evolutionary history than those populations from the harsh changing environment. Despite this higher standing diversity in the benign and control treatments, regions of low diversity are still evident in

the genomes of these populations, implying purifying selection on the traits encoded at these sites (see Fig 2.2).

fix figure below so that it's three separate figures.

2.3.1.2 Genetic Architecture

The selective shifts in both benign and harsh changing environments result in qualitatively different architectural styles from the static control environment. The task arrangements evolved under both experimental treatments are much more scattered throughout the genome than in the control. Specifically, the bulk of the sites responsible for performing the fluctuating task (EQU) did not overlap with the backbone task (XOR), except for a core region, which represents portions of the tasks that are shared between XOR and EQU. (Fig 2.3)

clarify wording

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In contrast, the architecture of XOR and EQU remain tightly intertwined in the control, and site positions do not change substantially over the course of the experiment. In the benign treatment, many more regions that perform the fluctuating task (XOR) are scattered throughout the genome, but site positions remain relatively fixed throughout the run after an initial adaptive phase. In the harsh treatment, not only are the active sites scattered, but the positions of active sites change and proliferate wildly over time.

Interestingly, populations evolved in both the benign and harsh treatments also show development of a large reservoir of formerly functional, now vestigial, sites; that is, sites that remain unchanged from when they were previously active in performing a task, but were disabled by a mutation elsewhere and are thus now neutral. These vestigial pseudogene-like sites appear to be important for allowing the organisms to quickly re-adapt as the fluctuations in the environment restore the previously-rewarded functions. (Fig 2.4)

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2.3.1.3 Nearby mutational landscape

add figure of the nearby landscape showing how mutations relate 1 and 2 steps out., a couple of nice figures showing both changes in detrimental mutations and phenotypic distribution.

In order to identify the role that these pseudogene-like structures play, we performed a survey of the single-step mutational landscape surrounding the most abundant genotype at the end of the experiment for each replicate population. This landscape contained 3,025 distinct mutants (121 loci with 25 possible mutations per locus) in each of the 50 replicates per treatment, for a total of nearly 450,000 mutants surveyed.We found that the availability of reservoirs of vestigial sites shifted the change-evolved organisms' position in the mutational neighborhood, such that a task that was lost due to mutation remains more accessible via one or two additional mutational steps. (Fig 2.5, 2.6)

fix wording to clarify

Todo, clarify per Charles's comment above

We also measured the proportion of non-deleterious mutants in the nearby fitness landscape. We found that between all treatments, this proportion remained approximately the same. However, we found that the proportion of these mutants with different (potentially adaptive) phenotypes increased in the changing environments. In this way, the organisms from the changing environment treatments have an advantage over organisms from the control runs in terms of the short-term evolvability of the fluctuating task. This result indicates real adaptation, not only to resources in their local environment, but a direct adaptation to the environmental change. (Fig 2.7)

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Add to caption per Charles's comment above

2.3.2 Stochastic Changing Environments

Contrary to our expectations, stochastic changing environments were no more effective at promoting evolvability than cyclically changing environments. In all measures of evolvability,

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the stochastic treatments performed similarly to the cyclically changing environments. There was a significant reduction [TODO STATS] in the Phenotypic Diffusion Rate (D_P) between the cyclic and stochastic harsh changing environments. Overall, D_P showed much larger variances, but settled on a lower mean in the stochastic harsh treatment as compared to the cyclic harsh, indicating a much lower probability of the population producing offspring that would switch phenotypes neutrally. (Fig 2.8)

stats

Similarly, both the overall fraction of 1-step mutants that lost EQU, and the fraction of 2nd-step regaining of EQU, were slightly reducedin comparison to the cyclic treatments. This result indicates that stochastic harsh environment was slightly less effective at promoting evolution toward areas of the mutational landscape where such mutations were common. (Fig 2.9, 2.10)

stats

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The greatest differences between the cyclic and stochastic treatments appeared in the number of functional and vestigial sites. While the functional site counts in the stochastic environment were, overall, relatively similar to those in the cyclic environment, the pattern was different for the vestigial sites. In the stochastic harsh treatment in particular, there was a small, but significant reductionin the number of XOR+EQU overlapping functional sites as compared to the cyclic treatment. Further, there was an overall reduction in the number of EQU-only vestigial sites. These features may provide a clue, indicating that architectural features that would promote the retention of EQU were less prevalent in the harsh stochastic environmental treatment. (Fig 2.11)

stats

Together, from these measures, we conclude that stochastic environments exert slightly less evolutionary pressure to move toward regions of the mutational landscape that are more congenial to neutral phenotypic exploration and evolvability. We hypothesize that this dynamic may be due to the randomly-occurring environmental changes may either occur too rapidly for a response to selection, or too slowly, such that drift may cause the information

contained in vestigial sites to mutate away. While the environment, on average, experiences as many changes as in the cyclic experiment, the distribution of the length of those environment periods may be very different. Thus, we can conclude that our stochastic changing environment is not more effective than a cyclic changing environment, and under harsh conditions, may actually be slightly worse for promoting the evolution of evolvability.

2.4 Conclusion

In cyclic changing environments, the direction of selection shifts frequently, and periodically drives populations to not only explore new regions of the genetic landscape, but also to carry with them vestigial genetic information about previous environmental conditions. Thus, the resulting populations are not only adapted to the current environment, but also to the meta-environment of cyclic change. Because of their evolutionary history, the genomes contain vestigial fragments of genetic material that were adapted to prior environments. As this exploration proceeds, mutations accumulate in the population, each creating a link to a new region of the mutational landscape. As these links accumulate, they form a reservoir of mobility for the population to quickly shift to new phenotypes as dictated by current selective conditions. In this way, the accumulation of vestigial or pseudogene-like regions acts as an indirect adaptation to the larger pattern of changing selective forces.

By contrast, in static (non-changing) environments, the majority of neutral mutations do not connect to as many phenotypically-interesting regions of genotype-space. There are far fewer pseudogene-like regions available that could regain functionality should conditions change. Thus, populations evolved in static environments are less evolvable in the short-term.

add a sentence emphasiszing that it's an indirect effect.

Surprisingly, stochastically changing environments are slightly less effective at exploration than cyclic changing environments, even if, on average, the amount of time spent in each environment was equal. We hypothesize that this result is because of more opportunity for drift to destroy the information contained in vestigial regions, as well as potentially fewer

opportunities for populations to respond to selection.

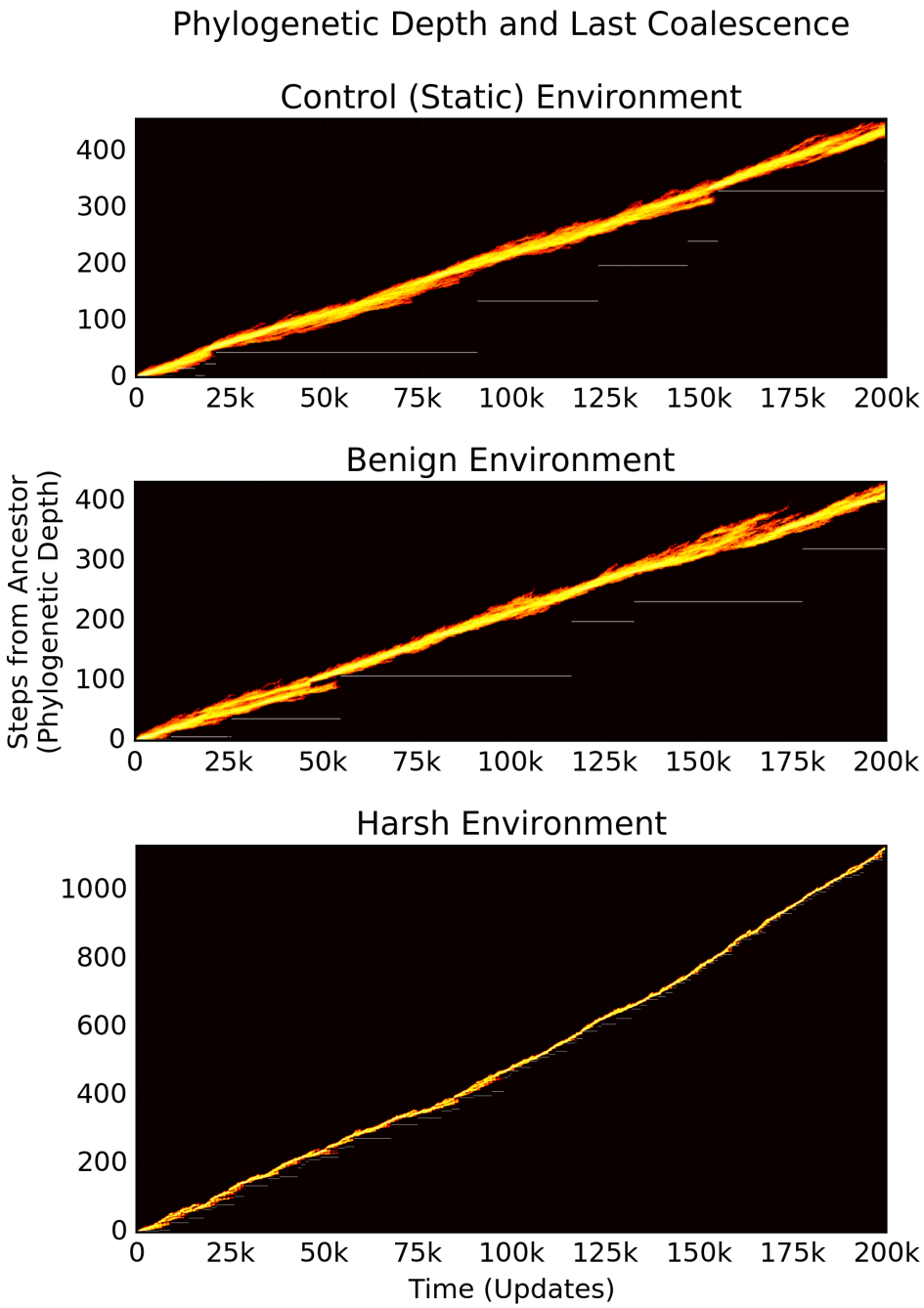


Figure 2.1: **Phylogenetic depth over time** of a sample population evolved in each of the three treatments of the cyclic changing environments. White horizontal lines mark the depth of the most recent common ancestor, and discontinuities in this line indicate that the most recent common ancestor has changed, and thus that a sweep occurred, or that a competing clade went extinct. The control treatments had a mean of 18 sweeps (STD=9.05), the benign treatments had a mean of 21 (STD=19.05), and the harsh treatments had a mean of 88 sweeps (STD=23.37). Note the difference in scales between y-axes: the control-evolved population has a maximum depth of 400 mutational steps from ancestor, while the harsh-evolved has upward of 1100.

