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Jordan A. Lewis Date

The influence of evolutionary reciprocity and genetic architecture on the evolution of host defense in *Caenorhabditis elegans*

By

Jordan A. Lewis
Doctor of Philosophy

Graduate Division of Biological and Biomedical Sciences
Population Biology, Ecology, and Evolution

Levi Morran, Ph.D.
Advisor

Chris Beck, Ph.D.
Committee Member

Nicole Gerardo, Ph.D.
Committee Member

David Cutler, Ph.D.
Committee Member

Lance Waller, Ph.D.
Committee Member

Accepted:

Kimberly Jacob Arriola, MPH, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Date

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An abstract of
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in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in
Graduate Division of Biological and Biomedical Sciences
Population Biology, Ecology, and Evolution
2022

Abstract

The influence of evolutionary reciprocity and genetic architecture on the evolution of host defense in *Caenorhabditis elegans*

By Jordan A. Lewis

Parasites are ubiquitous in nature and exert intense selection pressure on the populations they infect due to the fitness costs they impose on infected individuals. Consequently, over time host populations tend to evolve defenses to resist or tolerate their parasites. However, the characteristics of the defenses that evolve in host populations are often contextually dependent on the dynamics of their evolutionary interactions. Evolutionary theory and previous empirical research suggest a diverse array of factors can impact the evolution of host defenses. However, direct experimental testing of these predictions has been limited. This dissertation uses experimental evolution in the *Caenorhabditis elegans* – *Serratia marcescens* host-parasite system to elucidate these questions. In chapter II, I investigated the role of evolutionary reciprocity in shaping the breadth of parasites against which host defenses are effective (defense range). This was done by assaying experimental populations that had been exposed to *S. marcescens* at one of three levels of reciprocity (dead parasite, one-sided evolution, coevolution) against a range of other *S. marcescens* strains. In chapter III, I examined the impact of gene flow on adaptation by passaging hosts against parasites for 10 generations and controlling host gene flow and source population. Source populations had different genetic backgrounds (one the same as the sink population and two different) and two evolutionary histories (previously adapted or naïve). This allowed for examining the impact of genetic architecture and evolutionary history. Lastly, in chapter IV I reviewed the history and methodologies of selection experiments and identify the potential advantages of experimental evolution in applied biology. I end by identifying three areas where experimental evolution could assist research and development of industrial products. Overall, this dissertation contributes to a literature that describes the multitude of ways host defenses can be influenced by the dynamics of their interactions with their parasites. It also shows the ways in which the traits that make experimental evolution useful for basic science research can be more effectively used in applied biology.

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ACKNOWLEDGEMENTS

During the course of my life, and my education, I have been fortunate enough to be poured into, loved, taught and mentored by a host of great people. They are my family, friends, colleagues, mentors, and peers, and without them I would not have been able to successfully complete this dissertation. They truly are my "village", and I am the product of their collective work. If I had tried to make this section exhaustive, it would have been its own chapter, so instead I have tried to focus on those who have really motivated or assisted in my educational experience.

First, I want to thank my amazing parents, Marsha and David Lewis. My parents sacrificed so much for my brother and me growing up, and they set an example of selflessness, hard work, integrity, and trustworthiness. But, most of all, my parents have always encouraged and supported me in my endeavors. They taught me that I could do anything I put my mind to and always told me that my mind could take me wherever I wanted to go. They always wanted me to have it easier than they did and many times that allowed me to engage in other interests that have helped me become who I am today. I would also like to thank my little brother for always giving me motivation to set a positive example. Even though I do not have children, growing up with a brother who is 5 1/2 years younger than you infuses a natural "parental" element to the sibling dynamic. Jomar has always made me a proud big brother, and I am so excited to see what he does in the future. I would also like to thank my grandparents, Walter, Elizabeth, Freddie, and Rosa. None of my grandparents have college degrees, some did not finish high school. They worked agricultural and manufacturing positions, and they lived in rural South Carolina during times where a black man completing a PhD was unheard of. This dissertation is the product of the hard work they put in raising my parents and instilling the values that

contributed to my upbringing. They always encouraged me to put my education first and believed that I should take advantage of the opportunities that were not afforded to them. While three of them are not here to see me finish my Ph.D., they are always with me in spirit.

Secondly, in terms of relatives, I would like to thank my expansive family for the love and support the have showed me over my 27 years on this planet. Without my aunts, uncles, and cousins (close and not so close), I wouldn't be the person I am today. All of you own a piece of this work, because without you, I would not have completed it. A special thanks to those who did not live to see me finish this dissertation, but who would have been supporting me this whole way. Lastly, and related to my family, I want to thank everyone who supported me through my K-12 experience. While my parents and family did an amazing job raising me, they were supported by a host of coaches, teachers, family friends, and church members. I grew up in St. John AME church and that congregation has always been like family to me. I am thankful for how they have invested in me emotionally, spiritually, and physically over my life. I also want to thank the host of coaches and teachers that helped me develop my work ethic and grit.

Next, I would like to thank the people who were bought into my life through Winthrop University. I am a proud alumnus and maintain strong relationships with many of the people I came across during my 4 years in Rock Hill. First, I would like to thank the Winthrop Eagle Stem program, specifically Rachel Law and Dr. Clifton Harris. They recruited me into the program during a high school campus tour during my senior year, and without them I would not have enrolled at Winthrop. Over the course of my time at Winthrop, they both helped keep me focused on my goals and got me interested in research. Along those lines, I would also like to thank Dr. Matthew Heard and Dr. Victoria Frost, my friends and first research mentors. Looking back, I can see how much time and attention they gave me, and I will always be indebted to them

for giving me a great start in research. Further, they showed me what healthy lab dynamics and mentor-mentee relationships looked like, and that has continued to influence me today, both in terms of how I look for mentorship and how I mentor students. Luckily, our lab also had awesome undergraduates. My Frosty-Heard lab mates, Dr. Savannah Moritzky and (soon to be Dr.) Leigha Stahl, helped me stay the course and kept me motivated during our undergraduate days. It amazes me to see that we are all finishing the goals we set for ourselves back in 2015. Similarly, Winthrop's Biology faculty showed a love for research and teaching and went a long way to helping motivate me toward an academic career in biology.

I thank the Winthrop McNair Scholars program and, more specifically, Dr. Cheryl Fortner-Wood and Barbara Yeager. McNair was vital to helping me determine I wanted to get a PhD and to developing me as a scholar. Both, Barb and Dr. Fortner-Wood are vital to the success of the Winthrop program and their love and sincerity radiates in everything they do. I am happy to have been a part of the program and to have continued our relationship over the past few years. I would also like to thank my line brothers and my other fraternity brothers of Alpha Phi Alpha Fraternity, Incorporated for always being supportive, keeping me in mind, and giving me a group of high achieving young black men with whom I can walk through life with. To my line brothers specifically, I would like to thank you for always having my back and for the memories we created in college and as alumni. Lastly, for Winthrop, I would like to thank those who mentored me and helped me blossom as a leader. Over my time at Winthrop, I found myself in several offices multiple times a week to chat, ask for advice, vent, and receive pep talks. These moments were vital to my development as a student and are examples of why having good faculty and staff is so important. In particular, I would like to thank Angelo Jeter, Anthony Davis, Kinyata Adams Brown, Dr. Jason Hurlbert, and Dr. Gloria Jones.

Emory has also given me a host of supporters that have helped me develop into the person I am today. First, I would like to thank my advisor Dr. Levi Morran. Levi is the epitome of what a PhD advisor should be to a student. He is a friend, colleague, and mentor, but above all else, he is a good person. His character shines throughout his lab group and, if academia had more advisors like him, universities would be better places. Being a member of the lab has been an amazing experience and the connections I have made with undergraduates, graduate students, and post docs in the lab made it easier to come into work every day. Thanks to Michelle, (Dr.) Jenn, Liz, Emily, Sophie, McKenna, (Dr.) Kim, (Dr.) Signe, (Dr.) Amanda, Authur, Hannan, Dilys and Raythe. The same can be said for Dr. Nicole Gerardo who, while not my advisor, became an honorary one over the course of my degree due to her lab's closeness to ours. I would also like to thank my committee for the sage wisdom over the course of my degree and for the various ways each of them helped me during this process. From hunting salamanders in field ecology with Chris, to discussing South Carolina History with Lance, to Dave's insistence that I had helped Emory enough during my degree, each of you assisted in some way, and it is all appreciated it. I have also received incredible support from PBEE, both from faculty and students. My cohort has consistently achieved and helped motivate me in my studies. Dr. Kayla Stoy consistently reminds me of what a scientist should be and helps stoke my creative fires with her deep conceptual knowledge. (Soon to be Dr.) Sandra Mendiola has been a friend, a scientific peer, and a fellow BIPOC student interested in bettering the experiences of other black and brown students. In their own ways, they each have contributed to making me a better biologist.

A big thanks also goes to Dr. Amanda James, Naima Barton, and the Emory EDGE (Emory Diversifying Graduate Education) office. My work with EDGE recruiting students and working to support inclusion in graduate school has been vital to making me feel connected to

the university, and Amanda's advice and listening ear helped ground me many times. A special thanks goes out to my two adopted academic homes, the Center for Ethics, and the Institute for the Liberal Arts. Thanks go to Dr. Gerard Vong, Dr. Robyn Fivush, Dr. John Banja, and Dr. Ari Eisen. These faculty have been vital to my bioethics education, and the development of my love for it. These experiences in these centers, and their contribution to my teaching skills and experiences have been invaluable and have helped me develop as an educator and scholar. The Emory graduate community has also been great to be a part of, and the connections I have made here will undoubtedly help me moving forward. My experiences with the Laney Graduate Student Council and the Initiative to Maximize Student Development helped me find many of the friends I have made in school, and I am grateful to those groups. Thanks to (Dr.) Jamie, (Dr.) Freddie, (Dr.) Gaby (& DJ), and (soon to be Dr.) Erica, my support group during my early years of graduate school. Lastly, Emory also has given me three of my best friends and they have provided untold hours of emotional support and comradery. Taylor, (Dr.) Keith & (Dr.) Izzy have been my backbone through my PhD and have shown me what true friendship looks like. I am looking forward to our lifelong friendships and seeing how we all grow within our fields.

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CHAPTER I

Abiotic factors withstanding, much of what we observe in nature is the result of species interactions, or relationships amongst organisms of different species living in the same location (Vellend, 2010). Evolutionary change is primarily driven through these ecological interactions and thus the history of life is one of species interactions (Thompson, 1999). Ranging from wholly positive interactions where both organisms benefit (mutualisms), to those where both organisms receive negative effects (competition) (Figure 1.1), these interactions are fundamental and have long been of interest to biologists and naturalists (Darwin *et al.*, 1859; Wallace, 1864). Of these, antagonistic interactions, or those where one organism benefits and one is harmed, have often received the most attention, as all species engage in antagonistic interactions on some level. They also have a tremendous relevance to society, as antagonistic interactions drive various biological processes of interest to humans. For example, antagonistic interactions underlie the epidemiology of RNA viruses, as interactions between virus and hosts shape which mutations spread within a population (Moya, Holmes and González-Candelas, 2004). Indeed, antagonistic interactions are thought to be responsible for driving biological diversity and perhaps even the evolution of sexual reproduction itself (Hamilton, 1980; Anderson and May, 1982; Rainey *et al.*, 2000). However, direct hypothesis-testing of how these interactions proceed under different evolutionary dynamics is difficult, partially because of issues in isolating various evolutionary forces at work, especially concurrently. Thus, while experiments have been done to elucidate how antagonistic interactions are changed under various dynamics, numerous questions remain. The goal of this dissertation is to elucidate how host evolution is impacted by different evolutionary forces during adaptation in an antagonistic interaction with a parasite. I used the evolution of host defenses within a host-parasite interaction to investigate the impact of

evolutionary reciprocity and genetic architecture on adaptation. I accomplished this using experimental evolution and end the dissertation by identifying how the method can be of use in other fields of research.

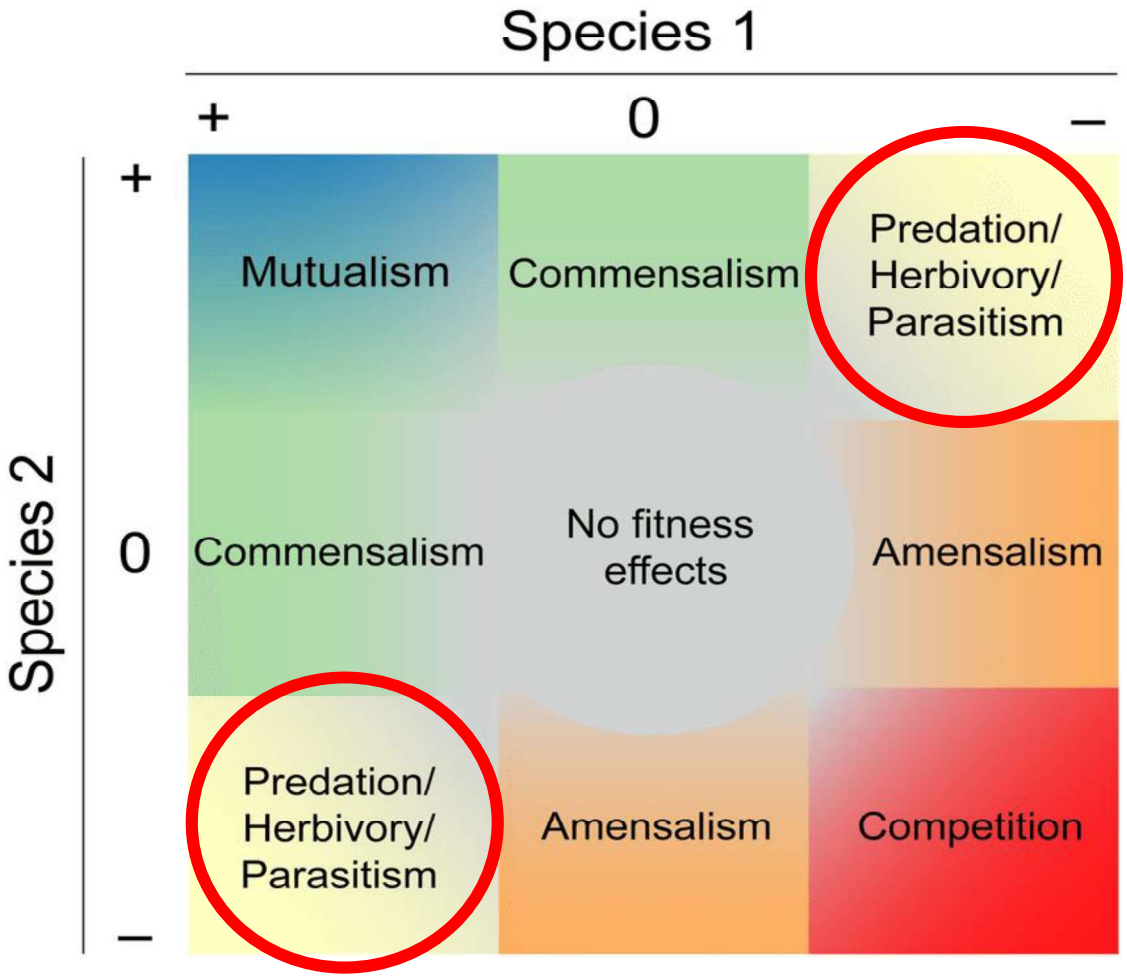


FIGURE 1.1. The Interaction Compass. A two-species interaction is illustrated with the terms defining each of the differently signed outcomes; the signs indicate individual fitness or population growth rate. A positive (+) sign thus indicates a positive effect of the interaction on the individual or population, a zero (0) sign indicates no effect, and a negative (–) sign indicates a negative effect. Moving away from the center increases the magnitude of the net effect of the interaction. Red circles indicate antagonistic interactions. **Adapted from:** (Pringle, 2016).

HOST-PARASITE INTERACTIONS & THE EVOLUTION OF HOST DEFENSES

Antagonistic species interactions encompass a wide variety of behaviors and can lead to selection on the species receiving the positive and the negative outcome. However, in no antagonistic interaction are species more closely linked than in host-parasite interactions. Parasites, organisms that live in or on their hosts and exploit them (Antonovics *et al.*, 2013), are ubiquitous in nature and exert intense selection pressure on their hosts. Due to the nature of the interaction, hosts and parasites are both under strong selective pressure to avoid, or initiate, infection (Anderson and May, 1982; Antonovics *et al.*, 2013). For example, around 40% of all animal species are parasites (Avise, Hubbell and Ayala, 2008), and organisms across the tree of life have evolved a variety of ways to avoid, resist or tolerate their parasites (de Roode, Lefèvre and Hunter, 2013). These mechanisms are collectively referred to as host defenses. However, despite the ubiquity of parasites in nature, and the benefits of host defenses, the characteristics of those defenses vary greatly over space and time (Laine, 2004) What might explain these spatial and temporal variations?

Theory suggests that host defenses will vary both in terms of the degree of defense against a given parasite (level) and in the breadth of parasites defenses are applicable against (range). Both the level and range of host defenses may be influenced by the nature of the interactions that produced them. As host population characteristics, parasite population characteristics, and environmental dynamics change, how host defenses are predicted to evolve changes as well. In particular, the level of evolutionary reciprocity within the host-parasite interaction, and the genetic architecture of the trait and the population may influence the evolution of host defenses.

EVOLUTIONARY RECIPROCITY & COEVOLUTION

Evolutionary reciprocity refers to the level of evolutionary change species are making in response to one another. In scenarios where species are reciprocally adapting, and evolutionary changes are tightly woven due to selective pressures, species are predicted to evolve differently than under other selection dynamics. For example, genetic changes within reciprocal adaptation are predicted to be less common outside of that interaction. Referred to as coevolution, these reciprocal adaptations are common throughout nature and operate in a number of crucial biological systems ranging from pollination and commensalisms to predator-prey and host-parasite interactions (Thompson, 1989). In fact, many interactions in the wild are thought to be a "mosaic" of interconnected coevolutionary hot and cold spots (Thompson, 2009). During coevolution, host and parasite populations are moving with each other over the adaptive landscape and changes within one species are tightly linked with changes in the other (Kawecki and Ebert, 2004; Thompson, 2009). Alternatively, host-parasite interactions can also be less reciprocal, especially if there are multiple parasites or hosts in the environment. This diffuse coevolution can lead to one-sided evolution, where one species A causes change in Species B, but with limited selection in species A due to B. Here, evolutionary changes between the populations are not linked and thus populations should evolve differently compared to reciprocally adapting host populations. In totality, this means that the characteristics of the defenses host populations develop in response to their parasites may vary depending on the level of reciprocity.

GENETIC ARCHITECTURE

Genetic architecture refers to the genetic effects that build and control a phenotypic character and variation in its properties (Young, 2000; Hansen, 2006). It includes any patterns of pleiotropy or epistasis, in addition to more foundational properties like allele number, the distribution of mutations, the distribution of allelic effects, and how dominance operates. In short, it completely describes the relationship from genotype to phenotype for a given trait. It can be studied at either an individual or population level. Ultimately, genetic architecture is of crucial importance during adaptation because allele effect sizes, the number of alleles conferring a trait, how that trait segregates, its epistatic effects in the genome, and how mutations and mutation frequencies act within the trait may all determine how it spreads during selection. As traits become more and more complex, it can become increasingly difficult to pinpoint which changes confer what results. Studies have suggested that the genetic architecture of a trait will also impact its evolvability, or the ability of the genetic system to produce and maintain potentially adaptive genetic variation (Hansen, 2006, 2013; Wilfert and Schmid-Hempel, 2008). In terms of host-parasite evolution and reciprocal adaptations, increasing complexity in genetic architecture may lead to more specialized responses.

EXPERIMENTAL EVOLUTION, *C. Elegans* & *S. Marcescens*

Experimental evolution is "the study of evolutionary changes occurring in experimental populations as a consequence of conditions (environmental, demographic, genetic, social, and so forth) imposed by the experimenter" (Kawecki *et al.*, 2012). In the past few decades, the methodology has risen in popularity alongside advances in genomic testing, which has allowed researchers to uncover the genetic and molecular basis of evolution. This has allowed more

direct testing of base evolutionary theory, as well as more specific research on adaptation, trade-offs accompanying evolutionary change, and the estimation of population level genetic parameters. Experimental evolution is common in a range of species including *Drosophila*, yeast, crickets, *E. coli*, *Pseudomonas*, *Daphnia*, *Arabidopsis*, and bacteriophages (Garland and Rose, 2009; Kawecki *et al.*, 2012). In this dissertation, I utilized experimental evolution in *Caenorhabditis elegans* and *Serratia marcescens* to investigate questions related to the evolution of host defenses.

