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The evolution of virulence in heterogeneous host populations

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The evolution of virulence in heterogeneous host populations

By

Patricia Signe White
B.S. & B.A., Indiana University, 2012

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An abstract of
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Abstract

Parasitism is a ubiquitous species interaction in which one partner gains a fitness benefit (i.e., increased survival and reproduction) to the detriment of the other by way of resource exploitation. Both partners impose reciprocal selection pressure on one another – hosts adapt to resist parasites, and parasites counteradapt to overcome host barriers – thus resulting in arms-race dynamics. However, host populations are complex, and the effects of factors such as genetic diversity and varying life stage on parasite evolution are largely unknown. Here, I empirically test theoretical predictions about the influence of host heterogeneity on the evolution of virulence (i.e., host mortality). I experimentally evolve populations of the roundworm *Caenorhabditis elegans* and its parasitic bacteria, *Serratia marcescens*. Host populations composed of multiple genotypes are expected to slow the rate of parasite adaptation, thereby reducing host mortality. I found that when evolving virulent *S. marcescens* parasites in populations with or without genetic diversity, host populations with genetic diversity were better able to reduce the effects of infection than in homogeneous host populations. In addition to genetic heterogeneity, the life stage at which a host is infected is predicted to influence infection dynamics. To determine if *C. elegans* life stages differed in ability to resist *S. marcescens*, I compared the dispersal life stage of *C. elegans* (dauer) with non-dauer life stages. I found that dauer is capable of avoiding *S. marcescens*, effects not found in non-dauer life stages. Further, host population size has been shown to be an essential factor in how quickly hosts can evolve resistance. I found that small populations of *C. elegans* are overcome by the random effects of genetic drift, and parasite resistance was either lost or unable to evolve at all. Overall, this dissertation provides evidence that many aspects of the host population can influence the outcome of host-parasite interactions. Host heterogeneity, life stage, and population size can all alter the evolutionary trajectories of host-parasite interactions. By understanding the factors that influence the evolution of virulence, we can better manage its effects in human populations, agriculture, and wildlife.

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CHAPTER I

INTRODUCTION

The biologist Donald A. Windsor once stated that "parasites are the true engines of evolution" (Windsor, 1998). Parasites are thought to be as old as life itself, evolving to exploit hosts as soon as there were hosts to exploit (Thompson, 1999). Indeed, species interactions are thought to have shaped much of the biological diversity on earth (Hatcher & Dunn, 2011). Parasitism is a ubiquitous type of species interaction in which one partner gains a fitness benefit to the detriment of the other, typically by way of resource exploitation (Crofton, 1971). Thus, parasitism has likely influenced (and still influences) a multitude of characteristics of biological life, including: sexual reproduction, which led to the ability to produce new genotypes and thus evade parasites (Hamilton, 1975); developmental and life history traits of hosts (Mennerat *et al.*, 2017); and spatial structure of hosts (Brockhurst *et al.*, 2003). Parasites have been predicted to make up more than 50% of all existing species (Price, 1977; May, 1988). Robert May suggested a 1:4 ratio of free-living hosts to parasites (May, 1992). Every free-living organism on earth has parasites, sometimes even parasites themselves (Koskella *et al.*, 2011; Morgan & Koskella, 2017). Thus, understanding the evolution of parasites is essential for understanding the evolution of life. This dissertation aims to understand how fundamental evolutionary processes shape host and parasite evolutionary dynamics.

Evolutionary forces

Host-parasite interactions are shaped by multiple evolutionary forces, including natural selection, mutation, genetic drift, and gene flow (i.e., migration). Natural selection is a

nonrandom process by which individuals with traits that permit an advantage in a particular environment are favored. Individuals with those traits are more likely to survive and reproduce (i.e., they have a high relative fitness), which allow genes that confer the beneficial trait to be passed on to their offspring. Thus, over time, the proportion of the population with the beneficial trait will increase. However, traits that are beneficial in one environment may be disadvantageous in a dissimilar environment (Ebert, 1994). For example, a parasite genotype may have high fitness in one host environment (e.g., a large population of hosts), whereas the same genotype may have low fitness another environment (e.g., a small population of hosts).

Mutation is a random process by which new alleles are created. It is the raw material on which natural selection acts (Loewe & Hill, 2010). If we consider a host-parasite interaction, mutation may generate new beneficial host genotypes that allow for parasite evasion. In the absence of other evolutionary forces, these beneficial alleles can be driven to fixation in the host population by natural selection, thus preventing further parasite infection (Bedhomme *et al.*, 2012). Mutation may then generate new parasite genotypes that allow for successful infection of the fixed host genotype (Koskella, 2018). Subsequently, natural selection can drive the beneficial parasite genotype to fixation, thus causing the extinction of the previously beneficial host genotype (Bonhoeffer & Nowak, 1994). This pattern of adaptation and counter-adaptation is known as a recurrent selective sweep, often referred to as an evolutionary arms race (Papkou *et al.*, 2016). Recurrent selective sweeps have been demonstrated empirically, such as in a long-term association between a bacterial host and its bacteriophage parasite (Buckling & Rainey, 2002).

In contrast with natural selection, genetic drift is a random process. Genetic drift occurs as a result of chance fluctuations in the frequency of alleles due to sampling error, such as the

random sampling of gametes or chance death of individuals before successful reproduction (Rudge, 2013). The probability that any given neutral allele will drift to fixation or extinction depends on the initial frequency of the allele in the population (Wright, 1931). The strength of genetic drift scales inversely with effective population size, thus smaller populations are often most susceptible to stochastic changes in allele frequencies (i.e., a small pool of alleles) (Paland & Schmid, 2003). However, the effects of drift may be overcome by selection, particularly when both population size and selection coefficient are large. Migration is a form drift which occurs frequently as host individuals (and their parasites) move between populations. The movement of alleles (i.e., hosts) out of and into host populations, also known as gene flow, influences the allelic diversity of that population. In turn, the presence or absence of particular alleles in a host population may influence parasite prevalence and virulence in the population (Hamilton, 1993). For example, if a host population has evolved resistance to a local parasite, then immigration of individuals with susceptible alleles may prevent local parasite extinction. Additionally, parasite local adaptation is expected to occur when host migration is limited (Greischar & Koskella, 2007). This has been shown to occur in host populations of the bacteria *Pseudomonas aeruginosa* and its phage parasite, in which low host migration allowed the phage to adapt to its local hosts (Chabas *et al.*, 2016).

Evolutionary forces frequently impact one another, as in the case of genetic bottlenecks, a type of genetic drift. A genetic bottleneck occurs when a subset of a larger population becomes isolated or leaves to establish a new population, such as on an island. Parasites go through numerous population bottlenecks throughout their life cycles, which can cause their effective population size to decrease and thus leave drift to be more influential. For example, bottlenecks occur when a small subset of parasite propagules is transmitted to a new host. Bottlenecks also

occur when the immune system culls a large number of the parasites within the host, or in the case that a parasite needs multiple hosts to complete its life cycle (Papkou *et al.*, 2016). All of these potential bottleneck events greatly reduce the parasite population, leaving it vulnerable to the effects of drift and potentially preventing adaptation (Papkou *et al.*, 2019). For example, when a vesicular stomatitis virus (VSV) was bottlenecked for multiple iterations, it was unable to as successfully infect its usual mice kidney cell hosts (Clarke *et al.*, 1993).

