EVOLUTION AND EVOLVABILITY IN CHANGING ENVIRONMENTS

By

Rosangela Canino-Koning

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Computer Science – Doctor of Philosophy

2017

ABSTRACT

EVOLUTION AND EVOLVABILITY IN CHANGING ENVIRONMENTS

By

Rosangela Canino-Koning

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. Evolvability is thought to be created through the interplay of modularity and robustness. Indeed, modularity and robustness build upon each other, layer by layer, to form a framework that produces more powerful adaptive effects while reducing the impact of deleterious mutations.

In this comprehensive exam, I will describe some research that I have completed and propose additional projects that explore the interplay among evolvability, robustness, and modularity. 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.

Modularity and robustness clearly have a complex interdependence in ongoing evolutionary dynamics, but they can evolve via simple mechanisms that are a byproduct of direct selection on other traits. Specifically, more modular and more robust genetic architectures are more likely to produce successful phenotypes, and thus they hitchhike to fixation in the population. In Chapter 2, I demonstrate how a cyclically changing environment provides a sufficient selective pressure to produce quasi-modular genetic architectures, independent of other features.

In the third chapter, I propose to study how modularity might also arise as a result of horizontal gene transfer. We hypothesize that organisms will choose to uptake gene fragments for food, even when there is a chance that those fragments might be integrated

into the genome. Furthermore, we expect that this uptake will result in higher modularity and, in turn, evolvability over time.

In Chapter 4, I propose to study how modularity and robustness affect the character of the local mutational landscape around evolved organisms. We hypothesize that populations made up of organisms possessing a robust phenotype will not only display more genomic diversity within that phenotype (since so many mutational possibilities will arrive back to the same phenotype), but the non-neutral mutants appearing in that population will display greater variety of distinct phenotypes as well.

In the fifth chapter, we show that sexual selection facilitates speciation through reduced gene-flow across groups. Specifically, we demonstrate that sexual selection is a strong early facilitator of reproductive isolation, and that post-zygotic isolation is not required to reduce gene flow between populations. Thus, sexual selection may aid in the fixation of evolvable traits in isolated populations.

In the final chapter, I conclude with a project plan, including a timeline for completion of the remaining experiments.

This thesis is dedicated to Kendall.

ACKNOWLEDGEMENTS

Your acknowledgements here

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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?

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

Within evolutionary computation, evolvability is also fundamental. The "representation problem", which influences every aspect of evolutionary search, can be characterized as a problem of how to design the underlying genetic encoding such that genomes can not only express complex solutions, but can also be mutated in meaningful ways[3]. In particular, good designs for genetic representations often involve increasing the probability that a recombination between potential solutions can produce a result that is not only viable, but more fit than either parent. The entire goal of the representation problem is to improve evolvability so that better solutions can be found. By definition, systems that exhibit good

characteristics in evolvability produce good solutions more quickly, while avoiding premature convergence[4] so adaptive evolution continues to as high a level as possible. Beyond the representation problem, many of the barriers to complexity are actually barriers to evolvability.

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

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

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

Finally, it is unclear whether evolvability is acted upon by direct selection, or whether it is a byproduct of other traits that are selected upon, or some combination of the two. At the individual level, its possible that some traits that support evolvability, such as robustness of developmental or cell processes[2] could have been selected for directly in response to adverse environmental conditions. However, at the population level, traits like neutral variation generation are more likely to have hitchhiked on the genomes of the adaptive variants that they produced. Finally, at the clade level, genetic structures that produced populations

of adaptive variants with robust and flexible genetic architectures would have been more successful at adaptive radiations[3], and thus go on to found whole branches of life with those traits[2].

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

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.

1.3 Historical Conceptions of Evolvability

1.3.1 Modern Synthesis

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

response to selection[6].

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

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

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

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

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

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

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

Using CV_A as the measure of genetic variability is superior to narrow-sense heritability because it scales additive genetic variance by the trait mean, rather than by total population variation. Thus, the additive variation component isnt overwhelmed by large population trait variance[17]. 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[18]. CV_A, however, being scaled by trait mean, predicts much higher response to selection for life-history traits[19, 6].

CV_A still suffers from significant drawbacks as predictors for adaptation and evolvability in a larger sense [19]. 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.

1.3.2 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.

1.3.3 Theoretical Frameworks for the Evolution of Evolvability

The Wagner and Altenberg paper on the evolution of evolvability significantly expanded the theoretical framework behind the evolution of the genotype-phenotype map[8]. The authors draw on knowledge from computational evolution to inform their perspective on evolvability, since the problem of evolvability is central to the representation problem in evolutionary computer science.

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

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

1.4 So, what do I mean by Evolvability?

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

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

1.4.1 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[20]), to the decoupling the mutational effects on distinct traits (functional modularity[8]), to the composition of groups of related trait complexes (variational modularity [21, 22]).

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[22]. Conversely, spatially modular genomic regions, because they are more self-contained, tend to better resist disruption from recombination, thus increasing robustness[20].

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[20]. 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[21].

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[23]. Reducing the incidence of large changes reduces the likelyhood of the development of entirely new traits as a result of relatively few mutations. Thus, the evolvability benefit of modularity may be mediated by the scale and degree to which it occurs.

1.4.1.1 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[24, 25].

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 [20]. 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

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

1.4.2 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, 26]. 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[27].

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 func-

tion 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.[28]

Finally, regulatory decoupling allows for more than one kind regulatory precursor to provide inputs for a process[28]. 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[29]. Acetate can be derived from either carbohydrates, lipids, or proteins, thus if any of those pathways are damaged by mutation, acetate can still be produced from other sources, and ATP production can continue.

It is worth noting that many of the architectural features that confer robustness to processes and genomes are based on arrangements of modular structures[30, 31]. In this way, much of robustness is facilitated by the evolution of modularity.

1.4.2.1 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[32]. 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 [32].

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[32]. 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 [32]. 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].

1.4.3 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 [32]) 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[33].

To calculate the **Genomic Diffusion Rate** (D_g) in the local neighborhood of a genotype, first calculate its *Fidelity* (F), or the probability of an offspring sharing this genotype with its parent, by measuring the probability that a single locus is not mutated ($1-\mu$) and raising it to the power of the genome length (l). Next, measure the proportion of 1-step mutants that are neutral or beneficial when compared to the parent (p_ν) as well as those that are detrimental or lethal (p_d), which must sum to one ($p_\nu + p_d = 1$). The *Neutral Fidelity* (F_ν) of a genotype is thus the probability that no harmful mutations occur, assuming no epistasis. Finally, subtracting Fidelity from Neutral Fidelity will yield the overall probability of producing an neutral offspring with a different genotype, yet neutral or better fitness (D_g).

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

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

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

Measures of neutral exploration, however, only show part of the picture. While some form of neutrality is necessary for exploring a fitness landscape, new phenotypes must be discovered to achieve higher local evolvability. In order to assess evolvability more specifically, we introduce a related measure, the **Phenotypic Diffusion Rate** (D_p), which represents the probability that an offspring will be fitness-neutral, but also express a different phenotype

than its parent. To do so, we must first measure the proportion of one-step mutants that are *phenotypically* neutral as compared to their parent ($p_{p\nu}$) and follow a similar procedure as above, first calculating the probability that a phenotype-changing mutation will occur (μ_{pheno}), then the phenotypic-level fidelity ($F_{p\nu}$).

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

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

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

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

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.

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

1.5 Digital Evolution

Digital Evolution uses self-replicating computer programs as model organisms to study evolutionary dynamics [34]. Unlike theoretical simulations, digital organisms have a fully

functional genome that direct them to self-replicate, mutate, and compete with their peers for resources and space in which to reproduce. Because digital organisms undergo genetic mutations (i.e., variation) that are passed on to their offspring (inheritance), and their survival is based on the actions they take (differential selection), they undergo evolution by natural selection.

Digital organisms do not suffer from many of the drawbacks of experimentation on natural organisms. Three of the advantages of digital organisms are particularly relevant for our study. First, the rates of reproduction in digital systems are much faster than in even the most rapidly-reproducing physical organisms; we can process generations of organisms in seconds, rather than the hours required for the fastest biological organisms under sustained conditions [35, 36], or the weeks to years needed for more complex multicellular organisms [37, 38].

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[33]; such exhaustive techniques are not feasible in even the simplest physical organisms. All of these factors make digital organisms ideal for studying the effects of changing environments on the mutational landscape.

1.5.1 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. Avida is a software platform for performing evolution experiments with digital organisms in a virtual world.

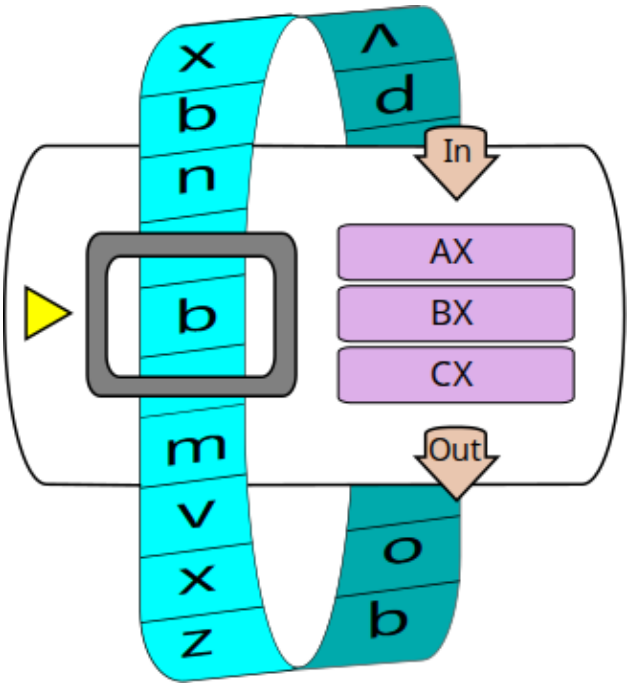

Figure 1.1: **A**n example virtual CPU from Avida, with a circular genome (blue), three registers (purple), input and output handlers (tan), and an instruction pointer (yellow) indicating the next instruction to be executed.