Figure 1.2. | An infected Caenorhabditis elegans. A nematode infected with Serratia.

C. elegans is a bacterivorous, soil-dwelling nematode that is widely used in biological research and studies of evolution (Félix and Braendle, 2010; Frézal and Félix, 2015). It is particularly useful as a model for host-parasite interactions, as the worm gut can be colonized by a range of bacteria (Stiernagle, 1999; Darby, 2005). Studies have shown that *C. elegans* often come into contact with microorganisms in the soil and they can distinguish between bacterial species (Mallo *et al.*, 2002). Further, the worms only have innate immune systems, and they show different immune responses depending on the microorganism they interact with. As worms feed on bacteria, some survive the worm "grinder" and successfully colonize the gut (Mallo *et al.*, 2002; Schulenburg and Ewbank, 2004; Teotónio *et al.*, 2017), leading to infection and

eventually death after sufficient bacterial replication. Over time, worm populations subjected to selection from bacteria will adapt to their populations and show elevated host defenses (Penley, Ha and Morran, 2017; Teotónio *et al.*, 2017). The bacterium *S. marcescens* is an opportunistic pathogen that often causes hospital-acquired infections, but is also relatively common in the environment (Hejazi and Falkiner, 1997). Most importantly, the bacteria is known to be highly virulent toward *C. elegans* worms. Given that *C. elegans* has a short generation time (three days), can lay 300 eggs over its lifetime (Schafer, 2005), are able to survive in the thousands on a petri dish, and can be cryogenically frozen for direct comparison (Stiernagle, 1999), this makes it an ideal system to directly study host-parasite evolution. Further, the system allowed me to isolate and test specific variables as they relate to evolutionary reciprocity, genetic architecture, and the evolution of host defense.

SUMMARY OF DISSERTATION CHAPTERS

In chapter II, I investigated the influence of evolutionary reciprocity on the defense range of host *C. elegans* populations. I took *C. elegans* populations that had been evolved with *S. marcescens* SM2170 in one of three ways and tested their ability to defend against a range of *S. marcescens* strains. Crucially, the three experimental treatments differed in the level of evolutionary reciprocity within their SM2170 interaction (coevolved parasite, stock parasite, heat-killed parasite). I showed that coevolved (high reciprocity) host populations, performed poorly against parasite strains except their coevolving parasite counterparts. Further, hosts that evolved to the stock SM2170 evolved defenses generally applicable against closely related SM2170 genotypes. Lastly, hosts that evolved with the heat-killed parasite performed well against non-SM2170 related hosts. These results show that intense evolutionary reciprocity between host and parasite

can lead to more specialized host defenses and thus a narrower defense range. This work was published in *Frontiers in Cellular and Infection Microbiology* in an article entitled "Antagonistic Coevolution Limits the Range of Host Defense in *C. elegans* Populations" (Lewis *et al.*, 2022).

In chapter III, I explored the role of gene flow to either impede or facilitate adaptation during adaptation to a parasite. I took genetically identical sink populations and exposed them to either live or heat-killed SM2170 for 10 generations. In the fifth generation, each treatment received gene flow from one of six sources. Each treatment varied in whether the migrant population had previously been exposed to SM2170 (naïve or adapted), and whether they had a shared or novel genetic background. I show that populations receiving gene flow during adaptation performed better than their counterparts that received no gene flow. However, the extent to which gene flow assisted adaptation depended on both previous exposure and genetic architecture, as populations receiving gene flow from novel or previously adapted populations recorded the lowest mortality rates. Overall, these results show that gene flow during adaptation can assist host populations and that evolutionary history matters.

In chapter IV, I reviewed selection experiments and placed experimental evolution (Laboratory Natural Selection) in context with directed evolution (DE) and artificial selection (AS). I then identified the strengths and weaknesses of experimental evolution, before reviewing its history in the basic sciences, and the history of DE and AS in the applied sciences. I ended by identifying three areas where experimental evolution could be of use in creating industrial or medical products. The areas I identified were identifying mechanisms of antibiotic resistance, developing new and more efficient processes in bioremediation, and various opportunities within the field of biofuel creation. This work was published in the *Journal of Evolutionary Biology* as "Advantages of laboratory natural selection in the applied sciences" (Lewis and Morran, 2022).

CHAPTER II
Antagonistic Coevolution Limits the Range of Host Defense in *C. elegans* Populations

Reprinted material from: Lewis JA, Penley MJ, Sylla H, Duran S and Morran LT (2022) Antagonistic Coevolution Limits the Range of Host Defense in C. elegans Populations. Front. Cell. Infect. Microbiol. 12:758745. doi: 10.3389/fcimb.2022.758745

ABSTRACT

Host populations often evolve defenses against parasites due to the significant fitness costs imposed by infection. However, adaptation to a specific parasite may alter the effectiveness of the host's defenses in general. Consequently, the specificity of host defense may be influenced by a host population's evolutionary history with parasites. Further, the degree of reciprocal change within an interaction may profoundly alter the range of host defense, given that antagonistic coevolutionary interactions are predicted to favor defense against specific parasite genotypes. Here, we examined the effect of host evolutionary history on host defense range by assessing the mortality rates of *Caenorhabditis elegans* host populations exposed to an array of *Serratia marcescens* bacterial parasite strains. Importantly, each of the host populations were derived from the same genetic background but have different experimental evolution histories with parasites. Each of these histories (exposure to either heat-killed, fixed genotype, or coevolving parasites) carries a different level of evolutionary reciprocity. Overall, we observed an effect of host evolutionary history in that previously coevolved host populations were generally the most susceptible to novel parasite strains. This data demonstrates that host evolutionary history can have a significant impact on host defense, and that host-parasite coevolution can increase host susceptibility to novel parasites.

INTRODUCTION

Parasites are ubiquitous in nature and are thought to be a key factor in the evolution and maintenance of genetic diversity within host populations (Hamilton, 1980; Anderson and May, 1982; Rainey *et al.*, 2000; Thompson, 2009). Parasites can impose strong selective pressure on host populations, due to the fitness advantage experienced by uninfected or tolerant individuals, and thus select for the evolution of elevated host defense over time. Generally, natural observations have aligned with expectations, as hosts have evolved a multitude of strategies for defending against infection(Roy and Kirchner, 2000; Ellis, 2001; Lemaitre and Hoffmann, 2007; Diamond *et al.*, 2009; Parker *et al.*, 2011; War *et al.*, 2012; de Roode, Thierry and Hunter, 2013; Weiss, Bayer and Yeaman, 2014). These observations have been further supported by experimental studies, which have demonstrated the ability of hosts to evolve defense against novel parasites in experiments across various systems. Some of these systems include beetles (Bérénos, Schmid-Hempel and Wegner, 2009), birds (Bonneaud *et al.*, 2011), *Daphnia* (Duncan and Little, 2007), *Drosophila* (Kraaijeveld and Godfray, 1997), isopods (Hasu, Benesh and Valtonen, 2009), moths (Fuxa and Richter, 1989; Boots and Begon, 1993), nematodes (Schulte, Makus, *et al.*, 2010; Penley, Ha and Morran, 2017), paramecium (Lohse, Gutierrez and Kaltz, 2006) and snails (Webster and Woolhouse, 1999; Koskella and Lively, 2007). Despite the benefit of evolved host defenses and the ubiquity of parasites, natural populations experience considerable variance in levels of host defense over space and time (Allen. *et al.*, 2004; Laine, 2004).

Several mechanisms have been proposed to explain the widespread observations of variance in host defense (Koskella, 2018), including the costs associated with maintaining defenses (Sheldon and Verhulst, 1996; Strauss *et al.*, 2002; Lenski, 2007; Graham *et al.*, 2010;

Melnyk, Wong and Kassen, 2015; Cipollini, Walters and Voelckel, 2017)and parasite reciprocal adaptation (Ebert and Hamilton, 1996; Carius, Little and Ebert, 2001; Schulte, Carsten, *et al.*, 2010) as mechanisms with strong support. Another potential factor that may contribute to the temporal and spatial variance in host defense is the evolutionary history of host populations. Host defenses exist on a spectrum ranging from more general, and effective against a broad range of parasites, to more specific and tailored to a particular parasite genotype. A host population's evolutionary history with parasites may determine the degree to which broad or specific defenses are evolved or maintained. In particular, the evolution of highly specific host defenses may inhibit, limit, or alter the evolution and maintenance of more general defenses. Coevolutionary interactions can drive the evolution of highly specific host defenses and parasite infection strategies *via* reciprocal adaptation. Such specificity between host and parasite populations is known as local adaptation (Gandon and Van Zandt, 1998) and has been observed in natural and experimental parasite populations across various systems (Edmunds and Alstad, 1978; Ebert and Hamilton, 1996; Lively and Dybdahl, 2000; Greischar and Koskella, 2007; Hoeksema and Forde, 2008; Leimu and Fischer, 2008; Vos *et al.*, 2009; Morran *et al.*, 2014; Bellis *et al.*, 2021). While local adaptation is more often observed in parasite populations, host populations are capable of exhibiting local adaptation (Gandon *et al.*, 1996; Kawecki and Ebert, 2004). Importantly, host populations that reciprocally evolve in response to locally adapted parasites may also exhibit a degree of specificity in their defense (Adiba, Huet and Kaltz, 2010; Kniskern, Barrett and Bergelson, 2011; Lemoine, Doligez and Richner, 2012). This specificity in host defense may come at a cost and ultimately increase a host population's susceptibility to novel parasites. Therefore, a host population's evolutionary history with parasites may be a

factor that contributes to the maintenance of variation in host defense within and between populations.

Here, we aimed to determine the effects of evolved host defense on host interactions with novel parasites. Evolved host defenses are generally the result of coevolved or one-sided evolutionary interactions, which are predicted to produce different outcomes in terms of host defense range (Antonovics *et al.*, 2013). Given that both one-sided and coevolutionary interactions may determine the nature and specificity of host defense, it is critical to distinguish between the predicted effects of these different evolutionary histories on host defense. Coevolution can drive numerous reciprocal changes in hosts and parasites. The genotypes that evolve to confer host defense under coevolution are likely to be highly specific, diverging substantially between different host populations and providing resistance against the local parasite population (Perlman and Jaenike, 2003; Antonovics *et al.*, 2013). One-sided evolution, which can be accomplished *via* frequent infection of hosts that are incapable of transmitting the parasite (Holt and Gomulkiewicz, 1997), can favor the evolution of host resistance without permitting parasite reciprocal adaptation. The absence of a coevolutionary arms race can limit the degree of evolutionary change and divergence in the host population because evolved host defenses maintain their effectiveness over time and subsequent change is not favored. Thus, one-sided evolution is predicted to generate less host-parasite specificity than coevolutionary interactions, but still result in the evolution of elevated host defense overall.

Therefore, testing the effects of host population evolutionary history on host defense requires a host-parasite system capable of one-sided and coevolution, a known host evolutionary history, and a diverse set of parasite genotypes to assay host defense range. The free-living nematode *Caenorhabditis elegans*, and its bacterial parasite *Serratia marcescens* (G. V. Mallo *et*

al., 2002), provide a system suitable for this test. While *C. elegans* lack an adaptive immune system, their innate immune system exhibits specific responses to different bacterial species (Wong *et al.*, 2007), providing the opportunity to measure host defense range. In a previous study, obligately outcrossing populations of host *C. elegans* populations were experimentally evolved under conditions that facilitated coevolution or one-sided evolution with *Serratia marcescens* strain SM2170, or with heat killed SM2170 as a control (Morran *et al.*, 2011). The resulting coevolved and one-sided host populations adapted to their respective parasite populations, and the coevolved parasite populations showed clear signatures of local adaptation (Morran *et al.*, 2014). Control populations, as expected, did not adapt to SM2170. Thus, these experimentally evolved host populations experienced vastly different evolutionary histories with S. marcescens parasites.

In this study, we evaluated the impact of evolutionary history on the range of evolved host defense. We exposed populations of *C. elegans*, which had been previously evolved against *S. marcescens* SM2170 in three treatments (coevolved, one-sided & a no parasite control) (Morran *et al.*, 2011), to various genotypes of *Serratia* which either were, or were not, derived from SM2170. We predicted that coevolved host populations would exhibit greater specificity in their defense when compared to one-sided and control populations, and as a result the coevolved populations would be most susceptible to novel (non-SM2170 derived) parasite strains. Further, we predicted that the effectiveness of evolved host defense would generally decrease against parasite strains that were not derived from SM2170.

MATERIALS AND METHODS

Host Populations

All *C. elegans* host populations used in this study were derived from the obligately outcrossing and highly inbred PX386 strain, which is a derivative of the CB4856 strain (Morran, Parmenter and Phillips, 2009). To generate PX386, the *fog-2 (q71)* mutant allele, which prevents hermaphrodites from self-fertilizing (Schedl and Kimble, 1988), was backcrossed into an inbred CB4856 background for five generations and was subsequently inbred for ten additional generations (Morran, Parmenter and Phillips, 2009). Then five populations of PX386 were independently mutagenized with ethyl-methanesulfonate to generate genetically variable populations prior to selection (Morran *et al.*, 2011). Following backcrossing, populations were kept under standard laboratory conditions for 4 generations in order to purge the most deleterious mutations. These populations were maintained on 10cm Petri dishes filled with NGM Lite (Nematode Growth Medium-Lite, US Biological, Swampscott, MA, USA) seeded with 30μL of OP50 stored at 20°C.

The methods above were used to generate five independent and genetically unique populations. Previous experimental evolution of these *C. elegans* host populations is fully described in (Morran *et al.*, 2011). Briefly, each of the five genetically unique populations of obligately outcrossing *C. elegans* were divided into 3 treatments (one-sided, coevolved and control) and evolved with *S. marcescens* SM2170 on *Serratia Selection Plates* (SSPs) for 30 generations respectively (Figure 2.1). SSPs consist of a 10 cm Petri dish with a lawn of *Serratia* opposite a lawn of *E. coli*. Worms are placed directly on the *Serratia* lawn which ensures hosts encounter the parasite before reaching their relatively benign lab food source, OP50 *E. coli* (Morran, Parmenter and Phillips, 2009). Within the context of the experiment, *C. elegans* individuals must survive and reproduce for their offspring to be passaged to the next round of selection. Coevolved host populations are unique in that they were passaged along with parasite

populations. In these treatments, parasites were required to infect and kill a host to be passaged to the next round of selection, thus allowing for reciprocal evolution in host and parasite populations. One- sided populations were passaged using similar methods, except parasites were not passaged and a static ancestral SM2170 plated each passage. Control populations were passaged with heat killed SM2170. Following thirty generations of experimental evolution, multiple samples from each host and parasite population were frozen (Morran *et al.*, 2011). Prior to being used in this experiment, host populations were thawed and maintained under standard laboratory conditions for approximately 4 generations to permit recovery.

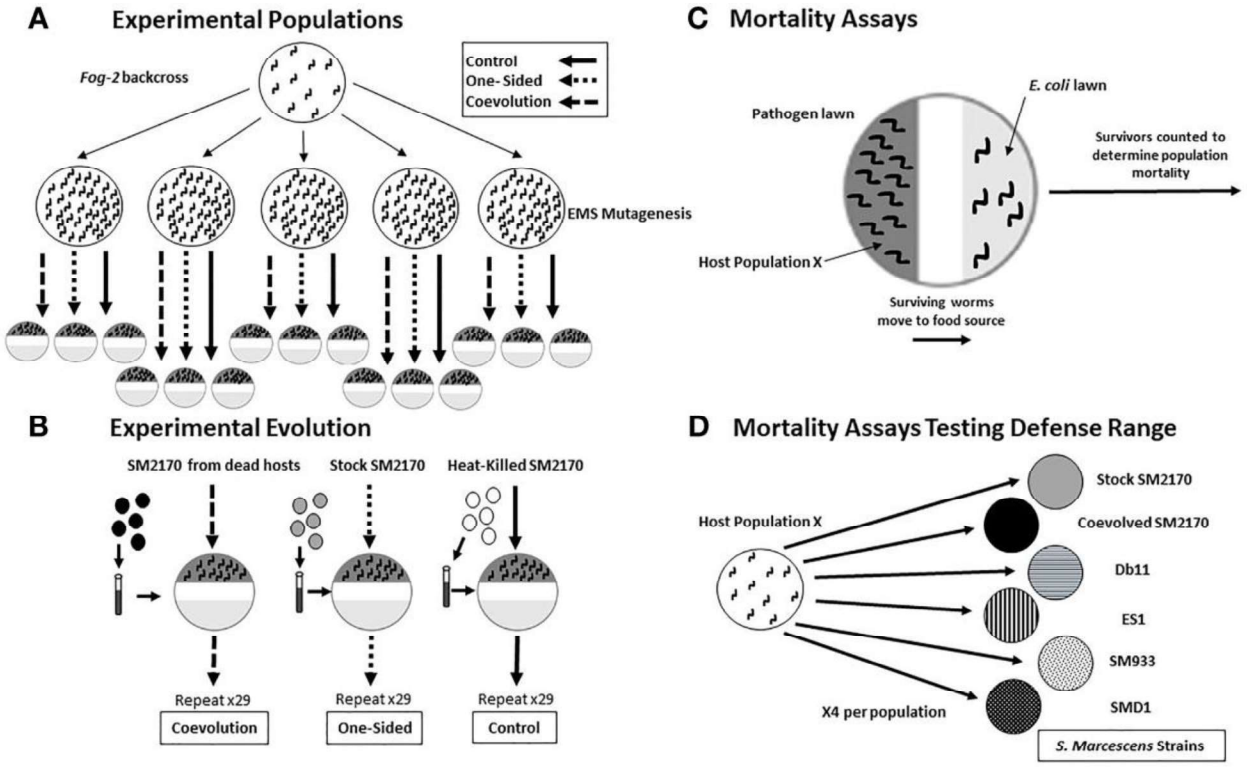


FIGURE 2.1 | Experimental Overview. (A) The fog-2 allele was backcrossed into inbred CB4856 strain C. elegans, which were subsequently mutagenized to induce variation. After several rounds of reproduction to purge deleterious mutations, worms were separated into 5 groups. Each group was then divided into 3 (control, one- sided, coevolution) and subjected to 30 rounds of exposure to their treatment parasite via Serratia Selection Plates. At the end of each round of selection, the surviving C. elegans are moved to the next plate to begin the process again. (B) In the control group, selection plates were seeded using heat killed Serratia SM2170. One-sided treatment plates received their bacterial lawns from a static stock of Serratia SM2170. Coevolving populations were seeded with SM2170 bacterial colonies removed from the guts of killed worms. (C) To determine population resistance, 200 worms were exposed to the same Serratia Selection Plate protocols as in (A). (D) To assess host range each of our experiential treatments were assayed against 6 Serratia strains. Each of the 5 replicate populations within each treatment were assayed 4 times. However, here worms were not moved to another plate, and instead were counted.

Parasite Populations

S. marcescens is an established bacterial parasite of *C. elegans* (G. V. Mallo *et al.*, 2002), with notable variance in mortality rate depending on strain (Schulenburg and Ewbank, 2004). In this experiment, populations were transferred from frozen stock to Luria Broth (LB) and grown overnight at 28°C. Colonies in LB were then used to seed 10cm Petri dishes filled with NGM-Lite and grown up at 28°C. Parasite mortality assays were completed using *S. marcescens* strains Db11, ES1, SMD1, SM2170, coevolved SM2170, and SM933.

The SM2170 genotype is highly virulent to *C. elegans* (Schulenburg and Ewbank, 2004). The ES1 strain was derived from SM2170, *via* passaging with *C. elegans* strain CB4856 under selection for increased virulence for 30 generations (Lynch, Penley and Morran, 2018). The coevolved SM2170 assays were conducted using parasite populations that were coevolved with hosts in the previous experiment and isolated after 20 passages (Morran *et al.*, 2011). Each of the five coevolved host populations thus has its own respective sympatric coevolved parasite population. Db11 is a streptomycin resistant derivative of Db10 (Flyg, Kenne and Boman, 1980) and has been shown to be moderately virulent in comparison to SM2170. The

other *Serratia* strains used for our treatments, SMD1and SM933, are strains available *via* Carolina Biological Supply (Burlington, NC). Importantly, Db11, SMD1, SM933 were not directly derived from SM2170 and thus represent novel parasite strains.

