SEPARATING HISTORICAL FLUKES FROM EVOLUTIONARY INEVITABILITIES: REPLAYING THE ORIGINS OF COGNITIVE BEHAVIORS

By

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ABSTRACT

While evolution has created a stunning diversity of complex traits in nature, understanding *how* a particular trait evolved remains a major challenge in evolutionary biology. Many dynamics can be at play during evolution, but are often summarized into three factors: adaptation (selective pressures), chance (stochastic events), and history (the genetic starting point for continued evolution). The interplay among these factors can be complex and difficult to disentangle. By conducting replay experiments on actively evolving populations, however, we can measure the role that each factor played in the evolution of a particular trait. Furthermore, these techniques allow us to study genotypes along the history of a linage in order to identify changes not only in phenotypic function, but in evolutionary potential.

Here I propose to leverage and expand upon these experimental techniques in digital systems. I plan to investigate the role that history plays in the evolution of early cognitive behaviors such as associative learning and memory usage in navigation. Are there common building blocks whose evolution facilitates more complex behaviors? Does the likelihood of a trait evolving increase gradually or in bursts? What other elements should we consider that might influence the evolution of a trait? To get at these questions, I experimentally measure "trait potentiation" as the probability of a trait arising for a given starting population and evolutionary conditions.

My use of digital systems allows me to conduct analytic replay experiments on a massive scale. Specifically, I can target individual points in evolutionary history and conduct replay experiments to measure their potentiation. By comparing potentiation before an after a given mutation, I am able to pinpoint specific mutations that affect potentiation. Points that increase potentiation inherently shift the evolution of a trait from requiring chance events to being able to rely on adaptive pressures. I will study lineages that ultimately lead to a trait of interest to determine if potentiation increases gradually or if the trait's appearance flips suddenly from fluke to inevitability.

I have two main aims in this dissertation proposal: 1) I want to understand the evolution of potentiation, and more broadly, how history interacts with adaptation and chance to produce complex traits and behaviors, and 2) I want to explore how cognitive behaviors evolve, and more specifically, why these behaviors so rarely arise in digital evolution systems. While these techniques have been refined over the last few decades, here I propose to conduct them on scales only feasible in digital systems. As described below, I will fully explore whole lineages, and even full fitness

landscapes, while disentangling the effects of different traits, environments, and representations. My goal is to develop a more holistic understanding of how potentiation changes during evolution.

I start this proposal (Chapter 1) with a review of the relevant background in adaptation, chance, and history, as well as my own perspective on how I view these topics. I also provide an overview of prior work on the evolution of cognitive behaviors in digital systems. Next (in Chapter 2) I demonstrate that single mutations can drastically increase the potentiation of associative learning, and follow that up with a proposal (Chapter 3) to expand the scale of this work to allow for deeper analyses and more powerful statistical comparisons. In Chapter 4, I take a step back and examine potentiation in a simplified bitstring model, which I propose as a mechanism to better understand the basics of potentiation and how it relates to epistatic interactions. Thinking about the role of history in evolution more broadly, in Chapter 5 I discuss published investigations into how the evolution of phenotypic plasticity, a common stepping stone for cognitive behaviors, shapes future evolutionary dynamics. In order to assess the robustness of earlier results, in Chapter 6 I propose to compare the associative learning potentiation work to new studies that I will conduct using my phenotypic plasticity environment as well as a new navigational behavior environment. Finally, in Chapter 7, I provide a timeline for this work and identify some additional assessments that should be performed in the future (or, perhaps, as alternatives to the chapters above). For example, the underlying representations of the digital organisms could be varied to investigate the generalizability of patterns across organism types.

Overall, I believe that these studies will help us gain a deep understanding about the evolution of potentiation, with strong implications for the evolution of evolvability and ideally the prediction of evolutionary outcomes.

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Chapter 1
Introduction

As many evolution-focused dissertations start, there is seemingly limitless diversity to the organisms that have evolved in nature. From microbes to megafauna, evolutionary processes have created a stunning array of traits and behaviors in organisms. But how did these features come to exist? And what general evolutionary trends can we abstract from these examples? These, of course, are grand challenges of evolutionary biology.

In addressing these challenges, biologists naturally examine the fossil record for clues on how evolution produced life as we know it. Looking to history, however, has many limitations: we are provided with only a single instance of evolution, the data we do have is incomplete, and we are not able to go back in time to conduct controlled experiments. In recent decades there has been a surge in experimental approaches to studying evolution. If we evolve populations in controlled laboratory conditions, we are able to evolve many populations in parallel, observe almost everything that occurs, and build a range of experimental conditions – thus addressing each of the problems above. In this dissertation, I will adopt this experimental mindset in an attempt to understand the role of historical contingency in evolution. I will use the possibilities revealed from counterfactual experiments to understand "life as it could have been" in our study systems. This techniques will help me separate the flukes from the inevitabilties in the dynamics that shaped the course of evolution as it was originally realized in these studies.

When looking at evolution in nature, we often encounter beneficial traits that were uniquely evolved in one type of organism, while organisms in the broader taxonomic unit found different survival strategies. For example, while many species of birds and insects exhibit self-powered flight, only one branch of mammals have: bats. Why was this behavior so rare, and what conditions led to the evolution of flight in only this one specific branch of mammals? In nature, there is only so much we can do; unfortunately we cannot travel back in time and “replay the tape of life” (as evoked by Gould (1990)) to observe if flight consistently evolved in bats, or if it was an uncommon stroke of luck. Could we, instead, use experimental evolution to ask similar questions? As I describe below, biologists have successfully employed this approach to understand the evolution of microbial traits, and I seek to further refine these techniques.

In thinking about the evolution of a particular trait, it is important to consider three different

factors: adaptation, chance, and history (Travisano et al., 1995). New or modified traits that provide a net benefit to the survival and reproduction of an organism are more likely to increase in frequency, thus illustrating the role that *adaptation* plays in shaping evolutionary outcomes. Of course, adaptation can only act upon genetic sequences that are available in the population. The stochastic nature of random mutations means that some genetic sequences will arise, while others will never even appear for selection to consider in the first place. The random appearance of sequences – or disappearance as misfortune can remove otherwise fit genotypes – highlights some of the roles of *chance* in evolution. The influence of chance is constrained, though, by the starting genotypes that mutations act upon; traits can appear or be modified only if such changes are available in the local genetic neighborhood. This distribution of genetic sequences in the population at the point under investigation in an evolutionary study can be described as the product of its *history*. It is the interplay of these three aspects that produce the complex dynamics that we observe in evolving populations.

Of course, what we call history is just a matter of temporal perspective. From the vantage point of a population in a given state, all of the dynamics that brought the population to that state are now all consolidated under the label of history. Looking forward, however, both adaptation and chance will be at play, with different mutations or combinations of mutations occurring at different probabilities, and the resulting combinations having differing survival potential. As time advances, these changes are again relegated to history, while new outlooks now exist for the population based on its new composition. As such, for any study where we examine the balance among these three factors, we need to be clear about the starting point from which our perspective will be based.

In this work, I focus on the balance between chance and adaptation and how that balance changes over the course of history. From one point along a lineage, the evolution of a given trait may be unlikely, subject to the whim of chance. From a later point along that lineage, that same trait's evolution may shift to being a near certainty, with adaptation more in control of the population's fate. Is there some way for us to predict these shifts in influence between adaptation and chance? Can we distinguish between a population that is being driven to a specific outcome from one that is simply adrift? And how do these shifts occur? Does chance give way to adaptation in small increments, or can an individual mutation dramatically alter the balance?

The challenge with addressing these problems using standard experimental evolution techniques

is that they require us to have speed, control, and data collection capabilities beyond what is currently possible. Specifically, we must be able to isolate all of the individual mutations along a lineage and replay evolution using each step as a new starting point. Furthermore, for each of these starting points, we need to be able to conduct enough replays to generate statistically powerful conclusions about evolutionary outcomes.

I leverage digital evolution to overcome these hurdles, , which gives many benefits over wetlab approaches, including greater speed, automatic high resolution data collection, and the ability to start an experiment with the exact conditions of our choosing. Of course, digital evolution also has its drawbacks. For example, there is a much lower limit to the complexity of the organisms and what we have been able to evolve *in silico*. Further, due to technological constraints, digital evolution is typically limited to scales of at most tens of thousands of organisms, while natural populations can be vastly larger. As such, evolving meaningfully complex traits from scratch in open-ended systems requires a better understanding of the underlying dynamics to be able to maximize evolvability.

While there are many complex traits that could be studied, here I focus on the evolution of early cognitive behaviors. This topic is of great interest to evolutionary biologists in understanding the origins of intelligent behaviors. The lack of obvious physical characteristics of intelligence makes it challenging to study the evolution of these traits by looking at the fossil record, and their complexity makes them difficult to re-evolve under laboratory conditions. Of course, this domain has also proven challenging for evolving digital systems, which is of little surprise as artificial intelligence as a field has encountered many hurdles in its quest to produce intelligent agents. Investigations into the origins of simple stepping stones to intelligence, however, have been much more fruitful. By looking at the role of history in the evolution of early cognitive behaviors, I aim to shed some light on how these behaviors arise, why they are so difficult to evolve, and how we might increase the complexity of intelligent behaviors that digital evolution systems can produce.

1.1 The role of history in evolution

While the ideas of evolution and natural selection have been around for well over one hundred and fifty years (Darwin, 1859), evolutionary biologists continue to argue about, test, and expand upon the different factors that contribute to evolution. Here I focus on the role of history in evolution, one of the three aspects succinctly identified by Travisano et al. (1995). While adaptation was the initial frontrunner, researchers argued for the importance of chance (Kimura, 1968; King and

Jukes, 1969; Mayr, 1983) and later history (Gould, 1990; Gould and Lewontin, 1979) in evolution.

It may appear obvious that history plays an important role in evolution, as the set of genetic sequences that could feasibly appear in the population relies on what sequences currently exist. Here, however, I mainly focus on the idea of "historical contingency" – the idea that small, often initially inconsequential changes can have a drastic effect on what ultimately evolves. As an example, consider a set of three genes, A, B, and C, that together give rise to a highly beneficial trait. All three genes are equally beneficial in isolation, while AB is slightly more beneficial but AC and BC are both detrimental to fitness. In this scenario, populations that have fixed either A or B in isolation have a beneficial pathway to the combined trait, ABC. If instead a population has fixed C by itself, the ABC trait becomes much harder to evolve, as both intermediate steps are deleterious; a double mutation is then needed to reach the trait without losing fitness. Even in this simple example, the initial fixing of C has no penalty when it occurs, but it shifts the possibilities of what is likely to evolve in the future. For a thorough review of the ideas and complications of historical contingency, as well as empirical investigations into its role in evolution, see (Blount et al., 2018).

While work has been done to study the role of historical contingency in the evolution of natural populations (e.g., (Keller and Taylor, 2008; Losos et al., 1998)), here I base my work on empirical studies of historical contingency in experimental evolution. Early work in *Escherichia coli* produced two drastically different results. Researchers found no influence of the initial value of fitness reflected in the populations' final evolved fitness, but in the same experiment, they found that the final evolved cell size of a population was highly contingent in the initial cell size of that replicate (Travisano et al., 1995).Building off this framework, Flores-Moya et al. (2012) found evidence that history plays a key role in the evolution of growth rate and toxin cell quota in algae. Recently, Smith et al. (2022) have shown that, while history does play a role in the evolution of *E. coli*, the interactions between adaptation, chance, and history can heavily depend on traits under investigation and the environment being studied. By leveraging clever experimental evolution studies, these researchers have shown that it is possible to disentangle the influence of adaptation, chance, and history on evolution in a particular system.

Digital systems have also been used to study historical contingency's role in evolution. By comparing normal evolutionary replicates to those where deleterious mutations were automatically reverted, Covert III et al. (2013) found that initially-deleterious mutations can increase the complex-

ity of evolved traits. Separately, Yedid et al. (2008) found that, using a controlled extinction event, pre-extinction presence of a complex trait factored into the re-evolution of the trait after the event. The seminal work of Travisano et al. (1995) was also replicated in the digital evolution system Avida and expanded to look at the contributions of adaptation, chance, and history over time, thanks to the perfect record keeping of digital systems (Wagenaar and Adami, 2004). More recently, Bundy et al. (2021) leveraged the speed of digital evolution to test the how the depth of history affects future evolution, dealing with generation counts well beyond what is currently feasible in microbial systems. Finally, Braught and Dean (2007) recreated the initial *E. coli* and Avida experiments using neural networks and demonstrated an interaction between adaptation, chance, history, and the Baldwin effect; they found that learning can influence the impact of the three factors. These studies show that not only are these techniques viable in digital systems, but that digital systems can *expand upon them* to conduct research that would otherwise be impossible.

1.1.1 Potentiation

While this dissertation proposal focuses on investigations of the role of history in the evolution of cognitive behaviors, most chapters emphasize the concept of "potentiation". Here we define potentiation as the likelihood that a target trait evolves from a given initial genotype or population.

The foundational work for measuring potentiation comes from Blount et al. (2008). The authors empirically tested whether the novel citrate metabolism in one of the Long Term Evolution Experiment populations (Lenski et al., 1991) was due to a fluke mutation or the accumulation of a potentiated genetic background. To do so, they founded multiple "replay" populations from various points along the lineage that originally evolved to metabolize citrate. They found that the metabolization of citrate was more likely to evolve from samples further along the lineage, providing support that genetic potentiation was a key factor.

Essentially, this framework is applying the analysis of Travisano et al. (1995) along each step of a lineage, and by varying the amount of evolutionary history present, we can identify shifts in the contributions of adaptation and chance in the evolution of the target trait. Increases in potentiation indicate an increased contribution of adaptation, as the trait is now more likely to evolve. This could happen if the population has moved such that a more-adaptive (or less un-adaptive) pathway to the target trait now exists. Decreases in potentiation, on the other hand, indicate a stronger reliance on chance and could be the result of convergence to a local optima in the fitness landscape. The

selective pressure of this optima could leave the population reliant on fluke mutations or genetic drift to escape and potentially find the target trait. Ultimately, a difference in potentiation between two points on a lineage indicates that the genetic changes between them, which are considered history in the context of the later point, are important in whether the target trait ultimately evolves. This opens the possibility of examining what mutations fall in this window, how they affected the organism overall, and if their appearance in the lineage was due to adaptation or chance.

Since that initial study, researchers have conducted similar experiments (now called analytic replay experiments) in various systems and looking at various traits. These include evolvability in *E. coli* (Woods et al., 2011), novel receptor usage in Phage λ (Meyer et al., 2012), and, recently, the epistatic interactions in yeast (Vignogna et al., 2021). Across these systems, the researchers showed that the accumulated genetic background is profoundly important in the eventual evolution of the target trait. While these techniques are relatively new, they offer valuable insight into how the interplay of adaptation, chance, and history can influence what subsequently evolves. These studies look backward, empirically testing what changes led to the final evolved behaviors, but this is deeply intertwined with concepts such as predictability in evolution. As such, I expect research in the near future to begin weaving these findings into the broader tapestry of evolutionary dynamics.

1.2 The evolution of cognitive behaviors

Previous studies of historical contingency in digital organisms have primarily explored the evolution of Boolean logic functions (Bundy et al., 2021; Wagenaar and Adami, 2004). Here I instead focus on the evolution of cognitive behaviors, which are more intuitive to understand as phenotypic traits, though their internal mechanisms can still be opaque.

Cognition focuses on sensing external information, dynamically processing it, and using the results to select a behavioral response. The specific definition of cognition is debatable, but all examples of cognitive behaviors in this work use past experiences to make more effective choices (i.e., they require memory). As such, these behaviors are integrating information over time, and I argue that clearly categorizes them as cognitive. While many interesting behaviors fall under this cognitive umbrella, in this dissertation I focus on two of the most simple: (1) remembering environmental cues and associating them with optimal behaviors (Chapters 2 and 3), and (2) monitoring local resource availability to identify when to shift between feeding on the current nutrient patch and searching for a new patch (Chapter 6).

A myriad of cognitive behaviors exist in animals, and debatably many exist in plants and microbes as well (Dussutour, 2021; Loy et al., 2021). Evolving these behaviors *in silico* is therefore critical if we want to create useful agents or to study more complex evolutionary dynamics found in nature. However, evolving cognitive behaviors in digital systems has traditionally been difficult. It is a challenge worth pursuing, though, and replay experiments to disentangle historical contingency provide a new opportunity to make progress. At the same time, the complex nature and multiple required components of cognitive behaviors create a valuable scenario for deepening our understanding of historical contingency. As an example, a mutation that provides an organism with the capacity for memory may be initially deleterious (or at best neutral) if the machinery to utilize that memory is not in place. However, if that machinery were to appear, the lack of that memory might render it useless. It is only in combination that these two traits form a beneficial behavior. As such, either these traits must arise simultaneously for adaptation to be able to act upon the combination, or one must persist by chance until the other provides it with utility. These possibilities raise the question: In lineages that successfully evolve cognitive behaviors, do we see an "all-or-nothing" simultaneous evolution of multiple interacting components, persistence of one component without benefit, or other dynamics such as exaptation of other traits? The work I propose here will illuminate critical steps in the evolution of cognitive behaviors, providing useful information for future attempts to evolve them while also establishing a framework to ask larger questions about the interplay of adaptation, chance, and history.

As mentioned, evolving these behaviors *in silico* can be a monumental challenge. I wish to make two key notes. First, there have been many studies focused on the interplay of learning and evolution (for a historical example, see (Hinton et al., 1987)), but here we are solely focused on evolving cognitive behaviors and not the downstream effects after cognition appears. The interactions between learning and evolution have long been theorized and studied (Baldwin, 1896), and this broad area of literature is generally outside the scope of this dissertation proposal. Second, it is important to note that here we are evolving these behaviors *from the ground up*, with little to no built-in machinery to assist in the evolution of cognition. Many representations such as Markov brains and recurrent neural networks have aspects like memory built in (Hintze et al., 2017). These representations and the work that has been done with them are invaluable, but here we start at a low level, requiring even simple building blocks like memory to be evolved. While every digital

system must make assumptions and use abstractions in designing the framework of organisms, I argue that requiring memory be evolved moves us closer to the challenges faced by early organisms in nature. Ultimately, these dynamics will need to be studied under a broad range of conditions and representations in order to draw generalized conclusions.

1.2.1 Challenges in the evolution of cognitive behaviors

One common hurdle in evolving cognitive behaviors is one familiar to all researchers in evolutionary computation: deceptive fitness landscapes (Lehman and Miikkulainen, 2014; Silva et al., 2016; Whitley, 1991). Here, we refer to fitness landscapes as genotype-to-fitness maps and deception as local optima that prevent evolution from reaching the target trait or global optimum. Deceptive landscapes are an issue in many areas of evolutionary computation, but they become especially problematic when evolving cognitive behaviors. The local optima that cause the issue are often behaviors that do not use memory, but still manage to do well enough to dominate a population (Risi et al., 2010). These local optima restrict the exploratory capabilities of the population and prevent the discovery of the target cognitive behaviors, even if they would otherwise be superior if given the opportunity. For example, bet-hedging techniques will often arise where organisms stochastically choose between two behaviors; if picking the correct behavior half of the time is sufficient for a net boost in fitness, such strategies will dominate.

Further, the evolution of cognitive behaviors suffers from the "bootstrap problem", where no positive fitness gradient exists between initial conditions and genotypes that exhibit cognitive behaviors (Gomez and Miikkulainen, 1997; Mouret and Doncieux, 2009; Silva et al., 2016). While this dissertation proposal argues for the importance of history in evolution, there is no denying that selection is a powerful driver of evolution. As such, an issue arises when stepping stones to cognitive behaviors are often not advantageous and thus not selected when they first appear. As described above, the capacity for memory will only be useful in conjunction with machinery that makes use of the stored information. Such situations are especially common with cognitive behaviors, where several components are all required to click into place all at once for any of them to be useful. Each such instance makes the final behavior exponentially less likely to evolve. In these cases, additional evolutionary incentives must often be employed to bootstrap the necessary building blocks to eventually reach these behaviors, as I describe below.

1.2.2 Previous work

The challenges inherent to evolving cognitive abilities in digital systems have encouraged researchers to develop various approaches to overcome them. Researchers have augmented the fitness function of organisms to reward them for memory usage or other indicators of cognitive abilities; this has been met with success in neural networks solving T-mazes (Ollion et al., 2012) and in Markov brains integrating over time (Schossau et al., 2016). These approaches fall under the general umbrella of behavioral decomposition, where organisms are independently evaluated on multiple aspects of a task. Variations in this idea can be seen in evolving the learning process separately from memory (Nordin et al., 1998) or evolving distinct components to solve subtasks (Duarte et al., 2012). Instead of evolving multiple components, some researchers have found success evolving a single system that is tested in progressively more difficult environments (Gomez and Miikkulainen, 1997). These incremental evolution approaches are not a panacea, however, and have been demonstrated failing at improving the evolution of cognitive behaviors (Christensen and Dorigo, 2006).

Beyond incremental evolution, others have argued that due to the deceptive nature of the fitness landscapes in these problems, one method is to abandon the objective, whether wholesale or to some lesser degree, and instead to encourage the exploration of novel behaviors. This has been demonstrated in the neuroevolution of memory usage (Lehman and Miikkulainen, 2014). Additionally, Carvalho and Nolfi (2016) demonstrated that, while we often think of reactive, non-cognitive behaviors as local optima that hinder the evolution of cognitive behaviors, in some circumstances they can be effective stepping stones instead.

Most of the work in this proposal build upon the Avida digital evolution framework (Ofria and Wilke, 2004), which was previously used to study the evolution of cognitive behaviors. Grabowski et al. (2010) demonstrated that Avida organisms can evolve rudimentary memory in a simple path-following environment. In a special case, this path following task even saw the evolution of counting in an odometric strategy (Grabowski et al., 2013). Pontes et al. (2020) expanded on this work to show that organisms can evolve to associate random nutrient cues with the different turning directions, an early form of associative learning. It is off of the foundation these works that I conduct Chapters 2 and 3 of this proposal.

In this work, I aim to uncover trends in the role that history played in the evolution of these

cognitive behaviors. After identifying which mutations played key roles in making the evolution of the behavior inevitable along a lineage, we will analyze those mutations in greater detail. Ideally, we may even be able to leverage this information in future attempts at evolving the behavior to increase our ability to target specific complex traits. Additionally, by looking at potentiation across different environments and genetic representations, we can better understand how the decisions made about our experiment (e.g., how to structure the environment and what representation to use) alter the ultimate probability of successfully evolving the target behavior.

1.3 Completed and proposed work

In this section I provide a breakdown of what is covered in each of the remaining chapters. Table 1.1 is provided for an overview at a glance.

Chapter	System	Environment	Focus	Status
2	Avida 5	Associative learning	Potentiation	Submitted
3	Avida 5	Associative learning	Potentiation	In progress
4	Bitstring	NK landscapes	Potentiation	Proposed
5	Avida 2	Cyclic logic 6	Evolutionary consequences	Published
6	Avida 5	Cyclic logic 6 + Patch harvesting	Potentiation	Proposed

Table 1.1: An overview of the study system, focal evolutionary dynamic, and current status for each chapter in this dissertation proposal.