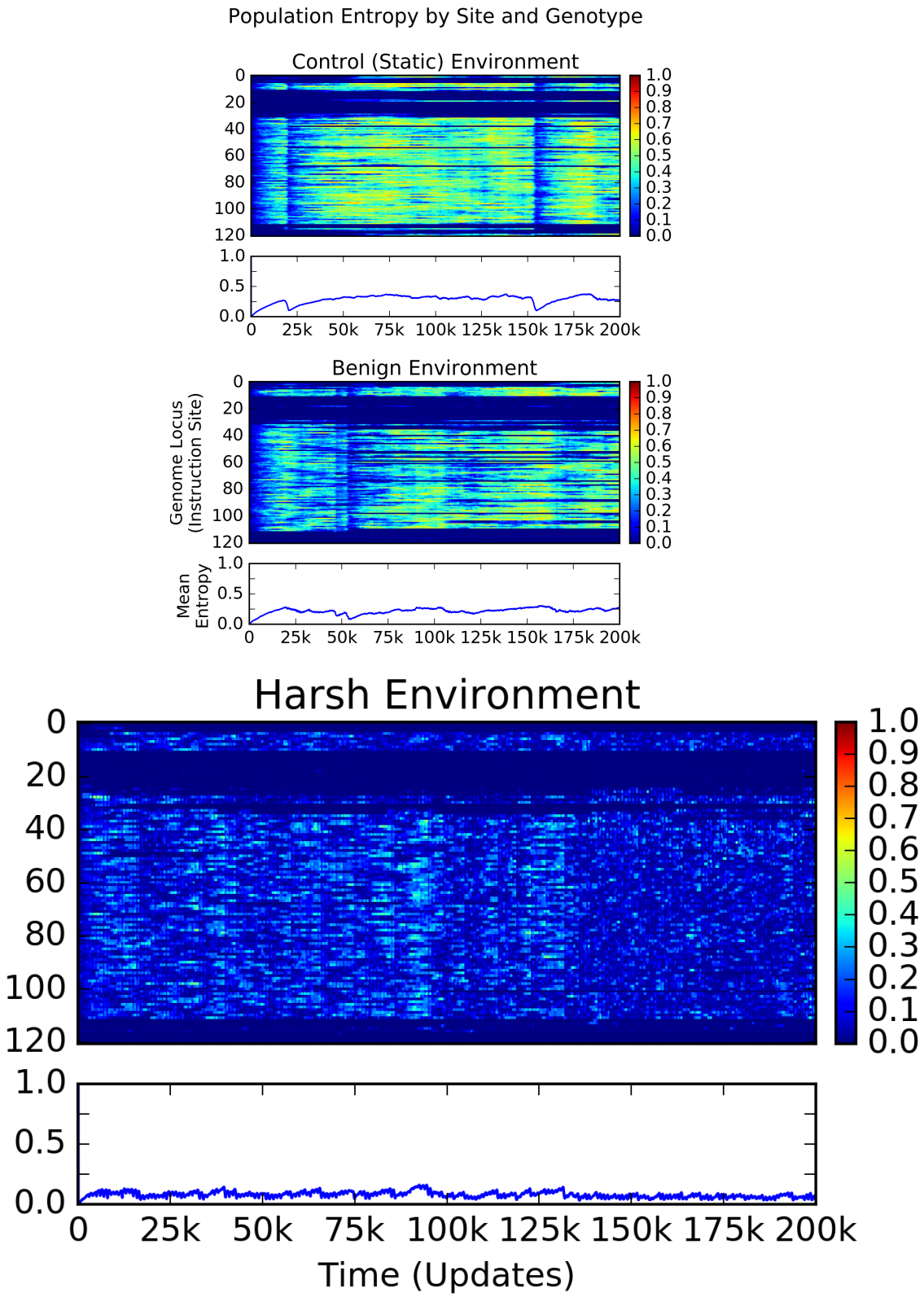


Figure 2.2: **Population Per-site Entropy over time** of a representative sample population. Each vertical slice represents the per-site entropy of the population at each update, both by genetic locus (upper), and overall population mean (lower). Hotter colors (red/orange/yellow) indicate greater diversity at this locus, while cooler colors (blues) indicate the a locus is more consistent across the population. Mean population entropy indicates the relative diversity of the population at any given time, while the per-site entropy shows where in the genomes the population diversity is located.

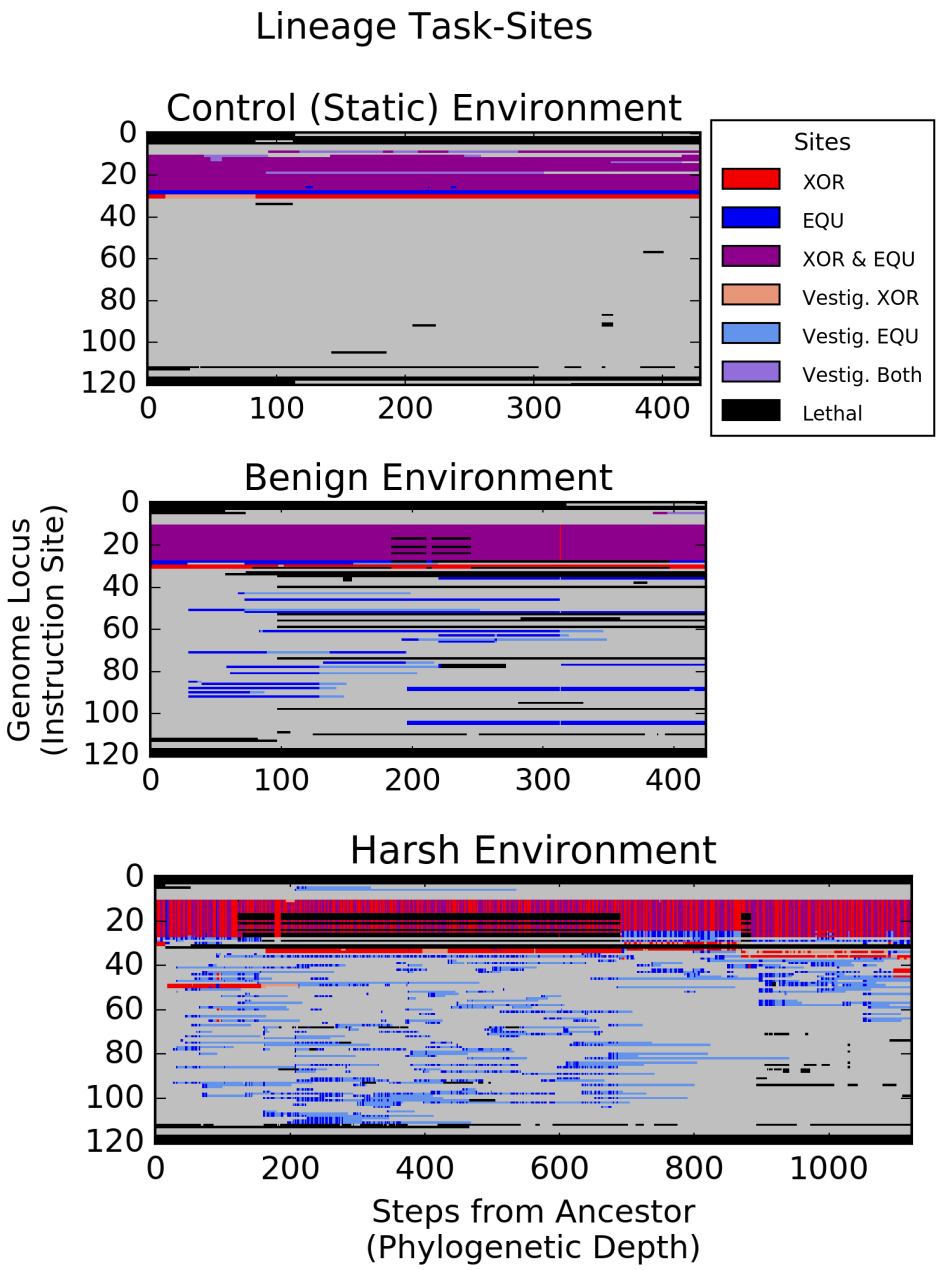


Figure 2.3: **Varying genetic architecture of XOR and EQU over time** for the final dominant genotype in a randomly selected replicate. Proceeding from the left of each figure, each vertical slice represents an organism along the line-of-descent to the final dominant. Positions along the Y-axis represent each genome locus; loci in an organism are colored based on the tasks that they code for. Sites in **red** are active sites that code for the XOR task only, sites in **blue** are active sites for the EQU task only, and **purple** sites code for both XOR and EQU. Knockouts to the sites in black are lethal to the organism. Sites in the lighter colors (tan, light blue, lavender) represent vestigial sites for XOR only, EQU only, or both tasks, respectively. As we proceed from left to right, we can see the evolutionary history of the final dominant genotype.

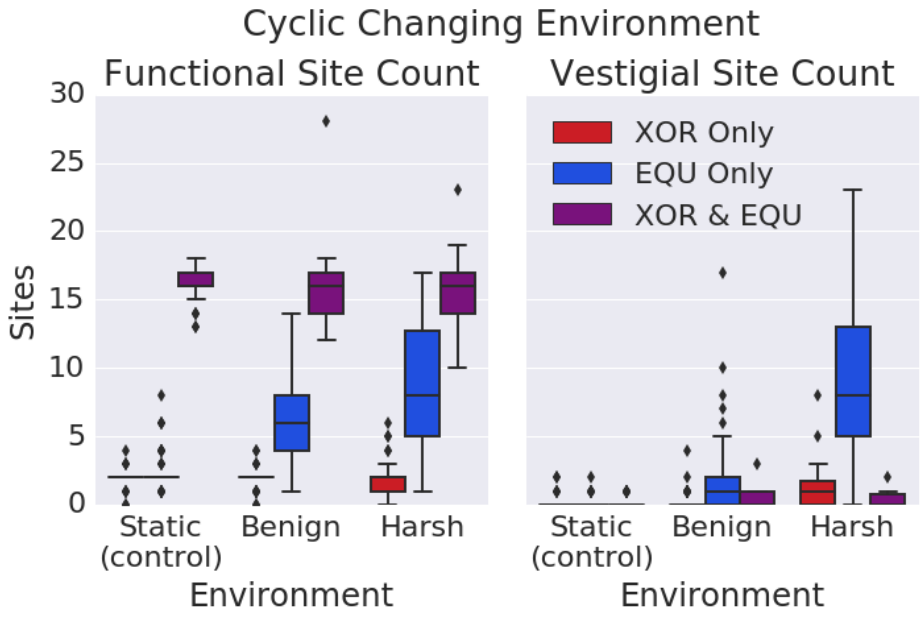


Figure 2.4: **Number of functional and vestigial sites by treatment**. The harsh environment has a significantly larger number of vestigial sites for the fluctuating (EQU) task compared to the benign treatment or control, while having a comparable number of functional sites (One-Way ANOVA F(X,YYY) = ZZ.ZZ, p << 0.000QQ).

