EVOLUTION AND EVOLVABILITY IN CHANGING ENVIRONMENTS

By

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ABSTRACT

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prep a layman's abstract if I have time

rework abstract

This thesis is dedicated to Kendall Koning.
I would never have gotten this far without you.

ACKNOWLEDGMENTS

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KEY TO ABBREVIATIONS

CE   Changing Environment

CV_A Additive Genetic Coefficient of Genetic Variation

D_g Genomic Diffusion Rate

D_p Phenotypic Diffusion Rate

KW   Kruskal Wallis

h^2 Narrow-sense Heritability

H^2 Broad-sense Heritability

HGT   Horizontal Gene Transfer

m_S Spatial Modularity

ME   Mutational Event

MRI   Mutation Rate Increase

r_P Phenotypic Robustness

r_G Genotypic Robustness

MWW   Mann Whitney Wilcoxon

TODO LIST

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,

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good designs for genetic representations often involve increasing the probability that a re-combination 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 time-scales. 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, against the current environment, 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.

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 them 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].

Evolution has many subtle forms that are produced due to evolution in different types of changing environments. In this dissertation, we show how changing environments not only drive exploration of the mutational landscape, but also select for populations whose genetic architectures are qualitatively different than those from populations evolved in static environmental conditions under purely directional selection. We argue that alternating the direction of selection acts as an engine for evolvability, promoting the acquisition of new traits, increasing the rate of exploration of the mutational landscape, and promotes the use and maintenance of certain kinds of evovability-promoting mutations.

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 this dissertation, 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 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 dissertation.

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

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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 likelihood 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 [41].

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 studies. 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 [42, 43], or the weeks to years needed for more complex multicellular organisms [44, 45].

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.

It is also worth noting that certain kinds of experimental evolution experiments are simply intractable in biological systems. For example, experiments comparing the fitness effects and outcomes of different kinds of mutations would be extremely difficult, time-consuming, and data-poor at a population-level scale. In contrast, these kinds of experiments can be easily performed using digital evolution models.

1.6.1 Avida

Throughout the rest of this dissertation, I use the Avida digital evolution platform to explore the effects of changing environments on the evolvability of populations of digital organisms, both in the short and long-term. Avida is a software platform for performing evolution experiments with digital organisms in a virtual world.
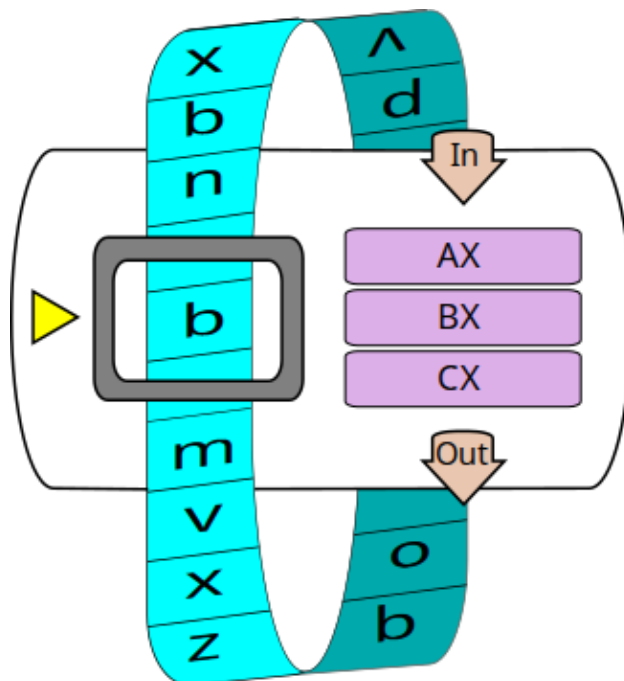
Figure 1.1: **An example virtual CPU from Avida**, with a circular genome (blue), three registers (purple), input and output handlers (tan), and an instruction pointer (yellow) indicating the next instruction to be executed.

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 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 dissertation may be found at https://github.com/voidptr/avida, https://github.com/voidptr/dissertation, and https://github.com/voidptr/dissertationdata.

git repos with data files and ipython notebooks

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 [46, 47, 48, 49] and in evolutionary computation settings [50, 51, 52]. 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 [53].

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 [54] 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 [55, 56, 57, 58]. 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 [59]. 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 may be equally accessible. Some genome regions may be more resistant to mutation than others. For example, in E. coli, the mismatch repair (MMR) pathway has been shown to preferentially repair coding regions over non-coding regions [60]. Alternately, some kinds of mutations may be more likely to occur than others. A mutation accumulation (MA) study of S. typhimurium found a strong bias toward GC-to-TA transversions rather than GC-to-AT transitions [61]. These kinds of effects thereby skew the probabilities of some kinds mutations occurring that might lead into certain regions of the mutational landscape. These kinds of differential probabilities may therefore moderate a population's diffusion through the mutational landscape.

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, 62]. This differential response to selection may therefore constrain 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

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

Table 2.1: **Experimental Treatments - Cyclic and Stochastic Changing Environments**

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

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.

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 1,000 updates, or roughly 30 generations. We chose this cycle length after surveying a series of possible values in order to determine an optimal 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

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[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.

selective pressure for modularity.

2.3 Results and Discussion

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

Each population was seeded with organisms that performed both the `EQU` fluctuating task, and the `XOR` backbone task. We measured the execution of the `EQU` task, and observed that in the static control treatment, `EQU` is fixed in the population and remains so throughout the run. In contrast, we observed a periodic dip in the execution of `EQU` in the benign changing environment during the non-rewarded phase of the cycle, followed by a rapid recovery when rewards are reinstated. Finally, in the harsh treatment, we observed abrupt disappearance of `EQU` performance, followed by rapid recoveries, coinciding with phases of reward and

punishment. As expected, these results suggest that the populations are responding to the selective pressures to perform EQU when it is rewarded, and to lose functionality when it is not rewarded or when it is punished.
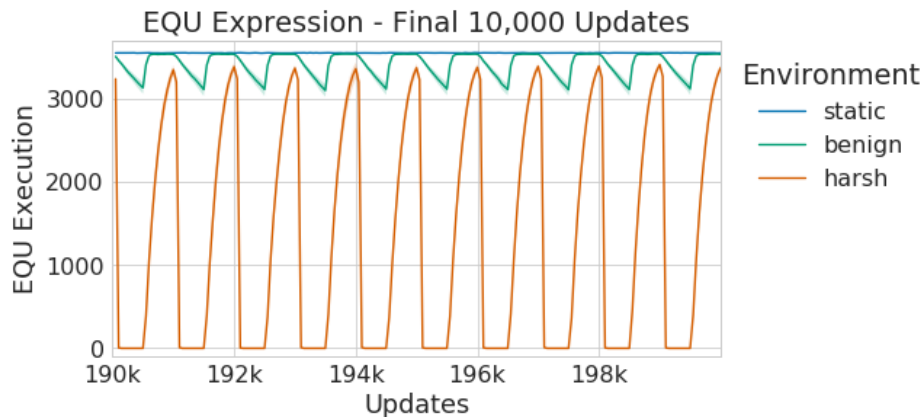
Figure 2.1: **Number of organisms performing EQU task**. We measured the execution of the EQU task in all treatments. By the end of the run, we observed fixation in the control treatment. In the benign treatment, we observed periodic dips in execution that coincide with phases of non-reward. Finally, in the harsh treatment, we observed abrupt disappearance of EQU in the punishment phase, followed by rapid recovery of EQU performance during the reward phase.

2.3.1.2 Evolutionary History and Population Structure

We then surveyed the evolutionary history and population structure of the evolving populations. 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 2.2)

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
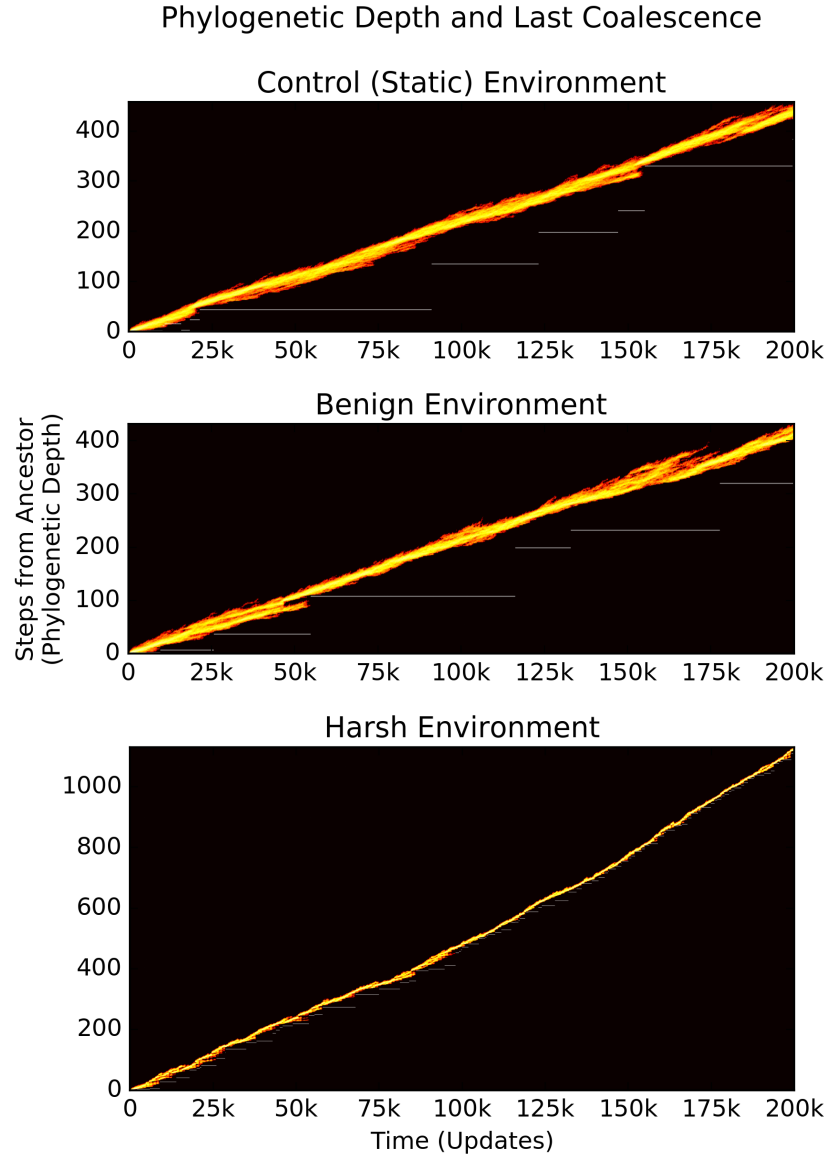
Figure 2.2: **Phylogenetic depth over time** of a sample population evolved in each of the three treatments of the cyclic changing environments. White horizontal lines mark the depth of the most recent common ancestor, and discontinuities in this line indicate that the most recent common ancestor has 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.

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 2.3).

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2.3.1.3   Genetic Architecture

The alternating selection in both benign and harsh changing environments results in qualitatively different architectural styles as compared with those genomes evolved in the static environment. The task arrangements evolved under both experimental treatments are much more scattered throughout the genome than in the control, which is tightly compacted. Specifically, the bulk of the sites responsible for performing the fluctuating task (EQU) did not overlap with the backbone task (XOR), except for a small core region, which represents portions of the tasks that are shared between XOR and EQU. That is, in the changing environment treatments, we see many more sites that only code for a single task, whereas in the static treatment, the majority of functional tasks sites code for both XOR and EQU. (Fig 2.4)

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In terms of site placement over time, functional task site locations in the control treatment did not change substantially over the course of the experiment. In the benign treatment, many more regions that performed the fluctuating task (XOR) were scattered throughout the genome, but site positions remained relatively fixed throughout the run after an initial adaptive phase. In the harsh treatment, however, not only are the active sites scattered, but the positions of active sites change and proliferate wildly over time.

In addition to the variation in site placement, populations in the benign and harsh changing environment treatments had significantly more functional sites devoted to performing just the EQU task (Wilcoxon Rank Sum Test: $Z = -5.57$ and -6.96, respectively, $p \ll 0.001$). 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
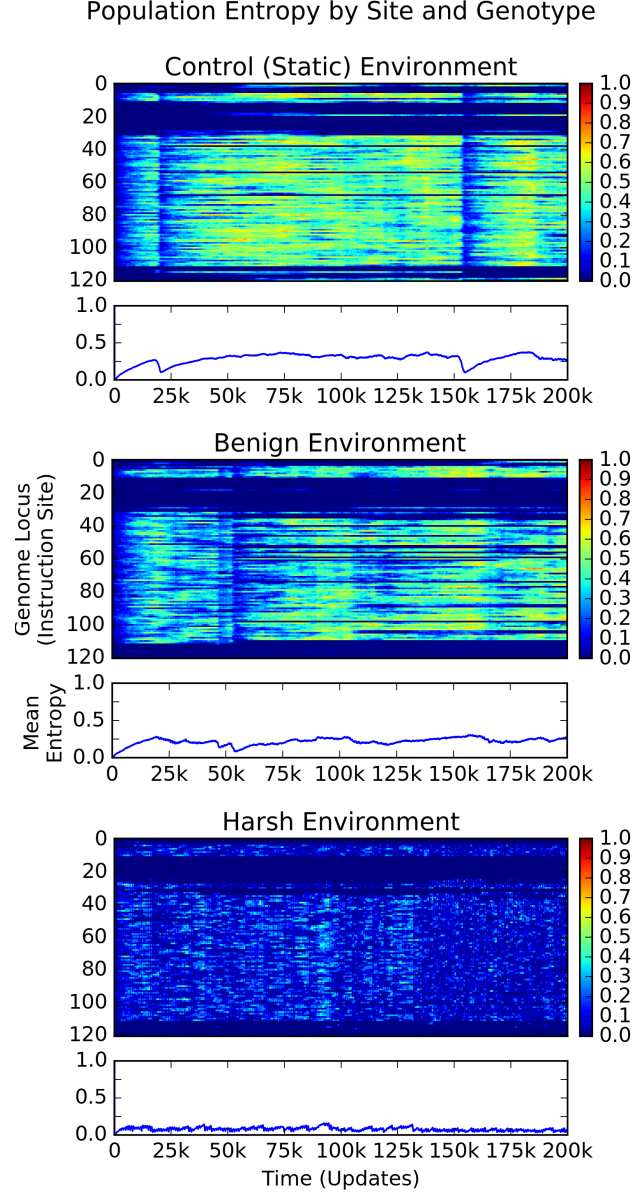
Figure 2.3: **Population Per-site Entropy over time** of a representative sample population. Each vertical slice represents the per-site entropy of the population at each update, both by genetic locus (upper), and overall population mean (lower). Hotter colors (red/orange/yellow) indicate greater diversity 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.

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

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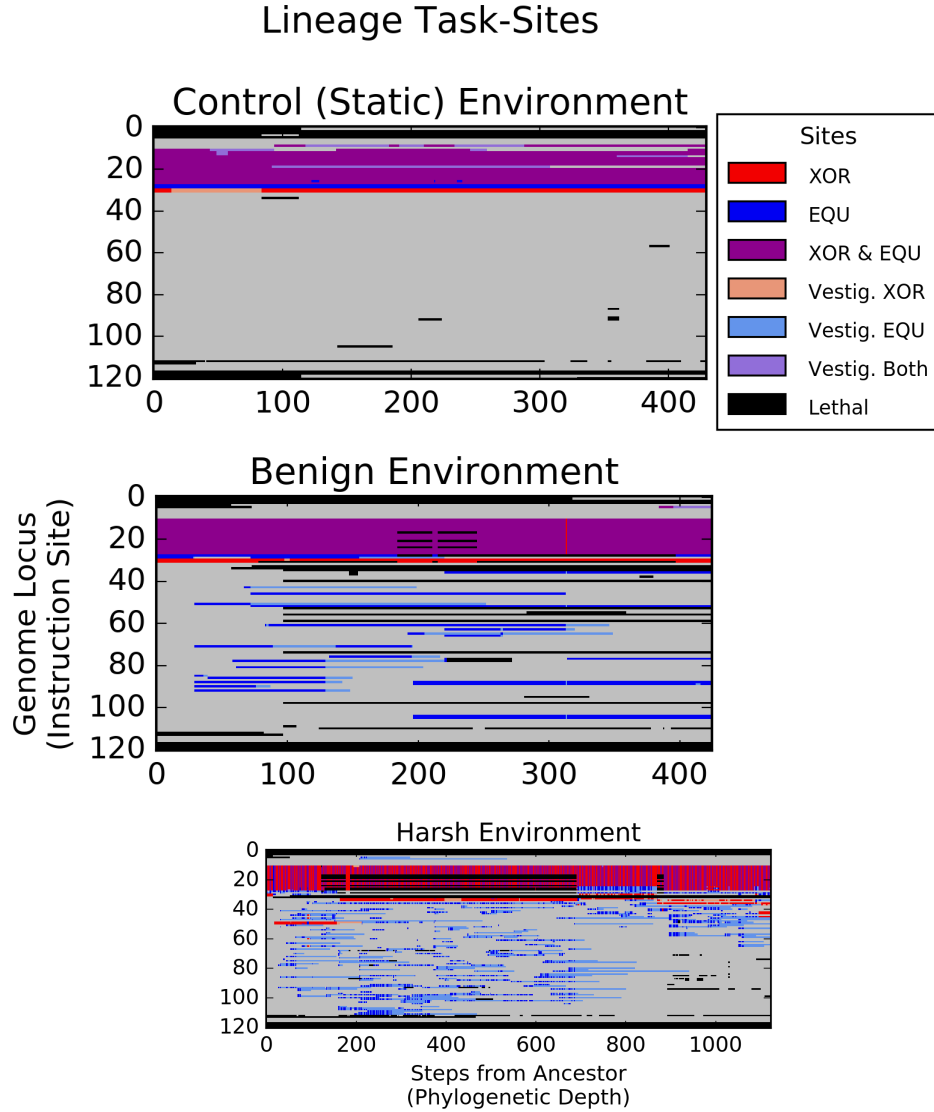
Figure 2.4: **Varying genetic architecture of XOR and EQU over time** for the final dominant genotype in a randomly selected replicate. Proceeding from the left of each figure, each vertical slice represents an organism along the line-of-descent to the final dominant. Positions along the Y-axis represent 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.

sites may be important for allowing the organisms to quickly re-adapt as the fluctuations in the environment restore the previously-rewarded functions. (Fig 2.5)

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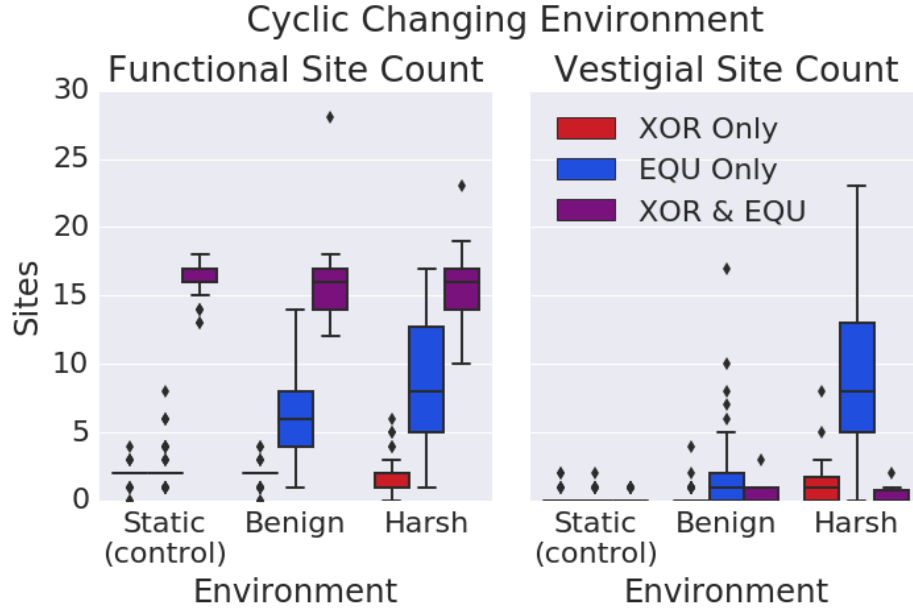
Figure 2.5: **Number of functional and vestigial sites by treatment**. Both the benign and harsh changing environments had significantly more sites devoted to performing only the EQU function (Wilcoxon Rank Sum Test: Z = -5.57 and -6.96, respectively, p << 0.001). The harsh environment has a significantly larger number of vestigial sites for the fluctuating (EQU) task compared to the benign treatment or control (Wilcoxon Rank-Sum Z = -6.57 and -8.33, p << 0.0001).