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 er-

rors (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 paper may be found at https://github.com/voidptr/avida and https://github.com/voidptr/ce_evolvability.

CHAPTER 2

CHANGING ENVIRONMENTS PROMOTE RAPID ADAPTATION IN DIGITAL ORGANISMS

2.1 Background

Fitness landscapes are a mathematical tool to map genetic sequences to expected evolutionary fitness. Many studies have examined the important role that different types of fitness landscapes play on evolutionary dynamics and outcomes, both in biological populations [39, 40, 41, 42] and in evolutionary computation settings [43, 44, 45]. 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 (i.e., the 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 [46].

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 [47]. Similarly, sexual populations tend toward regions of the fitness landscape with more modularity [20] and more negative epistasis [48] 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 [49, 50]. 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

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

In the short-term, this kind of evolvability determines a population's response to selection. This kind of evolvability depends primarily on the organization and interrelation of information in the genome; that is, the genetic architecture, and the resulting genotype-to-phenotype map [8]. An example of evolvable architecture can be found in some bacterial

genomes that contain highly mutable genome regions, called contingency loci. Small sets of insertions or deletions to these regions create transcription frameshifts that alter the expression of nearby coding regions, thus allowing populations to easily switch phenotypes via minor mutations. Contingency loci are most often seen in the genomes of pathogens, which are subject to frequent environmental shifts caused by the host immune system [51]. Thus, these populations are able to produce large amounts of heritable variation despite the reduction in population diversity resulting from population bottlenecks.

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

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

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 [32]. Robust genetic architectures that can tolerate more mutations without altering their phenotype reside in mutational landscapes that connect to more neutral mutants. Similarly, architectures that more easily switch

phenotypes in response to mutation without substantial reduction in fitness, reside in more evolvable regions of genotype-space.

It is worth noting that not all regions of the mutational landscape are equally accessible. Some genome regions may be more resistant to mutation than others [52], thereby altering [RCK TODO] the probabilities of mutations occurring that lead into certain regions of the mutational landscape. This kind of differential probability may therefore moderate a population's diffusion through the mutational landscape.

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, 53]. This differential response to selection therefore constrains the ability of populations to diffuse across a fitness landscape.

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

2.1.2 Changing environments create more paths to different kinds of phenotypes

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

In changing environments, however, the direction of selection is not fixed and peaks are not stable. Instead, as the environment changes, populations are driven to explore new regions of the mutational landscape [54, 55]. As they proceed, populations accumulate and carry with the genetic material acquired in prior explorations and adaptations, and use this history as raw material for new adaptation [56]. Indeed, earlier work has shown

that changing environments promote evolvability in many contexts, without compromising robustness [57, 47]. 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 [58].

In this chapter, 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. In particular, we show that populations evolved under harsh, cyclically-changing environments have many more changes along their phylogenetic histories than those evolved in static or benign changing environments. Organisms evolved in these populations also contain reservoirs of pseudogene-like vestigial loci that were acquired and deactivated through repeated adaptation and fixation cycles. As a result, populations evolved in these harsh cyclically-changing environments are low in standing neutral diversity at the population level, but they still connect locally with many more phenotypically-interesting regions of the mutational landscape than more diverse populations evolved in static or benign environments.

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

Table 2.1: **E**xperimental Treatments

Treatment Name		**Cycle**	**Phase 1**	**Phase 2**	
			Tasks	**Tasks**	**Cycle?**
Control		n/a	XOR and EQU (constant)	XOR and EQU (constant)	N
CCE-Benign	A	Cyclic Benign	XOR (constant) EQU (fluct 0/+)	XOR (constant) EQU (fluct 0/+) Logic 77 (constant)	Y
	B			XOR (constant) EQU (constant) Logic-77 (constant)	N
CCE-Harsh	A	Cyclic Harsh	XOR (constant) EQU (fluct -/+)	XOR (constant) EQU (fluct -/+) Logic 77 (constant)	Y
	B			XOR (constant) EQU (constant) Logic-77 (constant)	N
SCE-Benign‡	A	Stochastic Benign	XOR (constant) EQU (fluct 0/+)	XOR (constant) EQU (fluct 0/+) Logic 77 (constant)	Y
	B			XOR (constant) EQU (constant) Logic-77 (constant)	N
SCE-Harsh‡	A	Stochastic Harsh	XOR (constant) EQU (fluct -/+)	XOR (constant) EQU (fluct -/+) Logic 77 (constant)	Y
	B			XOR (constant) EQU (constant) Logic-77 (constant)	N

Experimental treatments. Four types of cyclic changing environment. Each is split into two treatments for phase two, one continuing with the changing environment, and one without. ‡Results of the phase-2 long-term evolvability experiments in stochastic environments may be found in the supplementary materials. [RCK TODO - Tidy up this table]

second phase addresses the relationship between short-term and long-term evolvability. 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 equal-length periods of reward and punishment. Each cycle extends for 1000 updates, or roughly 30 generations. 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.

In phase 1, we set up the system to detect organisms that performed XOR or EQU, two challenging bit-wise 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.

The second phase of the experiment continues the evolution of these populations, but introduces them to an entirely new set of rewarded bit-wise logical tasks: the Logic-77

environment. These new tasks use three bit-wise inputs rather than two, and are each rewarded with a constant 1.2-fold bonus to execution. This provides a mild selective pressure to evolve these task, but the benefits to performing them do not overwhelm the existing selective pressure to continue performing XOR or EQU.

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

[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.

2.3.1 Cyclic Changing Environments

2.3.1.1 Evolutionary History and Population Structure

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

The populations that evolved in the control and benign environments displayed more genetic diversity as compared to those evolved in the harsh cyclic environment, which underwent a bottleneck at each cycle shift. Because a selective sweep reduces current diversity within a population, the smaller number of sweeps in the benign and control treatments led populations in them to have higher standing diversity for most of their evolutionary history than those populations from the harsh changing environment. Despite this higher standing diversity in the benign and control treatments, regions of low diversity are still evident in the genomes of these populations, implying purifying selection on the traits encoded at these sites (see Fig 2.2).

2.3.1.2 Genetic Architecture

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

In contrast, the architecture of XOR and EQU remain tightly intertwined in the control, and site positions do not change substantially over the course of the experiment. In the benign treatment, many more regions that perform the fluctuating task (XOR) are scattered

throughout the genome, but site positions remain relatively fixed throughout the run after an initial adaptive phase. In the harsh treatment, not only are the active sites scattered, but the positions of active sites change and proliferate wildly over time.

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

2.3.1.3 Nearby mutational landscape

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

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

2.3.2 Stochastic Changing Environments

Contrary to our expectations, stochastic changing environments were no more effective at promoting evolvability than cyclically changing environments. In all measures of evolvability, the stochastic treatments performed similarly to the cyclically changing environments. There was a significant reduction [TODO STATS] in the Phenotypic Diffusion Rate (D_P) between the cyclic and stochastic harsh changing environments. Overall, D_P showed much larger variances, but settled on a lower mean in the stochastic harsh treatment as compared to the cyclic harsh, indicating a much lower probability of the population producing offspring that would switch phenotypes neutrally. (Fig 2.8)

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

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

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

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

2.3.3 Long-Term Evolvability in Cyclic Changing Environments

The second phase of our experiments demonstrates that the harshness of fluctuation has a dramatic effect on long-term evolvability, landscape exploration and task discovery.[2] In the first phase of the experiments, despite the logic-77 tasks not being rewarded, both changing-environment treatments discover more new tasks than the control. The harsh changing environment treatments in particular discover significantly more logic-77 tasks than either the benign treatment, or the control. (Fig 2.12. We hypothesize that this effect may be due to the large phylogenetic depth of the harsh-evolved populations, where the repeated bottlenecks drive the populations along a kind of forced march across the mutational landscape.

Despite the initial success of the harsh-evolved populations' random walk across the fitness landscape, once selection for the logic-77 tasks is engaged at the start of phase 2, the benign and control treatments out-perform the harsh-evolved populations in task discovery. In particular, those populations where the harsh changing environment continues, significantly under-perform in task exploration as compared to the benign-evolved populations, and are comparable to the control rate. (Fig 2.13). We hypothesize that this effect is due to the relative differences in the strength of selection between the harsh changing environment and the directional selection toward the logic-77 tasks. The pressure to gain and lose the

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

fluctuating task is much stronger than the pressure to acquire the logic-77 tasks, thereby depressing the rate at which they are found, and keeping it comparable to the pre-phase 2 rate. In contrast, the benign environment, with its comparatively weaker strength of selection for EQU task gain and loss, experiences a comparatively stronger selective pressure to acquire logic-77 tasks, while still benefiting from the increased exploration rate conveyed by the benign changing environment. (Fig 2.14 lower left)

Of additional interest is the comparison of task discovery rates between the benign changing environment populations A and B (Fig 2.14 lower right). The A population continues to be subject to the benign changing environment, where as the B population is only subject to directional selection toward the evolution of XOR, EQU, and the Logic-77 tasks. The task discovery rate for the A population is slightly higher than for B, but the effect is not statistically significant. Both, however, still perform better than the control treatment. Thus, this result is suggests that there are architectural features conferred by the changing environment that are helpful for long-term evolvability, even after the changes have stopped, and that this effect is distinct from the direct effects of the changing direction of selection. Further research is needed to fully untangle these effects.