Measuring Adaptation and Defense Specificity

Here we use host mortality as a measure of host defense, and a representative measure of host fitness (Penley, Ha and Morran, 2017). Mortality assays were conducted using SSPs identical to those used during experimental evolution (Figure 2.1C), with the exception of the parasite strain used. A mortality rate was calculated for every treatment against *S. marcescens* Db11, SMD1, SM933, SM2170, and the coevolved SM2170 populations. Approximately 200 L4 *C. elegans* were suspended in M9 buffer and transferred to a lawn of SM2170 on a 10 cm Petri dish (NGM-Lite agar). The average number of individuals transferred was calculated by determining densities of *C. elegans* in the buffer and taking the mean of plated controls. After 48 hours of exposure, we counted the number of surviving worms on the plate. Mortality rates are calculated by dividing the number of dead nematodes by the total number transferred (Morran *et al.*, 2011). It is important to note that while the majority of resistant worms move from the *Serratia* lawn to the opposite *E. coli* lawn, some individuals remain in the parasite lawn and those individuals are also counted. Every population (5) in each treatment (3) was replicated 4 times per bacterial strain (technical replicates). Mean mortality rates were analyzed using a generalized linear model (GLM) fitted with a normal distribution and identity link function, testing for effects of Bacteria, Treatment (coevolved, one-sided, control), and the interaction between Bacteria and Treatment. Contrast tests were used to compare mean mortality between treatments *post hoc*. Additionally, we tested for overdispersion using a Pearson test, and did not detect a significant level of

overdispersion. Finally, we performed a binomial GLM after converting our mortality data into a binomial distribution (alive vs dead). The results of our binomial analysis were qualitatively similar to the normal GLM, thus we report the stats of the GLM fitted with the normal distribution. We used JMP Pro (v13) for the GLM analyses.

RESULTS

We first tested for the evolution of elevated host defense in our coevolved and one-sided host populations against the coevolved parasite populations derived from SM2170. We found that the coevolved hosts exhibited lower rates of mortality than the control hosts when exposed to the coevolved populations of SM2170 (Figure 2.2; x_1^2= 5.174, *P* =.023), indicating the coevolved hosts adapted to their respective antagonists during experimental coevolution. Interestingly, the one-sided hosts also performed significantly better than the control groups against coevolved parasites (Figure 2.2; x_1^2= 5.174, *P* =.023). We then found that the one-sided evolution hosts adapted to the SM2170 strain as they exhibited reduced mortality in comparison to both the control and coevolved populations when exposed to SM2170 (Figure 2.2; x_1^2= 9.798, *P* =.002). Thus, the coevolved and one-sided evolution hosts evolved greater levels of host defense and exhibited unique evolutionary trajectories relative to one another and the controls.

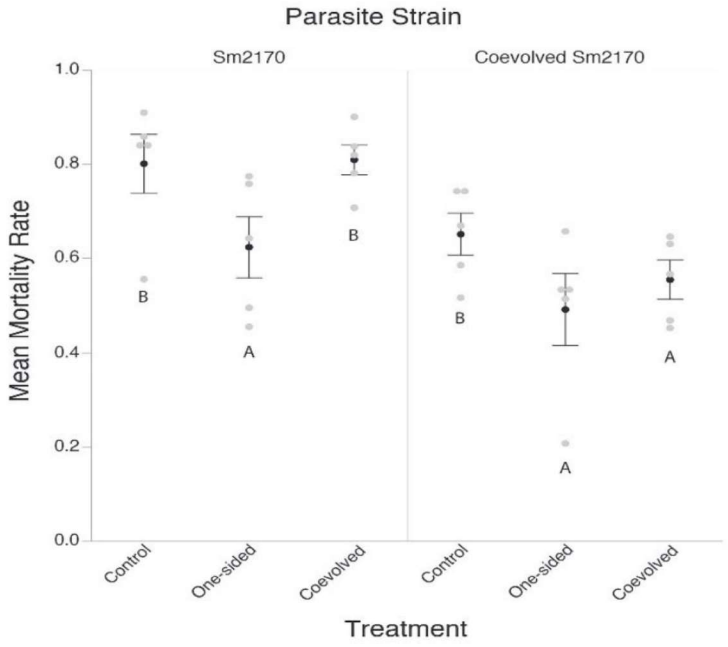


FIGURE 2.2 | Host Mortality across related parasites. For each mortality assay 200 Worms were exposed to S. marcescens for a period of 48 hours using Serratia Selection Plates. Surviving worms were counted and the mortality is expressed as (worms plated – worms counted)/worms plated). Black circles represent the average mortality rate across all host populations for each bacterial treatment group. White circles represent the average mortality rate across all replicates for one host population. Points which share letters are statistically indistinguishable from each other, and only apply within their respective column. Error bars represent standard error. Letters are differentiated by a= 0.05.

To determine the impact of host evolutionary history on the specificity of evolved defense, we compared mortality rates of three groups of hosts with different evolutionary histories (control, coevolved & one-sided) against four strains of *S. marcescens* (Db11, ES1, SMD1, and SM933). Overall, we found a significant difference in the mortality exhibited by hosts with different evolutionary histories. Specifically, coevolved hosts exhibited higher overall mortality rates than one-sided (x^2_{71}= 8.44, *P* =.004) and control (x^2_{71}= 4.85, *P* =.028) host populations (Supp. 1 & 2). However, the dynamics of host mortality responses varied significantly between parasite strains (Figures 2.2 & 2.3; x^2_{10}= 26.221, *P* =.004).

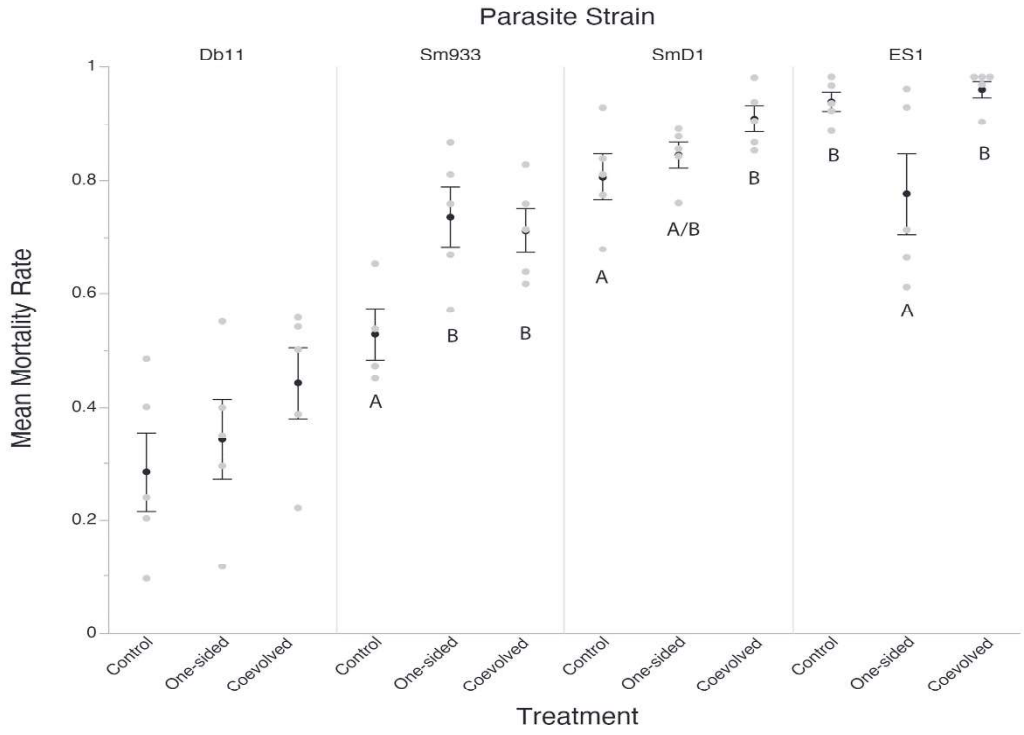


FIGURE 2.3 | Host Mortality across unrelated parasites. For each mortality assay 200 Worms were exposed to S. marcescens for a period of 48 hours using Serratia Selection Plates. Surviving worms were counted and the mortality is expressed as (worms plated – worms counted)/worms plated). Black circles represent the average mortality rate across all host populations for each bacterial treatment group. White circles represent the average mortality rate across all replicates for one host population. Points which share letters are statistically indistinguishable from each other, and only apply within their respective column. Error bars represent standard error. Letters are differentiated by a= 0.05.

In the presence of ES1, which is derived from SM2170, one-sided host populations experienced lower mortality than control and coevolved populations (Figure 2.3; x_1^2= 4.86, *P* =.028). Conversely, control hosts exhibited reduced mortality against the SM933 relative to the coevolved and one-sided evolution hosts (Figure 2.3; x_1^2= 9.68, *P* =.002). Then, coevolved hosts performed significantly worse on DB11 relative to both control and one-sided evolution hosts (Figure 2.3; x_1^2= 5.64, *P*= .018). Finally, SMD1 did not inflict significantly different mortality

rates regardless of host evolutionary history (Figure 2.3; x_1^2= 2.16, *P* =0.142). Therefore, host evolutionary history significantly altered host defense against novel parasite strains.

DISCUSSION

In this study, we investigated the effects of a host population's evolutionary history with parasites on the subsequent defense range of those hosts. Our data support the hypothesis that a host population's defense range can be altered by its past evolutionary interactions with parasites. Further, they suggest that coevolution and reciprocal adaptation can have a significant effect on host defense, beyond adaptation, to a coevolving antagonist. Among our host populations, coevolved hosts displayed elevated defense against only their co-evolved parasitic partner relative to the control hosts (Figures 2.2, 2.3). Otherwise, the coevolved host populations were overall more susceptible to novel parasites. This aligns with theory suggesting coevolution can lead to highly specific host defenses, due in part to the "arms-race" dynamics surrounding the evolution of those selected traits (Antonovics *et al.*, 2013). This idea is further supported by the coevolved hosts performance against SM2170, where they experienced mortality rates statistically similar to the *S. marcescens* naive control populations (Figure 2.2). Importantly, the SM2170 strain was the ancestral strain for each of the coevolved parasite populations, and yet the hosts maintained a very limited ability to defend against the strain. Thus, an evolutionary history of reciprocal adaptation with the parasite resulted in a very high degree of specificity over a relatively short period of time.

As expected, one-sided host populations displayed elevated defense when assayed with SM2170, the same genotype they had been exposed to for 30 generations. However, the one-sided host populations also showed elevated defense against all SM2170 derived parasite

genotypes, exhibiting the lowest mortality rates against ES1 and rates similar to coevolved hosts against the coevolved parasites (Figure 2.2). This provides further evidence that one-sided populations adapted to their parasites, and points towards those defenses as having some general applicability against similar parasite genotypes. This also aligns with theory, as one-sided evolution is predicted to favor any genetic combination in the host which provides adequate defense, rather than a genotype-specific response (Antonovics *et al.*, 2013). Interestingly, the control populations consistently experienced lower mortality rates when assayed against parasites that were not derived from SM2170 (Figure 2.3). Taken into context with the comparatively high mortality rates control populations experienced when exposed to SM2170 derived genotypes, this validates the control populations by showing a lack of defense evolution during experimental evolution and points toward a certain degree of evolutionary naiveté being beneficial for general defense. In other words, a lack of evolutionary history with parasites seems to confer an overall greater ability to defend against novel parasite strains. Therefore, host evolutionary history with a parasite, or lack thereof, can be an important factor shaping host defense range.

One limitation of this experiment is that coevolution was done in the presence of a single parasite population. This distinction is important, as research suggests infections in the wild commonly consist of multiple strains or species (Petney and Andrews, 1998; COX, 2001; Telfer *et al.*, 2008; Balmer and Tanner, 2011). Further, multi-genotype infections can alter the fitness of both hosts and parasites, thus having implications on their respective evolutionary trajectories (Alizon *et al.*, 2013; Lange *et al.*, 2014; King *et al.*, 2016). Thus, passaging host populations on single genotype bacterial lawns may have biased evolution in ways which are not applicable to some natural settings. However, while multi-strain infections are commonplace, particular strains

may still disproportionately drive the host's adaptive response, particularly those which invoke the most drastic fitness costs. Indeed, in some natural systems host defense evolution is predominantly driven by interactions with one highly virulent parasite, despite the presence of other parasites (Lively, Craddock and Vrijenhoek, 1990; Paczesniak *et al.*, 2019). An additional limitation of this experiment is that experimental evolution itself may have biased our results by relaxing the strength of non-parasite selective pressures on host populations (Kawecki *et al.*, 2012). As such, genes conferring defense may have risen in frequency which would not have in nature due to adverse pleiotropic effects. However, as such effects would be constant among all treatment groups, this still allows for the identification of relevant differences between treatments groups. Further, parasites can dictate host evolutionary trajectories through strong selection pressure. This may allow sufficiently beneficial defense alleles to increase in frequency despite other pleiotropic effects (Otto, 2004; Olson-Manning, Wagner and Mitchell-Olds, 2012), or closely linked deleterious alleles (Hartfield and Otto, 2011). An additional limitation of this experiment is that all host populations were derived from the same genetic background. As such, while treatments can respond differently to selection pressures during experimental evolution, the responses of the host populations are not fully representative of all possible genotypes. This work demonstrates that the evolutionary history of host populations can shape host defense range. However, such effects of evolutionary history may differ between host genetic backgrounds, which could account for some of the variation in host defense within and between host populations in nature.

In this experiment, we showed that evolution with a parasite can have a profound impact on the characteristics of host defenses. Specifically, the amount of evolutionary reciprocity within the interaction can influence the effective range of host defenses, with increased

reciprocity resulting in more narrow ranges (Figures 2.2, 2.3). This aligns with research showing that parasites evolved with homogenous host populations exhibited more narrow host ranges (Gibson, Baffoe-Bonnie, *et al.*, 2020; Gibson, White, *et al.*, 2020; White *et al.*, 2020). Therefore, host and parasite populations with an immediate evolutionary history of coevolution may often be constrained in genotypic space, pigeonholed by combinations of alleles that were previously advantageous but are contemporarily unfavorable. Further, host-parasite interactions may alter the evolutionary trajectories of host populations in many ways, including reducing levels of genetic variation in host populations (White *et al.*, 2021) and favoring the evolution of certain traits beyond host defense (Lively, 1987; Morran *et al.*, 2011). Generally, evolutionary history influences the evolutionary trajectory of a population because the genetic background of the population is determined to some extent by past interactions. This is made more complex due to pleiotropy and epistasis (Tyler *et al.*, 2009; Hansen, 2013). These phenomena can impact how a population adapts to a given environment (Østman, Hintze and Adami, 2012; Hansen, 2013) and determine the underlying genetic architecture of host defense (Wilfert and Schmid-Hempel, 2008; Lambrechts, 2010). Thus, host-parasite interactions can have implications that extend far beyond the direct outcome of the interaction itself.

Future adaptation may be limited by past adaptation, perhaps constraining the evolution of novel defense, or increasing rates of extinction in tightly co-evolved hosts that encounter significantly different parasites. Coevolutionary interactions can dominate a population's evolutionary trajectory as reciprocal adaptation occurs, but a population's evolutionary path can also be influenced by coevolution after the interaction has ended. Within the context of the wider phenomena of host defense varying within and between populations, and over space and time, this suggests evolutionary history does matter. It is said that host-parasite interactions reflect a

"mosaic" of coevolution, with various coevolutionary processes occurring between populations across a landscape (Thompson, 2009). It is clear that coevolution in the past can influence the composition of the present and, perhaps, future coevolutionary mosaic.

FUNDING & ACKNOWLEDGMENTS

This work was funded by the National Science Foundation (Graduate Research Fellowship Program 2017246734 to JL and DEB-1750553 to LTM). Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors(s) and do not necessarily reflect the views of the National Science Foundation.

SUPPLEMENTAL MATERIALS

Table 2.S1. Whole table summary for GLM of host mortality across all *S. marcescens* strains.

Model	-LogLikelihood	L-R ChiSquare	DF	Prob > ChiSq
Difference	65. 37	130.74	17	<.0001
Full	-76. 09			
Reduced	-10. 72			

Table 2.S2. Effect tests for GLM of host mortality across all *S. marcescens* strains.

Source	DF	L-R ChiSquare	Prob > ChiSq
Bacteria	5	121.35	<.0001
Treatment	2	9.13	0.0104
Bacteria * Treatment	10	26. 22	0.0035

CHAPTER III
Gene Flow Accelerates Adaptation to a Parasite

Reprinted material from: Lewis JA, Kandala P, Penley MJ, and Morran LT (2022). Gene Flow accelerates adaptation to a parasite. *Submitted.*

ABSTRACT

Gene flow into populations can increase levels of additive genetic variation and introduce novel beneficial alleles, thus facilitating adaptation. However, gene flow may also impede adaptation by disrupting beneficial genotypes, introducing deleterious alleles, or creating novel dominant negative interactions. While theory and fieldwork have provided insight on the effects of gene flow, direct experimental tests are rare. In this experiment, we evaluated the effects of gene flow on adaptation to the bacterial parasite, Serratia marcescens, in the host nematode Caenorhabditis elegans. We evolved hosts against parasites for 10 generations and controlled host gene flow and source population. We used source populations with different genetic backgrounds (one ~equal to the sink population and two different) and evolutionary histories (previously adapted to S. marcescens or naïve). Overall, we found that populations receiving gene flow exhibited greater increases in parasite resistance than those with no gene flow. Additionally, gene flow from previously adapted populations resulted in greater increases in resistance than gene flow from naïve populations, particularly with gene flow from novel genetic backgrounds. Overall, this work demonstrates that gene flow can facilitate adaptation. Further, the genetic architecture and evolutionary history of source populations can alter the sink population's response to selection.

INTRODUCTION

Gene flow, the movement and establishment of alleles into a novel population (Endler, 1977), is a fundamental evolutionary force. Gene flow is predicted to have a multitude of effects on the evolutionary trajectories of sink populations (Garant, Forde and Hendry, 2007). Depending on the quantity and effect sizes of the specific alleles introduced, gene flow has the potential to either facilitate or impede adaptation. At the extremes, when migrants come from a "genetically identical" population, gene flow can be effectively understood as a simple increase in effective population size (Wright, 1931) and therefore largely facilitates adaptation. At the other extreme, when gene flow comes from a different "species", gene flow generally results in dramatic fitness reductions, often via the introduction of dominant negative interactions (Turelli and Orr, 2000; Turelli, Barton and Coyne, 2001), and little opportunity at all for adaptive evolution. In between these extremes, understood outcomes often depend on precise modeling assumptions, which are rarely guided by experimental studies.

Models investigating the impact of gene flow on adaptation have often focused on the disruptive effects of gene flow in preventing local adaptation within populations, and adaptive divergence between populations (Wright, 1931; Slatkin, 1987). Gene flow works to disrupt these processes by reducing the genetic differences between populations, reducing the frequency of locally advantageous alleles, and disrupting beneficial associations between genes for adaptation or reproductive isolation (Coyne and Orr, 2004; Garant, Forde and Hendry, 2007). This is especially true in models of symmetrical gene flow where alleles move between populations that are concurrently adapting. Theory also predicts that gene flow will constrain adaptation when selection is not strong enough to maintain high frequencies of advantageous alleles due to the influx of maladapted alleles from source populations (Haldane, 1930). Empirical studies have

provided support for these ideas. For instance, experiments in insects (Ross and Keller, 1995; Nosil and Crespi, 2004; Nosil, 2009), spiders (Riechert, 1993), birds (Blondel *et al.*, 2006), mammals (Hoekstra, Krenz and Nachman, 2005; Sullivan *et al.*, 2014), fish (Lu and Bernatchez, 1999; Ferchaud and Hansen, 2016), reptiles (King and Lawson, 1995; Calsbeek and Smith, 2003), and plants (Sambatti and Rice, 2006; Papadopulos *et al.*, 2011), have shown an inverse relationship between divergence and gene flow. Some illustrative examples are studies investigating adaptive divergence in sticklebacks (*Gasterosteus aculeatus*), where researchers found that populations in lake environments connected via gene flow showed less morphological divergence than those living in isolated lakes (Hendry and Taylor, 2004; Ferchaud and Hansen, 2016). Other studies have supported the ability of gene flow to impede the process of local adaptation (Storfer, 1999; Fedorka *et al.*, 2012), like research examining phenotype mismatching in the parsnip webworm *Depressaria pastinacella*. Researchers determined that gene flow in worm populations led to increased trait mismatch frequency when grazing on allopatric wild parsnips (*Pastinaca sativa*) (Zangerl and Berenbaum, 2003). Similarly, research in wild blue tits (*Cyanistes caeruleus*) provided evidence that populations in mismatched habitats experiencing gene flow displayed higher rates of maladaptation than other populations (Blondel *et al.*, 2006).