2.3.1.4 Nearby mutational landscape

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In order to identify the role that these larger functional site footprints and pseudogene-like structures play, we performed a survey of the single-step mutational neighborhood surrounding the most abundant genotype present at the end of the experiment for each replicate population. Each neighborhood 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 measured the fraction of mutants that lost each of the rewarded tasks. (Fig 2.6.

We found that in both the benign and harsh treatments, there were many more mutations
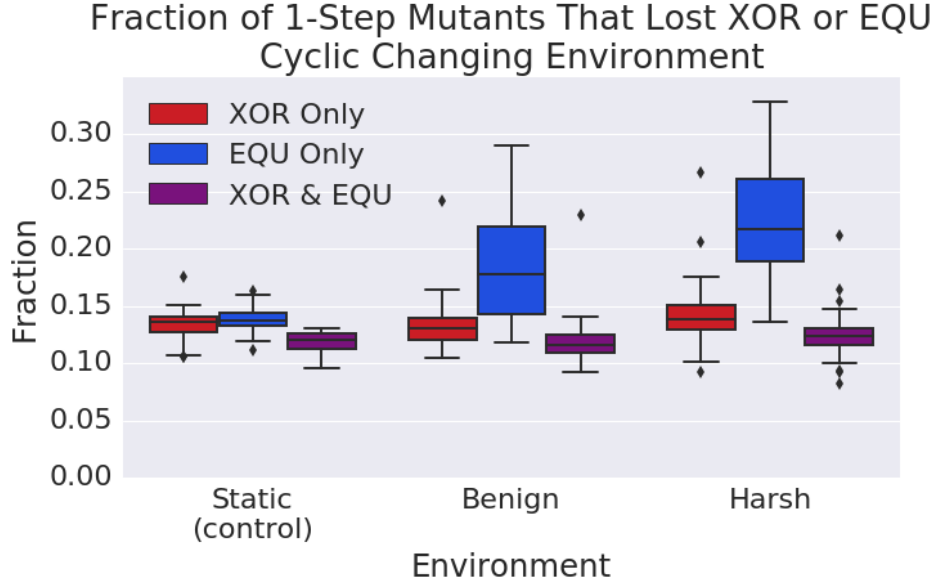
Figure 2.6: **A survey of the single-step mutational neighborhood** around organisms that performed the fluctuating task. Note that in both the benign and harsh treatments, there were significantly more mutants that lost the EQU task as compared to the control (Wilcoxon Rank Sum Test: $Z = -5.46$ and -7.80 respectively, $p \ll 0.0001$). This result indicates that it was easier for the organisms in both treatments to turn off the EQU task in response to one mutation.

that resulted in loss of the fluctuating task as compared to the control (Wilcoxon Rank Sum Test: $Z = -5.46$ and -7.80 respectively, $p \ll 0.0001$). An increase in task loss in the harsh treatment is to be expected, but why would the benign treatment lose EQU nearly as easily as the harsh treatment? One possibility is selective pressure to lose the task. There is no explicit pressure for task loss, merely an absence of reward. Even so, there is certainly an implicit penalty for performing a complex task for which there is no reward. Another possibility is drift. Indeed, in Figure 2.1, we observe a steady downward trend in execution of EQU when rewards are withdrawn. Then, as the reward returns, new mutations are applied that reactivate the task, and overall performance recovers quickly. This pattern of loss and regain would over time tend to increase the length of the task. Indeed, as noted in Figure 2.4, there is a rapid increase in task length as EQU is cyclically lost and regained.

However, is increased task length enough to account for increased task vulnerability to mutation? In order to begin to address this question, we calculated the correlation between

task length and the fraction of mutants that lost each of the tasks. We discovered a strong correlation between the number of functional sites and the number of task-losing mutants for the EQU task, both alone, and overlapping with XOR (Pearson's R: $r_2 = 8.77$ and 7.94, respectively, $p = << 0.001$) (Fig 2.7). We also found a weaker, but still significant correlation between the number of XOR-only functional sites and loss of the XOR task (Pearson's R: $r_2 = 4.77$, $p << 0.001$). This result confirms our intuition that the longer the task, the more targets there are for mutation to disable the task.
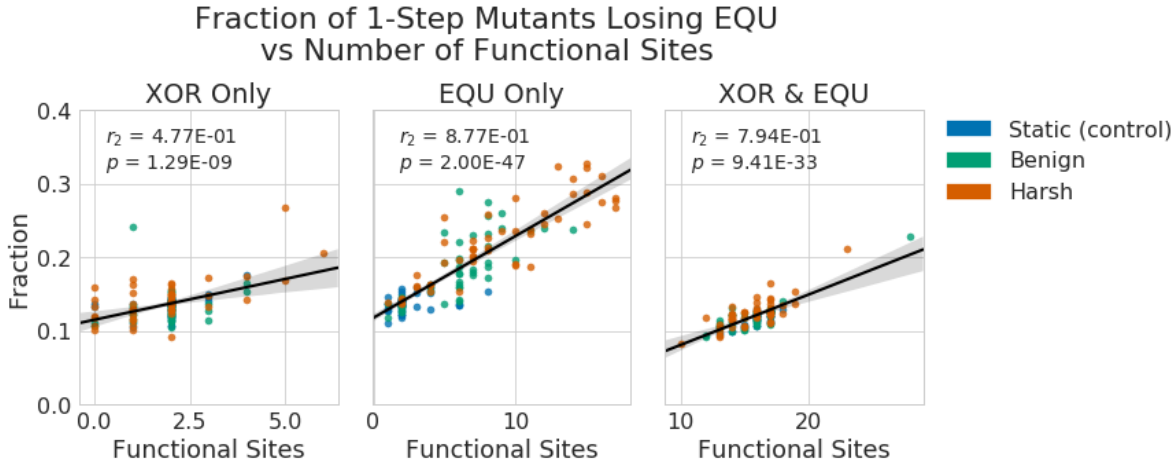
Figure 2.7: **Correlation between task length and mutational task loss** in the 1-step neighborhood across all treatments. Note the strong correlation between the length of the EQU task and the fraction of mutants that lost EQU (Pearson's R: $r_2 = 8.77$, respectively, $p << 0.001$). Further, consider the weaker correlation between XOR task length, and fraction of mutants that lost XOR. This suggests that EQU is even less robust to mutation compared to XOR than can be accounted for by task length alone.

Further, the lower correlation between length and task loss for the XOR suggests that it is not only task length, but some other architectural feature that makes the XOR task more robust to mutation, and the EQU task more fragile. Even so, the question of what kinds of architectural features account for this differential robustness remains open.

We then measured the proportion of second step mutants that regained EQU after having lost it in the single-step survey. We found that changing environments shifted shifted the populations' position in the mutational landscape, such that when a task that was lost due to mutation, that task could be regained via one or two additional mutations elsewhere.

That is, once a mutation caused the loss of a task, a different mutation could reactivate the task. (Fig 2.8).
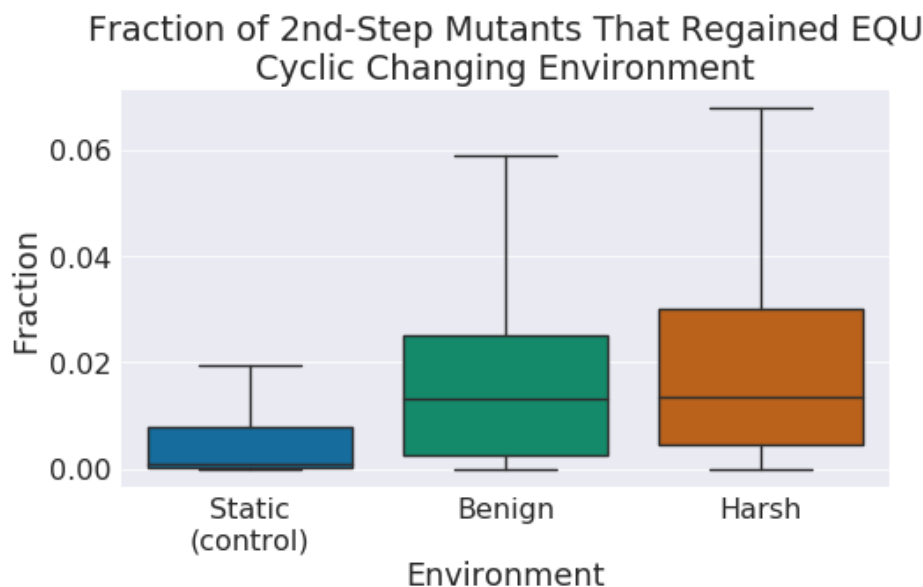
Figure 2.8: **A survey of the two-step mutational neighborhood** of the organisms that lost EQU function in the one-step survey. We found that in both the harsh and benign treatments, there were significantly more organisms that regained function in response to mutation than the control. (Wilcoxon Rank Sum Test: Z = -47.9 and -57.82 respectively, p << 0.0001). This result indicates that it was easier for the organisms in both fluctuating environments to regain the task in response to one additional, non-reversion mutation.

We speculate that this effect is due to the availability of reservoirs of formerly vestigial sites. How such reservoirs might perform these functions remains an open question. New mutations may either re-enable the old functional sites, or recruit vestigial functionality to perform the task elsewhere. Potentially, these vestigial sites are not altogether dormant at all. They might individually appear vestigial in the context of a single knockout survey, but they might also be related to other sites in a network of backup functionality that becomes activated in response to mutation. More research is needed to explore what role these feature play.

As an overall measure of neutral exploration, we also measured the proportion of non-deleterious mutants in the nearby fitness landscape - the Genomic Diffusion Rate. We

found that this proportion remained approximately the same between all treatments (Kruskal Wallis: H(2) = 1.44, p = 0.49). However, we found that the Phenotypic Diffusion Rate, the proportion of these mutants with different (and potentially adaptive) phenotypes, increased in the changing environment treatments as compared to the controls (Wilcoxon Rank Sum Test: Z = -8.02, -8.39, respectively, p << 0.001). In this way, the organisms from the changing environment treatments have an advantage over organisms from the control runs in 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 2.9)
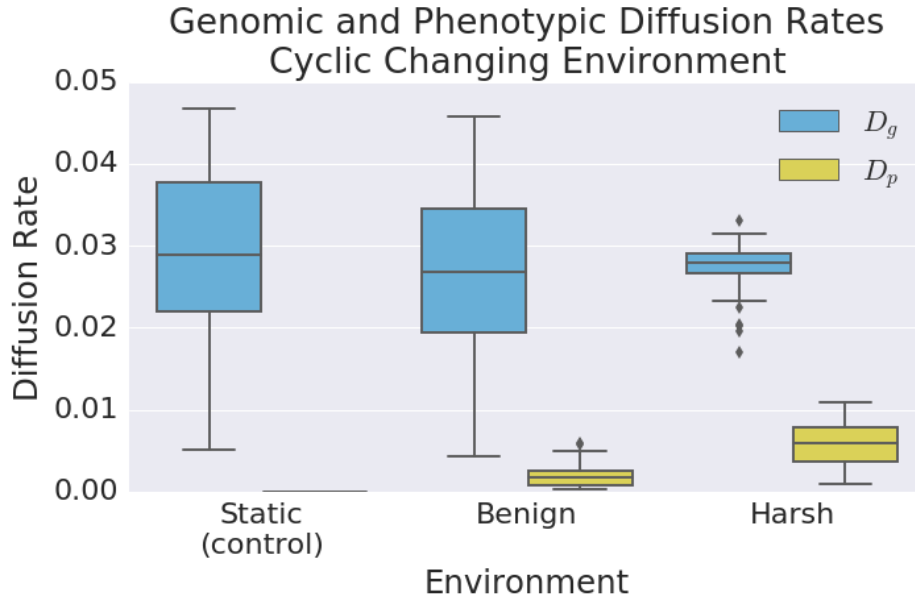
Figure 2.9: **Genomic and Phenotypic Diffusion Rates**, showing the probabilities of producing offspring that are genotypically (D_g) or phenotypically (D_p) distinct from the parent, while not reducing fitness. Note that while overall neutral exploration capacity remains relatively stable between treatments (Kruskal Wallis: H(2) = 1.44, p = 0.49), phenotypic exploration capacity is increased in both treatments, but especially in the Harsh treatment. (Wilcoxon Rank Sum Test: Z = -8.02, -8.39, respectively, p << 0.001). This result indicates that changing environments promote the phenotypic evolvability of populations.
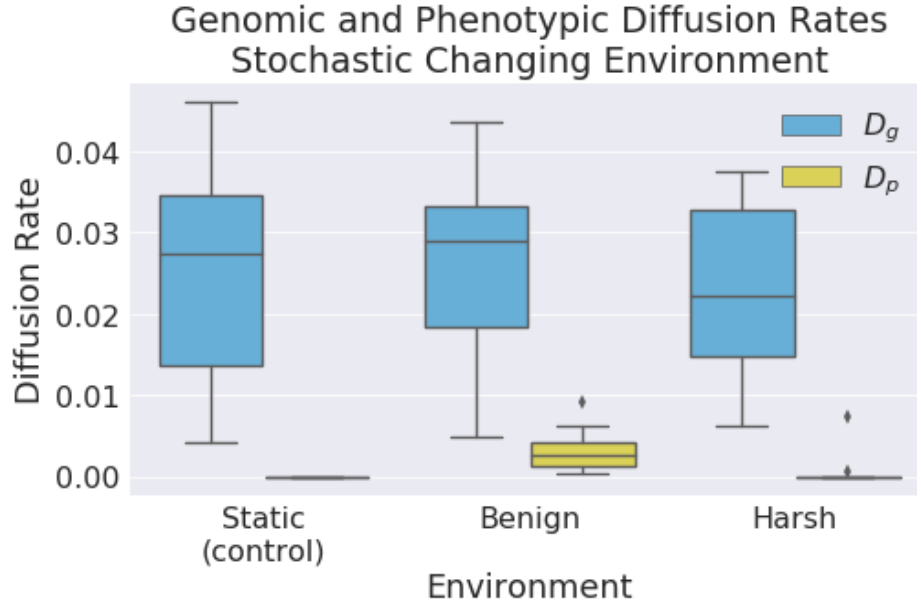
Figure 2.10: **Genomic and Phenotypic Diffusion Rates** in stochastic changing environments, showing the probabilities of producing offspring that are genotypically and phenotypically different from the parent, while remaining fitness neutral or better. As in the cyclic environment, D_g remains stable for the Benign treatment, but drops slightly in the Harsh as compared to the control, though this drop isn't statistically significant (Kruskal Wallis H(2) = 1.11, p = 0.57). Harsh D_p however, is significantly lower than seen in the cyclic environment treatment (Wilcoxon Rank-Sum Test: Z = -6.19, p << 0.0001). This result shows that harsh stochastic environments may not be as effective as cyclic environments at increasing the probability that organisms will produce phenotypically different, yet neutral offspring.

