EVOLUTION AND EVOLVABILITY IN CHANGING ENVIRONMENTS

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ABSTRACT

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The specific meaning of the term "evolvability" is heavily debated, but most definitions can be summarized as: the potential of populations and genomes to produce adaptive variation and complex structures in response to mutation and selection. Changing environments are thought to play a significant role in shaping and promoting evolvability through alternating selective pressures.

In this dissertation, I will discuss my recent research on the interplay between changing environments, evolvability, genetic architecture, and the evolution of horizontal gene transfer (HGT), an information-rich mutagenic function that is ubiquitous in nature. Before delving into my own research, however, I begin in the first chapter by providing a survey of current literature on each of these topics, with emphases on how they are believed to arise, how they affect subsequent evolution, and how they relate to each other.

Genetic architecture and population dynamics clearly have a complex interplay in ongoing evolutionary dynamics. Evolutionary history, population diversity, modularity, and task size all play a role in determining the location and characteristics of populations in genotype space, and alter the genotype to phenotype map that permits neutral genetic variation. All of these features contribute to evolvability. In Chapter 2, I demonstrate how changing environments provided a sufficient selective pressure to produce quasi-modular genetic architectures that allow for rapid adaptation to the meta-environment of environmental change.

Horizontal gene transfer is a highly regulated, ubiquitous, and ancient mechanism for exchanging genetic material between unrelated organisms. In the third chapter, I explore conditions which may have led to the evolution of horizontal gene transfer through transformation, and identify mechanisms that might support its continued performance.

In Chapter 4, I compare the fitness and phenotypic effects of the HGT process against other types of increasingly less information rich mutational operators. I demonstrate that not only is HGT selected for in harsh changing environments, but that other mutagenic instructions that contain less information, or provide lesser fitness benefits are not similarly selected for.

In the fifth chapter, I explore the long-term evolutionary potential of populations evolved in changing environments by evolving two different populations, one evolved in a minimal changing environment, and the other in a rich changing environment, and exposing them to a brand new environment. I demonstrate that while populations adapted to harsh changing environments are indeed able to adapt quickly to previously seen environmental changes, that these populations do not fare as well in brand new environments. Rather, benign changing environments perform best in measures of task discovery and exploration.

In the final chapter, I conclude with a synthesis of my results, along with implications for the field, as well as identification of some new directions for pursuing my research into changing environments.

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

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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

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

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

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

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

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

Evolvability, in its details, must mean different things at different evolutionary scopes and 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 regions further away 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 the genetic material acquired in prior explorations and adaptations, and use this history as raw material for new adaptation [19]. Indeed, earlier work has shown that changing environments promote evolvability in many contexts, without compromising

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

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 evolvability-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 Alberch [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 Altenberg's 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

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, or limited by environmental perturbation, 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. Given a uniform mutation rate, Fidelity can be calculated 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 one-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 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 (or better), 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.

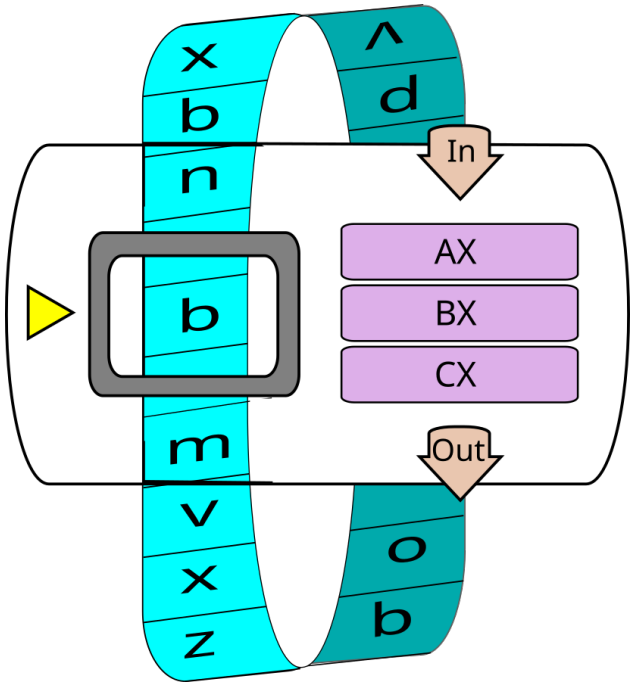

Figure 1.1: **An example virtual CPU from Avida**, with a circular genome (blue), three registers (purple), input and output handlers (tan), and an instruction pointer (yellow) indicating the next instruction to be executed.

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/dissertation_data`.

1.7 Statistical Methods

Most of the statistical techniques used in this dissertation are non-parametric, and focused on differentiating between sample distributions. In general, we applied Wilcoxon Rank-Sum tests [46] to distinguish between pairs of distributions, as well as Kruskal-Wallis [47] for identifying whether we could reject the null hypothesis of sameness between several different distributions. We assume all distributions are independent, and that compared distributions have similar shapes. In all situations where there were multiple comparisons of a given distributions, we applied Bonferonni corrections [48] before assessing statistical significance.

In certain cases, we report mean and median values of distributions. In these cases, we also report the standard deviation or 95% confidence intervals.

In specific cases, we also apply Spearman's rank-order correlation coefficient ρ (or r_s) [49] to measure correlations between data sets. In all cases, data points are matched from within a replicate.

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 [50, 51, 52, 53] and in evolutionary computation settings [54, 55, 56]. 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 [57].

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 [58] 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 [59, 60, 61, 62]. 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 capacity for mutations to generate adaptive variation in a genome.

In the short-term, this kind of evolvability determines a population's response to selection, and 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 [63]. Thus, these populations are able to produce large amounts of heritable variation despite their reduction in 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 where mutations are more likely to cause phenotype switching without substantial reductions 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 *Escherichia coli*, the methyl-directed mismatch repair (MMR) pathway has been shown to preferentially repair coding regions over non-coding regions [64]. Alternately, some kinds of mutations may be more likely to occur than others. A mutation accumulation (MA) study of *Salmonella typhimurium* found a strong bias toward GC-to-TA transversions rather than GC-to-AT transitions [65]. These kinds of effects thereby skew the probabilities of some kinds mutations occurring that might lead into certain neighborhoods 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, 66]. 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) (Eq 1.9) and the **Phenotypic Diffusion Rate** (D_p) (Eq 1.12), as normalized across changing environments (Eq 1.13).

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

2.2.1.1 Cyclic and Stochastic Changing Environments

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

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.

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 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

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

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

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.

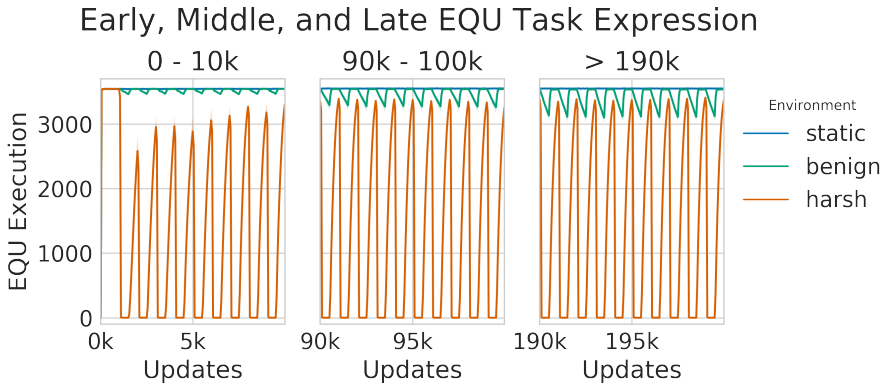

Figure 2.1: **Number of organisms performing `EQU` task**. We measured the execution of the `EQU` task in all treatments. By the end of the run, we observed fixation in the control treatment. In the benign treatment, we observed increasing periodic dips in execution that coincide with phases of non-reward as the experiments progressed. In the harsh treatment, we observed adaptation, resulting in 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 (see Fig 2.4). Because a selective sweep reduces current diversity within a population, the smaller number of sweeps in the benign and control treatments led populations in them to have higher standing diversity for most of their evolutionary history than those populations from the harsh changing environment. Despite this higher standing diversity in the benign and control treatments, regions of low diversity are still evident in the genomes of these populations, implying purifying selection on the traits encoded at these sites (see Fig 2.3).

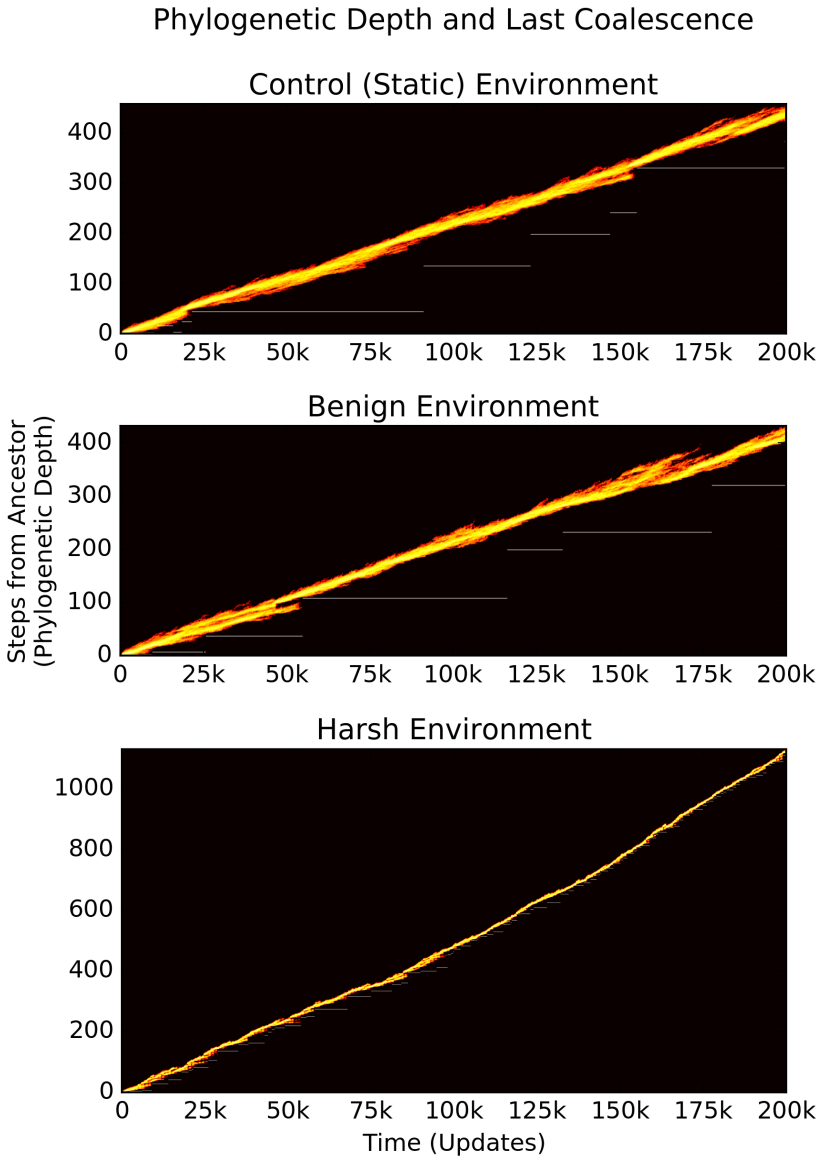

Figure 2.2: **Phylogenetic depth over time** of a sample population evolved in each of the three treatments of the cyclic changing environments. Phylogenetic depth is the number of mutational steps from the original ancestor, and is a rough analog for generations. White horizontal lines mark the depth of the most recent common ancestor, and discontinuities in this line indicate that the most recent common ancestor has changed, and thus that a sweep occurred, or that a competing clade went extinct. The control treatments had a mean of 18 sweeps (STD=9.05), the benign treatments had a mean of 21 (STD=19.05), and the harsh treatments had a mean of 88 sweeps (STD=23.37). Note the difference in scales between y-axes: the control-evolved population has a maximum depth of 400 mutational steps from ancestor, while the harsh-evolved has upward of 1100.

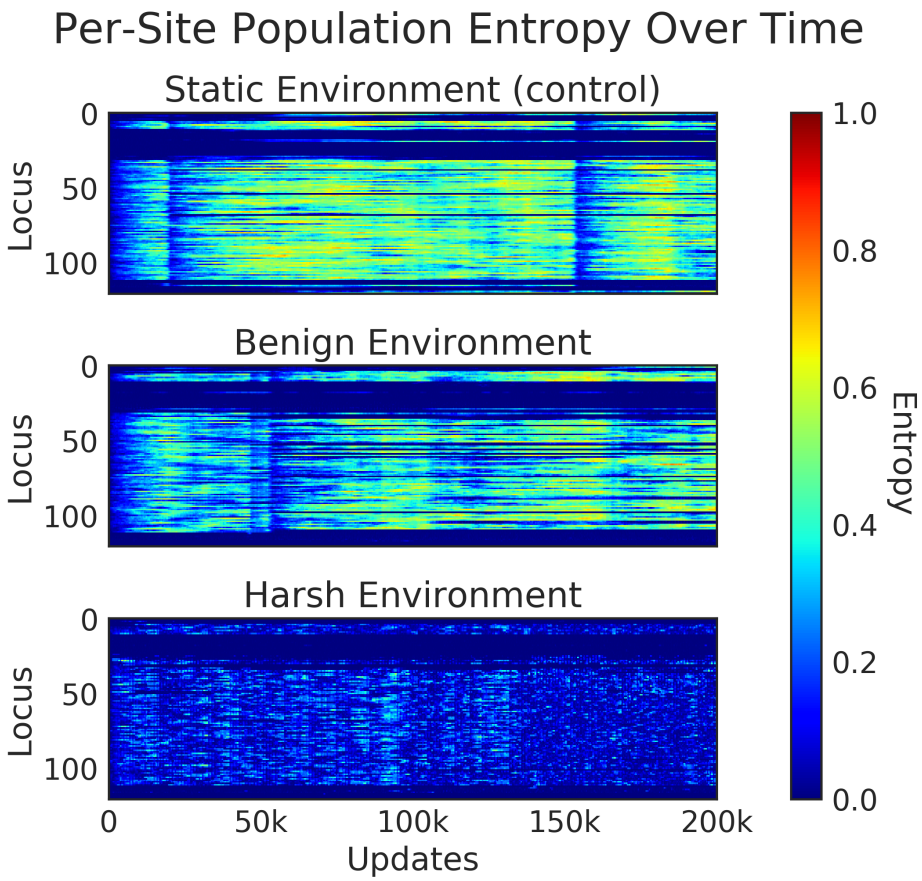

Figure 2.3: **Per-site entropy over time** of a representative sample population. Each vertical slice represents the per-site entropy of the population at each update by genetic locus. Hotter colors (red/orange/yellow) indicate greater diversity at this locus, while cooler colors (blues) indicate the a locus is more consistent across the population.

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

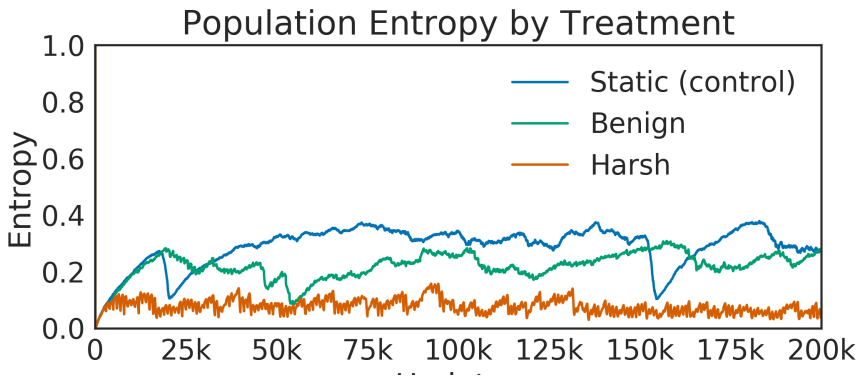

Figure 2.4: **Population entropy over time** of the representative sample population in Figure 2.3. Mean population entropy indicates the relative diversity of the population at any given time, while the per-site entropy (see Fig 2.3) shows where in the genomes the population diversity is located.

the static treatment, the majority of functional tasks sites code for both `XOR` and `EQU`. (See Figs 2.5, 2.6, and 2.7)

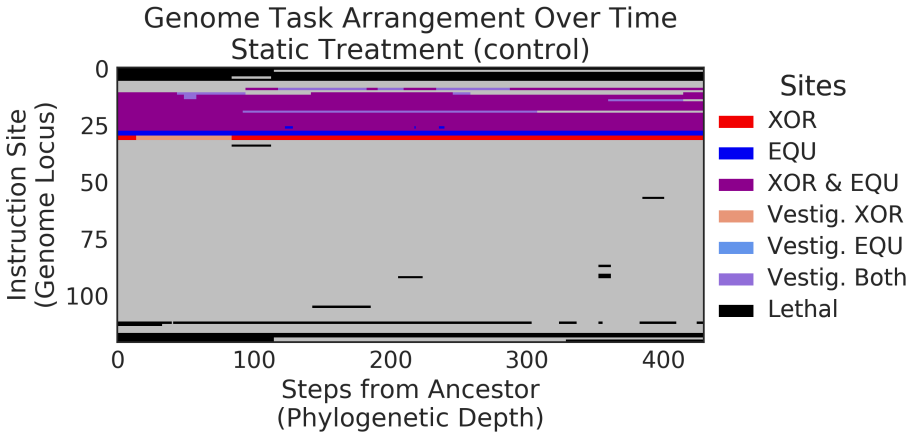

Figure 2.5: **Genetic architecture of XOR and EQU over time in static environment** for the final dominant genotype in a randomly selected replicate. Proceeding from the left of each figure, each vertical slice represents an organism along the line-of-descent to the final dominant. Positions along the 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. `XOR` and `EQU` overlap almost completely throughout the run.

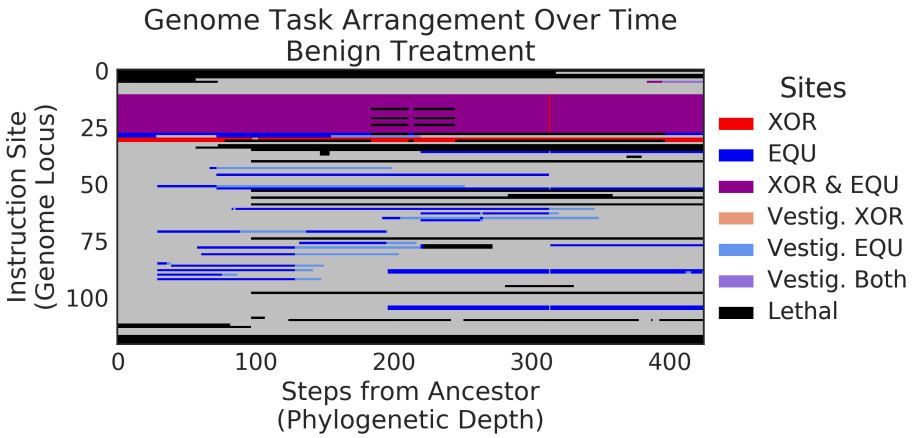

Figure 2.6: **Genetic architecture of XOR and EQU over time in benign environment** for the final dominant genotype in a randomly selected replicate. Proceeding from the left of each figure, each vertical slice represents an organism along the line-of-descent to the final dominant, and as in Figure 2.5, colors represent tasks performed by each genome locus. In this genome, XOR and EQU evolve to overlap much less than in the control.