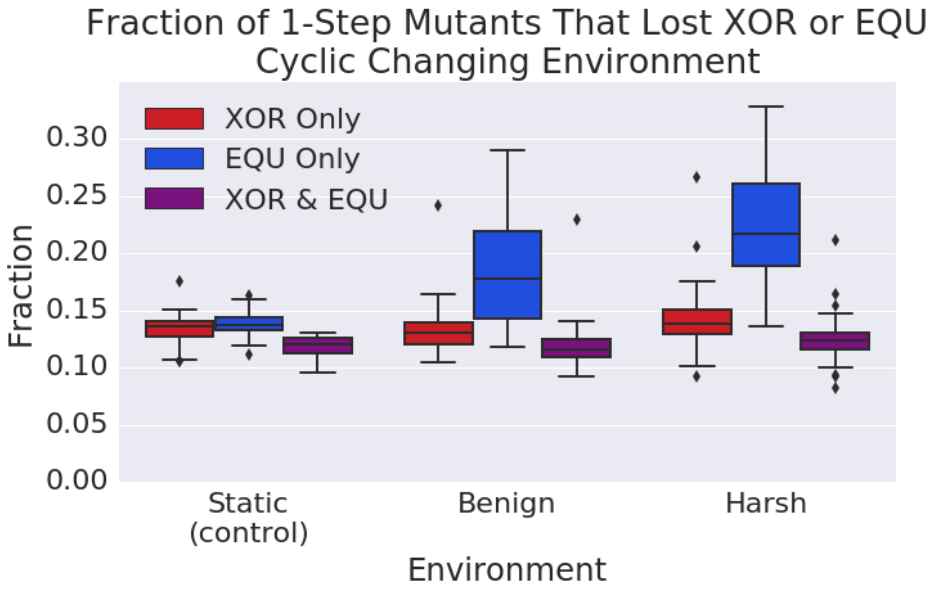


Figure 2.5: **A survey of the single-step mutational neighborhood** around organisms that performed the fluctuating task. Note that in both the benign and harsh treatments, there were significantly more mutants that lost the EQU task as compared to the control (Wilcoxon Rank Sum Test: Z = X.XX and Y.YY respectively, p << 0.000ZZ). This result indicates that it was easier for the organisms in both treatments to turn off the EQU task in response to one mutation.

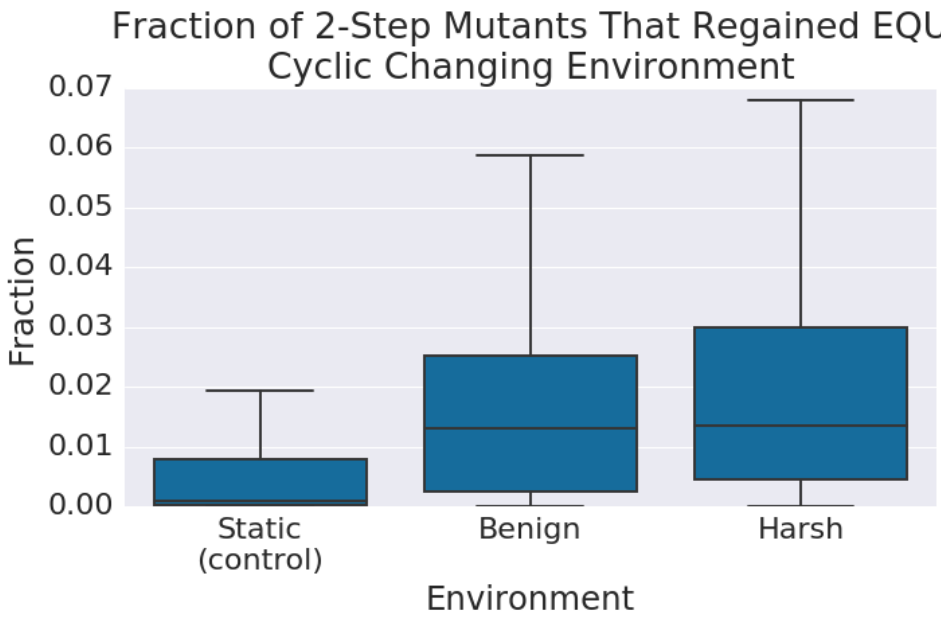


Figure 2.6: **A survey of the two-step mutational neighborhood** of the organisms that lost EQU function in the one-step survey. We found that in both the harsh and benign treatments, there were significantly more organisms that regained function in response to mutation than the control. (Wilcoxon Rank Sum Test: Z = X.XX and Y.YY respectively, p << 0.000Z). This result indicates that it was easier for the organisms in both fluctuating environments to regain the task in response to one additional mutation.

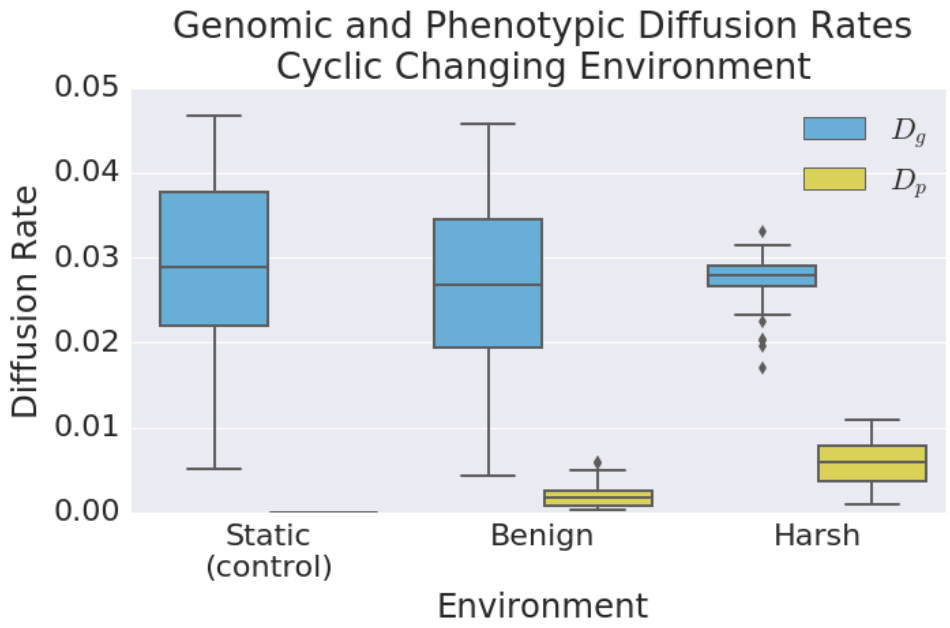


Figure 2.7: **Genomic and Phenotypic Diffusion Rates**, showing the probabilities of producing offspring that are genotypically (D_g) or phenotypically (D_p) distinct from the parent, while not reducing fitness. Note that while overall neutral exploration capacity remains relatively stable between treatments, phenotypic exploration capacity is increased in both treatments, but especially in the Harsh treatment. (Wilcoxon Rank Sum Test: Z = XX and XX respectively, p << 0.0001). This result indicates that changing environments promote the phenotypic evolvability of populations in particular.

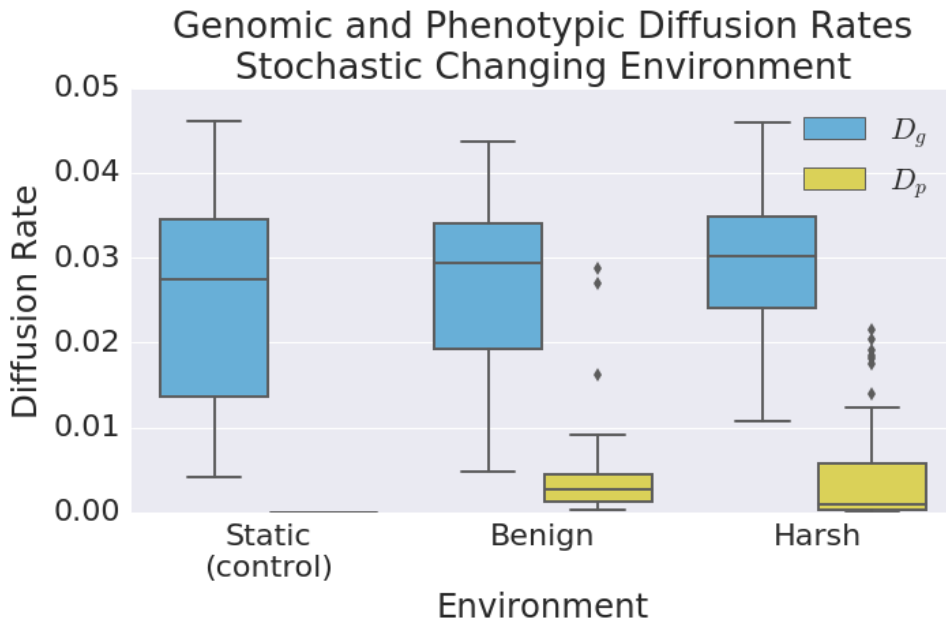


Figure 2.8: **Genomic and Phenotypic Diffusion Rates** in stochastic changing environments, showing the probabilities of producing offspring that are genotypically and phenotypically different from the parent, while remaining fitness neutral or better. As in the cyclic environment D_g remains stable, at comparable levels [todo stats], however, the mean is significantly lower[TODO stats]. This result shows that stochastic environments are not as effective as cyclic environments at increasing the probability that organisms will produce phenotypically different, yet neutral offspring.

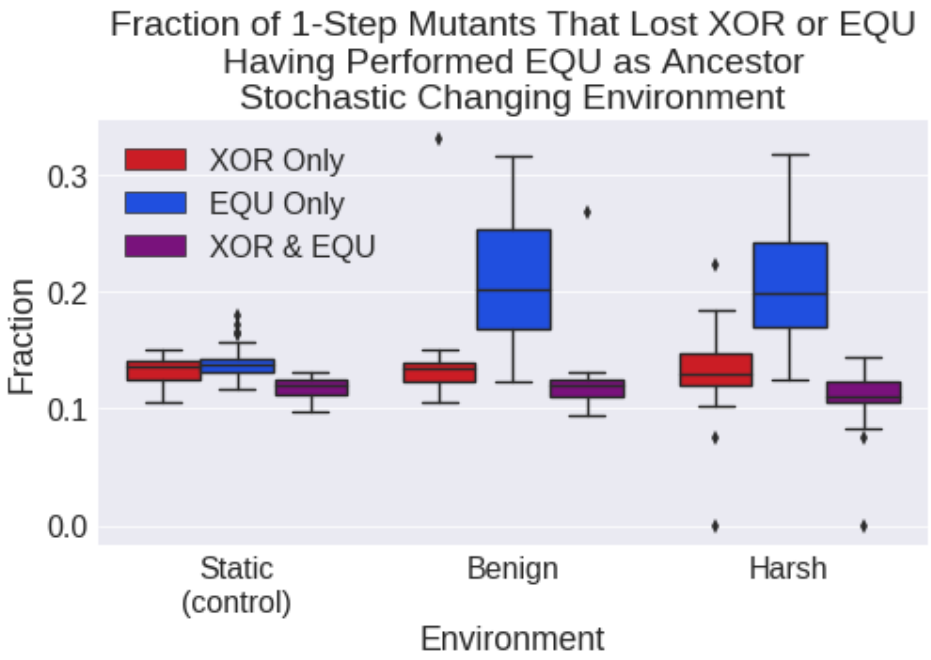


Figure 2.9: **A survey of the single-step mutational neighborhood** in the stochastic changing environment around organisms that performed the fluctuating task. Again, in the static and benign treatments, values are comparable to the cyclic changing environment [TODO stats]. However, in the harsh treatment, the means for both loss of the fluctuating task (EQU) and loss of both task were slight reduced [TODO stats]. This result indicates that in the context of a harsh treatment, stochastic environmental change is less effective at moving organisms to areas of the fitness landscape where they can more easily switch task expression.