2.3.2 Stochastic Changing Environments

Contrary to our expectations, stochastic changing environments were, overall, no more effective at promoting evolvability than cyclically changing environments. Harsh environments performed slightly worse, whereas benign environments were slightly better, though neither result was consistently statistically significant. There was a slight but significant reduction in the Phenotypic Diffusion Rate (D_p) between the cyclic and stochastic harsh changing environments; D_p settled on a lower median (Mdn = 0.0) in the stochastic harsh treatment as compared to the cyclic harsh (Mdn = 0.0058) (Wilcoxon Rank-Sum Test: Z = -6.19, p << 0.0001), indicating a lower probability of the population producing offspring that would

switch phenotypes neutrally. In the Benign treatments, however, Stochastic environments fared slightly better than cyclic, though this result was only barely statistically significant (Wilcoxon Rank-Sum Test: Z = 2.2, p<0.03). (Fig 2.10)

Despite the reduced D_p in the harsh treatment, both the overall fraction of 1-step mutants that lost EQU, and the fraction of 2nd-step regaining of EQU, were only very slightly reduced in comparison to the cyclic treatments, and this effect was not statistically significant(Fig 2.11, 2.12). This result indicates that stochastic harsh environments are certainly no more effective at promoting evolution toward areas of the mutational landscape where such mutations were common, and may, in fact perform worse.
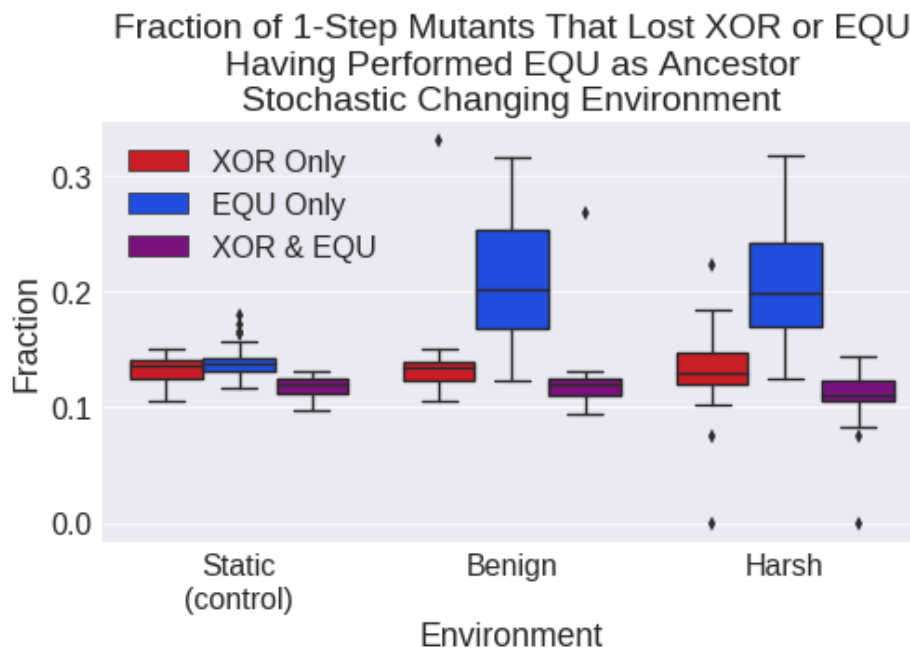
Figure 2.11: **A survey of the single-step mutational neighborhood** in the stochastic changing environment around organisms that performed the fluctuating task. Again, in the static and harsh treatments, values are comparable to the cyclic changing environment . However, in the benign treatment, the mean for the loss of the fluctuating task (EQU) was slightly higher in the stochastic treatment (Mdn = 0.2) than the cyclic (Mdn = 0.17), though the effect is barely statistically significant (Wilcoxon Rank-Sum Test: Z = -2.4, p = 0.015). This result indicates that stochastic environmental change is equivalently effective at moving organisms to areas of the fitness landscape where they can more easily switch task expression.

In contrast, we observed a very slight, but statistically significant increase in the fraction

of 1-step mutants that lost EQU in the stochastic treatment versus the cyclic (Wilcoxon Rank-Sum Test: Z = -2.4, p = 0.015). This effect was matched by a slight increase in the fraction of 2-step mutants that regained EQU in the benign stochastic treatment vs cyclic (Wilcoxon Rank-Sum Test: Z = -18.42, p << 0.0001). This indicates that in stochastic changing environments, benign environments might possibly perform better than harsh environments for promoting evolvability. However, because the effect sizes were so small, we cannot conclusively find that stochastic environments performing any differently than cyclic at promoting evolvbility.
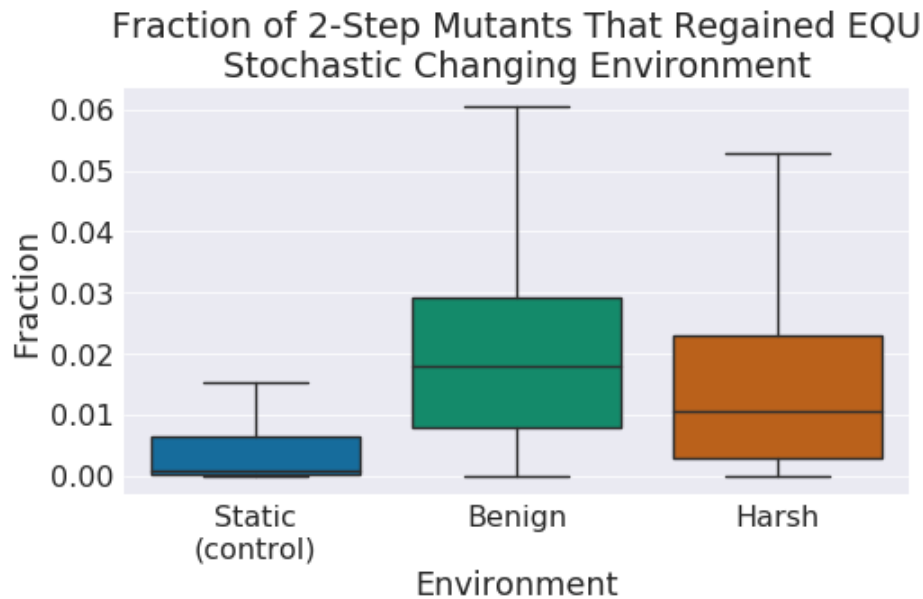
Figure 2.12: **A survey of the two-step mutational neighborhood** in the stochastic changing environment of the organisms that lost EQU function in the one-step survey. We found that the fraction of organisms regaining the fluctuating task (EQU) from a single additional mutation in the harsh treatment (Mdn = 0.013) were reduced compared to the cyclic harsh treatment (Mdn = 0.01) (Wilcoxon Rank-Sum Test: Z = 12.75, p << 0.0001) The opposite, however, was true of the Benign treatments. As in Fig 2.11, we find that stochastic outperforms cyclic (Wilcoxon Rank-Sum Test: Z = -18.43, p << 0.0001) This result confirms that the harsh stochastic environment is probably less effective than the cyclic harsh at promoting evolvability, but that the opposite may be true for a benign environment.

Few differences between the cyclic and stochastic treatments also appeared in the number of functional and vestigial sites. Both the numbers of functional and vestigial sites in the

stochastic environment were similar to those in the cyclic environment. In the stochastic harsh treatment, there was a small, but significant reduction in the number of XOR+EQU overlapping functional sites (Mdn = 14) as compared to the cyclic treatment (Mdn = 16) . (Fig 2.13)
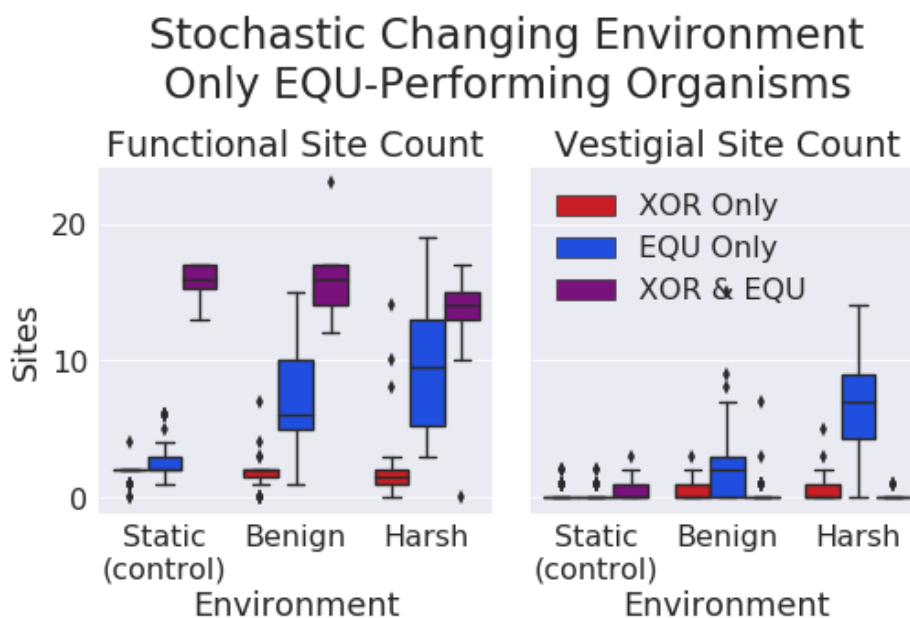
Figure 2.13: **Number of functional and vestigial sites by treatment** in a stochastic changing environment. The vestigial site counts for EQU-only performing organisms remain comparable to the cyclic environment (Wilcoxon Rank-Sum Z = 0.46, 1.45, and -1.10, p = 0.6, 0.14, and 0.2). The functional sites were also comparable, however, there was a slight, but statistically significant decrease in the number of XOR & EQU overlapping sites in the stochastic vs cyclic environments (Wilcoxon Rank-Sum Test: Z = -3.05, p<0.01).

Together, from these measures, we conclude that stochastic environments exert similar evolutionary pressure to move toward regions of the mutational landscape that are more congenial to neutral phenotypic exploration and evolvability. However, the combination of the slight improvements in benign stochastic environments, matched by slight decreases in effectiveness in harsh stochastic environments, suggests that the periodicity of the cyclic environment provides a slight advantage to adapting to harsh environments. In the benign environments, however, the stochastic nature may be less of a disadvantage. We hypothesize that this dynamic may be due to the randomly-occurring environmental changes either

occurring 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. We 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.

These results suggest, therefore, that architectural features that help with evolvabilty are more likely the result of repeated hitchhiking on adaptive mutants. In particular, we observed that much of the task-loss associated with the harsh changing environment could be attributed to increasing task length which is a result of the continuous addition of new mu-

tations activating and deactivating the task. Despite this correlation, however, we observed a potential difference in robustness between the XOR and EQU tasks, which suggest that a kind of anti-robustness may also be selected for as a result of the changing environments.

Surprisingly, stochastically changing environments are not more 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.

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 (transformation via natural competence [63]), or else receive them via bacterial conjugation [64] or viral infection (transduction [65, 66]). In the case of transformation, the fragments may either be decomposed inside the recipient cell for their nutrients, or recombined 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" [67]. For example, HGT appears to be the primary mechanism by which antibiotic resistance is conferred [68, 69] 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 Horizontal Gene Transfer in nature

In prokaryotes, "transformation" is an HGT mechanism by which organisms spontaneously uptake the DNA of dead organisms in the environment. Competent organisms benefit in several ways. 1) DNA is composed of a 5-carbon sugar, a phosphate, and nitrogenous bases, materials that are useful for DNA synthesis and repair. 2) The organisms may also benefit from taking up gene fragments that confer new adaptive functionality into the genome [70]. However, it is unclear whether the origins of transformation functions were developed solely in

order to obtain nutrients (the Grazing Hypothesis), or if the acquisition of new functionality was selected for as well. While grazing for gene fragments as nutrients certainly conveys an advantage, the possibility of integrating these gene fragments is likely to be deleterious to organisms more often than it is beneficial [71]. For example, since the DNA would be originating from dead organisms, DNA fragments may be of low quality, and recombine deleterious mutations, or even remove competence altogether [71]. Alternately, errors in homologous recombination may apply fragments to random locations in the genome.

Although advances in molecular biology are allowing researchers to investigate the mechanisms of HGT and even reconstruct specific inferred cases of HGT (as reviewed in Bock 2015 [72]), the processes themselves are ancient and nearly ubiquitous [73]. This very ancientness makes studying the early evolution of HGT in physical organisms exceedingly challenging, as we lack easily tractable systems that differ only in whether or not HGT exists within them. Therefore, empirical studies in natural systems remain exceedingly rare.

In order to test the Grazing Hypothesis of the origin of HGT, and to address the question of whether there are circumstances where gene fragment integration may be beneficial, subjected populations of evolving digital organisms to a harsh changing environment, where there is a strong selective pressure to quickly switch phenotype. We supplied organisms with an instruction that performs Horizontal Gene Transfer (`HGT-Uptake`). That is, the instruction triggers uptake of a genetic fragment from the environment, and there is a probability 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, we collect fragments of their genomes in reservoirs. These fragments 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.

look for and hunt down passive voice

We have implemented a configurable probability that, when a fragment is taken up, it is not metabolized; instead, homologous recombination may occur (Figure 3.1). We performed experiments to derive bonus levels, fragment sizes, and recombination probabilities to arrive at a maximum use level for the HGT instruction. See Appendix B for more details.

For all experiments described in this chapter, we used a 10% recombination probability. Please refer to Appendix B for more information about the selection of this probability value. We also required three instructions as the minimal homologous match length on either side of the fragment. Three instructions on either side of the fragment (26^3 unique sequences) approximates the number of unique values keyed by 7 nucleotides (4^7 unique sequences). For the purposes of homologous recombination in plasmid cloning in E. coli, 20bp is enough to assume identity [74].

3.2.1.1 Environmental Conditions

All experiments in this chapter compared outcomes between static environments, and harsh changing environments. In a similar manner to the experiments listed in the previous chapter (Chapter 2, Table 2.1), task rewards in the harsh changing environment switched 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. There are four
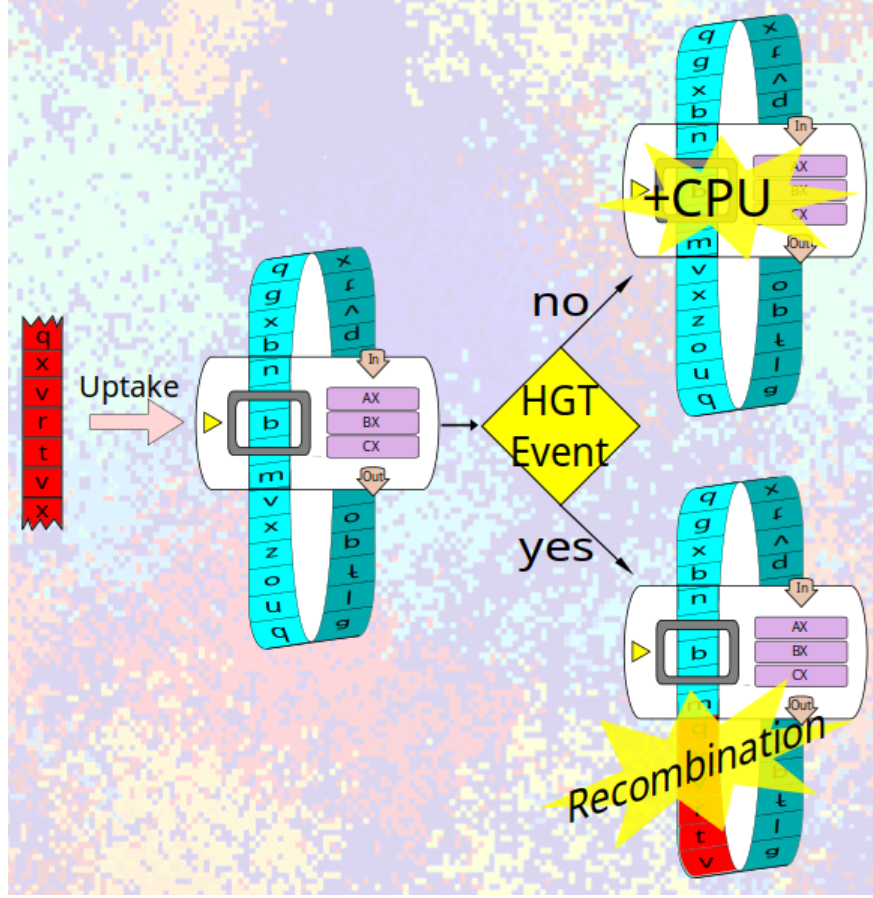
Figure 3.1: **The HGT process**. Organisms can execute instructions that trigger uptake from the environment. When uptake occurs, there is an experimenter-defined chance that either it will yield a boost to speed of execution or, alternatively, that the fragment will be integrated into the genome.

pairs of tasks of equivalent complexity, and we randomly allocated one from each pair to an experimental group. We also assigned EQU, which is the most complex task, and has no equivalent, to a random group. Thus, for the first phase of the cycle, we punished the NOT, AND, OR, NOR, and EQU instructions at -2^1, -2^2, -2^3, -2^4, and -2^5 respectively, while rewarding NAND, ORN, ANDN, and XOR at 2^1, 2^2, 2^3, and 2^4. In the second phase of the cycle, these rewards flipped, such that we rewarded NOT, AND, OR, NOR, and EQU, and punished NAND, ORN, ANDN, and XOR (Table 3.1).

Each complete cycle lasted 1,000 updates, and the whole experimental run extended for 200,000 updates. The static environment rewarded executions of all the Logic-9 tasks at

Table 3.1: **Logic-9 Rewarded Task Groupings**

	Tasks	Reward Phase 1	Reward Phase 2
Group 1	NOT	2^1	-2^1
	AND	2^2	-2^2
	OR	2^3	-2^3
	NOR	2^4	-2^4
	EQU	2^5	-2^5
Group 2	NAND	-2^1	2^1
	ORN	-2^2	2^2
	ANDN	-2^3	2^3
	XOR	-2^4	2^4

Logic-9 tasks were divided into to groups, with one task from each pair of tasks of equivalent complexity assigned to a group. The EQU task, which has no complexity equivalent, was arbitrarily assigned to the first group. During the first half of a cycle, we rewarded the first group of tasks and punished the second group (see Reward Phase 1). During the second half of the cycle, reversed the pattern, and rewarded the second group, and punished the first group (Reward Phase 2).

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 3.2), 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.

For the second set of hypotheses, where we identify the mechanisms that promote the use of HGT, we manipulated the content of the reservoirs to contain fragments drawn from specific phases in the cyclically changing environment, such that fragments either matched or did not match the environment (Table 3.3. We then measured HGT use, as well as average fitness effects of the HGT mutations, and the fraction of mutations that led to beneficial

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

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

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 3.3: **Experimental Treatments - Effects of HGT**

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

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

phenotype switches.

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table

3.3   Results and Discussion

Our results, discussed in detail below, show that both an uptake bonus and changing environment promote the use of HGT, and that the increases in uptake are largely additive. Further, we found that while on average, HGT mutations are neutral, that the majority of the benefits conveyed by HGT in changing environments come from fragments originating in periods of the cycle where the environment matched the current environment. This result indicates that it is the information content of the fragment that is valuable. Finally, we find that providing only fragments from the matching cycle elevates uptake rates significantly.