Virulence evolution: causes and effects

None of these evolutionary forces occur in isolation, and all are important to consider when analyzing host-parasite dynamics. Though all forces will come into play to varying degrees in this body of work, I will focus mainly on the effects of genetic drift and natural selection on host-parasite evolution in the context of a host metapopulation. Specifically, I will focus on understanding the interplay of evolutionary forces on the evolution of virulence. Virulence in and of itself is detrimental to both parasites and hosts. It is not a property belonging to either partner alone, but is instead a result of the interaction between the two (Ebert & Hamilton, 1996). Virulence is often defined as a reduction in host fitness due to parasite harm, and is often measured in terms of increased mortality and decreased fecundity (Regoes *et al.*, 2000; Bolker *et al.*, 2010; Alizon & Michalakis, 2015). For the purposes of this dissertation, I define virulence as the infection-induced host mortality rate (Anderson & May, 1982; Lenski & May, 1994; Day, 2002). Virulence may be thought of us a result of a trade-off between the parasite's within-host growth rate and its between-host transmissibility (Kubinak & Potts, 2013). Host harm comes from the parasite rapidly exploiting host resources. As I will later discuss, the definition of parasite virulence as it pertains to this body of work is limited to mortality because of the effects

of the parasite used in my experiments. The parasite causes rapid mortality, and significantly reduces host fitness (infection often occurs before reproduction). In addition to parasite virulence, host resistance also plays an important role in the host-parasite interaction outcomes. Host resistance is defined as the host's ability to reduce the probability of infection or growth, thus lowering the likelihood of fitness loss (e.g., mortality) (Dieckmann *et al.*, 2002). Multiple types of resistance can occur, including avoidance (preventing initial contact with a parasite), qualitative resistance (reducing probability of infection), and quantitative resistance (reducing parasite growth upon infection) (Gandon & Michalakis, 2000).

Heterogeneity of hosts

Importantly, the evolutionary trajectories of host-parasite interactions are influenced by the dynamics of the host population. Host populations can be complex, and this complexity may alter the rate or degree to which parasites are able to adapt to them. Host heterogeneity takes many forms: genetic (Keith & Mitchell-Olds, 2013), demographic (Claessen & de Roos, 1995), behavioral (Chang *et al.*, 2012), and spatial (Züst *et al.*, 2012; Moreno-Gámez *et al.*, 2013). In this dissertation, I focus on genetic and life stage heterogeneity (which is a form of both behavioral and demographic heterogeneity) in the context of host population size. Though not covered in-depth here, it is important to note that spatial heterogeneity plays an important role in many host-parasite systems (Lion & Boots, 2010; Moreno-Gámez *et al.*, 2013; Webb *et al.*, 2013; Carlsson-Granér & Thrall, 2015).

Genetic heterogeneity

Individuals within host populations are rarely uniform in their ability to resist parasite infection. Commonly, this difference results from genetic heterogeneity. A common form of

genetic heterogeneity (also referred to as diversity) includes allelic diversity for immune function (e.g., resistance or tolerance) (Thrall *et al.*, 2001; James *et al.*, 2009). The amount of resistance alleles and their relative abundances in the host population may determine how rapidly parasites are able to adapt to their hosts (Osnas & Dobson, 2012). In general, the higher a host population's average overall quantitative resistance, the stronger selection will be for greater virulence to overcome host barriers (Gandon *et al.*, 2001).

Life stage heterogeneity

In addition to genetic heterogeneity, the life stage of the host upon infection is an important determinant of infection success. Host populations often have varied demographic compositions; very few populations have equal ratios of life stages throughout the population, and different life stages may be differentially susceptible to infection (Tate & Rudolf, 2012). Various defense strategies may be employed at different times in the host's life, allocating resources differently during development. In particular, juveniles tend to suffer high rates of parasite-induced mortality, especially in arthropods (Ben-Ami, 2019). For example, larvae of the Indian meal moth *Polodia interpunctella* suffer high mortality rates when infected with a granulosis virus, whereas adults are not affected (Boots, 1998). Dispersal life stages typically occur during juvenile development. If host dispersal is necessary for population persistence, then there is likely to be strong selection for parasite avoidance by a host when it immigrates into a new patch and is met with novel parasites (Gibson & Morran, 2017). In this case, we may expect to see the opposite effect than in the previous moth-virus example: the dispersal stage may have a lower parasite load or suffer less mortality than in the adult stage (Osnas *et al.*, 2015). Further, when a small subset of the population disperses, its small size may leave it to more prone to the pressures of drift.

Population size

So far, I have discussed the importance of host genotype and host life stage as influences on infection dynamics. We must also consider the effects of host population size on the ability of hosts to evolve and maintain resistance. In reality, depending on many factors, host population size is unlikely to be identical on all patches in the metapopulation. Host population size has been shown to be essential in how quickly a parasite population can evolve resistance, as small population sizes are more prone to the effects of drift (Carlsson-Granér & Thrall, 2002). Population size can vary greatly in nature, particularly when populations are broken up into local patches (Carlsson-Granér & Thrall, 2006). As mentioned previously, drift has a larger effect when population sizes are small. Host resistance may be more easily lost in small populations of hosts, making it difficult for host populations to escape parasitism. However, drift is not always detrimental, and in some cases, drift may preserve beneficial alleles at random. The long-term persistence of a population of hosts can be shaped largely by its population size.

The experimental system: *Caenorhabditis elegans* and *Serratia marcescens*

All told, multiple aspects of host heterogeneity are important when considering the patterns and mechanisms of virulence evolution. Two important types of heterogeneity are explored in this dissertation: (1) host genetic heterogeneity and (2) life stage heterogeneity, both in the context of varying population size. To test predictions of how heterogeneity impacts said evolution, I performed three experiments exploring the dynamics between a nematode host, *Caenorhabditis elegans*, and its bacterial parasite, *Serratia marcescens.*

C. elegans is a nematode roundworm found naturally in decomposing organic matter, such as in human compost piles and in rotting fruit in orchards. It has long been a model

organism in genetics, developmental biology, and neuroscience due to its fast replication time, hermaphroditic reproductive mode resulting in high population growth rate, and its ease of laboratory husbandry. Because of these factors, large populations of *C. elegans* are amenable to manipulation and can be easily controlled for genetic and phenotypic variables of interest. Until recently, however, little attention had been paid to its natural history and ecology (Barrière & Félix, 2005b; Félix & Braendle, 2010; Blaxter & Denver, 2012; Félix & Duveau, 2012; Cutter, 2015; Frézal & Félix, 2015; Schulenburg & Félix, 2017). At last we have a basic understanding of *C. elegans* population dynamics in nature. It is now possible to use this growing body of literature to better inform experimental design and interpretation of results.

C. elegans has natural associations with many types of microbes, including bacteria in the family *Enterobacteriaceae*, which includes both *Escherichia coli*, the worms' benign food source, and *Serratia marcescens*, a parasite (Samuel *et al.*, 2016). These two bacterial species are used in each experiment outlined in this dissertation. It is important to note that these interactions may not be entirely novel, since all three species (*E. coli, S. marcescens*, and *C. elegans*) have been found in association. However, genotypes (and subsequently, phenotypes) of each three may vary dramatically, and the extreme variation encountered in these experiments may not occur naturally. This fact may prevent us from drawing direct application from the results found here to what occurs in nature. Instead, I focus on what is biologically possible in the realm of host-parasite evolution to test theoretical predictions in light of natural associations.