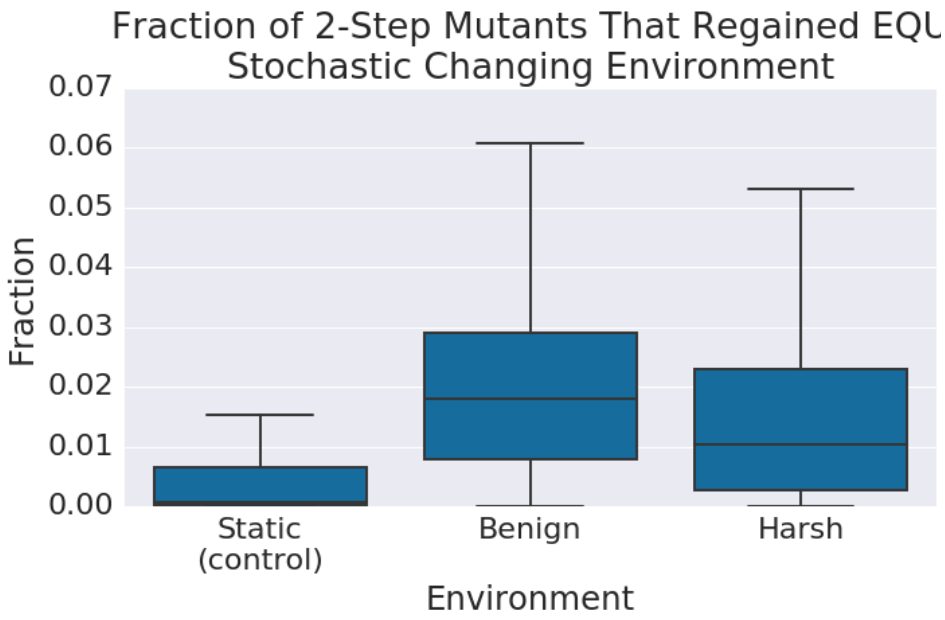


Figure 2.10: **A survey of the two-step mutational neighborhood** in the stochastic changing environment of the organisms that lost EQU function in the one-step survey. Similarly to the result in Fig 2.9, we found that the fraction of organisms regaining the fluctuating task from a single additional mutation in the harsh treatment were reduced compared to the cyclic harsh treatment[TODO stats]. This result confirms that the harsh stochastic environment is less effective than the cyclic harsh at promoting evolvability.

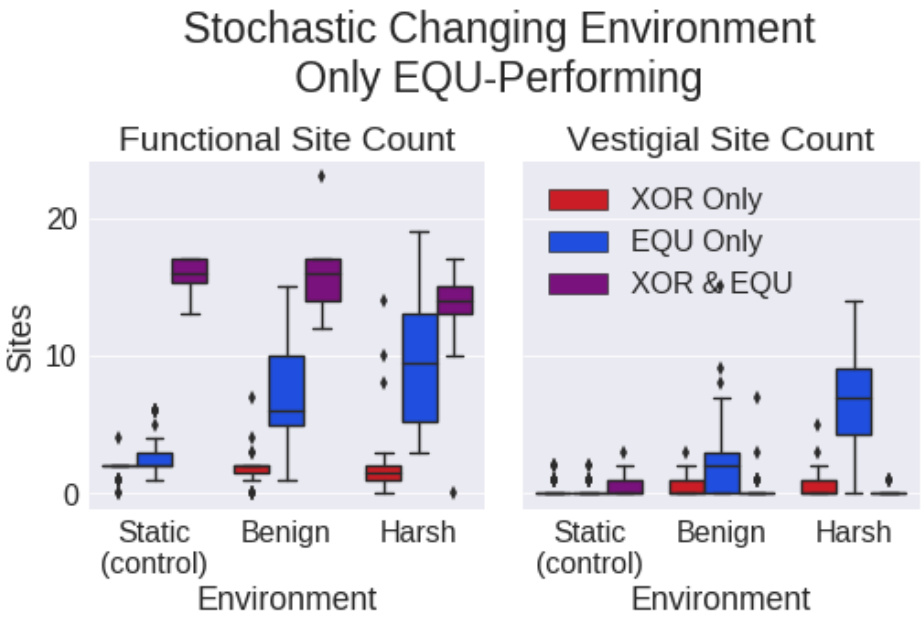


Figure 2.11: **Number of functional and vestigial sites by treatment** in a stochastic changing environment. The vestigial site counts remain comparable to the cyclic environment [TODO stats], however, there was a reduction in functional site counts for XOR+EQU overlapping sites in the stochastic harsh environment as compared to the cyclic harsh environment [TODO stats], as well as an overall reduction in the number of vestigial sites.

CHAPTER 3

CHANGING ENVIRONMENTS AND THE EVOLUTION OF HORIZONTAL GENE TRANSFER

3.1 Background

Horizontal Gene Transfer (HGT) is a broad term for the non-reproductive transfer of genetic material between organisms. Organisms may uptake genes directly from the environment (natural competence [59]), or else receive them via bacterial conjugation[60] or viral infection (transduction [61, 62]). The integrated fragments may either be decomposed inside the recipient cell for their nutrients, or integrated into their genomes.

HGT has had a profound impact on the evolutionary history of both prokaryotes and eukaryotes, with one study showing approximately 81% of genes in the sample "being involved in HGT at some point in their history" [63]. For example, HGT appears to be the primary mechanism by which antibiotic resistance is conferred [64, 65] since most antibiotics are sourced from the environment, and the organisms that develop them are themselves resistant to the compounds. However, the origins and evolution of HGT mechanisms remain unclear.

3.1.1 Origins of HGT in nature

In prokaryotes, natural competence is an HGT mechanism by which organisms spontaneously uptake the DNA of dead organisms in the environment. These organisms benefit in several ways. 1) DNA is composed of a 5-carbon sugar, a phosphate, and nitrogen bases, materials that are useful for DNA synthesis and repair. 2) The organisms may also benefit from uptaking gene-fragments that confer new adaptive functionality into the genome [66]. However, it is unclear whether the origins of uptake functions were developed solely in order to obtain nutrients, or if the acquisition of new functionality was selected for as well. While grazing

More about how this question is still open

for gene-fragments as nutrients certainly conveys an advantage, the possibility of integrating these gene fragments is likely to be disruptive to organisms more often than it is beneficial [67].

In order to test this hypothesis, and address the question of whether there are circumstances where gene-fragment integration may be beneficial, we subjected populations of evolving digital organisms to a harsh changing environment, where there is a strong pressure to quickly switch your phenotype. We supplied organisms with an instruction that performs Horizontal Gene Transfer. That is, the instruction triggers uptake of a genetic fragment from the environment, and there is a chance that, rather than metabolizing the fragment for a bonus to execution speed, the fragment will instead be homologously recombined into the organism's genome. We show that in harsh changing environments, without any kind of bonus, organisms increase use of HGT as compared to execution in a static environment.

3.2 Methods

In this chapter, we use Avida to test hypotheses about the origins of Horizontal Gene Transfer.

3.2.1 HGT in Avida

In Avida, HGT is triggered by the `HGT-Uptake` instruction that, when executed, attempts to uptake a genome fragment from the individual cell reservoirs in the environment. As organisms die in Avida runs with HGT enabled, fragments of their genomes will accumulate in reservoirs. The fragments in the reservoirs will deteriorate over time, with older genomes disappearing from the reservoir as new ones enter. Fragments for uptake will be randomly selected from the reservoir.

Upon uptake, there is a probability that the fragment is not metabolized; instead, homologous recombination may occur (Figure 3.1). Bonus levels, fragment sizes, and recombination probabilities were experimentally derived to arrive at a maximum use for the HGT

fixed-width font for HGT-Uptake and other Avida instruction names

instruction. See Appendix B for more details.

For all experiments described in this chapter, we used three [1] instructions as the minimal homologous match length, and a 10% recombination probability.

Citation TODOs

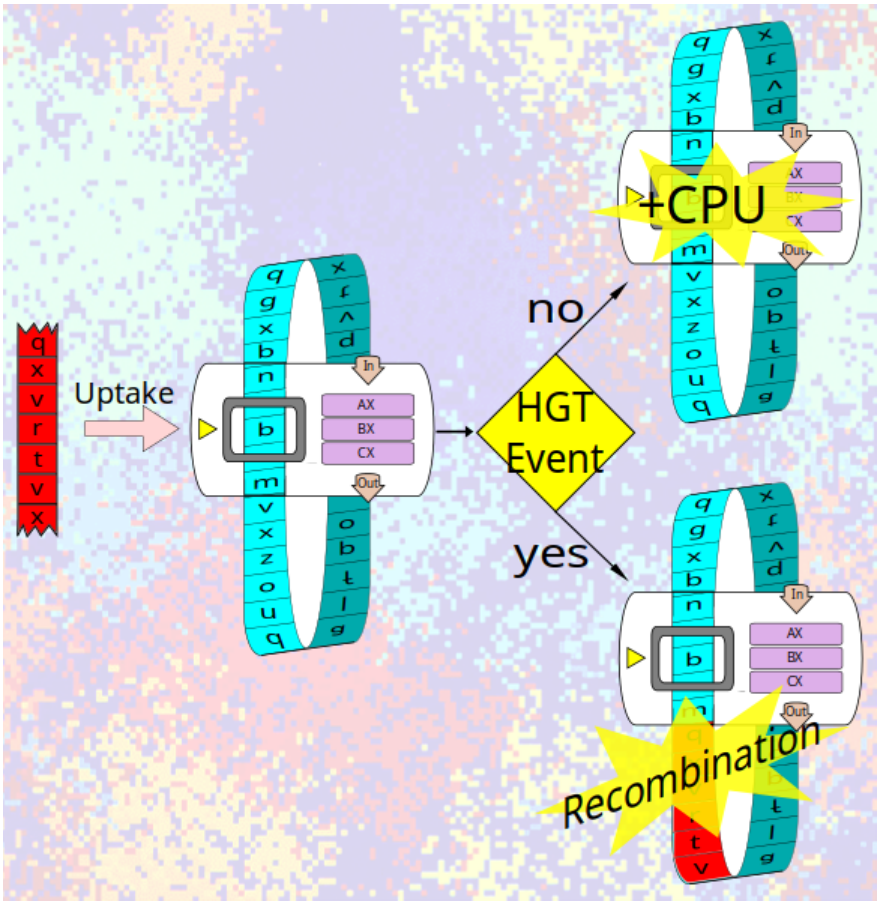


Figure 3.1: The HGT process. Organisms can execute instructions that trigger uptake from the environment. When uptake occurs, there is an experimenter-defined chance that either it will yield a boost to speed of execution or, alternatively, that the fragment will be integrated into the genome.

[1] This value is derived from a calculation associating the average size of one avida instruction to about 100 nucleotides [TODO CITE]. In natural systems, for homologous recombination to occur, an average of 300 nucleotides must match on both ends of the fragment[TODO CITE]

3.2.1.1 Environmental Conditions

All experiments in this chapter compare outcomes between static environments, and harsh changing environments. Similarly to the experiments listed in the previous chapter (Table 2.1), task rewards in the harsh changing environment switch from a positive to a negative reward. For the HGT experiments, we did not establish a backbone task that was always rewarded. Rather, we divided the Logic 9 environment into two halves, and alternated positive and negative rewards for each task in each set. Thus, for the first phase of the cycle, the NOT, AND, OR, NOR, and EQU instructions were punished at -2^1, -2^2, -2^3, -2^4, and -2^5 respectively, while NAND, ORN, ANDN, and XOR were rewarded at 2^1, 2^2, 2^3, and 2^4. In the second phase of the cycle, these rewards flip, such that NOT, AND, OR, NOR, and EQU are rewarded, whereas NAND, ORN, ANDN, and XOR are punished.

Each complete cycle lasts 1000 updates, and the whole experimental run continues for 200,000 updates.

The static environment rewards executions of all the Logic 9 tasks at their default levels, with no reward switching.