3.3.1   Changing environments elevate HGT use

We measured HGT fragment uptake in four conditions (see Table 3.2), plus of pair of non-HGT controls. Without a bonus, in a static environment, fragment uptake was depressed to a low level as compared to the rate of the non-HGT control, where the `HGT-Uptake` instruction does nothing (Wilcoxon Rank-Sum Test: Z = 9.74, p << 0.0001). This indicates

that HGT in a static environment is largely deleterious (Figure 3.2). However, in a no-bonus harsh changing environment, fragment uptake was elevated compared to the static environment (Wilcoxon Rank Sum-Test: Z = -8.44, p << 0.0001). This shows that in the context of a harsh changing environment, the integration of new genetic material is more beneficial than in the static environment. We also found that, regardless of whether the environment is static or changing, when a bonus to fragment uptake was provided (analogous to the nutritive benefit granted by natural competence in biological organisms), fragment uptake also increased (Wilcoxon Rank Sum Test: Z = -8.44, and -7.47, p << 0.0001).
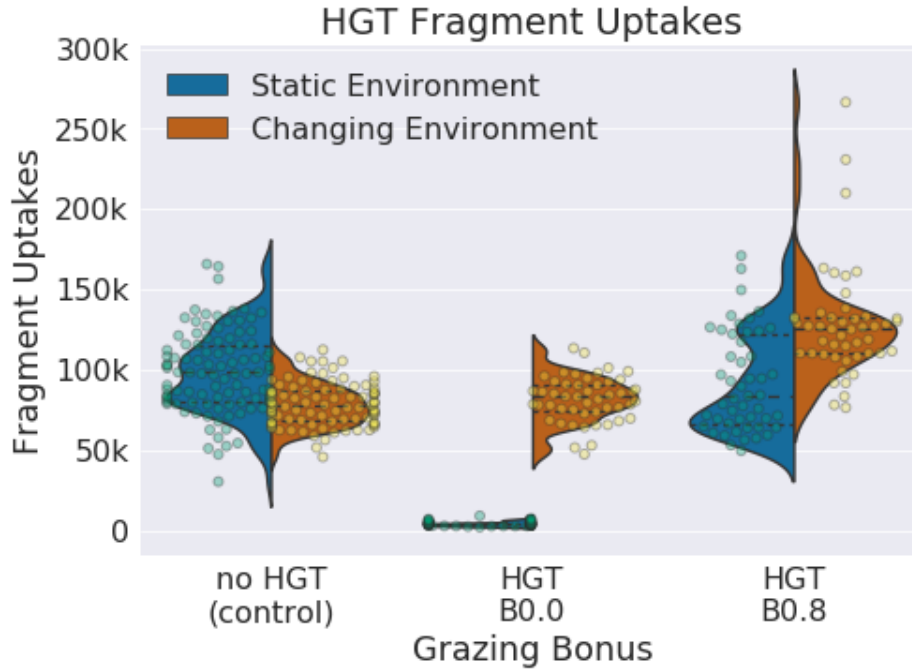
Figure 3.2: **Number of HGT fragment uptakes** in static and changing environments, without a grazing bonus, with a grazing bonus, and a no HGT control, where the `HGT-Uptake` instruction is inert. HGT uptake increased in response to a changing environment, and also in response to a grazing bonus. Both the grazing environment and a changing environment resulted in an even larger increase of fragment uptakes than either the changing environment, or the bonus alone (Wilcoxon Rank Sum Test: Z = -4.93 and -7.47 respectively, p << 0.0001).

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 increased largely additively. This indicates that the benefits granted by a grazing bonus are generally independent of the benefits conveyed by integrating new genetic material.

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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.

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

3.3.2.1 Fitness Effects

In order to understand the basis of the beneficial nature of HGT in changing environments, we measured the fitness effect of individual fragments on individual organisms within a population. We calculated the average fitness effects of fragments of the non-replaced HGT control treatment at the end of the last environmental cycle, and generated a distribution of fragments, and sorted the fragments by age and fitness-effect (Figures 3.3). We observe a clear pattern of more beneficial fitness effects from fragments of organisms originating in the matching cycle phase.

To quantify this observation, we performed experiments where we replaced the fragments in the reservoir with fragments originating in the matching phase, the off-phase, and mixture of both phases as a control. We measured the mean and median fitness effects of fragments in these treatments (Figure 3.4). We found that HGT mutations were, on average, neutral, or nearly neutral in all the treatments. Both the non-replaced control (Mdn = 0.0, 95% CI [0, 0]) and the replaced-"both" treatment (Mdn = -0.008, 95% CI [-0.009, -0.008]) were neutral or nearly neutral. The median fitness effect in the "on-phase" treatment was mildly deleterious (Mdn = -0.054, 95% CI [-0.055, -0.052]), while the mean was more strongly
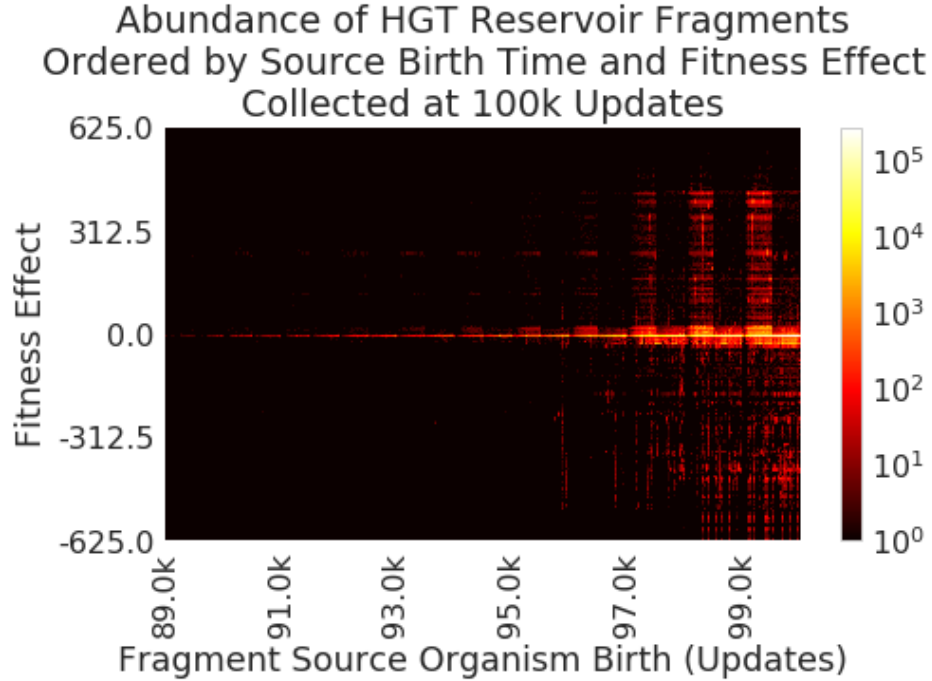
Figure 3.3: **Origin and fitness effect distributions of HGT fragments**, sampled midway through the experiment at 100,000 updates, and aggregated across all replicates. We applied each fragment in each cell's reservoir, one at a time, to the organism in the cell, then recorded the fitness effect. The x-axis is the birth update of fragment's original donor organism. The y-axis is a fragment's fitness effect. The color of each point represents the number of fragments that originate in that birth update a given fitness value. Hotter values indicate more fragments from that time origin at that fitness effect. Most fragments with a positive fitness effect (the upper half of the figure) appear in bands that correspond with the matching phase of an earlier cycle.

beneficial (M = 1.19, SD = 19.7). In contrast, in the "off-phase" treatment, the median fitness effect was, again, mildly deleterious (Mdn = -0.0005, 95% CI [-0.0006, -0.0004]), but with a much more deleterious mean (M = -23.06, SD = 101.8). This suggests that while, on average, HGT mutations remain nearly neutral, a few fragments from the matching phase were strongly beneficial, while the opposite was the case for the non-matching phase.
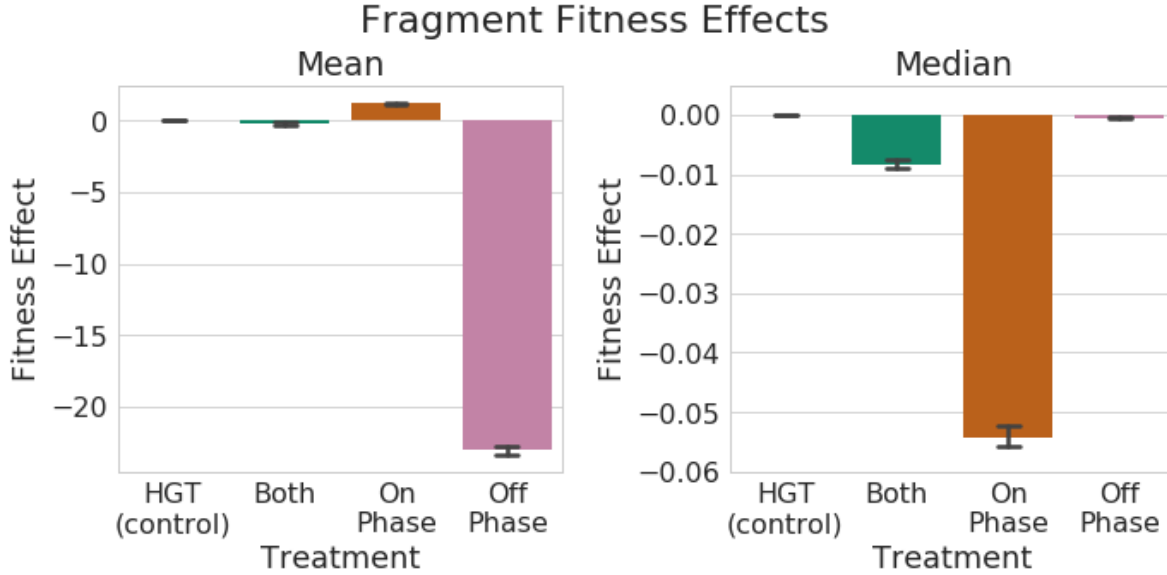
Figure 3.4: **Mean and Median fitness effects of fragments** in reservoirs, with 95% confidence intervals. Fragments in reservoirs are replaced by fragments based on the origin of donor organism. For the On-Phase treatment, We only permitted fragments in reservoirs that originated in a matching phase of the current cycle. For the Off-Phase treatment, we only permitted fragments from non-matching phases. Plus two controls: first, where no filtration takes place, and "Both" where fragments are injected from a mixed set of origins. The Off-Phase treatment had a significantly lower fitness effect than either of the controls (Wilcoxon Rank-Sum Test: Z = 143 and 31 respectively, p << 0.001), while the On-Phase treatment had a significantly better fitness effect (Wilcoxon Rank-Sum Test: Z = 105 and 3 respectively, p < 0.002).

3.3.2.2 Beneficial Phenotype Switching

In our environments, mutations that convey phenotypic change should have the largest impact. Specifically, 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. We quantified 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 saw that a significantly larger proportion of fragments in the "on-phase" treatment produced beneficial phenotype changes (Mdn = 0.09, 95% CI [0.07, 0.11]), as compared to fewer in the normal HGT control (Mdn = 0.03, 95% CI [0.03, 0.04]) and "both" treatments (Mdn = 0.04, 95% CI

[0.035, 0.05]) (Wilcoxon Rank Sum Test: Z = -6.42 and -6.12 respectively, p << 0.001). In contrast, we observed virtually no beneficial phenotype changes originating in fragments from the "off-phase" treatment (Mdn = 0.009, 95% CI [0.006, 0.01]). This result was significantly worse than both the control and the "both" treatments, which produced non-zero beneficial phenotype switches (Wilcoxon Rank-Sum Test: Z = 7.11 and 7.35 respectively, p << 0.001)
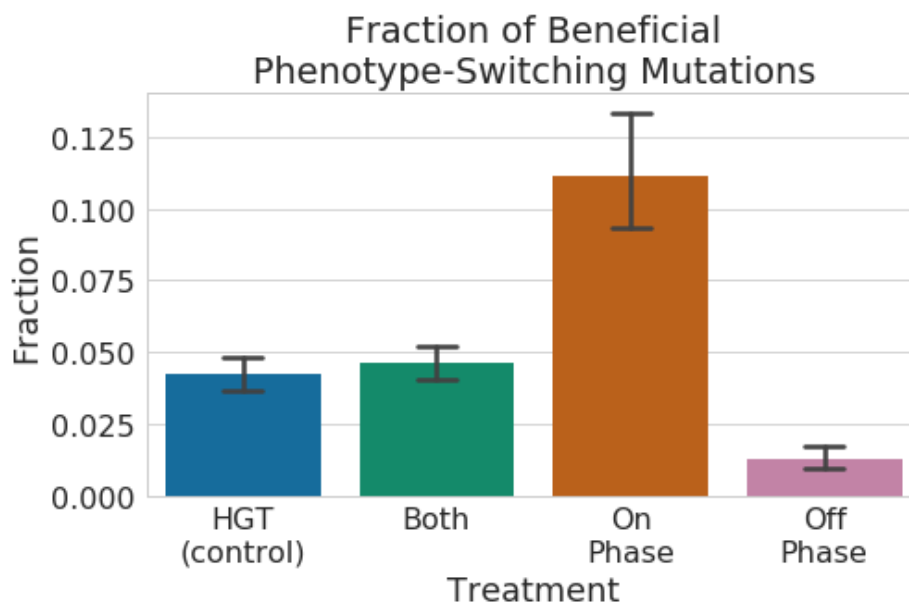
Figure 3.5: **Fraction of all fragments that produced a beneficial phenotype changes**, with 95% confidence intervals. The "On-Phase" treatment had significantly more fragments that convey a beneficial phenotype change than either of the controls (Wilcoxon Rank Sum Test: Z = -6.42 and -6.12 respectively, p << 0.001), while the "Off-Phase" had significantly fewer (Wilcoxon Rank-Sum Test: Z = 7.11 and 7.35 respectively, p << 0.001).

These results suggest that most direct benefit of using HGT derives from taking up 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, we 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 observed just such an increase. Thus we can conclude that HGT in our 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 was available, HGT use was not significantly depressed as compared to the control treatment. This suggests that despite the lack of exclusively matched environmental information, that fragments could still provide some benefit.
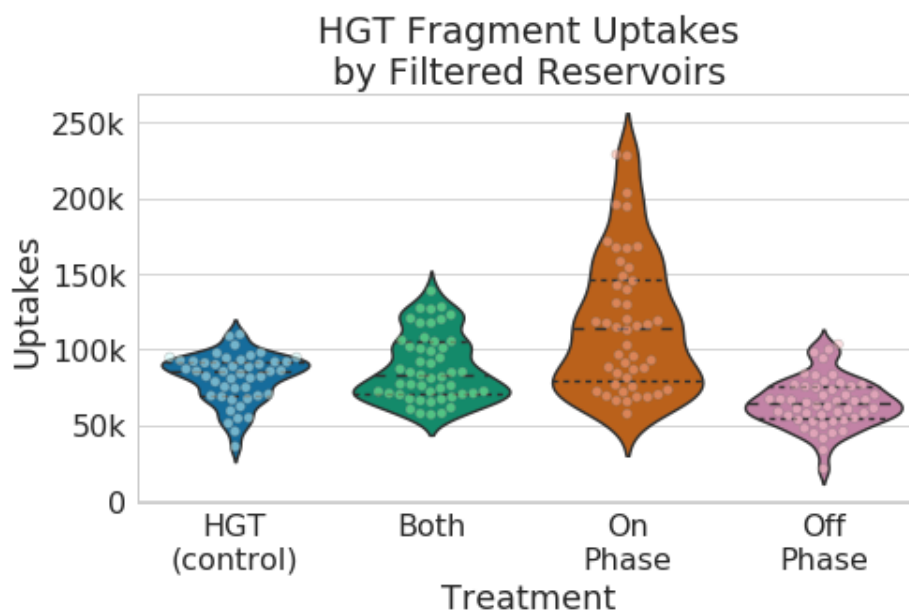
Figure 3.6: **Number of uptaken fragments** in the filtered reservoir treatments. The "On-Phase" treatment has a significantly larger number of HGT fragment uptakes, as compared to control, "both", and "off-phase" treatments (Wilcoxon Rank-Sum Test: Z = -3.9**, -3.2*, and -6.5**, respectively, $p < 0.002$* and $p \ll 0.001$**) This shows that HGT is significantly more beneficial when fragments match the current phase of the cycle. Thus, this suggests that the primary benefit from HGT is not mutational disruption, but the information that fragments convey about the current environment.

3.4 Conclusion

Our experiments confirm our expectations about the evolution of horizontal gene transfer, and match what we would expect to see in nature. First, we find that grazing bonuses, such as those conveyed by the uptake of nutrient-rich DNA fragments in natural systems, cause an elevation of use of the horizontal gene transfer instruction, regardless of the potential risk

of recombining uptaken gene fragments.

Further, when there is direct selection for phenotypic change - such as in a changing environment - we observe an increase in the use of the HGT instruction, even when no grazing bonus is offered. This indicates that the possibility of recombination itself is being selected for. Beyond this, we find that it is not only the fact of the recombination that is beneficial, but the information contained in the fragment. We found that most of the benefit conveyed by HGT derived from fragments that match the current environment, but also that when we filter so that only those fragments are available, that use of the HGT instruction increases even further.

In natural systems, this pattern in evident in the acquisition of antibiotic resistance in bacteria, where resistance is acquired by the spread of resistance genes via horizontal gene transfer. Therefore, our results provide a key insight into how horizontal gene transfer mechanisms could be selected for, and evolve in natural systems.

It is worth noting that most prior studies of HGT have been observational studies that look at existing traits and phylogenies and attempt to make inferences about the evolution of those traits on that basis. Performing experimental evolution studies of the evolution of HGT in organic systems is currently intractable. Digital evolution, on the other hand, allows us to explore and test many hypotheses about the evolution of HGT, such as the explicit benefits and consequences of different types of mutations. It gives us an opportunity to compare granular fragment grazing bonuses and quantify their effects on evolutionary outcomes. These experiments are ones that we would not be able to perform any other way.

CHAPTER 4

HORIZONTAL GENE TRANSFER VS OTHER TYPES OF MUTATION

4.1 Background

In both natural and artificial systems, populations evolve mechanisms to reduce or eliminate mutations [75], despite evidence that higher mutation rates are optimal for adaptive evolution [76]. In artificial systems, prior research has failed to show a substantial increase in endogenously-controlled mutation rates, even when such an increase would be beneficial for long-term adaptive evolution, such as in a changing environment [76]. However, Horizontal Gene Transfer through transformation seems to be an exception to this rule.

In nature, transformation is a highly regulated and refined mechanism for taking up DNA fragments from the environment [77, 78, 79]. Clearly, evolution is acting to maintain and preserve these functions, despite the risk of harmful recombinations. In the previous chapter, we showed that HGT is under positive selection in harsh changing environments. Despite the fact that the fitness effects of recombination tend to be neutral to slightly deleterious, HGT use is upregulated. What makes HGT different from other types of mutations? Is it the fitness effect? The information content of fragments? The probability of phenotypic changes? Furthermore, we need to ask what makes HGT special as compared to other types of mutations in these respects. And can any of the differences the we uncover account for the increased selection pressure for HGT as compared to other mutation types?

In order to address these question, we created instructions that create different kinds of mutational effects with similar per-instruction impact to HGT. Each new instruction controls for one specific aspects of HGT to determine how important it is as compared to others. We performed experiments with these new mutagenic instructions under the identical conditions as our evaluation of HGT, in static vs. changing environments. We found that not only are these new instructions triggered at significantly lowered rates as compared to HGT, but that

the rate at which they are used correlates significantly with the amount of useful information expected to be conveyed by the mutation.

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 collected from reservoirs in the environment.