Furthermore, *C. elegans* is an excellent system to study how life stage upon infection may change host-parasite dynamics. Specifically, *C. elegans* has a juvenile alternative life stage known as dauer, which is a dispersal phenotype. Dauer takes place of larval stage 3 (L3) in a normal life cycle (Figure 1). Dauer larvae develop when food sources are limited and when

worm crowding is high (Hu, 2007). Dauer have been found associated with various species of arthropods (in an interaction known as phoresy), which are thought to help with dauer dispersal to new food patches (Petersen *et al.*, 2015). If this is indeed the case, there is likely strong selection pressure for dauer to discriminate between benign and parasitic microbes. Once individual worms begin the transition out of dauer (say, if a bacterial bloom is found), they cannot stop or reverse the transition. The individual must resume development. There are rarely environments that are completely devoid of parasites. Even when a new habitat is relatively safe (the ratio between good and bad food sources is high), parasites still exist, which may elevate immunity while in dauer. Based on population genomics, it has been estimated that 3-10 individuals move from patch to patch, which suggests continuous bottlenecking of *C. elegans* populations (Richaud *et al.*, 2018). This work suggests that genetic drift may be a strong force in *C. elegans* populations, potentially preventing local adaptation.

Figure 1.1

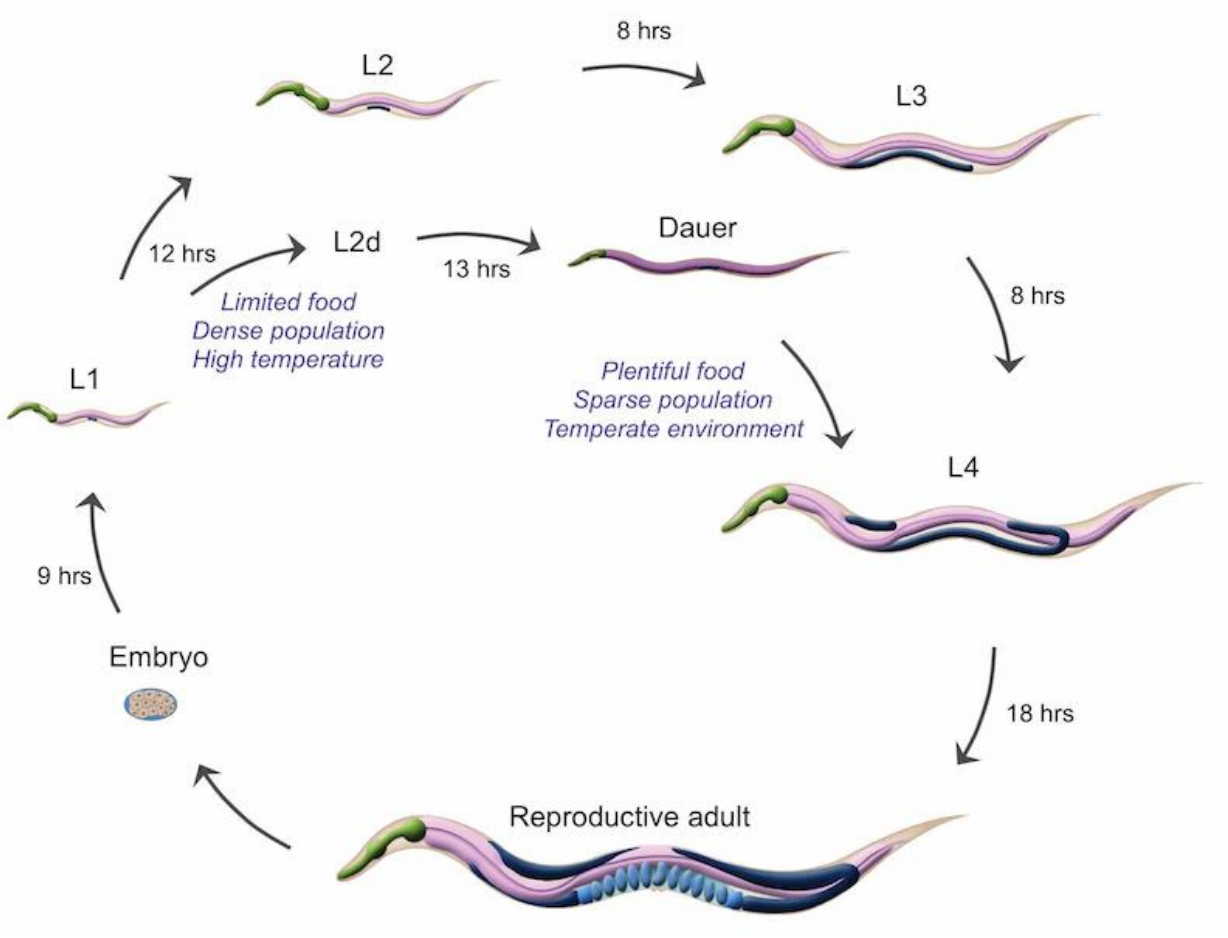


Figure 1.1 Life cycle of *Caenorhabditis elegans.* Figure adapted from Wolkow & Hall, 2015.

Interestingly, worldwide samples comparing hundreds of *C. elegans* isolates have shown relatively low global diversity via chromosome-scale selective sweeps. Further, worldwide isolates have shown that there are commonly shared haplotypes on four of six chromosomes (Andersen *et al.*, 2012). It is thought the most recent selective sweep occurred within the past couple centuries, and perhaps was brought about by agricultural changes and human migration (Haber *et al.*, 2005). *C. elegans* populations can be thought of as a large metapopulation with patchily distributed local populations (Sivasundar & Hey, 2003; Barrière & Félix, 2005a). Small

effective populations may be unable to evolve or maintain resistance to local parasites, bringing about local extinction (Cutter, 2015). Movement to new patches by phoretic hosts from disparate patches, as well as occasional outcrossing, may provide genetic rescue of locally maladapted populations.

C. elegans is an ideal model organism to test predictions about host heterogeneity, life stage heterogeneity, and population size. It is easy to grow in laboratory conditions, as is its parasitic bacterium, *S. marcescens*. This system allowed me to control variables easily and to better understand effects of parameters in isolation. It is nearly impossible to control for the complexity of nematode-bacteria interactions in nature. Bringing this system into the lab allows for a simple system for which to test fundamental questions relating to host and parasite evolutionary dynamics. The rest of this dissertation explains in depth how I used this system to bring clarity to host and parasite evolution.

CHAPTER II

Genetic drift limits host adaptation to a virulent parasite

Abstract

Different evolutionary forces frequently counteract one another, resulting in a limited ability to respond quickly to selection pressures in a given environment. Host-parasite interactions may often be subject to these opposing forces, which likely influence the evolutionary trajectories of both partners. Natural selection and genetic drift are two major evolutionary forces acting in host populations. Further, population size is a major determinant in the relative strengths of these forces. In small populations, even when selection pressure is strong, drift may work to undermine the persistence of beneficial alleles, preventing hosts from adapting to their parasites. Here, we investigate two questions: (1) can selection pressure for increased resistance on initially susceptible host populations overcome the effects of drift in small populations, and (2) can resistance be maintained when selection for resistance is high, but host population size is small? To answer these questions, we experimentally evolved the host *Caenorhabditis elegans* against its bacterial parasite, *Serratia marcescens* for 13 generations. First, we found that strong selection for resistance can be insufficient to outweigh the effects of random genetic drift in small populations, resulting in high host mortality. Second, in small populations of hosts that were initially resistant, we found that selection for continued resistance is unable to be maintained. We compared these results with selection in large host populations and found that in both cases host populations were able to gain or maintain high resistance against the parasite. These results show that in many circumstances, strong selection pressure for survival is not sufficient to counteract the random effects of drift. In consideration of *C. elegans* natural

population dynamics and severe bottlenecking during dispersal, we suggest that these forces are likely at play in nature, and that other forces, such as rescue effects of metapopulation, may be contribute to the persistence of *C. elegans* in nature.