Despite the potential for gene flow to disrupt adaptive evolution, research also suggests a more complex and multifaceted role of gene flow with regards to adaptation. Theory suggests (Haldane 1930), and experiments support, strong selection maintaining divergence and adaptive traits despite gene flow (Danley *et al.*, 2000; de Leon *et al.*, 2010; Sullivan *et al.*, 2014; Fitzpatrick *et al.*, 2015; Dennenmoser *et al.*, 2017; Kolora *et al.*, 2021). For example, field work in water snakes (*Nerodia* spp.) (Rautsaw *et al.*, 2021) and laboratory evolution of fission yeast (Tusso *et al.*, 2021) and silent crickets (Zhang *et al.*, 2021), has shown that significant adaptive

divergence is possible despite gene flow. Further, some studies indicate that gene flow has the potential to facilitate adaptation in populations, depending on the strength and direction of selection over time and space. Several mechanisms may explain the potential benefits of gene flow for adaptative change (Garant, Forde and Hendry, 2007; Tigano and Friesen, 2016). First, gene flow increases the standing genetic variation of a sink population, thus giving selection additional material on which to act (Ingvarsson and Whitlock, 2000). This is important, as adaptation from standing variation has various advantages to adaptation from new mutations, and some studies indicate that standing variation is the primary driver of adaptation in many contexts (Barrett and Schluter, 2008; Karasov, Messer and Petrov, 2010). One example of this is the genetic rescue of inbreeding *Drosophila* populations during experimental evolution, in which gene flow alleviated deleterious behavioral traits and decreased fecundity (Jørgensen, Ørsted and Kristensen, 2022). Additionally, gene flow can lead to adaptive introgression by facilitating the spread of beneficial alleles (Hedrick, 2013; Hawkins *et al.*, 2019; Taylor and Larson, 2019). Beneficial alleles may be introduced to the sink population at relatively high frequencies, thus increasing their probability of fixation relative to standing genetic variation or novel mutations. For instance, recent work investigating the spread of pesticide resistance in two-spotted spider mite (*Tetranychus uticae*) populations of Beijing suggests that introgression through gene flow is likely responsible for the spread of a major resistance mutation (Shi *et al.*, 2019).

Ultimately, the fate of incoming alleles may be determined by the genetic architectures of both the migrants and the sink population (Tigano and Friesen, 2016). Allele effect size (Griswold, 2006; Yeaman and Otto, 2011; Yeaman and Whitlock, 2011), linkage between alleles (Bürger and Akerman, 2011; Feder *et al.*, 2012), how recombination impacts traits (Samuk *et al.*, 2017), and the number of loci involved in conferring an adaptive trait (Mackay, 2001) all

contribute to the allele frequencies present within a population. Therefore, the outcome of selection in the presence of gene flow likely depends on the evolutionary history, and ultimately genetic architecture, of both the sink and source populations. Given the many differing predictions from models on the effects of gene flow on a population's evolutionary trajectory, and the dependency of those predictions on difficult to estimate parameters, we ask "can we build a living system that will experimentally determine when gene flow tends to facilitate vs impede adaptive evolution?" Here, we use the *Caenorhabditis elegans - Serratia marcescens* host – parasite system to test the effects of gene flow on adaptive evolution, as the system permits control of both gene flow and a population's evolutionary history.

In this experiment, we evaluated the effects of one-way gene flow on host adaption to a parasite. We exposed obligately outcrossing *C. elegans* host populations to either live or heat killed *S. marcescens* SM2170 and gene flow from one of several source populations. Importantly, these source populations varied in whether they had previously adapted to SM2170 and in their genetic background relative to the sink population. In a previous experiment, Morran *et al*., 2011 divided populations of *C. elegans* into isolated groups and independently mutagenized them before separating each group into two treatments. Importantly, each treatment evolved with *S. marcescens* SM2170 in a different way (dead parasite vs. static live parasite) and adapted differently. Hosts passaged against dead parasites showed no improvement in their ability to defend against SM2170, while those passaged against live SM2170 evolved to record lower morality rates over time (Morran, Parmenter and Phillips, 2009; Morran *et al.*, 2011). Combined with the shared genetic backgrounds between treatments from the same isolated groups, this allowed us to examine the effects of gene flow (received or did not), source population lineage (novel or shared background), and source population evolutionary history

(naïve or adapted), on adaptation within the sink population. By investigating one-way gene flow, rather than symmetric sustained flow between populations, we directly tested the impact of alleles entering the population and their impact on adaptation in differing source and sink genetic backgrounds. We hypothesized that gene flow would facilitate adaptation to live SM2170 and that gene flow from populations that had previously adapted would increase the rate of adaptation. Further, we expected these results to be dependent on the migrant's genetic background, with shared source and sink backgrounds providing the greatest benefits from gene flow. We hypothesized that the shared backgrounds will allow resistance alleles gained via gene flow to integrate into backgrounds more similar to those they evolved within (Griswold, 2006; Hansen, 2006).

MATERIALS AND METHODS

Host & Parasite Populations

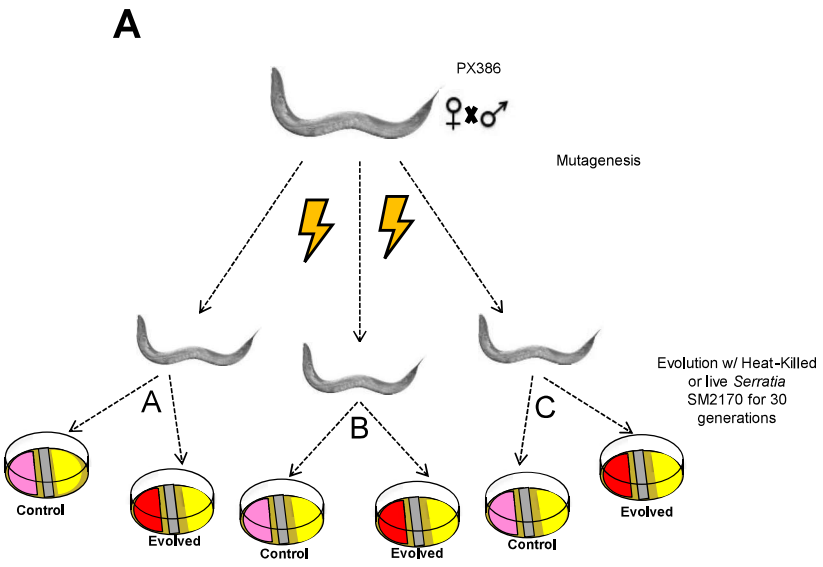
C. elegans host populations were generated from a population derived from the highly inbred and obligately outcrossing PX386 strain. Briefly, this strain was derived from the CB4856 strain (Morran, Parmenter and Phillips, 2009) and has been backcrossed with the fog-2 (q71) mutant allele, which prevents hermaphrodites from self-fertilizing (Schedl and Kimble, 1988). In a previous experiment, a population of PX386 nematodes were divided into five populations and independently mutagenized with ethyl-methanesulfonate (EMS) to generate genetically variable populations prior to selection (Morran *et al.*, 2011). Following this process, populations were kept under standard laboratory conditions for four generations in order to purge the most deleterious mutations. These populations were maintained on 10cm Petri dishes filled with NGM Lite (Nematode Growth Medium-Lite, US Biological, Swampscott, MA, USA) and seeded with

30µL of *Escherichia coli* OP50 stored at 20°C. The CB4856 strain used to create the PX386 strain was acquired from the Caenorhabditis Genomics Center (CGC, University of Minnesota).

The five independently mutagenized populations were subsequently divided into 15 host populations and exposed to either live or heat killed *S. marcescens* SM2170 across three treatment groups. These groups included one-sided evolution against a fixed ancestral SM2170 genotype, coevolution against a copassaged SM2170 genotype, or evolution against heat killed SM2170. The 15 populations were divided so that each of the five mutagenized populations was subjected to each of the three treatments. Following experimental evolution, these populations were frozen and stored at -80 °C, and then thawed for this experiment. After experimental evolution, mortality rates were generated for each of the populations by assessing their ability to resist infection from SM2170. Full statistical analysis is available in (Morran *et al.*, 2011; Penley, Ha and Morran, 2017) and is not reported here. Briefly, populations that were passaged with live SM2170 adapted to their hosts while those passaged with heat killed SM2170 did not, which is indicated by the comparatively low mortality rates recorded by the live evolution groups.

In this study, we used the populations that had been passaged with live SM2170 (SM2170 adapted) and those that had been passaged with heat killed SM2170 (SM2170 naïve). Specifically, the six groups we used represent three of the original independently mutagenized populations, each subjected to both heat killed and live SM2170 (Figure 1: B). Thus, each of the groups has a partner group with a linked genetic background but has undergone different experimental evolution with SM2170. In every treatment group, sink populations were comprised of Group 1 naïve worms; thus, shared background adapted gene flow came from the

Group 1 adapted worms. Gene flow from novel backgrounds came from Groups 2 and 3, with each having both adapted and naïve populations (Figure 1: A).



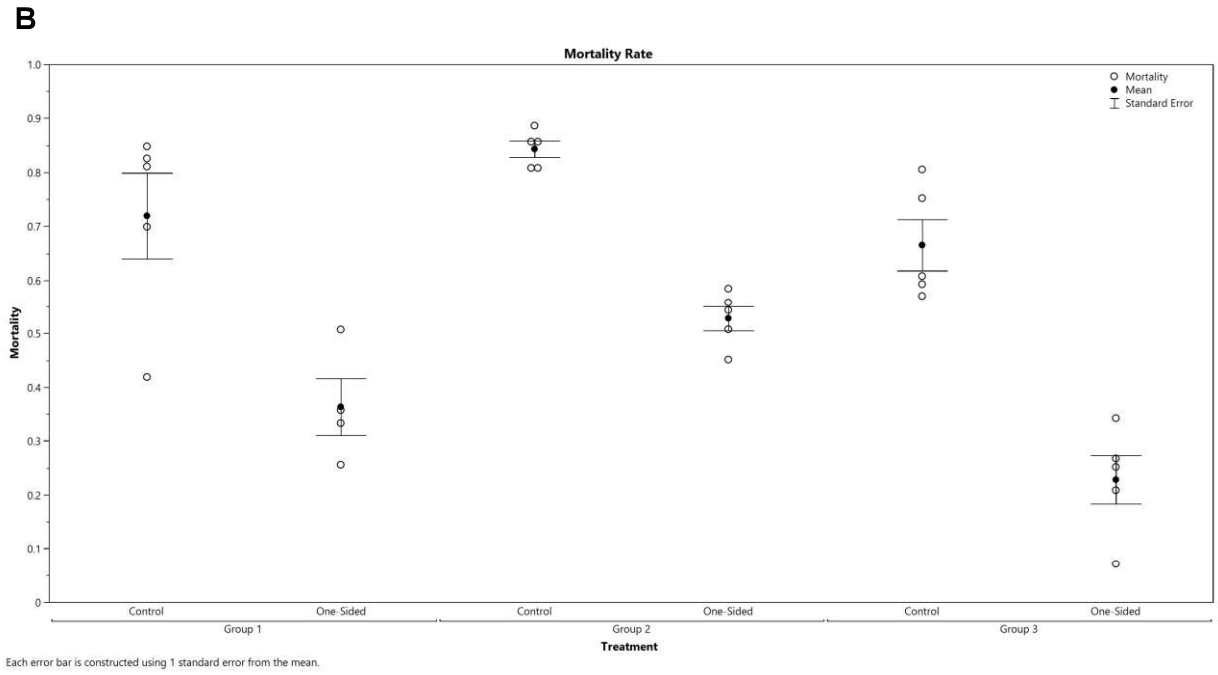


Figure 3.1 | Migrant Evolutionary History. A. Experimental evolution history of each background. Populations were divided into 3 groups and then mutagenized to infuse standing variation. Each group was then split into two treatments and exposed to either heat killed or live SM2170 for 30 generations. B. Average mortality rate for each migrant population following their previous experimental evolution. Each open circle represents a single mortality assay replicate and black circles represent the average mortality rate for the treatment population. Error bars represent the standard error of the treatment average mortality rate.

The bacterial parasite *S. marcescens* SM2170 is known to be highly virulent toward *C. elegans* hosts (Schulenburg and Ewbank, 2004). Hosts become infected via feeding on petri dishes and death typically follows in 48 hours. The strain of SM2170 used here was acquired from S. Katz at Rogers State University (OK, USA). *E. coli* OP50 is the primary food source of *C. elegans* and was acquired from the CGC. Both the OP50 and SM2170 bacterial strains were transferred from frozen stock to Luria Broth (LB) and grown overnight at 28°C; they were then used to seed 10cm Petri dishes filled with NGM-Lite and grown at 28°C overnight. Prior to each round of selection, colonies were selected from these Petri dishes and grown in 5 ml test tubes of LB for 24 hours at 28°C.

Experimental Evolution of Host Populations

Experimental evolution was conducted using *Serratia* Selection Plates (SSP) as previously described (Penley, Ha and Morran, 2017). Briefly, SSPs consist of a 10cm Petri dish filled with autoclaved NGM Lite. One side of the plate is seeded with 35µl of E. *coli* while the other side is seeded with 35µl of either live (one-sided) or heat killed (control) *S. marcescens*. 20 µl of ampicillin (100 mg/mL) was streaked across the plate between the bacterial lawns to prevent the spread of *S. marcescens* during the experiment. During experimental evolution, *C. elegans* were placed directly into the parasite bacterial lawn (alive or heat-killed) and required to

crawl through it to safely reach their food source (Morran, Parmenter and Phillips, 2009). After 48 hours, living individuals were transferred from the *E. coli* food source to a normal petri dish to build their population numbers. These plates also contained streptomycin to control the spread of *S. marcescens* and were seeded with the streptomycin resistant *E. coli* strain OP50-1 as a food source for the worms. Following three days on the dish, approximately 1000 individuals were moved from the OP 50-1 to the next round of selection on SSPs. These methods were used for each of the 70 host populations in this experiment for 10 consecutive rounds of selection (Figure 2). All host populations, except those which did not receive gene flow, received their migrants as they began their 5th passage on SSPs. During this step, only 950 individuals were moved from the last round of selection instead of the normal count of 1000 individuals, and each population received approximately 50 migrants. The number of migrating individuals was chosen to enable sufficient gene flow into sink populations to reduce the strength of genetic drift relative to selection (Hartl and Clark, 2006).

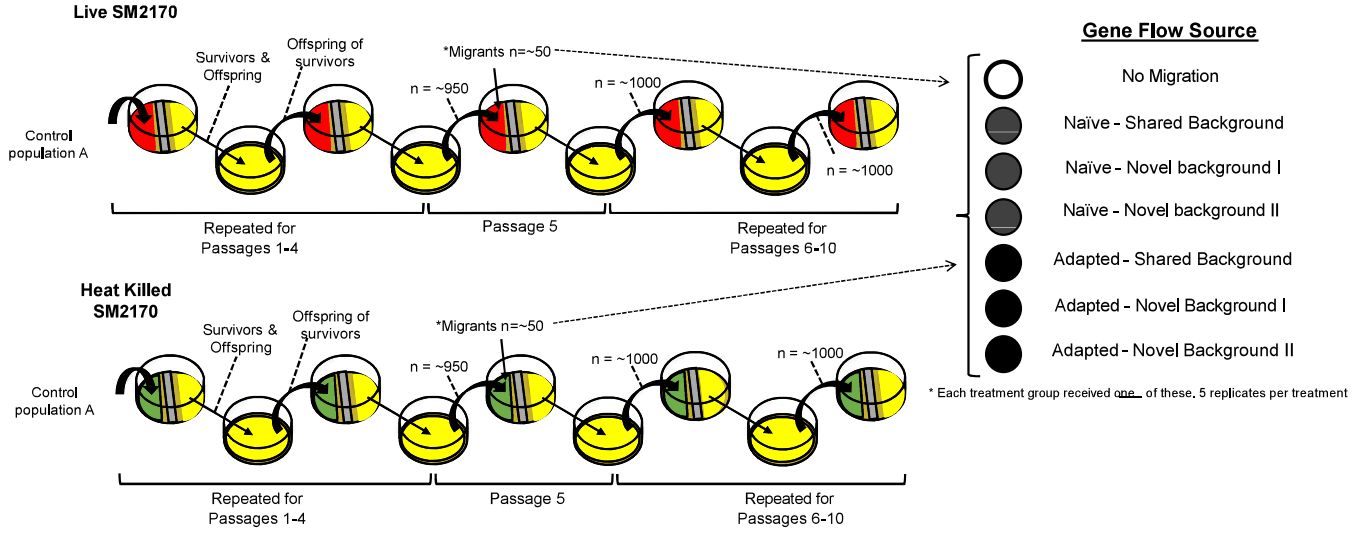


Figure 3.2 | Experimental Overview. Host populations began with ~1000 individuals from a previous experiment where they had been exposed to Heat-Killed SM2170 for 30 generations (Morran *et al.*, 2011). Populations were then passaged on Serratia Selection Plates with either live or heat killed SM2170 for 4 passages. In each round of passaging ~1000 individuals were moved randomly. On passage 5, experimental populations received 50 migrants from one of 6 source populations, with 2 groups receiving no migrants (heat-killed and live SM2170 controls). Groups receiving migrants received 950 individuals from their previous round of passaging, while control groups received the usual 1000. After passage 5, populations were passaged for 5 additional generations.

Mortality Assays & Statistical Analysis

Mortality assays were conducted following experimental evolution to determine how successfully hosts had adapted to their parasites. Mortality assays were conducted on SSPs using methods similar to those used during experimental evolution. Approximately 200 individuals were placed onto the *S. marcescens* lawn and exposed for 48 hours. Following 48 hours, living individuals were counted and the mortality rate was determined using the formula $1 - (\frac{number\ of\ living\ worms}{number\ of\ worms\ plated})$. When performing mortality assays, each of the five populations in each treatment had four technical replicates, totaling 280 assay plates between the treatment populations. All statistical analyses were performed in JMP Pro (v.16) (SAS Institute, Cary, North Carolina). Differences in mean mortality rates were compared using generalized linear models (GLM) fitted with a normal distribution and identity link function. We tested for the effects of bacterial treatment (live or heat killed), gene flow (no gene flow, adapted gene flow with a shared background, adapted gene flow with a novel background, naïve gene flow with a shared background, or naïve gene flow with a novel background), and the interaction between the two. We did not detect overdispersion using a Pearson test. We then performed contrast tests to compare differences between the groups.

RESULTS

First, we sought to investigate the impacts of the previous experiment (Morran *et al.*, 2011) on the *C. elegans* populations used in this study. This served two purposes. One, observing differences between mortality rates in the evolved and control treatments supports our use of them as "naïve" and "adapted" gene flow. Second, observing different mortality rates between background groups would support differences in their inherent resistance to SM2170, and that the initial infusion of variation via independent mutagenesis produced variable populations. Using data from Penley, Ha, and Morran (2017), we found that hosts that had been passaged with live parasites exhibited significantly lower mortality rates when exposed to SM2170 (x_1^2= 21.327, P = x < .0001; Table 1). We also found different levels of parasite resistance across the different backgrounds (Table 1), (x_1^2= 21.709, P = x < .0001; Table 1). Together, these results enabled us to use these populations to examine the impact of one-way gene flow on adaptation (Figure 1).

Table 3.1 | Statistical Values for Previous Migrant Adaptation

Effect Tested	Chi-square	Prob>Chi-square	Degrees of Freedom	Bacteria Treatment(s)
Treatment	21.327	$3.872e^{-6}$	1	Heat Killed & Live SM2170
Background Group	21.709	$1.932e^{-5}$	2	Heat Killed & Live SM2170
Treatment * Background Group	2.077	0.3540	2	Heat Killed & Live SM2170

To investigate the results of the evolution conducted within this study, we first tested for the evolution of elevated defenses in host populations exposed to live SM2170 relative to those passaged with heat killed SM2170. We found that host populations passaged with live SM2170 exhibited significantly lower mortality rates when exposed to SM2170 than did host populations which had been passaged with heat killed SM2170 (x_1^2= 7.022, *P* = 0.008.; Figure 3). This is indicative of adaptation to the parasite in our live treatments, and a lack of such adaption in our heat-killed treatments. These results support selective pressure from the live parasite as being the primary driver of host adaptation within our experimental treatments. Next, we tested the effect of gene flow on host adaptation to SM2170. We found that, across all treatments, there was no statistical difference between groups that had received gene flow and those that had not (x_6^2= 1.488, *P* =0.9603; Figure 3). However, the interaction of bacterial treatment and gene flow status was statistically significant (x_6^2= 22.278, *P* =0.0011; Figure 3), indicating that the effect of gene flow on host adaptation was context dependent. These results can be found in table 2. We then ran further contrast tests to further contextualize the relationship between gene flow status, gene flow source, and bacterial treatment.