2.4 Conclusion

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

selective conditions. In this way, the accumulation of vestigial or pseudogene-like regions acts as an 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.

Surprisingly, stochastically changing environments slightly less effective at exploration than cyclic changing environments, even if, on average, the amount of time spent in each environment was equal. We hypothesize this 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.

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

2.4.1 Limitations of Cyclic Changing Environments

Changing environments produce a set of selective pressures that speed up exploration of genotype space, while also building reservoirs of partial functionality that may be co-opted in the evolution of more complex structures. These features make changing environments useful for both their explanatory power in natural evolution, and as practical tools in the Artificial Life toolkit. Ultimately, however, cyclic changing environments only re-tread existing phenotypic ground, and though genotypic exploration is faster than under purely directional or stabilizing selection, the space explored remains constrained by the type of phenotypes

that are selected. Despite this constraint, however, we see that, particularly under harsh conditions, a lot of novel genotypic ground may be explored, even without direct selection for novelty.

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

Understanding the mechanisms by which select environmental conditions alter fitness landscapes is vital to understanding the forces that promote evolvability and increase complexity. In particular, understanding the role of vestigial sites may help us untangle how robustness can promote evolvability. Are these vestigial sites inactive remnants, reservoirs of function, or are they part of a complex compensatory framework supporting and buffering the expression of the phenotype? Or both? Changing environments provide one view into these dynamics, but we must explore further to find other mechanisms for exploring and exploiting genotype space.

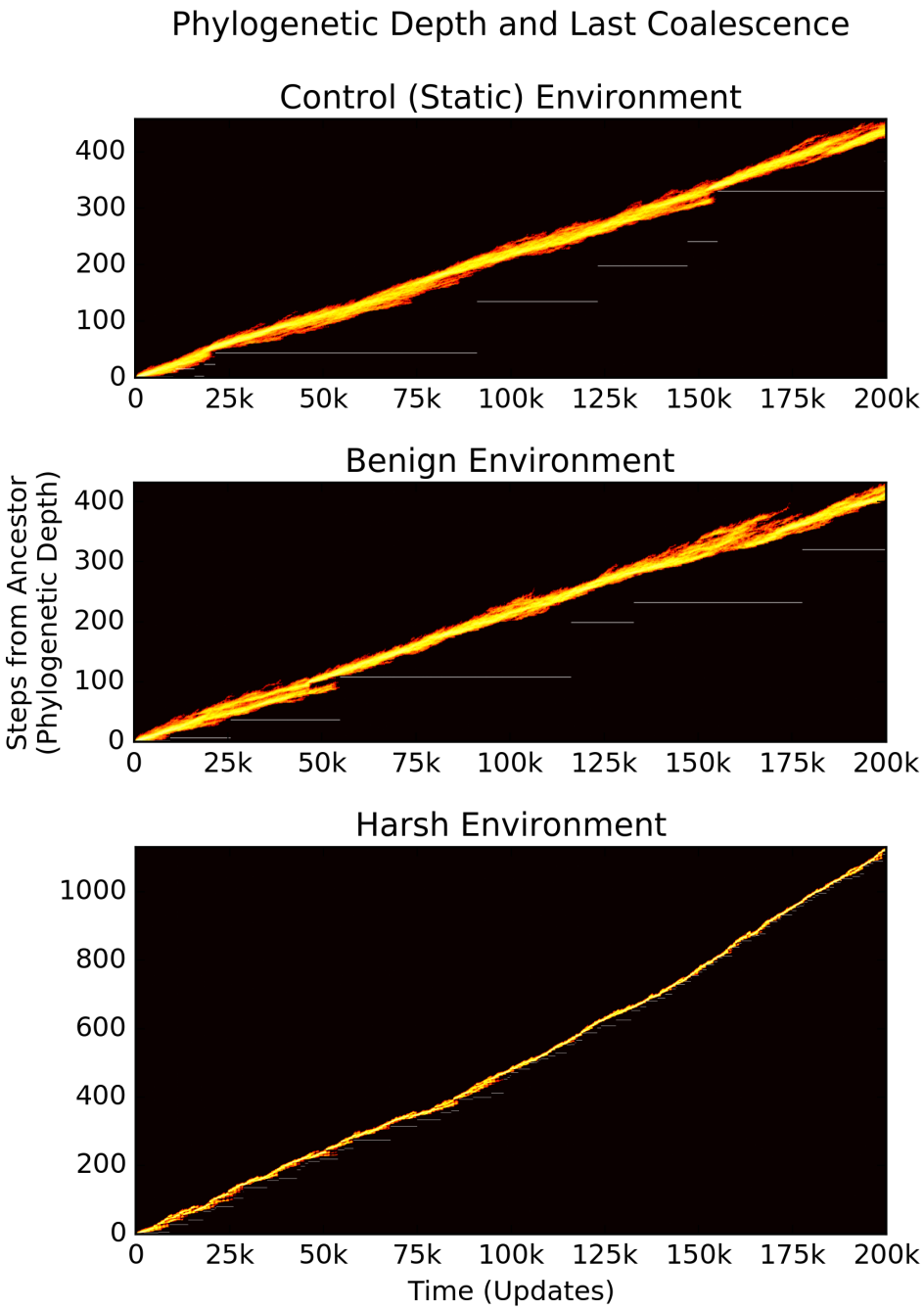

Figure 2.1: **P**hylogenetic depth over time of a representative sample population evolved in each of the three treatments of the cyclic changing environments. White horizontal lines mark the depth of the most recent common ancestor, and discontinuities in this line indicate that the most recent common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

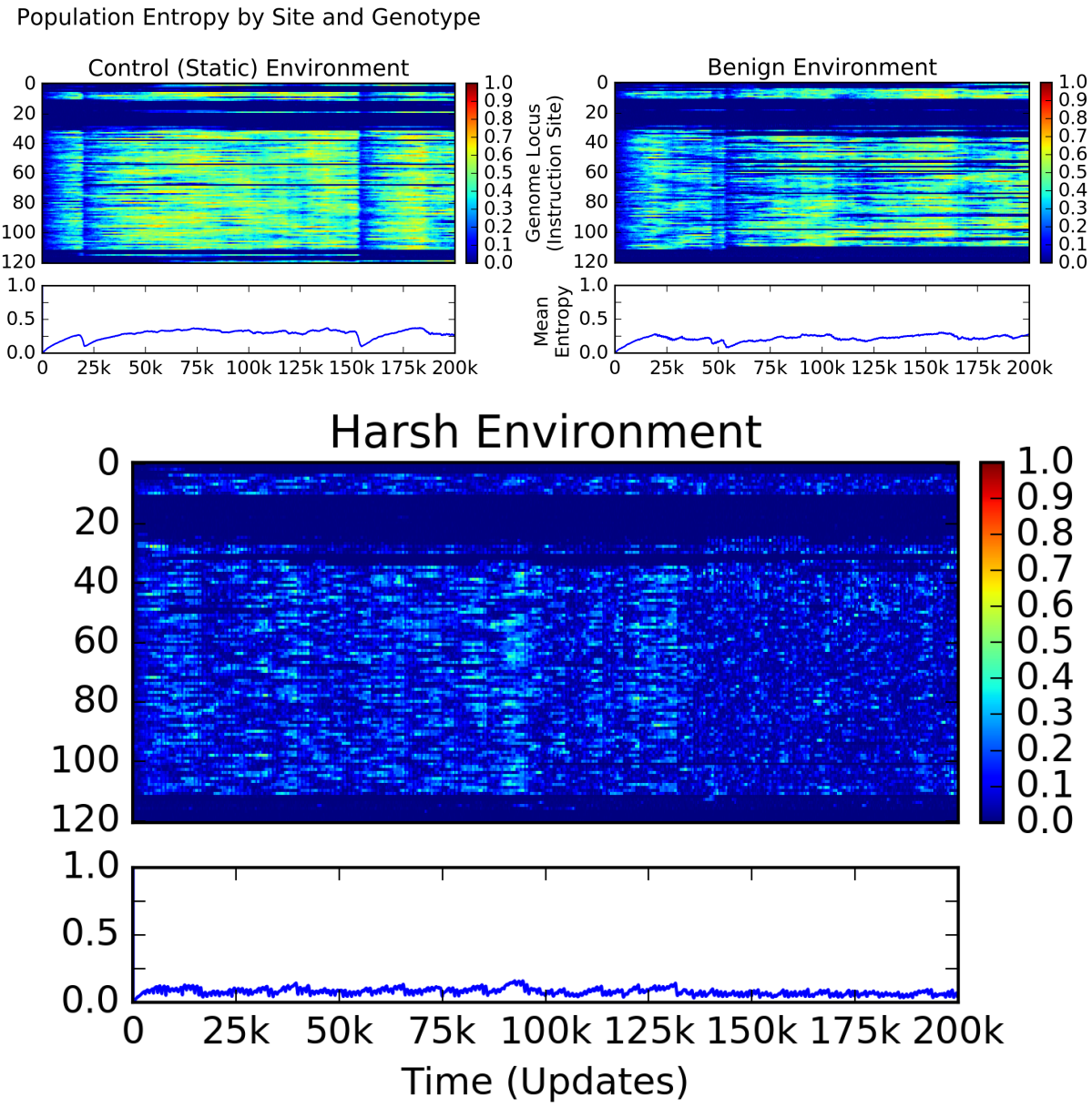

Figure 2.2: **P**opulation Per-site Entropy over time of a representative sample population. Each vertical slice represents the per-site entropy of the population at each update, both by genetic locus (upper), and overall population mean (lower). Hotter colors (red/orange/yellow) indicate greater diversity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