Introduction

Genetic drift has been shown to be a powerful evolutionary force in many natural systems (see Templeton *et al.*, 1990, 2001 and Young *et al.*, 1996). The bulk of research on host-parasite interactions has focused instead on gene flow and selection, but drift also has the potential to influence co-evolutionary dynamics between hosts and parasites. Few empirical studies have tested the influence of drift itself on a host population's ability to withstand infectious disease. Many experiments have investigated the potential effects of drift, including genetic load, loss of heterozygosity, and inbreeding on parasite resistance. Though not testing drift *per se*, these experiments can still make predictions about the effects of drift. In general, drift inhibits adaptation in host populations due to random extinction or fixation of beneficial or detrimental alleles, preventing hosts from evolving resistance to their parasites. Further, studies that look at genetic bottlenecking, a form of drift, often consider the parasite population and not the host population, but both are important in order to comprehensively understand host-parasite coevolutionary dynamics (Papkou *et al.*, 2016).

Genetic drift is predicted to be strongest in small populations, which in turn tend to have low genetic diversity. Parasites are predicted to infect the most common host genotype, and small populations are more likely to be homogeneous and thus incur a high disease burden, which has been shown to occur in natural populations of fish (Lively *et al.*, 1990) and in experimental populations of ryegrass (Polans & Allard, 1989; Barrett & Charlesworth, 1991). A variety of

factors determine population size, including disease epidemics, resource limitation, and life history traits. Here, we ask whether population size can influence the efficacy of selection for increased host resistance to a bacterial parasite. To answer this question, we performed and analyzed two experiments in which strong selection for resistance against *Serratia marcescens* was imposed on different sizes of *Caenorhabditis elegans* host populations.

In nature, dynamics of *C. elegans* populations are likely driven by factors such as dispersal ability, effective population size, availability of benign bacteria as food resources, defense against antagonistic microbes, rare outcrossing events, and competition among clones, other populations, and other species (Félix & Braendle, 2010). The population dynamics of *C. elegans* have been characterized by cycles of boom-and-bust (Frézal & Félix, 2015). The populations grow to high numbers as they consume bacteria on decomposing organic matter, i.e. "boom." The "bust" occurs when the food source has been consumed, at which time most individuals in the population will die. Because *C. elegans* are hermaphroditic, they can reproduce without mates and thus independently colonize new areas. Based on a population genomics study of multiple natural populations in France and Germany, it has been estimated that a new patch is colonized by ~3-10 genetically unique dauer individuals (Richaud *et al.*, 2018).

Populations of C. elegans have been found in association with an array of parasites, including viruses (Franz *et al.*, 2014), microsporidia (Troemel *et al.*, 2008), bacteria (Samuel *et al.*, 2016), and fungi (Maguire *et al.*, 2011). Resistance to these pathogens is likely important for continued survival and reproduction of *C. elegans* (Félix & Duveau, 2012; Balla & Troemel, 2013), but given the low population size in any given patch in nature, resistance against parasites may be difficult to selectively maintain over time. Sufficiently strong selection on alleles conferring resistance may be enough to drive beneficial alleles to high frequencies even in small

populations. Thus, our goal was to determine whether strong selection on alleles for survival against a parasite could overcome random drift acting on small populations. First, we experimentally evolved resistant and susceptible *C. elegans* populations of variable sizes against the bacterial parasite, *Serratia marcescens*. We then asked, relative to larger populations, (1) can small populations with low initial resistance evolve greater resistance and (2) can small populations with high initial resistance maintain their resistance? Overall, we expected that (1) susceptible small populations may not evolve increased resistance over time, and that (2) resistant small populations may not effectively maintain their resistance over time. In contrast, we predicted the large populations would respond to selection by evolving increased resistance in susceptible populations and maintaining high levels of resistance in the resistant populations.

Methods

Host and parasite strains

The host populations were derived from an obligately outcrossing *C. elegans* strain, PX386. PX386 is homozygous for the *q71* allele at the *fog-2* locus, resulting in obligate outcrossing. PX386 was mutagenized to create a genetically variable obligately outcrossing population (Morran *et al.*, 2011). Replicate populations of these genetically variable populations were then evolved for 30 host generations either against the parasitic bacteria, *Serratia marcescens* Sm2170, or against heat-killed (HK) *S. marcescens* Sm2170 (Morran et al. 2011 and see Supplemental Figure 1). All strains were cryopreserved at the end point of the experiment with freezing solution at -80ºC. Here, our starting populations were from the end point (generation 30) of the previous experiment (Morran *et al.*, 2011). We chose two starting populations derived

from the same genetically variable ancestral population, one population that was exposed to live Sm2170 parasites for 30 generations and another that was exposed to heat-killed Sm2170 for 30 generations. At the end of 30 generations, the population exposed to live Sm2170 exhibited significantly decreased mortality rates from 80% to 36%, thus we refer to this population as the "resistant" population (Penley & Morran, 2017). Conversely, the population exposed to heat-killed parasites did not exhibit a significant change in mortality rates after experimental evolution (from 80% to 72%) thus we refer to this population as "susceptible" (Penley & Morran, 2017). The resistant and susceptible host populations were then used as progenitors for the replicate populations in our experiments. These populations were thawed and then allowed to grow and reproduce for 2-3 generations prior to selection.

Experimental Evolution

Susceptible populations

The susceptible progenitor worm population was divided among four treatments: large populations (500 individuals) or small populations (50 individuals) plated on either live Sm2170 or HK Sm2170. Each treatment had 4 replicates. Only the host was allowed to evolve, whereas the parasite was replenished each passage from ancestral clones. Experimental evolution began by placing the appropriate number of hosts (see treatments above) on Serratia selection plates (SSPs). SSPs were divided into even thirds: 1/3 of the plate was seeded 35 μl of Sm2170, 1/3 with 35 μl of the benign food source (*Escherichia coli* OP50), and 1/3 an unseeded strip between the two bacteria with 20 μl of 100mg/mL ampicillin antibiotic (see Supplemental Figure 2). The ampicillin removed external bacteria accumulated on the worms as they crawled across the plate

to the OP50 side, thereby preventing the spread of Sm2170 throughout the entire plate. After worms were plated directly onto SSPs with either live or HK Sm2170, they were left on the treatment plates for 5 days. Then, after 5 days on SSPs, worms surviving on OP50 (which included overlapping generations) were then removed from the treatment plate and plated onto an OP50-seeded plate without Sm2170. Thus, we created strong selection pressure for increased survival (resistance) within the susceptible populations. The OP50-seeded plates were left for 2 days to allow for growth of the populations in the absence of the parasite. A subset of these worms, based on the treatment, was then used to seed the next passage. The appropriate number of individuals were transferred to SSPs via liquid transfer in M9 buffer. A total of 6 experimental passages were completed, accounting for 6 generations of selection and approximately 13 total *C. elegans* generations.

Resistant populations

The resistant progenitor worm population was divided among four treatments: large populations (500 individuals) or small populations (25 individuals) plated on either live Sm2170 or HK Sm2170. Each treatment had 4 replicates. Again, only the host was allowed to evolve. The resistant host populations were evolved concurrently with the susceptible populations in the same conditions as described above. The only difference was in the size of the small population, here 25 individuals were transferred (Supplemental Figure 1).