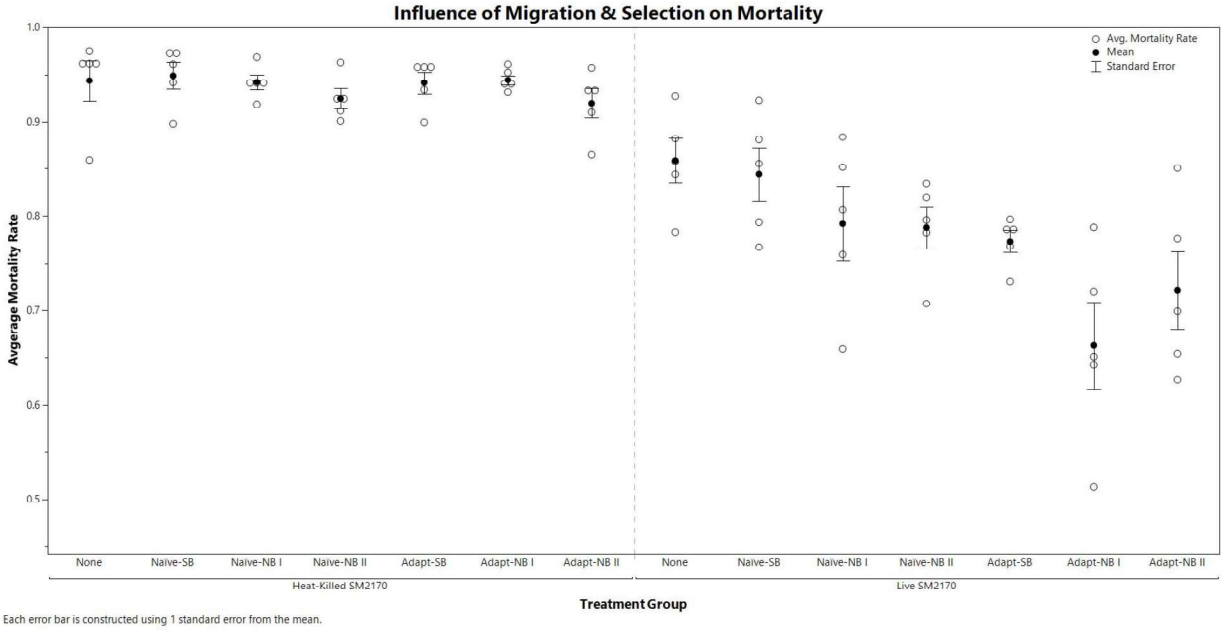


Figure 3.3 | Host Mortality Rates. For each mortality assay 200 worms were exposed to SM2170 for a period of 48 hours using Serratia Selection Plates. Surviving worms were counted and the mortality is expressed as ((worms plated – worms counted)/ worms plated). Each open circle represents the average mortality rate of 3 replicate assays for a given replicate population. Black circles represent the average mortality rate of all host populations within a given treatment. Error bars represent the standard error of the treatment average mortality rate.

To begin, we tested the impact of gene flow on adaptation in populations that had been passaged with live SM2170. We found that populations that received gene flow during exposure to SM2170 exhibited significantly lower mortality rates when compared to populations which did not receive gene flow while being passaged on live SM2170 (x_1^2= 14.345, P = x < 0.001; Figure 3). This demonstrates the ability of gene flow to facilitate host adaptation. Next, we examined the impact of gene flow source resistance on host mortality rates for sink populations passaged in the presence of live SM2170. We found that populations that received gene flow from previously adapted populations exhibited significantly lower mortality rates when compared to host populations that received gene flow from naïve populations (x_1^2= 20.798, P = x

< 0.0001; Figure 3). This is indicative of beneficial alleles being transferred from previously adapted populations, and further supports the idea that these populations evolved elevated resistance to SM2170 during the previous experiment (Morran et al. 2011). We then evaluated the impact of source population genetic background on the resulting adaptation of the sink population. We found that populations that received gene flow from sources that did not share their genetic background (novel populations) exhibited greater resistance against SM2170 than those that received gene flow from the shared background, irrespective of whether they were naïve or adapted (x_1^2= 11.505, *P* = x < 0.001; Figure 3). We further tested for differences between host populations that received gene flow from adapted populations with shared genetic backgrounds versus those that received migrants from adapted novel genetic backgrounds. Here, we found that sink populations adapted at greater rates when receiving gene flow from previously adapted source populations with novel backgrounds (x_1^2= 8.500, *P* = x < 0.0035; Figure 3). Lastly, we found that populations that received gene flow from naïve populations with novel backgrounds exhibited lower mortality rates than those which received naïve gene flow from shared backgrounds (x_1^2= 3.860, *P* = 0.0492; Figure 3). This further supports a general benefit of the variation brought in via gene flow. A summary of these contrast results can be found in Table 3.

Table 3.2. | Statistical Values for Treatment Comparisons

Effect Tested	Chi-square	Prob>Chi-square	Degrees of Freedom	Bacteria Treatment(s)
Serratia	7.022	0.0081	1	Heat Killed & Live SM2170
Gene Flow	1.488	0.9603	6	Heat Killed & Live SM2170
Serratia * Gene Flow interaction	22.278	0.0011	6	Heat Killed & Live SM2170

No Gene Flow vs. Gene Flow	14.345	0.0001	1	Live SM2170
Adapted vs. Naïve Gene flow	20.798	$5.104e^{-6}$	1	Live SM2170
Novel vs. Shared background Gene Flow	11.505	0.0007	1	Live SM2170
Naïve Shared background vs. Naïve Novel background	3.860	0.04817	1	Live SM2170
Adapted Shared background vs. Adapted Novel Background	8.500	0.0035	1	Live SM2170

DISCUSSION

In this study, we investigated the impact of gene flow and source population on the adaptation of hosts to parasites. In this experiment system gene flow facilitates adaptation to selective pressures in the environment in all conditions. However, the level of the effect, is dependent on the genetic architecture of both the source and sink populations. Host populations that evolved against live SM2170 and received gene flow of any kind experienced lower mortality rates than populations that did not receive gene flow, pointing toward a selection-dependent positive effect of one-way gene flow on adaptive evolution (Figure 3). Importantly, we also saw no effect of gene flow in the absence of live SM2170, showing that there was no general fitness benefit of gene flow without selection. This suggests the fitness increases we see in the live parasite treatments are not merely the result of an increase in effective population size (ex: reduction of inbreeding depression) but are the result of adaptation to the selective pressure of SM2170. Further, the impact of gene flow on sink population adaptation varied based on the evolutionary history of the migrants, with populations responding differently depending on whether their migrants where naive or resistant to SM2170, and if they had a shared or different genetic background. This supports the idea that gene flow is context-dependent and creates the possibility that future experiments may be able to determine when differences in genetic

architecture become sufficiently large to cause gene flow to impede, rather than universally help, adaptation (as seen here).

While differences in genetic architecture clearly mattered, in this experiment all host populations were, overall, genetically very similar. Host populations used in this experiment were all derived from a CB4856 (Hawaii) background (Morran, Parmenter and Phillips, 2009). Therefore, while EMS mutagenesis infused the populations with genetic variation, the groups started with an essentially identical background. This makes it even more notable that we recorded such defined evolutionary differences based on genetic background despite the relatively small number of genetic differences between populations. This suggests to us that more divergent backgrounds could cause a more serious impediment for adaptation. Consistent with this idea, various studies provide evidence that natural populations of *C. elegans* may commonly suffer from outbreeding depression (Dolgin *et al.*, 2007; Anderson, Morran and Phillips, 2010; Gimond *et al.*, 2013; Snoek *et al.*, 2014), suggesting that differing populations of *C. elegans* in nature may already be diverged to the point that gene flow likely impairs adaptation. Given research on outbreeding depression in *C. elegans*, and our use of obligate outcrossing populations, we may have biased our populations toward receiving a benefit from gene flow. As such, these results may be more applicable to other species of *Caenorhabditis* that outcross more frequently (Cutter, Morran and Phillips, 2019). However, most studies investigating outbreeding depression in *C. elegans* have been done in the lab and not under selection, so selection may be able to overcome outbreeding depression experienced by populations. For example, recent work investigating genetic diversity amongst CB4856 populations across the Hawaiian Islands, found evidence that the high levels of genetic diversity

within CB4856 may be due to historical gene flow with other *C. elegans* strains (Crombie *et al.*, 2019).

While models are far more common in this realm than experiments, this result is fundamentally consistent with other experiments of one-way gene flow to sink populations. A previous experiment evaluating the role of gene flow in increasing adaptive potential found that populations of *Drosophila* that received gene flow following isolation showed a 30-40% increase in trait response during laboratory evolution (Swindell and Bouzat, 2006). However, the trait in question, bristle number, is likely to be under long term stabilizing selection (or perhaps even neutral at the timescales of this experiment), and thus the *Drosophila* result may be somewhat dependent on the artificial nature of the selection. The results here suggest that gene flow leading to an increased capacity for evolutionary change in an adaptive trait (parasite resistance) is also possible and can increase the rate of adaptive evolution. For host populations receiving migrants from previously adapted populations, another mechanism to facilitate adaptation was likely the spread of advantageous alleles via gene flow (Hedrick, 2013). We observed a benefit to gene flow from adapted populations compared to those that were SM2170 naïve (Figure 3), suggesting that the resistance alleles carried by the migrants were responsible for the increased rate of adaptation to the parasite.

Conceptually, the one-way gene flow utilized here is perhaps most analogous to assisted gene flow (ASG), or the purposeful movement of gametes already adapted to an environment to populations currently undergoing adaptation to a changing environment (Aitken and Whitlock 2013). ASG has perhaps most famously been used to restore populations of the Florida panther (*Puma concolor*)(Johnson *et al.*, 2010; Hostetler *et al.*, 2013) and has been suggested as a potential technique to combat species extinction due to climate change in a range of organisms

(Aitken and Whitlock, 2013). For certain species, like long-lived forest trees, this may present the most effective strategy to mitigate species loss (Aitken and Bemmels, 2016). Resistance alleles may also be able to spread this way; however, their impact on the population will also depend on the nature of the evolutionary interaction that the population is engaged in. For example, in antagonistically coevolving systems where populations are chasing moving peaks across the fitness landscape (Thompson, 2009), interactions between genes are also important, and so genetic architecture will impact the fate of an immigrating allele (Hansen, 2006; Bürger and Akerman, 2011; Akerman and Bürger, 2014). This adds an additional layer of complexity and has been reflected in studies of gene flow in coevolving systems, as they show a multitude of effects ranging from positive to negative (Garant, Forde and Hendry, 2007). Here, in the absence of a coevolving parasite, we find a greater benefit of gene flow from novel backgrounds when compared to those with similar backgrounds (Figure 3).

One shortcoming of this study is our use of one-directional gene flow as opposed to two-way gene flow between adapting populations. In terms of its impact on variation, gene flow generally works to increase variation within populations while decreasing it between populations (Slatkin, 1987; Hendry, Day and Taylor, 2001; Lenormand, 2002; Garant, Forde and Hendry, 2007). Many of the presumed deleterious effects of gene flow on adaptation, like the breakdown of local adaptation, are dependent on the swapping of alleles between populations and a degree of environmental antagonism in their fitness effects (Dias, 1996). As such, one-directional gene flow may be biased toward positive effects during adaptation. Another related shortcoming is that gene flow only occurred once during our experiment. Populations were allowed to adapt to their parasites, received gene flow, and were subsequently allowed to adapt again. This may have allowed selection to limit the spread of maladapted alleles more effectively, thus allowing for

greater fitness benefits. Under continual gene flow, following the classic Island-mainland model, maladapted alleles may persist longer in the population, leading to less adaptation in the host population (Lenormand, 2002). However, continuous gene flow may have also added adaptation depending on the primary adaptive mechanism working in the sink population. For example, in populations receiving previously adapted migrants, a continuous flow of preadapted alleles may have caused greater proliferation of those alleles in the hosts, eventually leading to higher population fitness.

In this experiment, we provide evidence that gene flow into a population can assist during adaptation to a fixed bacterial parasite. Gene flow can assist either through the supply of greater standing variation or through the migration of advantageous alleles. Both seem to be impacted by the genetic background of the source population and its similarity to the sink population. This aligns with past research that has indicated potential advantages to gene flow during the adaptive process, both in terms of fitness and general adaptive capacity. Perhaps more importantly, it also adds to a substantial body of literature detailing the multifaceted role gene flow can play in the evolution of populations and the variety of variables that control those outcomes. Ultimately, despite the breadth of work investigating the role of gene flow in evolution, this highlights the complexity involved in its effects and the need to further elucidate its mechanisms.

ACKNOWLEDGMENTS

This work was funded by the National Science Foundation (Graduate Research Fellowship Program 2017246734 to JL and DEB-1750553 to LTM). Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors(s) and do not necessarily reflect the views of the National Science Foundation. We would also like to thank Elizabeth Chong for logistical support.

CHAPTER IV
Advantages of laboratory natural selection in the applied sciences

Reprinted material from: Lewis, J. A., & Morran, L. T. (2022). Advantages of laboratory natural selection in the applied sciences. *Journal of Evolutionary Biology*, 35, 5– 22. https://doi.org/10.1111/jeb.13964

ABSTRACT

In the past three decades, laboratory natural selection has become a widely used technique in biological research. Most studies which have utilized this technique are in the realm of basic science, often testing hypotheses related to mechanisms of evolutionary change or ecological dynamics. While laboratory natural selection is currently utilized heavily in this setting, there is a significant gap with its usage in applied studies, especially when compared to the other selection experiment methodologies like artificial selection and directed evolution. This is despite avenues of research in the applied sciences which seem well suited to laboratory natural selection. In this review, we place laboratory natural selection in context with other selection experiments, identify the characteristics which make it well suited for particular kinds of applied research and briefly cover key examples of the usefulness of selection experiments within applied science. Finally, we identify three promising areas of inquiry for laboratory natural selection in the applied sciences: bioremediation technology, identifying mechanisms of drug resistance and optimizing biofuel production. Although laboratory natural selection is currently less utilized in applied science when compared to basic research, the method has immense promise in the field moving forward.

INTRODUCTION

Empirical studies of natural selection in real time are a fairly recent development in biology (Garland and Rose, 2009). Charles Darwin co-founded the theory of evolution via natural selection, but generally believed that, apart from selective breeding, evolution was too slow to observe in real time (Darwin, 1859). However, in the last half-century we have seen a significant increase in studies which characterize evolution in real time, both in the field and in the laboratory (Reznick, Bryga and Endler, 1990; Losos, Warheitt and Schoener, 1997; Reznick *et al.*, 1997; Losos, Schoener and Spiller, 2004; Lenski, 2017). Direct observations of evolution have contributed to the shift in evolutionary biology from a historical science built on observation into a true experimental science wherein hypotheses are regularly directly tested via experimental manipulation. Laboratory natural selection, in particular, has been able to provide novel insights into several key outstanding hypotheses (Moya, Galiana and Ayala, 1995; Reboud and Bell, 1997; Burch and Chao, 1999; Ratcliff *et al.*, 2012) and illuminate the mechanisms underlying fundamental evolutionary processes (Dodd, 1989; R. *et al.*, 2006; Hollis, Fierst and Houle, 2009). Laboratory natural selection fits into a broader category of experiments we refer to as Selection Experiments, which includes all experiments that utilize selection pressure to change populations over time (Fuller, Baer and Travis, 2005; Kawecki *et al.*, 2012). This category consists of three methods: laboratory natural selection (LNS), artificial selection (AS) and directed evolution (DE) (Arnold, 1998; Fuller, Baer and Travis, 2005; Kawecki *et al.*, 2012). All three, while having commonalities, differ in specific methodological mechanisms, and those differences are imperative to identifying which questions are appropriate for each method. We define LNS, sometimes used interchangeably with experimental evolution, as the study of evolutionary changes in experimental populations as a consequence of conditions

(environmental, demographic, genetic, social, etc.) imposed by the experimenter (Kawecki *et al.*, 2012; Cooper and Cristina, 2018) (Figure 4.1). Generally, other forces like genetic drift and mutation are not excluded from operating; however, LNS methodologies are biased towards testing or observing adaptive traits and thus specifically impose natural selection (Cooper and Cristina, 2018). It is important to note that although LNS is sometimes called experimental evolution, we use LNS to specify experiments which are designed to enable selection to act as the dominant, but not sole, force.

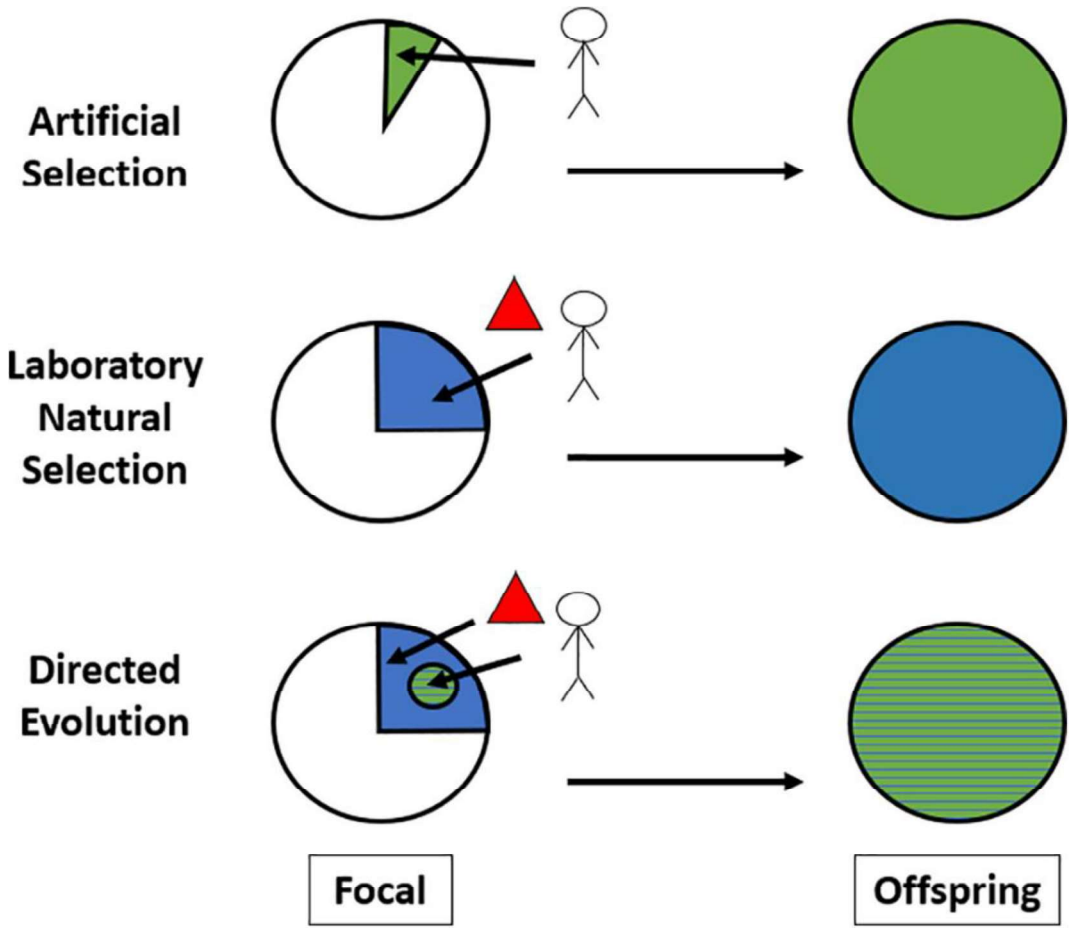


FIGURE 4.1 | Diagram of the methodological differences between artificial selection (AS), laboratory natural selection (LNS) and directed evolution (DE). Large circles represent populations, and red triangles represent selection pressure. Blue represents the portion of organisms in the population which are favored based on selection pressure, whereas green represents those selected by the researcher. In AS, the researchers determine which portion of organisms reproduce. In LNS, researchers place selection pressure on populations and the most fit reproduce. In DE, selection pressure manifests through function assays, creating a favored population (blue) and researchers determine which organisms from the favored pool will reproduce (green). Each of these methods can involve mutagenesis to infuse additional genetic variation, but this is most common in DE and LNS

AS can be defined as researcher-imposed selection, where only individuals with desired trait specifications are allowed to reproduce (Hill, 2001). AS differs from LNS in that the relationship between a given trait and fitness is determined by the experimenters, as opposed to fitness being determined within the context of the experiment like LNS. Importantly, both LNS and AS can be carried out on populations composed of natural standing genetic variation or standing genetic variation infused via mutagenesis. DE incorporates elements of LNS and AS coupled with frequent periods of mutagenesis to drive evolutionary change. Specifically, DE consists of a core three-step procedure of mutagenesis, screening/selection and researcher-imposed choice of contributing progenitor(s) for the next generation (Bloom and Arnold, 2009). A key component of DE is the repeated process of mutagenesis and identification of targets (Lutz, 2010; Cobb, Chao and Zhao, 2013), as creation of target libraries are a means to generate and test many different variant genotypes from a chosen progenitor (Cirino, Mayer and Umeno, 2003; Muteeb and Sen, 2010; Badran and Liu, 2015; Hendel and Shoulders, 2021). Thus, DE combines the selection process of LNS with the predetermined fitness relationship of AS, but fuels rapid evolutionary change by frequent mutagenesis.