3.2.2 Experimental Design

For the treatments corresponding to the first set of hypotheses on the origins of horizontal gene transfer, we subjected four populations of evolving digital organisms with HGT to harsh changing environments (Table 3.1), plus a fifth non-HGT control. The treatments correspond to the combination of two factors: static vs changing environment, and grazing bonus vs no bonus.

rename table

For the second set of hypotheses, where we identify the mechanisms that promote the use of HGT, we manipulate the content of the reservoirs to contain fragments drawn from specific phases in the cyclically changing environment, such that fragments either match or do not match the environment (Table 3.2. We then measure HGT use, as well as average

Table 3.1: **Experimental Treatments - Evolution of HGT**

Treatment	**Changing Env. Type**	**HGT Action**	**Bonus**
Control	Static	None	n/a
HGT B0.0 (Natural Competence No Bonus)	Static	10% Recombination Probabilty	n/a
HGT B0.0 CE (Natural Competence No Bonus)	Harsh Cyclic	10% Recombination Probabilty	n/a
HGT B0.8 (Natural Competence with Bonus)	Static	10% Recombination Probabilty otherwise Bonus Allocation	$2^{0.8}$ per Uptake
HGT B0.8 CE (Natural Competence with Bonus)	Harsh Cyclic	10% Recombination Probabilty otherwise Bonus Allocation	$2^{0.8}$ per Uptake

Experimental treatments. Four treatments corresponding to the combination of two factors: Static vs Changing Environment, and Grazing Bonus vs No Grazing Bonus, plus a non-HGT control, where the HGT instruction is inert.

Table 3.2: **Experimental Treatments - Effects of HGT**

Treatment	Fragment Source
HGT	organism death
HGT-Both	sampled from organisms from both phases
HGT-OnPhase	sampled from organisms from the matching phase
HGT-OffPhase	sampled from organisms from the non-matching phase

Experimental treatments. Four treatments corresponding to the sources of fragments. The first treatment uses the default fragment source (dead organisms from the environment). The second treatment samples a population for organisms corresponding to both phases, and injects those into the reservoirs. The third and fourth treatments sample the population, but only inject fragments from the matching and non-matching phases, respectively.

fitness effects of the HGT mutations, and the fraction of mutations that lead to beneficial phenotype switches.

rename table

3.3 Results and Discussion

Our results show that both an uptake bonus and changing environment promote the use of HGT, and that the effects are largely additive.

fill this in with a general overview of the argument

3.3.1 Changing environments elevate HGT use

We measured HGT fragment uptake in four conditions (see Table 3.1), plus of pair of non-HGT controls. Without a bonus, in a static environment, fragment uptake remains low as compared to the non-HGT control. This indicates that HGT in a static environment is largely deleterious (Figure 3.2). However, in a harsh changing environment, fragment uptake is elevated. This shows that in the context of a harsh changing environment, the integration of new genetic material is beneficial. We also found that, regardless of whether the environment is static or changing, when a bonus to fragment uptake is provided (analogous to the nutritive benefit granted by natural competence in biological organisms), fragment

uptake also increases.

In order to investigate the relationship between the effects granted by a nutritive bonus and the benefit of fragment recombination in a changing environment, we selected a bonus level that increased fragment uptake in a static environment to a level comparable to the increase seen in changing environments without a bonus. We then combined these factors, giving a bonus, plus using a changing environment. We saw that the resulting uptake level increases largely additively. This indicates that the benefits granted by a grazing bonus are largely independent of the benefits conveyed by integrating new genetic material.

Thus, not only is HGT evolution possible absent a bonus, the benefit stacks with that of a grazing bonus, proving a more likely scenario by which HGT might evolve.

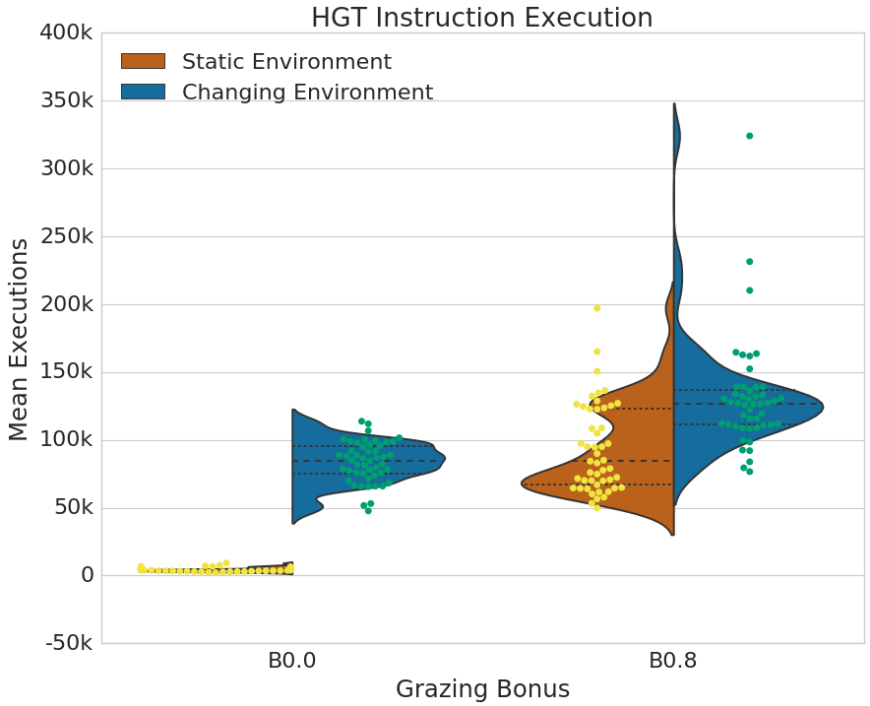


Figure 3.2: bleh

3.3.2 HGT derives most benefit from on-cycle fragments, but not all

In order to understand the basis of the beneficial nature of HGT in changing environments, we performed a series of treatments where we replaced the fragments in the cell reservoirs with fragments that originated at the end of the matching phase of the cycle, the off-phase of the cycle, and from both phases, plus a non-replaced control. (Figures 3.3, 3.6, 3.4, 3.5) We then measured the levels of HGT use, the fitness effect of HGT mutations, as well as the fraction of beneficial phenotype-switching mutations. In the both-phase and non-replaced control, the mean fitness effect was largely neutral, while the on-phase fragments had a significantly positive mean fitness benefit, and the off-phase fragments were largely negative. Further, the control, both-phase and on-phase had a larger fraction of beneficial phenotype-switching mutants, whereas the off-phase treatment had few to no beneficial phenotype switches. This indicates that it is not only genetic disruption that is beneficial, but that the majority of the benefit of HGT derives from uptaking and integrating useful fragments of information from time-periods where organisms were already adapted to the environment, thus bypassing the need to evolve a new phenotype from scratch.

3.3.2.1 Expected Fitness Effects

We measured the average fitness effects of fragments in a randomly selected replicate of the non-replaced HGT control treatment at the end of the last environmental cycle, and generated a distribution of fragments sorted the fragments by age and fitness-effect (Figures 3.3). We can see a clear pattern of more beneficial fitness effects from fragments of organisms originating in the matching cycle phase.

In order to quantify this result, we performed experiments where we replaced the fragments in the reservoir to contain only fragments originating in the matching phase, the off-phase, and mixture of both phases. We measured the mean expected fitness effects of fragments in these treatments (Figure 3.4). We found that HGT mutations are, on average, neutral in both the non-replaced control and the replaced-"both" treatment. The average

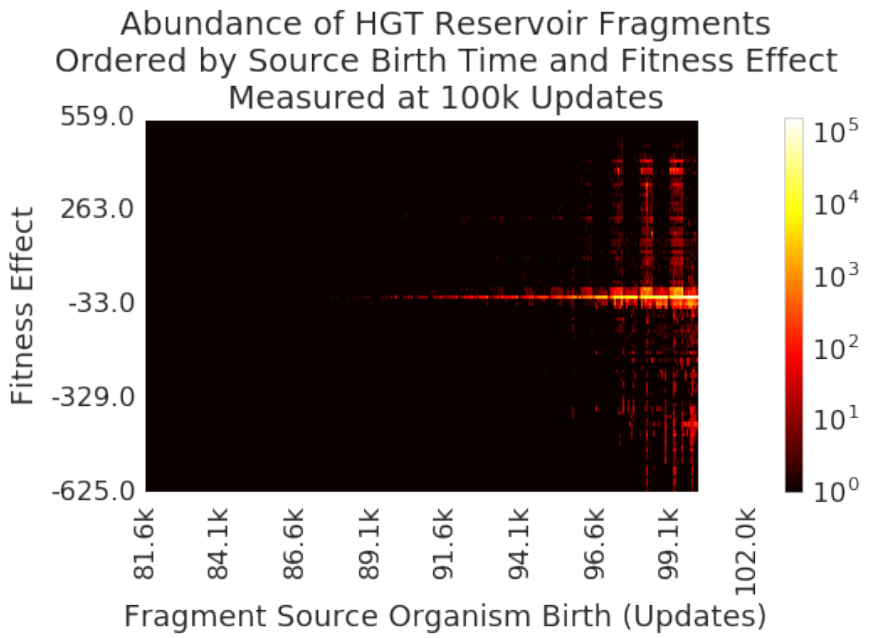


Figure 3.3: bleh

fitness effect in the "on-phase" treatment was mildly beneficial, while in the "off-phase" treatment, the effect was strongly deleterious.

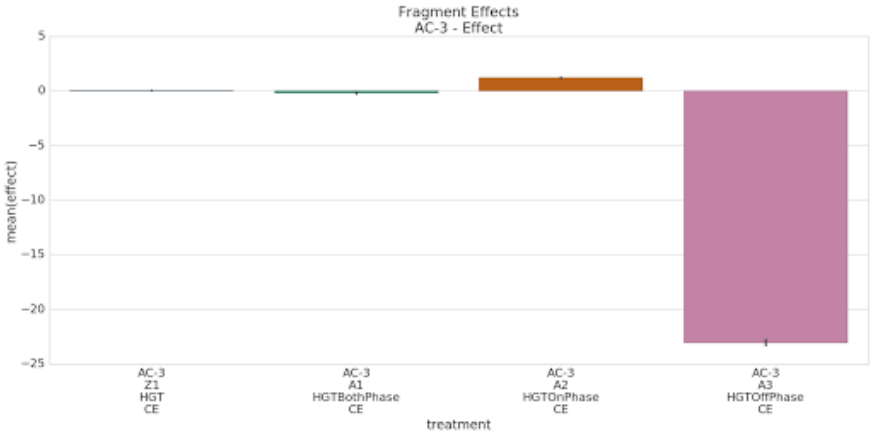


Figure 3.4: bleh

In our environments, the largest fitness benefits should occur when a mutation leads to the acquisition of a new rewarded task, or the loss of a punished task. Mutations that convey phenotypic change should have the largest impact. We identified the phenotypic effect of each

fragment by counting the number of times that fragments produced a beneficial phenotype change, vs all HGT mutations (Figure 3.5). We see that a significantly larger proportion of fragments in the "on-phase" treatment produce beneficial phenotype changes, as compared to fewer in the normal HGT and "both" treatments, and virtually zero in the "off-cycle" treatment.