Mortality assays

At the end of the 6th passage (~13 host generations), we measured the mortality rates of the evolved worms when infected with live Sm2170. Populations from each treatment were exposed

to live Sm2170 for mortality assays. Mortality assays were conducted on SSPs (see Supplemental Figure 3). 200 worms were plated onto the Sm2170 side. After 48 hours, the number of dead worms were counted, and from this the mortality rate was calculated (# of dead / 200). Mortality assays were performed twice for each replicate population.

Statistics

To determine differences in mean mortality rates between each population, we used JMP Pro13 (SAS, Cary, NC) to perform a generalized linear model with an exponential distribution and a reciprocal link function. Statistics were performed on the mean mortality rates of each treatment. The model effect factor included treatment as a single factor (large populations with live Sm2170, large populations with heat-killed Sm2170, small populations with live Sm2170, small populations with heat-killed Sm2170, and the ancestor). Post-test Tukey contrast tests were used to determine significance between treatments and are reported as Chi-square and p-values.

Results

Susceptible Populations

The ancestral host mortality rate of the susceptible populations when infected with Sm2170 was 72% (Penley & Morran, 2017). We selected for increased resistance (greater survival) over 6 passages of experimental evolution. We predicted that this selection would increase overall resistance against Sm2170, and thus reduce host mortality rates. However, we expected the efficacy of selection to be greater in the large (500) populations than in the small (50) populations, and that in the small populations, drift may inhibit resistance from evolving. Heat-

killed Sm2170 treatments with both large (500 individuals) and small (50 individuals) populations showed no significant difference in mortality compared to the ancestor ($X_2 = 0.015$, $p = 0.9$). The large populations evolved on live Sm2170 exhibited a significant decrease in mortality, indicating that increased resistance evolved ($X_2 = 9.87$, $p < 0.001$, Figure 2.1). Of the 4 replicates in the small population treatment evolved on live Sm2170, two replicates went extinct, thus skewing the mean mortality rate closer to 100% (Figure 2.1). Of the two remaining populations, one evolved an increase in mortality, and one evolved a decrease in mortality, thus a wide range of outcomes was observed in the small populations exposed to live parasites. Overall, large populations evolved significantly reduced mortality (i.e. increased resistance) compared to the small populations ($X_2 = 17.93$, $p < 0.0001$, Figure 2.1), but the mortality rate of the small population evolved on Sm2170 did not differ significantly from the ancestor ($X_2 = 0.08$, $p = 0.78$). These results are in line with the predictions that random genetic drift can overcome strong selection in small populations.

Figure 2.1

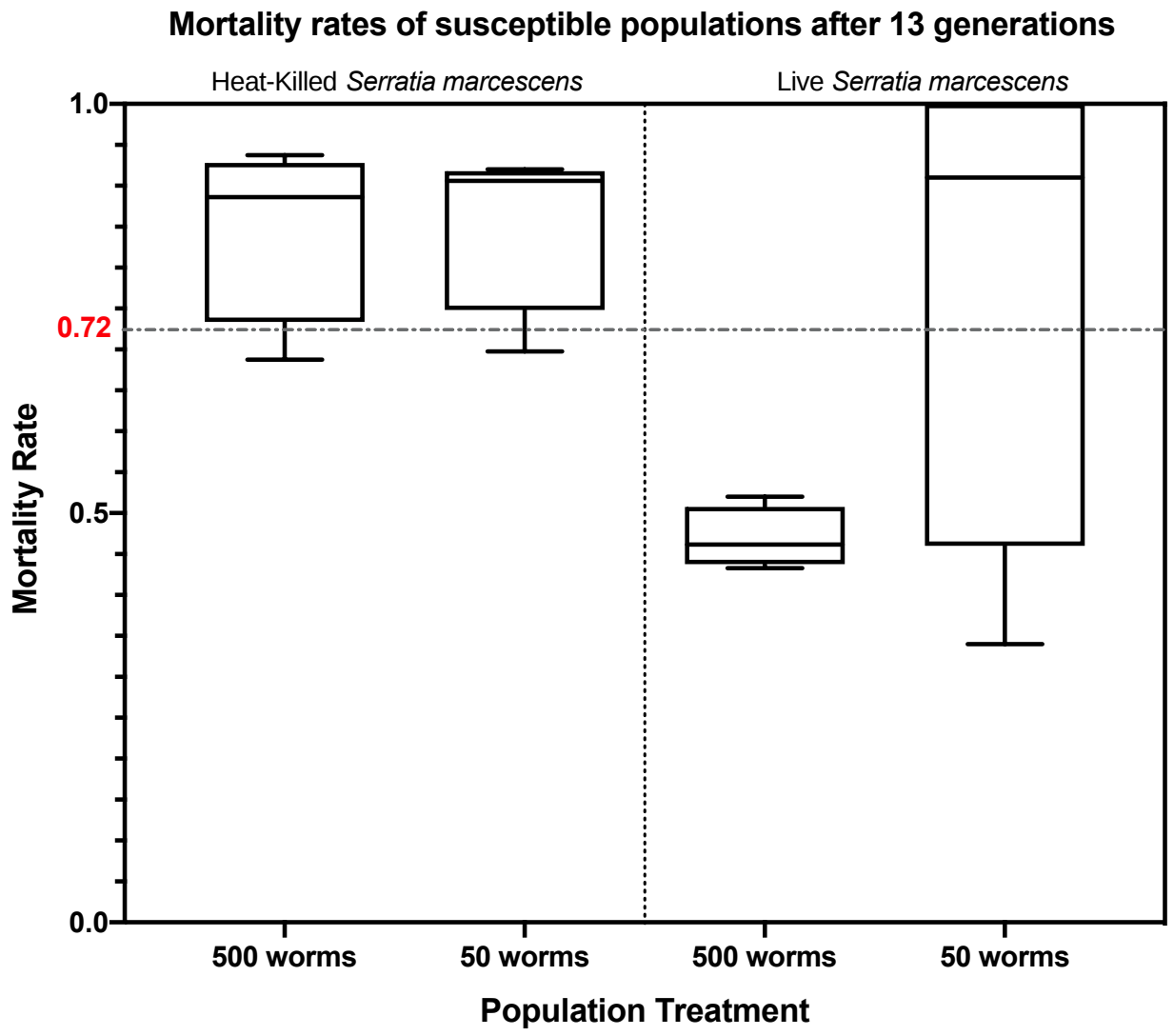


Figure 2.1. The mortality rates of evolved populations of the susceptible population *C. elegans* hosts when exposed to either heat-killed Sm2170 (left panel) or live Sm2170 (right panel). Populations exposed to either bacterial treatment differed in their size range, including 50 individuals in the small populations and 500 in the large populations. Each box-and-whiskers bar represents four replicate populations on which mortality assays were performed twice. Bars show standard error of the mean (SEM), and median values.