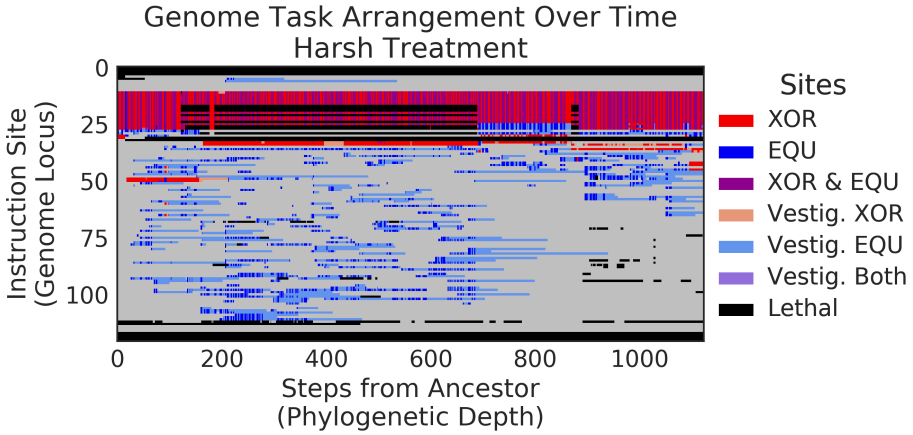

Figure 2.7: **Genetic architecture of XOR and EQU over time in harsh environment** for the final dominant genotype in a randomly selected replicate. Proceeding from the left of each figure, each vertical slice represents an organism along the line-of-descent to the final dominant, and as in Figures 2.5 and 2.6, colors represent tasks performed by each genome locus. In this genome, XOR and EQU evolve to overlap even less than in the control and benign treatments, with the EQU-only task sites becoming increasingly scattered throughout the genome.

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 << 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 remain unchanged from when they were previously active in performing a task, but were disabled by a mutation elsewhere and are thus now neutral. These vestigial pseudogene-like sites may be important for allowing the organisms to quickly re-adapt as the fluctuations in the environment restore the previously-rewarded functions. (Fig 2.8)

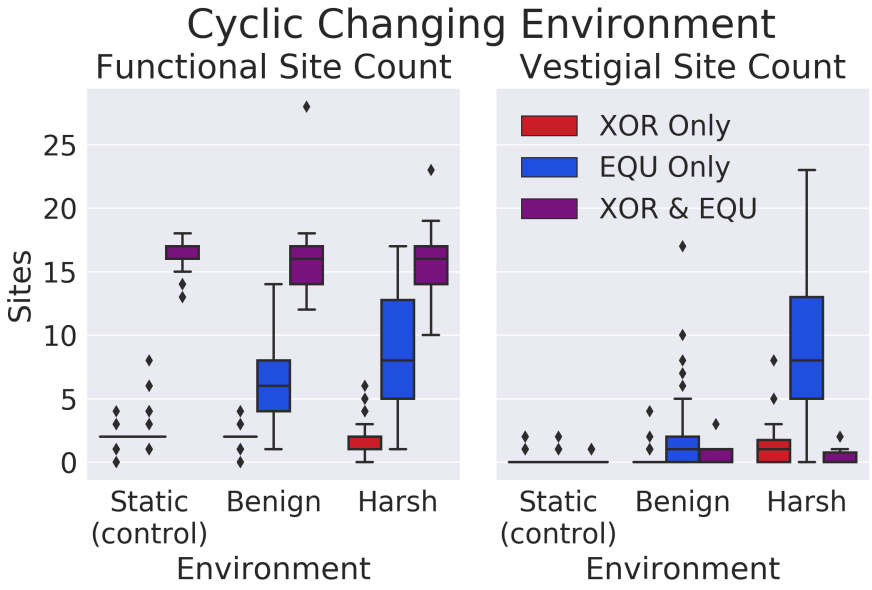

Figure 2.8: **Number of functional and vestigial sites by treatment**. Both the benign and harsh changing environments had significantly more sites devoted to performing only the EQU function (Wilcoxon Rank Sum Test: Z = -5.57 and -6.96, respectively, p << 0.001). The harsh environment has a significantly 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

In order to identify the role that these longer task 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 2.9.

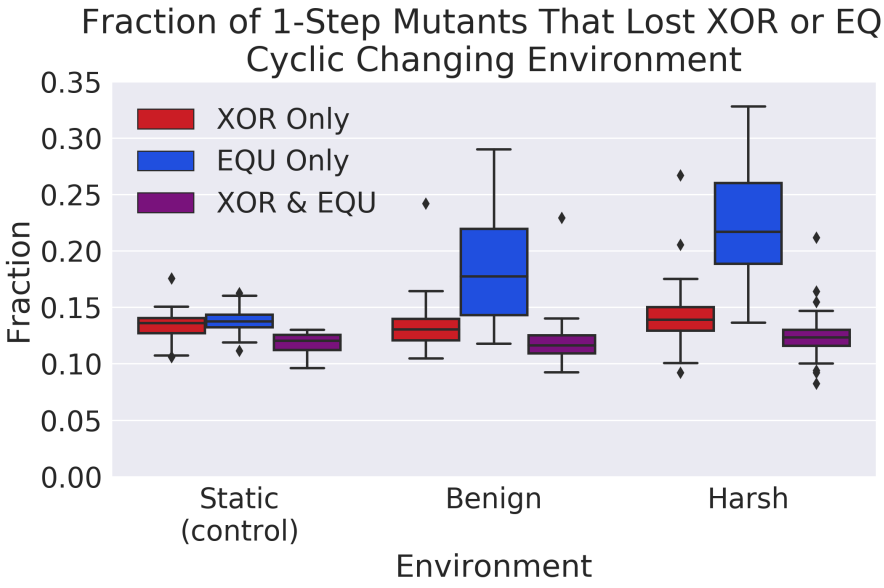

Figure 2.9: **A survey of the single-step mutational neighborhood** around organisms that performed the fluctuating task. Note that in both the benign and harsh treatments, there were significantly more mutants that lost the EQU task as compared to the control (Wilcoxon Rank Sum Test: Z = -5.46 and -7.80 respectively, p << 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.

We found that in both the benign and harsh treatments, there were many more mutations 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 << 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 2.7, 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 (Spearman's Rho: r_s = 8.72 and 6.45, respectively, p = << 0.001) (Fig 2.10). We also found a weaker, but still significant correlation between the number of XOR-only functional sites and loss of the XOR task (Spearman's Rho: $r_s = 3.85$, p << 0.001). This result confirms our intuition that the longer the task, the more targets there are for mutation to disable the task.

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 2.11).

We speculate that this effect is due to the availability of reservoirs of formerly vestigial

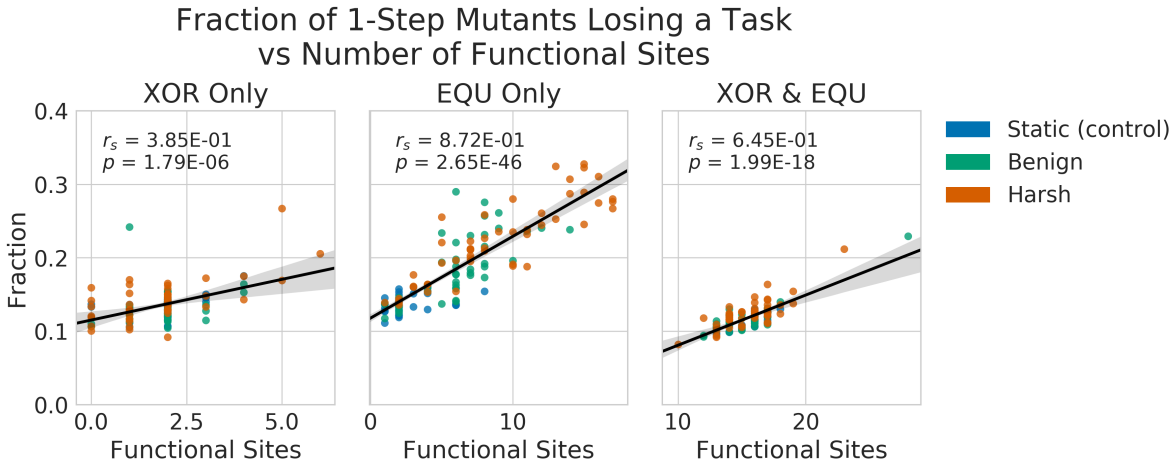

Figure 2.10: **Correlation between task length and mutational task loss** in the 1-step neighborhood across all treatments. Note the strong correlation between the length of the EQU task and the fraction of mutants that lost EQU (Spearman's Rho: $r_s = 8.72$, p $<<$ 0.001). Further, consider the weaker 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.

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

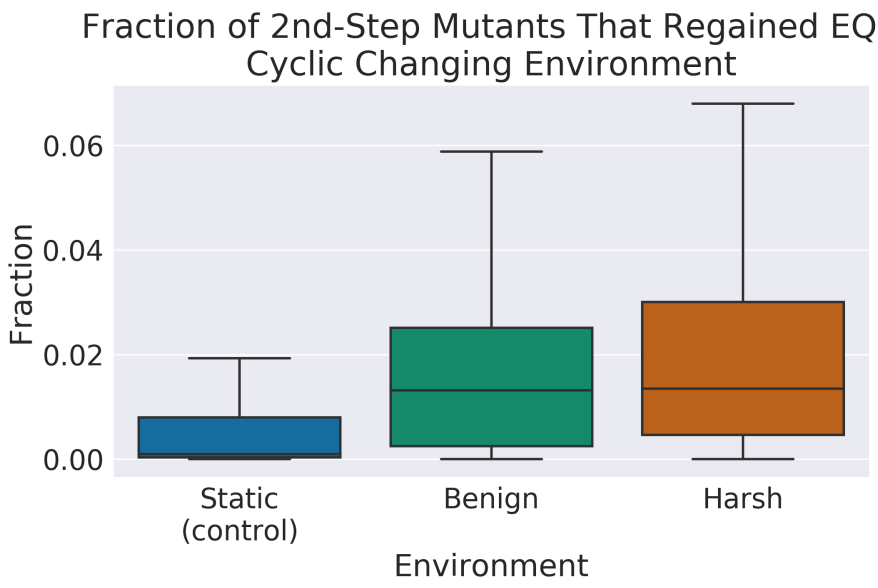

Figure 2.11: **A survey of the two-step mutational neighborhood** of the organisms that lost EQU function in the one-step survey. We found that in both the harsh and benign treatments, there were significantly more organisms that regained function in response to mutation than the control. (Wilcoxon Rank 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.

in the short-term evolvability of the fluctuating task. This result is consistent with real adaptation, not only to resources in their local environment, but a direct adaptation to the environmental change. (Fig 2.12)

What might account for this adaptation? Similar to the relationship between the number of functional sites of a task, and the number of single-step mutants that lost that task (See Fig 2.10), we hypothesize that the reacquisition of tasks in the 2nd-step survey may be mediated by the amount of useful task material present in the genome. We performed a multiple linear regression, predicting the mean fraction of mutants that regained EQU, by the number of functional and vestigial sites contained in the original genome (See Table 2.2 and Fig 2.13).

We can predict approximately 47% of the variation in mean number of second step mutants that regained EQU based on the number of functional and vestigial sites. Most

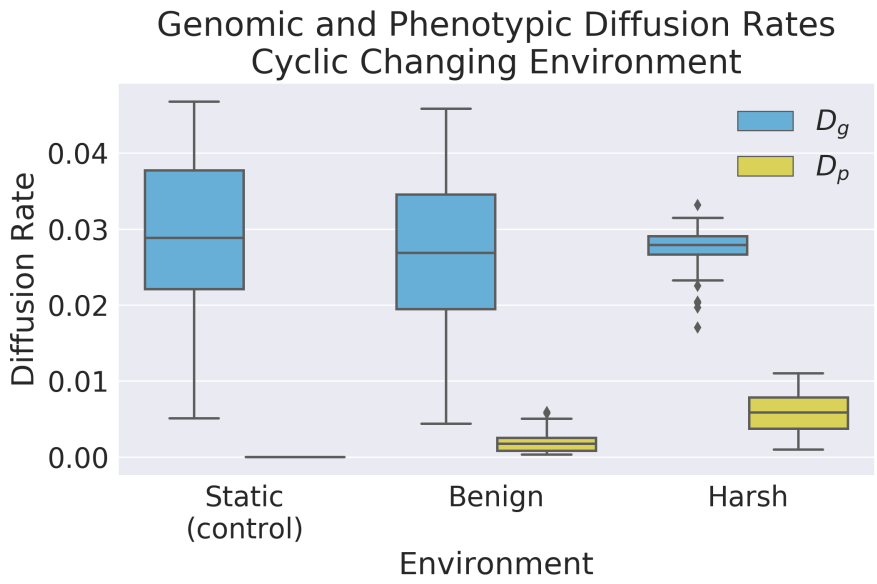

Figure 2.12: **Genomic and phenotypic diffusion rates**, showing the probabilities of producing offspring that are genotypically (D_g) or phenotypically (D_p) distinct from the parent, while not reducing fitness. Note that while overall neutral exploration capacity remains relatively stable between treatments (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 is consistent with changing environments promoting the phenotypic evolvability of populations.

of the variation is predicted by the number of functional sites, though vestigial sites do contribute a small amount. This result is consistent with the role of task length, and thus the number of informational sites, being important for regaining task function. We could not, however, account for all variation, indicating that there are other factors, possibly in robustness or modular architecture of tasks, that are important to this kind evolvability.

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

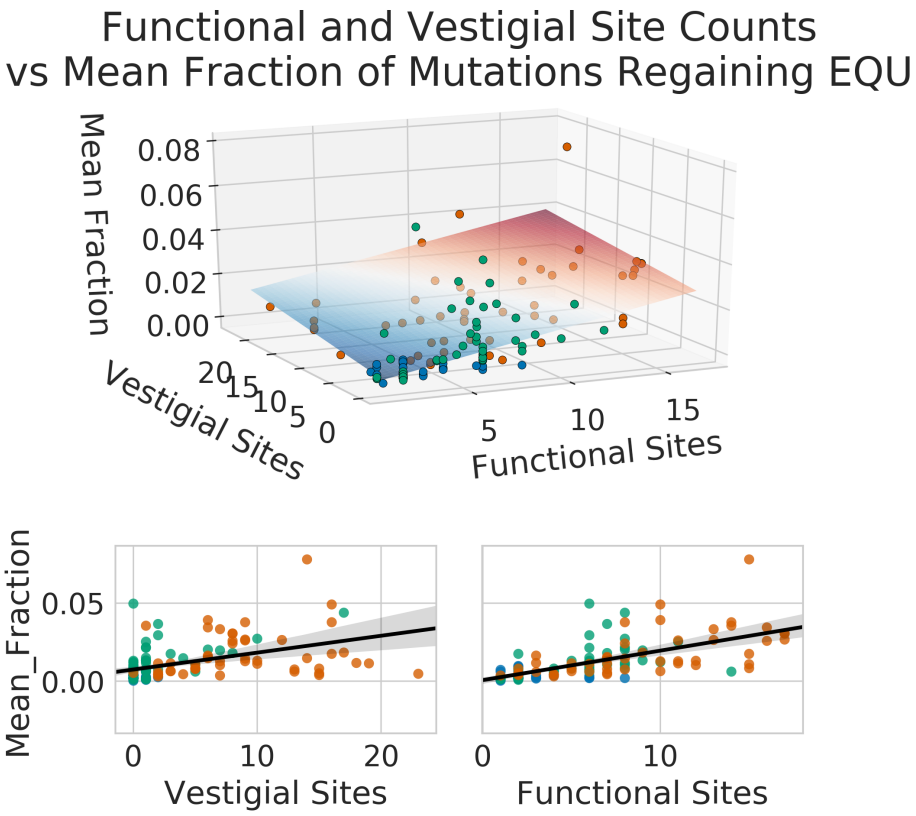

Figure 2.13: **Multiple linear regression predicting mean fraction of second-step mutants** from the number of functional and vestigial sites in the original organism. See Table 2.2.

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 2.14)

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 2.15, 2.16). This result suggests that stochastic harsh environments are certainly

Table 2.2: **OLS Regression Results - Mean Fraction of Mutants Regained EQU vs Number of Functional and Vestigial Sites**

Dep. Variable:	Mean_Fraction	**R-squared:**	0.472
Model:	OLS	**Adj. R-squared:**	0.464
Method:	Least Squares	**F-statistic:**	61.65
No. Observations:	141	**Prob (F-statistic):**	7.39e-20
Df Residuals:	138	**Log-Likelihood:**	467.28
Df Model:	2	**AIC:**	-928.6
		BIC:	-919.7

	coef	**std err**	**t**	**P>|t|**	**[0.025**	**0.975]**
Intercept	0.0003	0.001	0.243	0.808	-0.002	0.003
Func_Sites	0.0016	0.000	8.115	0.000	0.001	0.002
Vest_Sites	0.0005	0.000	3.180	0.002	0.000	0.001

Omnibus:	79.316	**Durbin-Watson:**	1.928
Prob(Omnibus):	0.000	**Jarque-Bera (JB):**	437.211
Skew:	1.970	**Prob(JB):**	1.15e-95
Kurtosis:	10.675	**Cond. No.**	15.0

Multiple linear regression, predicting Mean fraction of second-step mutants that regained EQU, based on the number of Functional and Vestigial sites in the original genome.

no more effective at promoting evolution toward areas of the mutational landscape where such mutations were common, and may, in fact perform worse.

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 result suggests 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 perform any differently from cyclic at promoting evolvability.

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

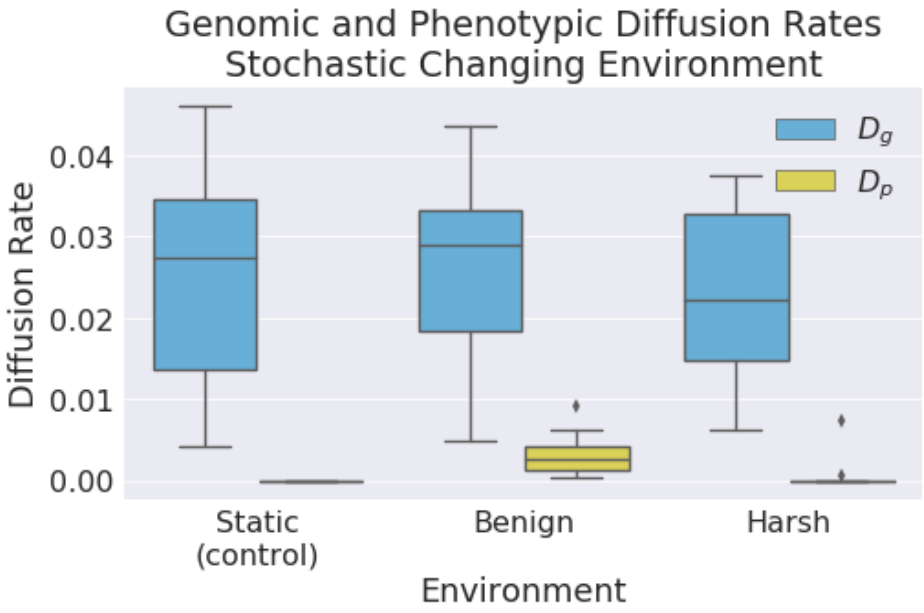

Figure 2.14: **Genomic and phenotypic diffusion rates** in stochastic changing environments, showing the probabilities of producing offspring that are genotypically and phenotypically different from the parent, while remaining fitness neutral or better. As in the cyclic environment, D_g remains stable 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.