As in many experimental evolution studies, we can run multiple replicates and count how many evolve a specific behavior. In **Chapter 2**, I start to investigate the question: As an individual replicate progresses, can we identify if the evolution of a target behavior has become either impossible or inevitable? I focus on retrospective analyses of four successful lineages in Avida, measuring the likelihood that associative learning ultimately re-evolves when restarting from each step (*i.e.*, I track *potentiation* over time). I find that potentiation can increase suddenly, even with a single phylogenetic step. These potentiating mutations are hard to pin down, however, as some mutations are clearly related to associative learning while the effects of other mutations remain unclear.

I propose to extend this work in **Chapter 3**, expanding well beyond four case-study lineages, collecting more comprehensive data, and attempting to draw statistically powerful conclusions. By

extracting summary statistics about the changes in potentiation, we can identify patterns and collect more data about the different types of mutations shown to promote potentiation. Additionally, these potentiation measurements will provide a basis of comparison for future work on this topic, both in this proposal and beyond.

But what are the underlying mechanisms for a mutation to increase potentiation? While some mutations may simply move toward the target trait in genotype space, others appear to move *away* from that behavior while still increasing potentiation. In **Chapter 4**, I will shift to a more tractable system to fully explore these possibilities, while also testing the generality of my earlier results. Specifically, I will quantify potentiation in bitstrings evolving on NK landscapes. In addition to providing greater speed and expanded analysis possibilities, analyzing NK landscapes will allow us to examine the relationship between epistatic interactions and potentiation. As such, this simplified model will provide the first glimpse into how potentiation dynamics change as we vary representations and environments, putting the associative learning results in a broader context and setting the stage for generalized hypotheses of potentiation. Furthermore, NK landscapes can be made small enough to allow exhaustive analysis of potentiation across the entire landscape, not just along a single lineage. These additional data will allow me to conduct more comprehensive analyses of potentiation dynamics.

Chapter 5 is previously-published work that examines phenotypic plasticity and whether or not it potentiates associated traits. I analyzed the effects of reactive phenotypic plasticity, a common stepping stone for cognitive behaviors, on future evolution. I found that adaptive plasticity stabilizes new tasks once they evolve, but does not seem to increase the probability of them evolving in the first place. Specifically, in a fluctuating environment, plasticity shifts the evolutionary dynamics (evolutionary change, retention of novel tasks, deleterious mutation accumulation, etc.) closer to those of a static environment. While this chapter does not directly investigate cognitive behaviors, it does indicate that plasticity increases potentiation through stabilizing new traits. Furthermore, it indicates that lineages that evolve reactive plasticity before evolving cognitive behaviors may benefit from similar stabilizing dynamics.

In **Chapter 6**, I ask how patterns in potentiation generalize across environments. I will do this by comparing the potentiation of associative learning in Chapter 3 and in NK landscapes in Chapter 4 to two new environments: the evolution of optimal plasticity in the cyclical environment

(from Chapter 5) and a cognitive multi-patch harvesting behavior. If we see similar trends in potentiation across these four environments, that would support the hypothesis that potentiation follows predictable dynamics, that are likely to generalize to natural systems.

Chapter 7 includes my final thoughts on this dissertation proposal. I discuss my perspective on where this work fits into the existing literature and where it might lead in the future. Additionally, dissertations are only accepted if they are finished, so this chapter also includes a proposed timeline for when the various components of each chapter will be conducted.

Chapter 2
Historical contingency in the evolution of intelligence: Potentiating mutations facilitate the evolution of associative learning in digital organisms

Authors: Austin Ferguson and Charles Ofria

Note: This chapter is presented as submitted for the 2023 Artificial Life Conference. Chapter 3 proposes to extend this work, looking at many more replicates. As such, this chapter may be subsumed into that chapter for the final dissertation.

2.1 Introduction

How likely is the evolution of a particular trait? Researchers have long been interested in predicting evolutionary outcomes, but the inherent stochasticity in the process makes this goal exceptionally challenging. In order to make more accurate predictions, we would need to better understand how and why the underlying probabilities of potential outcomes change over time. Looking purely retrospectively at evolution in nature, this type of analysis is not possible (at least not without a time machine). Leveraging the flexibility and controls available in experimental evolution, however, allows us to empirically test questions that were previously only hypothetical (Kawecki et al., 2012). Here, we focus on Stephen Jay Gould's idea of "replaying the tape of life" (Gould, 1990). The idea is simple: If we were to start life over again from the same initial conditions, would evolution follow the same pathway? Alas, Gould remarked that this experiment is unfortunately impossible.

While it may be impossible to replay the *entire* tape of life, practitioners of experimental evolution have conducted this experiment on a smaller scale. Travisano et al. (1995), Wagenaar and Adami (2004), and Blount et al. (2008) introduced and refined methods of investigating the role of historical contingency in evolving populations: parallel and analytic replay experiments. By evolving multiple populations from the same starting organisms, researchers can identify the range and distribution of outcomes. These populations can be evolved simultaneously (parallel replays), however many microbial and digital populations allow us to preserve a "fossil record", opening up another possibility. Analytic replay experiments systematically revive historical populations to re-evolve them, allowing researchers to identify alternative possibilities after the fact (Blount et al., 2018). When one strain of *E. coli* in Dr. Richard Lenski's long-term evolution experiment

(Lenski et al., 1991) unexpectedly evolved the ability to digest citrate, Blount et al. (2008) used analytic replay techniques on previously frozen samples (spaced across the lineage) to identify the potentiation of this unlikely evolutionary outcome. In their replay experiments, restarts from earlier time points never re-evolved citrate utilization, but successful re-evolution of the behavior in restarts from later time points indicated that the population had become potentiated. In later work, Blount et al. (2012) used genetic sequencing and manipulation to identify the specific potentiating mutations associated with this increased probability.

Analytic replay experiments provide a powerful new tool for understanding the role of history in evolution. In addition to studying the evolution of *E. coli* citrate metabolization, analytic replay experiments have also been used to study the evolution of novel receptor usage of Phage λ into *E. coli* (Meyer et al., 2012), and colistin resistance in *Pseudomonas aeruginosa* (Jochumsen et al., 2016). For a review of these experiments and other uses of analytic replay experiments, see (Blount et al., 2018).

In this work we use digital evolution, specifically the evolution of self-replicating computer programs in the Avida Digital Evolution Platform (Ofria and Wilke, 2004), which has previously been used to conduct replay experiments. Yedid et al. (2008) employed this technique to investigate the re-evolution of traits following an extinction episode, while Covert III et al. (2013) used analytic replay experiments to study the importance of individual deleterious mutations in the evolution of complex traits.

We selected associative learning as a model complex behavior to study potentiation in this work. Associative learning is a non-trivial capability seen in most complex organisms. It serves as an evolvable, yet rare, trait in digital evolution systems like Avida (Pontes et al., 2020). For a digital organism to exhibit associative learning, they must be capable of sensing their environment, taking an action, and storing information in memory. The evolution of associative learning has been studied via experimental evolution in both digital (McGregor et al., 2012; Pontes et al., 2020) and natural systems (Dunlap and Stephens, 2014; Mery and Kawecki, 2002), and yet many questions remain about how it might evolve. While many more complex forms of learning are used, associative learning remains an important building block for the others and insights about how it arises will be informative to understanding the evolution of intelligence.

In this work, we begin to analyze how the likelihood of evolving a complex trait changes along a

successful lineage. Using analytic replay experiments, we identified individual mutations that cause drastic increases in the potentiation of associative learning. We then analyzed those mutations and their mutational neighborhoods to begin characterizing how a mutant is potentiating. While these replay experiments are informative and useful for exploring counterfactual evolutionary possibilities, they are also computationally intensive. As such, we start by focusing on a set of case-study lineages to develop an initial framework for understanding how potentiation can occur.

Analyzing four successful lineages, we find that potentiation can increase suddenly, even due to a single mutation. Since these lineages were selected because they successfully evolved associative learning, potentiation generally increases in each, though some decreases do occur. Potentiating mutations vary in initial effect, making them challenging to detect. Retrospective analysis allows us to identify them, however, and begin hypothesizing about the dynamics that allow these mutations to potentiate associative learning. This work demonstrates using analytical replay experiments for quantifying potentiation along a lineage and establishes baselines and techniques for future studies.

2.2 Methods

Here we describe the digital evolution system and experiment setup used to conduct this work.

2.2.1 The Avida Digital Evolution Platform

This work uses an early version of version 5.0 of the Avida Digital Evolution Platform (Ofria and Wilke, 2004), currently under development as part of the Modular Agent Based Evolver 2 (MABE2) framework (https://github.com/mercere99/MABE2). In Avida, populations of self-replicating computer programs perform tasks to compete for CPU cycles, creating an evolution testbed that can support a wide array of experimental controls. Avida has been used for numerous studies include on the evolution of complexity (Lenski et al., 2003a; Zaman et al., 2014), associative learning (Grabowski et al., 2010; Pontes et al., 2020), and historical contingency via replay experiments (Covert III et al., 2013; Yedid et al., 2008). Fundamentally, Avida is designed to have tools necessary to conduct work at the scale required for replay experiments.

Avida genomes consist of assembly-like instructions that transfer data between registers, make basic comparisons, perform mathematical operations, *etc.* We use an extended instruction set that includes environment-specific and extra flow control instructions (Anonymous, 2023)

We used Avida populations on a 60x60 toroidal grid, resulting in a population cap of 3,600 organisms. Offspring are placed in a grid cell next to their parent, overwriting any existing organism

in that cell; the parent organism is also reset.During reproduction, point mutations occur in offspring at a rate of 0.0075 per instruction, while single-instruction insertion and deletion mutations occur at a rate of 0.05 per reproduction. Organisms reproduce by executing the `Repro` instruction. To prevent organisms from immediately replicating,organisms must execute 1,500 instructions before the `Repro` instruction can be activated.

Associative learning

We created an Avida environment to test the evolution of associative learning, inspired by the Avida path following environment. (Pontes et al., 2020). At any point, an organism exists in one of four states: *forward*, *left*, *right*, or the error state, *backward*. Each state is named for the action an organism must take to obtain an associated nutrient. Organisms are given a `Sense` instruction, which will give them the nutrient cue of their current state. The *forward* and *backward* states have fixed cues (0 and -1, respectively), while each organism is assigned random cue values for *left* and *right* in the range of $[1, 10^6]$ at birth. Organisms can genetically encode *forward* and *backward*, but must learn *left* or *right* to perfectly solve the task. Each path begins with one of four preset starting sequences, chosen randomly for each organism at birth, followed by additional random states. The four preset paths are the "one-fixed turn" paths from (Pontes et al., 2020), where organisms are guaranteed to encounter a *left* state before a *right*.

If the organism is not in an error state and executes the appropriate instruction, they are rewarded and move to the next state. If an organism executes the wrong instruction (e.g., the `left` instruction in the *right* state), it is penalized and placed in the error state. While in the error state, the organism must execute the `Backward` instruction to return to the previous state and be allowed to try again; it will be penalized for any other action. A cooldown is applied, however, such that executing the `Backward` instruction causes the organism to wait for the equivalent of 10 additional instruction executions. Organisms are scored based on the number of valid states they successfully traversed minus the number of incorrect moves made, with a maximum score of 300. Fitness is calculated as 1.25^{score}, so each additional correct movement grants a 25% boost in fitness.

In this environment, optimal behavior requires associative learning in the form of imprinting. Since the paths are guaranteed to have a *left* state before a *right* state, the optimal behavior is to find and store the first positive cue value as the *left* cue. Combined with genetically-encoded *forward* and *backward* logic, storing and using the *left* cue is enough for organisms to identify the *right* cue

through a process of elimination. Other possible behaviors involve error correction (assuming all turns are one direction, then correcting when wrong), bet-hedged learning (assuming more about the paths, e.g., that there are no instances of two lefts in a row, or specializing on a subset of the four possible paths), and various mixed strategies.

To categorize the behavior of a genotype, we evaluate it in 100 trials to ensure we observe how it performs in all four environments with different random cues. We then classify each of the 100 trials. Trials are classified as learning if the organism correctly handles greater than 90% of the states they were in, error correction if they always successfully navigated one turn state but not the other, and "low activity" if they failed to successfully navigate at least 25 states. To be categorized as learning or error correction, *all* 100 trials of that genotype must be of that class. If one or more trials were low activity, the genotype was categorized as "bet-hedged learning" or "bet-hedged error correction". If a genotype displayed at least one learning trial and at least one error correction trial, they were classified as "mixed bet hedging". Finally, all remaining genotypes were categorized as "low activity". This categorization system was used across all three phases of this work.

2.2.2 Experiment framework

To identify mutations that substantially increased the likelihood that learning evolved, we split the work into three phases (see Figure 2.1 for an overview). First, we seeded 200 initial parallel replicates in the associative learning environment with a default ancestor only capable of reproduction. Each replicate was given 250,000 updates, where one update is the time it takes for all organisms to execute 30 instructions, on average. We identified the most abundant genotype in each final population to represent the replicate and classified its behavior. We then extracted the "dominant lineage", stretching from the ancestor to the representative genotype.

To begin analyzing changes in potentiation, we ran exploratory replays replicates on four lineages capable of learning. For each, we seeded independent replicates for every 50^{th} step in the lineage, up to step 1,000. All replay replicates evolved in the same associative learning environment, and replays were given the same number of updates as had occurred after that genotype first appeared (e.g., replays for a genotype that appeared at update 150,000 would be evolved for the remaining 100,000 updates). Potentiation was measured as the portion of replay replicates that evolved learning. Because replays were seeded with a single organism, some replay populations went extinct before ever reproducing and thus were not factored in (the minimum number of finished re-

play replicates from a given lineage step was 38, while three case study lineages had a minimum of 48).

While the exploratory replays provide an overview of how potentiation changed, we dug deeper by running targeted replays to further explore windows of increasing potentiation. Specifically, we found the 50- or 100-step "potentiation window" that sees the largest increase in potentiation in the exploratory results, and seeded additional replays for every step in that range. These targeted replays were conducted identically to the exploratory replays, only they did not skip steps. Though computationally expensive, these replays illuminated the impact every genotypic change had on potentiation. Running 50 replay replicates per step still results in considerable noise, but we were able to identify mutations that clearly and substantially increased potentiation using these targeted replays.

We hand-analyzed algorithms in all potentiating mutations, here defined as single lineage steps that result in a potentiation increase of 25 percentage points or more. Further, we assessed genotype fitness in context of their lineage to identify if potentiation mutations were beneficial, deleterious, or neutral. Finally, we characterized the local fitness landscape of each genotype (one- and two-mutations out), measuring the presence and fitness of nearby genotypes that would be capable of learning.

2.2.3 Data and software availability

Both the data and the software used to conduct this work are available in the supplemental material (Anonymous, 2023). Analyses were conducted in the R statistical computing language (R Core Team, 2021) using the *dplyr* package to summarize data (Wickham et al., 2022). Data was visualized using the *ggplot2* package (Wickham et al., 2020).

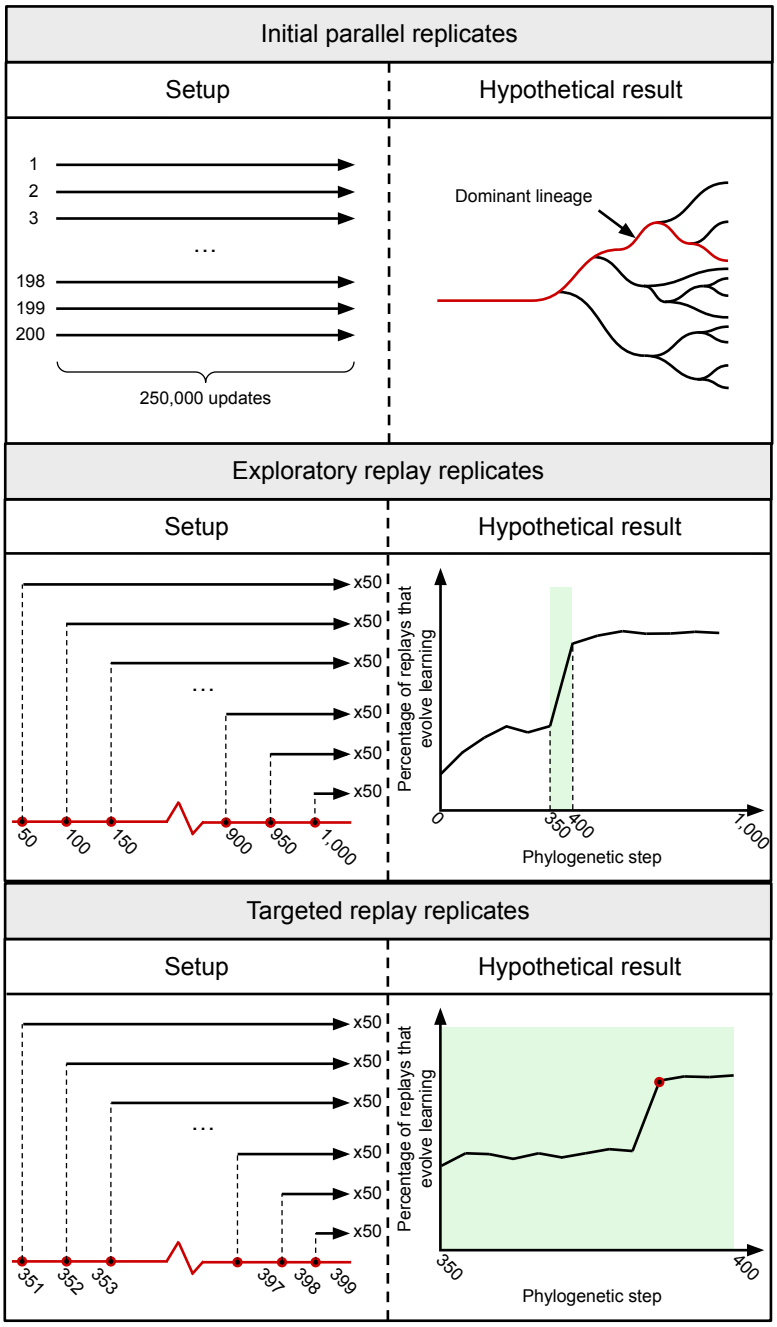

Figure 2.1: Illustration of the experimental design and hypothetical results. The top panes show the 200 initial parallel replicates seeded with the ancestral genotype and evolved for 250,000 updates. We extracted the lineage of the most abundant genotype in the evolved population (the dominant lineage), shown in red. Next, we conducted exploratory replays (middle panes) by launching replay replicates at regular intervals along focal lineages. These exploratory replays give a coarse-grained view of how potentiation changed over a lineage. We identified the window with the largest potentiation gained, shown as the shaded region. Finally, we ran targeted replay replicates for every step in this potentiation window. These fine-grained replay replicates show mutations that resulted in large potentiation increases (shown here with a red dot).

2.3 Results

Here we discuss the generation and analysis of the initial replicate runs, followed by the more detailed results from each of the four case study lineages.

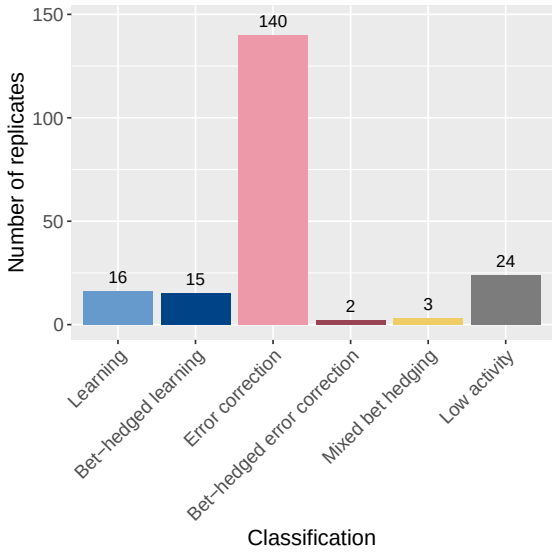

Figure 2.2: Behavior classification of the final dominant genotypes from the 200 initial parallel replicates.

2.3.1 Evolution of learning in the initial replicates

In the first phase of this study, we evolved 200 independent replicates for 250,000 updates (about 3600 generations) in the associative learning domain, each starting with a default ancestor.The distribution of evolved behaviors is shown in Figure 2.2. Only 16 of 200 replicates exhibited associative learning. An additional 15 replicates evolved forms of bet-hedged learning, with two of those replicates gaining and then losing associative learning along their lineage. The majority of replicates relied on some form of error correction, either as a sole strategy (140), a bet-hedged variant (2), or as a fallback due to limited learning (3). Finally, 24 replicates failed to navigate enough states to categorize them, leading us to label them as "low activity".

We analyzed all 16 replicates that evolved and maintained learning, identifying the length of their lineages from onset of evolution until learning stabilized, no longer showing further improvement. Given the substantial computational power required for each time point studied, we performed replay experiments only on the shortest three such lineages (lineages A-C), plus the shortest lineage that exhibited error correction at some point in its evolutionary history (lineage D). Selecting these

particular replicates to replay has the potential to bias the results, as discussed below.

2.3.2 Case studies of individual lineages

Below we present the results of the replay experiments performed on the four focal lineages and provide a step-by-step analysis of how key mutations altered both immediate fitness and evolutionary potential (potentiation). Where possible, we explained how these mutations altered the underlying algorithms. For each lineage, potentiation across both exploratory and targeted replays can be found in Figure 2.3.

Lineage A

Our first case study is one of the shortest lineages and it contains a quick jump to learning (at step 537) from low activity, with only a brief time spent in bet-hedged learning. Exploratory replays for this lineage revealed a stark jump in potentiation between 400 and 500 steps from the ancestor, which we call the potentiation window. From step 400 to step 450, potentiation increased from 20% of replicates to 44%, and increased again to 84% of replicates by step 500. Before this window, potentiation fluctuated around the original 8% found starting from the default ancestor. After the selected window, potentiation increased once more and then fluctuated between 94% and 100%.

With this in mind, we seeded 50 replays for each genotype in the potentiation window. Even with the noise due to a small sample size, we identified two mutations that conferred sizable increases in potentiation: steps 432 and 484. The mutation at step 432 brought potentiation above 50% for the first observed time in the lineage. Surprisingly, potentiation then decreased (on average) back to a local minimum of 20% of replicates at step 480. Finally, the mutation at step 484 substantially increased potentiation to 92%, where it stayed for all subsequent replays.

Even though the largest jump in potentiation occurred at step 484, learning did not appear in the lineage until step 537. That said, only steps 516 and 525 caused any change in behavior; all other interim mutations occurred in unexecuted regions of the genome. The potentiating mutation at step 484 made a key instruction in the main loop of the genome redundant. It had no immediate effect on fitness, but later (in intermediate step 516) allowed the redundant instruction to be replaced by a right turn that granted a small fitness increase as organisms could now navigate until they reached the second left turn. Step 525 further improved navigation, but used a comparison that made an unfounded assumption on whether the left or right random cue is larger. When the assumption was

correct organisms were capable of learning the cues, however the assumption is only correct 50% of the time, so this genotype is categorized as bet-hedged learning. Finally, step 537 swapped that comparison with one that makes no assumptions about cue values, enabling the genotype to learn in all environments.