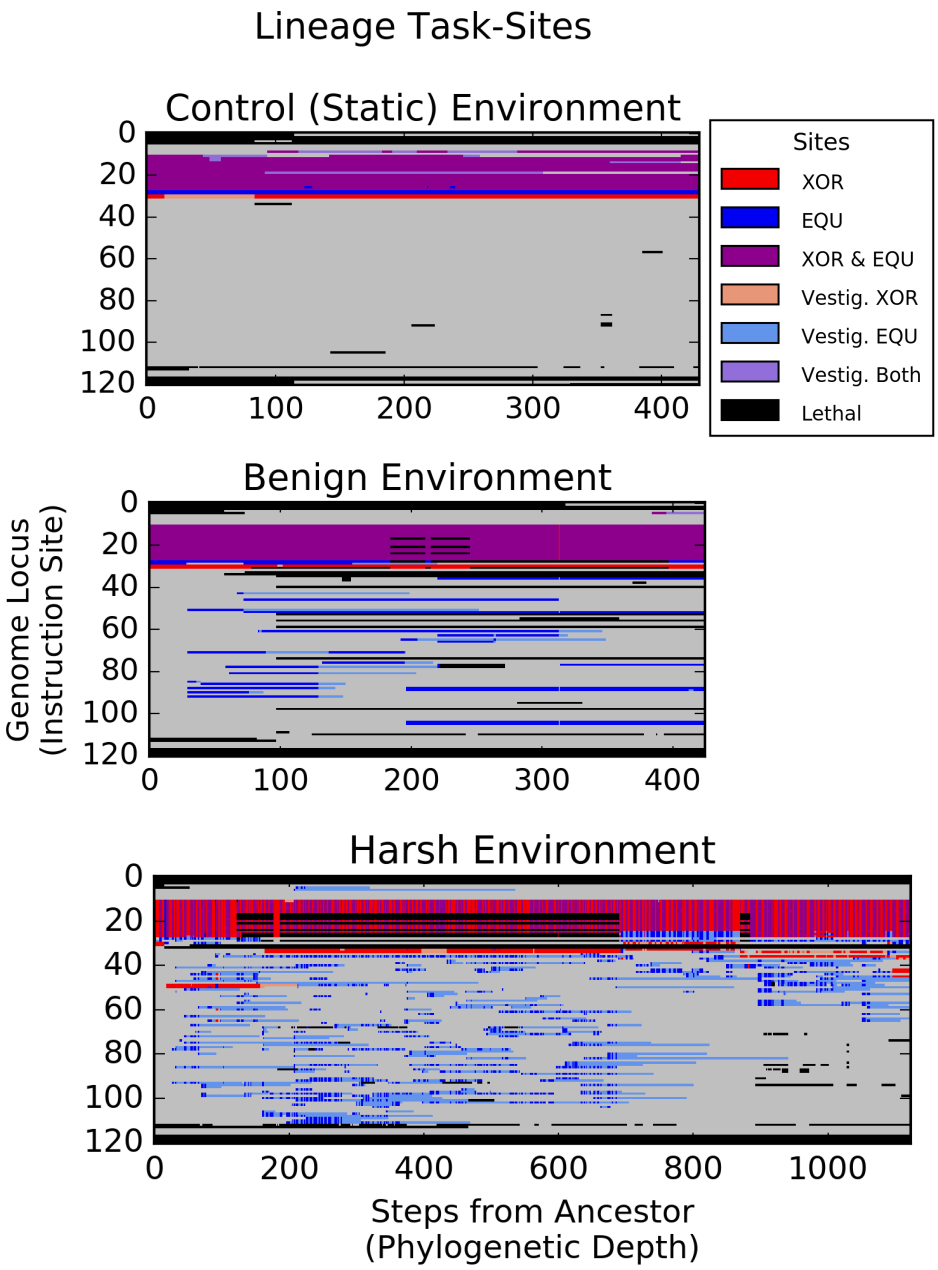

Figure 2.3: **V**arying genetic architecture of XOR and EQU over time for the final dominant genotype in a randomly selected replicate. Proceeding from the left of each figure, each vertical slice represents an organism along the line-of-descent to the final dominant. Positions along the Y-axis represent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

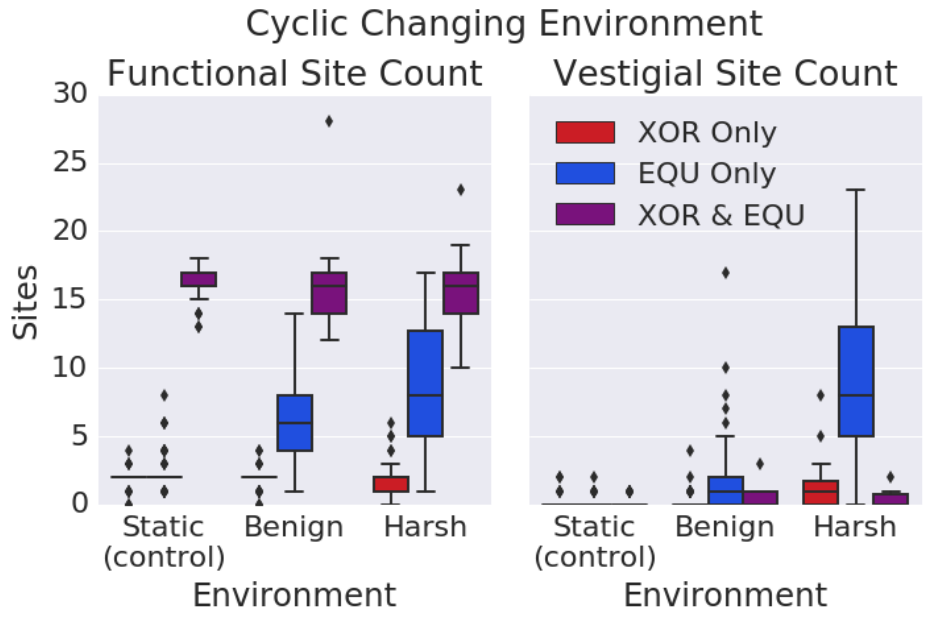

Figure 2.4: **N**umber of functional and vestigial sites by treatment. The harsh environment has a significantly larger number of vestigial sites for the fluctuating (EQU) task compared to the benign treatment or control, while having a comparable number of functional sites (One-Way ANOVA F(X,YYY) =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

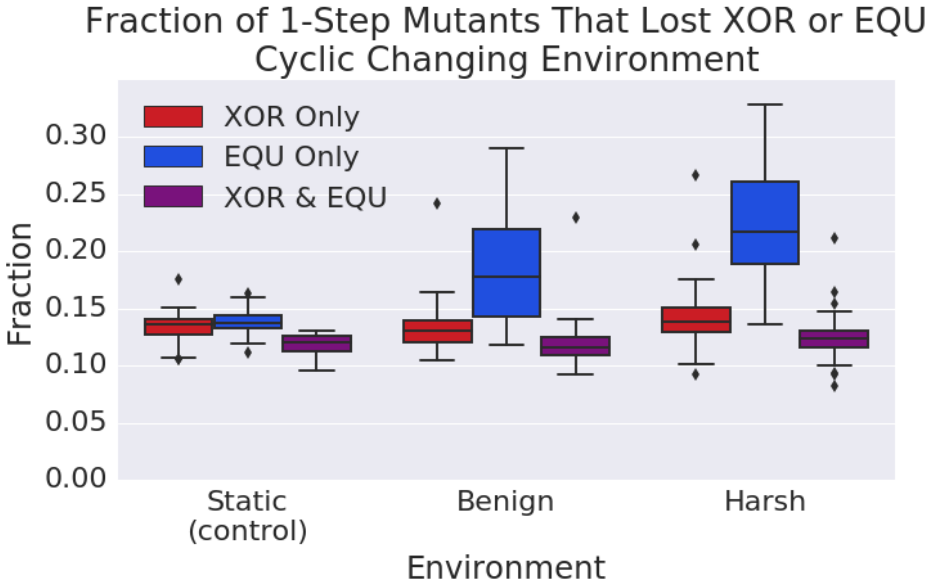

Figure 2.5: **A** survey of the single-step mutational neighborhood around organisms that performed the fluctuating task. Note that in both the benign and harsh treatments, there were significantly more mutants that lost the EQU task as compared to the control (Wilcoxon Rank Sum Test: Z = X.XX and Y.YY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