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 2.17)

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

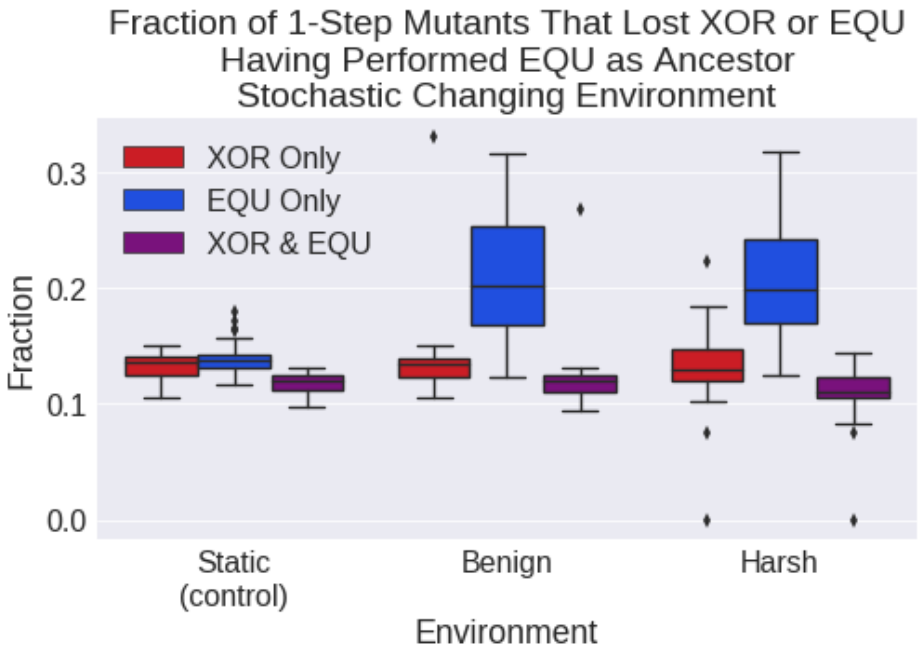

Figure 2.15: **A survey of the single-step mutational neighborhood** in the stochastic changing environment around organisms that performed the fluctuating task. Again, in the static and harsh treatments, values are comparable to the cyclic changing environment . However, in the benign treatment, the 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 is consistent with stochastic environmental change being equivalently effective at moving organisms to areas of the fitness landscape where they can more easily switch task expression.

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.

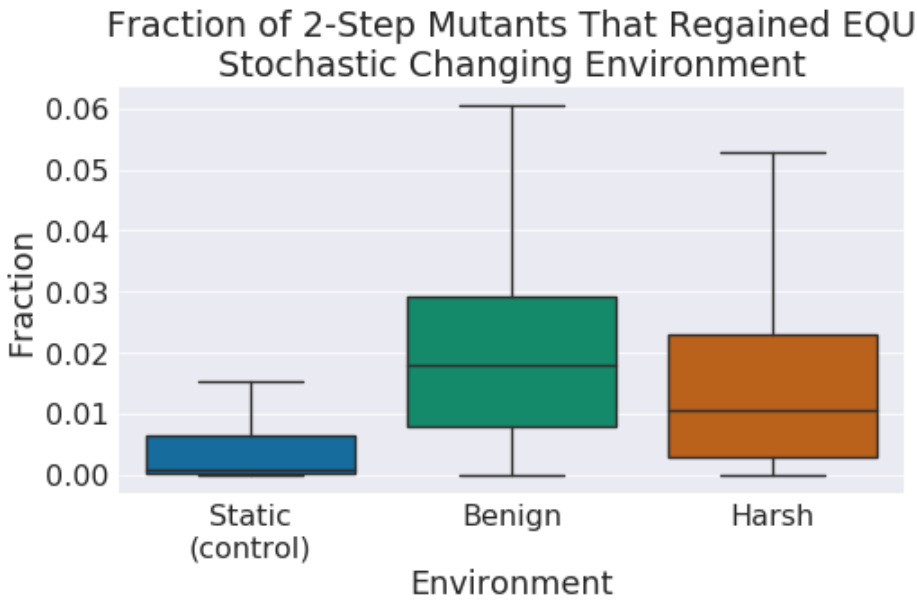

Figure 2.16: **A survey of the two-step mutational neighborhood** in the stochastic changing environment of the organisms that lost EQU function in the one-step survey. We found that the fraction of organisms regaining the fluctuating task (EQU) from a single additional mutation in the harsh treatment (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 2.15, 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.

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

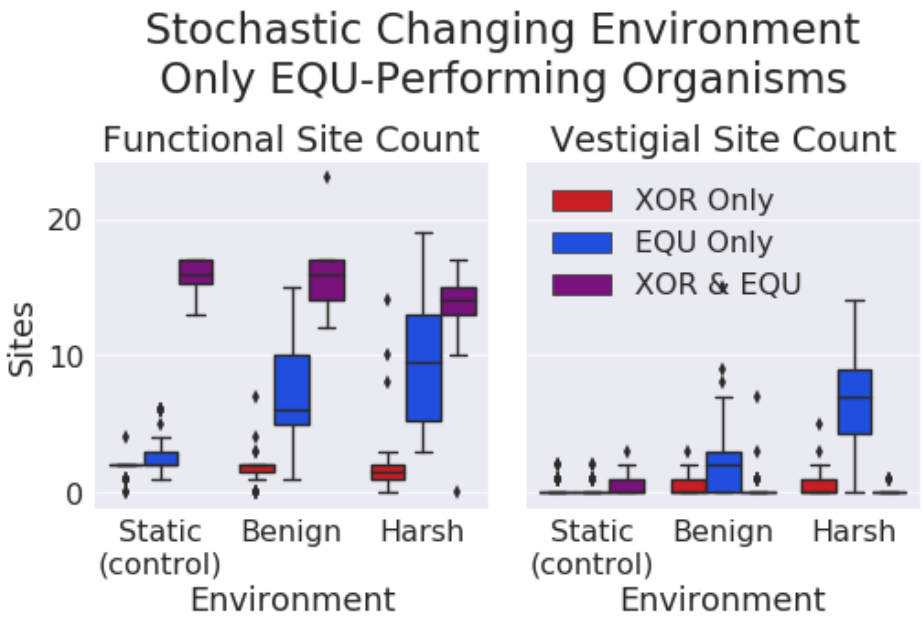

Figure 2.17: **Number of functional and vestigial sites by treatment** in a stochastic changing environment. The vestigial site counts for EQU-only performing organisms remain comparable to the cyclic environment (Wilcoxon Rank-Sum Z = 0.46, 1.45, and -1.10, p = 0.6, 0.14, and 0.2). The functional sites 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).

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 evolvability 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 mutations 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 [67]), or else receive them via bacterial conjugation [68] or viral infection (transduction [69, 70]). 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" [71]. For example, HGT appears to be the primary mechanism by which antibiotic resistance is conferred [72, 73] 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 [74]. 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 [75]. 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 [75]. 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 [76]), the processes themselves are ancient and nearly ubiquitous [77]. 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, we 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.

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 [78].

Homologous recombination requires a pair of valid matching sites in the genome, which are selected as follows: We search for a set of sites that match the first three instructions of the uptaken fragment, starting at the beginning of the genome. We search the whole genome, until we find a matching site 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, recombination fails. If recombination succeeds, it replaces the

content of the organism's genome between the selected beginning and end match sites with the content of the fragment. This may result in the genome growing or shrinking, depending on the distance between the matches, and the length of the fragment.

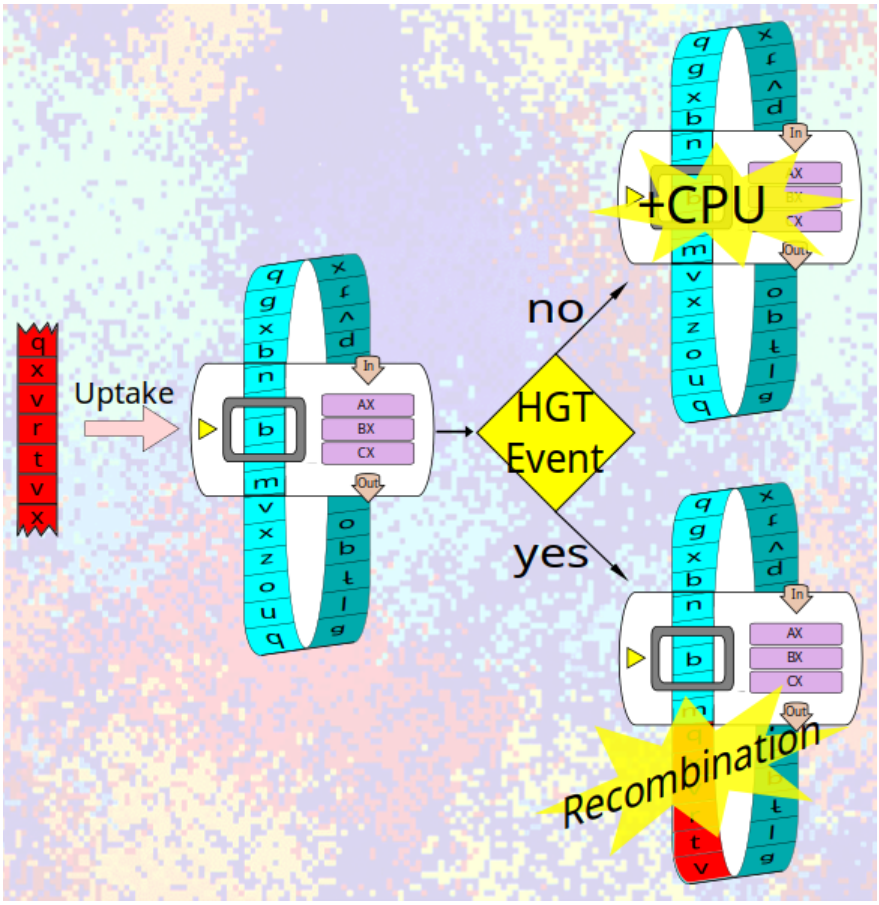

Figure 3.1: **The HGT process**. Organisms can execute instructions that trigger uptake from the environment. When uptake occurs, there is an experimenter-defined chance that either it will yield a boost to speed of execution or, alternatively, that the fragment will be integrated into the genome.

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

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).

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 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 (See Table 3.1).

Each complete cycle lasted 1000 updates, and the whole experimental run extended for 200,000 updates. The static environment rewarded executions of all the Logic-9 tasks at their default levels, with no reward switching.

3.2.2 Experimental Design

For the treatments corresponding to the first set of hypotheses on the origins of horizontal gene transfer, we subjected four populations of evolving digital organisms with HGT to harsh changing environments (Table 3.2), plus a pair of 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 phenotype switches.

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 is consistent with the information content of the fragment being 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 result is

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.

consistent with HGT in a static environment being 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, $p << 0.0001$).

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 (for more details of this bonus selection, please see Appendix B). We then combined these factors, giving a bonus plus a changing environment. We saw that the resulting uptake level increased largely additively. This result suggests that the benefits granted by a grazing bonus are generally independent of the benefits conveyed by integrating new genetic material.

Thus, not only is HGT evolution possible absent a bonus, the benefit stacks with that of

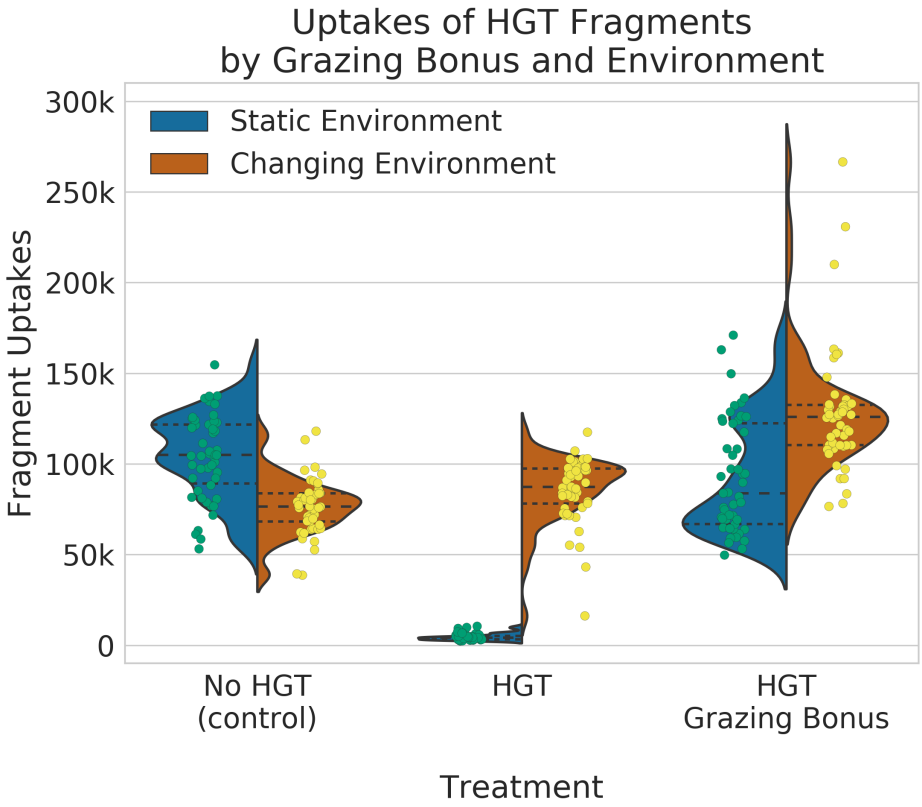

Figure 3.2: **Number of HGT fragment uptakes** in static and changing environments, without a grazing bonus, with a grazing bonus, and a no HGT control, where the `HGT-Uptake` instruction is inert. HGT uptake increased in response to a changing environment, and also in response to a grazing bonus. The grazing environment and a changing environment combined 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, $p << 0.0001$).

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.

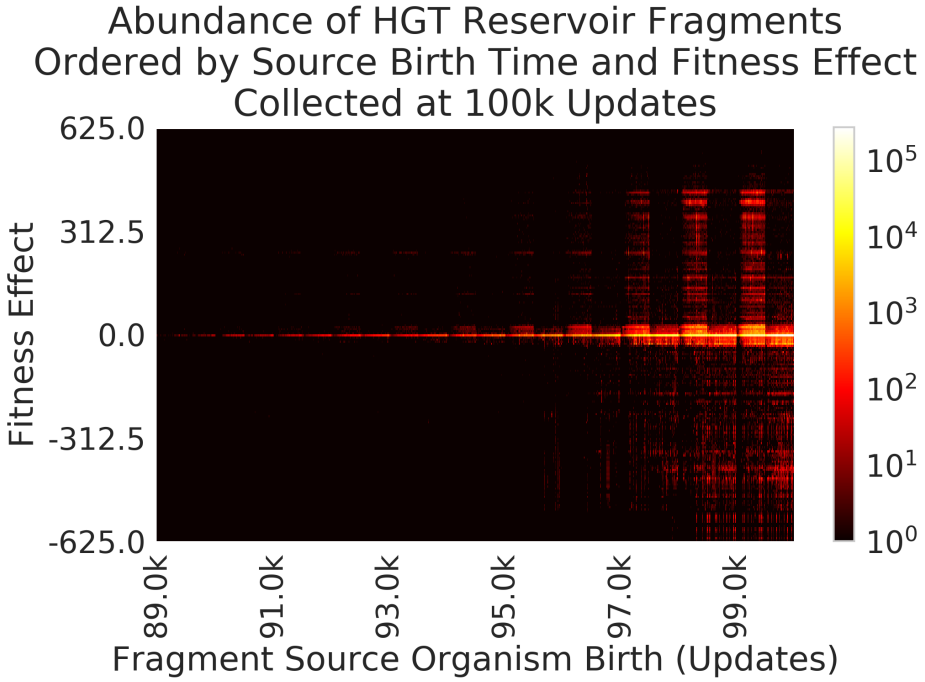

Figure 3.3: **Origin and fitness effect distributions of HGT fragments**, sampled midway through the experiment at 100,000 updates, and aggregated across all replicates. We applied each fragment in each cell's reservoir, one at a time, to the organism in the cell, then recorded the fitness effect. The 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.

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 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.

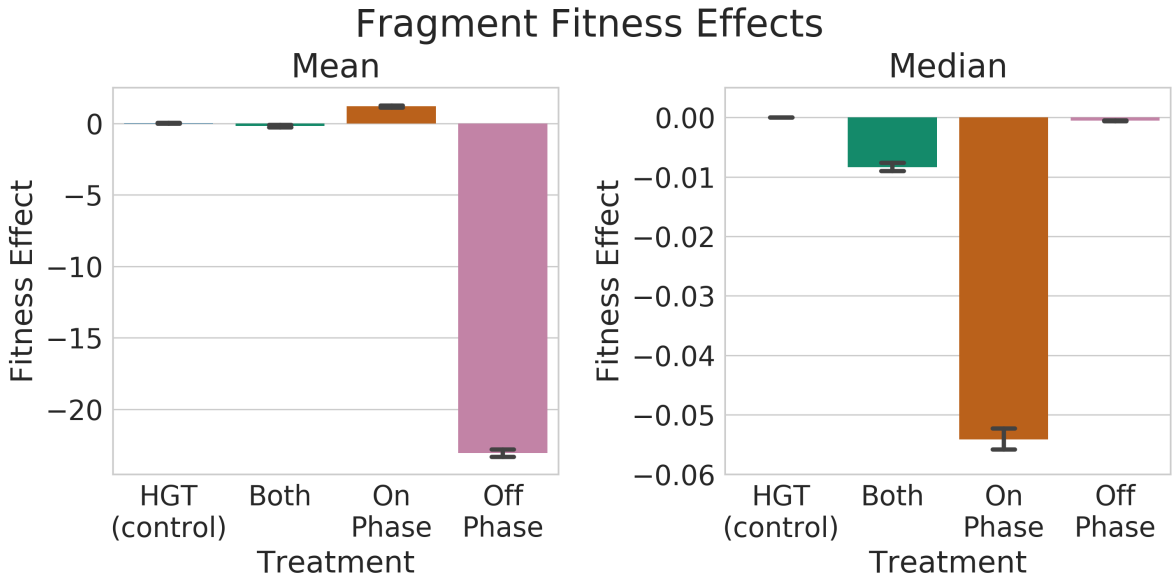

Figure 3.4: **Mean and median fitness effects of fragments** in reservoirs, with 95% confidence intervals. Fragments in reservoirs are replaced by fragments based on the origin of donor organism. For the On-Phase treatment, We only permitted fragments in reservoirs that originated in a matching phase 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.