Looking at the local mutational neighborhood, the potentiating mutation at step 484 increased the number of two-step mutations that conferred learning from 2 to 9 (of approximately 56 million). Additionally, the fitness of the learning mutations in the local neighborhood increased by three or four orders of magnitude.

What about the earlier potentiation that was gained and then lost? The mutation that substantially increased potentiation at step 432 introduced a comparison that had no immediate fitness effect. This comparison remained unimportant until step 525 when it became integral in introducing bet-hedged learning.Neither step 432 nor its predecessor had access to learning within a two-step mutational range. Thus, it is likely that the potentiation comes from that comparison given that we observed it being utilized for learning later on.

Why then, did potentiation decrease between steps 432 and 484? At step 432 (and indeed before it), the algorithm had a section where if register B was non-zero, then B stored the cue associated with a left turn. While this information was likely to make the evolution of learning easier, it was unused at that time. As such, the mutations between steps 432 and 484 dismantled that machinery, requiring a replacement to be built before learning could evolve.

Lineage B

Similar to Lineage A, this lineage transitioned from low activity to learning through a brief period of bet-hedged learning. Exploratory replays on this lineage reveal that learning was potentiated almost immediately; by step 150 potentiation had climbed above 95%, where it stayed for the rest of the lineage. As such, the potentiation window included steps 50 through 150.

Unlike Lineage A, the targeted replays reveal a general trend of increasing potentiation, with step 104 as a notable outlier. Mutations from steps 50 through 103 slowly increased potentiation from 4% to 44%, but the mutation at step 104 jumped it to 80%. From there, another slow increase continued to raise potentiation to a peak of 98% at step 150.

Learning did not appear until step 195, over 90 steps beyond the largest potentiating mutation. Given that 34 interacting mutations altered the encoded algorithm, the mechanistic pathway to

achieve learning is more complicated than can be broken down in this work. However, the potentiating mutation at step 104 modified the execution flow of the genome, which appears to have been later used essential for the later evolution of learning.

While two mutations occurred at step 104, only one caused a functional change: an instruction to swap data between registers was mutated to a left turn. Prior to this mutation, the genome encoded a left turn later on, after which the execution became trapped in an endless loop. The potentiating mutation was immediately beneficial; it allowed organisms to take the left turn earlier, which, in turn, allowed them to avoid the loop. In avoiding the infinite loop, a large portion of the genome was now skipped and remained so when learning evolved 91 steps later. Looking at the local fitness landscape, learning was neither present in the potentiating step's landscape nor in the step before. We hypothesize that the potentiation came from the change in structure of the genome, that skipping the execution of those instructions avoided a pitfall and freed up execution time that may have been useful in evolving learning.

Lineage C

Lineage C has the biggest single-mutation potentiation increase (64 percentage points), and that mutation was deleterious when it occurred at step 279 along the lineage. Learning later appeared at step 305.

The potentiating step mutated a no-operation instruction into a conditional flow control instruction. At step 278 the genotype was capable of bet-hedged learning, but the mutation at 279 knocked out all instances of learning, reclassifying the lineage as "low activity." The next step restored some fitness, and then step 285 interacted with the mutation at 279 to not only restore fitness, but to dramatically improve it. Ultimately, the potentiating mutation allowed the algorithm to more precisely discriminate between the left and right cues. Prior to the mutation, it used a less-than comparison, which only functioned correctly in tests where the "turn left" cue was less than the "turn right" cue. The potentiating mutation switched it to an equality comparison, which alleviated the assumption that one cue will be larger than the other.

Interestingly, the potentiating mutation both lowered the number of learning mutations available in the local fitness landscape and decreased the average fitness of the learning mutants that do exist.

Lineage D

Of the four replicates we analyzed, Lineage D is the only one that evolved error correction before learning. Like the earlier lineages, the exploratory replays show that almost all potentiation comes from a single window. In this case, potentiation grew from 34% of replicates to 96% between steps 500 and 550. Targeted replays are especially noisy for this lineage, but generally show an increase in potentiation, especially in the latter half of the window. The largest jump in potentiation occurred at step 548, near the end of the window. Prior mutations also showed notably increased potentiation, but in each case later mutations appeared to lower potentiation again. Specifically, steps 542 and 543 appear to have higher potentiation than the points around them, with potentiation dipping back below 50% again before the largest jump at 548.

Out of all four lineages, D has the fewest steps between the largest potentiating step (548) and the first appearance of learning (556). At the time, the potentiating mutations at 548 caused no discernible change in fitness even though they increased potentiation by 50 percentage points. Two mutations occurred at step 548: a point mutation swapped a flow control instruction for a math instruction and an insertion mutation added a comparative conditional instruction into the main execution loop. At step 548 the genotype encoded a naive error correction algorithm: after setup, organisms could always handle *turn right* states, but always failed *turn left* states, recovered, and then continued. At step 556, the algorithm started sensing the environment, albeit *too* often. A mutation swapped a sensing instruction with a math instruction, and this combined with the prior comparison instruction from step 548 to allow the organism to move left when needed. The organism still blundered when it encountered a state sequence of *left*, *forward*, *left*, but it quickly recovered. Since it can associate the cues with greater than 90% accuracy, we classify it as learning. The local fitness landscape supports the idea that the comparison instruction was useful to the evolution of learning, as the potentiating mutation increased the number of learning genotypes in the two-step mutational neighborhood from under 800 to over 100,000.

Looking back at the apparent false start at steps 542 and 543, it is not clear what algorithmic changes these mutations conferred. These steps did, however, alter the set of learning behaviors that fell within the local mutational neighborhood. At step 541, there were only 324 two-step mutations that conferred learning, and only three of those resulted in a substantial fitness increase (a merit $> 10^{15}$). After steps 542 and 543, that number rose such that over 900 two-step mutations could

confer learning, with over 500 resulting in a substantial fitness increase (including over 200 that reached a merit $> 10^{25}$. We may be unsure of the exact effects of these mutations on the mechanics of the algorithm, but the changes in the local fitness landscape are profound.

Figure 2.3: Potentiation of associative learning for all case studies, shown as the percentage of replicates that evolve associative learning when evolution is replayed starting at that point.For each case study, the left plot shows the results of the exploratory replays. We identified a window of increased potentiation in each lineage, indicated by the shaded region. Within that window, we conducted targeted replays for every step along the lineage. The results of those targeted replays are shown in the plots on the right. The color of the points corresponds to the behavior exhibited at that step of the lineage. A dotted line in the targeted replays indicates the mutation that confers the most potentiation.

2.4 Discussion and Conclusion

2.4.1 Potentiation can rise suddenly

We have documented several cases where wherein single mutations dramatically increased the probability of associative learning later evolving.Of the four lineages analyzed, each had a single step in the lineage that resulted in a substantial increase in potentiation (ranging from 36 to 64 percentage points). Indeed, two of the lineages had an additional potentiating mutation that resulted in an increase of over 30 percentage points. Limiting ourselves to exploratory replays, each lineage has a 50-step window that resulted in a potentiation increase of at least 40 percentage points.

While four lineages are insufficient to make any strong claims, these results demonstrate that it is *possible* for single mutations to drastically increase potentiation, and provide compelling evidence that they may, in fact, be common. In Lineages B and D, however, we do also observe regions with smaller, incremental increases in potentiation. Further studies are clearly necessary to more fully understand the general patterns and processes by which potentiation rises across different representations and environments.

2.4.2 Potentiation can decrease along a successful lineage

In two of the lineages we analyzed (A and D), we see evidence of potentiation decreasing over spans of the lineage. With only 50 replay populations per lineage step, our results are noisy and it is difficult to isolate what is occurring during these periods of potentiation decline. While we were unable to identify any "anti-potentiating" mutations with effects as large as the positive potentiation mutations, it is possible for a single step in a lineage to greatly decrease potentiation.

Since we limited our analyses to runs where associative learning arose in the original replicate, we did not expect a preponderance of anti-potentiating mutations, but were intrigued to see evidence of them, even if at low effect. These same analytic replay experiments could be applied to lineages that failed to evolve the target behavior, to see if potentiation on that behavior experiences sudden drops. Similarly, our replays targeted windows with substantial increases in potentiation; other windows would be more likely to include decreases. Finally, failed replays from starting points with otherwise high potentiation must have failed for a reason; they too could be used as a likely source (albeit more artificial) of anti-potentiating mutations.

2.4.3 Potentiating mutations can appear innocuous when they first occur

We analyzed the mutational step in each of the four lineages that conferred the greatest increase in potentiation. Of those four mutational events, two were neutral, one was deleterious, and one was beneficial. Even among these few replicates, there is no obvious pattern in the properties of potentiating mutations. Of the two neutral mutations, one made an instruction redundant while the other added a conditional instruction that had no effect when it was initially introduced. The deleterious and beneficial mutations both caused the execution flow to loop back earlier than it did before. Additionally, the number of mutations between the potentiating mutation and the appearance of learning varied wildly between lineages, ranging from 8 steps up to 91. The potentiating mutations in these four lineages are unique, and at the current time there is no pattern emerging among them. So, how are these mutations any different from other mutations? Untangling this mystery could be critical for predicting evolutionary outcomes or accelerating adaptive evolution.

2.4.4 We can identify *how* a mutation is potentiating

There are many mechanisms by which a mutation could facilitate the evolution of associative learning. For example, the mutation could provide a building block that is helpful to perform the task. But for a mutation to be potentiating it must notably increase the probability of associative learning appearing in the future. Any change, no matter how helpful, that was already likely to occur would not be considered potentiating. Indeed, it is the earlier mutations that made that change so likely that would be potentiating. Of course, those mutations are also more challenging to identify.

We have three of hypotheses for how a mutation could be potentiating: (1) It moves through genetic space in a useful direction, providing access to associative learning in the local fitness landscape. (2) It improves the eventual value of associative learning, increasing the likelihood of the trait being selected if it does appear. (3) It is a "gateway" mutation to another region of the fitness landscape that does not grant immediate access to associative learning, but does unlock a pathway to get there.

Across the potentiating mutations we analyzed, we have found evidence for each of these hypotheses. The largest potentiating mutation in Lineage D supports Hypothesis 1, as it is the first time in the lineage that learning is only one mutation away. The main potentiating mutation in

Lineage A and the earlier potentiation mutations in Lineage D support Hypothesis 2 as both cause drastic increases in the fitness benefit of learning mutations in the two-step neighborhood. It is worth noting that the mutation in Lineage A still only has a few two-step mutations with learning, while the mutation in Lineage D has many. Finally, Lineages B, C and the early potentiating mutation from Lineage A all provide support for Hypothesis 3. The mutations from Lineages A and B both have *zero* learning mutations in their two-step neighborhoods. Interestingly, Lineage C sees a *decrease* in the number of learning mutations in the local neighborhood and in the fitness of those learning mutations.

Hypothesis 3 has many possible mechanisms by which it may work. For example, new traits may produce a single, clear, beneficial pathway of improments to follow. Alternatively, a new building block may open a larger region with many different ways of evolving associative learning. Finally, the mutation may actually damage existing functionality or remove existing interactions that were impeding further evolution. While all three hypotheses have some support, future work can begin to uncover if a certain hypothesis is seen more often, or what conditions might result in each scenario.

2.4.5 Outlook

This work is only an early step, focused on developing techniques and expectations for performing fine-grained analyses of replay experiments. Next, we must expand beyond four lineages, to collect broader, more systematic replay data, automating as much of the process as possible. We conducted this study on associative learning in Avida, but the underlying techniques must be examined broadly in other environments and substrates to ensure that our results are not unique to Avida or the evolution of associative learning. Within the current study system, there are many questions that remain unanswered: We focused on large *increases* in potentiation, but are there more obvious signals associated with *decreases*? How much of the noise that we see in our data is due to limiting ourselves to 50 replicates, and how much of it is do to actual shifts in potentiation with each mutation? What does potentiation look like in replicates that fail to evolve learning? Finally, it would be valuable to compare the specific evolutionary pathways the different replays take. Do they follow the same trend or do they differ? This would allow us to understand if, for example, a potentiating mutation funnels evolution in a fixed direction.

Ultimately, these analytic replay techniques provide us with a tool for examining evolution

in a prospective fashion, not just the retrospective approach that we are traditionally limited to. They will allow for the development of new evolutionary theory and predictive capacity that will be invaluable, both for understanding how meaningful complexity is produced in the natural world and for improving evolutionary applications.

Chapter 3
In-progress - A deeper exploration of potentiation in associative learning

Authors: Austin Ferguson and Charles Ofria

Status: This is a direct extension of Chapter 2. It is marked in-progress as all software has been implemented and additional explorations beyond Chapter 2 have been conducted to identify useful changes to parameters and the environment. The actual data collection, however, has yet to begin. Note that this chapter may make Chapter 2 redundant in the final dissertation. Both are included here, however, as Chapter 2 serves as preliminary results for this chapter.

3.1 Introduction and Background

Previous experimental evolution studies in microbial systems (as summarized in Blount et al. (2018)) have demonstrated that analytic replay experiments can show how potentiation for a particular trait changes over the course of a lineage (Blount et al., 2008; Jochumsen et al., 2016; Meyer et al., 2012; Woods et al., 2011). These experiments typically focus on, at most, a few lineages. This limitation is shared by Chapter 2 of this dissertation proposal, which focuses on the potentiation of associative learning in four case study lineages. While these four lineages provided me with examples of what is possible in terms of potentiation, the small sample size leaves me unable to extract generalizations.

Here I propose to expand these techniques to look at potentiation more systematically *across many additional replicates*. By leveraging the associative learning environment and replay experiment pipeline introduced in Chapter 2, I plan to scale up this study by replaying genotypes from at least fifty associative learning lineages. This larger scale will provide me with enough data to identify patterns in how learning becomes potentiated in this system, and allow me to propose hypotheses about underlying evolutionary processes.

The biggest challenge that I faced in Chapter 2 was one of CPU limitations; each replay run required substantial computational effort. Specifically, each lineage studied required 20 starting points for the initial scan and at least 49 additional starting points once the potentiation window was identified. For each of these 69 starting points, I performed 50 replicate runs, for a total of 3450 Avida runs per lineage analysis. The limited number of replay experiments was satisfactory for an initial exploration, but now that I have identified the required data and worked out which analyses

will be the most informative, a larger scale study is clearly warranted.

In the preliminary data, I saw some lineage regions where potentiation jumped sharply with a single mutation, while potentiation grew more gradually in other regions. Indeed, some regions actually show a decline in potentiation. While I continue to believe that the large jumps in potentiation will be the most interesting to study (and to tease apart the underlying source of the potentiation change), investigations in these other regions may also prove fruitful. To that end I plan to conduct a small number of additional analyses on ten replicates that did not achieve associative learning. In these replicates I will examine overall potentiation patterns, with an emphasis on both jumps and drops.

The Avida genotype-phenotype map is known to be complex (Fortuna et al., 2017), with many different types of epistasis coexisting. Many different factors could come into play that promote or restrain the evolutionary potential toward a complex task. My hypothesis is that large jumps in potentiation will be due to these epistatic interactions. In the same way, epistasis could trap lineages in a region of genotype space where learning is effectively unreachable. As such, I also hypothesize that in lineages that fail to evolve learning, we will often see drops in potentiation that mirror the jumps evident in my prior work.

3.2 Proposed work

Since this work builds directly off of Chapter 2, I will use the same study system with minor refinements to the protocol. Here I elaborate on the aims of the work, discuss the protocol modifications used, and identify additional analyses and statistics to be performed.

The overarching goals of this work are threefold. First, I plan to further demonstrate the power and flexibility of this experimental design for making lineage-based replay experiments tractable. Second, I will collect more extensive quantitative baseline data for future studies to compare against (Chapters 4 and 6). Third, I will explore these data from a more qualitative perspective to refine my ideas for theoretical and conceptual models that I propose to build in Chapter 4.

3.2.1 Changes to replay experiments

As in Chapter 2, here I will conduct analytic replay experiments on lineages that evolved associative learning. The difference is that this work will take place at a greater scale, which will, ideally, allow me to identify trends and collect statistics on the potentiation measures. To do this, I will expand from an analysis of four lineages to an analysis of fifty lineages.

I will employ the same two replay phases, with slight modifications. For the exploratory phase, I will no longer sweep the entire early portion of the lineage. Instead, I will initially limit the exploration to the section of the lineage just before associative learning evolved. In the four study lineages from the previous chapter, I observed that the largest single-step increase in potentiation was always within 100 phylogenetic steps of the first discovery of associative learning. As such, I will refine the protocol to start the exploratory phase only 200 steps before associative learning appeared, rather than at the start of the lineage. This will require only four exploratory replays (50, 100, 150, and 200 steps before learning appeared). If the potentiation at the earliest replay is already above 20%, I will extend the exploratory phase for another 200 steps back (continuing to extend in the unlikely event this is needed).

The targeted replays will be selected and progress as before, replaying every genotype in the window with the largest increase in potentiation. I will also formalize the instances where two neighboring windows are both used in the targeted replays by including windows with a potentiation change within 10 percentage points of the maximal window. These changes should still identify the same potentiation windows as before, while saving substantial computational resources and thus allowing us to include more experimental replicates. Not only am I running fewer replay replicates per lineage, but the replays I do conduct start later along the lineage, requiring fewer updates to reach the 250,000 total updates. The tradeoff here, however, is that the larger number of replicates will make it infeasible for every lineage to be hand-analyzed to qualitatively describe the influence of potentiating mutations. As such, my analyses will be mostly quantitative and I will only hand-inspect potentiating mutations in exceptional cases.

In addition to the replays of fifty lineages that evolved associative learning, I will also replay ten lineages that did *not* evolve associative learning. Specifically, I will replay five lineages that evolved error correction and five that evolved bet-hedged learning. This set of replays will be too small for full comparisons, but any insight they provide will be invaluable for future work (Chapter 4). While learning did not evolve, it is possible that the potentiation for learning did increase. Is this potentiation different than potentiation in a successful lineage? It should be noted that there is no learning in these lineages, and as such exploratory replays cannot be based on that point. Instead, I will revert back to the methodology of Chapter 2 for these explorations, performing a uniform sweep across the lineage. The targeted replays will focus on windows of potentiation *loss*

instead of potentiation gain, but will otherwise function identically.

3.2.2 Changes to associative learning task

Chapter 2 guaranteed that organisms would see a left turn before a right turn. This was accomplished using the "one-fixed turn" maps from (Pontes et al., 2020). In the time since, exploratory runs have seen associative learning evolve on purely random paths, with no guarantee of turn order or, indeed, no set path starts at all. This is the first time this has been demonstrated in Avida, as associative learning never arose in "random start" paths previously (Pontes et al., 2020). I propose to switch to these truly random paths, which much more cleanly demonstrate associative learning. This change *requires* trial and error for a genotype to successfully learn arbitrary paths, while other strategies were possible for experiments with set paths.

While this change to random paths strengthens the type of associative learning that evolves, it comes at a cost. Chapter 2 saw 8% of replicates evolve associative learning. The exploratory runs that saw associative learning evolve on random paths saw it evolve in only 1 to 2% of replicates. This decrease in frequency means that more initial replicates are needed to collect a suitable number of learning lineages, however there is no difference in the replays conducted once those lineages have been identified. As such, this will require more computational resources up front, but since the majority of resources go into the replays themselves, I argue this is a reasonable tradeoff.

While the organism score calculations will not change from Chapter 2, how the score is used will change slightly. Chapter2 calculated the fitness of an organism as 1.25^{score}, but here I will switch to 2^{score}, meaning every additional correct movement always doubles fitness. This is the standard rate in Avida, and exploratory runs have shown that this change increases the percentage of replicates that evolve associative learning in the random path environment described above.

3.2.3 Potentiation measures

This work aims to collect a suite of quantitative characteristics about the potentiation of each lineage. Here I describe each measurement to be recorded and discuss my expectations of what I might observe.

I will collect two measures of potentiation gain for each lineage. First , I will record the *maximum observed single-step potentiation gain* of the lineage. Based on the surprising result of all four lineages in Chapter 2 seeing large potentiating mutations, I expect to see consistently large values for this measurement. To quantify the number of potentiating events, I will also record the

number of potentiation gain windows in each lineage. I define these windows as subsequent replays in the exploratory phase that see at least 10 percentage points of potentiation gain. Based on the results of the previous chapter, I expect some lineages will experience only a single window of potentiation gain while other lineages experience several.

Next, once I identify potentiating steps in a lineage, I will record three additional characteristics of each step. The *fitness effect* is a categorical measure that asks if the step was beneficial, neutral, or deleterious relative to the previous genotype in the lineage. I originally hypothesized that most potentiating mutations were neutral or deleterious, but one lineage in Chapter 2 experienced a beneficial potentiating mutation. As such, I expect most mutations to be neutral or deleterious, with a non-negligible fraction being beneficial. The *behavioral phenotype* of the focal step will be analyzed, asking what behavior (see Figure 2.2) was exhibited before and after the mutations occurred. I expect most potentiating mutations to not affect the behavior directly, but to potentiate subsequent mutations that change the behavior. Additionally, we can then perform cross-behavior analyses to statistically compare potentiation measures across behavioral backgrounds. Lastly, the *distance to learning* will measure the number of genotypes between the potentiating mutation and the first appearance of learning in the lineage. Further, I will differentiate overall distance from the *meaningful distance to learning*, ignoring mutations to non-executed regions of the genome. Results from Chapter 2 lead me to expect considerable variation in this measure.

Finally, I will also collect the same measurements on anti-potentiating mutations and windows (those that confer at least a 10 percentage point *loss* in potentiation). I do not expect to observe many examples of these mutations or windows in lineages that evolved associative learning. However, any instances we do find are likely to be very informative, as they show time periods that shifted the lineage away from learning, but werer ultimately recovered from. The main reason for collecting these measures is for the replayed lineages that did *not* evolve associative learning. The expectations for these values are typically the inverse of their potentiation gain counterparts. For example, I expect anti-potentiating phylogenetic steps to most often be beneficial, as they are moving the lineage toward a non-learning local optima.

3.2.4 Analyses

Most data, such as the maximum single-step potentiation of each lineage, will be analyzed as a distribution. Qualitative observations of these distributions will be made, but since these are the

first data of their kind we do not have a baseline to compare them against. I will, however, compare across our categorical variables. I will compare the potentiation increases of deleterious, neutral, and beneficial mutations to see if significant differences exist between fitness effects. Similarly, I will compare across behavior backgrounds to determine if the pre-learning strategy exhibited by a lineage affects the chateristics of potentaition. For example, do potentiating mutations that occur in an error correcting phenotype confer more potentiation than those in a bet-hedged learning phenotype?

Beyond examining the collected potentiation characteristics, I will perform two additional analyses. First, I will analyze the local mutational neighborhood of genotypes along a lineage (up to two steps away). I will measure how often learning was found, the fitness of those genotypes, and if the intermediate genotypes are beneficial. Analyzing the local neighborhoods in conjunction with the potentiating mutations can provide us with insight into how those mutations caused their change in potentiation. This was shown on a smaller scale in Chapter 2, where some potentiating mutations introduced associative learning to the local landscape for the first time, others increased the number of learning genotypes, and some increased the fitness benefit of learning in the local landscape. This analysis can identify epistatic interactions, as introducing learning to the local landscape for the first time demonstrates an interaction that was not possible before. It should be noted, however, that the two-step limit on the neighborhood is an arbitrary limit imposed by the infeasibility of fully enumerating beyond that distance. As such, I must be careful what claims I make about the relationship between the local neighborhood and the theoretical underpinnings of potentiation. However, an expansion of the local neighborhood analysis is possible in the simplified model of Chapter 4.