Although elements could be combined across these methodologies, LNS is most unique relative to AS and DE due to the nature of differential reproduction across the three methodologies (Figure 4.1). Further, the mechanisms of selection employed by AS and DE often impose stronger selection and limit effective populations sizes relative to LNS, which can significantly alter the evolutionary trajectories of populations under each regime. AS and DE both rely on researcher-imposed truncated selection, where researchers ultimately control which individuals contribute to the next generation based on their measurement or score for some desired trait or ability. This process constitutes a form of specific selection, where all the individuals in a population whose trait does not attain a certain value are removed. In short, researchers are selecting on a specific trait towards a specific direction of phenotypic space, enforcing a predetermined relationship between those traits and fitness. This method is designed to artificially facilitate recurrent rapid selective sweeps, which can generate immediate responses to selection but will erode standing genetic variation and reduce effective population sizes. Importantly, population level, between-lineage variation, is lost even with the recurring mutagenesis in DE. Mutagenesis within DE is an effective means of exploring numerous allelic variants originating from a single genotype or lineage, but this process is not sufficient to maintain large effective population sizes. We note that although these definitions are useful for a comparison of selection experiments, the margins of these methodologies can blur in particular systems and contexts. However, the usefulness of these definitions lies in their ability to serve as basic conceptual cores which illustrate the primary mechanisms behind the evolutionary changes in each experimental method.

Within the context of AS and DE, fitness is essentially a binomial distribution, where organisms either meet the experimenter determined threshold for a specific trait or they do not.

Both AS and DE may involve direct experimenter intervention or experiments may rely on automated systems to carry out selection. Regardless of the mechanism, the experimenter determines the relationship between a given trait and an organism's fitness. Conversely, fitness within LNS is much more continuous, and individuals are selected within the experiment based on relative fitness, regardless of which trait(s) confer that fitness. Importantly, this means populations are being selected on based on their ability to reproduce under particular stimuli rather than their absolute value for a given trait, and regardless of which traits are driving increased fitness. The population genetic consequences of LNS can mirror those of DE and AS under very strong selection; however, LNS is often conducted under somewhat more biologically relevant parameters that mitigate the strength of selection and the subsequent population genetic consequences relative to DE and AS. Ultimately, the endpoints of both DE and AS are inherently limited by the vision of researchers and their ability to predict an optimal evolutionary path. Although DE and AS are likely to be more efficient than LNS in terms of the magnitude of fitness change per generation, at least initially, LNS is comparatively unencumbered and trades efficiency for evolutionary freedom. This trade-off permits greater opportunities for neutral evolution, increased opportunity for drift and mutation to operate, greater liberty for populations to explore the adaptive landscape and ultimately, an increased probability of evolving novel adaptive phenotypes relative to AS and DE (Kauffman, 1993; Gavrilets, 2004; Poelwijk *et al.*, 2007; Bloom and Arnold, 2009).

Laboratory natural selection, AS and DE have all been utilized in multiple fields of the natural and physical sciences, but one key discrepancy exists regarding their usage. LNS is comparatively underutilized in the applied sciences. AS and DE have been mostly used for applied purposes since their inception, with examples like animal and crop domestication

(Clutton-Brock, 1995; Bruford, Bradley and Luikart, 2003; Zohary, Hopf and Weiss, 2012; Wilkes, 2014), and more recent applications like directed enzyme evolution (Arnold, 1998). In fact, evolutionary biology as a whole was once viewed as a field with relatively little non-academic usefulness except for crop and animal improvement (Bull and Wichman, 2001). Clearly that view has proven to be inaccurate over the last several decades (Crandall *et al.*, 2000; Fuller, Baer and Travis, 2005; Garland and Rose, 2009; Davies and Davies, 2010; Hawkins *et al.*, 2019; Jiahui Chen *et al.*, 2021). Nonetheless, some methodologies in evolutionary biology have not yet been fully utilized in applied biology.

In contrast to AS and DE, LNS has primarily been used by researchers in the basic sciences to answer conceptual questions, like the evolution of multicellularity (Ratcliff *et al.*, 2012), or the evolutionary basis of ageing (Rose, 1984). Although some of this underutilization can be attributed to the history and development of each method, there are multiple niches LNS could fill in the applied sciences, which include bioremediation, biofuel production and the identification of drug targets. We contend that LNS is currently underutilized in applied science studies and that by focusing predominantly on basic rather than applied questions we are potentially missing insightful results. Here, we illustrate the strengths and weaknesses of LNS and briefly cover a few notable selection experiments. Further, we detail the main advantages LNS has over other selection methodologies with regards to applied science and address three potential future applications.

WHY IS LABORATORY NATURAL SELECTION ADVANTAGEOUS FOR APPLIED SCIENCE?

As with any method or technique, LNS has strengths and weaknesses that need to be considered before usage (Boxes 1 and 2). Many of these attributes help determine which systems and questions are suitable for the technique and will provide insight into the value of the methodology for applied research. Several reviews have been written on LNS and evaluated its strengths and weakness, both in general and in particular systems (Fuller, Baer and Travis, 2005; Buckling *et al.*, 2009; Burke and Rose, 2009; Garland and Rose, 2009; Dunham, 2010; Kawecki *et al.*, 2012; Teotónio *et al.*, 2017; McDonald, 2019; Bram *et al.*, 2022) Those reviews provide greater detail on some of the concepts mentioned in Boxes 1 and 2.

Although many applied problems could be addressed to some extent by AS and DE, LNS is uniquely suited to provide powerful and unique responses. This can be attributed to two key characteristics and consequences of its methodology: the maintenance of greater genetic and phenotypic diversity in populations and exploring the fitness landscape via neutral and near-neutral mutations. These characteristics give LNS the opportunity to explore possibilities that are generally inaccessible to other selection experiment methodologies.

[Note: In this print boxes appear in text. In publication, boxes are separate figures and are not a part of the main text of the document.]

BOX 4.1 | Strengths of Laboratory Natural Selection

Experimental Control of Isolating Variables. In LNS researchers typically isolate variables, which allows single factors to be evaluated individually, or in combinations with other factors systematically. By "simplifying" the system, LNS practitioners are able to understand the importance of individual aspects to the dynamics of a whole system, and test for synergistic or antagonistic effects in multivariate experiments. When a product is the end goal, isolating variables can allow for efficient directional selection where populations adapt in response to specific pressures.

Population Size & Replicates. Stochasticity is inherent to evolution. Many processes which drive evolution, like genetic drift and gene flow, have effects that scale inversely with population size (Gillespie, 2004). The potential effects of these stochastic processes on small populations, when compared to larger populations, can dramatically alter the evolutionary trajectory of those populations over time. Such stochastic events have the power to alter experimental data, and the potential to misrepresent evolutionary mechanisms when comparing them to natural populations (particularly large natural populations). LNS can control for these issues, to an extent, by utilizing model systems with very large population sizes and minimizing the role of stochastic forces in the experiment via randomization. Additionally, researchers can manipulate the impact stochastic processes can have on the population by directly manipulating population size and testing the robustness of results across population sizes.

'Fossil' Records. Natural fossils can give important information about historical distributions of organisms and morphology changes over time. However, data from fossils is limited due to incomplete records and the inability to determine details related to molecular, behavioral and social processes. In many systems, LNS practitioners can create detailed 'fossil' records via cryogenic preservation of ancestral populations. These techniques are common with multiple systems including *Caenorhabditis* nematodes (Stiernagle, 1999; Teotónio *et al.*, 2017), *Escherichia coli* (*E.coli*) (Lenski *et al.*, 1991; Elena and Lenski, 2003; Blount, Lenski and Losos, 2018), various species of yeast (Zeyl, 2006; Dunham, 2010), and multiple insect species (Leopold and Rinehart, 2010; Štětina *et al.*, 2018), including limited success with *Drosophila* (Steponkus *et al.*, 1990; Peter *et al.*, 1992; Koštál *et al.*, 2016). Populations can be thawed and used for comparative assays and can be "replayed", giving context for odds of certain events and the role of contingencies. This method was used in the LTEE to determine the role of contingencies in CIT+ evolution (Blount, Borland and Lenski, 2008) (Box 3).

Detailed Genomics. Rather than using techniques post hoc to determine genetic changes that happen in a population, via LNS researchers can completely sequence focal, intermediate, and ending populations (Charlesworth *et al.*, 2000; Elena and Lenski, 2003; Kacar *et al.*, 2017). This allows detailed information on gene frequency changes and, when combined with cryopreservation of populations, can uncover the exact sequence of mutations or gene changes that lead to novel or improved phenotypes over the course of the experiment. This can be especially advantageous when attempting to determine the levels and impact of epistasis and hitchhiking, or non-additive mutation buildup over time.

Well characterized Model Systems. Many of the model systems used in LNS are widely used across biology and are well characterized. Many techniques have already been developed in other disciplines and are freely available. This makes voyaging into a new system more assessable for those new to LNS or LNS veterans who wish to utilize new systems. Many are classical systems like *Drosophila* or *E. coli*, which are used in a wide variety of fields and support extensive networks of researchers. For example, the nematode *C. elegans* is used in many areas of biological inquiry and is well characterized in many respects including genomic data, behavioral and social interactions, lab methods and have diverse strains available (Stiernagle, 1999; Teotónio *et al.*, 2017).

BOX 4.2 | Weaknesses of Laboratory Natural Selection

Isolating Variables & Population Size. In many studies, researchers attempt to uncover the processes that govern biological mechanisms or systems. Thus, it can be important that researchers consider all factors affecting a system and how those factors can interact in synchronization. Most experimental environments are typically not equivalent to a microcosm of true conditions, meaning researchers could miss key factors. This is especially true in when researchers are unsure which dynamics are truly influential. Likewise, while researchers can attempt to minimize stochasticity by using large population sizes, for some organisms, experimental population sizes can never approach those seen in nature. This can alter effects of drift and response of selection, making it more difficult to illuminate natural dynamics. This was seen in the case of DDT resistance in *Drosophilla* where laboratory studies supported polygenic effects, but wild resistance was linked to genes of large effects (Schmidt *et al.*, 2017).

Time Scale. Generation time, defined as the average time between generations in a population or the average age of reproduction in individuals, is extremely important in evolutionary biology. Heritable traits being disproportionally transferred to offspring is the heart of evolution by natural selection, and generation time can determine how quickly changes take place. While evolution can be observed in real time over relatively short timescales, many processes may need longer time to operate. This is especially true when considering the amount of time hypothesized to accompany processes like speciation and macroevolution. Even comparatively small changes in populations, like the creation of novel genotypes, can take thousands of generations to produce (Rozen and Lenski, 2000). When comparing this amount of time with the average length of many research studies, it is easy to see the unattractiveness of experimental evolution in many contexts. The constraints of time can also lead to bias in which organisms are suitable to study certain phenomena. Generation time tends to bias studies toward those with shorter average lifespans and generation times like bacteria, phages viruses and fungi. While these may yield more data, it may be more difficult to extrapolate results to more complex organisms. Further, the operating costs of certain systems may make long-term studies prohibitive.

Role of Fortuity. Mutations provide the mechanism through which raw genetic material is altered for natural selection to create disparities in fitness. While mutation rates may increase or decrease between different portions of genomes, there is inherent stochasticity in which loci incur mutations and the nature of those mutations. This is especially true with respect to multiple mutations in a single individual. Due to the nature of the mutational process and the potential for interaction between mutations, via epistasis and non-additive effects, the appearance of novel genotypes may rely on extremely rare sequences of events. Importantly, these effects are more likely to impact asexual species due to clonal interference (Muller, 1964; Gerrish and Lenski, 1998).

Adaptation, Isolating Selection Pressure, & Laboratory Conditions. Many of the organisms utilized in LNS have been bred in laboratory for generations and may have adapted to laboratory conditions (Kawecki *et al.*, 2012). Laboratory-induced pressures like social or sexual behavior changes due to confined spaces or numerous other laboratory conditions can impact results significantly and must be considered (Kawecki *et al.*, 2012). Examples include density dependent mating increase in *Drosophila* (Williams, Rose and Bradley, 2004) and infection multiplicity from contamination (Ebert and Mangin, 1997). This can be problematic when applying laboratory results to the field. Finally, testing fitness in experiments is typically difficult as fitness is multifaceted and hard to generalize and measure (Rausher, 1992; Orr, 2009; Wagner, 2010).

Maintenance of diversity

In both AS and DE, traits are evaluated by experimenters and subsequently selected for propagation based on trait measurements. The end goal of this methodology is to constantly drive the trait towards a particular intended value or magnitude. This means that any variation which arises in the population is quickly eliminated unless it meets one of two criteria: (1) A variation that moves the trait or assay value in the desired direction and with sufficient magnitude or (2) a variation that is selectively neutral and stochastically arises in an individual who also has a variation meeting criterion 1. This paring process generally involves exceptionally strong selection and can functionally dispose of the majority of de novo neutral or nearly neutral variation, as screening is biased towards mutations of immediate and large effects. Although DE does often include mutagenesis to replenish and/or expand upon, a population's standing genetic variation, the de novo mutations only build off a single or small group of genetic backgrounds. DE experiments seldom use methodologies which allow mutants to contribute to the next generation in a manner proportionate to their fitness (Bloom and Arnold, 2009). Therefore, variation is highly restricted, and many neutral allelic combinations are not maintained from generation to generation.

Conversely, LNS is typically designed to impose moderate to strong selection across a gradient of fitness values, which is permissive to any individual that is sufficiently fit to persist or produce offspring that persist. By often imposing relatively weaker and less specific selection relative to DE and AS (yet still stronger than what is encountered in nature), LNS permits the maintenance of larger effective population sizes and thus greater overall potential for evolutionary change. The maintenance of neutral or non-additive mutations in particular is important, as those mutations may serve as intermediate steps towards complex novel

phenotypes which require some degree of historical contingency in the genome (Blount, Lenski and Losos, 2018). In the context of the Breeder's equation (Falconer and McKay, 1996), AS and DE can impose large selection differentials, which can generate rapid and substantial responses to selection. However, such intense selection can also significantly reduce genetic variance across the genome within a population and impede subsequent responses to selection by limiting the heritability of traits (Lande, 1979).

Exploring the fitness landscape

Quantitative traits can be viewed on a fitness landscape, which can be heuristically represented on a topographical map. All possible genetic combinations which confer the trait are visualized on the *X*- and *Y*-axes, and the overall fitness conferred by specific genotypes correlates to the height of the peaks, or depths of valleys, on the *Z*-axis (Wright, 1932; Svensson and Calsbeek, 2012). In the event, the starting population is not proximate to the maps global peak, relying on strong specific selection as occurs with AS and DE, can prevent populations from achieving global peak fitness. This is because movement between peaks likely involves mutations beyond those with immediate fitness benefits. For populations traversing the landscape, the relatively weaker selection used in LNS can permit greater exploration than specific selection by allowing mutations which are effectively neutral to persist. Ultimately, greater exploration of the landscape may result in the evolution of greater mean fitness, as populations have the ability to evolve towards global fitness peaks as opposed to local fitness peaks (Figure 4.2). Conversely, the stronger and more specific selection in AS and DE can restrict access to evolutionary pathways by selecting for strong immediate effects and purging variation (Bloom and Arnold, 2009). Therefore, AS and DE may ultimately limit the universe of possible phenotypes, whereas

LNS provides researchers with much greater leeway to explore genotypic and phenotypic space as means to identify global fitness maxima. When conducting evolution for the purposes of creating a defined product, whether industrial, medical or agricultural, reaching these maxima can lead to increased efficacy and thus greater utility.

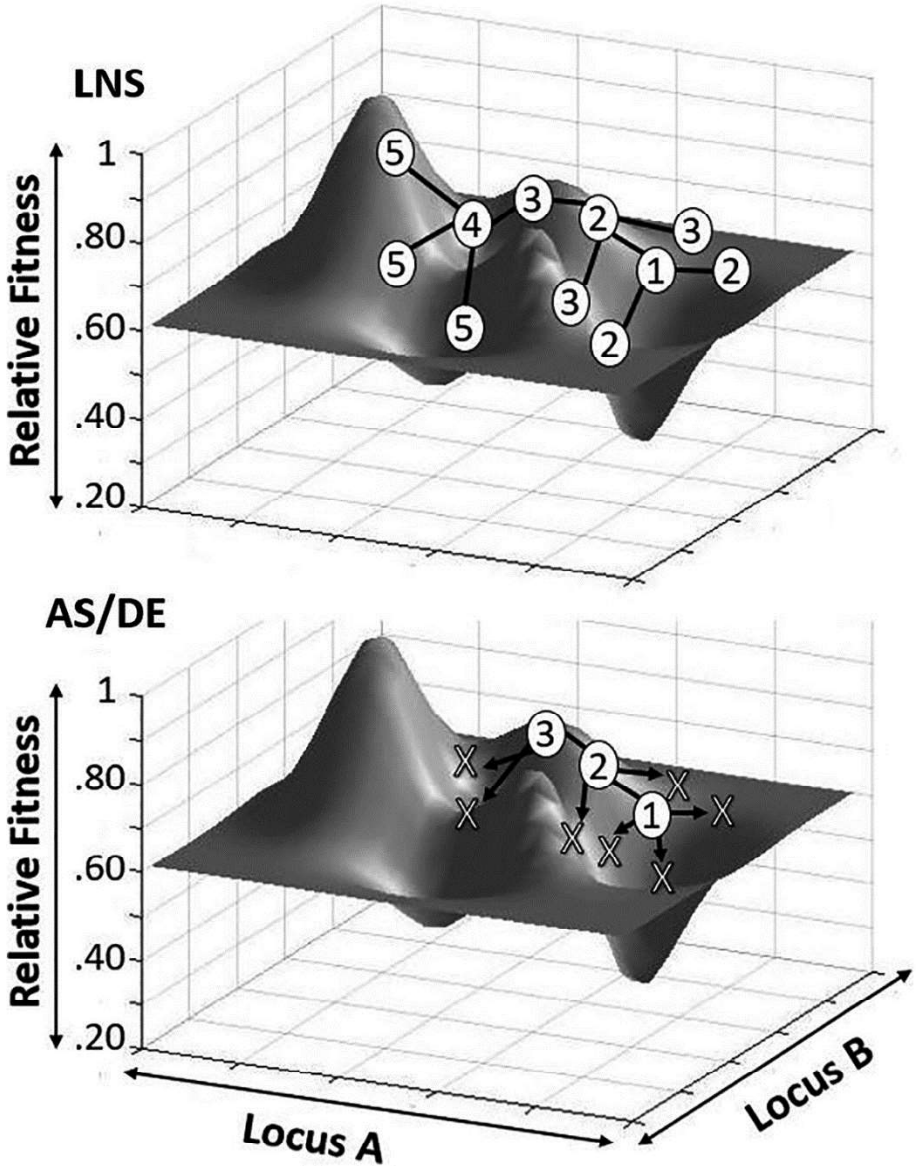


FIGURE 4.2 | Evolutionary trajectories for each of the 3 evolution experiment methodologies. Xs represent evolutionary dead ends imposed by the experimenter, whereas numbers represent generations passed under the respective protocol. In AS and DE, existing traits (point 1) are directionally selected to climb that traits fitness peak. With AS using controlled breeding strategies from *de novo* or existing variation and DE explicitly using artificially manipulated variation. In both cases, the trait being selected for climbs the peak it currently rests on and cannot navigate up the global peak unless it starts there. In LNS, traits can move from local peaks to the global peak via mutation and/or drift, as long as those intermediates are not at a significant fitness disadvantage while traversing the area between peaks. In LNS, all dead ends are due to fitness disadvantage, not experimenter-imposed selection. Figure made using MATLAB 8.0 and Statistics Toolbox 8.1, The MathWorks, Inc., Natick, Massachusetts, USA

The importance of historical contingencies

A clear example of the importance of neutral intermediate steps and the traversal of the fitness landscape is the evolution of the CIT+ phenotype in the Long-Term Evolution Experiment (LTEE). Briefly, the LTEE is an ongoing experiment consisting of 12 (initially) genetically identical populations of *Escherichia coli* (*E. coli*) which have been passaged thousands of times in the past 30 years (Lenski *et al.*, 1991). It is one of the most well-known long-term experimental studies and has generated a number of insightful findings (Box 3). CIT+ refers to a novel phenotype which is able to metabolically utilize citrate present in the culture medium (Blount, Borland and Lenski, 2008). The evolution of the CIT+ phenotype is perhaps the most impactful finding of the LTEE, as it is among the best experimental evidence for the role of historical contingency in evolution.