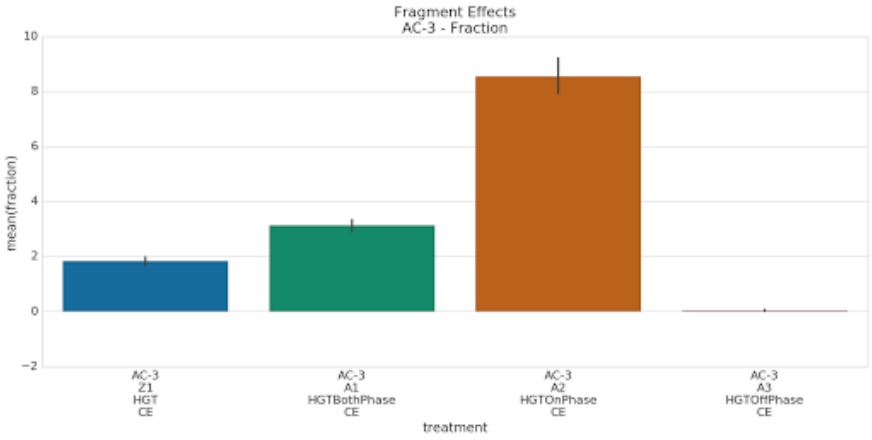


Figure 3.5: bleh

These results suggests that any direct benefit of using HGT derives from uptaking fragments that match the cycle phase of the environment of the affected organism, and thus likely contains information that relates to that environment.

Further, if fragments from the matching phase are indeed beneficial, you would expect to see an increase in HGT use in those treatments, as compared to those where the fitness effects are mixed or deleterious. And indeed (Figure 3.6), we do observe just such an increase. Thus we can conclude that HGT in changing environments is most beneficial when the fragments in the environment contain information that would be beneficial in that environment. However, even when no such information is available, HGT use is not significantly depressed as compared to the control treatment. This suggests that despite the lack of exclusively match environmental information, that fragments could still provide some benefit.

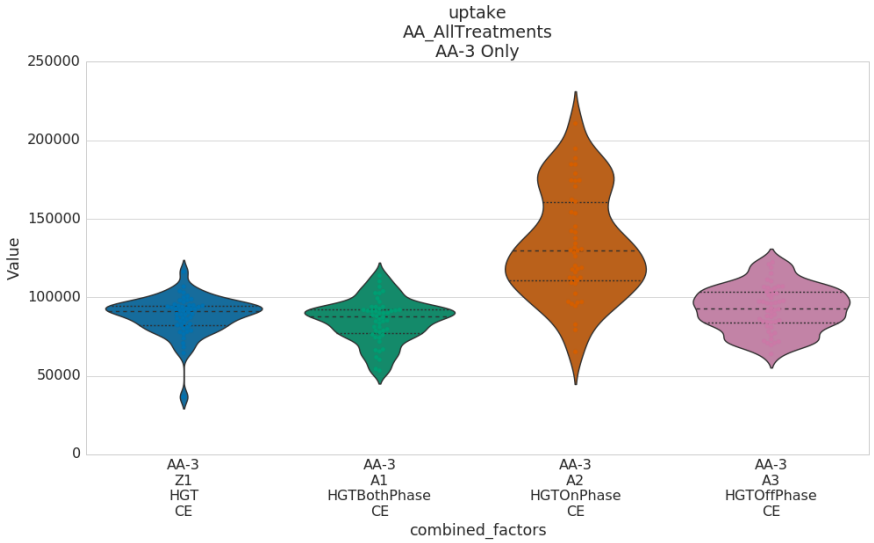


Figure 3.6: bleh

3.3.3 Information content of fragments predicts HGT benefit

fill this in

3.4 Conclusion

fill this in

CE selects for evolvability * When sufficiently beneficial mechanisms exist, and * When change is directly selected for, as in a changing environment * Because HGT is much more beneficial than other types of mutation Under Stress, Natural Competence increases * In natural organisms, Natural Competence tends to be up-regulated, and recombination rates increase [cite]. * This effect may occur because of loss of fidelity of DNA copying and repair mechanisms due to stress. * Avida has no such mechanism, so lack of fidelity does not account for an increase in HGT use. Instead, HGT can only increase due to selection.

CHAPTER 4

HORIZONTAL GENE TRANSFER VS OTHER TYPES OF MUTATION

4.1 Background

Prior research has failed to show an increase in endogenously-controlled mutation rates, when such an increase would be beneficial for long-term evolution, such as in a changing environment[TODO CITE]. Why should HGT mutations be different? In order to address this question, we created instructions that create different kinds of mutational effects with similar per-instruction impact to HGT. We then performed experiments with these mutagenic instructions under the same conditions as our evaluation of HGT, in static vs changing environments. We not only found that these instructions are triggered at significantly lowered rates as compared to HGT, but that the use rate correlates significantly with the amount of useful information conveyed by the mutagen.

add more with some coverage of what research has already been done in this area, and where this all fits in the context

4.2 Methods

In this chapter, we use Avida to compare mutational effects of HGT to other types of mutations. As in the previous chapter, HGT is triggered by the *HGT-Uptake* instruction, and fragments are uptaken from reservoirs in the environment.

All experiments in this chapter compare outcomes between static environments, and harsh changing environments. Similarly to the experiments listed in the previous chapter, task rewards in the harsh changing environment switch from a positive to a negative reward between the divded set of tasks in the Logic 9 environment. Please refer to Table 3.1 for more details. Each complete cycle lasts 1000 updates, and the whole experimental run continues for 200,000 updates. Also as in the previous chapter, the static environment rewards executions of all the Logic 9 tasks at their default levels, with no reward switching.

4.2.1 Experimental Design

4.2.1.1 Alternative Mutagens

In order to perform experiments comparing HGT to other types of mutagens, we modified the functioning of the HGT instruction to perform a new set of mutagenic functions which should have the same raw, per-instruction effect as the normal HGT instruction. All mutagens have the same 10% execution probability and viable recombination site requirements as the default HGT instruction.

- **HGT (Intact Fragment)**: The default HGT operation. A fragment is uptaken from the environment. There is a 10% probability that the fragment is recombined into the organism's executing genome. The recombination occurs homologously - that is, three instructions at the beginning and end of the fragment must match exactly with locations on the genome. The first viable match is used. If no match is found, the recombination fails. If recombination succeeds, it replaces the content between the selected beginning and end match sites. This may result in the genome growing or shrinking, depending on the distance between the matches, and the length of the fragment.

- **HGT Shuffle**: A fragment is uptaken from the environment, and a viable recombination site is found. Prior to recombination, the fragment is shuffled, and inserted at the selected site.

- **HGT Random**: A fragment is uptaken from the environment, and a viable recombination site is found. Prior to recombination, the fragment is entirely replaced with an equal number of randomly selected instructions, and inserted at the selected site.

- **Mutation Event - Sampled**: A fragment is uptaken from the environment, and a viable recombination site is found. Rather than recombine, a number of point mutations are applied, matching the number of instructions in the fragment. The mutations

applied are sampled from the selected fragment. This mutagen is analogous to the the **HGT Shuffle** mutagen, except that instead of having a shuffled fragment be applied in a single location, the instructions from the fragment are sampled and applied randomly throughout the genome.

- **Mutation Event - Random**: A fragment is uptaken from the environment, and a viable recombination site is found. Rather than recombine, a number of random point mutations are applied, matching the number of instructions in the fragment. This mutagen is analogous to the **HGT Random** mutagen, except that, again, its effects are applied randomly throughout the genome.

- **Mutation Rate Increase**: A fragment is uptaken from the environment, and a viable recombination site is found. Rather than recombine, the point-mutation rate for the organism is increased such that it will experience an additional number of point mutations matching the number of instructions in the uptaken fragment.

- **Die**: A fragment is uptaken from the environment, and a viable recombination site is found. Rather than recombine, the organism dies.

4.2.1.2 Comparing HGT to Other Mutation Types

For the experiments comparing the mutational effects of different types of mutations, we provided populations of evolving digital organisms with instructions that endogenously trigger different kinds of mutation events (see above), then subjected them to both static and harsh changing environments (Table 4.1).

4.3 Results and Discussion

Our research shows that only mutations containing useful information are elevated in response to changing environments. Intact-fragment HGT is used most, with shuffled-fragment

[TODO - more discussion of what each treatment is intended

Table 4.1: **Experimental Treatments - Mutation Types**

Treatment	Environment	Mutagen
HGT	Static	HGT
HGT CE	Changing	
HGT-Shuffle	Static	HGT-Shuffle
HGT-Shuffle CE	Changing	
HGT-Random	Static	HGT-Random
HGT-Random CE	Changing	
ME-Sampled	Static	Mutation Event - Sampled
ME-Sampled CE	Changing	
ME-Random	Static	Mutation Event - Random
ME-Random CE	Changing	
MRI	Static	Mutation Rate Increase
MRI CE	Changing	
Die	Static	Die
Die CE	Changing	

Experimental treatments. Two types of environment (static vs changing environment), for each mutagen. No bonus was given for performing any of the mutagen instructions.

used less, and the other, non information-bearing mutagen types used at the lowest levels.

fill this in with an introduction to the argument we are making

4.3.1 Other mutation types are not elevated in response to HGT

In order the compare the use of HGT aginst other kinds of mutation, we compared rates of non-bonus HGT fragment uptake against a execution of series of other mutation types, in both static and changing environments.4.1 As expected, in the static environment, endogenously controlled performance of all types of mutation (including HGT) is strongly suppressed. However, in harsh changing environment, HGT use dominates the other mutation types. Of particular interest is the decreasing use of HGT-like fragment insertions as information is destroyed, first by shuffling fragments (HGT-Shuffle), and then by replacing all fragment instructions with randomly selected instructions (HGT-Random). The latter performs comparably to the remaining mutation types: Mutation Events: Biased and Random, Mutation Rate Increase, and the Die control. This indicates that the information content of the frag-

ment is an important predictor of the use of HGT.