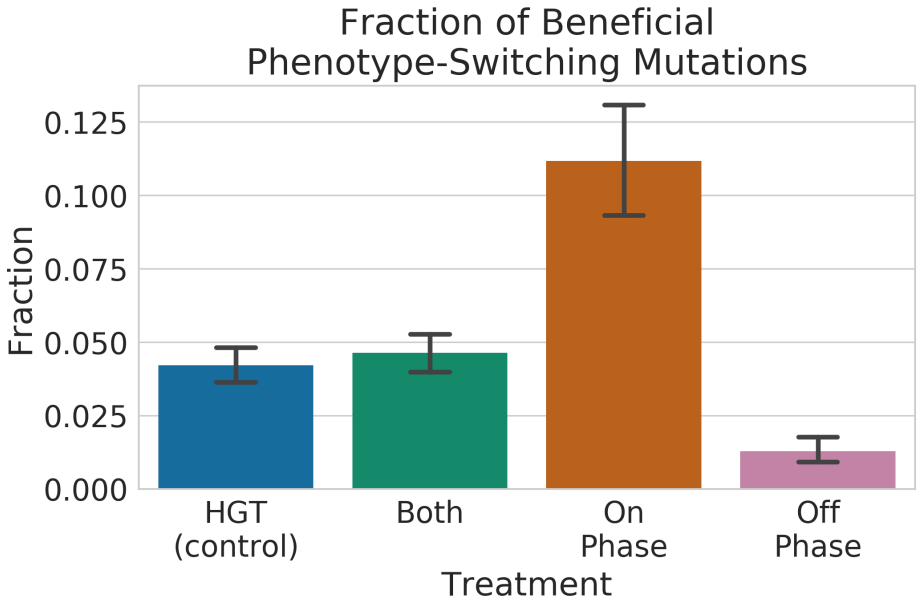

Figure 3.5: **Fraction of all fragments that produced a beneficial phenotype changes**, with 95% confidence intervals. The "On-Phase" treatment had significantly more fragments that convey a beneficial phenotype change than either of the controls (Wilcoxon Rank Sum Test: Z = -6.42 and -6.12 respectively, p << 0.001), while the "Off-Phase" had significantly fewer (Wilcoxon Rank-Sum Test: Z = 7.11 and 7.35 respectively, p << 0.001).

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)

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

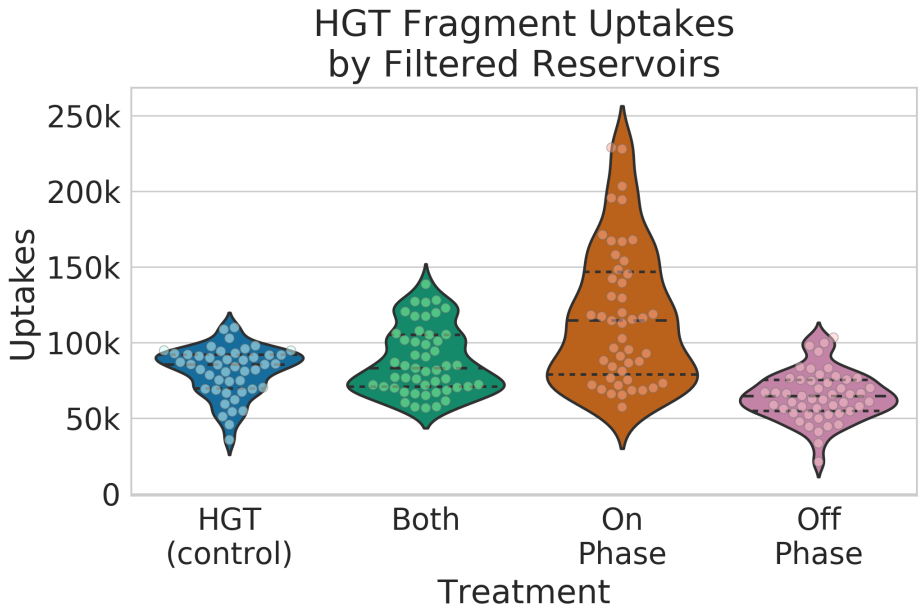

Figure 3.6: **Number of uptaken fragments** in the filtered reservoir treatments. The "On-Phase" treatment has a significantly larger number of HGT fragment uptakes, as compared to control, "both", and "off-phase" treatments (Wilcoxon Rank-Sum Test: Z = -3.9**, -3.2*, and -6.5**, respectively, p < 0.002* and p << 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.

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.

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 is consistent with the possibility of recombination itself 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. Even so, HGT use is still modestly elevated even when no new information is provided by fragments. This suggests that it is not just novel information that conveys a benefit. Rather, it is possible that HGT might be useful for other functions, such as error correction.

In natural systems, evidence for phenotype change via HGT is apparent in the evolution of antibiotic resistance in bacteria, where resistance is acquired largely 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 [79], despite evidence that higher mutation rates are optimal for adaptive evolution [80]. 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 [80]. 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 [81, 82, 83]. 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 up-regulated. What makes HGT different from other types of mutations? Is it the beneficial fitness effects of HGT mutations? The information content of fragments relating to a new environment? 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?

We hypothesize that those instructions that convey the largest amount of information, the best fitness outcomes, and the best likelihood of beneficial phenotype change will be used more than those that convey less information or lead to worse fitness outcomes.

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 aspect 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 1000 updates, and the whole experimental run continues for 200,000 updates. Also as in the previous chapter, the static environment rewards executions of all the Logic-9 tasks at their default levels, with no reward switching.

4.2.1 Experimental Design

We conducted a total of 14 treatments in order to isolate the advantages of HGT-Uptake and to identify the underlying reasons why this instruction is under positive selection. Each treatment differed by either environmental condition (static vs. changing) or the available mutagenic instruction.

4.2.1.1 Alternative Mutagenic Instructions

In order to perform experiments comparing HGT to other types of mutagenic instructions, 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. All instructions also have the same viable recombination site requirements as the default HGT instruction (as detailed in Chapter 3, Methods), in order to be able to compare use rates across treatments.

The specific set of possible instructions are:

- **HGT (Intact Fragment)**: The default HGT operation, as detailed in Chapter 3, Methods. We uptake a fragment from the environment. We apply a 10% probability that the fragment is recombined into the organism's executing genome. A valid recombination site is required for recombination to occur.

- **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 recombination 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 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 each 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. The Die instruction is a control, to identify a minimal level of use where populations would suppress the use of the instruction as much as possible.

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).

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` (Mutation Event - Sampled)
ME-Sampled CE	Changing	
ME-Random	Static	`ME-Random` (Mutation Event - Random)
ME-Random CE	Changing	
MRI	Static	`MRI` (Mutation Rate Increase)
MRI CE	Changing	
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 mutagenic instructions.

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 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 experimental results (detailed below) show 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 mutagenic instruction 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 expected 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

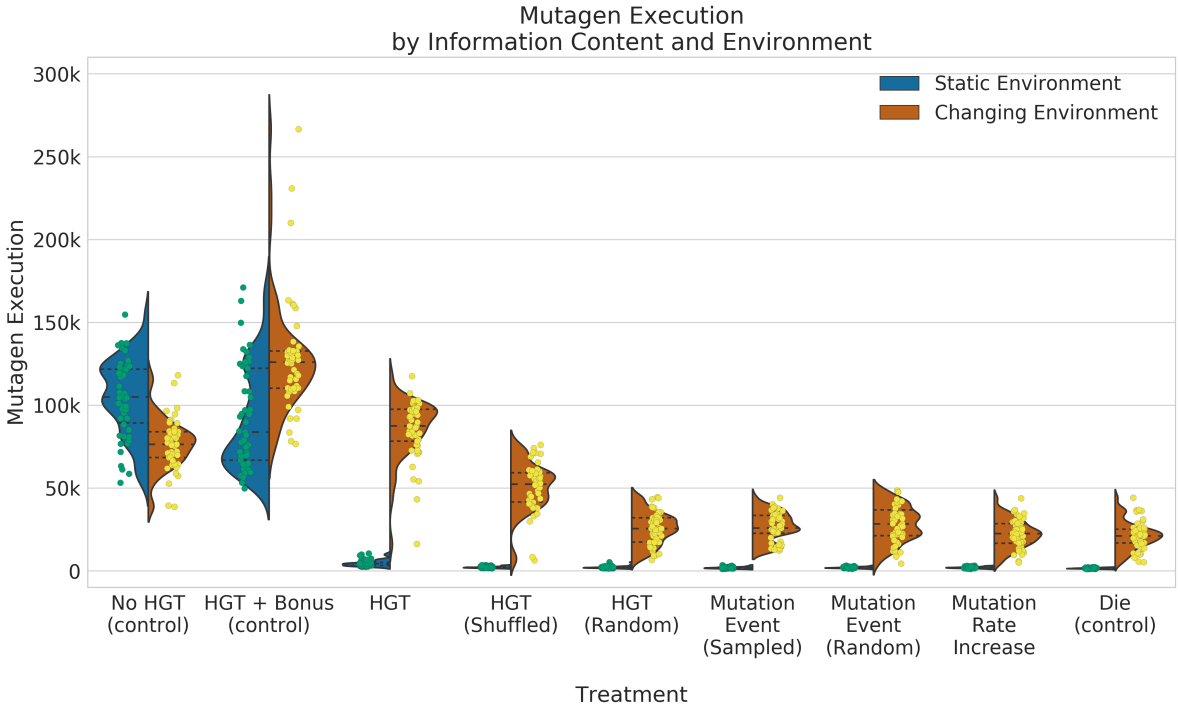

Figure 4.1: **Executions of mutagenic instructions**. In static environments, use of all mutagenic instructions was strongly suppressed (Wilcoxon Rank Sum Test: Z = -22.65, p<<0.001). In changing environment treatments, the `HGT` and `HGT-Shuffled` instructions were used at elevated rates, compared to 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 ranking of the use of the HGT-like instructions by order of information content.

The baseline intact-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 finally 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: `ME-Sampled` (Mdn = 25,798.03, CI 95% [24335.01, 30824.09]), and `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 are used 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 primary difference between the `ME-Sampled` and `HGT-Shuffle` treatments is the localization of the mutation effect (the `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 and substantially different (Wilcoxon Rank Sum Test: Z = 7.19, p << 0.001). This result suggests that localization of the HGT mutation plays an important role, over and above any useful enrichment of instructions originating in a living organism.

We speculate that this effect may also synergize with the level of modularity of 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

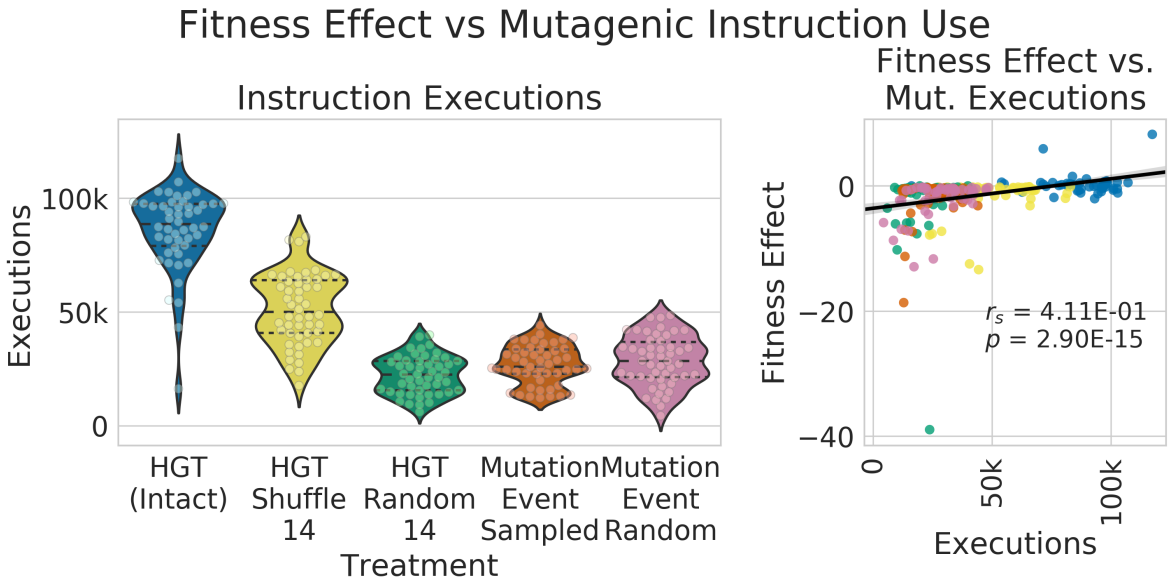

Figure 4.2: **Mutagenic instruction use vs fitness effect of mutation**. The use of the mutagenic instruction correlates with the fitness effect of that instruction (Spearman's Rho: $r_s = 0.41$, p $<<$ 0.001). Because mutagenic instructions are integrated into the genomes, those instructions that produce 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 [80]. 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 (Spearman's Rho: $r_s = 0.41$, 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

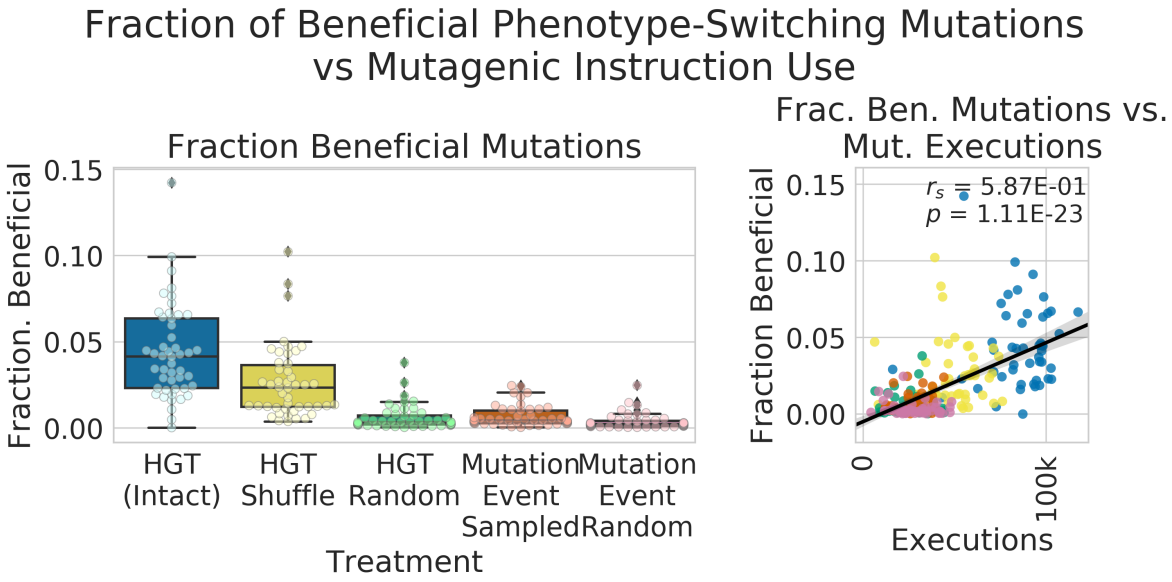

Figure 4.3: **Fraction of beneficial phenotype-switching mutations vs mutagenic instruction use**. We observe a strong correlation between the fraction of mutations that produce beneficial phenotype switches, and the use of the mutagenic instruction (Spearman's Rho: $r_s = 0.59$, p = << 0.001). This 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 (Fig 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 (Spearman's Rho: $r_s = 0.59$, p = << 0.001). This correlation is much stronger than the correlation with fitness effect, suggesting 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 these effects predict the levels of use of that mutagenic instruction.

In particular, we observed a substantial increase in the use of the `HGT` instruction in response to a changing environment. What could account for this increase? If this increase were driven purely by an increase in the effective mutation rate, we would expect to see a similar increase in all instructions that increase this mutation rate. Instead, we do not observe such an increase in the use of the `MRI` instruction, which explicitly increases the mutation rate in just such a way. Instead, the use of this instruction was not noticeably different from the level of the `Die` instruction control.

Another possibility that might account for increase in the use of the `HGT` instruction would be the benefit of limiting mutations to a single contiguous region. In this case, we would expect to see an increase in the rate of the `HGT-Random` instruction over the level of the `Die` control. We do observe just such an increase, but while it is statistically significant, it is not substantial compared to the elevation we observe of the `HGT` instruction.

One other possibility is that there is a benefit to biasing mutation toward known-useful instructions, such as those collected from living organisms. In this case, we would expect to see a similar increase in use of the `HGT-Shuffle` instruction. We do observe a substantial increase of this instruction over `HGT-Random`, however, the increase of `HGT` is much higher.

This is consistent with some benefit occurring from a biased instruction content, but does not account for the entirety of the effect.

Thus, the most likely possibility is that it is specifically the information content of the fragment that accounts for the extra increase of `HGT` over `HGT-Shuffle`. We found a strong correlation between the fraction of mutations that produce a beneficial phenotype change, and the use of that mutagenic instruction. Thus, in the context of a changing environment, where there are strong selective pressures to change your phenotype, the information corresponding to the target environment would be very valuable.

This result is consistent with prior research that shows that populations will tend to depress the use of non-information-rich mutagens, even though, in the long-term, higher mutation rates are likely to be optimal [80]. This result is further evidence that selection tends to act in the short term. Thus, even though certain kinds of mutations 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.

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. Even using the latest techniques in molecular biology, experiments comparing the effects of different kinds of mutations, measuring their use, and isolating their fitness effects are impossible at population-level scales [84]. Digital evolution allows us to perform the kinds of experiments that otherwise could not be done in natural systems.

CHAPTER 5

CHANGING ENVIRONMENTS AND LONG TERM EVOLVABILITY

5.1 Background

For longer evolutionary timescales, beyond the limited scope of direct response to selection against an environment, evolvability is concerned 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 needing to cross 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 convey short-term evolvability may not be the same as those that confer longer-term evolvability [13]. For example, features such as anti-robustness 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.

Finally, there is some evidence that the types of selection regimes typically used in experiments with changing environments and evolvability might preferentially favor individual evolvability (the probability of an individual's offspring accessing novel phenotypes) over population-level evolvability (the probability of the population at large accessing novel phenotypes) [85, 86]. Adaptive selection, that is, selection toward a particular goal, has been shown to depress population diversity even while it increases individual evolvability in changing environment regimes. In contrast, divergent selection, such as frequency-dependent selec-

tion, increases standing diversity, and thus evolvability at the population level [85]. Therefore, it is not clear that the kinds of selective pressures that promote short-term adaptation in changing environments would, in turn, promote exploration and exploitation of novel environments.