The second additional analysis is a reversion assay of genotypes between the main potentiating step and the first appearance of learning. For each step along that region of the lineage, I will revert the mutations found in the potentiating step. This will help identify when that mutation actually became relevant to fitness and behavior, including the possibility that potentiating mutations are not always active in the first learning behavior but instead were stepping stones along the way. Based on explorations during Chapter 2, I expect that most potentiating mutations persist until the evolution of learning and continue to play a vital role in that behavior. This analysis will indicate if any potentiating mutations are transient, serving only as a bridge to a more promising

region of the fitness landscape.

3.2.5 Broader impacts

In this chapter I have proposed to extend Chapter 2, diving deeper into potentiating mutations in the evolution of associative learning in Avida. I proposed to conduct similar analyses but at a much larger scale, collecting enough data for us to identify possible trends in potentiation and to allow for future comparative studies.

Every combination of evolutionary substrate and environment has its quirks, and this particular combination of associative learning and Avida will impact the form that potentiation takes. However, quantitative cross-lineage comparisons have, to my knowledge, never been performed and likely will not be feasible in living systems for quite some time. As such, this work will provide an initial baseline for how potentiation changes along lineages. This data will be invaluable for conceptualizing theory behind how potentiation change (Chapter 4) and for direct comparisons with other environments (Chapter 6). The value of this data is not limited to the confines of the other chapters in this dissertation proposal, however. It will also provide a solid baseline for comparisons in other areas of evolutionary biology. I will ensure the data is hosted on a well-established scientific data repository (e.g., Open Science Framework, Dryad) so other researchers can use it for their own comparisons. As an example, researchers in microbial systems will, for the first time, have data on how potentiation changes in one model. While the differences in systems will undoubtedly create differences in potentiation, we must start somewhere, and that is exactly what I propose to do with this work.

In addition to shedding light on how potentiation changes, this work may also change how we view evolving associative learning or Avida itself. If, for instance, I find that potentiating mutations are often doing something trivial but is difficult to do in Avida (e.g., adding a new instruction into an existing loop), that can inform how we shape future digital evolution systems. On the flip side, if I see that potentiation drastically increases with memory availability, that tells us that selecting not just for performance on the task, but also for memory itself (Ollion et al., 2012; Schossau et al., 2016), may increase the rate at which learning evolves.

Chapter 4
Proposed - Epistasis and potentiation landscapes: Insights from a simplified model

Authors: Austin Ferguson and Charles Ofria

Status: Proposal. A small sample of preliminary data has been collected to help estimate time requirements and to verify that we do see meaningful levels of potentiation in NK landscapes.

4.1 Introduction

Chapter 2 found evidence of individual mutations that conferred huge gains in potentiation. Because all four lineages exhibited these potentiating mutations, I expect them to be found in most lineages that evolve associative learning in that system. Epistatic interactions must play a key role in these mutations, but are they enough to create the full potentiation dynamics that I have shown? Further, does the level of epistasis in a system affect how potentiation changes?

Here I propose investigating potentiation in a much simpler model by evolving bitstrings in an NK landscape. While this model will remove much of the complexity found in Avida, NK landscapes allow me to tune the level of epistasis by varying the K parameter. If similar potentiation dynamics appear in this simple system, they will provide strong evidence that epistasis alone is sufficient as a causative agent for the potentiation results in previous chapters. If, instead, the potentiation results are drastically different, I will have narrowed down the set of possible factors, giving us additional information to develop further hypotheses and for building the next system to study potentiation.

As in previous chapters, I measure potentiation of a genotype by seeding multiple evolutionary replicates with that genotype and then calculating the percentage of those replicates that evolve the target trait (Blount et al., 2012). Normally we focus on a particular phenotype as our target trait. Given the simplicity of NK landscapes, however, I define the target trait as the globally optimal genotype. If this simple target is insufficient to produce potentiation results due to the global optimum being too small of a target, I will explore other mechanisms for defining the target trait, but preliminary results (discussed below) indicate that this is not likely to be an issue.

Indeed, preliminary results in these NK landscapes have shown that potentiation varies across genotype space. With this confirmation, NK landscapes offer several benefits in the study of potentiation. First, as with most bitstring models, they are substantially faster than more complex systems like Avida. This speed improvement drastically reduces the time required to gather data,

which has been the largest hurdle in previous studies of potentiation. Second, by reducing the complexity of the system, we can perform deeper analyses (e.g., larger mutational neighborhood studies, including exhaustive landscape analyses in some cases). While work in Avida can examine only a portion of genotypes along each lineage, full enumeration of the potentiation landscape is possible when using short bitstrings. Combined with the ability to tune the amount of epistasis in NK landscapes, these attributes make it tractable for me to systematically examine potentiation.

I have three main aims in this chapter: 1) to compare potentiation in NK landscapes to that found in the associative learning Avida domain, 2) to investigate the effect that epistasis has on potentiation, and 3) to continue building our intuition of potentiation and expanding our repertoire of measurements and analytical tools. The first two aims can be accomplished by calculating potentiation in NK landscapes and conducting comparison analyses. The third aim, however, requires more discussion.

Through the lens of adaptation, chance, and history, potentiating mutations decrease the influence of chance and increase the influence of adaptation in reaching the target trait. I hypothesize that these mutations can take many different forms, at least in how they are commonly observed. However, I propose that, while our limited observations of these potentiating mutations may vary considerably, at a fundamental level they are all moving toward pathways where adaptation can drive the population toward the target trait. This disconnection between the underlying mechanics and the observed values arises from the epistasis of the system and our inability to analyze across large genetic differences. In a complex system, for any mutational neighborhood analysis of a given distance, even if a target trait is observed, it is not necessarily most easily reached by a direct path, nor is it even guaranteed that there is not an easier target to reach at a further distance. In a small enough NK landscape, however, we can fully test these dynamics, identifying how useful local landscape information is for overall prediction of evolutionary outcomes. Therefore, I will conduct mutational neighborhood analyses of various distances to determine if this technique is useful for characterizing potentiating mutations, and if so, which distances perform the best across epistatic strengths.

Beyond mutational neighborhoods, I will utilize the simplicity of the NK landscape to benchmark several other analyses. I will draw from the substantial body of literature that exists for analyzing these simpler fitness landscapes (Horn and Goldberg, 1995; Malan and Engelbrecht, 2013).

Specifically, I will calculate the basin of attraction (Østman and Adami, 2014) for each optima to determine how the specific basins a genotype is in relates to its potentiation. I will also analyze the relationship between potentiation and fitness of each genotype to test my hypothesis that potentiating mutations are often neutral or deleterious. Additionally, I will employ analytical search techniques to find the most likely path between a genotype and the target trait. By comparing the probability of this path to the potentiation of the genotype, I will build intuition on the explanatory power of the most likely path versus the many viable paths to the target that may exist. This will help inform how multiple paths combine to create the potentiation that we actually measure.

Taken together, these comparisons and analyses will improve our understanding by inspecting the generalizability of potentiation trends and testing the metrics we use to characterize potentiation. This work will also provide additional data for future studies, starting the process of expanding our study systems beyond Avida. Finally, I expect this study to either demonstrate the explanatory power of using NK landscapes to understand potentiation dynamics, or it will identify the existence of more complex dynamics that underlie potentiation and thus broaden the features of landscapes that need to be considered to conduct potentiation analyses.

4.2 Proposed work

Here, I explain the system to be implemented and the experiments to be conducted.

4.2.1 Potentiation in an NK landscape

Since I expect that epistatic interactions play a key role in potentiating mutations, the simple bitstring model must contain some level of epistasis. Fortunately, this is an inherent property of NK landscapes (Kauffman and Levin, 1987), where N is the length of the bitstring and K is the number of additional bits that epistatically interact to determine the fitness of each position in the bitstring. As such, not only are NK landscapes epistatic, but the degree of epistasis is a parameter I can vary, allowing for clean comparisons of dynamics at different levels of epistasis.

Quantifying potentiation requires a target trait, as we must designate whether lineages are "successful". All genotypes in the NK landscape, as I consider it, map to a scalar value. I will construct the landscapes such that, for each bit position, each unique combination of $K + 1$ bits grants a score between 0 and 1 that is random but set for the duration of the landscape. The total score of a genotype is then the sum of scores for all positions in the bitstring. Since I use floating-point numbers, the randomness of the bit position scores allows me to assume that a unique

global optimum exists in each landscape. I therefore define the successful trait as having exactly this optimal bitstring. Conveniently, the distance to the target trait is just the Hamming distance between the optimal bitstring and the genotype in question.

I will conduct evolution like a traditional synchronous-generation genetic algorithm. I will seed the initial population of bitstrings with the genotype being tested, and evaluate each bitstring on the landscape. After this evaluation, I will select parents via a mix of elite selection (to ensure the optimal genotype persists if it is discovered) and tournament selection. Finally, all parents will be copied and possibly mutated, creating the next generation to be evaluated. I will then repeat this process until a stopping criterion is met.

Quantifying the potentiation of a genotype is done by seeding some number of evolutionary replicates with that genotype and measuring the percentage of those replicates that evolve the target trait (as originally introduced in Blount et al. (2008)). Typically, this is only measurable via multiple replay experiments that restart evolution at specific points along a lineage. Because I am using bitstrings, I am able to enumerate potentiation for *all* possible genotypes in a landscape, creating a "potentiation landscape". This requires nontrivial effort, as there are 2^N possible genotypes in an N-length bitstring, and analyzing each genotype requires multiple evolutionary replicates started from it (here I use 50). This approach limits the size of landscapes that I can enumerate. As such, I propose to start by analyzing landscapes where $N \in \{10, 12, 14, 16\}$ bits. For each of these N values, I will also vary $K \in [0, N-1]$. To ensure we observe the diversity landscapes can have at a particular combination of N and K, I will enumerate the potentiation landscape for 30 landscapes at each pair of values.

4.2.2 Proposed comparative analyses

I plan to conduct two comparisons of potentiation: one between the NK landscape and the associative learning Avida domain, and the other across different N and K values within the NK landscape. However, the measurements taken in the associative learning domain were looking at potentiation along a lineage, not over the entire landscape (see 3.2.3 for the list of measurements). I will calculate the lineage metrics in the NK landscape by rerunning replicates at particular genotypes, tracking the evolving phylogenies. Specifically, I will target genotypes with low potentiation values, below 10%, in order to create a fair comparison with the low initial potentiation seen in the associative learning results. Here, however, we do not need to perform replay replicates along these

lineages, as we already know the potentiation of every possible genotype. We merely need to map genotypes along the lineage with their potentiation values. Once potentiation has been assigned to every genotype along the lineage, we can calculate the potentiation measurements as normal.

After these measurements have been collected, I will compare the distributions of each with those found in the associative learning environment of Chapter 3. As an example, I will test if there is a significant difference in the largest single-step potentiation gain between the two systems. Not all measurements translate to this system, but largest single-step potentiation gain/loss, the distance to the target trait from the potentiating mutations, and distributions of fitness effect apply equally to both systems. This will provide the first comparison of potentiation across environments and representations. If the distributions are similar, that would be strong evidence that there are general trends in potentiation. If, instead, significant differences exist, that will also provide information on what our simplified model might be missing.

Next, I plan to perform a similar analysis across K values in the NK landscape. Since K controls the level of epistasis in the landscape, these tests will highlight the effect that epistasis has on our potentiation measurements. I will perform this analysis for each of the N values tested. I will compare the relevant potentiation measurements across the set of K values, seeing which ones statistically differ. For example, I expect increased epistasis to make the landscapes more deceptive and thus increase the effect size of potentiating mutations. Likewise, I will examine the consistency of the effect of K on potentiation, by comparing fixed K values across each N.

For both of these comparisons, I will conduct a Kruskal-Wallis test across all groups to see if any significant difference is found (Kruskal and Wallis, 1952). If a difference is present, I will then conduct pairwise Mann-Whitney-Wilcoxon tests to determine which pairs of groups differ (Wilcoxon, 1945). Finally, I will apply Holm-Bonferroni corrections for multiple comparisons where needed (Holm, 1979).

4.2.3 More advanced analyses

Finally, there are some analyses that are tractable in the NK landscape that were infeasible in Avida. Indeed, the power of being able to generate a potentiation landscape suddenly makes many additional analyses possible. Local optima can be counted in the NK model, allowing me to investigate the relationship between potentiation and the number of local optima in the landscape. Similarly, the enumeration of both the fitness and potentiation landscapes will allow me to examine

correlations between potentiation and fitness for individual genotypes. Due to local optima, I expect potentiation to decrease with fitness, but I also expect this distribution to be bimodal as genotypes close to the global optimum should have both high fitness and high potentiation. For any optimum, we can determine the set of genotypes that have a path to that optimum that monotonically increases in fitness (i.e., the basin of attraction for that optimum (Østman and Adami, 2014)). We can then see if crossing into the set of genotypes that have a path to the global optimum increases potentiation. However, the global optimum entering that set may not be enough; we may not see jumps in potentiation until the we reach a genotype where *only* the global optimum is in that set.

In Chapter 2, I inspected the two-step mutational neighborhood in an attempt to identify how potentiating mutations interacted with the target trait. The limited scope of that analysis brings its usefulness into question. In this NK landscape, however, we can exhaustive examine each genotype in its relationship to the local mutational neighborhood. This will allow me to investigate how often changes to the n-step mutational neighborhood translate to meaningful differences in potentiation. We can calculate the fraction of n-step mutants that are both beneficial and closer to the target trait. How well does this correlate with potentiation? If we see that increasing this fraction for one- and two-step neighbors correlates well with potentiation, then this may be useful in more complex systems. If there is no strong signal, then this analysis may not be worth continuing in the future.

I will also test if the probability of the most likely path from a given genotype to the target trait is a strong indicator of potentiation. By enumerating the fitness landscape, I can compare the fitness between one genotype and all N of its one-step mutants. Assuming a mutation occurs, I can use the fitness values of each mutant to create the probability that each mutation would be selected (this varies with several factors, such as the selection scheme in use). By doing this for every genotype, I can create a graph of the entire genotype space with genotypes as nodes and their transition probabilities as weighted edges. Next, I will perform a negative log transformation on each weight. This will transform the probabilities into positive values, with smaller probabilities converting to larger values. Due to the nature of logarithms ($log(ab) = log(a) + log(b)$), summing these log-transformed values is the equivalent to multiplying the underlying probabilities. As such, this approach allows us to perform a search from a given genotype to the target trait using Dijkstra's algorithm. This analysis will return the *most likely* path through genotype space to get from that genotype to the target. I will calculate this value for each genotype, and then analyze its relationship

with potentiation. Since multiple adaptive paths between a genotype and the target can exist, I expect that, in many cases, this measure fails to accurately predict potentiation. Future work can then expand on this analysis by finding the k shortest paths to the target trait, and seeing how many paths are required to accurately approximate potentiation (Eppstein, 1998).

4.2.4 Broader impacts

This work will be the first empirical measure of patterns in potentiation across systems. Compared to Chapter 3, if we see similar patterns in potentiation, then we can start to consider that these patterns may be generally applicable, at least in digital evolution but potentially into natural systems. If not, we can dive into the differences in patterns to begin asking if other systems will be more like one system or the other. Additionally, this work will provide insight into how we measure potentiation and characterize potentiating mutations, and these landscapes will provide a second data set for future comparative studies.

If I find evidence of interesting potentiation dynamics in this system, that will help establish NK landscapes as a useful tool for investigating potentiation. An NK landscape where $K = 0$ is just a single hill to climb, and as such I expect all genotypes in that landscape to have 100% potentiation. If I see potentiation vary more across landscapes as K increases, that will provide support for the hypothesis that epistatic interactions are key to potentiation. They might not be the only factor though, and future models may need to expand beyond the traditional NK model. If, for instance, I do not see large jumps in potentiation, I may need stronger binary "on/off" forms of potentiation. A simple example would be a bitstring environment where the first half of the bitstring is evaluated on one NK landscape and the second half on another, with the target trait being optimal genotypes in both landscapes. If I limit fitness such that the second half of the bitstring only contributes to fitness if the first half is at the global maximum, then I would expect to see a drastic increase in potentiation when the first half reaches that optimal genotype. This model would more directly simulate required building blocks in the evolution of the target trait. There are many ways that we could extend the traditional NK model, and this work will help shape those future studies, if needed.

4.3 Preliminary results

To ensure that potentiation in NK landscapes is not entirely trivial, I ran some very early preliminary data. I selected $N = 10$ and enumerated potentiation in 50 landscapes each of $K \in \{0, 1, 2, 3\}$.

For each landscape, I ran 50 replicates from every genotype (1,024 genotypes per landscape) to quantify potentiation at that point.

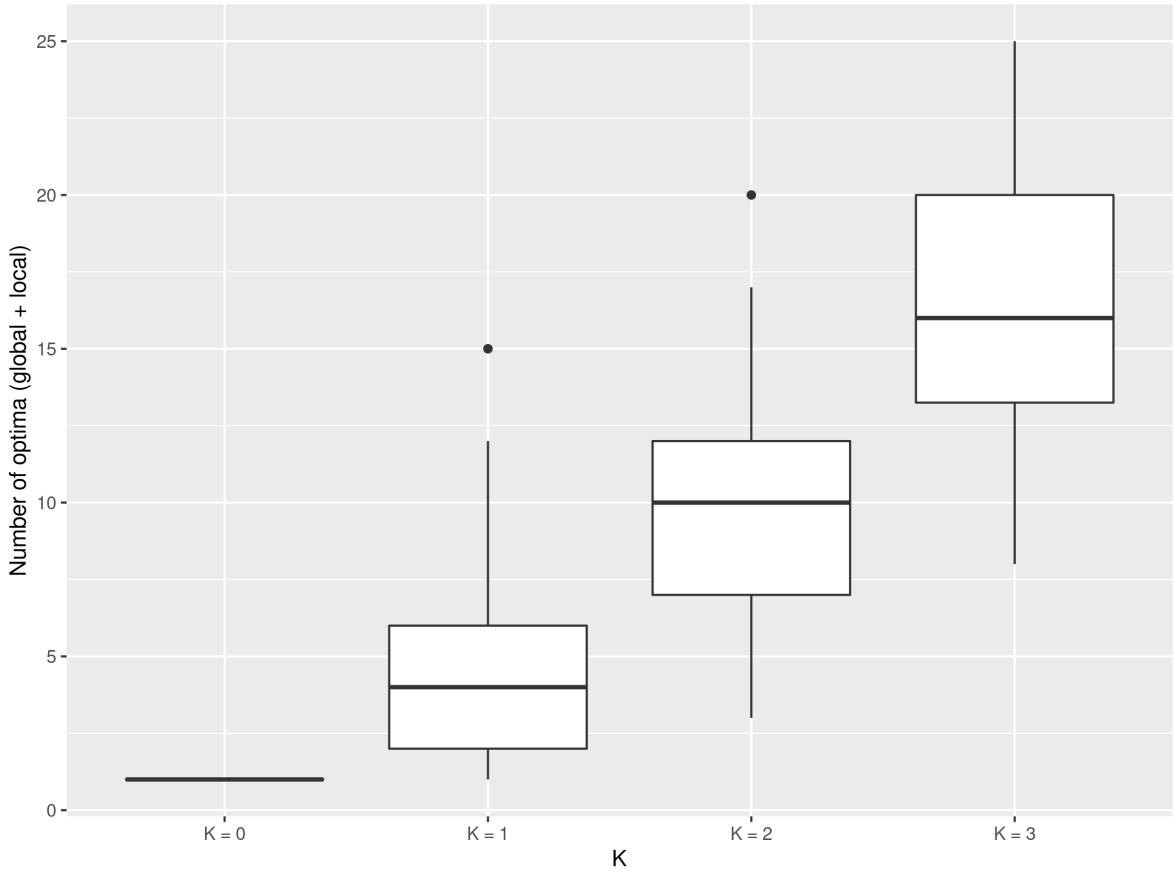

Figure 4.1: Boxplots showing the number of peaks in all 50 landscapes of a given K value.

First, Figure 4.1 shows the number of peaks at each K value. Here we define a peak as a genotype that has higher fitness than the N genotypes around it; this definition includes local optima as well as the global optimum (Østman and Adami, 2014). NK landscapes are often described as "tunably rugged", with larger K values creating more rugged landscapes due to increased epistatic interactions. We see exactly that here, as the number of peaks increases smoothly as we increase K.

Figure 4.2 shows the overall distribution of potentiation across all genotypes in all landscapes at each K value. First, $K = 0$ shows 100% potentiation for every genotype in every landscape, which matches expectations as individual bits can be optimized independently (i.e., there is zero epistasis) and thus the landscape is a single hill to climb. As K increases, we see some mass of the

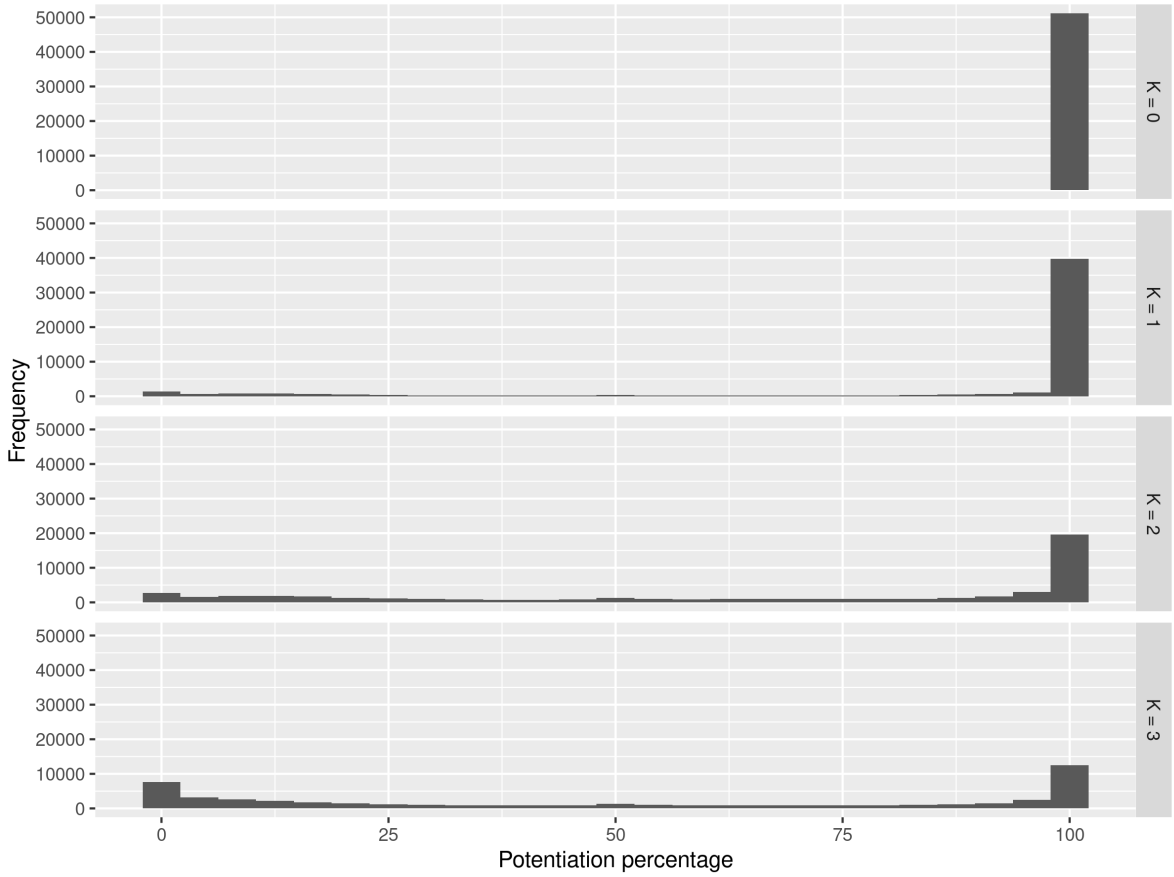

Figure 4.2: Histograms of potentiation, with one row per K value. Each histogram includes the potentiation of all 1,024 genotypes in all 50 landscapes for that K value.

distribution shift from 100% potentiation to the lower values, especially the very low values around 0%. This also meets my expectation, as we have shown that increasing K increases the number of local optima and thus creates more opportunities for populations to become "stuck" and unable to reach the global optimum. When it comes time to compare potentiation in this model to that in Avida, we do see genotypes with potentiation levels similar to those found in Avida, indicating that we can conduct those comparisons fairly.