Also of note is that the only difference between the Mutation Event: Sampled and HGT-Shuffle treatments is the localization of the mutation effect (HGT-Shuffle fragment insertion is applied to a single location, whereas the shuffled instructions are scattered randomly throughout the genome), however their use rates are significantly different. This indicates that not only is information content important, but that localization (clustering) of the mutation effect also matters.

stats

caption

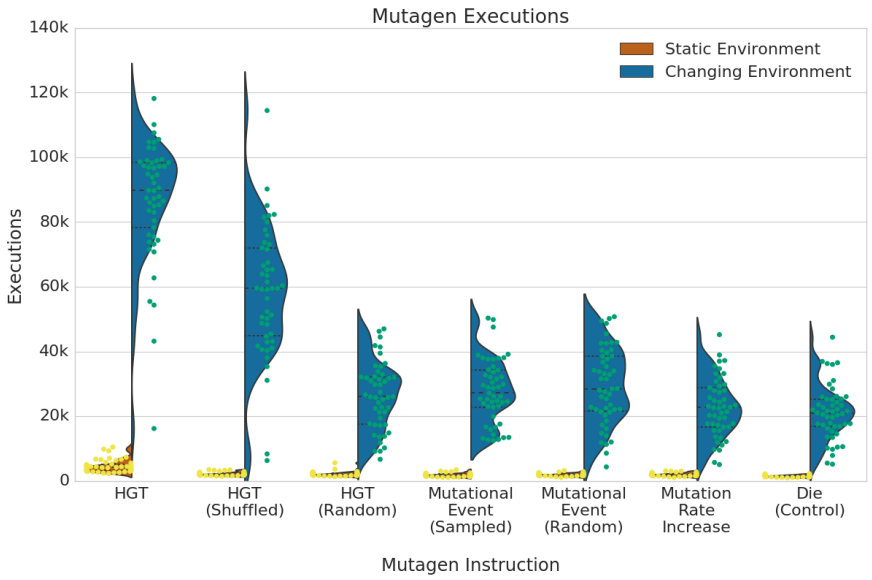
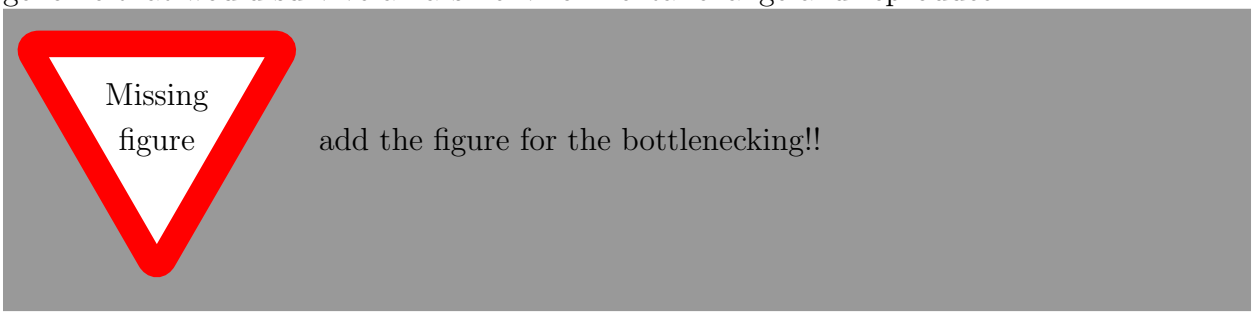


Figure 4.1: bleh

4.3.1.1 Changing Environments Elevate All Instruction Use Due to Repeated Bottlenecks

Above, we observe that the Die mutagen control is also elevated in the changing environments treatment. This counterintuitive effect is due to the repeated bottlenecking of the populations subjected to the harsh changing environment.4.2. We observe that under increasingly harsh bottlenecks, rates of execution of the "die" instruction increase to levels comparable to what is seen in the changing environment treatments. Because not all instructions contained

in an organism's genome are neccessarily executed (due to genetic flow-control structures), it is possible for any instruction to remain dormant. Thus, not all organisms with the "die" instruction in their genome will express it. Thus, the selective pressure of the environmental change may ultimately outweigh that of the die instruction if it is initially dormant in a genome that would survive a harsh environmental change and reproduce.



stats

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Figure 4.2: bleh

4.3.2 Mutation fitness effect correlate with mutagen use

Prior research has failed to show an increase in endogenously-controlled mutation rates, when such an increase would be beneficial for long-term evolution, such as in a changing environment. Why should HGT mutations be different? In order to address this question, we measured the average fitness effect of each mutation, and compared it to that mutation's usage rate in changing environments. We observed a correlation between the average fitness effect, and the use of the mutagen. We observed many more positive or neutral fitness effects from HGT-like instructions than the other mutagen types, which exhibited primarily neutral or negative mutation effects. This indicates that mutagen use is under direct selection4.3

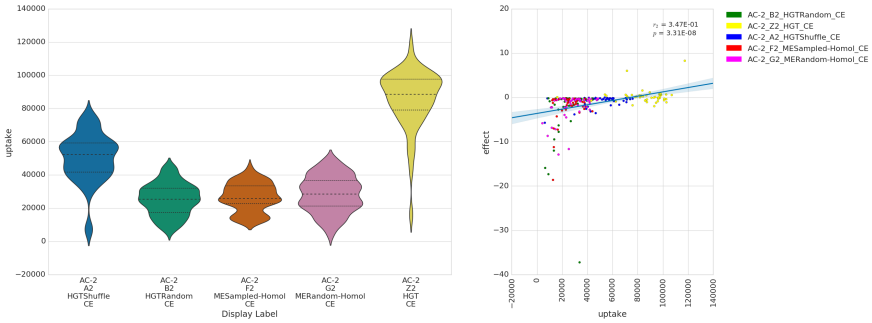

Figure 4.3: bleh

4.3.3 HGT mutations increase evolved probability of beneficial phenotype switching

Further, we measured the proportion of mutations that produce beneficial phenotype changes.4.4 In the context of a changing environment, a mutation that switches your phenotype to match the environment should be strongly selected for. Indeed, we found that this proportion was significantly and substatially higher for HGT-like mutations than other mutation types, and that there was strong correlation between the proportion of beneficial phenotype-switching mutations and mutagen use. This correlation is much stronger than the correlation with fitness effect, indicating that the magnitude of the fitness effect is less important to selection than the sign of the effect.

stats

caption

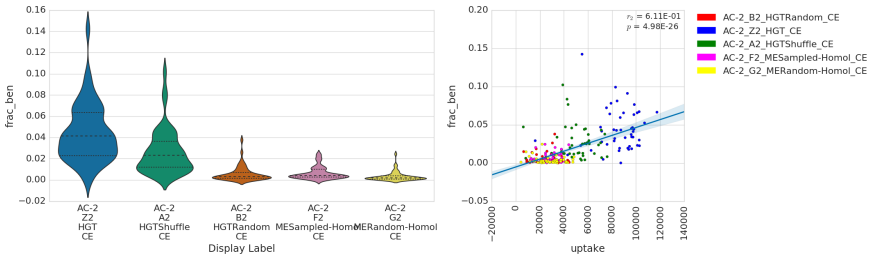

Figure 4.4: bleh

4.4 Conclusion

prose this out

CE selects for evolvability

* Because HGT is much more beneficial than other types of mutation Under Stress, Natural Competence increases

CHAPTER 5

CHANGING ENVIRONMENTS AND LONG TERM EVOLVABILITY

5.1 Background

For longer timescales, evolvability is concerned more with variability generation and exploration of neutral spaces. Populations that exhibit this kind of evolvability would possess genomes with genetic architectures that more easily traverse the mutational landscape along neutral roads and thereby discover new fitness peaks while avoiding crossing fitness valleys. This kind of evolvability would allow populations to more easily colonize new ecological niches and form new clades[2, 12].

Despite some common features, the relationship between short-term and long-term evolvability is not obvious. Architectural features and evolutionary pressures that might convey short-term evolvability may not be the same as those that confer longer-term evolvability[13]. For example, features that promote rapid adaptation to a harsh fluctuating environment might reduce fitness in constant or benign fluctuating environments as compared to that of wild-type invaders. Alternately, the adaptation to harsh fluctuating environments and the resulting bottlenecks would potentially reduce diversity to the point where large amounts of neutral novelty generation could not occur.

fill this in about the prior research done in this area

5.2 Methods

info about using Avida, but how we used existing code that matches with earlier chapters

5.2.1 Experimental Design

In order to examine the dynamics and mechanisms of evolving populations in changing environments, we performed two sets of experiments (cyclic vs stochastic changing environments), each divided into two phases (short-term evolvability vs. long-term evolvability). The cyclic environments are designed to simulate predictable cycles of change, such as day/night or seasonal cycles, whereas the stochastic environments represents less predictable oscillations in environmental states, such as random weather patterns, or climactic changes. The first phase of each set of experiments allows organisms to adapt to a predictable set of environments, whereas the second phase introduces the change-evolved populations to a completely new environment. Thus, the first phase explores short-term evolvability dynamics, and the second phase addresses the relationship between short-term and long-term evolvability. See Table 5.1

5.2.1.1 Cyclic and Stochastic Changing Environments

We took populations evolved under the experimental conditions detailed in Chapter 2, and introduced them to an entirely new set of rewarded bitwise logical tasks: the Logic-77 environment. These new tasks use three bitwise inputs rather than two, and are each rewarded with a constant 1.2-fold bonus to execution. This reward structure provides a mild selective pressure to evolve these task, but the benefits to performing them do not overwhelm the existing selective pressure to continue performing XOR or EQU.

Again, For all of the experiments described in this section, we held the individual genomes at a fixed length of 121 instructions[1], but tested the new genomes for mutations after each successful replication event at a substitution probability of 0.00075 per site. We configured the Avida world to have local interactions on a toroidal grid that is 60-by-60 cells (3600 cells

[1]As part of our initial controls, we hand-wrote an organism with separated sections that performed XOR and EQU. This hand-written organism had 121 instructions and as such we used this genome length as a constraint for the evolve organisms as well.

Table 5.1: **Experimental Treatments**

Treatment	**Changing Env. Type**	**Rewarded Tasks**				
		Phase 1 (0-200,000 Updates)		**Phase 2 (200,000-400,000 Updates)**		
		XOR	EQU	XOR	EQU	Logic-77 (all)
Control	None (static)	constant 2^3	constant 2^5	constant 2^3	constant 2^5	constant $2^{0.3}$
Benign	Cyclic	constant 2^3	benign fluctuating 0 or 2^5	constant 2^3	benign fluctuating 0 or 2^5	constant $2^{0.3}$
Benign Quiesce	Cyclic	constant 2^3	benign fluctuating 0 or 2^5	constant 2^3	constant 2^5	constant $2^{0.3}$
Harsh	Cyclic	constant 2^3	harsh fluctuating -2^5 or 2^5	constant 2^3	harsh fluctuating -2^5 or 2^5	constant $2^{0.3}$
Harsh Quiesce	Cyclic	constant 2^3	harsh fluctuating -2^5 or 2^5	constant 2^3	constant 2^5	constant $2^{0.3}$

Experimental treatments. Four types of cyclic changing environment, plus a static control. Each is split into two phases. The first phase is a normal changing environment similar to those found in Chapter 2, Table 2.1. The second phase introduces an additional set of tasks (Logic 77) that are rewarded at a lower rate.

in total), and we seeded the initial populations with an ancestor that was previously evolved to perform XOR and EQU under a static reward. The genetic architecture for performing XOR and EQU is tightly intertwined in this ancestral organism, as it was evolved with no selective pressure for modularity.

TODO fill this in

5.3 Results and Discussion

5.3.1 Long-Term Evolvability in Cyclic Changing Environments

The second phase of our experiments demonstrates that the harshness of fluctuation has a dramatic effect on long-term evolvability, landscape exploration and task discovery.[2] In the first phase of the experiments, despite the logic-77 tasks not being rewarded, both changing-environment treatments discover more new tasks than the control. The harsh changing environment treatments in particular discover significantly more logic-77 tasks than either the benign treatment, or the control. (Fig 5.1. We hypothesize that this effect may be due to the large phylogenetic depth of the harsh-evolved populations, where the repeated bottlenecks drive the populations along a kind of forced march across the mutational landscape.

stats

caption

Despite the initial success of the harsh-evolved populations' random walk across the fitness landscape, once selection for the logic-77 tasks is engaged at the start of phase 2, the benign and control treatments out-perform the harsh-evolved populations in task discovery. In particular, those populations where the harsh changing environment continues, significantly under-perform in task exploration as compared to the benign-evolved populations, and are comparable to the control rate. (Fig 5.2). We hypothesize that this effect is due to the relative differences in the strength of selection between the harsh changing environment and the directional selection toward the logic-77 tasks. The pressure to gain and lose the fluctuating task is much stronger than the pressure to acquire the logic-77 tasks, thereby depressing the rate at which they are found, and keeping it comparable to the pre-phase 2 rate. In contrast, the benign environment, with its comparatively weaker strength of selection for EQU task gain and loss, experiences a comparatively stronger selective pressure to

[2]Outcomes between the stochastic and cyclic changing environments were qualitatively similar in the second phase, therefore we will focus on the cyclic changing environment. More detail on the results of the stochastic environments in phase 2 may be found in the supplemental materials.