Resistant Populations

The resistant host populations had an initial mortality rate of 36% when infected with Sm2170, significantly lower than in the susceptible population (Penley & Morran, 2017). Here, we continued to select for increased survival in the subsequent 6 passages of experimental evolution. We predicted that this selection would either maintain or further reduce the mortality rate. We expected for selection on resistance to be strong in the large (500) population. In the small (25) populations, we expected drift may lead to either high or low mortality by random sampling. Similar to the small susceptible populations evolved with live parasites, described above (Figure 1), we saw extinction of two of the four replicate small populations passaged against live Sm2170. One population maintained resistance, and one population exhibited increased mortality, but not to the level of extinction (Figure 2.1). Mortality rates of worms evolved on heat-killed treatments in either large or small populations did not differ significantly from the ancestor ($X_2 = 2.24$, $p = 0.13$). For large populations that evolved on live parasites, there was no significant difference in mortality compared with the ancestor, suggesting that resistance was not lost when selection persisted in large populations ($X_2 = 2.14$, $p = 0.14$). However, we see that the small populations exposed to live parasites had a mortality rate significantly greater than the ancestor, which indicates that resistance previously gained was then subsequently lost due to genetic drift ($X_2 = 5.77$, $p = 0.02$, Figure 2.2).

Figure 2.2

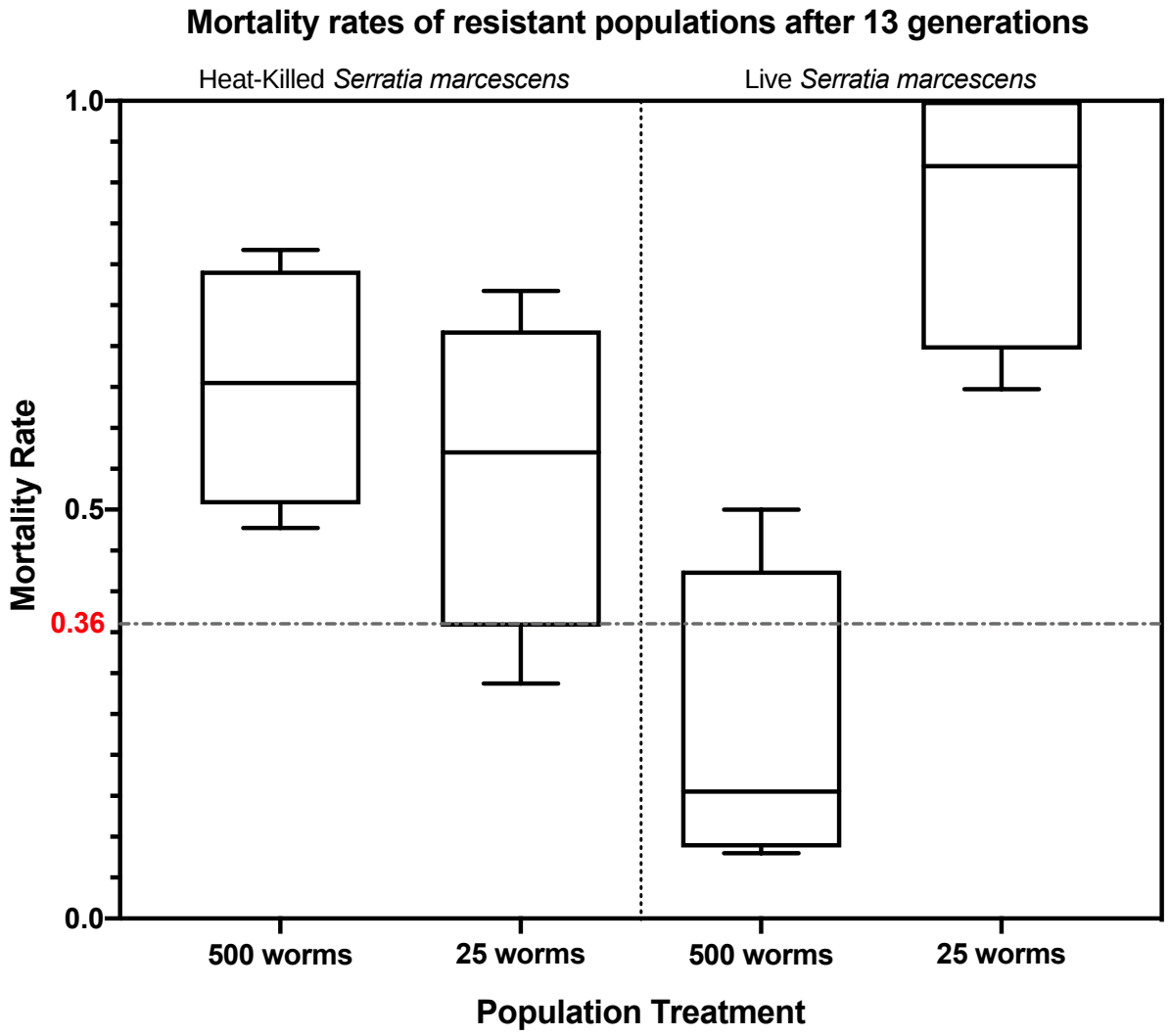


Figure 2.2. The mortality rates of evolved populations of the resistant *C. elegans* hosts when exposed to either heat-killed Sm2170 (left panel) or live Sm2170 (right panel). Populations exposed to either bacterial treatment differed in their size range, including 25 individuals in the small populations and 500 in the large populations. Each box-and-whiskers bar represents four replicate populations on which mortality assays were performed twice. Bars show standard error of the mean (SEM), and median values.

Discussion

Here, we assessed the effects of population size on the evolution and maintenance of resistance to the parasite *S. marcescens* in host populations of *C. elegans*. We found that host population size significantly altered the evolutionary trajectories of host populations. Despite strong selection imposed by the parasites, multiple small host populations went extinct within six passages of experimental evolution and did not consistently evolve or maintain resistance to the parasite (Figures 2.1 and 2.2). Conversely, the large host populations readily evolved greater resistance or maintained high levels of resistance when under selection by the parasites (Figures 2.1 and 2.2). Together, these results strongly suggest that genetic drift was the primary evolutionary force determining the evolutionary trajectories of the small host populations.

Our experiments show that effective population sizes, and drift by proxy, are an important determinant of the evolution and maintenance of resistance in host populations (Papkou *et al.*, 2016). This finding is relevant when considering the natural history, life history, and ecology of *C. elegans* (Barrett *et al.*, 2008; Schulenburg & Félix, 2017). *C. elegans* are found mostly as hermaphrodites in nature with low genetic diversity both within populations and across spatial scales (Barrière & Félix, 2005b; Andersen *et al.*, 2012). This low diversity combined with the necessity of dispersal to new food sources is likely to be a major determinant of whether or not *C. elegans* is able to persist in a new environment (Cutter, 2015). Thus, worms often may not survive new food patches, which may harbor parasites, due to their low effective population size and potentially limited ability to respond to local selective pressures (Félix *et al.*, 2013). However, very few experiments have actually tested adaptation of *C. elegans* to their local

parasites directly. Rather, much of what we know about genome-wide diversity comes from inferences of small-scale population genomics projects (Gibson & Morran, 2017). It may be that in nature, *C. elegans* populations are rescued from extinction by migration of genotypes from multiple areas to one new patch, thus benefitting from the dynamics of the metapopulation which may be much larger than the number of individuals that colonize a single bacterial patch (Barrière & Félix, 2005a; Richaud *et al.*, 2018). Rather than rescue, metapopulations may also provide opportunities for recolonization of bacterial patches if local population extinction occurs (Hastings & Harrison, 1994; Bohrer *et al.*, 2005). Additionally, "local" adaptation may occur on a scale that is greater than a single patch of bacteria, in which case our small populations may not accurately reflect *C. elegans* population size (Cutter, 2015; Frézal & Félix, 2015). Finally, *C. elegans* can also exhibit parasite avoidance behaviors, so *C. elegans* defense against parasites in nature may often occur behaviorally as opposed to an immune response (Zhang *et al.*, 2005; Glater *et al.*, 2014).