Finally, Figure 4.3 shows how potentiation changes as we vary K and look at the distance from a genotype to the target trait. As we have already seen, $K = 0$ is a single hill and thus all genotypes are fully potentiated. Starting at $K = 1$, we see that while the median potentiation stays consistently high, the lowest quartile decreases as distance from the target increases. At $K = 2$, we see the whole distribution of potentiation shift to lower values as distance increases, with very few points at the maximum distance having high potentiation. This trend continues into

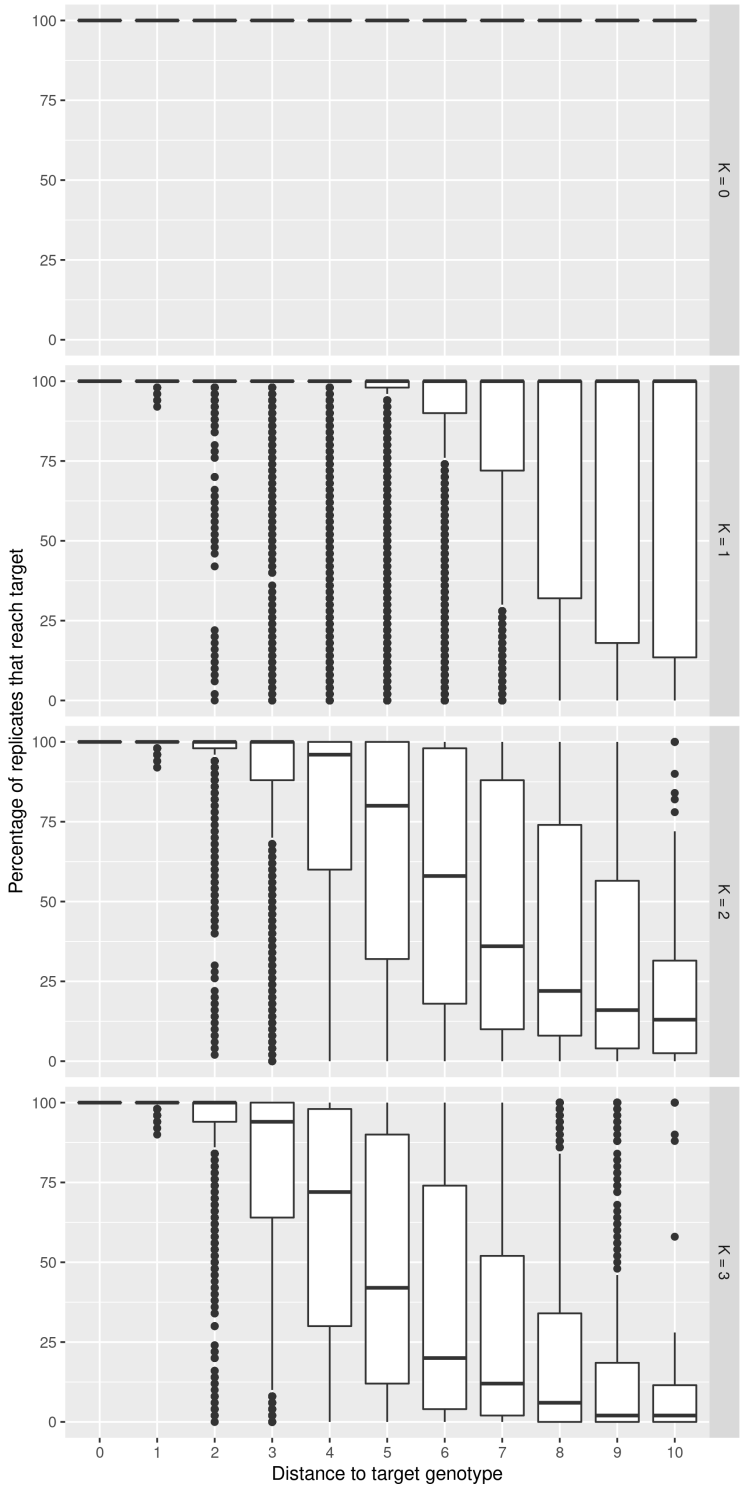

Figure 4.3: Boxplots showing the potentiation of genotypes at a given distance away from the global optimum. Rows show the various K values, and each boxplot shows all genotypes at that distance for all 50 landscapes for that K value.

$K = 3$, becoming more pronounced. Overall, Figure 4.3 meets my expectations. In the simpler landscapes, most genotypes have high potentiation, but as the landscapes become more rugged we see potentiation fall faster as we increase the distance to the target.

While these data are only a shallow glance into potentiation in NK landscapes, they provide reassurance that the system is worthy of examination. When performing the work in earnest, I will be analyzing landscapes with higher values of N and K. Additionally, I will be looking at the lineages of semi-potentiated genotypes, allowing me to compare the potentiation measures that will be recorded in Chapter 3. This brief glimpse reveals promise in the system; hopefully this promise holds true and NK landscapes can help us delve further into potentiation.

Chapter 5
Adaptive phenotypic plasticity stabilizes evolution in fluctuating environments

Authors: Alexander Lalejini, Austin J. Ferguson, Nkrumah A. Grant, and Charles Ofria

Note: This chapter is adapted from (Lalejini et al., 2021). The only change is the lead-in paragraph at the beginning.

5.1 Introduction

When considering the role of history in evolution, we must consider what effects "stepping stone" behaviors may have on future evolutionary dynamics. In cognitive behaviors, these earlier milestones may take the form of reactive strategies that respond to environmental stimuli but do not store or integrate information. Here we investigate the evolution of one such reactive behavior: adaptively plastic metabolism regulation in a cyclic environment. In replicates that evolve the desired behavior, we use them to seed new replicates in a different environment, analyzing how the plastic behavior affects attributes such as novel function gain and the accumulation of deleterious traits. This work examines how the evolution of particular behavior can influence future dynamics, which could sway what other behaviors can later evolve.

Natural organisms employ a wide range of evolved strategies for coping with environmental change, such as periodic migration (Winger et al., 2019), bet-hedging (Beaumont et al., 2009), adaptive tracking (Barrett and Schluter, 2008), and phenotypic plasticity (Ghalambor et al., 2007). The particular mechanisms that evolve in response to fluctuating environments will also shift the course of subsequent evolution (Schaum and Collins, 2014; Wennersten and Forsman, 2012). As such, if we are to understand or predict evolutionary outcomes, we must be able to identify which mechanisms are most likely to evolve and what constraints and opportunities they impart on subsequent evolution.

In this work, we focus on phenotypic plasticity, which can be defined as the capacity for a single genotype to alter phenotypic expression in response to a change in its environment (West-Eberhard, 2003). Phenotypic plasticity is controlled by genes whose expression is coupled to one or more environmental signals, which may be either biotic or abiotic. For example, the sex ratio of the crustacean *Gammarus duebeni* is modulated by changes in photoperiod and temperature (Dunn et al., 2005), and the reproductive output of some invertebrate species is heightened when infected

with parasites to compensate for offspring loss (Chadwick and Little, 2005).

Evolutionary biologists have long been interested in how evolutionary change is influenced by phenotypic plasticity because of its role in generating phenotypic variance (Gibert et al., 2019). The effects of phenotypic plasticity on adaptive evolution have been disputed, as few studies have been able to observe both the initial patterns of plasticity and the subsequent divergence of traits in natural populations (Forsman, 2015; Ghalambor et al., 2015, 2007; Hendry, 2016; Wund, 2012). In changing environments, adaptive phenotypic plasticity provides a mechanism for organisms to regulate trait expression within their lifetime, which can stabilize populations through those changes (Gibert et al., 2019). In this context, the stabilizing effect of adaptive plasticity has been hypothesized to constrain the rate of adaptive evolution (Ancel, 2000; Gupta and Lewontin, 1982; Huey et al., 2003; Paenke et al., 2007; Price et al., 2003). That is, directional selection may be weak if environmentally-induced phenotypes are close to the optimum; as such, adaptively plastic populations may evolve slowly (relative to non-plastic populations) unless there is a substantial fitness cost to plasticity.

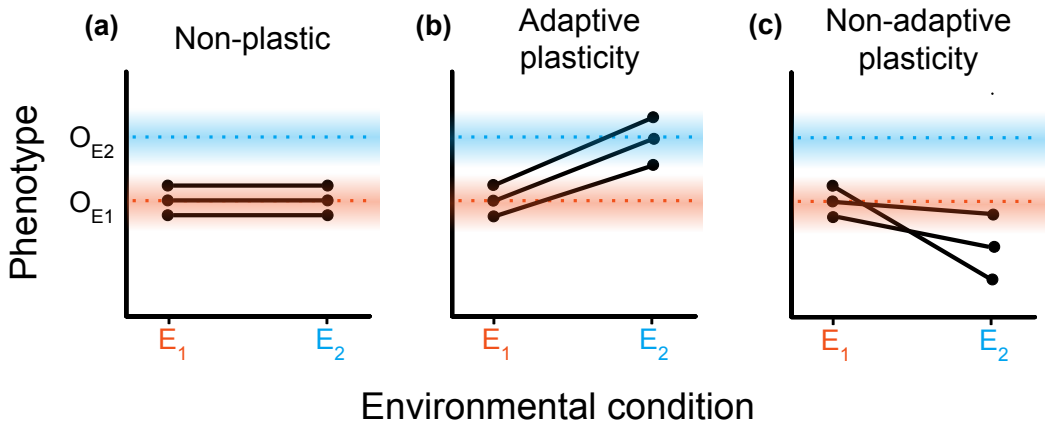

Figure 5.1: **Hypothetical reaction norms for populations comprising genotypes placed in different environments.** A reaction norm describes phenotypic change (or lack thereof) induced by environmental variation (West-Eberhard, 2008). In all panels, two environmental conditions (denoted E_1 and E_2) are shown on the x-axis. The y-axis indicates the phenotype expressed in each environment with O_{E1} and O_{E2} designating the optimal phenotype for E_1 and E_2, respectively. Each pair of points connected by a solid black line denotes a genotype, with the points themselves representing its hypothetical phenotypes in each environment. We present three scenarios for how populations could respond to a change from E_1 to E_2. (a) A non-plastic population where phenotypes do not change with environmental shifts. (b) An adaptively plastic population where phenotypes dynamically adjust to the new optimum. (c) A population exhibiting non-adaptive plasticity where environmental change induces phenotypes further away from the optimum.

Phenotypic plasticity allows for the accumulation of genetic variation in genomic regions that are unexpressed under current environmental conditions. Such cryptic ("hidden") genetic variation can serve as a source of diversity in the population, upon which selection can act when the environment changes (Levis and Pfennig, 2016; Schlichting, 2008). It remains unclear to what extent and under what circumstances this cryptic variation caches adaptive potential or merely accumulates deleterious alleles (Gibson and Dworkin, 2004; Paaby and Rockman, 2014; Zheng et al., 2019).

The "genes as followers" hypothesis (also known as the "plasticity first" hypothesis) predicts that phenotypic plasticity may facilitate adaptive evolutionary change by producing variants with enhanced fitness under stressful or novel conditions (Levis and Pfennig, 2016; Schwander and Leimar, 2011; West-Eberhard, 2003). Environmentally-induced trait changes can be refined through selection over time (*i.e.*, genetic accommodation). Further, selection may drive plastic phenotypes to lose their environmental dependence over time in a process known as genetic assimilation (Crispo, 2007; Levis and Pfennig, 2016; Pigliucci, 2006; Schlichting and Wund, 2014; West-Eberhard, 2005). In this way, environmentally-induced phenotypic changes can precede an evolutionary response.

Phenotypic plasticity may also "rescue" populations from extinction under changing environmental conditions by buffering populations against novel stressors. This buffer promotes stability and persistence and grants populations time to further adapt to rapidly changing environmental conditions (Chevin and Lande, 2010; West-Eberhard, 2003).

Disparate predictions about how phenotypic plasticity may shift the course of subsequent evolution are not necessarily mutually exclusive. Genetic and environmental contexts determine if, and to what extent, phenotypic plasticity promotes or constrains subsequent evolution. Figure 5.1 overviews how we might expect different forms of phenotypic plasticity to result in different evolutionary responses after an environmental change. In Figure 5.1a, we would expect non-plastic populations to experience strong directional selection toward the new optimum (O_{E2}) after the environment changes. We would expect an adaptively plastic population (Figure 5.1b) to remain relatively stable after the environment changes, as plasticity shifts organisms' phenotypes to the new optimum. In Figure 5.1c, we would expect the non-adaptively plastic population to experience strong directional selection on their response to the new environmental conditions; indeed, such maladaptive plasticity may even put the population at risk of extinction in the absence of beneficial mutations.

Experimental studies investigating the relationship between phenotypic plasticity and evolutionary outcomes can be challenging to conduct in natural systems. Such experiments would require the ability to irreversibly toggle plasticity followed by long periods of evolution during which detailed phenotypic data would need to be collected. Digital evolution experiments have emerged as a powerful research framework from which evolution can be studied. In digital evolution, self-replicating computer programs (digital organisms) compete for resources, mutate, and evolve following Darwinian dynamics (Wilke and Adami, 2002). Digital evolution studies balance the speed and transparency of mathematical and computational simulations with the open-ended realism of laboratory experiments. Modern computers allow us to observe many generations of digital evolution at tractable time scales; thousands of generations can take mere minutes as opposed to months, years, or millennia. Digital evolution systems also allow for perfect, non-invasive data tracking. Such transparency permits the tracking of complete evolutionary histories within an experiment, which circumvents the historical problem of drawing evolutionary inferences using incomplete records (from frozen samples or fossils) and extant genetic sequences. Additionally, digital evolution systems allow for experimental manipulations and analyses that go beyond what is possible in wet-lab experiments. Such analyses have included exhaustive knockouts of every site in a genome to identify the functionality of each (Lenski et al., 2003b), comprehensive characterization of local mutational landscapes (Canino-Koning et al., 2019; Lenski et al., 1999), and the real-time reversion of all deleterious mutations as they occur to isolate their long-term effects on evolutionary outcomes (Covert et al., 2013). Furthermore, digital evolution studies allow us to directly toggle the possibility for adaptive plastic responses to evolve, which enables us to empirically test hypotheses that were previously relegated to theoretical analyses.

In this study, we conduct digital evolution experiments to investigate how the evolution of adaptive phenotypic plasticity shifts the course of evolution in a cyclically changing environment. We use the Avida Digital Evolution Platform (Ofria et al., 2009). Avida is an open-source system that has been used to conduct a wide range of well-regarded studies on evolutionary dynamics, including the origins of complex features (Lenski et al., 2003b), the survival of the flattest effect (Wilke et al., 2001), and the origins of reproductive division of labor (Goldsby et al., 2014). Our experiments build directly on previous studies in Avida that characterized the *de novo* evolution of adaptive phenotypic plasticity (Clune et al., 2007; Lalejini and Ofria, 2016) as well as previous

work investigating the evolutionary consequences of fluctuating environments for populations of non-plastic digital organisms (Canino-Koning et al., 2019; Li and Wilke, 2004). Of particular relevance, Clune et al. (2007) and Lalejini and Ofria (2016) experimentally demonstrated that adaptive phenotypic plasticity can evolve given the following four conditions (as described by Ghalambor et al. 2010): (1) populations experience temporal environmental variation, (2) these environments are differentiable by reliable cues, (3) each environment favors different phenotypic traits, and (4) no single phenotype exhibits high fitness across all environments. We build on this previous work, but we shift our focus from the evolutionary causes of adaptive phenotypic plasticity to investigate its evolutionary consequences in a fluctuating environment. Specifically, we examine the effects of adaptive plasticity on subsequent genomic and phenotypic change, the capacity to evolve and then maintain novel traits, and the accumulation of deleterious alleles.

Each of our experiments are divided into two phases: in phase one, we precondition sets of founder organisms with differing plastic or non-plastic adaptations; in phase two, we examine the subsequent evolution of populations founded with organisms from phase one under specific environmental conditions (Figure 5.2). First, we examine the evolutionary histories of phase two populations to test whether adaptive plasticity constrained subsequent genomic and phenotypic changes. Next, we evaluate how adaptive plasticity influences how well populations produced by each type of founder can evolve and retain novel adaptive traits. Finally, we examine lineages to determine whether adaptive plasticity facilitated the accumulation of cryptic genetic variation that would prove deleterious when the environment changed.

We found that the evolution of adaptive plasticity reduced subsequent rates of evolutionary change in a cyclic environment. The non-plastic populations underwent more frequent selective sweeps and accumulated many more genetic changes over time, as non-plastic populations relied on genetic variation from *de novo* mutations to continuously readapt to environmental changes. The evolution of adaptive phenotypic plasticity buffered populations against environmental fluctuations, whereas repeated selective sweeps in non-plastic populations drove the accumulation of deleterious mutations and the loss of secondary beneficial traits. As such, adaptively plastic populations were better able to retain novel traits than their non-plastic counterparts. In general, the evolution of adaptive phenotypic plasticity shifted evolutionary dynamics to be more similar to that of populations evolving in a static environment than to non-plastic populations evolving in an identical

fluctuating environment.

5.2 Materials and Methods

5.2.1 The Avida Digital Evolution Platform

Avida is a study system wherein self-replicating computer programs (digital organisms) compete for space on a finite toroidal grid (Ofria et al., 2009). Each digital organism is defined by a linear sequence of program instructions (its genome) and a set of virtual hardware components used to interpret and express those instructions. Genomes are expressed sequentially except when the execution of one instruction (*e.g.*, a "jump" instruction) deterministically changes which instruction should be executed next. Genomes are built using an instruction set that is both robust (*i.e.*, any ordering of instructions is syntactically valid, though not necessarily meaningful) and Turing Complete (*i.e.*, able to represent any computable function, though not necessarily in an efficient manner). The instruction set includes operations for basic computations, flow control (*e.g.*, conditional logic and looping), input, output, and self-replication.

Organisms in Avida reproduce asexually by copying their genome instruction-by-instruction and then dividing. However, copy operations are imperfect and can result in single-instruction substitution mutations in an offspring's genome. For this work, we configured copy operations to err at a rate of one expected mutation for every 400 instructions copied (*i.e.*, a per-instruction error rate of 0.0025). We held individual genomes at a fixed length of 100 instructions (the default genome size in Avida); that is, we did not include insertion and deletion mutations. We used fixed-length genomes to control for treatment-specific conditions resulting in the evolution of substantially different genome sizes (Lalejini and Ferguson, 2021a)[1], which could, on its own, drive differences in evolutionary outcomes among experimental treatments. When an organism divides in Avida, its offspring is placed in a random location on the toroidal grid, replacing any previous occupant. For this work, we used the default 60 by 60 grid size, which limits the maximum population size to 3600 organisms. As such, improvements to the speed of self-replication are advantageous in the competition for space.

During evolution, organism replication rates improve in two ways: by improving genome efficiency (*e.g.*, using a more compact encoding) or by accelerating the rate at which the genome is

[1]We repeated our experiments without genome size restrictions and observed qualitatively similar results (see supplemental material, Lalejini and Ferguson 2021a).

expressed (their "metabolic rate"). An organism's metabolic rate determines the speed at which it executes instructions in its genome. Initially, an organism's metabolic rate is proportional to the length of its genome, but that rate is adjusted as it completes designated functions, such as performing Boolean logic functions (Ofria et al., 2009). In this way, we can reward or punish particular phenotypic traits (*i.e.*, Boolean logic functions).

Phenotypic plasticity in Avida

In this work, we measure a digital organism's phenotype as the set of Boolean logic functions that it performs (*i.e.* expresses) in a given environment. Sensory instructions in the Avida instruction set allow organisms to detect how performing a particular function would affect their metabolic rate (see supplemental material for more details, Lalejini and Ferguson 2021a). We define a phenotypically plastic organism as one that uses sensory information to alter which functions it performs based on the environment.

Phenotypic plasticity in Avida can be adaptive or non-adaptive for a given set of environments. Adaptive plasticity shifts net function expression closer to the optimum for the given environments. Non-adaptive plasticity changes function expression in either a neutral or deleterious way. In this work, optimal plasticity toggles functions to always perfectly match the set of rewarded functions for the given set of environments.

5.2.2 Experimental design

We conducted three independent experiments using Avida to investigate how the evolution of adaptive plasticity influences evolutionary outcomes in fluctuating environments. For each experiment, we compared the evolutionary outcomes of populations evolved under three treatments (Figure 5.2): (1) a **PLASTIC** treatment where the environment fluctuates, and digital organisms can use sensory instructions to differentiate between environmental states; (2) a **NON-PLASTIC** treatment with identical environment fluctuations, but where sensory instructions are disabled; and (3) a **STATIC** control where organisms evolve in a constant environment.

Each experiment was divided into two phases that each lasted for 200,000 updates[2] of evolution (Figure 5.2), which is equivalent to approximately 30,000 to 40,000 generations. In phase one of each experiment, we evolved plastic, non-plastic, and control organisms for use in phase two. In

[2]One update in Avida is the amount of time required for the average organism to execute 30 instructions. See (Ofria et al., 2009) for more details.