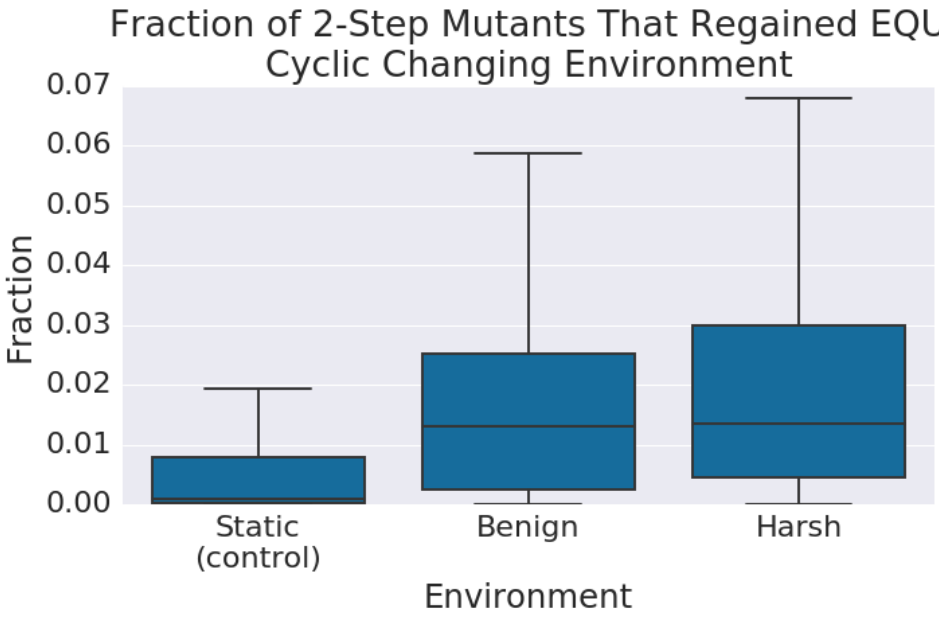

Figure 2.6: **A** survey of the two-step mutational neighborhood of the organisms that lost EQU function in the one-step survey. We found that in both the harsh and benign treatments, there were significantly more organisms that regained function in response to mutation than the control. (Wilcoxon Rank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

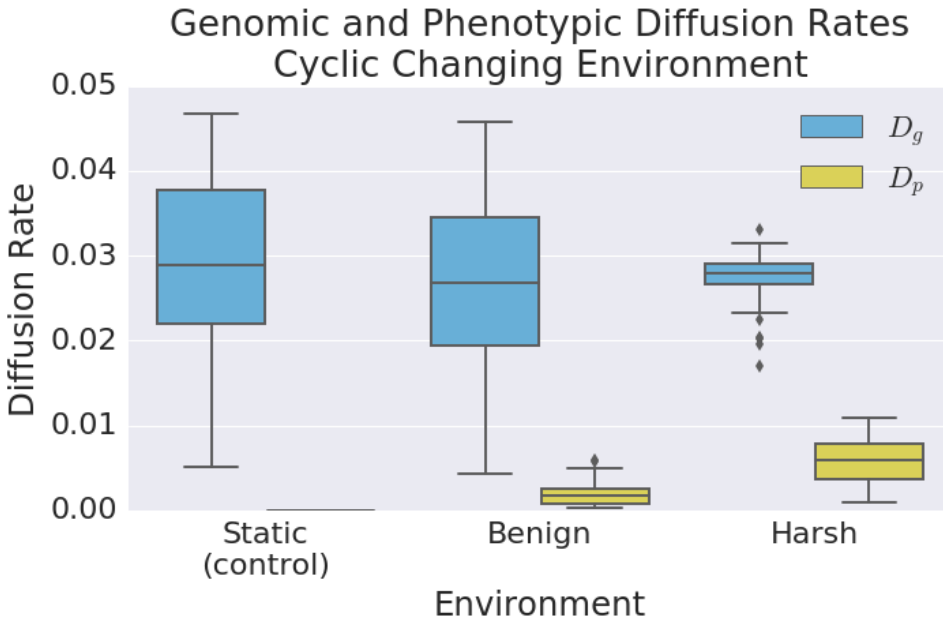

Figure 2.7: **G**enomic and Phenotypic Diffusion Rates, showing the probabilities of producing offspring that are genotypically (D_g) or phenotypically (D_p) distinct from the parent, while not reducing fitness. Note that while overall neutral exploration capacity remains relatively stable between treatments,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

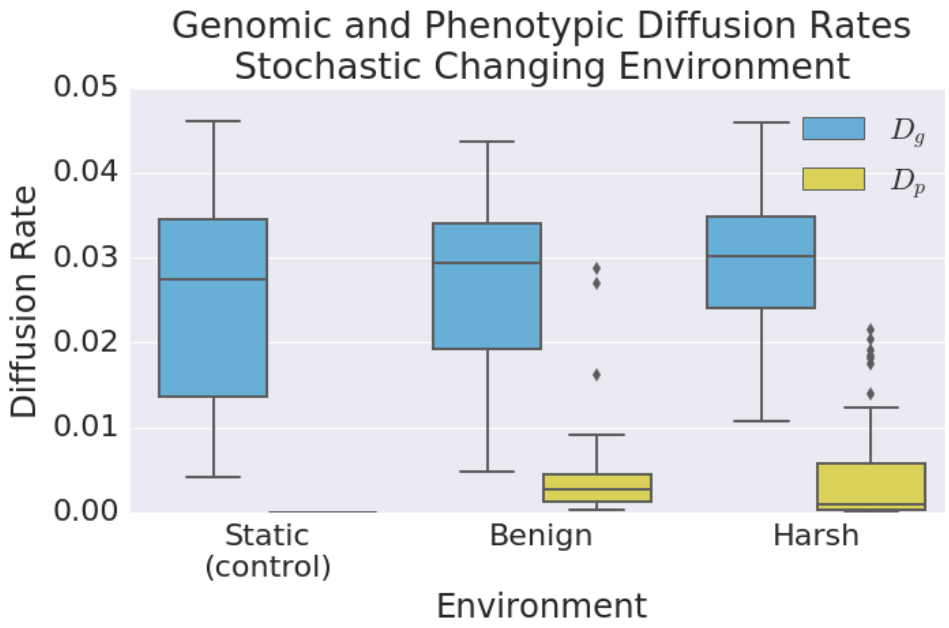

Figure 2.8: **G**enomic and Phenotypic Diffusion Rates in stochastic changing environments, showing the probabilities of producing offspring that are genotypically and phenotypically different from the parent, while remaining fitness neutral or better. As in the cyclic environment D_g remains stable,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

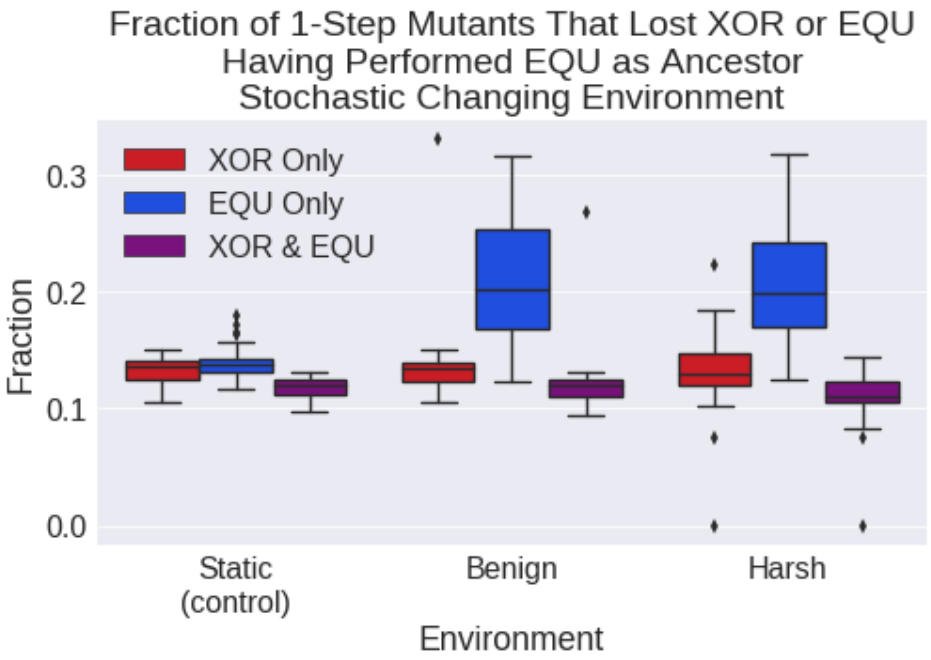

Figure 2.9: **A** survey of the single-step mutational neighborhood in the stochastic changing environment around organisms that performed the fluctuating task. Again, in the static and benign treatments, values are comparable to the cyclic changing environment (TODO STATS). However, in the harsh treatment,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