Host-parasite interactions are ubiquitous in nature, and all species interactions are driven by a combination of evolutionary forces. In addition to gene flow, selection and drift are two common mechanisms driving host evolution, but the degree to which they counteract or complement each other depends on host population size. Many studies of genetic drift have focused on bottlenecks of the parasite population (Papkou *et al.*, 2016), but we argue that in hosts with unique dispersal life stages in which populations continuously go through bottlenecks, drift is an essential driver of host-parasite evolution. Here, we tested the influence of both selection and drift on evolving host populations in consideration of natural dynamics. Our results indicate that drift has a significant influence on the evolution of hosts, even when selection pressure is strong and host

populations are genetically diverse. The better we understand how evolutionary forces interact and impact host evolution, the better we can understand host-parasite evolutionary trajectories.

Chapter II Supplemental Materials

Supplemental Table 1.
Resistant populations and ancestor: GLM with reciprocal logit and exponential distribution.
Treatment populations: live vs. HK Sm2170, small versus large population, ancestor vs. evolved

Whole Model Test

Model	**-Log Likelihood**	**L-R Chi Square**	**DF**	**Prob > Chi sq**
Difference	12.4042307	24.8085	4	<0.0001*
Full	33.7753718			
Reduced	46.1796024			

Post-test Tukey contrasts.

Resistant Pops.	HK 25	Live 500	HK 500	Ancestor
Live 25	X2 = 2.7079, p = 0.0999	X2 = 22.1243, p = 0.000002	X2 = 1.0809, p = 0.2985	X2 = 5.7724, p = 0.016
HK 25		X2 = 10.0505, p = 0.0015	X2 = 0.3733, p = 0.5412	X2 = 1.3689, p = 0.242
Live 500			X2 = 14.0427, p = 0.00018	X2 = 2.1435, p = 0.1432
HK 500				X2 = 2.6792, p = 0.1017

Supplemental Table 2.
Susceptible populations and ancestor: GLM with reciprocal logit and exponential distribution.
Treatment populations: live vs. HK Sm2170, small versus large populations, ancestor vs. evolved

Whole Model Test

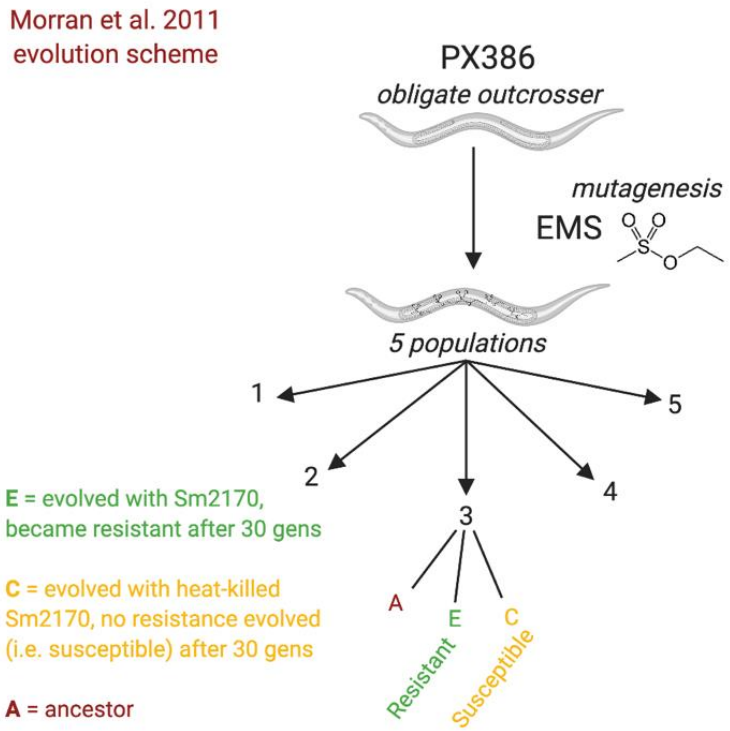
Model	**-Log Likelihood**	**L-R Chi Square**	**DF**	**Prob > Chi sq**
Difference	17.7375244	35.4750	8	<0.0001*
Full	411.276286			
Reduced	429.01381			

Post-test Tukey contrasts.

Susceptible Pops.	HK 25	Live 500	HK 500	Ancestor
Live 25	X2 = 0.4473, p = 0.5036	X2 = 17.9309, p < 0.0001	X2 = 0.3494, p = 0.5544	X2 = 0.0807, p = 0.7764
HK 25		X2 = 21.0638, p < 0.0001	X2 = 0.0060, p = 0.9381	X2 = 0.0194 p = 0.8892

Live 500			X2 = 23.1792, p < 0.0001	X2 = 9.8584, p = 0.0017
HK 500				X2 = 0.0083, p = 0.9273

Supplemental Figure 1.



Supplemental Figure 2. A scheme of strain origins. First, the strain was systematically inbred CB4856 to create PX382, then backcrossed the *q71* allele to be homozygous at the *fog-2* locus, resulting in an obligately outcrossing strain, PX386. PX386 was then mutagenized with ethyl methanesulfonate (EMS) to create approximately 1000 point mutations. Once mutagenized, a lineage population from the PX386 was divided into 5 ancestral populations and then experimentally evolved for 30 generations against either the Sm2170 parasite or heat-killed Sm2170 (Morran *et al.*, 2009, 2011).

Supplemental Figure 2.

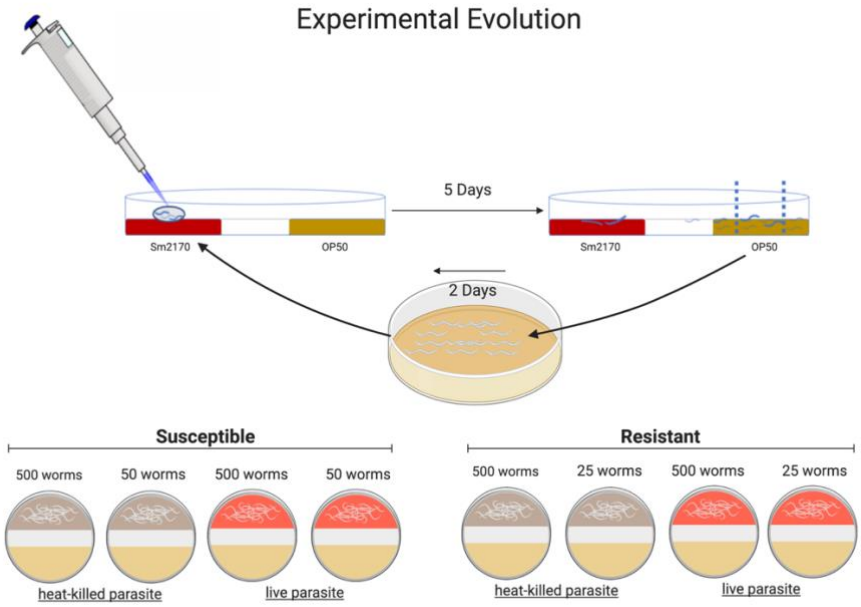
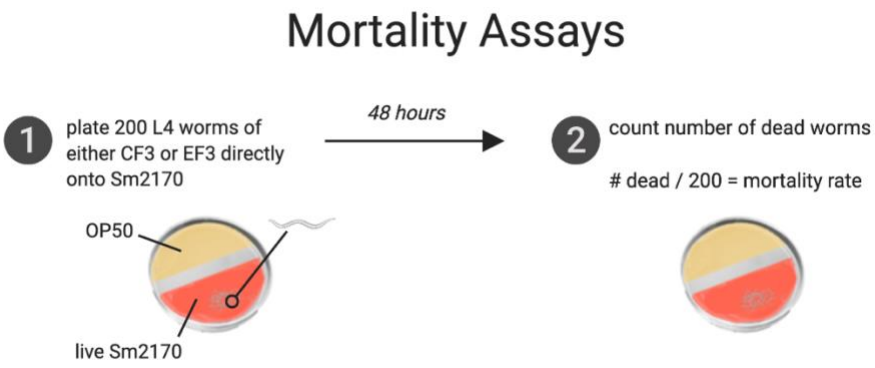