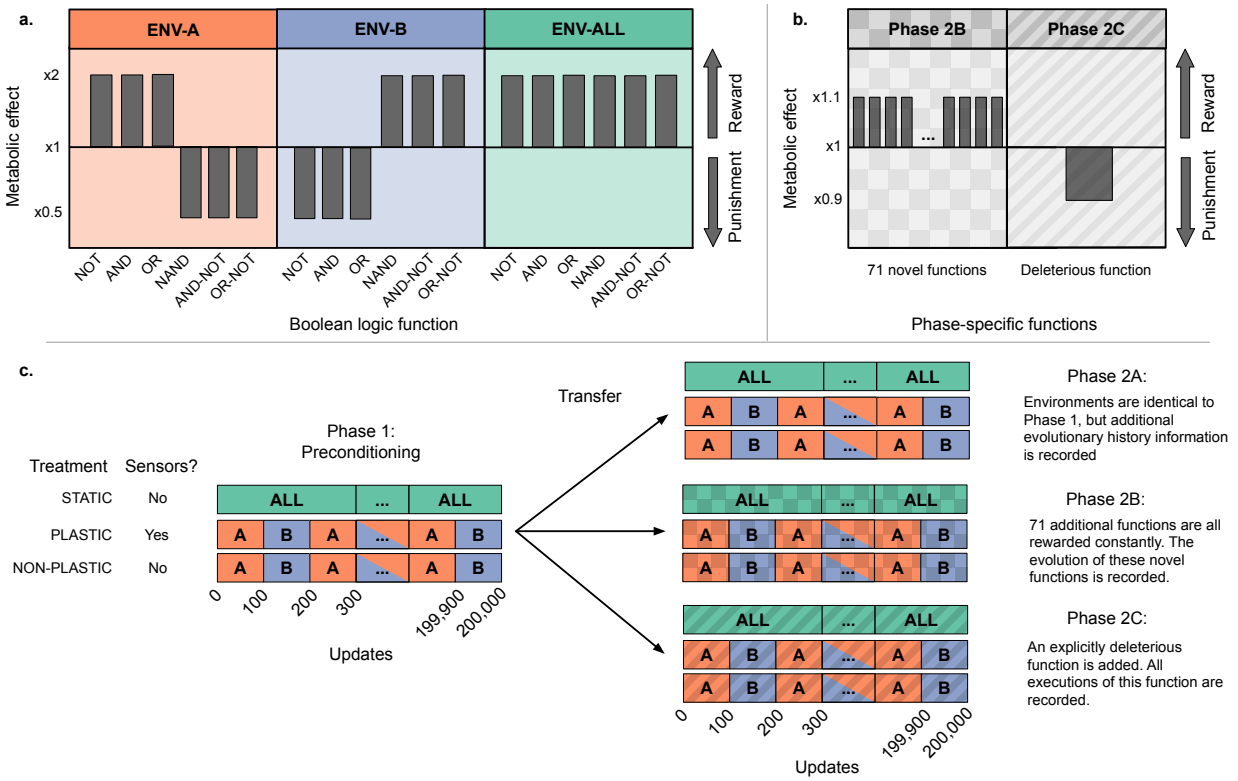

Figure 5.2: **Overview of experimental design.** The three plots in panel (a) show the environmental conditions used in every experiment and whether they reward or punish each base function. The two plots in (b) show the additional functions added in phases 2B and 2C. Panel (c) shows treatment differences and experimental phases. Treatments are listed on the left, with each treatment specifying its environmental configuration and whether sensors are functional. We conducted three independent two-phase experiments, each described on the right. Phases 2B and 2C are textured to match their function definitions in panel (b). Phase one is repeated for *each* experiment with 100 replicate populations per treatment per experiment. For each replicate at the end of phase one, we used an organism of the most abundant genotype to found the second phase population. All STATIC and NON-PLASTIC populations move on to phase two, but PLASTIC populations only continue to the second phase if their most abundant genotype exhibits optimal plasticity. Metrics are recorded only in phase two.

phase two, we founded new populations with these evolved organisms and examined their subsequent evolution under given combinations of treatment and experimental conditions. During phase two, we tracked and saved each population's evolutionary history as well as saving the full final population. Phase one was for preconditioning only; all comparisons between treatments were performed on phase two data.

Environments

We constructed three experimental environmental conditions, abbreviated hereafter as "ENV-A", "ENV-B", and "ENV-ALL". In ENV-A, organisms are rewarded for expressing the NOT, AND,

and OR Boolean logic functions, and organisms are punished for expressing the NAND, AND-NOT, and OR-NOT functions. ENV-B is the reverse of ENV-A; that is, in ENV-B, organisms are rewarded for expressing the NAND, AND-NOT, and OR-NOT functions and are punished for expressing the NOT, AND, and OR functions. In ENV-ALL, organisms are rewarded for expressing each of the NOT, AND, OR, NAND, AND-NOT, and OR-NOT functions. In all environmental conditions (ENV-A, ENV-B, and ENV-ALL), a rewarded function expressed by an organism doubles their metabolic rate, allowing them to execute twice as many instructions in the same amount of time. A punished function halves an organism's metabolic rate. Each Boolean logic function is a non-trivial trait to evolve, as they each require the coordination of multiple genetic instructions to express (Lenski et al., 2003b).

In both the PLASTIC and NON-PLASTIC treatments, the environment cycles between equal-length periods of ENV-A and ENV-B. Each of these periods persist for 100 updates (approximately 15 to 20 generations). Thus, populations experience a total of 1,000 full periods of ENV-A interlaced with 1,000 full periods of ENV-B during each experimental phase. Previous work has shown that this rate of change reliably allows for thot Alternative for Visual Comparison of Distributions. These provide a side-by-side display that contains the density curve, the origie evolution of adaptive phenotypic plasticity in Avida (Clune et al., 2007; Lalejini and Ofria, 2016).

Organisms in the PLASTIC treatments differentiate between ENV-A and ENV-B by executing one of six sensory instructions, each associated with a particular Boolean logic function; these sensory instructions detect whether their associated function is currently rewarded or punished. By using sensory information in combination with execution flow-control instructions, organisms can conditionally perform different functions depending on the current environmental conditions.

Experiment Phase 1 – Environment preconditioning

For each treatment, we founded 100 independent populations from a common ancestral strain capable only of self-replication. At the end of phase one, we identified the most abundant (*i.e.*, dominant) genotype and sampled an organism with that genotype from each replicate population to found a new population for phase two.

For the PLASTIC treatment, we needed to ensure that our observations during the second phase of each experiment reflected the evolutionary consequences of adaptive plasticity. To do so, measured the plasticity of each PLASTIC-treatment population's dominant genotype by in-

dependently testing that genotype in each of ENV-A and ENV-B and recording the phenotype expressed in each environment. We discarded PLASTIC-treatment phase one populations if the dominant genotype did not exhibit optimal plasticity (as defined in Section 5.2.1), which ensured that PLASTIC-treatment phase two populations were founded with an optimally plastic organism.

Experiment Phase 2A – Evolutionary change rate

We conducted experiment phase 2A to test for differences in evolutionary change—the accumulation of genetic and phenotypic changes—among populations evolving under each of our three treatment conditions (PLASTIC, NON-PLASTIC, and STATIC). Phase 2A continued exactly as phase one, except we tracked the rates of evolutionary change in each of the PLASTIC-, NON-PLASTIC-, and STATIC-treatment populations. Specifically, we quantified evolutionary change using four metrics (each described in Table 5.1): (1) coalescence event count, (2) mutation count, (3) phenotypic volatility, and (4) mutational robustness.

While environmental conditions during phases one and 2A are identical, these phases are distinct in how populations are founded: each phase one population is founded with a common ancestor capable only of self-replication, whereas each phase two population is founded with an organism that was evolved in its treatment-specific conditions. Thus, during phase two, phylogenies are rooted with an ancestor that is well-adapted to its treatment conditions, which, in turn, ensures that our observations can exclude dynamics associated with initial adaptation.

Experiment Phase 2B – Novel function evolution

We conducted experiment phase 2B to quantify the extent to which organisms evolved under PLASTIC-, NON-PLASTIC-, and STATIC-treatment conditions were able to acquire and retain novel functions. Phase 2B extended the conditions of phase one by adding 71 novel Boolean logic functions (Ofria et al., 2009), which were always rewarded in all treatments. The original six phase one functions (NOT, NAND, AND, OR-NOT, OR, and AND-NOT; hereafter called "base" functions) continued to be rewarded or punished according to the particular treatment conditions. An organism's metabolic rate was increased by 10% for each novel function that it expressed (limited to one reward per function). This reward provided a selective pressure to evolve these functions, but their benefits did not overwhelm the 100% metabolic rate increase conferred by rewarded base functions. As such, populations in the PLASTIC and NON-PLASTIC treatments could not easily escape environmental fluctuations by abandoning the fluctuating base functions.

During this experiment, we used three metrics to quantify novel function acquisition and retention in evolving populations (each described in Table 5.1): (1) final novel function count, (2) novel function discovery, and (3) novel function loss.

Experiment Phase 2C – Deleterious instruction accumulation

We conducted experiment phase 2C to quantify the extent to which organisms evolved under PLASTIC-, NON-PLASTIC-, and STATIC-treatment conditions acquired deleterious instructions via mutation. Phase 2C extended the instruction set of phase one with a `deleterious` instruction. When an organism executes a `deleterious` instruction, it performs a "deleterious" function, which reduces the organism's metabolic rate (and thus reproductive success) but does not otherwise alter the organism's behavior. We imposed a 10% penalty each time an organism performed the deleterious function, making the `deleterious` instruction explicitly harmful to execute. We did not limit the number of times that an organism could perform the deleterious function, and as such, organisms could perform the deleterious function as many times as they executed the `deleterious` instruction.

We tracked the number of times each organism along the dominant lineage performed the deleterious function. Specifically, we used two metrics (each described in Table 5.1): (1) final deleterious function count and (2) deleterious function acquisition count.

5.2.3 Experimental analyses

For each of our experiments, we tracked and analyzed the phylogenetic histories of evolving populations during phase two and generated a set of summary statistics (Table 5.1). For each phylogenetic history, we counted the number of times that the most recent common ancestor for the population shifted and used this value as the number of coalescence events. Next, at the final time point, we identified the most abundant genotype in the evolved population, and chose a *representative organism* from that genotype for further analysis. We used the lineage from the founding organism to the representative organism to summarize the evolutionary pathway of a population. This lineage represents the majority of the evolutionary history from the given population as long as the entire population traces back to the lineage in recent history. We manually inspected evolved phylogenies and found no evidence that any of our experimental treatments supported long-term coexistence. As such, analyses of the representative lineages reflect the majority of evolutionary history for a given population.

Some of our metrics (Table 5.1) required us to measure genotype-by-environment interactions. Importantly, in the fluctuating environments, we needed to differentiate phenotypic changes that were caused by mutations from those that were caused by environmental changes. To accomplish this, we produced organisms with the given focal genotype, measured their phenotype in each environmental condition, and aggregated the resulting phenotypes to create a *phenotypic profile*. Although organisms with different genotypes may express the same set of functions across environmental conditions, their phenotypic profiles may not necessarily be the same. For example, an organism that expresses NOT in ENV-A and NAND in ENV-B has a distinct phenotypic profile from one that expresses NAND in ENV-A and NOT in ENV-B. Because phenotypic profiles encapsulate function expression across all relevant environmental conditions (ENV-A and ENV-B), a change in phenotypic profile from parent to offspring indicates a mutationally-induced phenotypic change.

While most analyses employed here are retrospective metrics applied to lineages, digital evolution allows precise manipulations on individual organisms and genomes. Mutational robustness uses this technique when looking at the possible mutations on a representative genotype. Genomes in Avida are linear sequences of instructions, and as such, possible mutations can be simulated by substituting other instructions at the desired site. Indeed, the mutational robustness of a genotype examines all one-step mutations (*i.e.*, each mutation where exactly one instruction is substituted). This allows us to disentangle whether the frequency of mutationally-induced phenotypic changes observed along a lineage is a consequence of evolved genetic architectures versus the result of population dynamics that actively skewed the distribution of organisms along the lineage; that is, are genomes organized such that mutations are more likely to induce phenotypic changes, or are organisms with phenotypes different from their parents more likely to be successful and thus appear along the representative lineage?

5.2.4 Statistical analyses

Across all of our experiments, we differentiated between sample distributions using non-parametric statistical tests. For each major analysis, we first performed a Kruskal-Wallis test (Kruskal and Wallis, 1952) to determine if there were significant differences in results from the PLASTIC, NON-PLASTIC, and STATIC treatments (significance level $\alpha = 0.05$). If so, we applied a Wilcoxon rank-sum test (Wilcoxon, 1992) to distinguish between pairs of treatments. We applied

Bonferroni corrections for multiple comparisons (Rice, 1989) where appropriate.

5.2.5 Software availability

We conducted our experiments using a modified version of the Avida software, which is open source and freely available on GitHub (Lalejini and Ferguson, 2021a). Modifications to Avida included an improved phylogeny tracking system that enabled us to track coalescence events and the addition of custom sensory instructions specific to our experiments. We used Python for data processing, and we conducted all statistical analyses using R version 4 (R Core Team, 2021). We used the tidyverse collection of R packages (Wickham et al., 2019) to wrangle data, and we used the following R packages for analysis, graphing, and visualization: ggplot2 (Wickham et al., 2020), cowplot (Wilke, 2020), Color Brewer (Harrower and Brewer, 2003; Neuwirth, 2014), rstatix (Kassambara, 2021), ggsignif (Ahlmann-Eltze and Patil, 2021), scales (Wickham and Seidel, 2020), Hmisc (Harrell Jr et al., 2020), fmsb (Nakazawa, 2019), and boot (Canty and Ripley, 2019). We used R markdown (Allaire et al., 2020) and bookdown (Xie, 2020) to generate web-enabled supplemental material. All of the source code for our experiments and analyses, including configuration files and guides for replication, can be found in our supplemental material, which is hosted on GitHub (Lalejini and Ferguson, 2021a). Additionally, our experimental data is available on the Open Science Framework at https://osf.io/sav2c/ (Lalejini and Ferguson, 2021b).

Metric	Description
Coalescence event count	Number of coalescence events that have occurred, which indicates the frequency of selective sweeps in the population.
Mutation count	Sum of all mutations that have occurred along a lineage.
Phenotypic volatility	Number of instances where parent and offspring phenotypic profiles do not match along a lineage.
Mutational robustness	Proportion of mutations (from the set of all possible one-step mutations) that do not change the phenotypic profile of a focal genotype. We also measured *realized mutational robustness*, which is the proportion of mutated offspring along a lineage whose phenotypic profile matches that of their parent.
Final novel function count	Count of unique novel functions performed by the representative organism in a final population from experiment phase 2B. This metric can range from 0 to 71 and measures how well the fitness landscape was exploited at a given point in time.
Novel function discovery	Number of unique novel functions ever performed along a given lineage in experimental phase 2B, even if a function is later lost. This metric can range from 0 to 71 and measures a given lineage's level of exploration of the fitness landscape.
Novel function loss	Number of instances along a given lineage from experimental phase 2B where a novel function is performed by a parent but not its offspring. This metric measures how often a given lineage fails to retain evolved traits over time.
Final deleterious function count	Number of times the deleterious function is performed by the representative organism from a final population from experiment phase 2C.
Deleterious function acquisition count	Number of instances along a given lineage where a mutation causes an offspring to perform the deleterious function more times than its parent.

Table 5.1: **Metric descriptions.**

5.3 Results

5.3.1 Adaptive phenotypic plasticity slows evolutionary change in fluctuating environments

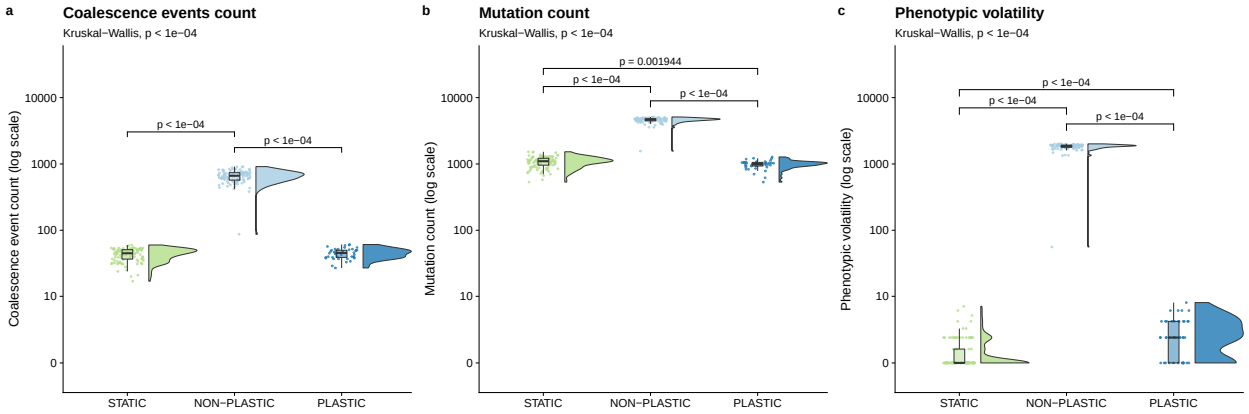

Figure 5.3: **Magnitude of evolutionary change.** Raincloud plots (Allen et al., 2019) of (a) coalescence event count, (b) mutation count, and (c) phenotypic volatility. See Table 5.1 for descriptions of each metric. Each plot is annotated with statistically significant comparisons (Bonferroni-corrected pairwise Wilcoxon rank-sum tests). Note that adaptive phenotypic plasticity evolved in 42 of 100 replicates from the PLASTIC treatment during phase one of this experiment; we used this more limited group to found 42 phase-two PLASTIC replicates from which we report these PLASTIC data.

In experimental phase 2A, we tested whether adaptive phenotypic plasticity constrained or promoted subsequent evolutionary change in a fluctuating environment. First, we compared the total amount of evolutionary change in populations evolved under the PLASTIC, NON-PLASTIC, and STATIC treatments as measured by coalescence event count, mutation count, and phenotypic volatility (Figure 5.3). According to each of these metrics, NON-PLASTIC populations experienced a larger magnitude of evolutionary change than either PLASTIC or STATIC populations. We observed significantly higher coalescence event counts in NON-PLASTIC populations than in PLASTIC or STATIC populations (Figure 5.3a). NON-PLASTIC lineages had significantly higher mutation counts (Figure 5.3b) and phenotypic volatility than PLASTIC or STATIC lineages (Figure 5.3c).

Changing environments have been shown to increase generational turnover (*i.e.*, how rapidly generations elapse) in Avida populations (Canino-Koning et al., 2016), which could explain why we observe a larger magnitude of evolutionary change at the end of 200,000 updates of evolution in NON-PLASTIC populations. Indeed, we found that significantly more generations of evolution

elapsed in NON-PLASTIC populations (mean of 41090 ± 2702 std. dev.) than in PLASTIC (mean of 31016 ± 2615 std. dev.) or STATIC (mean of 30002 ± 3011 std. dev.) populations during phase 2A (corrected Wilcoxon rank-sum tests, $p < 10^{-4}$).

To evaluate whether increased generational turnover explains the greater magnitude of evolutionary change in NON-PLASTIC populations, we examined the average number of generations between coalescence events and the realized mutational robustness of lineages (Table 5.1). A coalescence event indicates a selective sweep, which is a hallmark of adaptive evolutionary change. Realized mutational robustness measures the frequency that mutations cause phenotypic changes along a lineage. We expect that static conditions should favor fit lineages with high realized mutational robustness that no longer undergo rapid adaptive change and hence do not trigger frequent coalescence events. Under fluctuating conditions, however, lineages must be composed of plastic organisms if they are to maintain both high fitness and realized mutational robustness. Without plasticity, we expect fluctuating conditions to produce lineages with low realized mutational robustness and frequent coalescence events as populations must continually acquire and fix mutations to readapt to the environment.

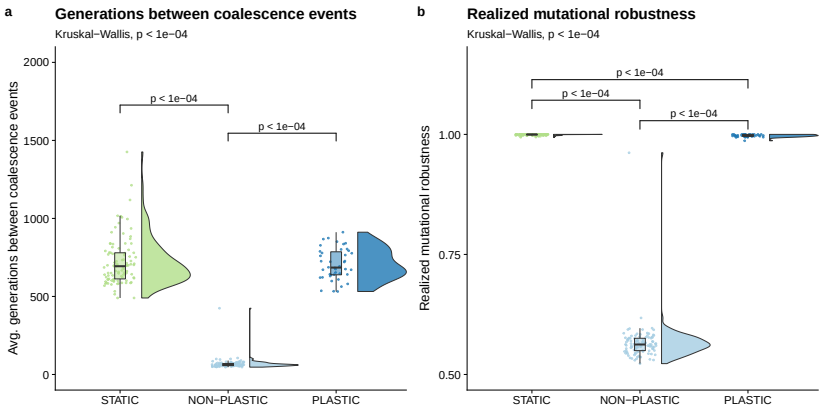

Figure 5.4: **Pace of evolutionary change.** Raincloud plots of (a) average number of generations between coalescence events, and (b) realized mutational robustness (Table 5.1). Each plot is annotated with statistically significant comparisons (Bonferroni-corrected pairwise Wilcoxon rank-sum tests).

On average, significantly fewer generations elapsed between coalescence events in NON-PLASTIC populations than in either PLASTIC or STATIC populations (Figure 5.4a). We also found that both STATIC and PLASTIC lineages exhibited higher realized mutational robustness relative to that of NON-PLASTIC lineages (Figure 5.4b); that is, mutations observed along NON-

PLASTIC lineages more often caused phenotypic changes in offspring. Overall, our results indicate that NON-PLASTIC populations underwent more rapid (and thus a greater amount of) evolutionary change than either PLASTIC or STATIC populations.

While both STATIC and PLASTIC lineages exhibited high realized mutational robustness, we found that STATIC lineages exhibited higher realized robustness than PLASTIC lineages (Figure 5.4b). Overall, there were rare instances of mutations that caused a change in phenotypic profile across all PLASTIC lineages. Of these mutations, we found that over 80% (83 out of 102) of changes to phenotypic profiles were cryptic. That is, the mutations affected traits that would not have been expressed in the environment that the organism was born into but would have been expressed had the environment changed.

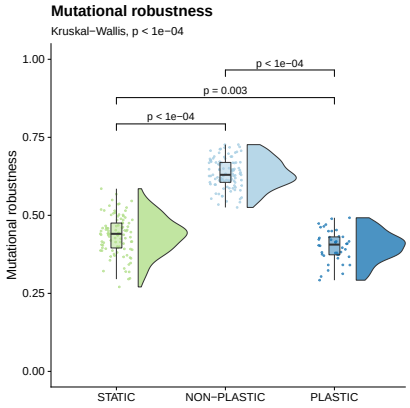

Figure 5.5: **Mutational robustness.** Raincloud plot of mutational robustness of each representative genotype (Table 5.1). The plot is annotated with statistically significant comparisons (Bonferroni-corrected pairwise Wilcoxon rank-sum tests).