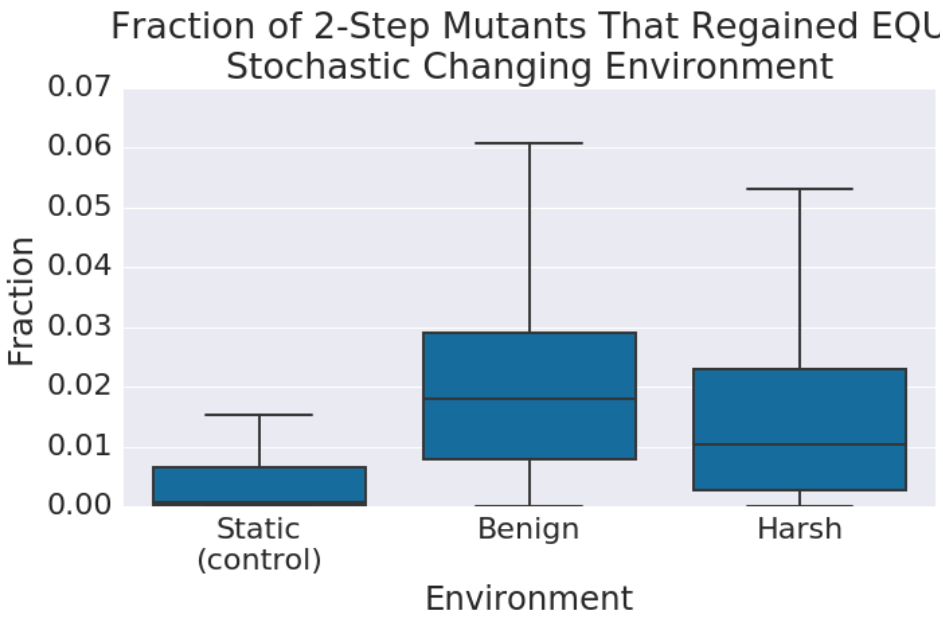

Figure 2.10: **A** survey of the two-step mutational neighborhood in the stochastic changing environment of the organisms that lost EQU function in the one-step survey. Similarly to the result in Fig 2.9, we found that the fraction of organisms regaining the fluctuating task from a single additional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

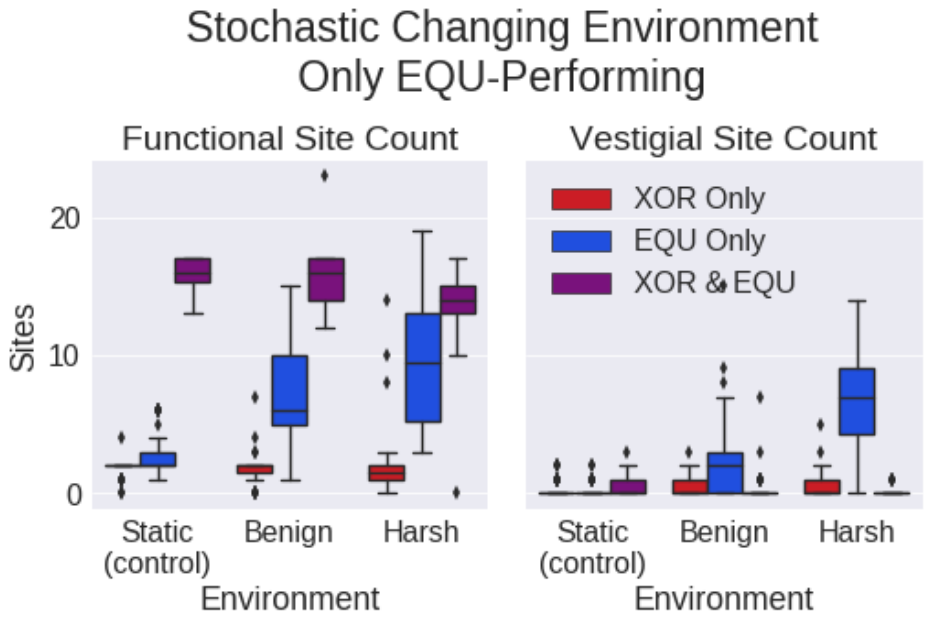

Figure 2.11: **N**umber of functional and vestigial sites by treatment in a stochastic changing environment. The vestigial site counts remain comparable to the cyclic environment (TODO STATS), however, there was a reduction in functional site counts for XOR+EQU overlapping sites in the stochastic harsh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

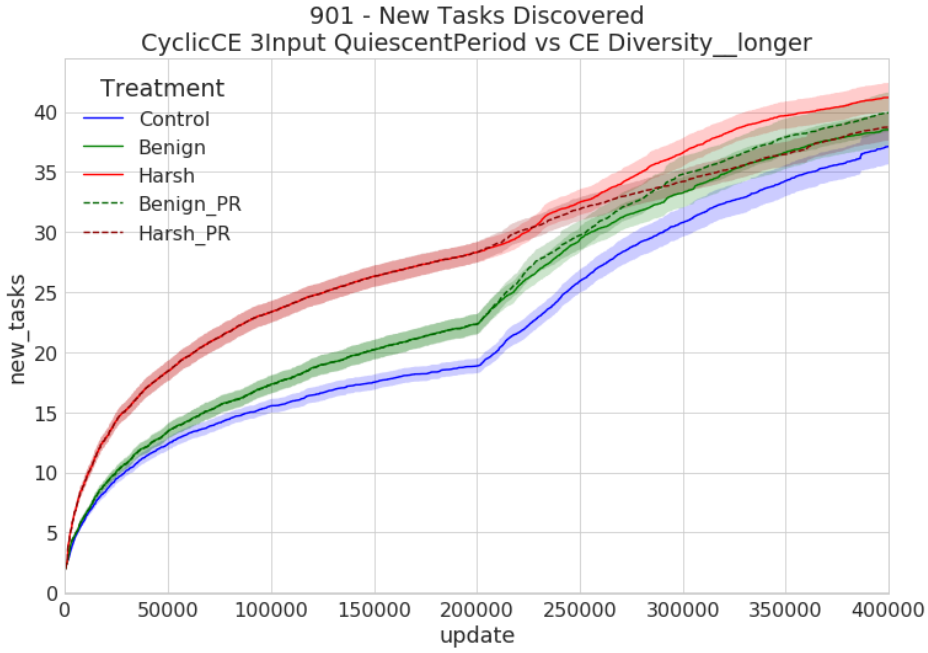

Figure 2.12: **N**umber of new logic-77 tasks discovered over time. [TODO DESCRIPTION AND STATS]

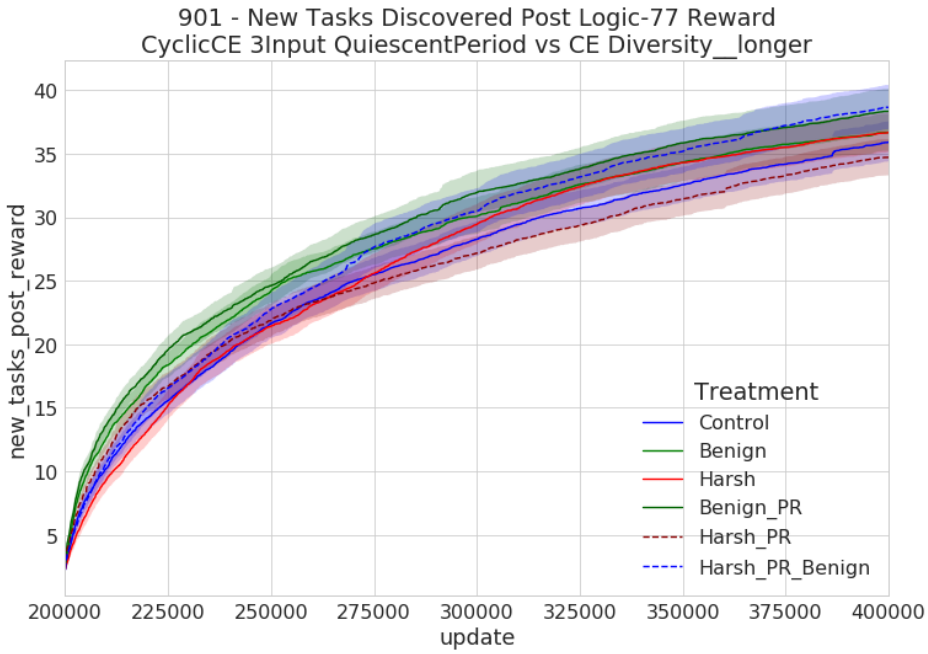

Figure 2.13: **N**umber of new logic-77 tasks discovered beginning in phase 2. [TODO DESCRIPTION AND STATS]