All experiments in this chapter compare outcomes between static environments and harsh changing environments. Similarly to the experiments in the previous chapter, task rewards in the harsh changing environment switch from a positive to a negative reward between the divided set of tasks in the Logic-9 environment. Please refer to Table 3.2 for additional details. Each complete cycle lasts 1,000 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 Mutagenic Instructions

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 mutagenic instructions have the same 10% probability of triggering their mutagenic effect. When relevant, all instructions also have the same viable recombination site requirements as the default HGT instruction.

The specific set of possible instructions are:

- **HGT (Intact Fragment)**: The default HGT operation. We uptake a fragment from the environment. We apply 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. We use the first valid match. We find valid matches by selecting a random location on the genome, then search from there. We loop around the whole genome, past the end and into the beginning, until we find a for the front three instructions of the fragment. Then, we begin searching for a match for the back three instructions of the fragment, starting at the edge of the front match. If a valid back-end match site is not found, we scan forward, looking for the next front-end match and repeat the process until all possible match sites on the genome are exhausted. 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**: We uptake a fragment from the environment, and a valid recombination site is found, as above. Prior to recombination, however, we shuffle the fragment. The identical set of instructions are inserted, but the order of each instruction randomized. We then insert the fragment at the selected site.

- **HGT Random**: We uptake a fragment from the environment, and find a valid re-combination site, as above. Prior to recombination, however, we replace the entire fragment with an equal number of randomly selected instructions, and insert this new sequence at the selected site.

- **Mutation Event - Sampled**: We uptake a fragment from the environment, and find a valid recombination site, as above. Rather than recombine, however, we apply a number of point mutations in random locations throughout the genome. The number of applied mutations matches the number of instructions in the fragment. The mutations applied are sampled, with replacement, from the selected fragment. This mutagenic

instruction 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**: We uptake a fragment from the environment, and find a valid recombination site, as above. Rather than recombine, we apply a number of random point mutations, matching the number of instructions in the fragment. This mutagenic instruction is analogous to the **HGT Random** mutagen, except that, again, its effects are applied randomly throughout the genome instead of at a single location.

- **Mutation Rate Increase**: We uptake a fragment from the environment, and find a valid recombination site, as above. Rather than recombine, we increase the point-mutation rate for the organism, such that it will experience an expected additional number of point mutations matching the number of instructions in the uptaken fragment.

- **Die**: We uptake a fragment from the environment, and find a valid recombination site, as above. Rather than recombine the fragment in, the organism instead dies. As with several of the other cases, the finding of a recombination site is simply to control for the frequency of success to ensure that this effect is trigged with the same probability as recombination.

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).

The goal of each treatment is to compare the use rates of each of the different mutagenic instruction across several factors. We can examine the effects of localization (concentrating

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

Treatment	Environment	Mutagenic Instructions
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	`ME-Sampled`
ME-Sampled CE	Changing	(Mutation Event - Sampled)
ME-Random	Static	`ME-Random`
ME-Random CE	Changing	(Mutation Event - Random)
MRI	Static	`MRI`
MRI CE	Changing	(Mutation Rate Increase)
Die	Static	`Die`
Die CE	Changing	

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

mutations in a single area) by comparing the `HGT-Random` vs the `ME-Random` treatments. We can measure the effect of time-concentration (all mutations happening at once vs spread out over time) by comparing the `ME-Random` and `MRI` treatments. We can examine the effect of information content by comparing the `HGT`, `HGT-Shuffle`, and `ME-Sampled` treatments. We can identify a basement-level for mutagenic instruction use by comparing against the use of the entirely deleterious `Die` instruction. Finally, we can compare all these factors with their environment by comparing the static vs changing environment treatments.

4.3   Results and Discussion

Our research shows that only mutagenic instructions containing useful information are elevated in response to changing environments. Intact-fragment HGT is used most, with shuffled-fragment (`HGT-Shuffle`) used less, and the other, non-information-bearing mutagen types used at the lowest levels. Further, we find a positive correlation between expected fitness and mutagenic instruction use. That is, mutagenic instructions that have higher ex-

pected fitness effects are used more. Finally, we see a strong positive correlation between the fraction of mutations from a mutagenic instruction that provide a beneficial phenotype-altering change, and the resulting use of that instruction.

4.3.1 Other mutation types are not elevated in response to HGT
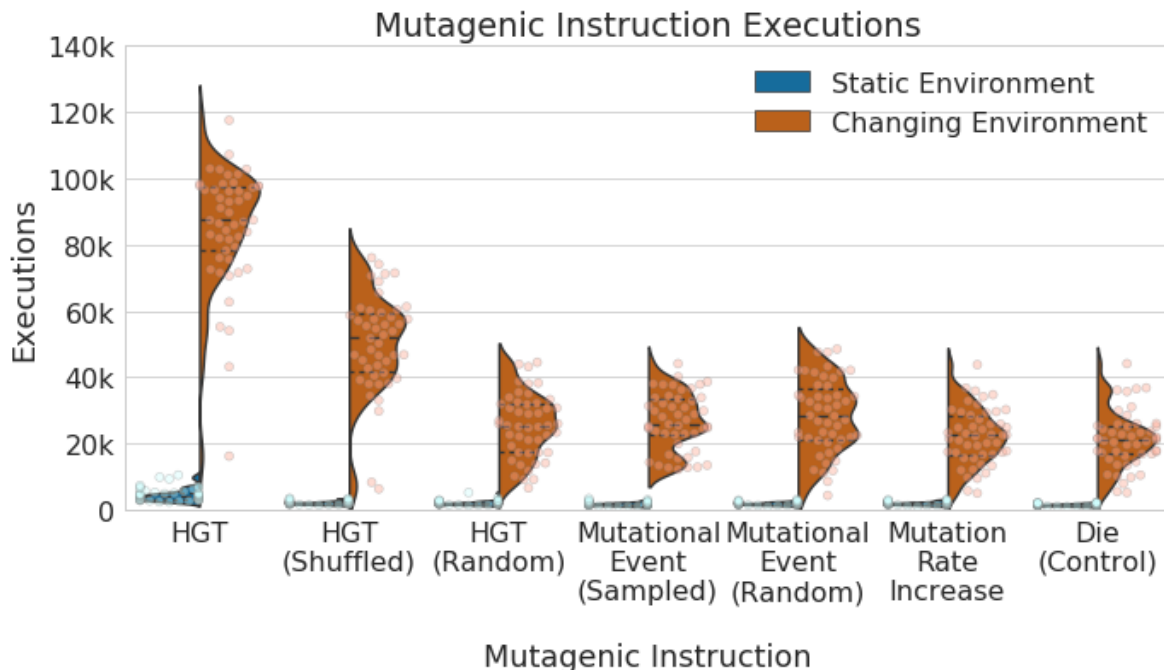
Figure 4.1: **Executions of mutagenic instructions**. In static environments, use of all mutagenic instructions was strongly suppressed (Wilcoxon Rank Sum Test: Z = -22.65, p<<0.001). In changing environment treatments, the `HGT` and `HGT-Shuffled` instructions were used at elevated rates, compared to the other mutagenic instructions, including the `Die` control (Wilcoxon Rank Sum Test: Z = 10.66, p<<0.001). The `HGT` instruction (Mdn = 87389.71, CI 95% [82982.98, 96000.3]) is used more than the `HGT-Shuffle` instruction (Mdn = 52220.3, CI 95% [45094.92, 57465.52]), which is used more than `HGT-Random` (Mdn = 25442.98, CI 95% [22099.45, 30490.08]), indicating that the information content of the mutation matters.

In order to compare the use of HGT against other kinds of mutations, we compared rates of non-bonus HGT fragment uptake against a series of other mutation types, in both static and changing environments (Fig 4.1). As expected, in the static environment, endogenously-controlled performance of all types of mutations (including HGT) is strongly suppressed

(Wilcoxon Rank Sum Test: Z = -22.65, p << 0.001). However, in harsh changing environments, use of HGT dominates the other mutation types (Wilcoxon Rank Sum Test: Z = 10.66, p << 0.001). Of particular interest is how the use of the HGT-like instructions are used most by order of information content. The baseline HGT instruction is used most (Mdn = 87389.71, CI 95% [82982.98, 96000.3]), followed by the `HGT-Shuffle` instruction (Mdn = 52220.3, CI 95% [45094.92, 57465.52]), and then followed by HGT-Random (Mdn = 25442.98, CI 95% [22099.45, 30490.08]), where no information remains. The latter (Mdn = 25,442.98, CI 95% [22099.45, 30490.08]) performs comparably to the remaining mutation event types: Mutation Events: `ME-Sampled` (Mdn = 25,798.03, CI 95% [24335.01, 30824.09]), `ME-Random` (Mdn = 28,369.85, CI 95% [22829.31, 33745.83]) (Kruskal-Wallis $H^2 = 1.91$, p = 0.3). The `MRI` and `Die` instructions performed at even lower levels (Mdn = 22493.75, CI 95% [19330.91, 24818.06] and 21021.12, CI 95% [17722.93, 23308.02]), respectively). This result is consistent with our hypothesis that the information content of the fragment is an important predictor of the use of HGT.

Also of note is that the only difference between the `ME-Sampled` and `HGT-Shuffle` treatments is the localization of the mutation effect (`HGT-Shuffle` fragment insertion is applied to a single location, whereas with `ME-Sampled`, the instructions are scattered randomly throughout the genome), however their use rates are significantly different (Wilcoxon Rank Sum Test: Z = 7.19, p << 0.001). This result indicates that not only is information content important, but that localization (clustering) of the mutation effect also matters. We speculate that this effect may synergize with the level of modularity in a genome. If a genome is even slightly modular, there are gradients of relatedness between loci that decrease with physical distance. A single mutation is enough to disrupt a function. Thus, if all mutations are concentrated in one region, they would be more likely to affect fewer total functions. Thus, concentrating mutations to a single area may limit the reach of the damage, so to speak, as opposed to hitting many different locations at once. We would expect this effect to increase as modularity of a genome increases.

4.3.2 Mutation fitness effect correlate with mutagenic instruction use
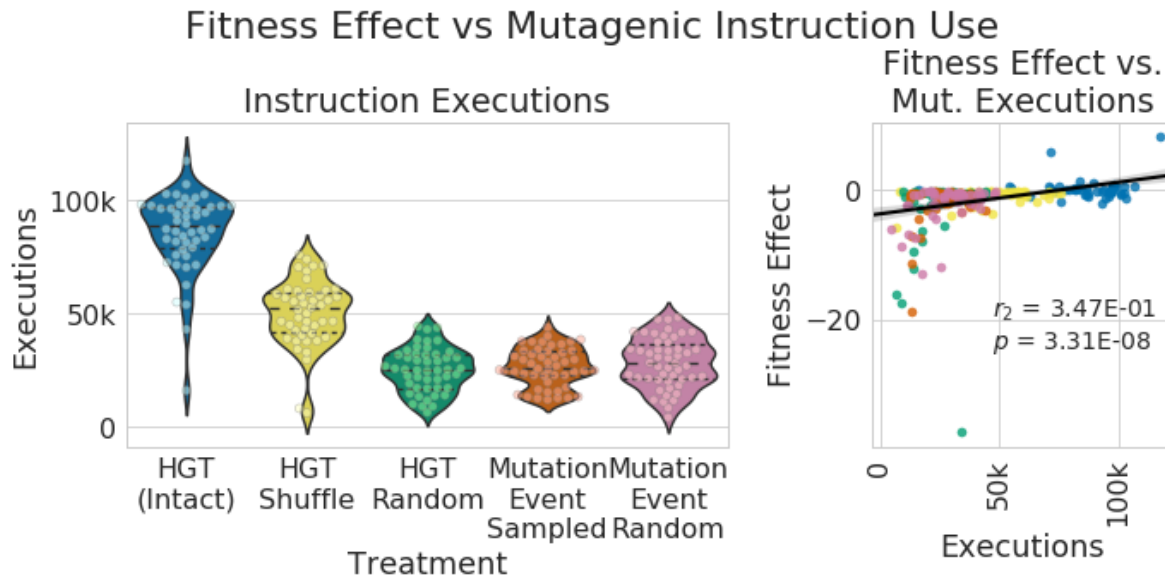
Figure 4.2: **Mutagenic instruction use vs fitness effect of mutation**. The use of the mutagenic instruction correlates with the fitness effect of that instruction (Pearsons R: r_2 = 0.35, p << 0.001). Because mutagenic instructions are integrated into the genomes, those instructions that produce a beneficial fitness effect are more likely to be selected for.

Prior research has failed to show a substantial increase in endogenously-controlled mutation rates, when such an increase would be beneficial for long-term evolution, such as in a changing environment [76]. 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 positive correlation between the average fitness effect, and the use of the mutagenic instruction (Pearson's R: r_2 = 0.35, p << 0.001). We observed many more positive or neutral fitness effects from the HGT instruction (Mdn = 0.08, CI 95% [-0.12, 0.37]) than the other mutagenic instruction types, which exhibited primarily neutral or negative mutation effects (Mdn = -0.62, CI 95% [-0.79, -0.52]) (Wilcoxon Rank Sum Test: Z = 7.09, p << 0.001). This result is consistent with mutagenic instruction use being under direct selection (Fig 4.2).

4.3.3 HGT mutations increase evolved probability of beneficial phenotype switching
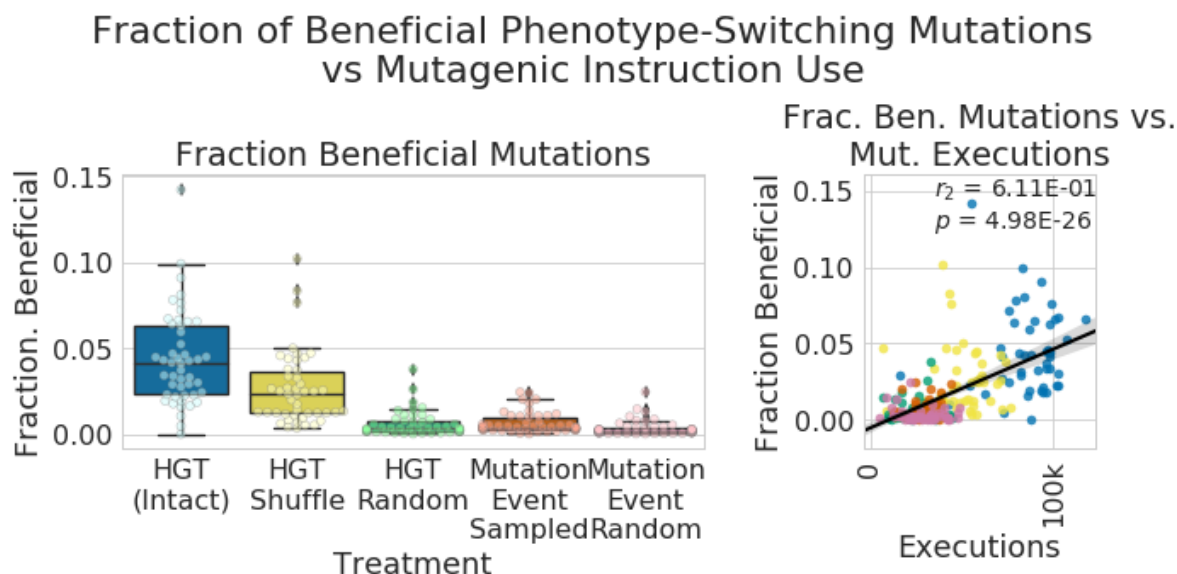
Figure 4.3: **Fraction of beneficial phenotype-switching mutations vs mutagenic instruction use**. We observe a strong correlation between the fraction of mutations that produce beneficial phenotype switches, and the use of the mutagenic instruction (Pearson's R: $r_2 = 0.61$, p = << 0.001). This correlation is much stronger than the correlation between fitness effect and mutagenic instruction use. As such, this result suggests that it is not only the absolute fitness effect that is selected for, but the evolvability benefit.

We measured the proportion of mutations that produce beneficial phenotype changes.4.3 In the context of a changing environment, a mutation that switches an organism's phenotype to match the environment should be strongly selected for. Indeed, we found that this proportion was significantly and substantially higher for HGT mutations (Mdn = 0.041, CI 95% [0.0291, 0.0463]) than other mutation types (Mdn = 0.004, CI 95% [0.0036, 0.0053]) (Wilcoxon Rank Sum Test: Z = 8.56, p << 0.001), and that there was strong correlation between the proportion of beneficial phenotype-switching mutations and use of mutagenic instructions (Pearson's R: $r_2 = 0.61$, p = << 0.001). 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.

4.4 Conclusion

Our experiments expand on our understanding of the evolution of horizontal gene transfer, and how it relates to other types of mutations in the context of changing environments. As expected, we find that the fitness effects of different types of mutations vary significantly, and that they predict the levels of use of that mutagen. In particular, we find a strong correlation between the fraction of mutations that produce a beneficial phenotype change, and the use of that mutagen. Thus, in the context of a changing environment, where there are strong selective pressures to change your phenotype, levels of HGT are elevated, while levels of other mutagens remain low.

This result matches prior research that shows that populations will tend to depress the use of mutagens, even though, in the long-term, higher mutation rates are optimal [76]. This result is further evidence that selection tends to act in the short term. Thus, even though certain kinds of mutagens and architectural features may promote long-term evolvability, these features are hitchhiking on short-term adaptive benefits. Thus, our research shows that even though evolvability may evolve, it is not necessarily selected due to its long-term consequences.

add a takeaway, as per the note below

As with the experiments described in the previous chapter, performing equivalent tests of horizontal gene transfer in an organic system would be extremely difficult, time consuming, and data poor. In particular, comparing the effects of different kinds of mutations, measuring their use, and isolating their fitness effects would be effectively impossible at a population-level scale. Digital evolution allows us to perform these kinds of experiments that otherwise could not be done in natural systems.

does this (below) read ok?

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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.

ch5 - background - fill this in about the prior research done in this area

Prior research has shown that...

In this chapter, we examine how different kinds of changing environments affect long-term evolutionary potential. We introduce change-evolved populations from prior experiments with changing environments, to an entirely new environment, where we can assess long-term variability generation in the form of new task discovery and exploration.

5.2 Methods

For most experiments described in this chapter, we used populations of Avida organisms that were originally evolved in other experimental treatments with changing environments. We used populations originally evolved in the simple environments described in Chapter 2 to survey the long-term evolutionary potential of populations originating in environments with a single fluctuating task. For more complex environments, we also evolved a new set of populations that originate in a complex changing environment, with alternating sets of fluctuating tasks, and variable length genomes.

5.2.1 Experimental Design

In order to examine the long-term dynamics and mechanisms of evolving populations in changing environments, we performed two sets of experiments. For each set of experiments, we took populations evolved in conditions where short-term evolvability pressures dominated, and introduced them to a completely new environment, with a new set of rewarded bitwise tasks to perform: Logic-77. Strictly speaking, the Logic-77 task set is a super-set of the basic Logic-9 task set, and includes all bitwise tasks for which there are up to 3 inputs. However, for the purposes of this chapter, we refer to those tasks which are only in the Logic-77 superset but not in the original Logic-9 task set, as "Logic-77". These new tasks use up to three bitwise inputs rather than a maximum of two in Logic-9.