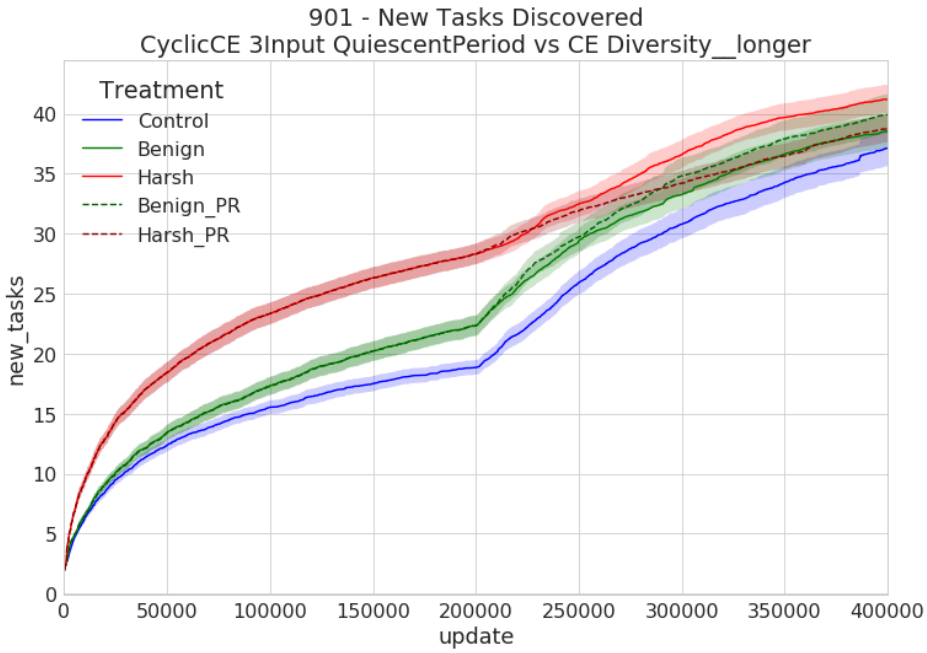

Figure 5.1: **Number of new logic-77 tasks discovered over time**. [TODO DESCRIPTION AND STATS]

acquire logic-77 tasks, while still benefiting from the increased exploration rate conveyed by the benign changing environment. (Fig 5.3 lower left)

stats

caption

Of additional interest is the comparison of task discovery rates between the benign changing environment populations A and B (Fig 5.3 lower right). The A population continues to be subject to the benign changing environment, where as the B population is only subject to directional selection toward the evolution of XOR, EQU, and the Logic-77 tasks. The task discovery rate for the A population is slightly higher than for B, but the effect is not statistically significant. Both, however, still perform better than the control treatment. Thus, this result is suggests that there are architectural features conferred by the changing environment that are helpful for long-term evolvability, even after the changes have stopped, and that this effect is distinct from the direct effects of the changing direction of selection. Further research is needed to fully untangle these effects.

stats

caption

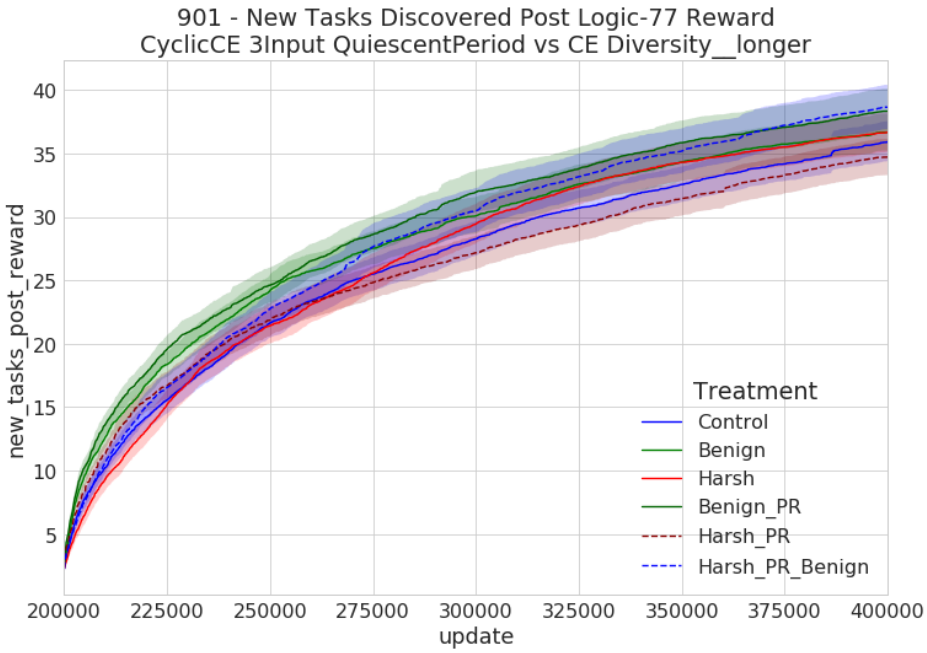

Figure 5.2: **Number of new logic-77 tasks discovered beginning in phase 2**.

5.4 Conclusion

Finally, we have shown that changing environments both directly and indirectly influence long-term evolvability by directly driving exploration across the fitness landscape, and also by creating genetic architectures that increase the rate of new task discovery in novel environments. We hypothesize that these effects are driven by the presence of the accumulation of pseudogene-like structures that provide cryptic functionality. In our experiments, the increased rate of task discovery is significant, though relatively small, but we expect that future experiments focused on this specific effect might yield more conclusive results.

fill this in, recapitulating the argument and framing

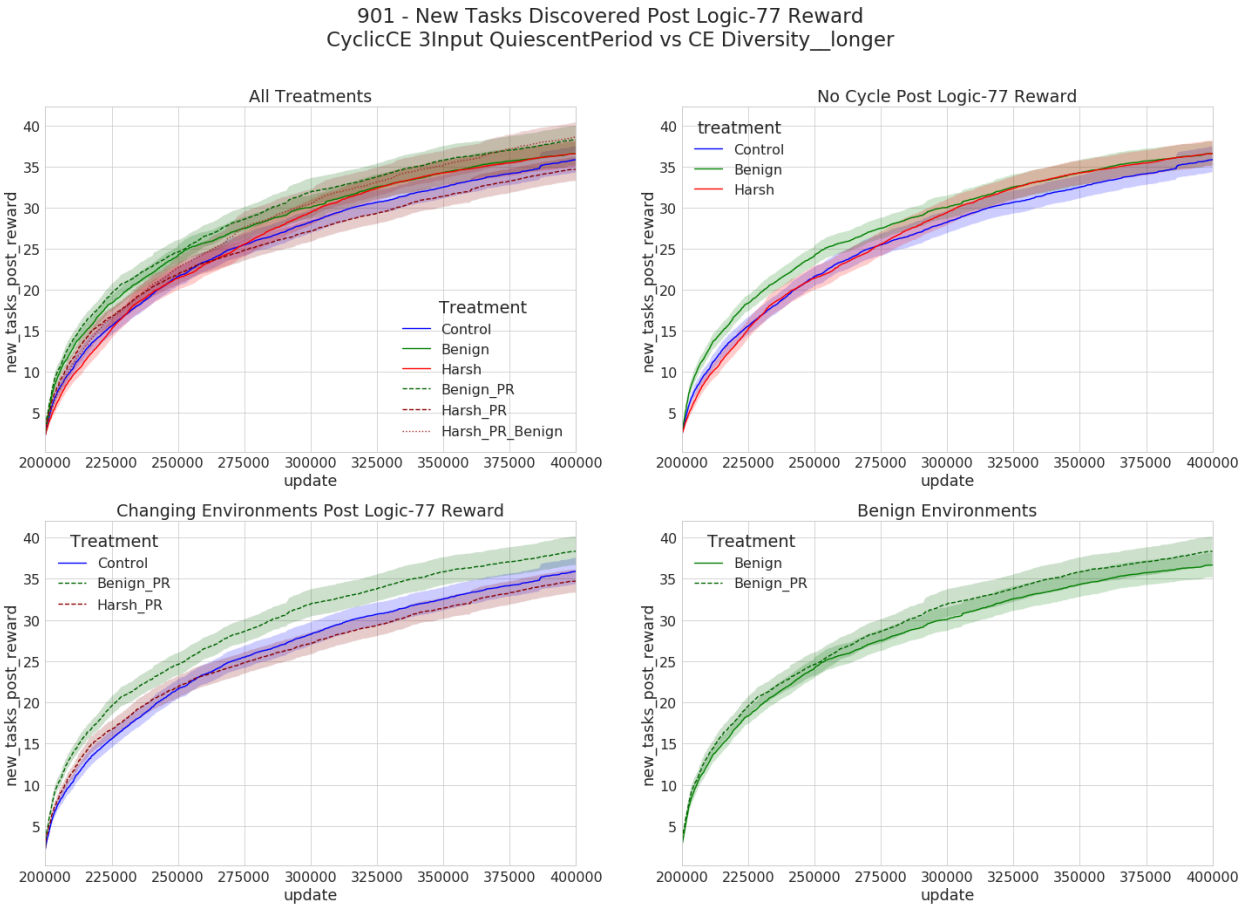

Figure 5.3: **Detail of task discovery rates over time.**

CHAPTER 6

CONCLUSION

FILL THIS IN - Synthesis of all results and how it all fits together to paint a picture of how CE works.

6.1 Limitations of Cyclic Changing Environments

Changing environments produce a set of selective pressures that speed up exploration of genotype space, while also building reservoirs of partial functionality that may be co-opted in the evolution of more complex structures. These features make changing environments useful for both their explanatory power in natural evolution, and as practical tools in the Artificial Life toolkit. Ultimately, however, cyclic changing environments only re-tread existing phenotypic ground, and though genotypic exploration is faster than under purely directional or stabilizing selection, the space explored remains constrained by the type of phenotypes that are selected. Despite this constraint, however, we see that, particularly under harsh conditions, a lot of novel genotypic ground may be explored, even without direct selection for novelty.

Even so, there must exist methods of exploring genotype space that do not suffer from these limitations at all. For example, perhaps repeated bottlenecking of populations could promote faster traversal of the fitness landscape in quasi-random directions. More ambitiously, perhaps these kinds of environments could be coupled with dynamically increasing open-ended complexity goals.

Understanding the mechanisms by which select environmental conditions alter fitness landscapes is vital to understanding the forces that promote evolvability and increase complexity. In particular, understanding the role of vestigial sites may help us untangle how robustness can promote evolvability. Are these vestigial sites inactive remnants, reservoirs of function, or are they part of a complex compensatory framework supporting and buffering

tweak below?

the expression of the phenotype? Or both? Changing environments provide one view into these dynamics, but we must explore further to find other mechanisms for exploring and exploiting genotype space.

APPENDICES

APPENDIX A

EXPERIMENTALLY DERIVING PARAMETERS FOR CHANGING ENVIRONMENT CYCLE LENGTHS

FILL THIS IN with cycle length sweeps

APPENDIX B

EXPERIMENTALLY DERIVING PARAMETERS FOR HGT RECOMBINATION PROBABILITY AND BONUS LEVELS

FILL THIS IN with bonus and probability sweeps

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