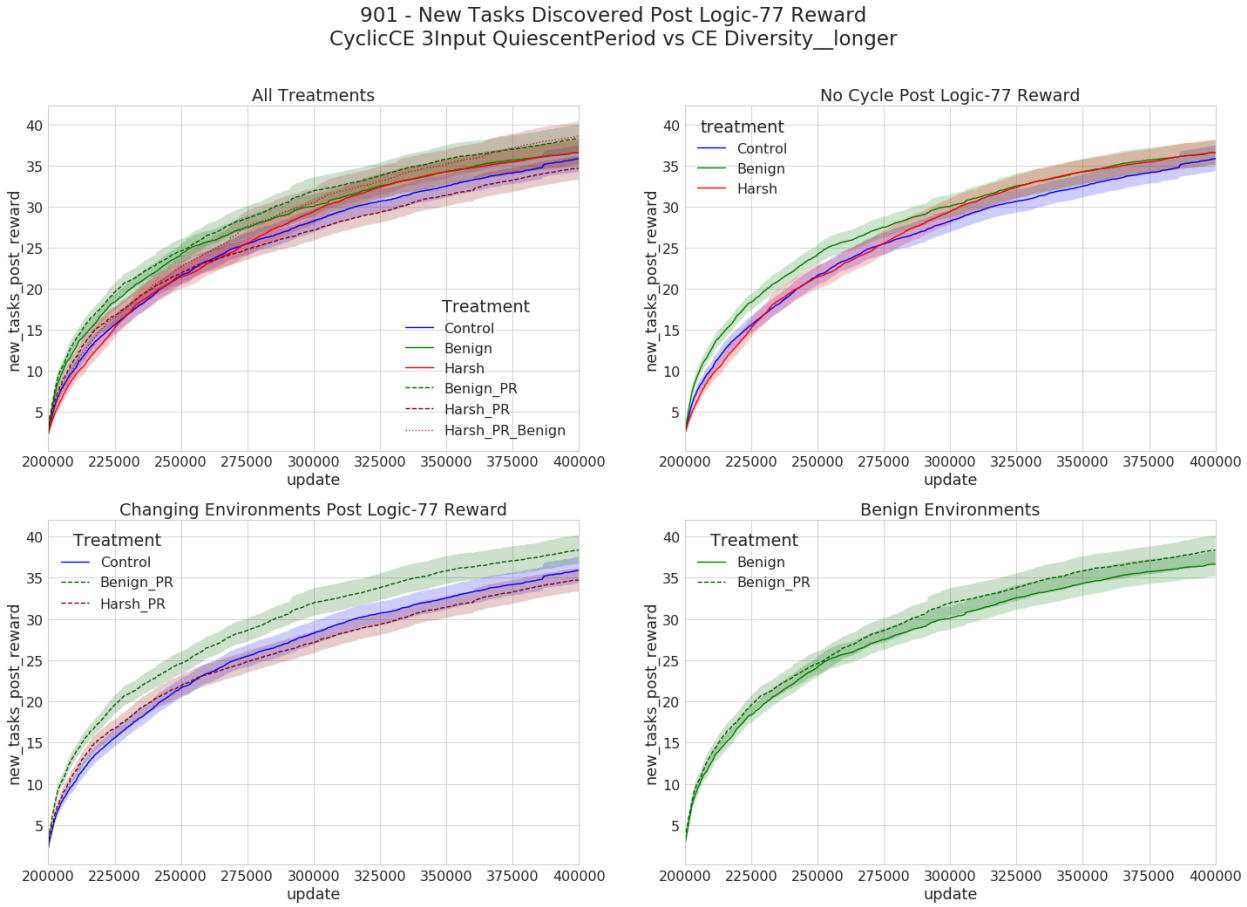

Figure 2.14: **D**etail of task discovery rates over time. [TODO DESCRIPTION AND STATS]

CHAPTER 3

CHANGING ENVIRONMENTS AND LONG TERM EVOLVABILITY

3.1 Background

3.2 Methods

3.3 Results and Discussion

CHAPTER 4

ALTERNATIVES TO THE GRAZING HYPOTHESIS: CHANGING ENVIRONMENTS PROMOTE THE EVOLUTION OF HORIZONTAL GENE TRANSFER

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

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

4.0.0.1 Origins of HGT in nature

In prokaryotes, natural competence is an HGT mechanism by which organisms spontaneously uptake the DNA of dead organisms in the environment. These organisms benefit in several ways. 1) DNA is composed of a 5-carbon sugar, a phosphate, and nitrogen bases, materials that are useful for DNA synthesis and repair. 2) The organisms may also benefit from uptaking gene-fragments that confer new adaptive functionality into the genome[66]. However, it is unclear whether the origins of these functions were developed solely in order to obtain nutrients, 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 clearly going to be disruptive to organisms more often than it is beneficial[67].

In this chapter, I explore the environmental pressures that promote the use of horizontal gene transfer, and attempt to disambiguate between the grazing and new-function hypotheses.

4.0.1 Methods

I propose to implement HGT within Avida and conduct experiments to test the hypotheses listed in the previous section (Figure 4.1). As organisms die in Avida runs with HGT enabled, fragments of their genomes will accumulate in reservoirs. The fragments in the reservoirs will deteriorate over time, with older genomes disappearing from the reservoir as new ones enter. Fragments for uptake will be randomly selected from the reservoir.

For the treatments corresponding to the first set of hypotheses, I will create two new mutagenic instructions to trigger uptake of fragments. One of these instructions will confer an energy bonus upon uptake, while the other will not. Both instructions will incur a random chance of recombining the fragment into the genome at a homologous location. If the insertion occurs, no energy bonus will be given.

The energy bonus level, uptake mutagenic chance, and fragment size will be configurable per treatment. I will perform initial exploration to find reasonable configuration values before proceeding to conduct experiments.

For experimental conditions corresponding to the second set of hypotheses, I will create a random mutation chance that triggers gene fragment uptake in the organism. This random chance will immediately integrate the fragment into the genome at a homologous location.

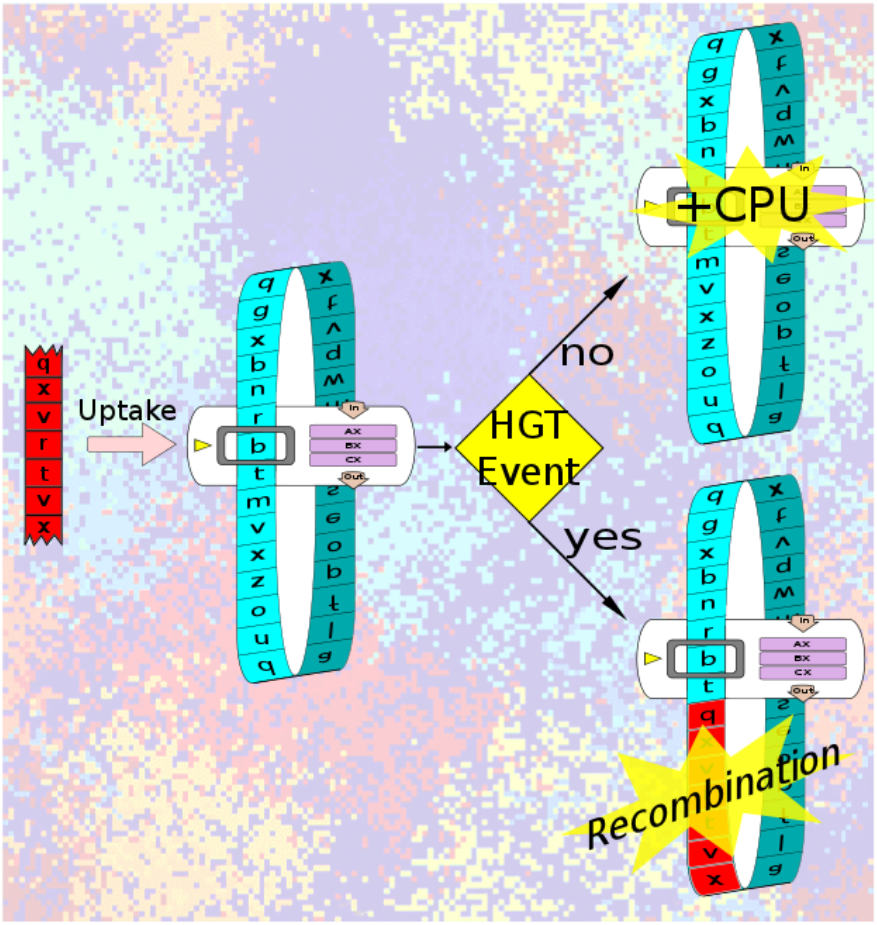

Figure 4.1: The HGT process. Organisms can execute instructions that trigger uptake from the environment. When uptake occurs, there is an experimenter-defined chance that either it will yield a boost to speed of execution or, alternatively, that the fragment will be integrated into the genome.

4.0.2 Results and Discussion

4.0.2.1 Changing environments elevate HGT use

Measured HGT fragment uptake in four conditions - 0Static, BStatic, 0CE, BCE. * No bonus, static - uptake remains low * Bonus, static - uptake increases * No bonus, CE - uptake higher than no bonus static * Bonus, CE - uptake higher than either BonusStatic or NoBonusCE

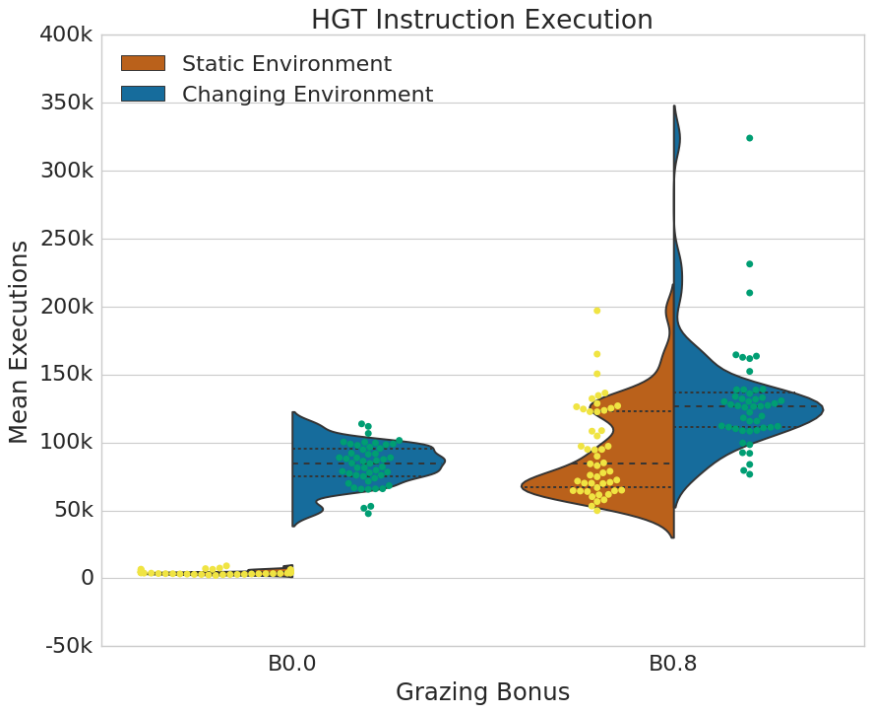

Figure 4.2: bleh

4.0.2.2 Other mutation types are not elevated in response to HGT

Measured use of mutagen instruction in static and changing environments * Die instruction (control) * Raised mut rate * Spatially-Dispersed Mutation Event * Localized Mutation Event * Shuffled-Fragment HGT * HGT

4.0.2.3 Mutation fitness effect correlate with mutagen use

Measured HGT fragment uptake in four conditions - 0Static, BStatic, 0CE, BCE. * Measured fitness effects of different types of mutagens * Spatially-Dispersed Mutation Event * Localized Mutation Event * Shuffled-Fragment HGT * HGT * CE

Fitness effects correlate with use of the mutagen.