We performed two sets of experiments, with five environmental treatments per set, and each treatment divided into two parts.

The first set of experiments examines the evolutionary dynamics of populations in simple changing environments, with a single fluctuating task, and are a continuation of the experiments described in Chapter 2. For the second set of experiments, we evolved population in a more complex changing environment where rather than evolving to turn a single task on and off, we evolved populations that switch back and forth between performing two halves

the Logic-9 environment. The environment and genetic structure of the second set of experiments derives from the experiments described in 3. However, these populations were evolved without a working HGT instruction.

The first part of each experiment is as described above, while the second part takes populations evolved in either the simple or complex changing environments, and adds the new Logic-77 tasks. Thus, the goal of our experiments is to evolve populations where short-term evolutionary pressures dominate, and then subject them to an entirely new environment, where we can measure their long-term evolutionary potential.

5.2.1.1 Environmental Treatments

For each of our experiment sets, we prepared four different types of changing environment treatments, plus a static control. The basic changing environment treatments are intended to examine how populations adapt to a brand new environment, while still juggling the alternating direction of selective pressures associated with cycling rewards and punishments. The quiescent treatments, in contrast, remove the reward alternation as we introduce rewards for performing the Logic-77 tasks. Thus, these treatments provide only directional selection for adaptation to the new environment. Therefore, we can isolate the effect of alternating directions of selection on adaptation to a new environment.

- **Static (Control)**: For the first 200k updates (part 1), we reward populations for performing either XOR and EQU for the simple environment (Table 5.1), or the whole Logic-9 task set (Table 5.3). For the second 200k updates (part 2), we add constant rewards for each of the additional tasks for the larger Logic-77 task set, at a 1.2-fold bonus to task execution.

- **Benign Changing Environment**: For the first 200k updates (part 1), we alternate rewarding and not rewarding populations for performing either the EQU task (the simple environment - Table 5.1), or we alternate rewarding each half of the Logic-9

task set (Tables 5.3 and 3.1). For the second part of the experiment, starting at 200k updates, we add constant rewards for each of the additional tasks for the larger Logic-77 task set, at a 1.2-fold bonus to task execution. The environmental fluctuation from the first phase continues through the end of the experiment.

- **Benign Quiescent Changing Environment**: For the first 200k updates (part 1), we alternate rewarding and not rewarding populations, as in the Benign Changing Environment above. For the second part of the experiment, starting at 200k updates, we add constant rewards for each of the additional tasks for the larger Logic-77 task set, at a 1.2-fold bonus to task execution. The environmental fluctuation from the first phase stop at 200k updates, and we instead reward the tasks of the first phase as we did in the Static treatment (all constant reward).

- **Harsh Changing Environment**: For the first 200k updates (part 1), we alternate rewarding and **punishing** populations for performing either the EQU task (the simple environment) (Table 5.1, or we alternate rewarding and punishing each half of the Logic-9 task set (Tables 5.3 and 3.1). For the second part of the experiment, starting at 200k updates, we add constant rewards for each of the additional tasks for the larger Logic-77 task set, at a 1.2-fold bonus to task execution. The environmental fluctuation from the first phase continues through the end of the experiment.

- **Harsh Quiescent Changing Environment**: For the first 200k updates (part 1), we alternate rewarding and **punishing** populations, as in the Harsh Changing Environment above. For the second part of the experiment, starting at 200k updates, we add constant rewards for each of the additional tasks for the larger Logic-77 task set, at a 1.2-fold bonus to task execution. The environmental fluctuation from the first phase stop at 200k updates, and we instead reward the tasks of the first phase as we did in the Static treatment (all constant reward).

5.2.1.2 Set 1 - Simple Cyclic Changing Environments

As descibed above, the first set of experiments consisted of four treatments, plus a control, and each treatment was divided into two parts. In the first part, for a duration of 200,000 updates (analogous to our earlier experiments, detailed in Chapter 2), we rewarded a pair of two-input bitwise logical tasks: XOR and EQU. We rewarded XOR at a constant rate, with an 8-fold bonus to execution, and we alternated rewarding and punishing (or non-rewarding) the EQU task with a reward that switches between 32-fold bonus to execution and either 0 reward for the benign treatments, or a -32-fold punishment in the harsh treatments. For the second part, lasting an additional 200,000 updates, we added a reward for executing the additional Logic-77 tasks. Each new Logic-77 task was rewarded with a constant 1.2-fold bonus to execution. This reward structure provided a mild selective pressure to evolve these task. However, the benefits to performing them do not overwhelm the existing selective pressure to continue performing the tasks rewarded by the original changing or static environment.

As in Chapter 2, we held the individual genomes at a fixed length of 121 instructions, and applied 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).

5.2.1.3 Set 2 - Complex Cyclic Changing Environment

The second set of experiments looked at the evolution of populations of organisms with variable-length genomes, evolved in a more complex cyclic changing environment. As in the simple environment experiments, we performed four experimental treatments, plus a static control (Table 5.3). For the first part of the experiments, we divided the Logic-9 tasks into two groups (Table 5.2) and alternated reward and punishment (or non-reward) between them. For the control treatment, we rewarded all of Logic-9. For the second part of our experimental treatments, we introduced rewards for performing the new Logic-77 tasks, either continuing alternating selection, or switching to constant reward.

Table 5.1: **Experimental Treatments - Simple Cyclic Changing Environment**

Treatment	Changing Env. Type	Rewarded Tasks				
		Part 1 (0-200,000 Updates)		Part 2 (200,000-400,000 Updates)		
		XOR	EQU	XOR	EQU	Logic-77
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 $2^{0.3}$

Four types of cyclic changing environment, plus a static control. Each is split into two phases. The first phase is a normal changing environment like 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.

5.2.2 Measuring Task Discovery and Task Performance

Task discovery and task performance are important measures not only for the adaptation of digital organisms to their local environment, but they also indicate the extent to which populations are more or less evolvable. Populations that are more evolvable should be able to acquire new tasks at a faster rate than less evolvable populations. If the evolvability of our populations is affected by evolution in a changing environment, then this effect should result in differential rates of task discovery and performance.

Table 5.2: **Complex Changing Environments - Logic-9 Rewarded Task Groupings**

	Tasks	Reward Phase 1	Reward Phase 2
Group A	NOT	2^1	-2^1
	AND	2^2	-2^2
	OR	2^3	-2^3
	NOR	2^4	-2^4
	EQU	2^5	-2^5
Group B	NAND	-2^1	2^1
	ORN	-2^2	2^2
	ANDN	-2^3	2^3
	XOR	-2^4	2^4

Logic-9 tasks are divided into to groups, with one task from each pair of tasks of equivalent complexity assigned to a group. The EQU task, which has no complexity equivalent, is assigned to the first group. During the first half of a cycle, we reward the first group of task and punish the second group (see Reward Phase 1). During the second half of the cycle, we reward the second group, and punish the first group (Reward Phase 2).

5.2.2.1 Task Discovery

We measured task discovery by counting the number of unique non-ephemeral tasks that have been discovered by a population, starting at a defined point in time. Once a task is discovered, it may not become "undiscovered." Thus, task discovery counts will always increase monotonically. We measure "Overall" task discovery, counting all possible tasks - including Logic-77 which were available, but not rewarded in the first phase of experiments - and also "Post-Reward" task discovery, where we begin counting new tasks from the beginning of the second phase of the experiment, once Logic-77 rewards are applied.

reword above

5.2.2.2 Task Performance

We measured task performance by counting the number of unique non-ephemeral tasks that a population performs. Each task may be performed only once per organism, yielding a maximum task count of 3600 at any given time. Thus, we define a non-ephemeral task as

Table 5.3: **Experimental Treatments - Complex Cyclic Changing Environment**

Treatment	Changing Env. Type	Rewarded Tasks		
		Part 1 (0-200,000 Updates)	**Part 2 (200,000-400,000 Updates)**	
		Logic-9	Logic-9	Logic-77
Control	None (static)	constant 2^{1-5}	constant 2^{1-5}	constant $2^{0.3}$
Benign	Cyclic	benign fluctuating A: 2^{1-5} or 0 B: 2^{1-4} or 0	benign fluctuating A: 2^{1-5} or 0 B: 2^{1-4} or 0	constant $2^{0.3}$
Benign Quiesce	Cyclic to Static	benign fluctuating A: 2^{1-5} or 0 B: 2^{1-4} or 0	constant 2^{1-5}	constant $2^{0.3}$
Harsh	Cyclic	harsh fluctuating A: -2^{1-5} or 2^{1-5} B: -2^{1-4} or 2^{1-4}	harsh fluctuating A: -2^{1-5} or 2^{1-5} B: -2^{1-4} or 2^{1-4}	constant $2^{0.3}$
Harsh Quiesce	Cyclic to Static	harsh fluctuating A: -2^{1-5} or 2^{1-5} B: -2^{1-4} or 2^{1-4}	constant 2^{1-5}	constant $2^{0.3}$

Four types of cyclic changing environment, plus a static control. Each is split into two parts. The first part is a complex changing environment similar to those found in Chapter 3, Table 3.2. The second part introduces an additional set of tasks (Logic-77) that are rewarded at a constant lower rate. The environments may or may not fluctuate in the second part, depending on the treatment.

one that a population is performing at equal to or more than 0.1% level, or 3.6 times per sampling.

reword above to explain the meaning of ephemeral more clearly

5.3 Results and Discussion

5.3.1 Task Discovery

add something there

5.3.1.1 Benign changing environments outperform harsh environments in task discovery

We found that once we began rewarding the Logic-77 tasks, populations evolving in harsh changing environments discovered many fewer tasks that those evolving in benign changing environments. This effect is consistent across both the simple (Wilcoxon Rank Sum Test: Z = 2.75, p < 0.01) and the complex environment sets (Wilcoxon Rank Sum Test: Z = 5.96, p << 0.001) (Figs 5.1, 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 selective pressures to gain or lose the fluctuating tasks represent up to a $2 * 2^5$-fold bonus over the course of a single cycle, whereas the new Logic-77 tasks can individually only offer a 1.2-fold bonus to execution speed. Thus, those organisms that promptly gain or lose a fluctuating task are more likely to survive, regardless of whether or not they have gained one of the new Logic-77 tasks. Thus pressures to gain and lose the fluctuating tasks are much stronger than the pressure to acquire new Logic-77 tasks, thereby depressing the rate at which they are found.

In contrast, the benign environment experiences a weaker strength of selection for EQU task gain and loss, in the form of a maximum 2^5-fold bonus directional selection pressure to gain the tasks, a no direct pressure to lose the task. Thus, when compared to the harsh treatments, the fraction of the total selective pressure for gaining new Logic-77 tasks is

greater in the benign treatment. This increased pressure, plus the benefit of an increased exploration rate conveyed by the benign changing environment, results in a higher overall task discovery rate.
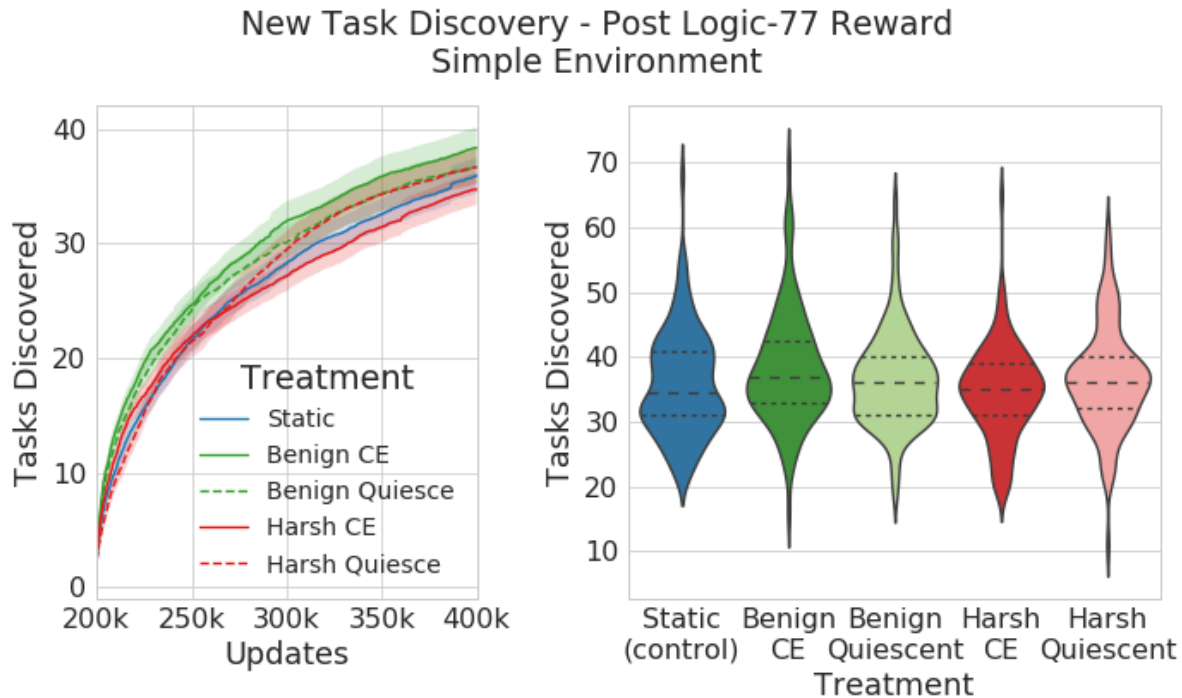
Figure 5.1: **Number of new logic-77 tasks discovered in post-reward in the simple environments**. The left plot shows a time-series of the number of non-ephemeral tasks discovered by populations by each treatment. The right plot shows the number of tasks discovered at the end of the experiments. While the individual values overlap their neigbors, the top and bottom-most treatments (Benign and Harsh) are significantly different from each other (Wilcoxon Rank Sum Test: $Z = 2.75$, $p < 0.01$).

Interestingly, in the quiescent treatments, where we removed the harsh environmental changes as we introduce Logic-77, we see that task exploration recovers somewhat and reaches a comparable level to the control (Wilcoxon Rank Sum Test: $Z = -0.91$, $p = 0.37$). This suggests that it is the alternating selective pressures of the harsh changing environment that depress task exploration, and not a penalty conveyed by architectural features that persist after selective pressures have changed.

expand above per Mike's comment

Also of interest is the comparison of task discovery rates between the benign chang-
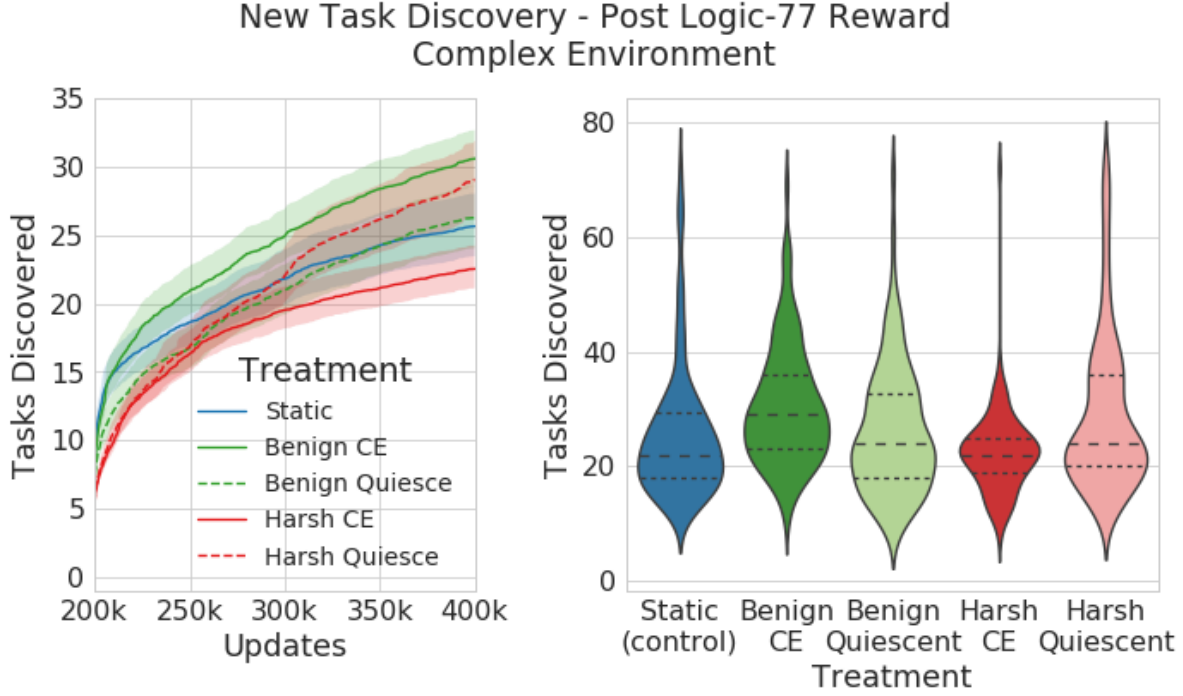
Figure 5.2: **Number of new logic-77 tasks discovered post-reward in the complex environments**. The left plot shows a time-series of the number of non-ephemeral tasks discovered by populations in each treatment. The right plot shows the number of tasks discovered by the end of the experiments. Overall, the number of Logic-77 tasks discovered in the complex environments (Mdn = 24.0, CI 95% [23.0, 25.0]) is significantly lower than those found in the simple environments (Mdn = 36.0, CI 95% [35.0, 36.0]) (Wilcoxon Rank Sum Test: Z = -16.15, p << 0.001). However, the same pattern holds, where the benign changing environment treatment discovers significantly more tasks than the harsh changing environment (Wilcoxon Rank Sum Test: Z = 5.96, p << 0.001).

ing environment populations BenignCE and BenignQuiescent. The BenignCE populations continue to be subject to the benign changing environment, whereas the Benign Quiescent populations are only subject to directional selection toward the evolution of the original and the Logic-77 tasks. In both environment types, task discovery rate for the BenignCE population (Simple: Mdn = 37.0, CI 95% [36.0, 38.0]; Complex: Mdn = 29.0, CI 95% [26.0, 31.0]) is slightly higher than for BenignQuiescent(Simple: Mdn = 36.0, CI 95% [34.0, 38.0]; Complex: Mdn = 24.0, CI 95% [22.0, 27.0]), but the effect is not statistically significant in the simple environment (Complex: Wilcoxon Rank Sum Test: Z = 3.15, p < 0.01; Simple: Wilcoxon Rank Sum Test: Z = 1.39, p = 0.16). Both, however, still perform better than

the control treatment (Simple: Mdn = 34.5, CI 95% [32.0, 38.0]; Complex: Mdn = 22.0, CI 95% [21.0, 25.0]), though this effect is only statistically significant between the BenignCE and Control treatments in the complex environment (Wilcoxon Rank Sum Test: Z = -4.33, p < 0.001). Thus, this result suggests that there may be architectural features conferred by evolution in the changing environment that are helpful for long-term evolvability, even after the environmental changes have stopped, and that this effect is distinct from the direct effects of the changing direction of selection.