In this chapter, we examine how two different kinds of changing environments affect long-term evolutionary potential. We introduce change-evolved populations from prior experiments, 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 the experiments described in this chapter, we evolve populations in both minimal and rich environments. Minimal environments have few rewarded tasks, and genomes are fixed length and limited in size. Rich environments allow rewards for many tasks, and genome lengths are allowed to vary. For our experiments with minimal environments, we used populations originally evolved in Chapter 2 to survey the long-term evolutionary potential of populations originating in environments with a single fluctuating task. For the rich environment experiments, we evolved a new set of populations that originate in a more complex changing environment, with alternating sets of fluctuating tasks, and variable length genomes.

5.2.1 Experimental Design

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. In order to examine the long-term dynamics and mechanisms of evolving populations in changing environments, we performed two sets of experiments, one set with populations evolving in a minimal environment, and the other in a rich environment.

Each set is composed of five environmental treatments per set, for a total of 10 treatments. Each treatment was divided into two stages, with the first stage focusing on short-term adaptation to a changing environment, and the second stage on long-term evolvability via adaptation to an expanded set of tasks.

For both set of experiments, we took populations evolved in conditions where short-term evolvability pressures dominated, and introduced them to a completely new environment, with an expanded set of rewarded bitwise tasks to perform: Logic-77. For the purposes of this chapter, we refer to those tasks which were selected for in stage 1 as the **basic task set**. The Logic-77 task set is a super-set of the basic task set, and includes all bitwise tasks for which there are up to 3 inputs, including those that were initially rewarded in stage 1. We refer to the additional tasks from Logic-77 - those which are not part of the basic tasks set, and that we reward only in stage 2 - as the **expanded task set**. The total Logic-77 task set is a combination of both the basic and expanded task sets.

The first set of experiments examines the evolutionary dynamics of populations in a **minimal** changing environments, with a single fluctuating task. These experiments are a continuation of the experiments described in Chapter 2. For the second set of experiments, we evolved populations in a **rich** 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 of the Logic-9 environment. The environment and genetic structure of the second set of experiments derives from the experiments described in Chapter 3. However, these populations were evolved without a working HGT instruction. The first stage of each experiment is as described above, while the second stage takes the evolved populations and adds the expanded task set.

5.2.1.1 Environmental Treatments

As outlined above, for each of our experiment sets, we prepared four different types of changing environment treatments, plus a static control. In the second stage of each treatment, we

examine how populations adapt to a brand new environment. The active changing environment treatments will still juggle the alternating direction of selective pressures associated with cycling rewards and punishments. The quiescent treatments, in contrast, remove the reward alternation in the second stage Thus, these treatments provide only directional selection for adaptation to the new environment. This allows us to isolate the effect of alternating directions of selection on adaptation to a new environment.

- **Static (Control)**: This treatment is a baseline for comparing adaptation to the expanded task set. For the first 200k updates (stage 1), we reward populations for performing either XOR and EQU for the minimal environment (Table 5.1), or the whole Logic-9 task set (Table 5.3). For the second 200k updates (stage 2), we add constant rewards for the expanded task set. Each new task is rewarded at a 1.2-fold bonus to task execution.

- **Benign Changing Environment**: This treatment shows the effects of a continuing benign changing environment on adaptation to the expanded task set. For the first 200k updates (stage 1), we alternate rewarding and not rewarding populations for performing either the EQU task (the minimal 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 stage of the experiment, starting at 200k updates, we add constant rewards for each of the new tasks in the expanded task set, at a 1.2-fold bonus to task execution. The environmental fluctuation from the first stage continues through the end of the experiment.

- **Benign Quiescent Changing Environment**: In contrast to the benign changing environment treatment, this treatment tests the abilities of populations initially evolved in a benign changing environment to adapt to the expanded task set, but without active environmental fluctuation during the adaptation. For the first 200k updates (stage 1), we alternate rewarding and not rewarding populations, as in the Benign Changing Environment above. For the second stage of the experiment, starting at 200k updates,

we add constant rewards for each of the new tasks in the expanded task set, at a 1.2-fold bonus to task execution. The environmental fluctuation from the first phase stops at 200k updates, and we instead reward the tasks of the first phase (the basic task-set) as we did in the static treatment (all constant reward).

- **Harsh Changing Environment**: This treatment shows the effects of a continuing harsh changing environment on adaptation to the expanded task set. For the first 200k updates (stage 1), we alternate rewarding and **punishing** populations for performing either the EQU task (the minimal 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 stage of the experiment, starting at 200k updates, we add constant rewards for each of the new tasks of the expanded 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**: In contrast to the harsh changing environment treatment, this treatment tests the abilities of populations initially evolved in a harsh changing environment to adapt to the expanded task set, but without active environmental fluctuation during the adaptation. For the first 200k updates (stage 1), we alternate rewarding and **punishing** populations, as in the Harsh Changing Environment above. For the second stage of the experiment, starting at 200k updates, we add constant rewards for each of the new tasks in the expanded task set, at a 1.2-fold bonus to task execution. The environmental fluctuation from the first stage stops at 200k updates, and we instead reward the basic tasks of the first phase as we did in the static treatment (all constant reward).

5.2.1.2 Set 1 - Minimal Cyclic Changing Environments

As described above, the first set of experiments consisted of four treatments, plus a control, and each treatment was divided into two stages. In the first stage, 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 treatment. For the second stage, lasting an additional 200,000 updates, we added a constant reward for executing the expanded task set. Each task in the expanded task set was rewarded with a constant 1.2-fold bonus to execution ($2^{0.3}$). 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 basic 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. This limitation in genome length, plus the limited set of tasks initially rewarded results in a less rich environment for evolution to occur. 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 - Rich Cyclic Changing Environment

The second set of experiments looked at the evolution of populations of organisms with variable-length genomes, evolved in a richer cyclic changing environment. As in the minimal environment experiment set, we performed four experimental treatments, plus a static control (Table 5.3). For the first stage of the experiments, we divided the basic 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 the Logic-9 basic task set. For the second

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

Treatment	**Changing Env. Type**	**Rewarded Tasks**				
		Stage 1 (0-200,000 Updates)		**Stage 2 (200,000-400,000 Updates)**		
		XOR	EQU	XOR	EQU	Expanded Task-set (Logic-77 minus XOR & EQU)
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 Quiescent	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 Quiescent	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 treatment is split into two stages. The first stage is a normal changing environment like those found in Chapter 2, Table 2.1. The second stage introduces an additional set of tasks (the expanded task set) that are rewarded at a lower rate.

stage, we introduced rewards for performing the new expanded task set tasks, either with continuing alternating selection (CE), or switching to constant reward (Quiescent).

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

Table 5.2: **Rich Changing Environments - Basic Task-set Rewarded Task Groupings**

Basic Task-set (Logic-9)	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

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

populations is affected by evolution in a changing environment, then this effect should result in differential rates of task discovery and performance. Task discovery and performance together describe the exploration and exploitation of the environment by a population.

5.2.2.1 Task Discovery

Task discovery represents the level of exploration of the fitness landscape. We measured task discovery by counting the number of unique non-ephemeral tasks that have been discovered by a population. Each task may be performed only once per organism, yielding a maximum task count of 3600 at any given time. We define a non-ephemeral task as one that is performed by at least than 0.1% of the population. Therefore, in order for a new task to be marked as discovered, it must be performed by at least 4 individuals at the time of sampling.

Once a task is discovered, it may not be undiscovered; task discovery counts will always

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

Treatment	**Changing Env. Type**	**Rewarded Tasks**		
		Stage 1 (0-200,000 Updates)	**Stage 2 (200,000-400,000 Updates)**	
		Basic Task-set (Logic-9) Groups A & B	Basic Task-set (Logic-9) Groups A & B	Expanded Task-set (Logic-77 minus Logic-9)
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 Quiescent	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 Quiescent	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 treatment is split into two stages. The first stage is a normal changing environment like those found in Table 2.1. The second stage introduces an additional set of tasks (the **expanded task set**) that are rewarded at a lower rate.

increase monotonically. We measure **overall** task discovery by beginning to collect unique tasks starting at the beginning of the run. For the overall measurement, we count all possible tasks - the entire Logic-77 task set - even though not all tasks are rewarded in the first stage of the experiment. We also measure **post-reward** task discovery, where we begin counting new tasks from the beginning of the second stage of the experiment, once we have begun rewarding execution of the **expanded task set**. Task discovery can range anywhere from a minimum of zero tasks discovered, to a maximum of 77.

5.2.2.2 Task Performance

In addition to counting the number of unique task discovered, we also measure task performance. We measure task performance by counting the total number of unique, non-ephemeral tasks that a population is performing at each sampling point. This measure represents the level of exploitation of the fitness landscape. This measure can range from 0 to a maximum of 77 task being performed by the population. This value will always be either equal to, or smaller than the number of tasks discovered, since a population can't perform a task it hasn't discovered yet.

5.3 Results and Discussion

5.3.1 Task Discovery

Task discovery is an important measure of long-term evolvability in that it quantifies the ability of populations to explore and adapt to entirely new environments. We measured task discovery in each of the changing environment treatments.

5.3.1.1 Benign changing environments outperform harsh environments in task discovery

We found that once we began rewarding the expanded task set, populations evolving in harsh changing environments discovered many fewer tasks that those evolving in benign changing

environments. This effect is consistent across both the minimal (Wilcoxon Rank Sum Test: $Z = 2.75$, $p < 0.01$) and the rich 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 expanded task set.

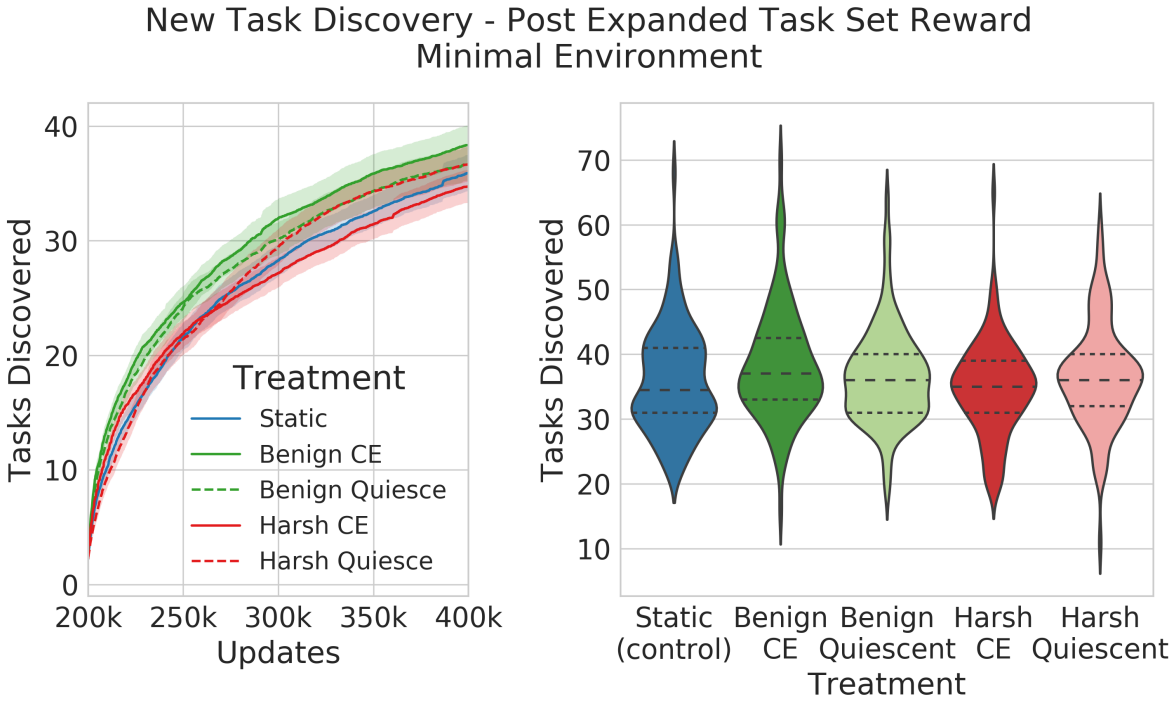

Figure 5.1: **Number of new expanded task set tasks discovered in post-reward in the minimal environments**. The left plot shows a time-series of the number of non-ephemeral tasks discovered by populations by each treatment. The right plot shows the number of tasks discovered at the end of the experiments. While the individual values overlap their neighbors, the top and bottom-most treatments (Benign and Harsh) are significantly different from each other (Wilcoxon Rank Sum Test: $Z = 2.75$, $p < 0.01$).

In the harsh changing environments, the selective pressure to gain or lose the fluctuating tasks represents up to a $2 * 2^5$-fold bonus over the course of a single cycle, whereas the expanded task set 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 **expanded task set** tasks.

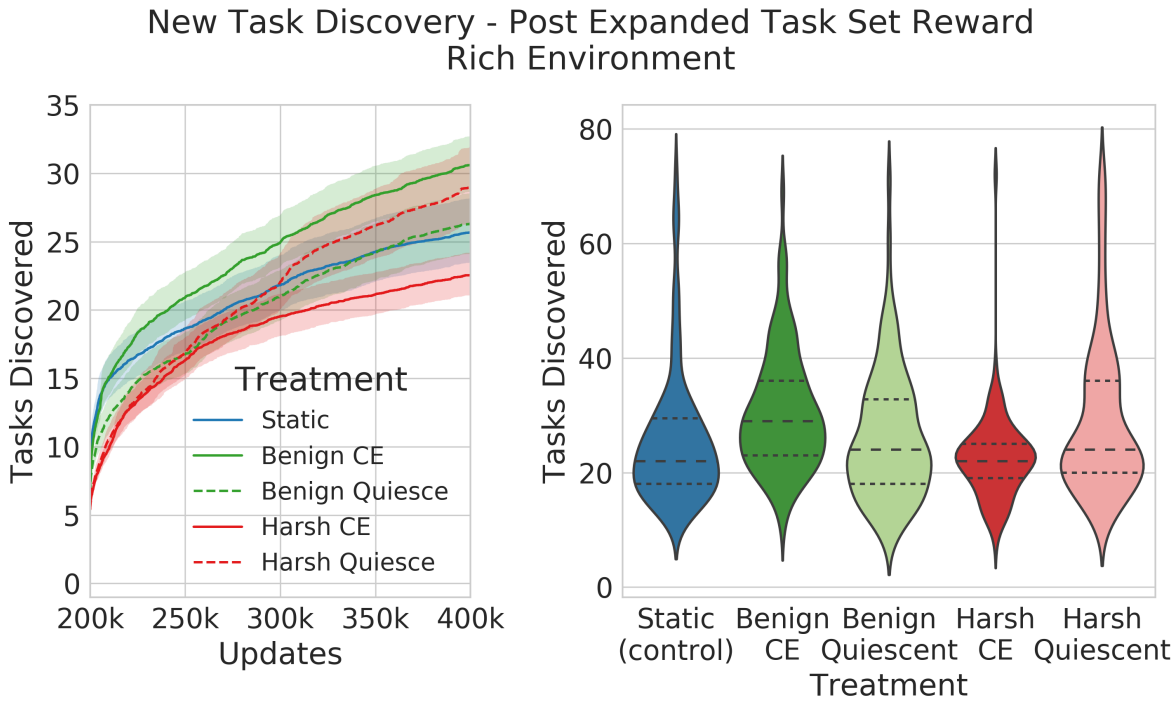

Figure 5.2: **Number of new expanded task set tasks discovered post-reward in rich environments**. The left plot shows a time-series of the number of non-ephemeral tasks discovered by populations in each treatment. The right plot shows the number of tasks discovered by the end of the experiments. Overall, the number of **expanded task set** tasks discovered in the rich environments (Mdn = 24.0, CI 95% [23.0, 25.0]) is significantly lower than those found in the minimal 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).

Thus pressures to gain and lose the fluctuating tasks are much stronger than the pressure to acquire new **expanded task set** 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, and no direct pressure to lose the task. Thus, when compared to the harsh treatments, the fraction of the total selective pressure for gaining new expanded tasks is greater in the benign treatment. This increased pressure, plus the benefit of an increased exploration rate conveyed by the benign changing environment, result in a higher overall

task discovery rates.

Interestingly, in the harsh quiescent treatment (HarshQuiescent) beginning in stage 2, we saw that task exploration recovered and achieved a comparable level to the control (Wilcoxon Rank Sum Test: Z = -0.91, p = 0.37). What could account for this recovery?

One possibility is that the introduction of the new tasks provided sufficient selective pressure to cause the increase in the discovery rate. In this case, we would expect to see a similar increase in task exploration in the harsh changing environment treatment (HarshCE).

Another possibility is that the alternating environment in the first part of the experiment created a diversity disadvantage in populations in those treatments. If this were the case, we would expect HarshQuiescent's task discovery to initially grow more slowly than the control, which would have suffered from no such disadvantage. Then, as diversity recovered, we would expect to see task discovery grow at comparable rates.

Finally, there is the possibility is that the alternating selection regime was directly responsible for depressing task exploration. In this case, once we stopped alternating task rewards, we would expect to see a significant difference in task discovery rates between the HarshQuiescent and HarshCE treatments.

Indeed, we found that the the HarshQuiescent treatment has a much higher task discovery rate than the HarshCE treatment. This is inconsistent with the hypothesis that the task rewards alone account for the recovery of the HarshQuiescent. Instead, this result is consistent with the possibility of a direct negative effect from the continuing alternating selection. We also found that there was a lag in task discovery compared to the control. This suggests that there was, at least initially, some population-level disadvantage occurring in the HarshQuiescent populations. We also observed that after the initially slow recovery phase, the quiescent treatment rapidly increased its task discovery rate, and exceeded that of the control. This is consistent with a recovery of diversity, plus, potentially some lingering architectural advantage for finding new tasks.

Also of interest is the difference in task discovery rates between the benign environment

populations BenignCE and BenignQuiescent. Once the expanded task rewards are introduced, the BenignCE populations continue to be subject to a benign changing environment, whereas the BenignQuiescent populations are only subject to directional selection toward the evolution of the original and expanded tasks. In both environment types, the task discovery rate for the BenignCE populations (Minimal: Mdn = 37.0, CI 95% [36.0, 38.0]; Rich: Mdn = 29.0, CI 95% [26.0, 31.0]) are slightly higher than for BenignQuiescent (Minimal: Mdn = 36.0, CI 95% [34.0, 38.0]; Rich: Mdn = 24.0, CI 95% [22.0, 27.0]), but the effect is not statistically significant in the minimal environment (Rich: Wilcoxon Rank Sum Test: $Z = 3.15$, $p < 0.01$; Minimal: Wilcoxon Rank Sum Test: $Z = 1.39$, $p = 0.16$). Both, however, still perform better than the control treatment (Minimal: Mdn = 34.5, CI 95% [32.0, 38.0]; Rich: Mdn = 22.0, CI 95% [21.0, 25.0]), though this effect is only statistically significant between the BenignCE and Control treatments in the rich environment (Wilcoxon Rank Sum Test: $Z = -4.33$, $p < 0.001$). What could account for this reversal of the pattern observed between HarshCE and HarshQuiescent?