Given that NON-PLASTIC lineages exhibited the lowest realized mutational robustness of our three experimental treatments, we sought to determine if this effect was driven by differences in evolved genetic architectures. Specifically, did the NON-PLASTIC genetic architectures evolve such that mutations were more likely to result in phenotypic change? Such a mutational bias would trade off descendant fitness in the same environment in exchange for a chance of increasing descendant fitness in alternate environments. This strategy would be an example of diversifying bet-hedging (*i.e.*, reducing expected mean fitness to lower variance in fitness) (Childs et al., 2010). Alternatively, the lower realized mutational robustness in NON-PLASTIC lineages could be due to survivorship bias, as we measured realized mutational robustness as the fraction of mutations observed along

successful lineages that caused a phenotypic change. That is, our measure of realized mutational robustness concentrates only on mutations that appear in the representative lineage (*i.e.*, "survivors" of selection), ignoring mutations that did not. Thus, lower realized mutational robustness in NON-PLASTIC lineages could simply be due to phenotype-altering mutations being more frequently favored by selection.

We analyzed the mutational robustness of representative genotypes by calculating the fraction of single-instruction mutations that change the phenotypic profile. We found that mutations to representative genotypes on NON-PLASTIC lineages are *less* likely to result in a phenotypic change than mutations to comparable genotypes on either STATIC or PLASTIC lineages (Figure 5.5). These data provide evidence against NON-PLASTIC lineages engaging in a mutation-driven bet-hedging strategy, and instead, are consistent with the hypothesis that lower realized mutational robustness in the NON-PLASTIC treatment was due to survivorship bias.

In general, adaptive plasticity stabilized PLASTIC-treatment populations against environmental fluctuations, and their evolutionary dynamics more closely resembled those of populations evolving in a static environment. We observed no significant difference in the number and frequency of coalescence events in PLASTIC and STATIC populations. We did, however, observe small, but statistically significant, differences in each of the following metrics: elapsed generations, mutation counts, phenotypic volatility, realized mutational robustness, and mutational robustness between PLASTIC and STATIC populations. We expect that these differences are a result of plastic organisms needing to simultaneously maintain both function and function-regulation machinery, resulting in more genetic components that can be broken by mutations; moreover, many of these components are under relaxed selection in periods between environmental changes. Overall, these differences were not substantial enough to play an obvious role in any of the dynamics we analyzed, but could be examined further in the future.

5.3.2 Adaptively plastic populations retain more novel functions than non-plastic populations in fluctuating environments

We have so far shown that adaptive plasticity constrains the rate of evolutionary change in fluctuating environments. However, it is unclear how this dynamic influences the evolution of novel functions. Based on their relative rates of evolutionary change, we might expect NON-PLASTIC-treatment populations to evolve more novel functions than PLASTIC-treatment populations. But,

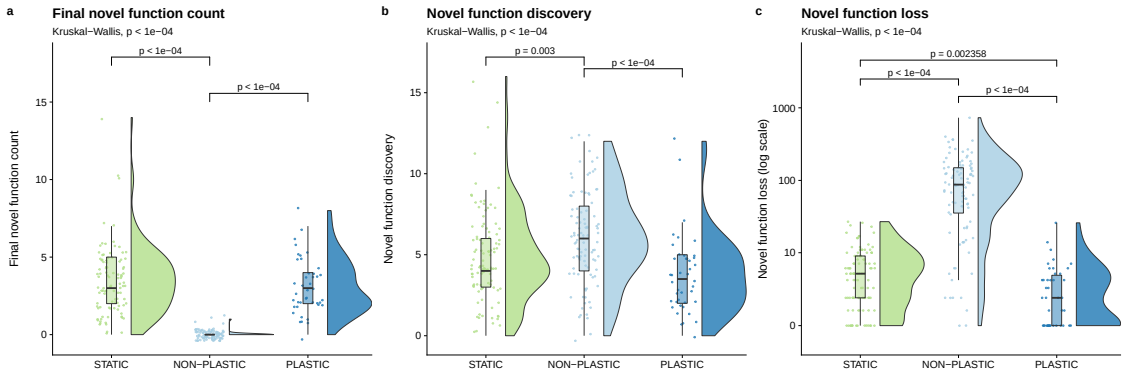

Figure 5.6: **Novel function evolution.** Raincloud plots of (a) final novel function count, (b) novel function discovery, and (c) novel function loss. See Table 5.1 for descriptions of each metric. Each plot is annotated with statistically significant comparisons (Bonferroni-corrected pairwise Wilcoxon rank-sum tests). Note that adaptive phenotypic plasticity evolved in 42 of 100 replicates from the PLASTIC treatment during phase one of this experiment; we used this more limited group to seed the resulting 42 phase-two PLASTIC replicates.

how much of the evolutionary change in NON-PLASTIC populations is useful for exploring novel regions of the fitness landscape versus continually rediscovering the same regions?

To answer this question, we quantified the number of novel functions performed by a representative organism in the final population of each replicate. We found that both PLASTIC and STATIC populations had significantly higher final function counts than NON-PLASTIC populations at the end of the experiment (Figure 5.6a). The final novel function count in PLASTIC and STATIC lineages could be higher than that of the NON-PLASTIC lineages for several non-mutually exclusive reasons. One possibility is that PLASTIC and STATIC lineages could be exploring a larger area of the fitness landscape when compared to NON-PLASTIC lineages. Another possibility is that the propensity of the NON-PLASTIC lineages to maintain novel traits could be significantly lower than PLASTIC or STATIC lineages. When we looked at the total sum of novel functions discovered by each of the PLASTIC, STATIC, and NON-PLASTIC lineages, we found that NON-PLASTIC lineages generally explored a larger area of the fitness landscape (Figure 5.6b). Although the NON-PLASTIC lineages discovered more novel functions, those lineages also exhibited significantly higher novel function loss when compared to PLASTIC and STATIC lineages (Figure 5.6c).

A larger number of generations elapsed in NON-PLASTIC populations than in PLASTIC or STATIC populations during our experiment (Lalejini and Ferguson, 2021a). Are NON-PLASTIC lineages discovering and losing novel functions more frequently than PLASTIC or STATIC lineages,

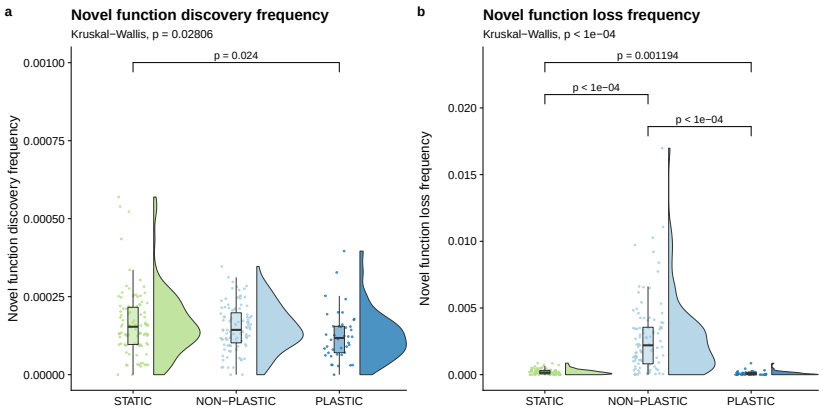

Figure 5.7: **Rate of novel function evolution.** Raincloud plots of (a) novel function discovery frequency and (b) novel function loss frequency. Each plot is annotated with statistically significant comparisons (Bonferroni-corrected pairwise Wilcoxon rank-sum tests).

or are our observations a result of differences in generational turnover? To answer this question, we converted the metrics of novel function discovery and novel function loss to rates by dividing each metric by the number of elapsed generations along the associated representative lineages. We found no significant difference in the frequency of novel function discovery between NON-PLASTIC and STATIC lineages, and we found that PLASTIC lineages had a lower frequency of novel function discovery than STATIC lineages (Figure 5.7a). Therefore, we cannot reject the possibility that the larger magnitude of function discovery in NON-PLASTIC lineages was driven by a larger number of elapsed generations. NON-PLASTIC lineages had a higher frequency of function loss than either PLASTIC or STATIC lineages, and PLASTIC lineages tended to have a lower frequency of novel function loss than STATIC lineages (Figure 5.7b).

Next, we examined the frequency at which novel function loss along lineages co-occurred with the loss or gain of any of the six base functions. Across all NON-PLASTIC representative lineages, over 97% (10998 out of 11229) of instances of novel function loss co-occurred with a simultaneous change in base function profile. In contrast, across all PLASTIC and STATIC dominant lineages, we observed that approximately 20% (29 out of 142) and 2% (13 out of 631), respectively, of instances of novel function loss co-occurred with a simultaneous change in base function profile. As such, the losses of novel functions in NON-PLASTIC lineages appear to be primarily due to hitchhiking or epistatic effects where a mutation that knocks out a maladaptive base function (after the environment changes) also knocks out a beneficial novel function.

5.3.3 Lineages without plasticity that evolve in fluctuating environments express more deleterious functions

Phenotypic plasticity allows for genetic variation to accumulate in genomic regions that are unexpressed, which could lead to the fixation of deleterious instructions in PLASTIC populations. However, in NON-PLASTIC lineages, we observe a higher rate of novel function loss, indicating that they may be more susceptible to deleterious mutations (Figure 5.7b).

Therefore, in experiment phase 2C, we tested whether adaptive phenotypic plasticity can increase the incidence of deleterious function performance. Specifically, we added an instruction that triggered an explicitly deleterious function and measured the number of times it was executed. Each execution of the `deleterious` instruction reduces an organism's fitness by 10%. At the beginning of phase 2C, the `deleterious` instruction is not present in the population, as it was not part of the instruction set during phase one of evolution. Accordingly, if a `deleterious` instruction fixes in a population, it must be the result of evolutionary dynamics during phase 2C, including cryptic variation, hitchhiking, or as a result of sign epistasis where a `deleterious` instruction knocks out an even more maladaptive trait.

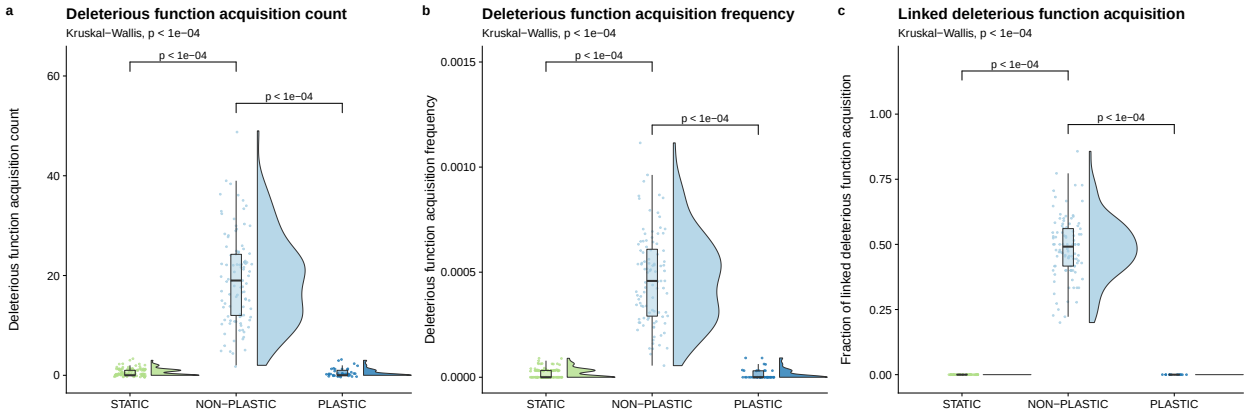

Figure 5.8: **Deleterious instruction accumulation.** Raincloud plots of (a) deleterious function acquisition, (b) deleterious function acquisition frequency, and (c) the proportion of mutations that increase deleterious function expression along a lineage that co-occur with a change in phenotypic profile. Each plot is annotated with statistically significant comparisons (Bonferroni-corrected pairwise Wilcoxon rank-sum tests). Note that adaptive phenotypic plasticity evolved in 43 of 100 replicates from the PLASTIC treatment during phase one of this experiment; we used this more limited group to seed the 43 phase-two PLASTIC replicates.

At the end of our experiment, no representative organisms from the PLASTIC or STATIC treatments performed the deleterious function under any environmental condition; however, repre-

sentative organisms in 14% of replicates of the NON-PLASTIC treatment performed the deleterious function at least once. NON-PLASTIC lineages contained significantly more mutations that conferred the deleterious function as compared to PLASTIC or STATIC lineages (Figure 5.8a), and these mutations occurred at a significantly higher frequency in NON-PLASTIC lineages (Figure 5.8b).

Next, we measured how often mutations that increased deleterious function performance co-occurred with changes to the base function profile within representative lineages. A `deleterious` instruction can fix in a population by having a beneficial effect that outweighs its inherent cost (*e.g.*, knocking out a punished function) or through linkage with a secondary beneficial mutation at another site within the genome. Across all NON-PLASTIC representative lineages, we found that approximately 49% (956 out of 1916) of mutations that increased deleterious function expression co-occurred with a change in the base function profile (Figure 5.8c). In all representative lineages from the PLASTIC treatment, only 18 mutations increased deleterious function expression, and none co-occurred with a change in base function profile (Figure 5.8c). Likewise, only 58 mutations increased deleterious function performance in all representative lineages from the STATIC treatment, and none co-occurred with a change in base function profile (Figure 5.8c). We did not find compelling evidence that the few mutations that increased deleterious function expression occurred as cryptic variation in PLASTIC lineages.

We repeated this experiment with 3% and 30% metabolic rate penalties associated with the deleterious function, which produced results that were consistent with those reported here (Lalejini and Ferguson, 2021a).

5.4 Discussion

In this work, we used evolving populations of digital organisms to determine how adaptive phenotypic plasticity alters subsequent evolutionary dynamics and influences evolutionary outcomes in fluctuating environments. Specifically, we compared lineages of adaptively plastic organisms in fluctuating environments to both non-plastic organisms in those same environments and other non-plastic organisms in static environments.

5.4.1 Evolutionary change

We found strong evidence that adaptive plasticity slows evolutionary change in fluctuating environments. Adaptively plastic populations experienced fewer coalescence events and fewer total

genetic changes relative to non-plastic populations evolving under identical environmental conditions (Figure 5.3). Whereas non-plastic populations relied on *de novo* mutations to adapt to each environmental fluctuation, plastic populations leveraged sensory instructions to regulate function performance. Indeed, in fluctuating environments, selection pressures toggle after each environmental change. We hypothesize that in non-plastic populations such toggling would repeatedly drive the fixation of mutations that align an organism's phenotypic profile to the new conditions. This hypothesis is supported by the increased frequency of coalescence events in these populations (Figure 5.4a) as well as increased rates of genetic and phenotypic changes observed along the lineages of non-plastic organisms.

Representative lineages in the non-plastic treatment experienced lower realized mutational robustness than plastic and static lineages (Figure 5.4b). We reasoned that this lower realized mutational robustness was due to non-plastic populations evolving a bet-hedging strategy where mutations are more likely to modify the phenotypic profile. However, when we switched from measuring the realized mutational robustness of representative lineages to measuring the mutational robustness of representative genotypes (*i.e.*, what fraction of one-step mutants change the phenotypic profile), we observed that non-plastic genotypes exhibited the highest mutational robustness of all three treatments (Figure 5.5). This result runs contrary to both our expectations and the results of other fluctuating environment studies in Avida (Canino-Koning et al., 2019). Canino-Koning et al. (2019) found that mutational robustness is negatively correlated with the number of function-encoding sites in the genome. In our work, most plastic and static genotypes encode all six base functions, while most non-plastic genotypes only encode functions from one environment; this results in fewer function-encoding sites, which may increase mutational robustness in non-plastic genotypes (relative to plastic and static genotypes). Regardless of the cause, this higher mutational robustness in non-plastic organisms indicates that bet-hedging is not driving the low realized mutational robustness observed in non-plastic lineages. Thus, we expect the lower realized mutational robustness in non-plastic lineages to be driven by survivorship bias. Because non-plastic lineages must rely on mutations to adapt to environmental changes, phenotype-altering mutations are often highly advantageous, and their selection decreases the realized mutational robustness of successful lineages.

To our knowledge, this study is the first in-depth empirical investigation into how the *de novo*

evolution of adaptive plasticity shifts the course of subsequent evolution in a cyclic environment. The relative rates of evolutionary change that we observed in non-plastic populations, however, are consistent with results from previous digital evolution studies. For example, Dolson et al. (2020) showed that non-plastic populations that were evolved in cyclically changing environments exhibited higher phenotypic volatility and accumulated more mutations than that of populations evolved under static conditions. Furthermore, Lalejini and Ofria (2016) visually inspected the evolutionary histories of non-plastic organisms evolved in fluctuating environments, observing that mutations along successful lineages readily switched the set of traits expressed by offspring.

Our results are also consistent with conventional evolutionary theory. A trait's evolutionary response to selection depends on the strength of directional selection and on the amount of genetic variation for selection to act upon (Lande and Arnold, 1983; Zimmer and Emlen, 2013). In our experiments, non-plastic populations repeatedly experienced strong directional selection to toggle which functions were expressed after each environmental change. As such, retrospective analyses of successful lineages revealed rapid evolutionary responses (that is, high rates of genetic and phenotypic changes). Evolved adaptive plasticity shielded populations from strong directional selection when the environment changed by eliminating the need for a rapid evolutionary response to toggle function expression. Indeed, both theoretical and empirical studies have shown that adaptive plasticity can constrain evolutionary change by weakening directional selection on evolving populations (Ghalambor et al., 2015; Paenke et al., 2007; Price et al., 2003).

5.4.2 The evolution and maintenance of novel functions

In fluctuating environments, non-plastic populations explored a larger area of the fitness landscape than adaptively plastic populations (Figure 5.6b). However, adaptively plastic populations better exploited the fitness landscape, retaining a greater number of novel functions than non-plastic populations evolving under identical environmental conditions (Figure 5.6a). In our experiment, novel functions were less important to survival than the fluctuating base functions. In non-plastic populations, when a mutation changes a base function to better align with current environmental conditions, its benefit will often outweigh the cost of losing one or more novel functions. Indeed, we found that along non-plastic representative lineages, 97% of the mutations associated with novel function loss co-occurred with phenotypic changes that helped offspring adapt to current environmental conditions.

Previous studies have shown that transitory environmental changes can improve overall fitness landscape exploration in evolving populations of non-plastic digital organisms (Nahum et al., 2017). Similarly, changing environments have been shown to increase the rate of evolutionary adaptation in simulated network models (Kashtan et al., 2007). In our system, however, we found that *repeated* fluctuations reduced the ability of non-plastic populations to maintain and exploit functions; that said, we did find that repeated fluctuations may improve overall function discovery by increasing generational turnover. Consistent with our findings, Canino-Koning et al. (2019) found that non-plastic populations of digital organisms evolving in a cyclic environment maintained fewer novel traits than populations evolving in static environments.

Our results suggest that adaptive phenotypic plasticity can improve the potential for populations to exploit novel resources by stabilizing them against stressful environmental changes. The stability that we observe may also lend some support to the hypothesis that phenotypic plasticity can rescue populations from extinction under changing environmental conditions (Chevin et al., 2010).

Our data do not necessarily provide evidence for or against the genes as followers hypothesis. The genes as followers hypothesis focuses on contexts where plastic populations experience novel or abnormally stressful environmental change. However, in our system, environmental changes were cyclic (not novel), and no single environmental change was *abnormally* stressful. Further, the introduction of novel functions during the second phase of the experiment merely added static opportunities for fitness improvement. This addition did not change the meaning of existing environmental cues, nor did it require those cues to be used in new ways.

5.4.3 The accumulation of deleterious alleles

We found that non-plastic lineages that evolved in a fluctuating environment exhibited both greater totals and higher rates of deleterious function acquisition than that of adaptively plastic lineages (Figure 5.8). There are several, non-mutually exclusive possibilities that could explain the fixation of explicitly deleterious instructions: random genetic drift, deleterious hitchhiking, epistatic effects, and cryptic variation (in plastic organisms). We find it unlikely that random genetic drift explains our observations. Each time an organism expresses a `deleterious` instruction, the organism incurs a 10% penalty to their replication rate, which results in strong purifying selection against mutations that cause offspring to execute `deleterious` instructions.

In asexual populations without horizontal gene transfer, all co-occurring mutations are linked. As such, deleterious mutations linked with a stronger beneficial mutation (*i.e.*, a driver) can sometimes "hitchhike" to fixation (Buskirk et al., 2017; Smith and Haigh, 1974; Van den Bergh et al., 2018). Natural selection normally prevents deleterious mutations from reaching high frequencies, as such mutants are outcompeted. However, when a beneficial mutation sweeps to fixation in a clonal population, it carries along any linked genetic material, including other beneficial, neutral, or deleterious mutations (Barton, 2000; Smith and Haigh, 1974). Therefore, deleterious genetic hitchhiking could have contributed to `deleterious` instruction accumulation along non-plastic lineages in changing environments.

Epistatic effects (*i.e.*, interactions between genes) could have also contributed to `deleterious` instruction accumulation along non-plastic lineages. On their own, mutations that increase `deleterious` instruction execution are maladaptive; however, if such a mutation were to also knock out an even more harmful function, that mutation may have a net beneficial effect. As such, mutations that confer increased `deleterious` instruction execution could directly drive a selective sweep.

Representative lineages from non-plastic populations in the cyclic environment exhibited higher mutation accumulation (Figure 5.3b), novel function loss (Figure 5.6c), and deleterious function acquisition (Figure 5.8a) than their plastic counterparts. In aggregate, we found that many (∼49%; 956 / 1916) mutations that increased `deleterious` instruction execution in offspring co-occurred with mutations that provided an even stronger benefit by adapting the offspring to an environmental change. We expect that an even larger fraction of these deleterious mutations were linked to beneficial mutations, but our analysis only counted mutations that co-occurred in the same generation. Our analyses did not distinguish between epistatic effects and deleterious hitchhiking; however, more fine-grained analyses of secondary effects of mutations that conferred `deleterious` instruction execution could be performed in future work to disentangle these two mechanisms.

Theory predicts that under relaxed selection deleterious mutations should accumulate as cryptic variation in unexpressed traits (Lahti et al., 2009). Contrary to this expectation, we did not find evidence of `deleterious` instructions accumulating as cryptic variation in adaptively plastic lineages. One possible explanation is that the period of time between environmental changes was too brief for variants carrying unexpressed `deleterious` instructions to drift to high frequencies

before the environment changed, after which purifying selection would have removed such variants. Indeed, we would not expect drift to fix an unexpressed trait since we tuned the frequency of environmental fluctuations to prevent valuable traits from being randomly eliminated during the off environment. Additionally, plastic organisms in Avida usually adjust their phenotype by toggling the expression of a minimal number of key instructions, leaving little genomic space for cryptic variation to accumulate.

5.4.4 Limitations and future directions

Our work lays the groundwork for using digital evolution experiments to investigate the evolutionary consequences of phenotypic plasticity in a range of contexts. However, the data presented here are limited to the evolution of *adaptively* plastic populations. Future work might explore the evolutionary consequences of maladaptive and non-adaptive phenotypic plasticity (*e.g.*, Leroi et al. 1994), which are known to bias evolutionary outcomes (Ghalambor et al., 2015).

Additionally in our experiments, sensory instructions perfectly differentiated between ENV-A and ENV-B, and environmental fluctuations never exposed populations to entirely new conditions. These parameters have been shown to influence evolutionary outcomes (Boyer et al., 2021; Li and Wilke, 2004), which if relaxed in the context of further digital evolution experiments, may yield additional insights. Our experiments also focused on asexually reproducing digital organisms. Sexual reproduction has been shown to be advantageous in rapidly changing environments, such as the cyclic environments used in our study (Misevic et al., 2010). Future work could investigate how sexual reproduction affects the evolutionary consequences of adaptive plasticity.