add note about how simple also perform more tasks than the complex. this is contrary to our expectation

5.3.1.2 Harsh changing environments drive populations across the mutational landscape

In the first phase of the experiments, despite the logic-77 tasks not being rewarded, both changing-environment treatments (BenignCE and HarshCE) discovered more new tasks than the control (Simple: Wilcoxon Rank Sum Test: Z = -5.75 and -11.15 respectively, p << 0.001; Complex: Wilcoxon Rank Sum Test: Z = -4.32 and -3.73 respectively, p < 0.001). In the simple environment, the harsh treatment in particular discovered substantially and significantly more Logic-77 tasks than either the benign treatment(Wilcoxon Rank Sum Test: Z = -8.0, p << 0.001) or the control, despite these tasks not being rewarded (Fig 5.3. 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. However, as the experiment proceeds, and Logic-77 task rewards are introduced, this effect disappears, and task discovery rates converge (Kruskal Wallis: H(2) = 6.97, p = 0.03).
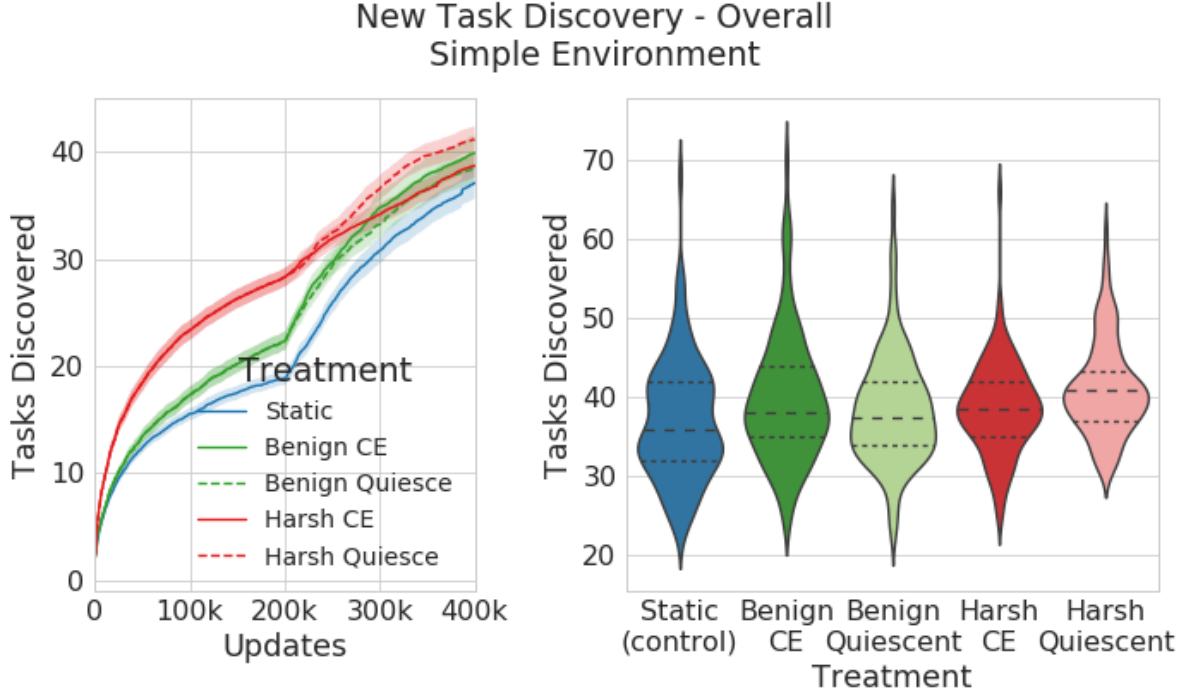
Figure 5.3: **Number of new logic-77 tasks discovered in simple environments, over the whole experiment**. The left plot shows a time-series of all new tasks discovered over the course of the entire run, including non-rewarded Logic-77 tasks. The right-hand plot shows the final count at the end of the run. Before we introduce rewards for performing Logic-77 tasks, the harsh changing environment discovers far more new tasks (Mdn = 28.0, CI 95% [27.0, 30.0]) than either of the other treatments (Mdn = 22.0, CI 95% [22.0, 23.0]) (Wilcoxon Rank Sum Test: Z = 8.61, p << 0.001). This occurs despite no reward being given for performing the Logic-77 tasks in the first part of the experiment.

5.3.2 Task Performance

5.3.2.1 Benign changing environments outperform harsh environments in task performance

Similar to task discovery, populations evolving in harsh changing environments performed far fewer distinct tasks than either the benign-evolved or control populations (Wilcoxon Rank Sum Test: Simple: Z = -11.22 and -11.15 respectively; Complex: Z = -4.32 and -3.73 respectively, p << 0.001) (Fig 5.5 and 5.6). While both the benign changing environemtn and benign quiesce populations seemed to outperform the control, this effect was only statistically significant in the complex environment (Wilcoxon Rank Sum Test: Z = 2.42 and
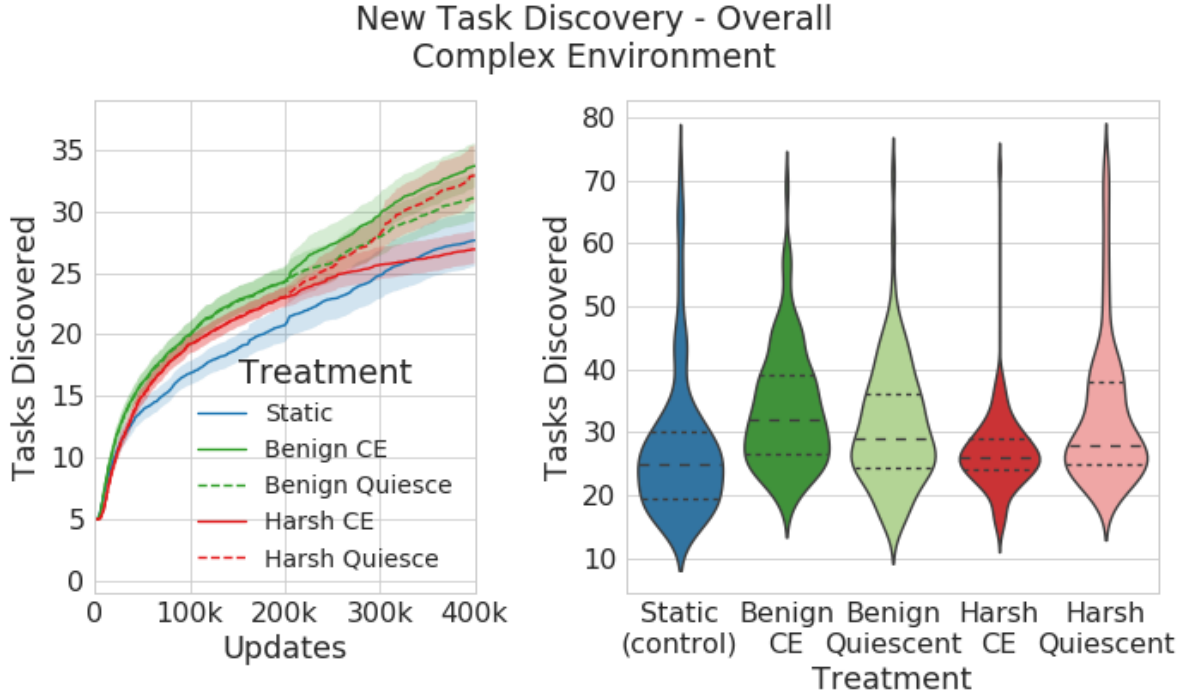
Figure 5.4: **Number of new logic-77 tasks discovered in complex environments, over the whole experiment**. The left plot shows a time-series of all new tasks discovered over the course of the entire run, included non-rewarded Logic-77 tasks. The right-hand plot shows the final count of tasks discovered by the end of the run. Again, both sets of changing environments (BenignCE and HarshCE) randomly discover more Logic-77 tasks than the control treatment before rewards for those tasks are offered (Wilcoxon Rank Sum Test: Z = -4.32 and -3.73 respectively, p < 0.001). This result suggests that the alternation of the direction of selection drives populations to explore new regions of the fitness landscape more effectively than directional selection alone.

2.37 respectively, p < 0.05). In the simple environment, the differences were not statistically significant (Kruskal Wallis: H(2) = 2.76, p = 0.25).

Interestingly, in the complex environments, the harsh quiescent treatments seemed to out-perform all other environment types in task performance, though the differences between harsh quiescent and both benign treatments are only barely statistically significant (Wilcoxon Rank Sum Test: Control Z = 4.33, HarshCE Z = -9.3, p < 0.001; BenignCE Z = 2.25, Benign Quiesce Z = 1.96, p < 0.05). This effect may be due to a lingering architectural effect of evolving in harsh changing environments.

expand speculation per mike's comment
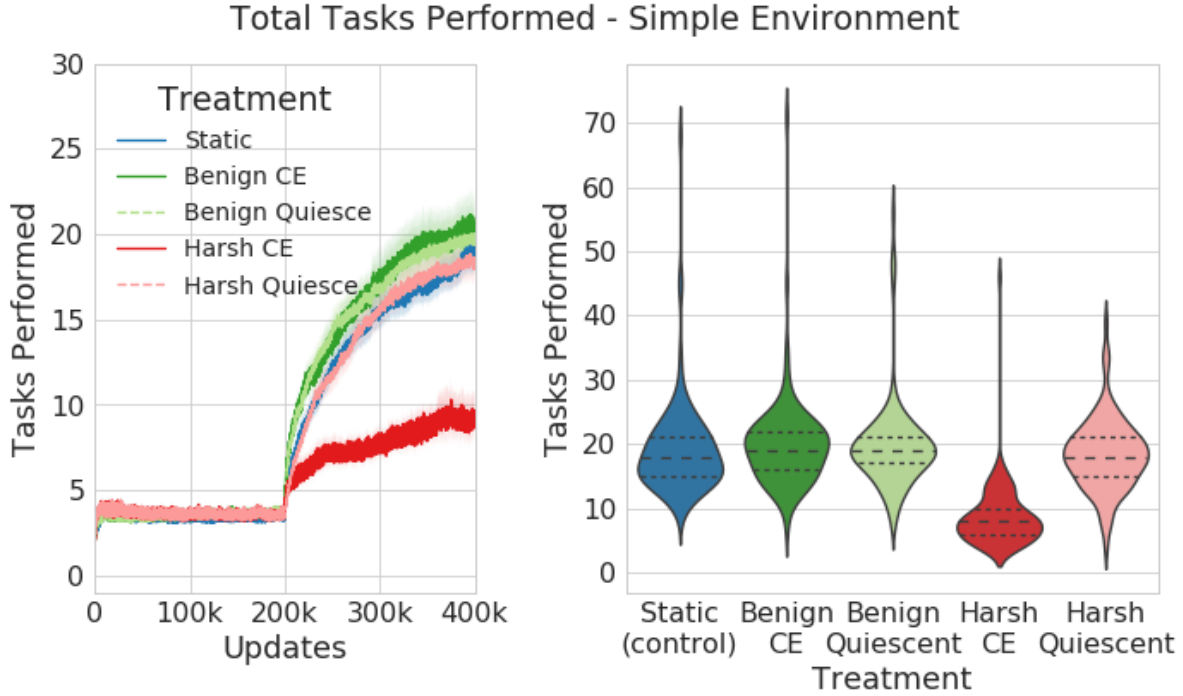
Figure 5.5: **Number of distinct tasks performed in simple environments**. The left plot shows a time-series of the number of distinct tasks performed by the treatment populations over time. The right-had plot shows the number of tasks performed at the end of the experiments. The harsh changing environment treatment performs substantially and significantly fewer tasks than any of the benign or control treatments (Wilcoxon Rank Sum Test: Z = -11.22 and -11.15 respectively, p << 0.001). The benign treatments perform best, but the differences are not statistically significant from the control (Kruskal Wallis: H(2) = 2.76, p = 0.25).

Further research is needed to identify a mechanism for this effect.

5.4   Conclusion

Our experiments demonstrate that the harshness of fluctuation has a dramatic effect on long-term evolvability, landscape exploration and task discovery. We find that while harsh changing environments were most effective at driving short-term adaptation, these environments depress long-term evolvability once we introduce a new environment. In all experiments, we find that benign environments produce the highest levels of task discovery, far outperforming harsh changing environments.

why does this happen?
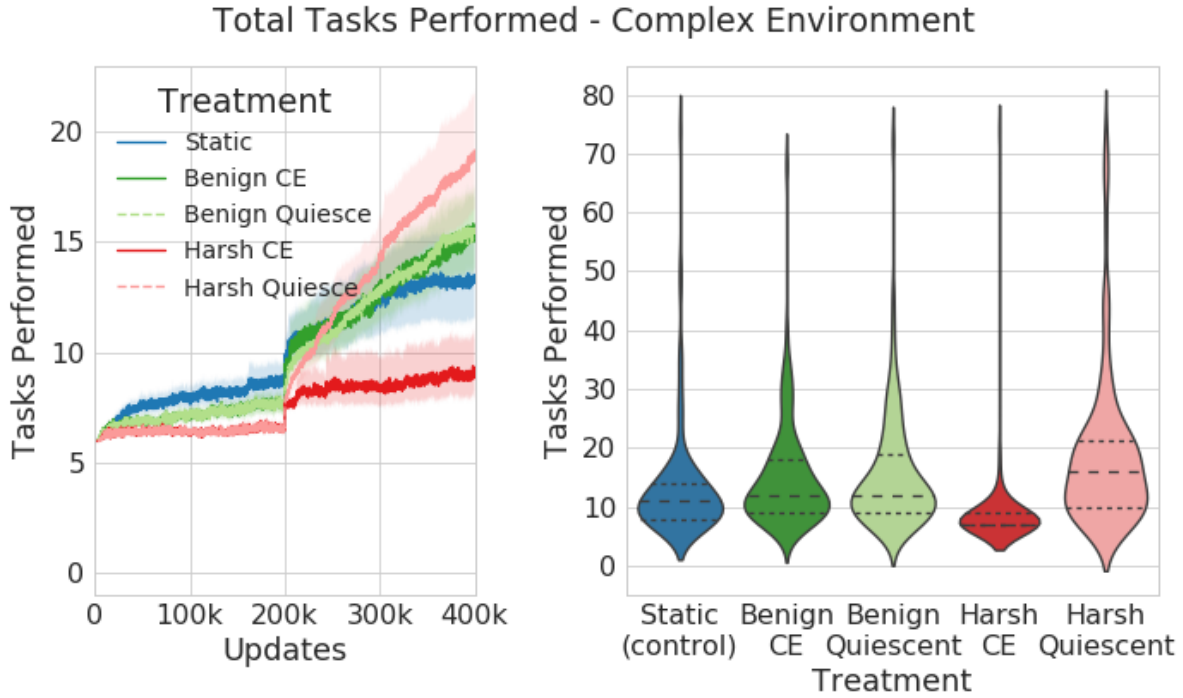
Figure 5.6: **Number of distinct tasks performed in complex environments**. The left plot shows a time-series of the number of distinct tasks performed by the treatment populations over time. The right-had plot shows the number of tasks performed at the end of the experiments. Similar to the simple environment, the harsh changing environment treatment performs substantially and significantly fewer tasks than any of the benign or control treatments (Wilcoxon Rank Sum Test: $Z = -4.32$ and -3.73 respectively, $p << 0.001$). Similarly to task discovery, the complex environment performs fewer tasks overall (Mdn $= 11.0$, CI 95% [10.0, 12.0]), compared to the simple environment(Mdn $= 17.0$, CI 95% [17.0, 18.0]) (Wilcoxon Rank Sum Test: $Z = -9.25$, $p << 0.001$). Interestingly, the Harsh Quiescent treatment performs the most tasks, though this effect is only slightly significantly different from the benign treatments (Wilcoxon Rank Sum Test: Control $Z = 4.33$, HarshCE $Z = -9.3$, $p < 0.001$; BenignCE $Z = 2.25$, Benign Quiesce $Z = 1.96$, $p < 0.05$).

clean this up (above)

Our experiments show 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 accumulated 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 about balancing of fitness effects.

CONCLUSION AND FUTURE DIRECTIONS

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

This result matches prior research that shows that populations will tend to depress the use of mutagens, even though, in the long-term, higher mutation rates are optimal [CITE].

This result is further evidence that selection tends to act in the short term. Thus, even though certain kinds of mutagens and architectural features may promote long-term evolvability, these features are hitchhiking on short-term adaptive benefits. Thus, our research shows that even though evolvability may evolve, it is not necessarily selected for in the long-term.

add section about the HGT bonus depressing task exploration in the long term evolution chapter

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 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.

tweak below?

APPENDICES

APPENDIX A

EXPERIMENTALLY DERIVING PARAMETERS FOR CHANGING ENVIRONMENT CYCLE LENGTHS

Cyclic changing environments have predictable cycles and thus have consistent periods of time that allow adaptation and fixation of traits. Too short a cycle, and traits are not able to fix. Too long a cycle and traits that are no longer selected for in one phase, but are useful in the other, become increasingly vulnerable to loss through genetic drift.

In order to identify an optical cycle time to support our experiments, we surveyed a series of cycle lengths, and measured task performance rates in both benign and harsh changing environments (Fig A.1).