One possibility is that some population-level diversity advantage is being conveyed by the activity of the benign changing environment. In this case, we would expect the BenignQuiescent task discovery rates to initially closely track that of the BenignCE treatments, and then as diversity equalized, to drop down to rates comparable to the control.

Another possibility is that the benign changing environment is directly promoting task exploration. In this case, we would expect to see a significant difference in task discovery rates between the BenignCE and BenignQuiescent treatments.

We observe that following the introduction of the **expanded task set** tasks, the BenignQuiescent treatment task rates quickly depart from the BenignCE task discovery rates. This result is inconsistent with the hypothesis of a population diversity advantage. If anything, task discovery levels lag behind the control treatment. Thus, we find that it is most likely that the benign changing environment is directly promoting task discovery, though the mechanism of this promotion remains unclear.

5.3.1.2 Harsh changing environments drive populations across the mutational landscape

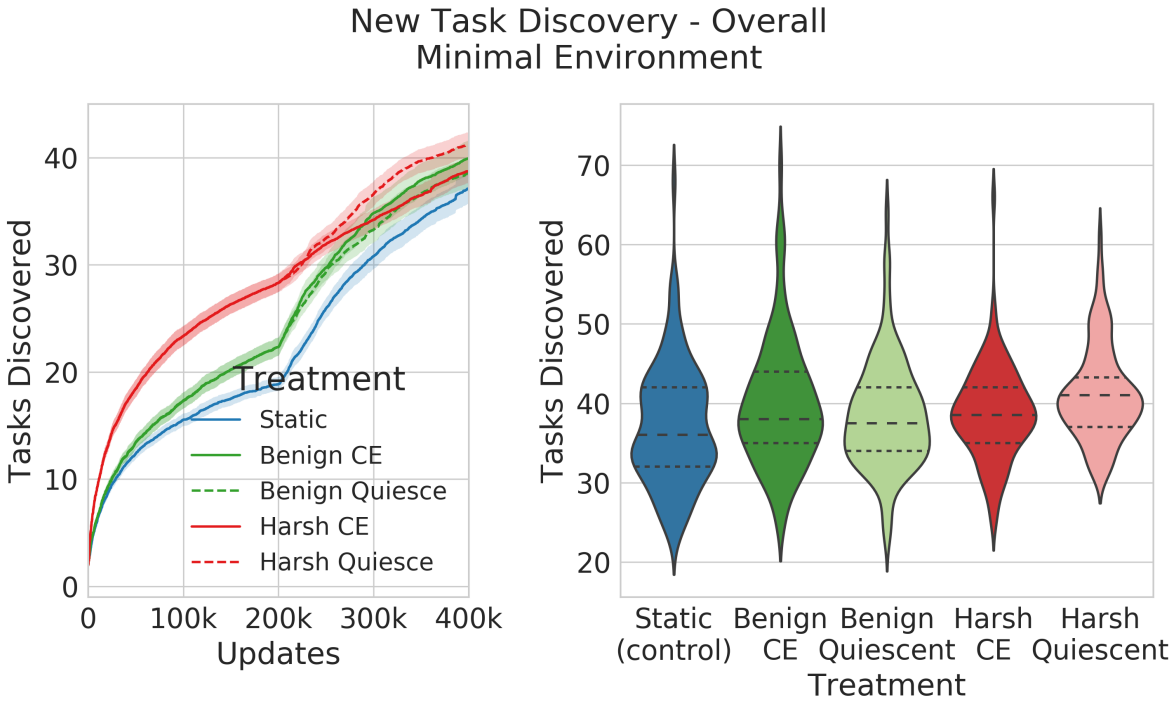

Figure 5.3: **Number of new expanded task set tasks discovered in minimal environments, over the whole experiment**. The left plot shows a time-series of all new tasks discovered over the course of the entire run, including non-rewarded **expanded task set** tasks. The right-hand plot shows the final count at the end of the run. Before we introduce rewards for performing **expanded task set** 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 **expanded task set** tasks in the first part of the experiment.

In the first part of the experiments, despite the **expanded task set** tasks not being rewarded, both changing-environment treatments (BenignCE and HarshCE) discovered more new tasks than the control (Minimal: Wilcoxon Rank Sum Test: Z = -5.75 and -11.15 respectively, p << 0.001; Rich: Wilcoxon Rank Sum Test: Z = -4.32 and -3.73 respectively, p < 0.001). In the minimal environment, the harsh treatment in particular discovered substantially and significantly more **expanded task set** tasks than either the benign treatment (Wilcoxon Rank Sum Test: Z = -8.0, p << 0.001) or the control, despite these tasks not

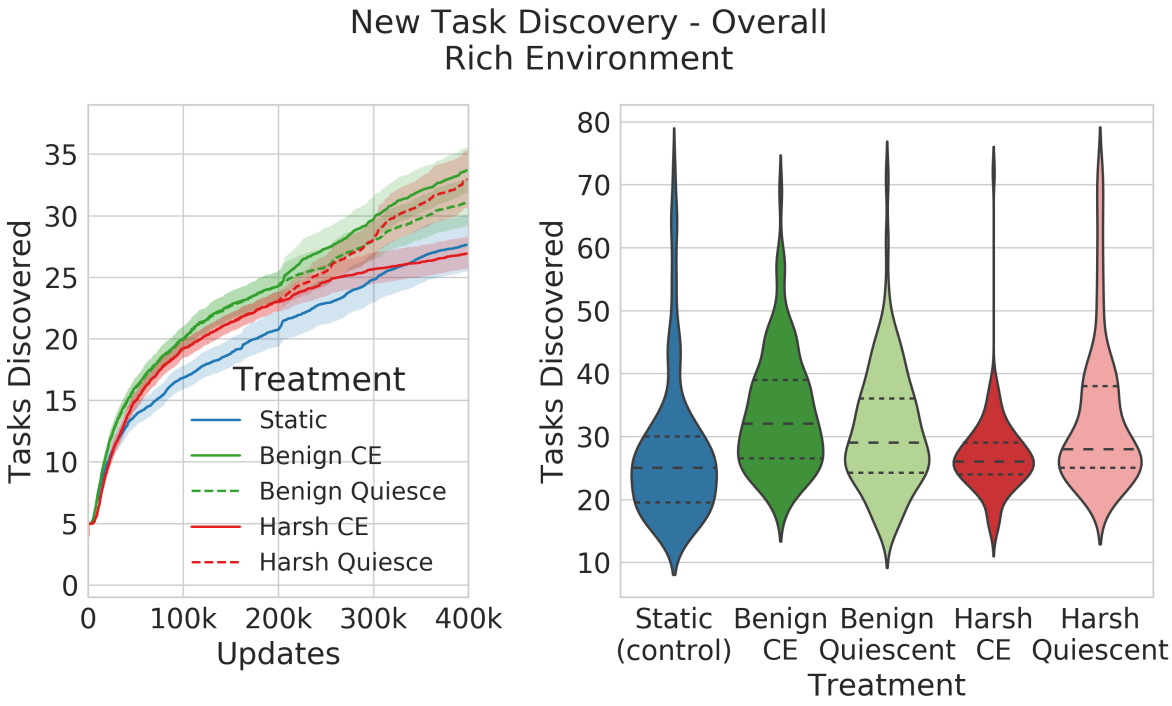

Figure 5.4: **Number of new expanded task set tasks discovered in rich environments, over the whole experiment**. The left plot shows a time-series of all new tasks discovered over the course of the entire run, included non-rewarded **expanded task set** tasks. The right-hand plot shows the final count of tasks discovered by the end of the run. Again, both sets of changing environments (BenignCE and HarshCE) randomly discover more **expanded task set** 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.

being rewarded (Fig 5.3. We speculate 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 **expanded task set** task rewards are introduced, this effect disappears, and task discovery rates converge (Kruskal Wallis: $H(2) = 6.97$, $p = 0.03$).

5.3.2 Task Performance

In additiona to task discovery, task performance is another an important measure of long-term evolvability, in that it quantifies exploitation and fixation of traits that are beneficial in new environments. We measured task performance in each of the changing environment treatments.

5.3.2.1 Benign changing environments outperform harsh environments in task performance

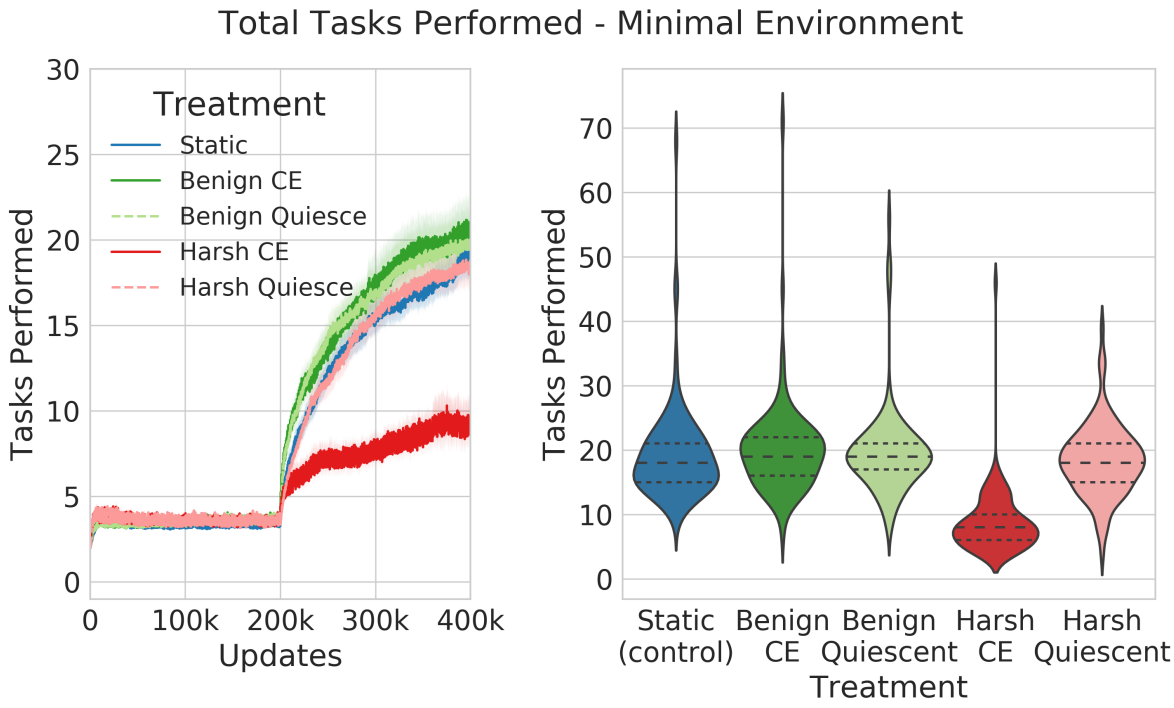

Figure 5.5: **Number of distinct tasks performed in minimal environments**. The left plot shows a time-series of the number of distinct tasks performed by the treatment populations over time. The right-had plot shows the number of tasks performed at the end of the experiments. The harsh changing environment 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).

Similar to task discovery, populations evolving in harsh changing environments performed

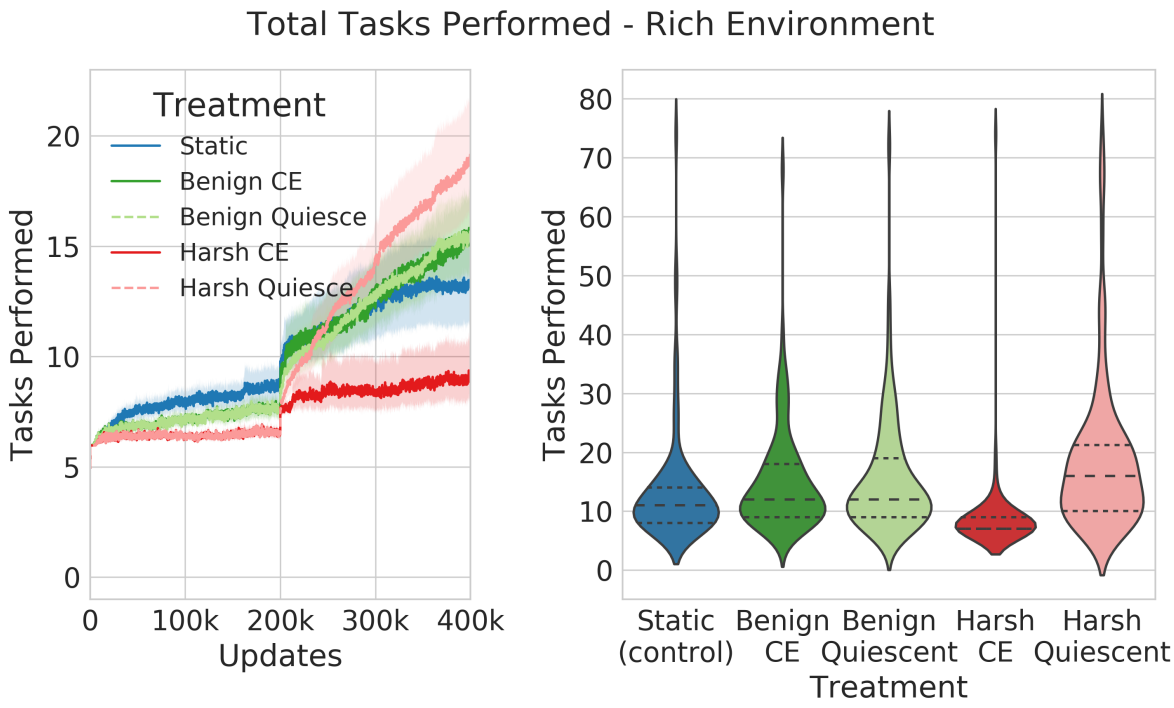

Figure 5.6: **Number of distinct tasks performed in rich environments**. The left plot shows a time-series of the number of distinct tasks performed by the treatment populations over time. The right-had plot shows the number of tasks performed at the end of the experiments. Similar to the minimal environment, 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 rich environment performs fewer tasks overall (Mdn = 11.0, CI 95% [10.0, 12.0]), compared to the minimal 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).

far fewer distinct tasks than either the control, or either benign populations (Wilcoxon Rank Sum Test: Minimal: Z = -11.22 and -11.15 respectively; Rich: Z = -4.32 and -3.73 respectively, p << 0.001) (Fig 5.5 and 5.6). While both the BenignCE and BenignQuiescent populations seemed to outperform the control, this effect was only statistically significant in the rich environment (Wilcoxon Rank Sum Test: Z = 2.42 and 2.37 respectively, p < 0.05). In the minimal environment, the differences were not statistically significant (Kruskal Wallis: H(2) = 2.76, p = 0.25).

Interestingly, in the rich environments, the HarshQuiescent treatments seemed to outperform all other environment types in task performance, though the differences between HarshQuiescent 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, $p < 0.05$). We speculate that this effect may be due to a lingering architectural effect of evolving in harsh changing environments. For example, because task length increases in changing environments, these tasks are more likely to experience mutations that could change their phenotype in a variety of ways. Thus, once the pressure to limit diversity is released, the population would be much more likely to produce a large amount of population variation in a short time. Thus, we would expect to see a significant increase in measures of task performance, but might also expect to see a relatively lower number of task being performed by the entire population. Further research is needed to identify the mechanisms responsible for this effect.

5.4 Conclusion

The relationship between short and long term evolvability is non-obvious. Architectural features and selective pressures that promote repeated re-adaptation to a known set of environments may not be beneficial for the acquisition of entirely new adaptive traits. For example, harsh changing environments act to depress both fitness and population diversity, which might make these populations less effective at adaptation when introduced into a new environment.

Indeed, our experiments show that harsh changing environments, with their strong selective pressures, suppress the ability of populations to acquire new, weakly-selected traits. However, benign changing environments, with their milder set of selective pressures, are able to leverage their accumulated heritage of dormant vestigial sites to rapidly respond to selection, and acquire new tasks at a faster rate than either harsh or non-change-evolved populations. However, despite the direct negative effects of harsh changing environments,

populations initially evolved in these environments are able to rapidly acquire new tasks if alternating selection is removed. This result suggests that there are important architectural features conveyed by these environments that are beneficial for new task acquisition.

Our results are consistent across both the minimal and rich environment types. This suggests that regardless of environmental and genomic complexity, changing environments are important drivers of adaptation and long-term evolvability, either directly by driving populations to move across the fitness landscape, or indirectly, by conveying lingering architectural advantages.

CHAPTER 6

CONCLUSION AND FUTURE DIRECTIONS

In this dissertation, we explore the evolutionary dynamics of changing environments, along with their impact on both short and long-term evolvability. We find that changing environments promote short-term adaptation by driving populations to regions of genotype space where more adaptive mutants are available within one or two mutational steps.

Harsh changing environments create selective pressures to switch phenotypes, rather than selecting only for increasing fitness. These kinds of selective pressures are well suited to promoting the evolution of information-rich mutation types like horizontal gene transfer via transformation. HGT in particular is an excellent adaptation to harsh changing environments, where there is a strong selective pressure for phenotype switching. Indeed, we observe that HGT is much more strongly selected for than other types of mutation operators with similar associated mutation rates. The fragments from the environment serve as reservoirs for dormant functionality, just as vestigial regions within genomes do, thus providing more opportunities for mutations to produce switches between phenotypes.

In the context of long-term evolutionary potential, the benefits of changing environments are less clear. Benign changing environments appear to perform at least, if not more efficiently, than purely directional selection at acquiring traits in a novel environment. While benign changing environments appear to convey some architectural advantages in terms of task discovery and performance, the effects are slight. This result may be due to the types of selection at play in our changing environments. The selective pressures in our changing environments are largely adaptive selective pressures, rather than divergent selective pressures. Divergent selection pressures, such as negative frequency dependence, have been shown to outperform adaptive selection pressures in measures of population-level evolvability [85]. Our results are consistent with these findings, and suggest that changing environments alone are insufficient for promoting long-term population-level evolvability.

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 exploratory power in natural evolution, and as practical tools in the Artificial Life toolkit. Ultimately, however, as alluded to above, cyclic changing environments only re-tread existing phenotypic ground, and though genotypic exploration can be 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, or divergent selection mechanisms such as negative frequency dependence to promote the maintenance of diversity in evolving populations.

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 merely inactive remnants, reservoirs of function, or are they part of a complex compensatory framework supporting and buffering the expression of the phenotype? Or all of these things? Changing environments provide one view into these dynamics, but we must explore further to find other mechanisms for exploring and exploiting genotype space.

6.2 Future Directions - Horizontal Gene Transfer and Long Term Evolvability

Our work in understanding how changing environments interact with evolvability and different kinds of mutations is obviously by no means complete. In particular, questions of how mutational operators like horizontal gene transfer or sexual reproduction interact with long-term evolutionary potential remain open.