Historical contingency refers to evolutionary outcomes which are affected by past events that seem to be inconsequential (Blount, Lenski and Losos, 2018). Data have shown that the CIT+ utilizing phenotype required multiple intermediate steps that individually conferred little to no fitness benefit. Further, these mutations accumulated over thousands of generations within a single flask population. To date, no other direct routes to CIT+ have evolved in the other *E. coli*

populations, and subsequent experiments have shown that frozen populations from the CIT+ flask which have been reanimated from different time points have different propensities for developing the phenotype (Blount, Borland and Lenski, 2008). Considering the various other evolution experiments which have generated parallel mutations in identical populations (Cooper *et al.*, 2014; Lind, Farr and Rainey, 2015), this suggests that other mutational paths to CIT+ are highly unlikely to occur and/or extremely complex. LNS allowed populations to traverse neutral space and reach a presumably distant novel phenotype. Had Lenski and his team decided to artificially or directly evolve populations for citrate usage, relying on specific selection, then CIT+ would likely have never evolved. Given that advantageous mutations are generally thought to be rare (Eyre-Walker and Keightley, 2007), populations may routinely find themselves separated from novel phenotypes by neutral fitness space. Thus, LNS may generally lead to more novelty by allowing populations to accumulate these, not only moving in fitness space, but also by building contingencies.

PAST AND CURRENT USES OF LABORATORY NATURAL SELECTION

Before examining how AS and DE have been used in applied science, and projecting where LNS may be advantageous in that realm, it is important to grasp the history of LNS in its own right. Perhaps the first documented LNS took place in the 1870s, when cleric William Dallinger corresponded with Darwin about his ongoing culture experiments with replicate microbe populations and their ability to adapt to warming temperatures (O'Malley *et al.*, 2015). While not LNS, two of the first experiments studying selection in real time were the 1896 Illinois Corn Experiment (Hill and Caballero, 1992) and a study by W.F.R. Weldon on selection in the wild in estuarine crabs (Weldon, 1901). These were followed by numerous other studies in the early to

mid-20th century (Falconer, 1992), which all contributed to the study of evolution in real time becoming more mainstream. Experimental testing of evolution is now so commonplace that it can be done in primary school biology laboratories and undergraduate teaching laboratories with relatively simple equipment (Krist and Showsh, 2007; Plunkett and Yampolsky, 2010; Fonseca *et al.*, 2012). However, LNS did not develop into a legitimate methodological approach as quickly as other selection experiments. The pioneering work of scientists in the latter half of the 20st century helped to legitimize the method and helped it rise in popularity within the field of evolutionary biology (Lynch, 1980, 1994; Rose, Passananti and Matos, 2004; Rose, 2005). In the past three decades in particular, LNS has experienced a dynamic increase in both the number and profile of studies which rely on the technique. With the rise of genetic methods making, it less expensive to gather population-level genomic data and the increase in the speed of the process itself, more and more precise evolutionary studies are possible. Prior to these sequencing techniques, it was difficult to pinpoint the underlying genetic changes in adaptation studies. Researchers can now evolve and resequence populations, providing numerous practical advantages when compared to the methods traditionally used in ecology and evolution (Schlötterer *et al.*, 2015). Further, genomic techniques can allow for more complex studies using genomic markers, as has been demonstrated in field studies of plant pathogens (Zhan and McDonald, 2013).

Much of the usefulness of LNS can be seen in its wide applications in the basic biological sciences, as it has been used to study a diverse array of biological questions. LNS has provided insights into many fundamental evolutionary processes that would have been difficult to unveil using other experimental methodologies. For example, researchers were able to evolve several characteristics hypothesized to be required for the emergence of multicellularity while

conducting LNS in yeast (Ratcliff *et al.*, 2012). The evolution of symbioses has also been investigated using LNS, as researchers have developed novel symbioses in the mouse gut using the typically pathogenic yeast *Candida albicans* (Tso *et al.*, 2018) and with legumes using the plant pathogen *Ralstonia solanacearum* (Marchetti *et al.*, 2010). The genetic consequences of colonization and its ability to facilitate evolutionary divergence were famously shown in the *Anolis* lizards of the Caribbean Islands (Losos, Warheitt and Schoener, 1997), and recent studies have been able to link specific mutations to phenotypic divergence in wild mice (Barrett *et al.*, 2019). Researchers have also elucidated the benefits of sexual reproduction using LNS, demonstrating that mutation accumulation in RNA viruses produced Muller's Ratchet dynamics (Chao, 1990) and that coevolving parasites favor biparental reproduction over uniparental reproduction (Morran *et al.*, 2011). Indeed, even fundamental mysteries like ageing have been evaluated using LNS, as researchers experimentally increased late-life mortality in *Drosophila* (Rose *et al.*, 2002). Finally, LNS has shown that rapid adaptation is plausible in natural populations (Reznick *et al.*, 1997). A more comprehensive list of findings can be found in (Kawecki *et al.*, 2012). Studies have also sought to evaluate long-term evolutionary effects using experiments which have been ongoing for hundreds of generations like the Park Grass experiment (Silvertown *et al.*, 2005; Silvertown *et al.*, 2006), long-term *Drosophila* experiments (Archer *et al.*, 2003; Phelan *et al.*, 2003; Burke *et al.*, 2010) and the previously discussed LTEE (Box 3). More recently, LNS has also been implemented via digital organisms using software like the AVIDA system (Lenski, Winkworth and Riley, 2003; Misevic, Lenski and Ofria, 2004; Abi Abdallah *et al.*, 2020). Undoubtedly as research continues to progress LNS will be employed in more basic science studies within these fields and others not listed.

BOX 4.3 |

Brief recap of the Long-Term Evolution Experiment (LTEE) with methods and main findings

The Long-Term Evolution Experiment, otherwise known as the LTEE, is a long-term experimental evolution project which has been mainly overseen by evolutionary biologist Richard Lenski at Michigan State University. The experiment consists of 12 genetically identical populations of *Escherichia coli* (*E. coli*) (Lenski *et al.*, 1991), which have been passaged thousands of times in the past 30 years. In that time, researchers have been able to observe and record data on evolution over the course of over 60,000 generations, along with a detailed and expansive 'fossil' record (Good *et al.*, 2017). Creating over 65 peer reviewed articles, the LTEE has allowed researchers to study multiple elements of evolutionary theory and dynamics. Arguably, the most important thing accomplished by the LTEE has been showing the feasibility and usefulness of experimental evolution (Fox and Lenski, 2015). The LTEE has been covered extensively in both academia and general science media; therefore, we only provide a brief recap of the methodologies and the main findings of the experiment.

Methodologies. The experiment was started with 12 genetically identical populations of *E. coli*, each in individual containers. Populations only reproduce asexually, have no known plasmids, and no viable prophage, all important details to ensure any changes are due to mutation, drift, and natural selection. Every day (22–26 h) 1% of each population is propagated via transfer using standard techniques. Every 500 generations (~75 days) population samples are frozen in a −80°C freezer. Fitness assays are done on evolved populations every 500 generations and compared to ancestral populations (Lenski *et al.*, 1991). This also allows populations to be "re-animated" from previous time points for further experimentation.

Main Findings. Over the course of the experiment, all populations showed fitness increases compared to the ancestral strains, with rapid increases taking place within the first 20,000 generations (Lenski, 2010). Half of the populations have evolved DNA repair defects which have increased the rate of mutation in those populations (Sniegowski, Gerrish and Lenski, 1997). However, even with this increased mutation rate, Lenski estimates that only 10–20 of the millions of mutations occurring over the first 20,000 generations reached fixation in those population (Lenski, Winkworth and Riley, 2003). All populations have evolved increased cell sizes which are associated with expression of a gene that is advantageous under the conditions of the LTEE (Philippe *et al.*, 2009). All populations have experienced a degree of specialization to the glucose medium in the experiment and now have reduced ability to grow on alternative sources compared to the ancestral strains. Population 2 evolved two distinct variants identifiable through colony morphologies, each with an advantage in varying stages of experiment transfers and co-exist with each other (Rozen and Lenski, 2000). Around generation 33,000 population 3 evolved the ability to utilize citrate present in the medium (CIT+), drastically increasing the growth rate of the population. Upon further experimentation, mutants were found as early as generation 31,500. Using the cryopreserved populations, experimenters "replayed" evolution and found that cells isolated from after generation 20,000 have a much higher chance of evolving the CIT+ phenotype (Blount, Borland and Lenski, 2008). This is considered among the strongest experimental evidence for the importance of historical contingencies in evolutionary history (Blount, Lenski and Losos, 2018). The importance of historical contingencies was also illustrated in LTEE populations via their evolution for antibiotic resistance, showing that populations do not reach the level of antibiotic resistance their ancestors had originally (Card *et al.*, 2019, 2021). More recently, fitness assays undertaken early in the LTEE have been redone to assess their repeatability, with more recent data sets aligning well with the original relative fitness values measured (Barrick, Deatherage and Lenski, 2020).

PAST ARTIFICIAL SELECTION AND DIRECTED EVOLUTION EXPERIMENTS

We have, thus far, described the historical and contemporary applications of LNS primarily in the basic sciences. Here, we turn towards the history and contemporary usage of AS and DE within the realms of applied science. This serves to contextualize the place of selection methodologies in the broader realm of methods in applied biology and provide illustrative examples of selection experiments.

Artificial selection has been taking place since the beginning of domestication and human transition to agriculture from nomadic lifestyles (Bhargava and Srivastava, 2019). The method works by removing natural selection pressures, like response to predation or fecundity, and instead allowing humans to dictate which traits are favored. Unknowingly in some instances and consciously in others, our ancestors began to artificially select organisms based on traits which benefited humans. Early examples include the domestication of crops like grain and maize (Zohary, Hopf and Weiss, 2012; Wilkes, 2014), and various species of farm and pet animals (Diamond, 2002; Driscoll, Macdonald and O'Brien, 2009; Larson and Fuller, 2014). Charles Darwin famously used domestication and breeding as major lines of evidence for natural selection in "The Origin of Species" (Darwin *et al.*, 1859)and subsequently conducted artificial selection experiments with pigeons (Secord, 1981; Bartley, 1992).

AS is the oldest and most well studied of all the selection experiments and has been discussed in depth in numerous reviews (Falconer, 1992; Hill and Caballero, 1992; Brakefield, 2003; Fuller, Baer and Travis, 2005). Many of these highlight the methods key strengths, including ease of implementation and relatively straight forward methodology. At its core, it only requires a sufficiently heritable trait that can be altered in a population over time by controlling which parents contribute to the next generation. One useful example of AS is the

Illinois Long Term Selection Experiment for Grain Protein and Oil Concentration (ILTSE), which is now one of the longest running experiments in any discipline (Moose, Dudley and Rocheford, 2004). The project sought to test whether selective breeding could produce distinct strains of maize, and strains with altered kernel chemical composition (Hopkins, 1899). Now with over 100 years and 100 generations of experimentation, the experiment has shown the power of long-term selection experiments as tools of both basic and applied biology.

The experiments began with the analysis of 163 ears of corn, which were screened for oil and protein concentration, and divided into 4 groups based on those analyses. Those groups have been subjected to recurrent directional selection every year since World War II (Moose, Dudley and Rocheford, 2004). Populations now span the extremes of kernel chemical composition, with some measuring over 20 standard deviations from the mean in the positive and 4 standard deviations in the negative for kernel oil content. Furthermore, strains also exhibit much higher levels of genetic variation than expected (Dudley and Lambert, 2003). The ILTSE has been studied heavily, especially among geneticists, plant breeders and evolutionary biologists. Results have been used to inform quantitative genetics applied to plant breeding strategies and to develop knowledge on some of the physiological determinants of kernel composition (Goldman, Rocheford and Dudley, 1993; Below *et al.*, 2010). The experiment also has had numerous important contributions to the fields of plant breeding and genetics (Jones, 1927; Crabb, 1947; Alexander *et al.*, 1967; Hymowitz *et al.*, 1974; Moreno-Gonzalez, Dudley and Lambert, 1975; Dudley, 1994; Lambert, Alexander and Mejaya, 2010). Lastly, the project has shown that (1) AS can lead to trait values beyond the extreme ranges of the initial starting population, and (2) fairly extreme phenotypic variation can be selected for in crops, which both have potential application

in studies seeking crop improvement in various characteristics via breeding (Tracy *et al.*, 2004; Floros *et al.*, 2010).

In contrast, DE can be seen as a combination of LNS and AS. Like LNS, researchers manipulate conditions to invoke an evolutionary response, but like AS, they then choose individuals from the favored subpopulations to reproduce based on a desired trait value. In terms of current applications, DE has made headlines in biotechnology via the development of enzymes for industrial and therapeutic uses (Wang *et al.*, 2021), leading to the 2018 Nobel Prize in Chemistry. The method has also been applied to DNA and RNA among other systems (Cobb, Si and Zhao, 2012). Rather than relying on traditional methods to create new molecular products, directed enzyme evolution combines induced recurrent mutation and selection assays to optimize function over time. In regard to creating new proteins, there are two major considerations: the nature of folding and the subsequent function. A functional protein can lose its function or be impaired by mutations that not only target the enzymatic domain, but also other domains that contribute to proper folding. Conversely, novel mutations may improve the function of a protein by altering the manner in which it folds. Rather than relying on projecting which steps will be advantageous, the combination of creating a mutant library and selectively screening has proven to be more effective and less time-consuming method for generating beneficial proteins (Romero and Arnold, 2009).

The process begins with the replication of DNA sequences that encode a desired functional protein. Many copies of that sequence are created via replication in microbes, and from there, sequences are changed via mutagenesis or recombination. Those microbes then read the DNA and make new proteins based on the mutated sequences. The resulting proteins are then assayed for function and researchers choose which proteins will contribute to the next generation

from the pool of successful "parents" (Dalby, 2011). Through reiterating this process multiple times and starting with proteins which are good candidates to respond to less complicated changes, researchers have been able to use this "random uphill climb" strategy to optimize protein function. Since its inception, the process has led to the creation of new proteins which have applications in an array of industries (Molina-Espeja *et al.*, 2016). Thus far, the largest successes using DE have been improving the binding affinity of antibodies for therapeutic usage and altering substrate specificity of currently used enzymes to make them more effective (Hawkins, Russell and Winter, 1992; Toscano, Woycechowsky and Hilvert, 2007; Shaikh and Withers, 2008).

More recently, DE has been used to increase the efficacy of bacteriophages through repeated rounds of mutagenesis and characterization of the mutation patterns which arise (Favor *et al.*, 2020). Researchers utilized an evolution platform called CAVE which uses iterative mutagenesis, physical characterization, and genomic analysis to steer evolution of a desired trait. After 30 rounds of selection with a thermal-selection filter, researchers were able to generate mutant libraries with highly increased thermal tolerance. The conceptual motivations behind the iterative mutagenesis have also been combined with LNS in laboratory to speed up dynamics in real time. This was accomplished via the use of a mutator strain of *Aeromonas veronii* in a zebrafish host–microbe system to investigate the role of extra-host factors in the evolution of host–microbe interactions (Robinson *et al.*, 2018).

We note that the use of mutagenesis or mutator strains permits DE to explore the fitness landscape associated with a certain trait more thoroughly than AS, and potentially LNS as well. Mutagenesis libraries, by definition, represent a large proportion of the genetic landscape within a few allelic changes of the progenitor strain. Therefore, DE is a highly effective means to refine

or modify a known trait, yet LNS has the potential to be a more effective method to solve applied problems involving complex traits or for discovering novel beneficial traits. Another consideration is that, in selecting for ability under a stimulus rather than absolute trait value, LNS limits the success of individuals with negative pleiotropy. This could assist or hinder populations depending on where they are on the fitness landscape. Overall, DE and AS have made significant contributions to applied biology. But, LNS can add to the utility of selection experiments in the applied sciences. Going forward, we focus on current areas of scientific inquiry in the applied sciences which are well suited for LNS, either alone or in collaboration with other selection techniques.

POTENTIAL APPLIED NICHES FOR LABORATORY NATURAL SELECTION

The potential of LNS in the applied sciences is vast. As opposed to AS and DE, LNS methodologies are more likely to be successful in creating complex novel phenotypes due to the nature of selection (Figure 4.1). In the context of applied biology, this could allow for the evolution of phenotypes which are inaccessible to other selection methodologies and thus products which may not have been generated (Figure 4.2). Although LNS may require more replication and likely generations of experimental evolution, it may often be a more comprehensive approach. Recent work has discussed the potential role applied evolutionary biology could play in addressing global issues related to agriculture, industry and the health of humans, animals, and ecosystems (Carroll *et al.*, 2014; Sandberg *et al.*, 2019; Matthews *et al.*, 2020). We assert that LNS can fill a niche alongside other approaches of applied evolutionary biology in addressing these issues. Here, we highlight three potential areas of research where

LNS could make tremendous strides in applied biology: identifying mechanisms of drug resistance, biofuel development and evolving organisms for bioremediation.

Mechanisms of resistance & identifying drug targets

The misuse and overuse of antibacterial drugs have caused a surge in drug-resistant bacteria in the past half-century (Read, Troy and Silvie, 2011). Indeed, some pathogen strains have become almost entirely resistant to traditional drug treatments which were effective less than two decades ago. Multiple studies have identified drug resistance as one of the biggest challenges of the future (Neu, 1992; Gary, 2008; Laxminarayan *et al.*, 2013; Ventola, 2015). The rise of drug resistance, while a public health and medical issue, is one created by well-understood host–pathogen interactions, which has led to rapid and intense evolutionary change. Although changes in how we prescribe antibiotics can slow the rise of resistant bacteria, it is unlikely to rapidly undo years of evolutionary change. Thus, researchers are tasked with creating new drugs to replace or compliment those currently in use. However, efforts to synthesize new drugs must be combined with effective treatment regimens which are capable of stopping the spread of resistance (Bush *et al.*, 2011). One strategy to help contain the emergence of resistance is the use of combinational therapies, where two or more drugs are used together to increase their efficacy (Tyers and Wright, 2019). This can involve either a multidrug cocktail (two antibiotics or an antibiotic and adjuvant) or different drugs given in a sequential pattern. Combination therapies are being pursued as treatments for numerous diseases, ranging from cancer (Bozic *et al.*, 2013; Mokhtari *et al.*, 2017) to COVID- 19 (Asakura and Ogawa, 2020; Lauriola *et al.*, 2020), and multidrug combinations have been used to limit the spread of antibacterial resistance with some

success (Joshi, 2011). Using LNS, we can develop combinational drug treatments which can help turn the tide, or at least buy time, in the war against drug-resistant bacteria.

Antibiotic resistance can emerge quickly, arising within the course of one infection in some cases (M. *et al.*, 2007; Lieberman *et al.*, 2011; Eldholm *et al.*, 2014). Resistance can be intensified by antibiotic-driven selection pressure on populations of pathogens, causing a rise in frequency among those with mutations which confer fitness advantages in the face of the antibiotic (Davies and Davies, 2010). Continual exposure to the antibiotic can result in selective sweeps and higher prevalence of resistance, progressively rendering treatments less effective. Combination therapies are thought to be effective because mutations conferring resistance to one drug may not be sufficiently advantageous when evolving in a multidrug environment (Mouton, 1999), generally analogous to the cost of resistance (Andersson and Levin, 1999). By changing the fitness landscape populations are traversing, researchers can constrain, or even bias, certain evolutionary paths. An example of this is the use of drugs which impart collateral sensitivity, whereby resistance to one drug confers sensitivity to the other (Barbosa *et al.*, 2017). Indeed, combinational therapies have even been explored as a potential avenue to reverse existing resistance by exploiting various interactions between drugs (Baym *et al.*, 2016; Baym, Stone and Roy, 2016).Conducting LNS with medically relevant microbes could be an immensely powerful tool in the creation of combination therapy protocols, as accurately mimicking clinical conditions may result in similar evolutionary outcomes. This would allow LNS to have more predictive power in comparison with other selection methodologies. Further, identifying these mechanisms via LNS can help better inform which combinations of drugs should be prescribed for the given mutations present in a patient.