We found that, of our surveyed values, a cycle time of 1,000 updates (roughly 30 generations) both provided adequate time for traits to evolve and fix (and thus be performed by the entire population), but also not enough time for drift to destroy alternate-phase traits, thus minimizing the time required to re-evolve the task. Cycle times that were too short never acquired and fixed adaptive traits in the entire population, while as cycles got longer, the fluctuating tasks would take much longer to re-evolve, indicating that the populations had drifted away from the regions of the mutational landscape that contained them (Fig A.2).
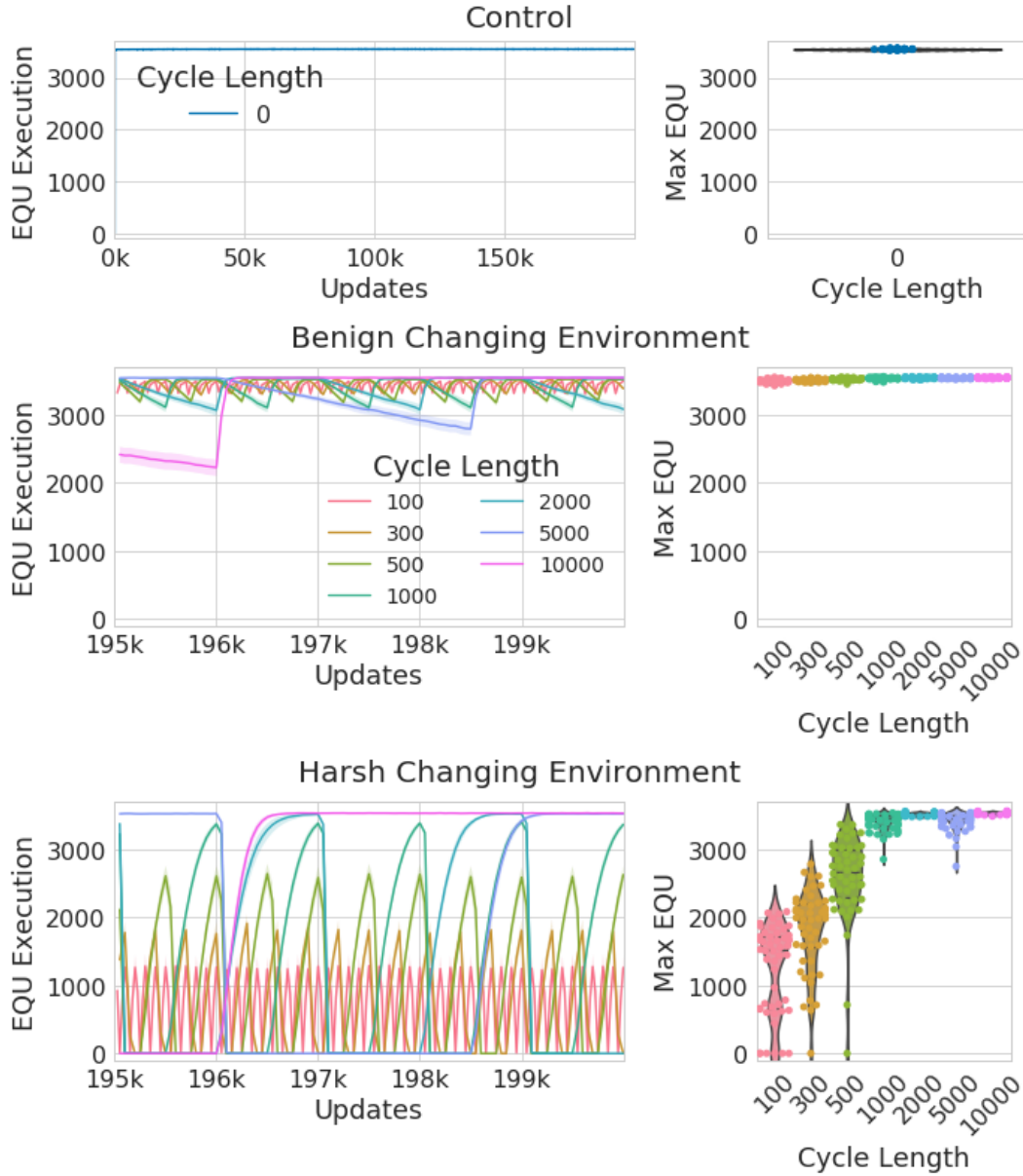
Figure A.1: **Survey of task execution** for a series of cycle-lengths. The top plots count the number of executions of the EQU instruction in the control treatment. Each organism only receives a bonus for executing EQU once. The seed population performed EQU, and it is never lost. The middle and lower plots show EQU execution in benign and harsh changing environments, respectively. Each line describes a different cycle-length treatment. The longer cycle lengths correspond with longer times to lose and regain EQU. Only treatments with cycles longer than 1000 updates achieve fixation, at a maximum value of 3600 EQU executions.
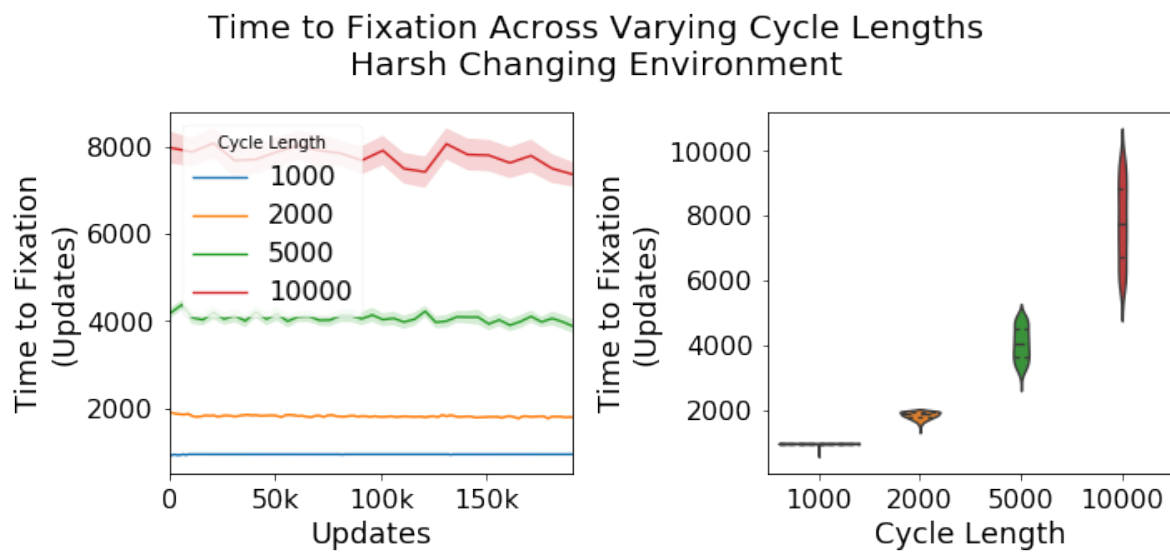
Figure A.2: **Survey of task time to `EQU` fixation for harsh changing environments** for a series of cycle-lengths. Only those treatments where EQU fixation occurs (cycle lengths greater than 1000 updates), were surveyed. The longer the cycle time, the longer it takes for fixation to occur.

APPENDIX B

EXPERIMENTALLY DERIVING PARAMETERS FOR HGT RECOMBINATION PROBABILITY AND BONUS LEVELS

B.1 Recombination Probability Sweep
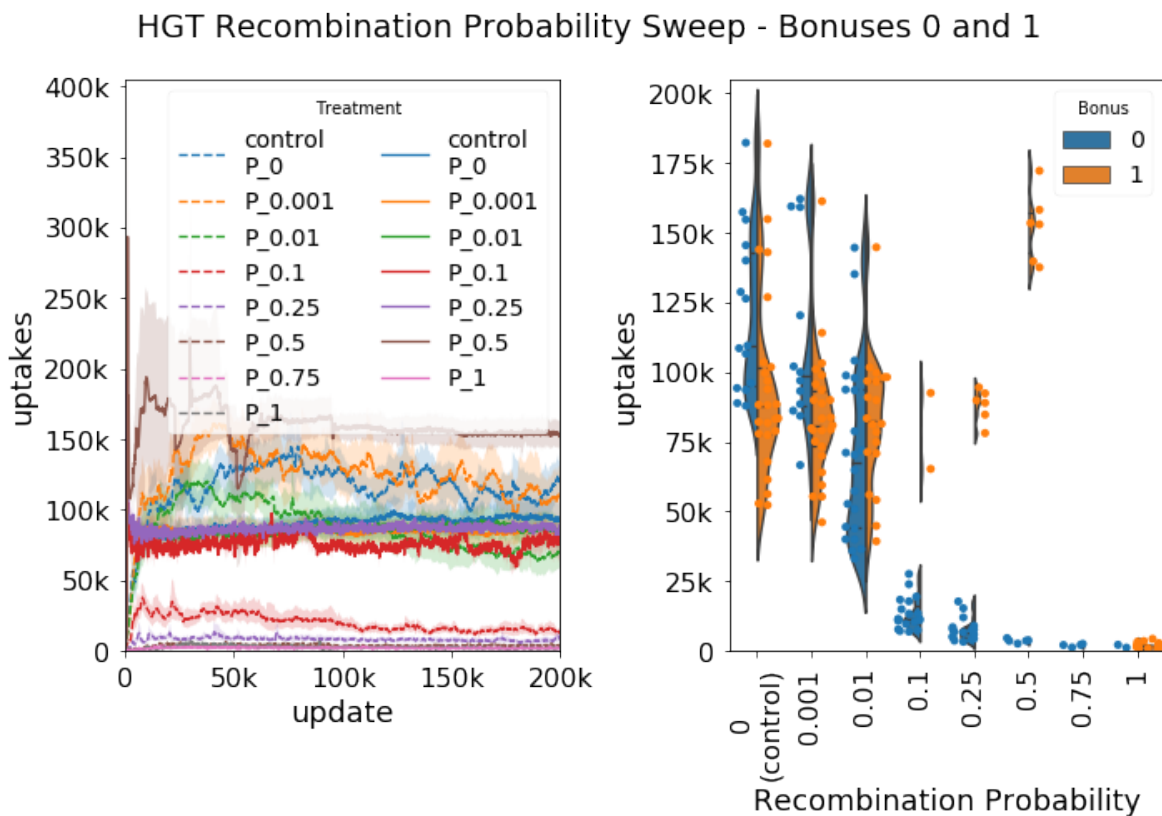
Figure B.1: **Survey of `HGT` use** for a series of recombination probabilities in a static environment. The left-hand figure shows a time-series of fragment uptakes, with and without bonus values. The right-hand plot shows the final uptake counts at the end of the runs. Those values at a recombination probability of less than 0.1 are not meaningfully depressed as compared to the bonus values. Values greater than 0.1 showed more suppression, and ultimately reached mutational melt-downs with few surviving populations.

In nature, the probability of horizontal gene transfer occurring varies wildly by species, environmental conditions, and mechanism of action. In order to identify an optimal probability of HGT uptake resulting in recombination, we performed a series of experiments

comparing different probabilities, coupled with a pair of basic grazing bonuses. Our initial grazing bonuses were based on the reward given for a similarly complex task - `NAND` - which only requires a single instruction, plus an `IO` instruction, to implement. `NAND` is typically rewarded with a bonus of 2^1 to execution speed. Thus, performing HGT, which only requires a single instruction - `HGT-Uptake`, was rewarded similarly.

The goal was to identify a probability of recombination that would, without a grazing bonus, result in a reduced HGT uptake level as compared to with a grazing bonus. Further, we wanted the recombination probability to not be so high as to result in mutational meltdown, thus frequently killing the populations. We found that a level of 0.1 probability met these characteristics. That is, recombination probabilities of less than 0.1 had similar HGT expression rates with and without a bonus, while probabilities above 0.1 tended to have many many fewer surviving populations (Fig B.1).

B.2   Bonus Sweep
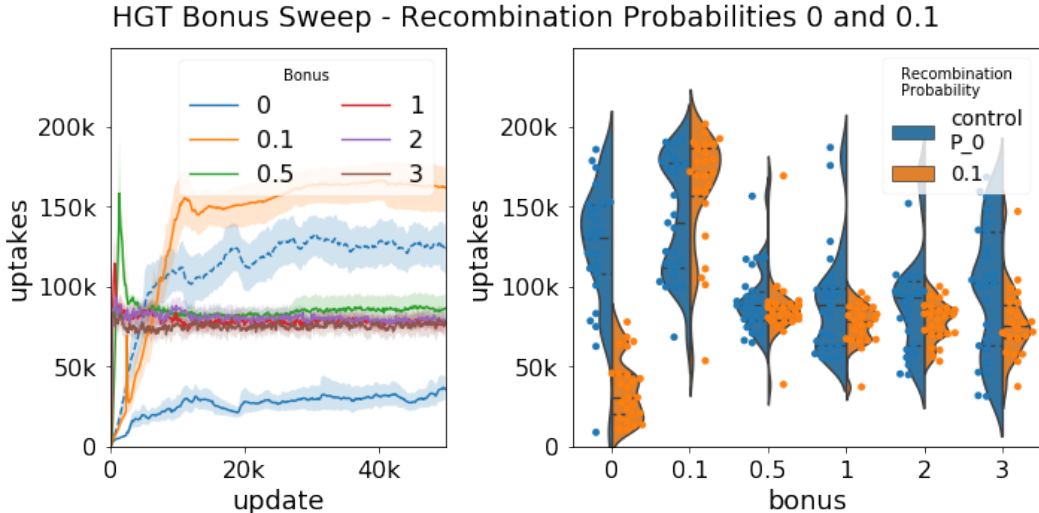
Figure B.2: **Survey of `HGT` use** for a series of bonus values in a static environment. The left plot shows a time-series of HGT fragment uptakes, while the right-hand plot shows final uptake values at the end of the experimental runs. With recombination probability at 0.1, bonus values of zero depress HGT use. A bonus of 0.1 increases uptake dramatically, indicating a very high use. Bonuses at 0.5 and greater show diminishing returns in increasing HGT use.

Similarly, in nature, the amount of nutritive benefit derived from taking up DNA fragments from the environment is difficult to quantify. In Avida, when populations are presented with instructions that provide a direct boost to execution speed, use of these instructions is strongly selected for. Populations rapidly fill their genomes with these instructions, to the exclusion of virtually all else. Thus, our goal in selecting potential bonus levels lay in finding a range of minimal value that would balance the presumably deleterious effects of the HGT uptake instruction, without completely overwhelming the balance of selective pressures we wished to apply to our experiments. As expected, and coupled with the recombination probability above, values around 2^1 seemed to perform the best (Fig B.2). Above a value of 2^2, there seem to be diminishing returns.

B.3 Comparable Bonus Values
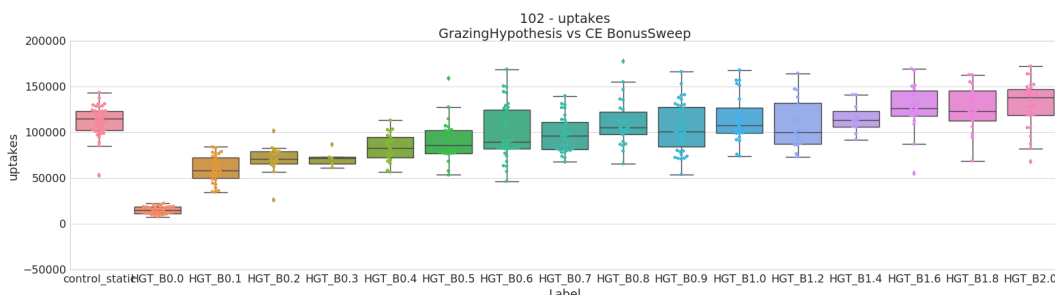
Figure B.3: **Survey of `HGT` use, across a series of comparable bonus values** in a static environment, with a recombination probability of 0.1. This figure shows a sweep of bonus values, demonstrating a largely linear progression of increasing HGT use as bonus values increase, thus balancing negative effects caused by recombination in a static environment.

For later experiments, in order to identify the relationship between HGT uptakes prompted by grazing bonus, and uptakes prompted by evolvability pressures in changing environments, we surveyed a series of bonuses to find a value that, in a static environment, would yield an HGT fragment uptake rate similar to that found in changing environments without a grazing bonus. We found that a bonus value of $2^{0.8}$ yielded similar HGT fragment uptake

levels (Fig B.3).

CHANGING ENVIRONMENTS ELEVATE ALL INSTRUCTION USE DUE TO REPEATED BOTTLENECKS
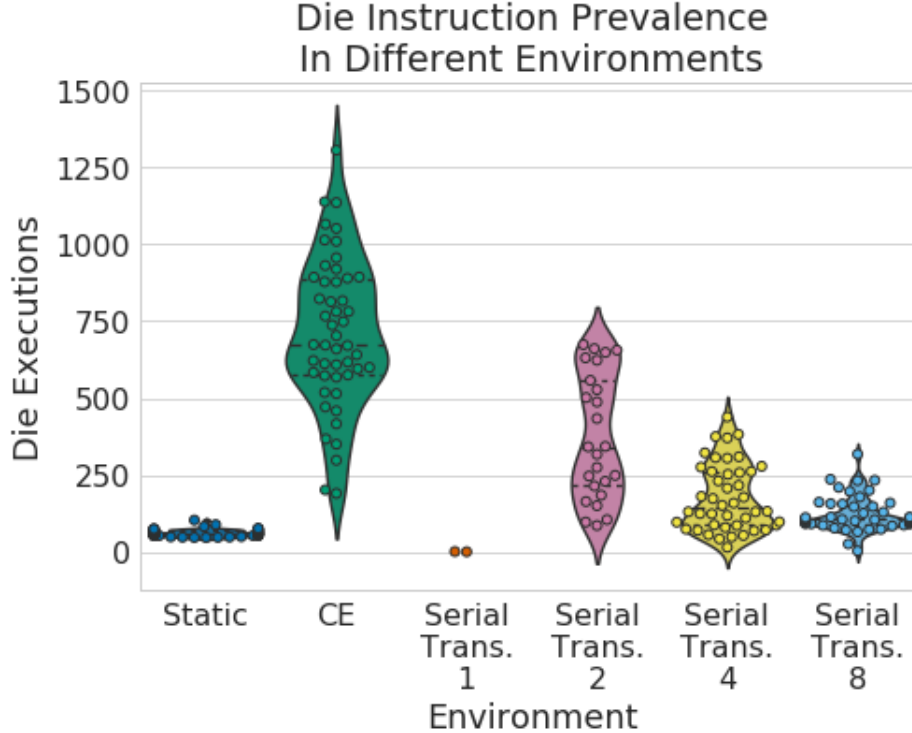
Figure C.1: **`Die` instruction executions** across a series of artificial bottlenecks. We observe that the `Die` instruction is elevated in a harsh changing environment as compared to a static environment. We apply a pair of bottlenecking procedures. In the "Serial Transfer" treatments, we kill the population, but select increasingly fewer organisms to seed from. As the number of seed organisms become fewer, the expression of the `Die` instruction increases. This indicates that founder effects are likely the cause of increasing expression of strongly deleterious traits in harsh changing environments.

In Chapter 4, we observed that the `Die` instruction control is elevated in the changing environments treatment, as compared to the control treatment. This counterintuitive effect is due to the repeated bottlenecking of the populations subjected to the harsh changing environment (Fig C.1). 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 necessarily 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 pressures of the environmental change may ultimately outweigh that of the `Die` instruction if it were initially dormant in a genome that survived a harsh environmental change and reproduced.

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