In addition to our experiments characterizing the long-term evolutionary potential of populations evolved in minimal and rich changing environments, we also subjected HGT-evolved populations to the same expanded task set environments as described in Chapter 5. Intriguingly, we found a strong negative correlation between the use of a grazing HGT bonus

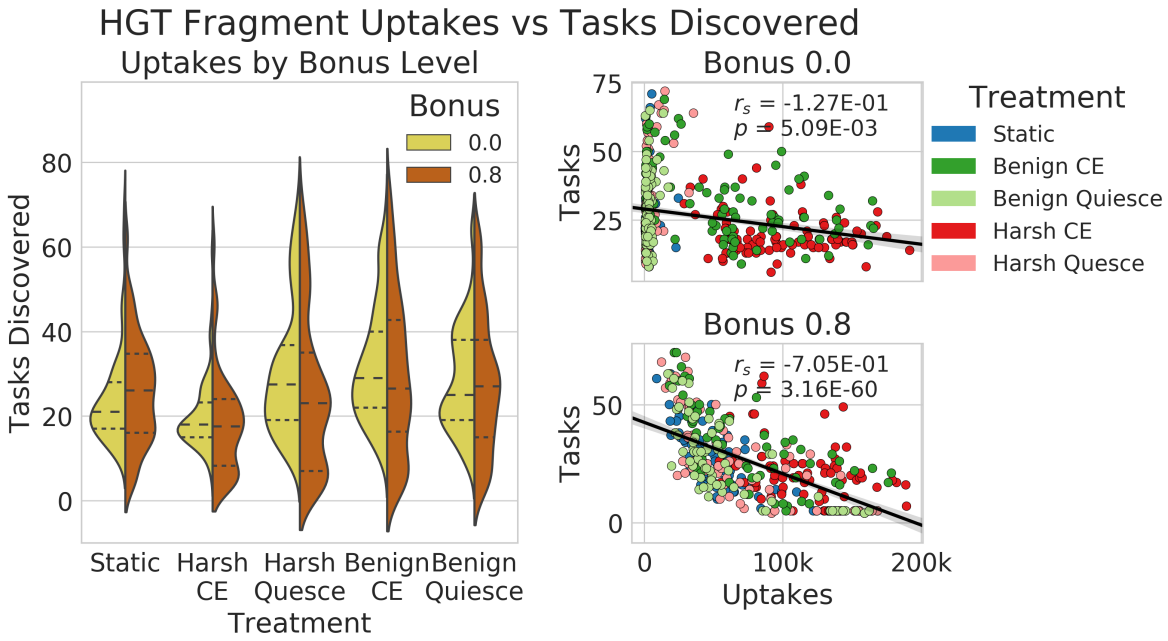

Figure 6.1: **Task discovery, with and without HGT grazing bonus**. Populations evolving with a grazing bonus had a strong negative correlation between task discovery and HGT use (Spearman's Rho: r_s = -0.71, p << 0.001).

and rates of task discovery (Spearman's Rho: r_s = -0.71, p << 0.001). In contrast, we found a much weaker and less robust correlation when no grazing bonus was given for HGT (Spearman's Rho: r_s = -0.12, p < 0.01) (Fig 6.1). This result is surprising because we

would expect that an information rich mutation operator would promote the discovery of new tasks by combining existing functionality in novel ways. However, in the case of the grazing bonus, while HGT does use generally increases, we also observed that this increase was negatively associated with discovery of new tasks. Why should mutation by HGT reduce task discovery? There are several factors that may account for this counterintuitive result.

First, evolving HGT with a grazing bonus may lead to fundamentally different genetic architectures than those found in treatments where HGT use gives no direct reward. Because the HGT instruction is relatively simple to acquire and confers a substantial execution bonus, those genetic architectures that use it might have evolved features that are more robust against HGT-caused mutations, while still being able to reap the benefits of the grazing bonus. This very robustness might then make these architectures slower to adapt to new environments.

Another possibility is that this correlation is related to treatment use patterns, where treatments under harsh alternating selection not only use HGT more, but also exhibit lower amounts of task discovery. In fact, this is the pattern that appears in the non-bonus HGT treatments. However, in the grazing bonus treatments, we observed an overall increase in the use of HGT across all treatment types, with no associated increase in task discovery. This relationship would therefore suggest that HGT does not itself have a significant effect on task discovery at all, and that instead this correlation is related purely to the fact that HGT levels increase due to a bonus, and thus have no strong effect on task discovery one way or another. If so, then this might also provide evidence for the possibility of architectural adaptation to HGT with a grazing bonus.

This result underlines how much we have yet to learn about how changing environments interact with different kinds of mutations that affect genetic architectures in different ways. Evolvability remains a complex and rich topic of study, and there is still much to learn.

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 1000 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).

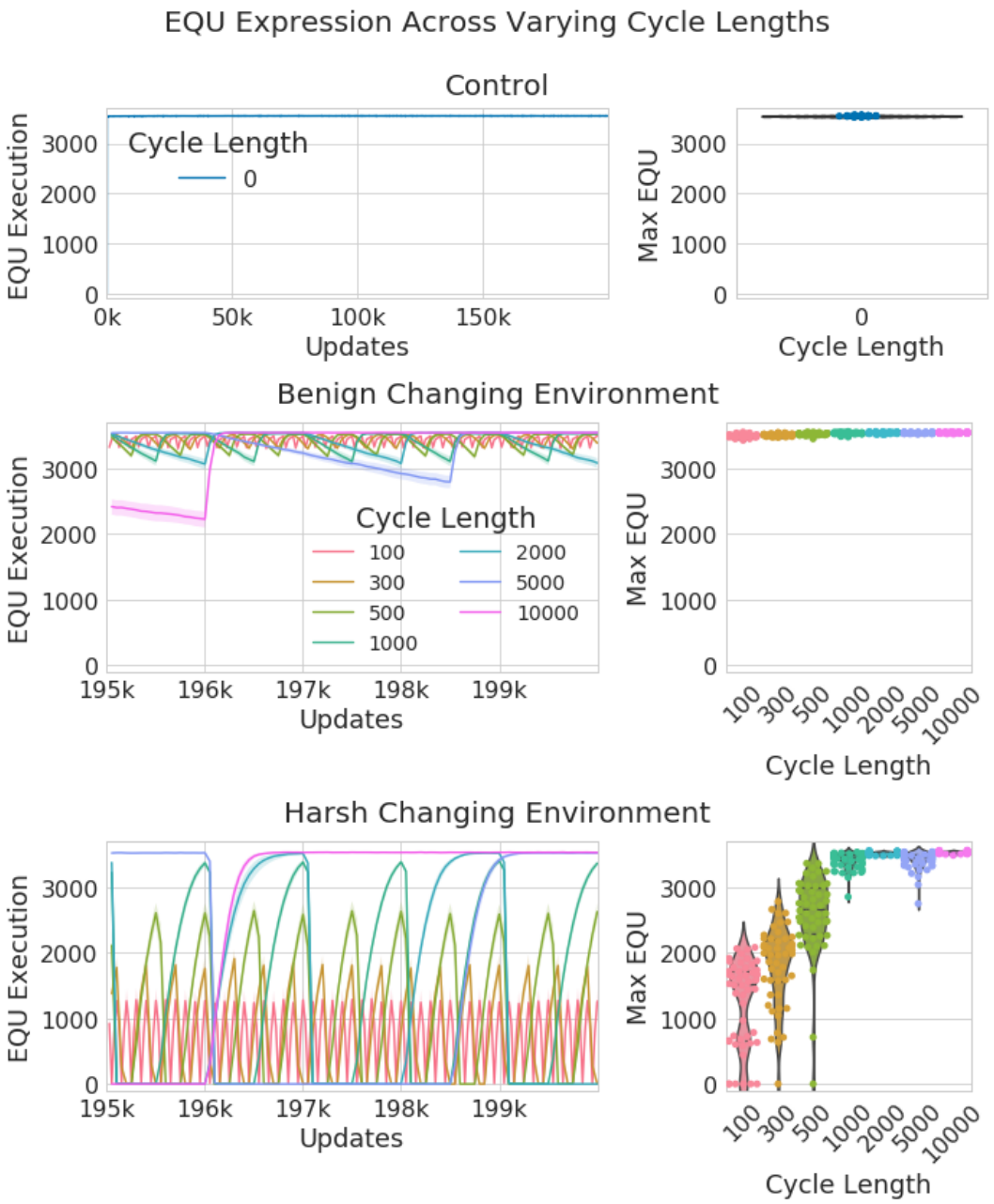

Figure A.1: **Survey of task execution** for a series of cycle-lengths. The top plots count the number of executions of the EQU instruction in the control treatment. Each organism only receives a bonus for executing EQU once. The seed population performed EQU, and it is never lost. The middle and lower 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.

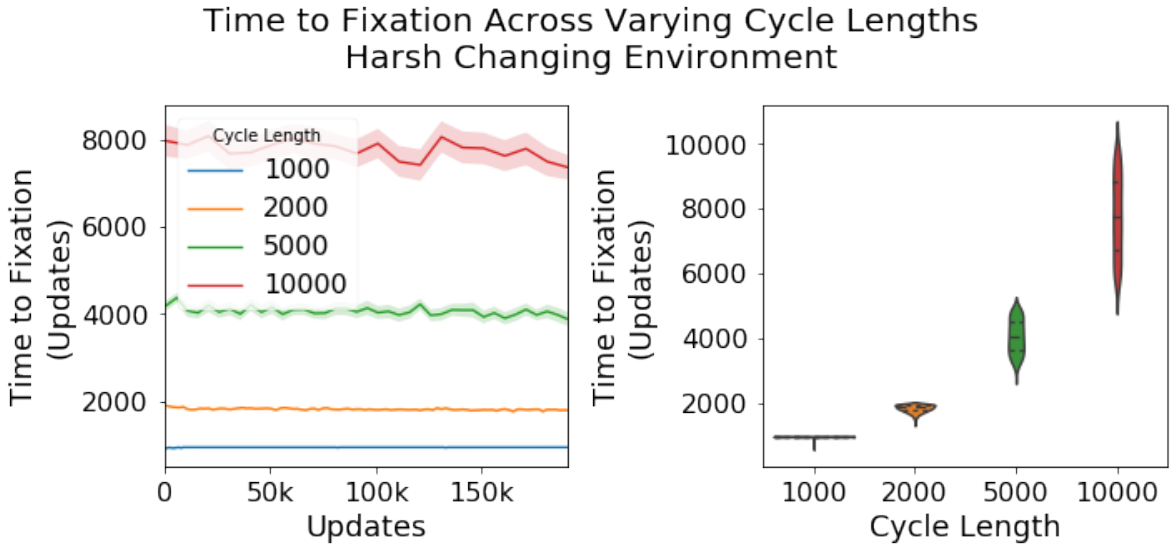

Figure A.2: **Survey of task time to `EQU` fixation for harsh changing environments** for a series of cycle-lengths. Only those treatments where `EQU` fixation occurs (cycle lengths greater than 1000 updates), were surveyed. The longer the cycle time, the longer it takes for fixation to occur.

APPENDIX B

EXPERIMENTALLY DERIVING PARAMETERS FOR HGT RECOMBINATION PROBABILITY AND BONUS LEVELS

B.1 Recombination Probability Sweep

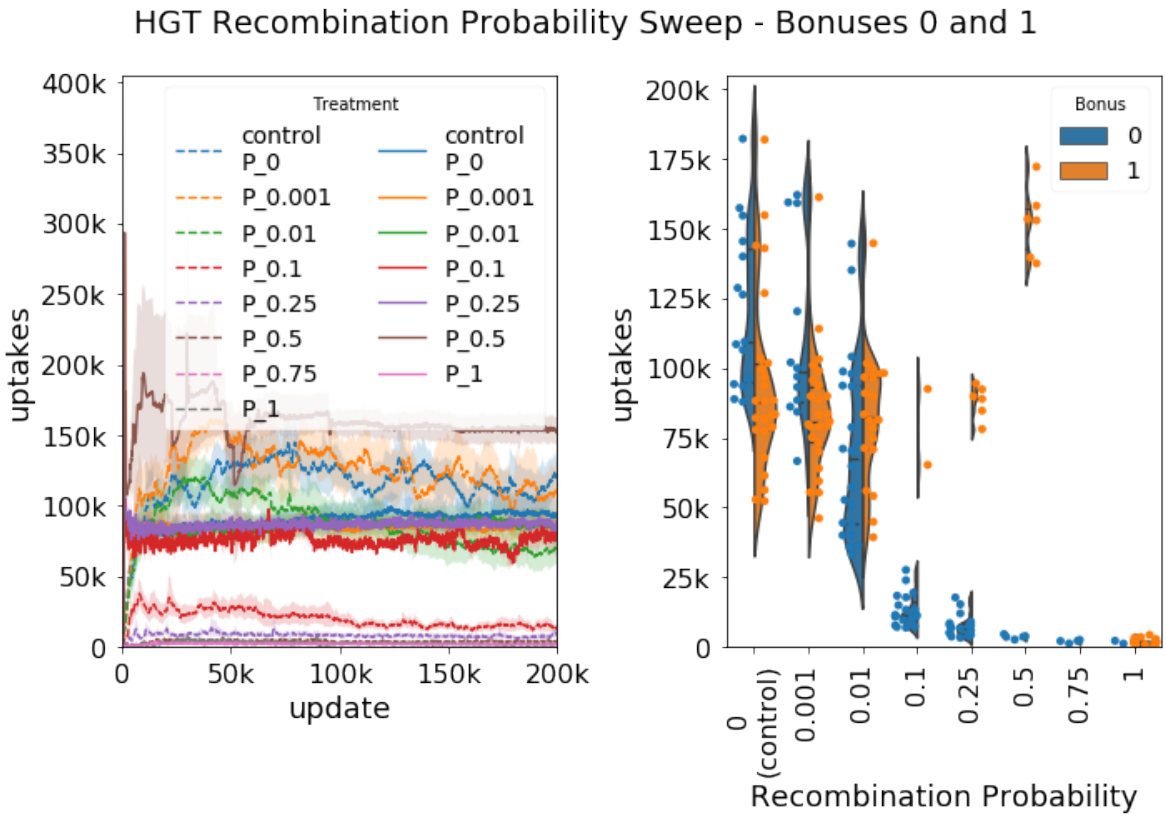

Figure B.1: **Survey of `HGT` use for a series of recombination probabilities** in a static environment. The left-hand figure shows a time-series of fragment uptakes, with and without bonus values. The right-hand plot shows the final uptake counts at the end of the runs. Those values at a recombination 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

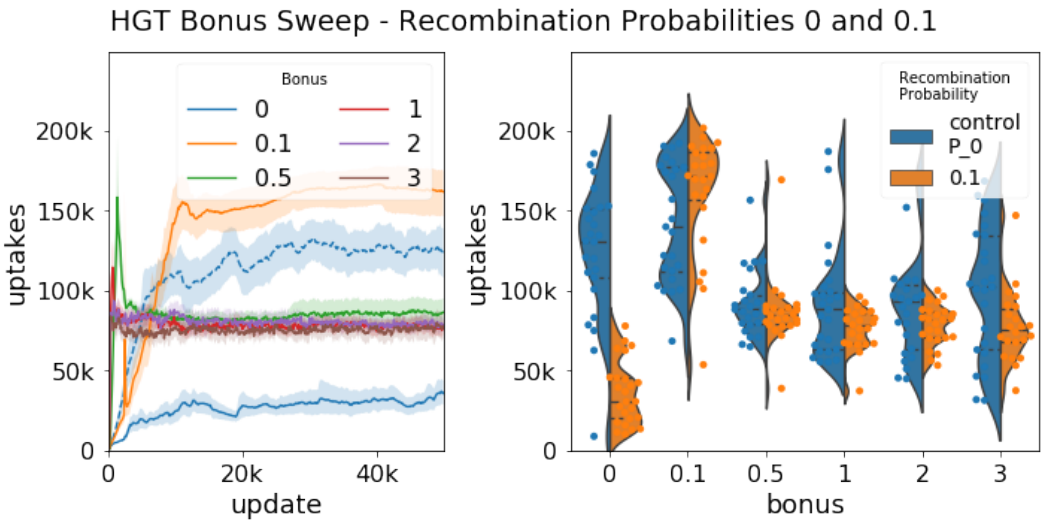

Figure B.2: **Survey of `HGT` use for a series of bonus values** in a static environment. The left plot shows a time-series of HGT fragment uptakes, while the right-hand plot shows final uptake values at the end of the experimental runs. With recombination probability at 0.1, bonus values of zero depress 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

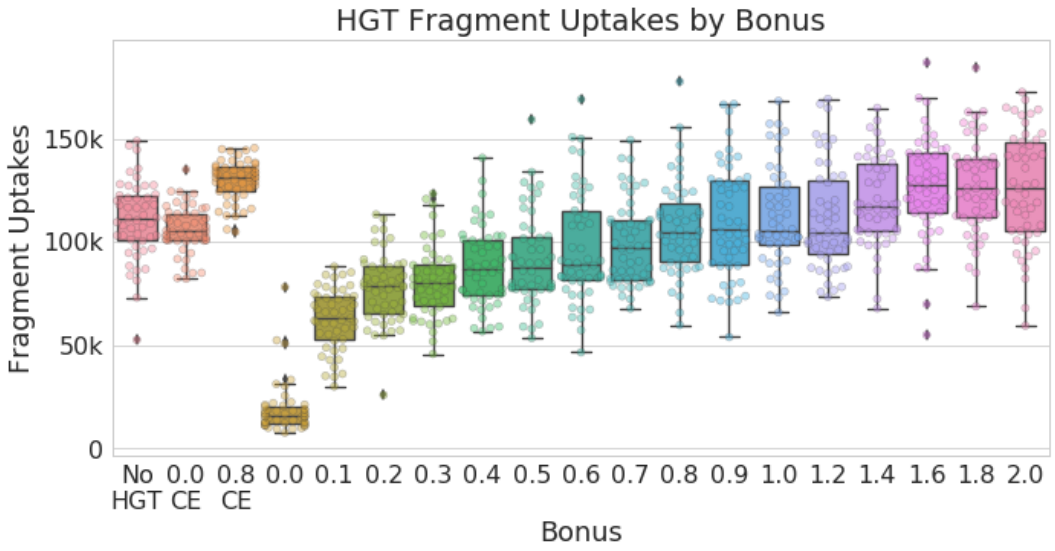

Figure B.3: **Survey of `HGT` use, across a series of comparable bonus values** in a static environment, with a recombination probability of 0.1. This figure shows a sweep of bonus values, demonstrating a largely linear progression of increasing `HGT` use as bonus values increase, thus balancing negative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

APPENDIX C

CHANGING ENVIRONMENTS ELEVATE ALL INSTRUCTION USE DUE TO REPEATED BOTTLENECKS

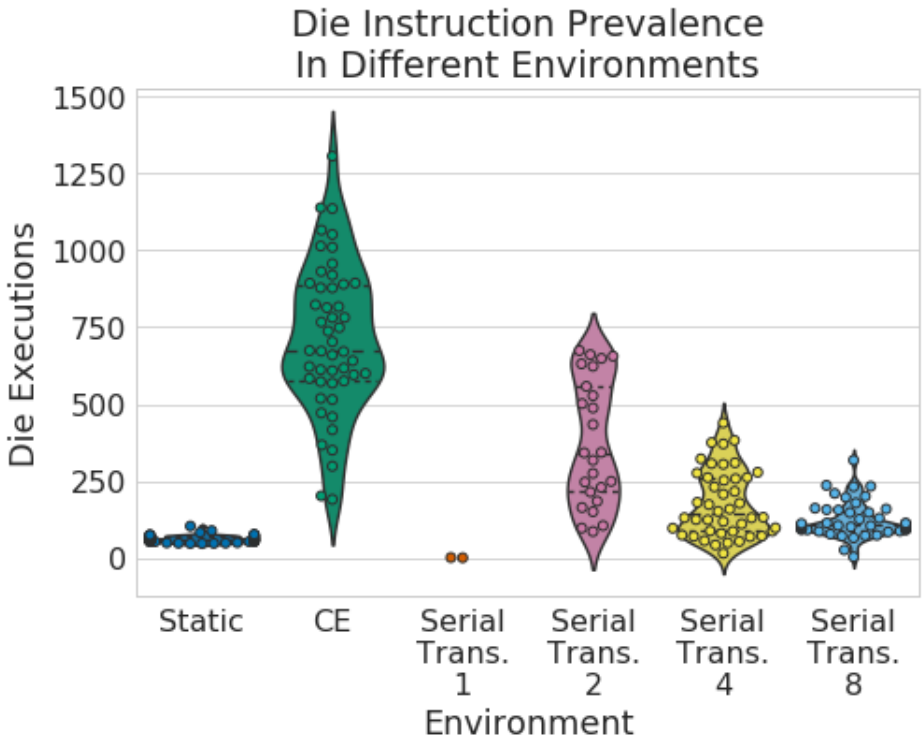

Figure C.1: **`Die` instruction executions** across a series of artificial bottlenecks. We observe that the `Die` instruction is elevated in a harsh changing environment as compared to a static environment. We apply a pair of bottlenecking procedures. In the "Serial Transfer" treatments, we kill the 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 is consistent with founder effects being 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.