We focused our analyses on the lineages of organisms with the most abundant genotype in the final population. These successful lineages represented the majority of the evolutionary histories of populations at the end of our experiment, as populations did not exhibit long-term coexistence of different clades. Our analyses, therefore, gave us an accurate picture of what fixed in the population. We did not, however, examine the lineages of extinct clades. Future work will extend our analyses to include extinct lineages, giving us a more complete view of evolutionary history, which may allow us to better distinguish adaptively plastic populations from populations evolving in a static environment.

As with any wet-lab experiment, our results are in the context of a particular model organism: "Avidian" self-replicating computer programs. Digital organisms in Avida regulate responses to en-

vironmental cues using a combination of sensory instructions and conditional logic instructions (`if` statements). The `if` instructions conditionally execute a single instruction depending on previous computations and the state of memory. As such, plastic organisms in Avida typically regulate phenotypes by toggling the expression of a small number of key instructions as opposed to regulating cohorts of instructions under the control of a single regulatory sequence (Lalejini and Ferguson, 2021a). This bias may limit the accumulation of hidden genetic variation in Avida genomes. However, as there are many model biological organisms, there are many model digital organisms that have different regulatory mechanisms (*e.g.*, Lalejini and Ofria 2018) that should be used to test the generality of our results.

As with most digital evolution experiments, our mutation rates were high and population sizes were small (3600 individuals) relative to experiments with microbes or conditions common in nature. As such, beneficial mutations can be generated rapidly and selective sweeps can occur quickly. Moreover, our analyses were limited to a single rate of environmental change and simple function reward structures, which likely influenced the rates of selective sweeps observed in our experiments. Future studies could address these limitations by increasing population sizes, decreasing mutation rates, investigating different function rewards and punishments, and altering the time spent in each environment.

Supplemental Material

The supplemental material for this article is hosted on GitHub and can be found online at https://github.com/amlalejini/evolutionary-consequences-of-plasticity (Lalejini and Ferguson, 2021a).

Data Availability Statement

The datasets generated and analyzed for this study can be found on the Open Science Framework at https://osf.io/sav2c/ (Lalejini and Ferguson, 2021b).

Chapter 6
Proposed - Patterns in potentiation across multiple environments

Authors: Austin Ferguson, Anselmo Pontes, and Charles Ofria

Status: Proposed. While data collection has not started, both environments I propose to explore have been ported from Avida2 to MABE2 and are ready to be used.

6.1 Introduction

Previous work has shown that the environment can influence the relative contributions of adaptation, chance, and history Smith et al. (2022). I have previously proposed testing how changes to the environment and underlying representation affect potentiation in successful lineages (Chapters 3 and 4). However, I will be unable to concretely identify if differences between those two systems are due to changing the environment or the representation. To remedy this issue, here I propose to quantify potentiation along successful lineages in two additional Avida environments. By keeping the representation constant, potentiation differences must therefore be a result of the change in environment or the targeted trait within.

I hypothesize that in many cases, potentiating mutations are highly epistatic, as epistasis is one key way for a seemingly innocuous mutation to have a drastic effect on later mutations. As such, studies of potentiation should employ representations capable of epistatic interactions. This is an advantage of using Avida; there are multiple possibilities for epistasis between instructions, and in previous work I have confirmed that these interactions are sufficient for complex potentiation dynamics (Chapter 2). By conducting studies of potentiation in multiple Avida environments, I will investigate how environmental differences influence potentiation as the underlying representation, and thus the potential for epistatic interactions, only slightly differ.

Specifically, I propose to investigate potentiation in a patch harvesting behavior and in phenotypic plasticity. In the patch harvesting environment described in (Pontes, 2021), I will target the successful harvesting of multiple patches in an organism's lifetime. In the cyclic logic 6 environment (Chapter 5), I will target optimal plasticity. While both target behaviors are complex, their details differ both with each other and with associative learning from previous work (Chapter 3). Ultimately, will these details influence how potentiation changes?

The patch harvesting behavior I am targeting is a basic cognitive behavior. In order to harvest multiple patches, organisms must switch between exploring to find new patches or exploiting the

patch they are currently on. Similar to the associative learning behavior in Chapter 3, this behavior requires several different components in order to function (information storage, memory retrieval and usage, etc.). As such, I expect the two behaviors to exhibit similar patterns in potentiation. The other behavior, optimal plasticity in the cyclic logic 6 environment, is a reactive behavior that does not require memory. It does, however, still consist of multiple components (performing tasks and regulating them due to environmental cues) that can be neutral or deleterious in isolation. Therefore, I also expect to see similar patterns in potentiation in the evolution of optimal plasticity.

This work will expand upon the previous chapters, providing additional examples of how potentiation changes as we vary the environment and target behavior. All together, these works will shape how we view potentiation, and will provide an expanding dataset for other researchers to compare against in the future.

6.2 Methods

Here I describe the two environments and the behaviors that we are targeting within them.

6.2.1 The patch harvesting environment

The first environment aims to model the patch harvesting behavior of early bilaterians as they traversed microbial mats. Fossil records of the paths taken by these animals provide evidence of complex behavioral patterns, potentially resulting from early cognitive behaviors (Carbone and Narbonne, 2014). This environment is a re-implementation of the one found in (Pontes, 2021). In this environment, organisms are rewarded for consuming nutrients and punished for time spent off the patch. Here, we specifically look at environments with multiple patches of nutrients, and our target behavior is the successful consumption of multiple patches. This behavior requires switching between at least two tasks (exploring or exploiting), and as such, it is a cognitive behavior comparable to associative learning in Chapter 3.

Our Avida organisms exist in the typical 60x60 toroidal grid, with parent organisms replicating into a neighboring cell. The evaluation of the organisms, however, occurs on a toroidal, two-dimenstional spatial grid that represents microbial mats like those encountered by the early bilaterians. These environments consist of various patterns of nutrients that the organisms can consume and empty tiles whose traversal causes the organisms to waste energy.

To facilitate the consumption of nutrients, organisms are granted four new instructions to navigate their environment. Movement is accomplished via three instructions: `Turn Left`, `Turn`

`Right`, and `Move Forward`. The right and left instructions rotate the organism 45 degrees in the appropriate direction. The move instruction always moves the organism a single tile in the direction it is pointing, including diagonally. Finally, the organisms are given a `Sense` instruction to pull information from their environment. Specifically, the sense instruction will give the organism a numeric cue depending on what floor tile it is currently on. Empty tiles return a negative one, while already-consumed nutrient tiles result in positive one. Nutrients are consumed as the organism moves onto that tile without requiring an additional action, and as such organisms will never sense a nutrient tile that has not been consumed.

Organisms are scored based on how well they consume nutrients and avoid empty tiles. The organism's score is increased by one for each nutrient consumed. For every empty tile visited, however, the organism's score is reduced by one to model the organism expending energy to move for no nutrient gain. Moving onto a previously-consumed tile has no effect on score, as if some residual nutrients could offset the energy consumption. Finally, as is often done in Avida, this score is used in an exponential. The performance of the organism is calculated as 2^{score}, which means an additional nutrient consumed always doubles performance, regardless of how many nutrients have been consumed in total. As is customary in Avida, this score is used to weight which organisms receive the most updates and thus execute their genomes faster.

While previous work has used multiple patch types (Pontes, 2021), here I propose to study only one: multiple disconnected patches. The smaller patches in this environment require organisms to switch patches once they have exploited their current patch in order to maximize their score. These two behaviors (exploration and exploitation) can be thought of as two states the organism can be in. As such, I expect this behavior to require memory, as a single bit of information is needed to store what state the organism is in. Figure 6.1 shows an example of the disconnected patches, as well as an evolved organism's successful trace through the environment. To easily categorize organisms that are capable of consuming multiple patches, I will require the organism to have consumed at least half each of two patches to be considered a "successful" behavior. Exploratory work has shown that this behavior does evolve, but only rarely. In 200 initial replicates, it appeared in only nine. Much like associative learning in previous work, this rarity provides substantial room for improvements in potentiation over the course of a lineage.

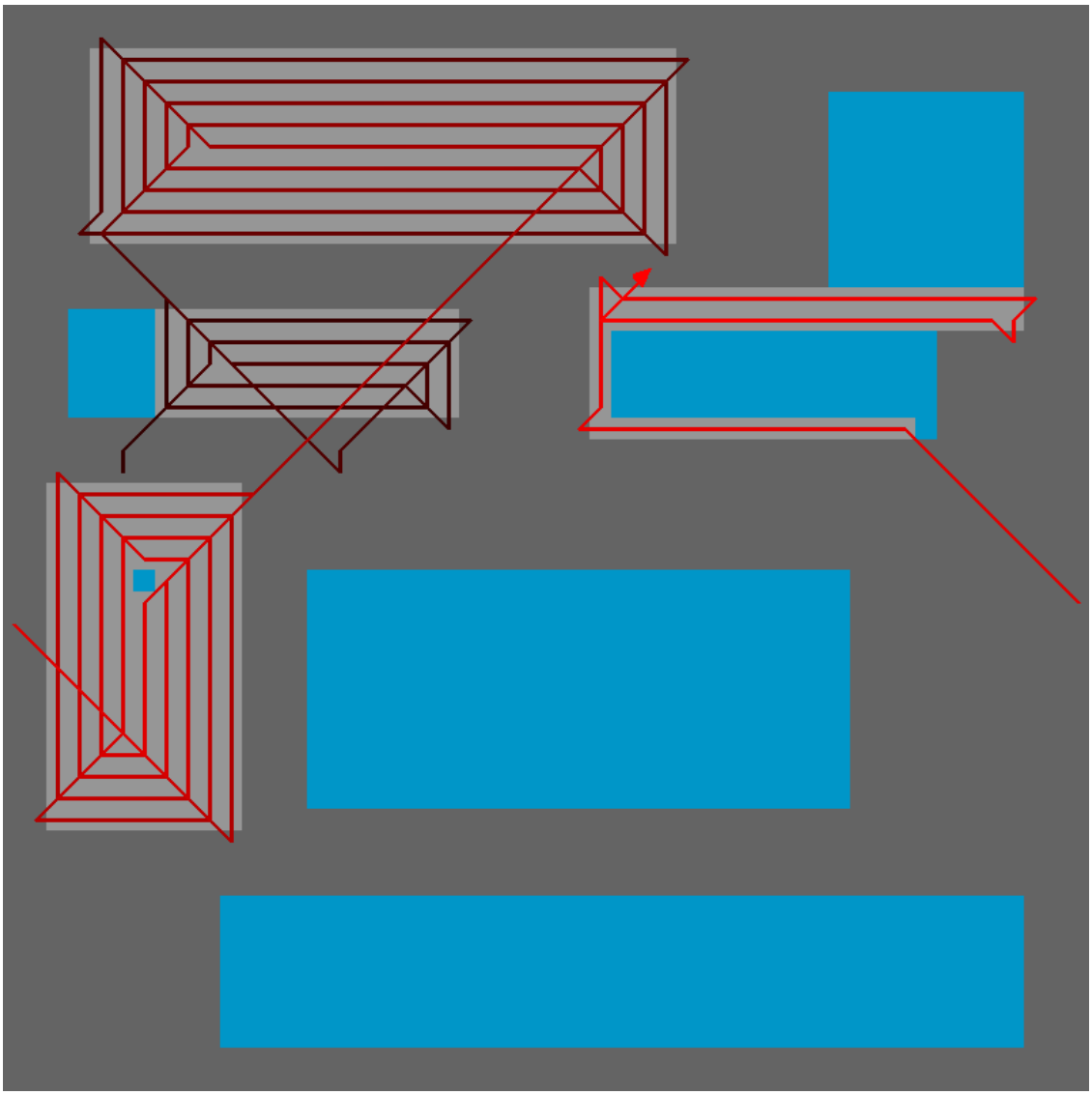

Figure 6.1: An example layout of disconnected patches in the patch harvesting environment. Blue tiles represent food that has yet to be consumed, light gray tile are food that has been consumed, and dark gray tiles show empty space which is costly to move into. The lines show the trace of an evolved organism (red triangle), with the start of the line in black and a slow fade to red as the trace becomes more recent. This organism has evolved a spiraling behavior and has successfully consumed three patches of food.

6.2.2 The cyclic logic 6 environment

There may be inherent similarities between the associative learning task of Chapters 2 and 3 and the cognitive behavior needed in the patch harvesting environment. As such, here I propose one final, non-cognitive environment to test potentiation: the cyclic environment from Chapter 5, which I will refer to here as "cyclic logic 6".

The cyclic logic 6 environment in Avida consists of two sets of bitwise logic tasks: one that is rewarded and one that is punished. Which set is rewarded, however, switches at regular intervals. Each organism receives a set of random input numbers, and then has the opportunity to perform computations and eventually output these values. If output values are deemed to be a successful logic operation on one or more inputs, then the organism is rewarded or punished according to the current state of the environment. The Avida organisms, however, are only given access to a bitwise `NAND` instruction, which they must use to build the other logic operations.

At any given time, three of the six logic tasks will be rewarded while the other three are punished. In this cyclic environment, this reward scheme is flipped at regular intervals. When performing a logic task for the first time, an organism's score is doubled if that task is rewarded or halved if the task is punished. Here I flip the rewards and punishments every 100 updates. I will give organisms access to a new instruction, `Sense`, so they can determine what environment they are in and can potentially regulate task expression accordingly. This environment is described in detail in Chapter 5, though here I am only using the PLASTIC environment from Phase 1 (see Figure 5.2, panels A and C). I will use the same two sets of tasks used in Chapter 5 (NOT, AND, and OR) and (NAND, AND-NOT, OR-NOT).

Due to the cycling of which tasks are rewarded, organisms must exhibit optimal plasticity to maximize fitness. As in the previous chapter, I define optimal plasticity as performing the three rewarded tasks and zero of the punished tasks. While organisms are born into a particular environment, I will test for optimal plasticity *post hoc*, evaluating the organism in both variations of the environment. Therefore, for an organism to exhibit optimal plasticity, they must be capable of perfectly regulating all six tasks depending on which environment they are in.

It is this optimal plasticity that I will target for the potentiation analysis. Compared to associative learning and multi-patch harvesting behaviors, optimal plasticity is a much more common

behavior to evolve from the ancestor, with Chapter 5 seeing greater than 40% of replicates evolve the behavior. While it is may be more common, this behavior is still nontrivial. To perform optimal plasticity, organisms must be capable of performing all six logic tasks, sensing the environment, and using the environment data to regulate the execution of the logic tasks. This task, however, can be solved reactively; it is unnecessary to store the environment state in memory. Still, I expect the complex nature of the environment to translate into interesting patterns in potentiation, not unlike those of associative learning seen in previous work.

6.3 Proposed work

In both environments, I will measure attributes of potentiation by conducting analytic replay experiments as described in Chapter 3. I will then compare the measurements to previous data from Chapters 3 and 4 to determine what differences exist between the different environments. This work will provide additional examples of potentiation and allow the first cross-environment comparisons while keeping the underlying representation the same.

Potentiation will be quantified using the exact same methods as Chapter 3. Initial evolutionary replicates seeded with the default ancestor will be conducted to find replicates that exhibit the target behavior (multi-patch harvesting or optimal plasticity). For each environment, I will run initial replicates until I reach 40 successful lineages. To measure the changes in potentiation along the dominant lineage of each replicate, I will conduct two phases of replay experiments. The first will seed replicates with the genotypes found 50, 100, 150, and 200 steps before the target behavior first appeared in the lineage. I will continue to go backward along the lineage until a step shows less than 10 percentage points of improvement over the success rate from the initial replicates. Once the exploratory replays have been conducted, I will identify potentiation windows (as in Chapter 3) and seed replay replicates for every genotype in the windows. As before, I will run fifty replay replicates for each genotype analyzed.

After conducting the replay experiments, I will collect the same potentiation measures described in Section 3.2.3. Since these are the same measurements recorded in Chapters 3 and 4, I can then statistically compare the distributions of each measurement across environments. The one exception is the behavioral background, which is unique to each task and thus can only be compared within a given environment. The cross-environment comparisons will be conducted first as a Kruskal-Wallis test to determine if significant differences exist across all of the environments (Kruskal and Wallis,

1952). If a difference is detected, I will then conduct pairwise Mann-Whitney-Wilcoxon tests to look for significant differences between each pair of environments (Wilcoxon, 1945). Finally, I will use a Holm-Bonferroni correction for multiple comparisons (Holm, 1979).

These comparisons will provide insight into how these potentiation dynamics vary when the environment changes but the representation stays the same. I expect only small differences between the associative learning behavior of Chapter 3 and the patch harvesting behavior examined here. Both cognitive behaviors require memory and have exist in environments containing non-cognitive alternative behaviors with high performance that might function as local optima. For the other environment, cyclic logic 6, I expect to see similar overall dynamics but a difference in the measured values. While the environment and behavior are drastically different from the other two, one particularly notable difference is that $> 40\%$ of replicates evolved optimal plasticity in the different experiments of Chapter 5. Therefore I do not expect the same levels of potentiation gain as we observed in the evolution of associative learning in Chapter 2, simply because the lineages start with a much higher level of potentiation. Alternatively, we must consider that the cyclic logic 6 environment is the only proposed environment that changes with time; these changes to selective pressures may repeatedly break down potential building blocks and thus increase the potentiation gain when they are finally utilized. Regardless of the outcome, this work will provide needed data on potentiation and more insight into how evolution is (or is not!) contingent on initially innocuous mutations.

Chapter 7
Concluding thoughts

7.1 Outlook

In this dissertation proposal I have outlined my plans to study how history interacts with adaptation and chance to produce cognitive behaviors. Specifically, I first demonstrated the power of analytic replay experiments *in silico*, and I proposed to expand these studies to create the first large, cross-lineage dataset of how potentiation changes along successful lineages. Next, I proposed shifting to a simpler bitstring model to further develop our intuition and analysis techniques for potentiation. Taking a step back, I examined how the evolution of phenotypic plasticity influences future evolutionary dynamics, finding that plasticity stabilizes evolution. Finally, I proposed to study potentiation in two additional environments to test the generalizability of my earlier findings as the study system changes.

This proposal originally had one more chapter: an alternative proposal to study potentiation in different representations. Specifically, I would repeat the potentiation study in the patch harvesting environment using Markov brains, recurrent neural networks, and Cartesian genetic programming. By keeping the environment the same, this would demonstrate how potentiation dynamics change with the representation. While this chapter has been cut for brevity, it remains an option if a proposed chapter encounters unsalvageable issues or if my committee prefers it to another chapter.

The nature of this work is mostly exploratory; prior work has measured potentiation in specific systems, but not enough work has been conducted to begin drawing general conclusions about potentiation. This is the gap I aim to fill. While all the work I have proposed is digital, by studying different environments and representations, I will create intuition about potentiation that is likely to extend beyond digital systems. Doing this work *in silico* is required, however, as the work that I have proposed is currently intractable to conduct *in vivo*. Regardless, here I expand on the previous work on microbial potentiation to help us understand how adaptation, chance, and history interact to create the diverse complexity we see in the world.

There are countless ways the work in this proposal could be extended. Since this area is so new and so difficult to study in wetlab systems, quantifying potentiation after varying *any* aspect of the system can provide new insights into how potentiation arises and the forms that it takes. For example, while the cyclic logic 6 environment of Chapters 5 and 6 changes over time, a more

systematic study of the effects of temporal changes on potentiation would be relevant for many natural systems, where both biotic and abiotic factors are constantly changing. I am particularly interested the fundamentals of potentiation, and as such I am curious how potentiation changes in other simple models like the NK landscapes of Chapter 4. Specifically, I am interested in the effect of switching from bitstrings to multi-allele representations. When targeting only single trait in a bitstring model, every single-bit mutation must bring you either closer or farther away from that trait. By switching to a multi-allele model, we could analyze mutations that are neutral with respect to the genetic distance to the target. Finally, the analyses here focus on target traits that are effectively optimal, but this is not required. How do potentiation dynamics change as we switch to targeting suboptimal traits, and how does the potentiation of one trait affect the potentiation of other traits? These ideas only begin to scratch the surface of possibilities in studying potentiation dynamics, and it will be exciting to see how this area of evolutionary biology develops over the coming decades.

7.2 Proposed timeline

I have outlined a substantial amount of work of be done in this proposal. The three proposed chapters require huge amounts of time just to generate the data, which is compounded by analysis and writing. I have attempted to trim the fat where possible, reducing the amount of computation needed for each chapter. Even still, my current plan is to defend late next spring. To have any hope of meeting that deadline, I need a plan. Therefore, I present my timeline for when I will perform the various tasks for each chapter Figure 7.1.

As stated above, all three proposed chapters, but especially Chapters 3 and 6, are very computationally expensive. Thus I have included two initial months of data collection for both, with an extra month allotted for any additional data collection that was identified as needed during analysis. The other chapter, Chapter 4, will deal with an enormous amount of data, but the bitstring model makes that data much faster to collect. As such, it receives only one month of initial data collection and one month for collecting any additional data.

Chapter 3 is not allotted any dedicated implementation time, as all the pieces are in place thanks to Chapter 2. This chapter is ready to begin data collection once the comprehensive examination has concluded. Chapter 4 is allotted a very conservative two months of implementation, as all the pieces (NK landscapes, enumeration and replay pipelines) exist and will only require tweaks and

	Chapter 3	Chapter 4	Chapter 5	Misc.
May 2023	Data Collection	Implementation		
June 2023	Data Collection	Implementation		
July 2023	Analysis	Data Collection		
August 2023	Data Collection	Analysis	Implementation	
September 2023	Analysis	Data Collection	Data Collection	
October 2023	Writing	Analysis	Data Collection	
November 2023	Writing		Analysis	
December 2023		Writing	Data Collection	
January 2024		Writing	Analysis	Writing
February 2024			Writing	
March 2024			Writing	
April 2024				Writing
May 2024				Defend!

Figure 7.1: My proposed timeline for completing the proposed chapters and my dissertation as a whole. The miscellaneous column includes writing the introduction and conclusion of the dissertation. Remainder of explanation in text.

testing. Finally, all the environments and Avida changes are already implemented in MABE2, and thus Chapter 6 is given a single month of dedicated implementation time to connect the pieces together.

Each project is given two months for analysis, one after the initial data collection and one more after any additional data collection is completed.

Finally, all of these projects will need to be written up. As things stand, I have allotted two months of writing time for each proposed chapter. I have also allotted two months to writing the other pieces of the dissertation (e.g., introduction, conclusion, and any needed appendices).

Fortunately, data collection is mostly hands-off once it has been started, and most analyses will be reused for the various projects, saving substantial time in the long run. Having different projects going simultaneously is my preferred way to work, as I often switch between them when I hit a rut, allowing me to come back later with a fresh mind. As such, I have attempted to structure this timeline such that I always have one active task (coding or writing) occurring concurrently with

data collection and analysis. While I could not easily show it in the figure, I will begin writing the introduction and methods to each chapter during the early data collection and analysis phases.

While it will be a ton of work, I look forward to it.

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