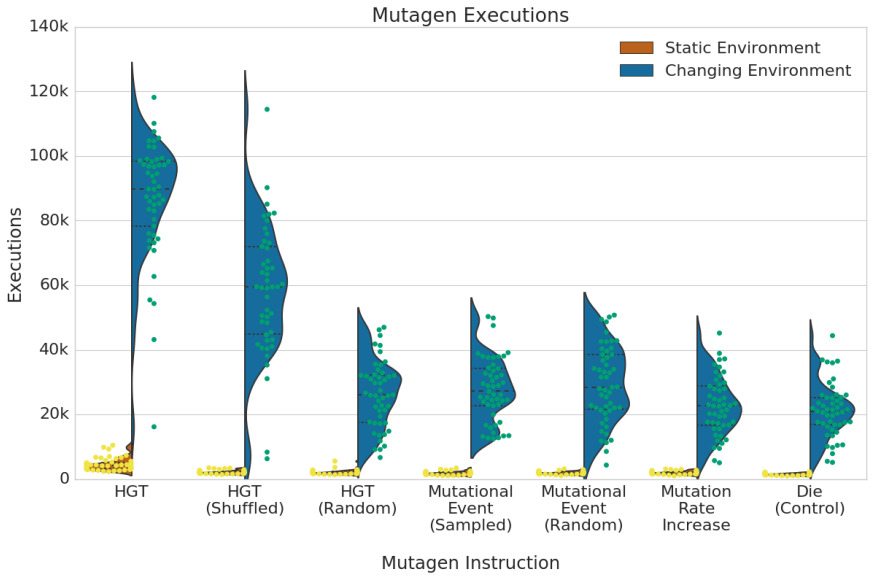

Figure 4.3: bleh

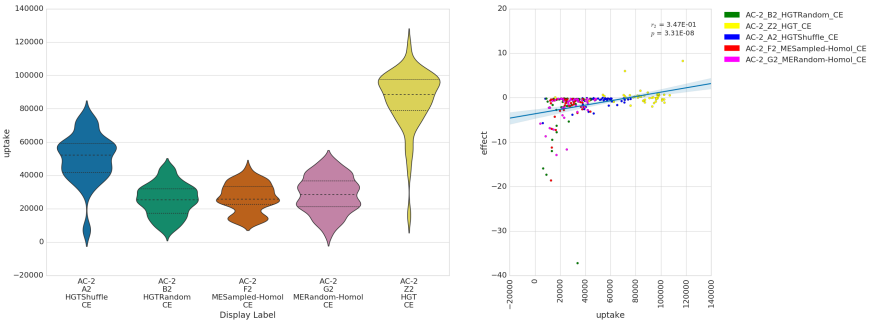

Figure 4.4: bleh

4.0.2.4 HGT mutations increase evolved probability of beneficial phenotype switching

Measured proportion of mutations that convey beneficial phenotype change (with an increase in fitness) for different types of mutagens * Spatially-Dispersed Mutation Event * Localized Mutation Event * Shuffled-Fragment HGT * HGT

HGT conveys greater proportions of beneficial phenotype-changing mutations than other mutagens.

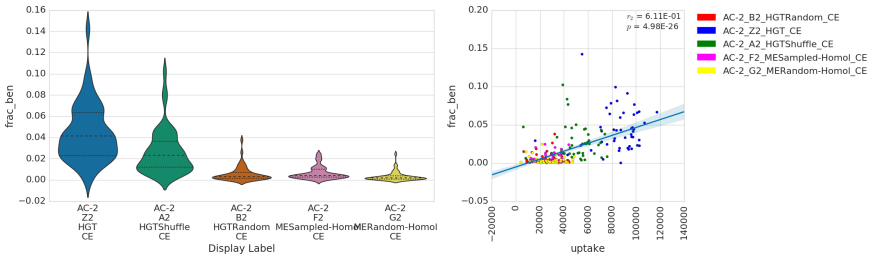

Figure 4.5: bleh

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

Measured fitness effects of HGT fragments that originated in a matching phase in a previous cycle vs fragments originating in the non-matching phase of the cycle.

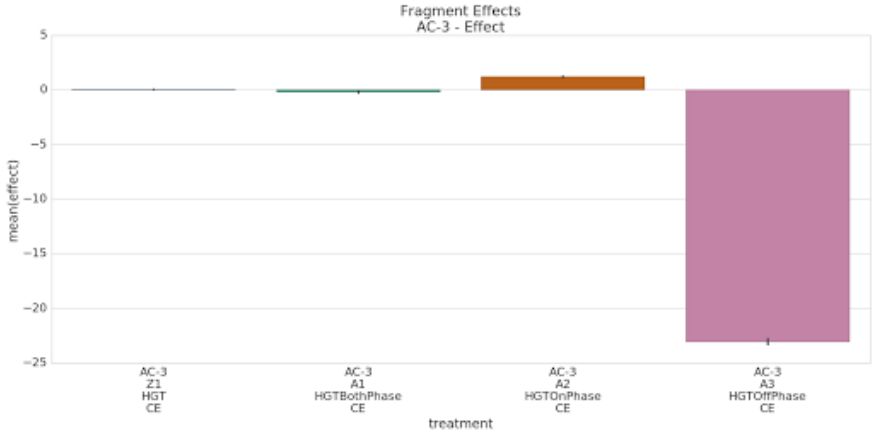

Figure 4.6: bleh

On-cycle fragments convey much of the benefit of HGT

4.0.3 Discussion

CE selects for evolvability * When sufficiently beneficial mechanisms exist, and * When change is directly selected for, as in a changing environment * Because HGT is much more beneficial than other types of mutation Under Stress, Natural Competence increases * In natural organisms, Natural Competence tends to be up-regulated, and recombination rates

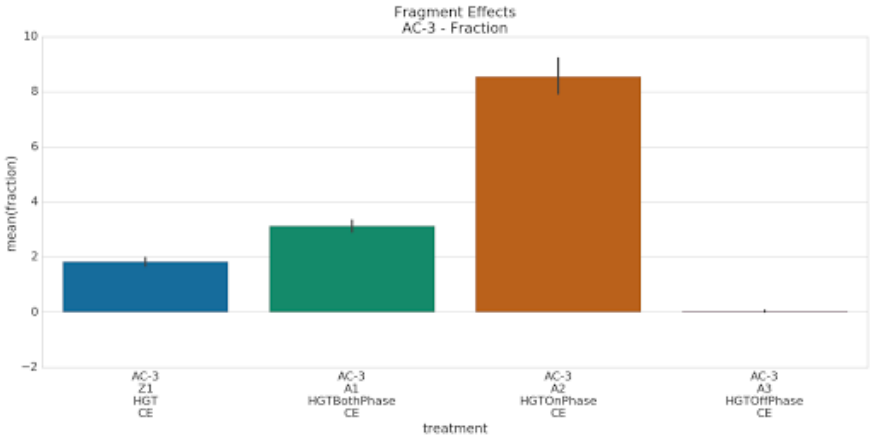

Figure 4.7: bleh

increase [cite]. * This effect may occur because of loss of fidelity of DNA copying and repair mechanisms due to stress. * Avida has no such mechanism, so lack of fidelity does not account for an increase in HGT use. Instead, HGT can only increase due to selection.

CHAPTER 5

HORIZONTAL GENE TRANSFER PROMOTES LONG TERM EVOLUTION

5.1 Background

5.2 Methods

5.3 Results and Discussion

CHAPTER 6

HORIZONTAL GENE TRANSFER AS MOST PREFERRED MUTATION

6.1 Background

6.2 Methods

6.3 Results and Discussion

APPENDIX

APPENDIX

YOUR FIRST APPENDIX

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

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

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

[20] 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.

[21] 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.

[22] 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.

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

[24] 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.

[25] 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.

[26] 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.

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

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

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

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

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

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

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

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

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

[36] 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.

[37] 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.

[38] 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.

[39] 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.

[40] 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.

[41] 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.

[42] 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.

[43] 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.

[44] 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.

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

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

[47] 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.

[48] 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.

[49] 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.

[50] 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.

[51] 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.

[52] 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.

[53] 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.

[54] 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.

[55] 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.

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

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

[58] 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.

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

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

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

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

[63] 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.

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

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

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

[67] 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.