BIBLIOGRAPHY

BIBLIOGRAPHY

[1] John Maynard Smith and Eors Szathmary. The major evolutionary transitions. *Nature*, 374(6519):227–32, March 1995.

[2] Marc Kirschner and John Gerhart. Evolvability. *Proceedings of the National Academy of Sciences*, 95(15):8420–8427, 1998.

[3] Richard Dawkins. 13 - The evolution of evolvability. In Sanjeev Kumar and Peter J. Bentley, editors, *On Growth, Form and Computers*, pages 239–255. Academic Press, London, 2003.

[4] Lee Altenberg. The evolution of evolvability in genetic programming. *Advances in genetic programming*, 3:47–74, 1994.

[5] Ronald Aylmer Fisher. *The genetical theory of natural selection.* Clarendon Press, Oxford, 1930.

[6] David Houle. Comparing Evolvability and Variability of Quantitative Traits. *Genetics*, 130(1):195–204, 1992.

[7] Terry Van Belle and David H. Ackley. Code Factoring And The Evolution Of Evolvability. In *Proceedings of the Genetic and Evolutionary Computation Conference*, GECCO '02, pages 1383–1390, San Francisco, CA, USA, 2002. Morgan Kaufmann Publishers Inc.

[8] Gunter P. Wagner and Lee Altenberg. Perspective: complex adaptations and the evolution of evolvability. *Evolution*, pages 967–976, 1996.

[9] Andreas Wagner. Robustness, evolvability, and neutrality. *FEBS letters*, 579(8):1772–1778, 2005.

[10] Hiroaki Kitano. Biological robustness. *Nature Reviews Genetics*, 5(11):826–837, November 2004.

[11] P. Alberch. From genes to phenotype: dynamical systems and evolvability. *Genetica*, 84(1):5–11, 1991.

[12] J. F. Y Brookfield. Evolution: The evolvability enigma. *Current Biology*, 11(3):R106–R108, February 2001.

[13] Massimo Pigliucci. Is evolvability evolvable? *Nature Reviews Genetics*, 9(1):75–82, 2008.

[14] Samir Okasha. *Evolution and the Levels of Selection*, volume 16. Clarendon Press Oxford, 2006.

[15] Stephen Jay Gould and Richard C. Lewontin. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proceedings of the Royal Society of London B: Biological Sciences*, 205(1161):581–598, 1979.

[16] Sewall Wright. Evolution in Mendelian Populations. *Genetics*, 16(2):97–159, March 1931.

[17] Nadav Kashtan, Elad Noor, and Uri Alon. Varying environments can speed up evolution. *Proceedings of the National Academy of Sciences*, 104(34):13711–13716, August 2007.

[18] Brian D. Connelly, Katherine J. Dickinson, Sarah P. Hammarlund, and Benjamin Kerr. Negative niche construction favors the evolution of cooperation. *Evolutionary Ecology*, pages 1–17, October 2015.

[19] Barbara McClintock. *The significance of responses of the genome to challenge*. Singapore: World Scientific Pub. Co, 1993.

[20] Anton Crombach and Paulien Hogeweg. Evolution of Evolvability in Gene Regulatory Networks. *PLOS Comput Biol*, 4(7):e1000112, July 2008.

[21] Claus O. Wilke, Jia Lan Wang, Charles Ofria, Richard E. Lenski, and Christoph Adami. Evolution of digital organisms at high mutation rates leads to survival of the flattest. *Nature*, 412(6844):331–3, July 2001.

[22] Matthew R. Goddard, H. Charles J. Godfray, and Austin Burt. Sex increases the efficacy of natural selection in experimental yeast populations. *Nature*, 434(7033):636+, March 2005. 636.

[23] Thomas F. Hansen and David Houle. Measuring and comparing evolvability and constraint in multivariate characters. *Journal of evolutionary biology*, 21(5):1201–1219, 2008.

[24] Trevor Price and Dolph Schluter. On the Low Heritability of Life-History Traits. *Evolution*, 45(4):853–861, 1991.

[25] Thomas F. Hansen, Christophe Plabon, and David Houle. Heritability is not Evolvability. *Evolutionary Biology*, 38(3):258–277, June 2011.

[26] Dusan Misevic, Charles Ofria, and Richard E Lenski. Sexual Reproduction Reshapes the Genetic Architecture of Digital Organisms. *Proceedings. Biological Sciences / The Royal Society*, 273(1585):457–464, February 2006.

[27] Gnter P. Wagner and Jianzhi Zhang. The pleiotropic structure of the genotypephenotype map: the evolvability of complex organisms. *Nature Reviews Genetics*, 12(3):204–213, 2011.

[28] E. Ravasz, A.L. Somera, D.A. Mongru, Z.N. Oltvai, and A.-L. Barabasi. Hierarchical organization of modularity in metabolic networks. (Reports). *Science*, 297(5586):1551+, August 2002. 1551.

[29] Thomas F Hansen. Is modularity necessary for evolvability?: Remarks on the relationship between pleiotropy and evolvability. *Biosystems*, 69(23):83–94, May 2003.

[30] Mihaela Pavlicev and Gnter P. Wagner. A model of developmental evolution: selection, pleiotropy and compensation. *Trends in Ecology & Evolution*, 27(6):316–322, June 2012.

[31] Jason G. Mezey, James M. Cheverud, and Gnter P. Wagner. Is the Genotype-Phenotype Map Modular?: A Statistical Approach Using Mouse Quantitative Trait Loci Data. *Genetics*, 156(1):305–311, September 2000.

[32] J. Arjan G. M. de Visser, Joachim Hermisson, Gnter P. Wagner, Lauren Ancel Meyers, Homayoun Bagheri-Chaichian, Jeffrey L. Blanchard, Lin Chao, James M. Cheverud, Santiago F. Elena, Walter Fontana, Greg Gibson, Thomas F. Hansen, David Krakauer, Richard C. Lewontin, Charles Ofria, Sean H. Rice, George von Dassow, Andreas Wagner, and Michael C. Whitlock. Perspective: Evolution and Detection of Genetic Robustness. *Evolution*, 57(9):1959–1972, 2003.

[33] James M. Whitacre. Degeneracy: a link between evolvability, robustness and complexity in biological systems. *Theoretical Biology and Medical Modelling*, 7(1):6, 2010.

[34] Andreas Wagner. Distributed robustness versus redundancy as causes of mutational robustness. *BioEssays*, 27(2):176–188, February 2005.

[35] Jack E. Baldwin and Hans Krebs. The evolution of metabolic cycles. *Nature*, 291(5814):381–382, June 1981.

[36] Jrg Stelling, Uwe Sauer, Zoltan Szallasi, Francis J. Doyle III, and John Doyle. Robustness of Cellular Functions. *Cell*, 118(6):675–685, September 2004.

[37] Gunter P. Wagner, Mihaela Pavlicev, and James M. Cheverud. The road to modularity. *Nature Reviews Genetics*, 8(12):921+, December 2007. 921.

[38] Andreas Wagner. Robustness and evolvability: a paradox resolved. *Proceedings of the Royal Society of London B: Biological Sciences*, 275(1630):91–100, 2008.

[39] Charles Ofria and Christoph Adami. Evolution of genetic organization in digital organisms. In *Evolution as Computation*, pages 296–313. Springer, 2002.

[40] P. McKinley, B. Cheng, C. Ofria, D. Knoester, B. Beckmann, and H. Goldsby. Harnessing Digital Evolution. *Computer*, 41(1):54–63, January 2008.

[41] Daniel C. Dennett. Darwin's dangerous idea. *The Sciences*, 35(3):34–40, 1995.

[42] Francis J. Ryan. Evolution observed. *Scientific American*, 189:78–82, 1953.

[43] Richard E. Lenski, Michael R. Rose, Suzanne C. Simpson, and Scott C. Tadler. Long-term experimental evolution in Escherichia coli. I. Adaptation and divergence during 2,000 generations. *The American Naturalist*, pages 1315–1341, 1991.

[44] Jennifer L. Anderson, Levi T. Morran, and Patrick C. Phillips. Outcrossing and the Maintenance of Males within C. elegans Populations. *Journal of Heredity*, page esq003, January 2010.

[45] S. C. Stearns, M. Ackermann, M. Doebeli, and M. Kaiser. Experimental evolution of aging, growth, and reproduction in fruitflies. *Proceedings of the National Academy of Sciences*, 97(7):3309–3313, March 2000.

[46] Frank Wilcoxon. Individual Comparisons by Ranking Methods. *Biometrics Bulletin*, 1(6):80–83, 1945.

[47] William H. Kruskal and W. Allen Wallis. Use of Ranks in One-Criterion Variance Analysis. *Journal of the American Statistical Association*, 47(260):583–621, December 1952.

[48] William R. Rice. Analyzing tables of statistical tests. *Evolution*, 43(1):223–225, 1989.

[49] Charles Spearman. The proof and measurement of association between two things. *The American journal of psychology*, 15(1):72–101, 1904.

[50] Aisha I. Khan, Duy M. Dinh, Dominique Schneider, Richard E. Lenski, and Tim F. Cooper. Negative Epistasis Between Beneficial Mutations in an Evolving Bacterial Population. *Science*, 332(6034):1193–1196, June 2011.

[51] Ivan G. Szendro, Martijn F. Schenk, Jasper Franke, Joachim Krug, and J. Arjan G. M. de Visser. Quantitative analyses of empirical fitness landscapes. *Journal of Statistical Mechanics: Theory and Experiment*, 2013(01):P01005, 2013.

[52] Daniel M. Weinreich, Nigel F. Delaney, Mark A. DePristo, and Daniel L. Hartl. Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science*, 312(5770):111–114, 2006.

[53] Joshua R. Nahum, Peter Godfrey-Smith, Brittany N. Harding, Joseph H. Marcus, Jared Carlson-Stevermer, and Benjamin Kerr. A tortoisehare pattern seen in adapting structured and unstructured populations suggests a rugged fitness landscape in bacteria. *Proceedings of the National Academy of Sciences*, 112(24):7530–7535, June 2015.

[54] P. Merz and B. Freisleben. Fitness landscape analysis and memetic algorithms for the quadratic assignment problem. *IEEE Transactions on Evolutionary Computation*, 4(4):337–352, November 2000.

[55] J. Humeau, A. Liefooghe, E.-G. Talbi, and S. Verel. ParadisEO-MO: from fitness landscape analysis to efficient local search algorithms. *Journal of Heuristics*, 19(6):881–915, June 2013.

[56] Leila Kallel, Bart Naudts, and Alex Rogers. *Theoretical aspects of evolutionary computing*. Springer Science & Business Media, 2013.

[57] Sergey Gavrilets. *Fitness landscapes and the origin of species (MPB-41)*. Princeton University Press Princeton, NJ, 2004.

[58] Dusan Misevic, Charles Ofria, and Richard E. Lenski. Experiments with Digital Organisms on the Origin and Maintenance of Sex in Changing Environments. *The Journal of Heredity*, 101(suppl 1):S46–S54, March 2010.

[59] Luis Zaman, Justin R. Meyer, Suhas Devangam, David M. Bryson, Richard E. Lenski, and Charles Ofria. Coevolution drives the emergence of complex traits and promotes evolvability. *PLoS Biol*, 12(12):e1002023, 2014.

[60] Justin R. Meyer, Devin T. Dobias, Joshua S. Weitz, Jeffrey E. Barrick, Ryan T. Quick, and Richard E. Lenski. Repeatability and Contingency in the Evolution of a Key Innovation in Phage Lambda. *Science*, 335(6067):428–432, January 2012.

[61] Guillaume Martin, Thomas Lenormand, and C. Goodnight. The fitness effect of mutations across environments: a survey in light of fitness landscape models. *Evolution*, 60(12):2413–2427, December 2006.

[62] Daniel J. Kvitek and Gavin Sherlock. Reciprocal Sign Epistasis between Frequently Experimentally Evolved Adaptive Mutations Causes a Rugged Fitness Landscape. *PLOS Genetics*, 7(4):e1002056, April 2011.

[63] Christopher D. Bayliss, Dawn Field, and E. Richard Moxon. The simple sequence contingency loci of Haemophilus influenzae and Neisseria meningitidis. *Journal of Clinical Investigation*, 107(6):657–666, March 2001.

[64] Heewook Lee, Ellen Popodi, Haixu Tang, and Patricia L. Foster. Rate and molecular spectrum of spontaneous mutations in the bacterium Escherichia coli as determined by whole-genome sequencing. *Proceedings of the National Academy of Sciences*, 109(41):E2774–E2783, October 2012.

[65] Peter A. Lind and Dan I. Andersson. Whole-genome mutational biases in bacteria. *Proceedings of the National Academy of Sciences*, 105(46):17878–17883, November 2008.

[66] Ashley J. R. Carter, Joachim Hermisson, and Thomas F. Hansen. The role of epistatic gene interactions in the response to selection and the evolution of evolvability. *Theoretical Population Biology*, 68(3):179–196, November 2005.

[67] Ines Chen and David Dubnau. DNA uptake during bacterial transformation. *Nature Reviews Microbiology*, 2(3):241+, March 2004. 241.

[68] Joshua Lederberg and Edward L. Tatum. Gene recombination in Escherichia coli. *Nature*, 158:558, 1946.

[69] Norton D. Zinder and Joshua Lederberg. Genetic Exchange in Salmonella. *Journal of Bacteriology*, 64(5):679–699, November 1952.

[70] E. S. Lennox. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology*, 1(2):190–206, July 1955.

[71] Tal Dagan, Yael Artzy-Randrup, and William Martin. Modular Networks and Cumulative Impact of Lateral Transfer in Prokaryote Genome Evolution. *Proceedings of the National Academy of Sciences of the United States of America*, 105(29):10039–10044, 2008.

[72] J. E. Davies. Origins, acquisition and dissemination of antibiotic resistance determinants. *Ciba Foundation Symposium*, 207:15–27; discussion 27–35, 1997.

[73] Jos L. Martnez. Antibiotics and Antibiotic Resistance Genes in Natural Environments. *Science*, 321(5887):365–367, 2008.

[74] Michiel Vos. Why do bacteria engage in homologous recombination? *Trends in Microbiology*, 17(6):226–232, June 2009.

[75] R. J. Redfield, M. R. Schrag, and A. M. Dean. The Evolution of Bacterial Transformation: Sex with Poor Relations. *Genetics*, 146(1):27–38, May 1997.

[76] Ralph Bock. Witnessing Genome Evolution: Experimental Reconstruction of Endosymbiotic and Horizontal Gene Transfer. *Annual Review of Genetics*, December 2015.

[77] Shannon M. Soucy, Jinling Huang, and Johann Peter Gogarten. Horizontal gene transfer: building the web of life. *Nature Reviews Genetics*, 16(8):nrg3962, July 2015.

[78] Ana Paula Jacobus and Jeferson Gross. Optimal Cloning of PCR Fragments by Homologous Recombination in Escherichia coli. *PLOS ONE*, 10(3):e0119221, March 2015.

[79] Sbastien Wielgoss, Jeffrey E. Barrick, Olivier Tenaillon, Michael J. Wiser, W. James Dittmar, Stphane Cruveiller, Batrice Chane-Woon-Ming, Claudine Mdigue, Richard E. Lenski, and Dominique Schneider. Mutation rate dynamics in a bacterial population reflect tension between adaptation and genetic load. *Proceedings of the National Academy of Sciences*, 110(1):222–227, January 2013.

[80] Jeff Clune, Dusan Misevic, Charles Ofria, Richard E. Lenski, Santiago F. Elena, and Rafael Sanjun. Natural Selection Fails to Optimize Mutation Rates for Long-Term Adaptation on Rugged Fitness Landscapes. *PLoS Comput Biol*, 4(9):e1000187, 2008.

[81] Jonathan M. Solomon and Alan D. Grossman. Who's competent and when: regulation of natural genetic competence in bacteria. *Trends in Genetics*, 12(4):150–155, April 1996.

[82] Patrick Seitz and Melanie Blokesch. Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. *FEMS Microbiology Reviews*, 37(3):336–363, May 2013.

[83] Laetitia Fontaine, Cline Boutry, Marie Henry de Frahan, Brigitte Delplace, Christophe Fremaux, Philippe Horvath, Patrick Boyaval, and Pascal Hols. A Novel Pheromone Quorum-Sensing System Controls the Development of Natural Competence in Streptococcus thermophilus and Streptococcus salivarius. *Journal of Bacteriology*, 192(5):1444–1454, March 2010.

[84] Fen Peng, Scott Widmann, Andrea Wnsche, Kristina Duan, Katherine A. Donovan, Renwick C. J. Dobson, Richard E. Lenski, and Tim F. Cooper. Effects of beneficial mutations in pykF gene vary over time and across replicate populations in a long-term experiment with bacteria. *Molecular Biology and Evolution*.

[85] Bryan Wilder and Kenneth Stanley. Reconciling explanations for the evolution of evolvability. *Adaptive Behavior*, 23(3):171–179, 2015.

[86] Joel Lehman, Bryan Wilder, and Kenneth O. Stanley. On the Critical Role of Divergent Selection in Evolvability. *Frontiers in Robotics and AI*, 3, 2016.