To identify the specific mutations which confer resistance, microbes are repeatedly exposed to an antibiotic, and surviving individuals are sequenced and/or assayed after each round. Experiments using this, or similar methodologies, to identify resistance genes or constrain their emergence, have become more commonplace as genetic tools have become more available and higher throughput (Wong, Rodrigue and Kassen, 2012; Kim, Lieberman and Roy, 2014; Fuentes-Hernandez *et al.*, 2015; Yen and Papin, 2017; Santos-Lopez *et al.*, 2019; Hansen *et al.*, 2020). One example of note is the use of a bioreactor and repeated antibiotic exposure to identify resistance conferring mutations in *Pseudomonas aeruginosa* (Mehta, Prater and Shamoo, 2018), an opportunistic pathogen with resistance to multiple antibiotics, which the CDC has identified as a serious threat (CDC, 2013; Murray *et al.*, 2015). Researchers developed an experimental bioreactor meant to mimic the environment of clinical adaptation, and progressively exposed susceptible *P. aeruginosa* populations to colistin, a last resort drug for Gram-negative bacterial infections (Tamma, Cosgrove and Maragakis, 2012). This adds to a body of work which has attempted to replicate bacterial habitats more accurately and conduct LNS outside of the traditional serial flask transfer method (Zhang, Qiucen *et al.*, 2011; Toprak *et al.*, 2012; Baym *et al.*, 2016; Baym, Stone and Roy, 2016). Highly favorable bioreactor conditions were maintained to avoid unwanted adaptive mutations and samples of the entire population were collected everyday (26 days) for deep metagenomic sequencing.

Using this methodology, researchers were able to generate resistance mutations similar to those found in clinical settings, including a hypermutator phenotype (Hammerstrom *et al.*, 2015), and trace multiple adaptive trajectories within the population. The ability of LNS to explore the fitness space of resistance more fully is a clear advantage and one that is not possible with other selection methods. Furthermore, experiments have shown that environments can impact the

topography of fitness landscapes (Flynn *et al.*, 2013; Ogbunugafor *et al.*, 2016; Li and Zhang, 2018), which support studies showing different evolutionary dynamics under different treatment parameters during antimicrobial evolution (Palmer and Kishony, 2013; Baym *et al.*, 2016; Baym, Stone and Roy, 2016; Maltas, Krasnick and Wood, 2020). Laboratory experiments like the previously mentioned resistance study (Mehta, Prater and Shamoo, 2018) may thus be more likely to achieve resistant phenotypes similar to those seen in patients, as they can simulate these conditions. Following this rationale, LNS can also be conducted *in* vivo, as has been done with malaria in mouse models (Mackinnon and Read, 2004; Barclay *et al.*, 2012; Acosta *et al.*, 2020). Being able to determine precise evolutionary trajectories and molecular basis of resistance shows the potential advantage of LNS in this realm of research.

Biofuel optimization

The past few decades we have seen substantial growth in sustainable fuel research. Much of this growth has been driven by two main lines of thinking. One is that fossil fuels are finite and our exploitation of them inevitably will end at some point (Höök and Tang, 2013). Secondly, the large increase in greenhouse gasses driven by fossil fuels has caused large scale environmental damage including climate change and ocean acidification (Doney *et al.*, 2009; Pachauri *et al.*, 2014). In the search for reliable sustainable fuel sources, some have proposed the use of carbon neutral biofuels to curb our petroleum appetite in the short term and potentially replace it totally in the long term. Biofuels are liquids, gas or solid fuels predominantly produced from biomass, which is material originating from plants or animals that is not used for food (Demirbas, 2008). Biofuel production utilizes enzymes to break down plant material and convert it into fuel. Commonly, starches in the plant are converted to glucose and then fermented to make ethanol.

One difficult portion of the process is that in many crops like corn, glucose is difficult to separate from fibers in the plant due to their resilient nature (Schubert, 2006). Separating out the sugar requires treatment with acids or other potentially environmentally harmful processes. Another issue is that in many countries, farmland is limited and crops which could be used for food are instead used to generate fuel. Further, many biofuel products are less energy dense than petroleum and current infrastructure is not built for these fuels (Arnold, 2008). Circumventing these difficulties may require growing plants with more easily assessable glucose stores, utilizing more efficient industrial processes and growing plants dedicated to fuel production.

Laboratory natural selection has the potential to improve the biofuel industry by addressing and improving upon many of these ideas and working in collaboration with other applied strategies like genetic engineering (Snow and Smith, 2012). Growing crops with more easily assessable glucose stores (i.e. weaker cellulose structures (Lynd, 2017) could be accomplished via induced mutations and subsequent selection, or by exerting selection pressure to encourage directional selection for increased glucose. Crops with richer stores would reduce the overall area of farmland needed to produce the same yield of fuel, assuming they do not require substantially more resource investment. Alternatively, LNS could be used to create microorganisms with more efficient enzymatic processes which may increase overall yields when converting plant matter. Similar to the evolution of a citrate utilizing phenotype in the long-term evolution experiment, providing a niche within the experiment which favors bacteria with more efficient processing mechanisms may create opportunity for evolutionary change. Both marine and terrestrial plant resources can be subjected to these methodologies (Gaurav *et al.*, 2017). Moving away from more traditional plant fibers and glucose, terpenes, aromatic compounds typically found in plant resin have been put forward as a genetically manipulatable

and readily available alternative (Mewalal *et al.*, 2017). Another possibility for biofuel production would be utilizing fuel sources from genetically engineered microorganisms.

One fuel of note is butanol, which was proliferating prior to the rise of petroleum in the mid-1900s and has received attention again more recently (Rathour *et al.*, 2018; Xue *et al.*, 2019). The production process relies on bacteria to create butanol via metabolic processes, but current output efficiency is not practical for wide scale industrial use. Using LNS, researchers can breed microbes with higher efficiencies in butanol production. Traditionally, butanol fermentation has been studied in *Clostridium* strains, as was first reported by Louis Pasture. However, *Clostridia* have been difficult to engineer and thus a host of other bacteria have been used as surrogates to produce the alcohol (Zhao, Zhang and Li, 2020). Although the LNS process may not be the most straight forward method to circumvent these engineering difficulties, it may have greater ability to generate rare genotypes which are inaccessible to other selection methodologies.

Overall, LNS is uniquely suited to address several key issues in biofuel optimization. LNS can widen the scale of possible phenotypes that can be created and alleviate the need to accurately predict specific enzymatic changes to optimize alternative fuels. However, LNS may also be less efficient and more time-consuming than AS or DE when optimizing existing phenotypes. Therefore, LNS could be specifically utilized when AS and DE are unable to drive significant progress. In summation, a synergistic research strategy combining all three selection methods may be the most fruitful path forward for biofuel production and usage.

Bioremediation

Bioremediation is the process of using microorganisms to destroy, or reduce the concentration of, hazardous waste (Boopathy, 2000). The technique is often used to aid in processes like wastewater, solid waste and heavy metal removal (Garima and Singh, 2014; Ojuederie and Babalola, 2017). The idea of using bioremediation to deal with dangerous and/or functionally non-degradable materials is not new, and in some ways, nature has already begun the process through species adaptation to human activities (Sih, Ferrari and Harris, 2011). For instance, the widespread evolution among microbes of the ability to degrade atrazine, a popular herbicide used since the 1950s (Udiković-Kolić, Scott and Martin-Laurent, 2012). It is not hard to envision scientists breeding organisms to utilize waste products and purge them from the environment. An example which clearly shows the potential for this is the development of nylonase by Flavobacteria. Nylon, a synthetic polymer, was first invented in the 1930s and quickly became widely used around the world. By 1975, scientist had discovered a strain of Flaviobacterium, dwelling in ponds which contained wastewater from a nylon factory in Japan, that could digest nylon products (Kinoshita *et al.*, 1977). Since then, scientists have been able to evolve other bacterial species to achieve similar capabilities by forcing them to live in resource-depleted environments which are rich in nylon (Prijambada *et al.*, 1995) and by plasmid transfer into *E. coli* (Negoro *et al.*, 1983). More recently, researchers have discovered bacteria which are capable of digesting the common plastic polymer polyethylene terephthalate, otherwise known as PET (Yoshida *et al.*, 2016). As a commonly used plastic, PET is a major source of pollution (Geyer, Lambeck and Law, 2017), especially in marine environments (Worm *et al.*, 2017). Researchers are now working with the bacterium and recent studies have shown promising results in characterizing and improving the enzymes responsible for digesting PET (Austin *et al.*, 2018).

The evolution of nylonase by microbes just some 50 years after the development of nylon has become a textbook example of the power of evolution and the immense potential it has for bioremediation. More recent successes like the aforementioned PET digesting bacterium *Ideonella sakaiensis 201-F6* (Yoshida *et al.*, 2016) continue to show that potential. Bacteria, fungi and plants are now being researched for their bioremediation potential. In regard to microbes, current efforts to improve bioremediation potential mostly center around molecular techniques like protein and metabolism engineering using both genomics and proteomics (Wood, 2008; Shukla, Singh and Sharma, 2010; Basu and Stolz, 2011). These include the use of transgenic plant species which synthesize enzymes originally found, or enhanced, within bacteria (Peng *et al.*, 2014). Other research approaches via systems biology or molecular engineering have potential to improve our bioremediation capacities (Dangi *et al.*, 2019); however, LNS should join these techniques on the cutting edge of bioremediation research. Just as researchers were able to use LNS to produce nylon digesting bacteria (Prijambada *et al.*, 1995), similar techniques could be used to create organisms to degrade plastics and other long-lasting materials. As previously mentioned in the biofuel section, evolving enzymes in their host organism can avoid issues typically caused by using surrogate bacteria in directed evolution. Here, LNS could serve to co-adapt the genome to the engineered products.

One of the biggest challenges with bioremediation techniques so far has been the lack of biodegradability for many common products including plastics, oil, and metals (Juwarkar, Singh and Mudhoo, 2010). Rather than waiting for researchers to determine the most efficient way to break down these materials, LNS gives the opportunity for natural selection to engineer chemical processes to deal with these pollutants. This could buy substantial time in preventing environmental damage and wildlife loss. Ultimately, an integrative approach combining the three

selection experiment methodologies could be most helpful to bioremediation. Similar to biofuels, in bioremediation LNS would be most helpful with the initial creation of novel phenotypes and optimization, as well as creating a diversity of viable phenotypes. However, once these are created, the strong selection imposed by the other selection experiments may be more advantageous. A synergistic approach would ideally use laboratory natural selection to create the needed enzymatic processes, then directed or artificial evolution to further optimize the reactions. Researchers could continue to use LNS to build neutral mutation load and explore genotypic space to find alternative fitness peaks; however, this would be purely exploratory unless combined with rational design studies. Nonetheless, LNS would be vital as the experimental method best suited to create the initial microbial phenotypes for bioremediation.

THE FUTURE OF LABORATORY NATURAL SELECTION IN APPLIED STUDIES

Evolution is a powerful process which has shaped the biological world over the course of natural history. Utilizing the power of evolutionary processes for human application is not a new idea. Many organisms we rely on have been altered over the course of time by applied evolution. Selection experiments, which encompass artificial selection, directed evolution, and laboratory natural selection, are all techniques which harness the power of evolution, however, of the three LNS is comparatively underutilized in the applied sciences. This is a missed opportunity, as LNS has the potential to be a useful tool in applied biological science. Unlike AS and DE, which impose strong selection and can create lower effective populations sizes, LNS allows for less stringent selection and thus the maintenance of greater genetic variation over time. By allowing mutations with neutral or small effect sizes to persist and accumulate in the population, LNS can increase the odds of novel phenotypes arising which require multiple negligible fitness

conferring mutations. This allows LNS to explore more of the fitness landscape. Functionally, this means that laboratory natural selection can remove the barrier of researcher knowledge or foresight which is needed in biological design studies.

Laboratory natural selection can provide solutions to some of the most important issues in the applied sciences today. Applying LNS strategies to the identification of drug resistance mechanisms, and the development of microbes for biofuels and bioremediation has the potential to yield fruitful results which could greatly impact society. Particularly because these are direct ways of mediating some of the most looming issues we will face in the future: diminishing fossil fuel resources and climate change, emerging antibiotic resistance, and dealing with anthropogenic pollution. Ultimately, AS, DE and LNS approaches should all be utilized harmoniously, and sometimes collaboratively, to address the broad issues we face as a society. Generally, not all systems will be well suited for the LNS methods discussed in this review, and their usage should be carefully analyzed and considered prior to being undertaken. However, the wide variety of systems and conceptual issues LNS has addressed is indicative of its power as a methodology and the immense potential it may hold in the applied sciences. Just as LNS has been advantageous to researchers in the basic sciences, it can join other selection methods in the forefront of applied studies.

ACKNOWLEDGEMENTS

The authors would like to thank Berry Brosi, Brandon Ogbunu, Lance Waller, Michael Zwick and Kayla Stoy for their helpful comments in the preparation of this manuscript. This work was funded by the National Science Foundation Graduate Research Fellowship Program (2017246734 to JL) and the National Science Foundation (DEB-1750553 to LTM). Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors(s) and do not necessarily reflect the views of the National Science Foundation.

CHAPTER V

SUMMARY AND DISCUSSION OF PREVIOUS CHAPTERS

The goal of this dissertation was to examine the role of genetic architecture and evolutionary reciprocity on the evolution of host defenses. Building on existing theory and experimental evidence, I used host-parasite experimental evolution in the *Caenorhabditis elegans- Serratia marcescens* system. Specifically, I examined how evolutionary reciprocity and genetic architecture influence the impact of gene flow on adaptation to a parasite, and the extent to which evolutionary reciprocity influenced host defense range. Here, I place these results in context with each other and describe future conceptual questions concerning these ideas and questions within the study system.

EVOLUTIONARY RECIPROCITY

Coevolution is predicted, and has been shown, to be an important driver within many ecological interactions (Anderson and May, 1982; Thompson, 1989, 2009). Further, the evolution of both sides of the coevolutionary interaction are predicted to proceed differently than they would going through less reciprocal interactions. In chapter II, I showed that highly reciprocal interactions lead to more specific host defenses, and thus hosts perform poorly against all but their coevolving parasite partner. In chapter III, while not evaluating a situation of high evolutionary reciprocity, I further investigated the evolution of "one-sided" host defenses in *C. elegans* in response to SM2170. In chapter II, I showed that one-sided hosts evolved elevated defenses to SM2170, and that those defenses seem to have a degree of general applicability to SM2170 derived parasite strains (ES1 and coevolved). Chapter III sheds light on the nature of these

defenses, as I show that adapted migration led to lower mortality rates in host populations and assisted adaptation to SM2170. However, adapted migration only resulted in decreased mortality rates in the presence of SM2170, not in its absence. This further supports advantageous defense alleles evolving in the one-sided treatments and not changes due to the evolution of other traits. Generally speaking, these results add to a body of literature showing the ability of coevolution to alter host defenses. These results also help illuminate the architecture of these traits. Overall, this work shows that evolutionary reciprocity can have a significant impact on the evolution of traits and thus the evolutionary trajectories of populations.

GENETIC ARCITECTURE

Genetic architecture refers to the genetic effects that build and control a phenotypic character and variation in its properties (Young, 2000; Hansen, 2006). It describes the relationship from genotype to phenotype for a given trait. Here, I investigated the role of genetic architecture from various angles. First, I looked at a collection of phenotypic traits (host defense level) and asked how its range varied based on the level of reciprocity in the interaction. Theory predicts that gene combinations that arise in highly reciprocal interactions are less likely to be shared outside of that interaction, and so by testing defense range we also proxy for the defense genotype (Young, 2000; Kawecki and Ebert, 2004; Lambrechts, 2010). I found that not only did coevolved hosts perform poorly against bacteria that were not coevolving with them, but that one-sided hosts evolved more general defenses. Further, control hosts tended to do well outside of SM2170 derived genotypes. This supports the idea that the changes taking place in the coevolving host populations were specific to their coevolving parasites, and that less-specific adaptations were developed by one-sided hosts. However, it also points to levels of innate resistance within the

CB4856 worm background (Mallo *et al.*, 2002; Lansdon, Carlson and Ackley, 2022), since control groups only performed well against non- SM2170 derived parasites.

Second, I took the phenotypic trait (defense to SM2170) and examined its ability to operate in different host backgrounds. Epistasis and pleiotropy can be important to trait function, and in chapter III, I had the opportunity to potentially observe effects by proxy through the investigation of gene flow into adapting populations. I found that populations that received gene flow from previously adapted populations displayed elevated defenses against SM2170 when compared to populations that received naïve gene flow. Given that migrants had previously adapted to SM2170, high mortality rate numbers in the sink population may have been indicative of alleles not pairing well with the genetic background of the migrants. However, it is important to note that populations that received adapted novel migration fared better than those that received adapted shared background migration. This is interesting, as it suggests that the genetic background the resistance alleles evolved in may not necessarily represent a global fitness peak, but a local one. Generally, this work shows the importance of genetic architecture in determining the impact of a trait migrating into a population and that co-evolution can change the genetic architecture underlying a phenotypic trait.

EXPERIEMTNAL EVOLUTION & ITS APPPLICATIONS

Experimental evolution, or the study of evolutionary changes occurring in experimental populations as a consequence of conditions imposed by the experimenter (Kawecki *et al.*, 2012), has risen in popularity as a methodology in the past decades . This is, in part, due to experimental evolution's ability to isolate variables at work within a population, allowing for direct hypothesis testing (Cooper and Cristina, 2018; McDonald, 2019). The increase of experimental evolution in

popularity has also led to speculation, and recommendations, for how it could be utilized in the future. For example, Hoang, Morran, and Gerardo 2016, propose experimental evolution as a useful, and underutilized, tool for studying the evolution and maintenance of mutualisms. Here, in addition to using experimental evolution to address questions related to genetic architecture, evolutionary reciprocity, gene flow, and adaptation, I also identified where experimental evolution could be used in applied science. In chapter IV, I showed why experimental evolution may be advantageous in the applied sciences and how it could be used in collaboration with other selection methodologies. I then reviewed past uses of the method and showed that it could lead to useful industrial or medical products in the realms of bioremediation, drug resistance mechanism identification, and optimizing biofuel production. As technology continues to improve and our genetic capabilities continue to advance with technologies like CRISPR (Hanna and Doench, 2020; Shivram *et al.*, 2021), the potential for experimental evolution to uncover insightful results also increases. Ultimately, the method has immense potential in a number of arenas.

FUTURE DIRECTIONS

There are various outstanding questions related to the evolution of host defenses, the role of genetic architecture, the role of evolutionary reciprocity, how gene flow influences the adaptive process, and how host defense range is determined by evolutionary interactions. For one, in chapter II the strains of bacteria used to assess host range were still *Serratia.* The degree to which the mechanisms used to defend against SM2170 could be used against other bacteria (for instance other gram negatives) remains untested. Next, the host populations in chapter III were all derived from a mutagenized background, and while this infused variation, the populations

were not that genetically dissimilar. This may have biased us to see a general result of variation. Lastly, gene flow is predicted to be most detrimental during adaptation to a coevolving parasite or during sustained gene flow between two locations (Garant, Forde and Hendry, 2007). Repeating the gene flow experiment with a coevolving parasite or with gene flow between adapting populations could prove to be more analogous to how gene flow typically acts during adaptation to a parasite. For both chapters II and III, the next step would be to sequence populations at various time steps to examine the genetic changes underlying adaptation in this system. While we know resistance alleles for SM2170 are housed on the 5th chromosome in CB4856 worms (unpublished QTL data), we do not know how many alleles underlie this resistance or how recombination impacts it. Whole genome sequencing of host populations from post migration (Chapter III), post coevolution (Chapter II), and post one-sided evolution (Chapter II) may reveal substantial information about the architecture of defense in *C. elegans.*

Lastly, experimental evolution, in and of itself, is a great tool to explore evolutionary hypotheses and directly rest the impact of variables on evolutionary phenomena. However, experimental evolution also has its own biases that may impact studies in a multitude of ways (Kawecki *et al.*, 2012)(Box 4.2). Other experimental systems may yield similar, or different, results depending on the nature of host defense evolution and parasite infection within the system. However, using experimental evolution also allowed for explicit testing in ways not possible in natural settings. Overall, experimental evolution should be combined with further field studies, mesocosm studies, mathematical modeling, and genomics to further elucidate the questions posed in this dissertation.

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