Figure 2. Experimental evolution design. *C. elegans* of either genotype (susceptible or resistant) or population size (large or small) were pipetted directly onto a Serratia selection plate (SSP) on the side with *S. marcescens* Sm2170 (or heat-killed Sm2170). The opposite side of the plate was seeded with a benign food source, *Escherichia coli* OP50, toward which they could move. In the middle of plate was a strip of 20µl 100mg/ml ampicillin antibiotic to remove any external Sm2170 before reaching the OP50. The worms were allowed to persist on the SSP for five days, at which time any living worms were removed and placed on a plate with only OP50. They were then allowed to defecate any remaining Sm2170 before being placed on the next round of SSPs. Top part of figure by Danial Arslan.

Supplemental Figure 3



Supplemental Figure 3. Mortality assays were performed using an identical set-up to the SSPs. 200 individual worms were. placed on evolved or ancestral Sm2170 and left for two days. At the end of day two, the number of dead worms were counted, and mortality rates were calculated.

CHAPTER III

Host heterogeneity mitigates virulence evolution

submitted to Biology Letters at the time of writing

Abstract

Parasites often infect genetically diverse host populations, and the evolutionary trajectories of parasite populations may be shaped by levels of host heterogeneity. Mixed genotype host populations, compared to homogeneous host populations, can reduce parasite prevalence and potentially reduce rates of parasite adaptation due to tradeoffs associated with adapting to specific host genotypes. Here, we used experimental evolution to select for increased virulence in populations of the bacterial parasite *Serratia marcescens* exposed to either heterogenous or homogenous populations of *Caenorhabditis elegans*. We found that parasites exposed to heterogenous host populations evolved significantly less virulence than parasites exposed to homogeneous host populations over several hundred bacterial generations. Thus, host heterogeneity impeded parasite adaptation to host populations. While we detected tradeoffs in virulence evolution, parasite adaptation to two specific host genotypes also resulted in modestly increased virulence against the reciprocal host genotypes. These results suggest that parasite adaptation to heterogenous host populations may be impeded by both tradeoffs and a reduction in the efficacy of selection as different host genotypes exert different selective pressures on a parasite population.

Introduction

Hosts and parasites are ubiquitous in nature. A long-standing goal in evolutionary biology is to understand the reciprocal selective pressures exerted by host and parasite interactions (Ebert &

Bull, 2007). Theoretical and empirical studies point to multiple factors that can determine the rate and magnitude of parasite adaptation to hosts as predicted by theory and supported by empirical work. These factors include host genetic heterogeneity (Regoes *et al.*, 2000; Morley *et al.*, 2017), host spatial structure (Haraguchi & Sasaki, 2000; Boots & Mealor, 2007), within-host or between-host competition (De Roode *et al.*, 2004; Mideo *et al.*, 2008), migration and gene flow of both host and parasite (Lively, 1996; Lion & Gandon, 2015), and host demography (Ebert & Mangin, 1997). Of particular interest is how host genotypes influence the evolutionary trajectory of parasites populations as they adapt to host populations.

Historically, host heterogeneity has been overlooked in theoretical models of infection dynamics (Bremermann & Pickering, 1983; May & Anderson, 1983), yet host heterogeneity is both biologically relevant and a potential source of selection driving parasite evolution. Host homogeneity is generally rare in natural populations, even in many asexual host populations (Dybdahl & Lively, 1995; Fontcuberta García-Cuenca *et al.*, 2016). Theoretical models of host heterogeneity predict that specialization on similar host genotypes results in reduced transmission between dissimilar genotypes, which leads to lower parasite prevalence (Kaltz & Shykoff, 1998; Morley *et al.*, 2017). Due to this trade-off, parasite prevalence tends to be mitigated compared with prevalence in homogeneous populations. This is also known as the monoculture effect (Ekroth *et al.*, 2019). Evidence for this trade-off has been found in agriculture systems (Elton, 1958; Garrett & Mundt, 1999; Zhu *et al.*, 2000; Pilet *et al.*, 2006) as well as in natural populations (e.g. plants (Schmid, 1994); prokaryotes (van Houte *et al.*, 2016); invertebrates: bumblebees (Baer & Schmid-Hempel, 1999, 2001), *Daphnia* (Altermatt & Ebert,

2008; Ganz & Ebert, 2010), snails (Campbell *et al.*, 2010); and vertebrates (Pearman & Garner, 2005)), in which prevalence differs between homogeneous and heterogeneous populations.

Heterogeneous populations may impede parasite adaptation and thus limit virulence. In some cases, high levels of host genetic diversity can even prevent parasite adaptation altogether, such as occurred in a study by Morley et al. (2017), in which a bacteriophage exposed to a genetically diverse bacterial host population failed to adapt to local hosts (Morley *et al.*, 2017). High levels of host diversity reduce the average rate at which parasites successfully infect hosts (Gandon & Nuismer, 2009). In addition, host diversity reduces the opportunity for specialization on a single host genotype. Both of these forces may limit the potential for adaptation and evolution of increased virulence. In this study, we asked whether heterogeneity per se is sufficient to alter parasite evolutionary trajectories by examining virulence evolution in populations with different ratios of host genotypes. Further, if homogeneity allows for greater virulence to evolve, is there a cost of adapting to one specific genotype when parasitizing novel hosts, resulting in a fitness loss for the parasite?

Here, we used experimental evolution to select for greater parasite virulence while passaging parasites through either genetically homogeneous or heterogeneous host populations. We predicted that the rate at which parasites evolved greater virulence would be impeded in heterogeneous host populations. Accordingly, we hypothesized that parasites evolved in homogeneous host populations would evolve greater virulence by specializing on a single host genotype. Further, we expected to see a cost of specialization when infecting a new host genotype, shown as reduced virulence against the new genotype. To test these predictions, we

evolved a clonal bacterial parasite, *Serratia marcescens*, strain Sm2170, in two genotypes of the host *Caenorhabditis elegans*. The *C. elegans* genotypes used were CB4856 and ewIR 68 (Doroszuk *et al.*, 2009). The two strains have genetically diverse backgrounds but identical portions of chromosome V, where many innate immune loci reside. CB4856 and ewIR68 were chosen to minimize tradeoffs of specialization as a means to better isolate heterogeneity as a variable. For our experimental treatments, we varied the ratio of the host genotypes in each host population. We then compared the mortality rates of the evolved parasites from each treatment to the ancestral parasites by infecting each host genotype separately.

Methods

(a.) Experimental Evolution

Using experimental evolution, we imposed selection for increased virulence on *S. marcescens* Sm2170 parasites exposed to either homogenous or heterogeneous host populations. Hosts were the *C. elegans* strains ewIR 68 and CB4586. *S. marcescens* is transmitted orally. Some live bacterial cells survive ingestion (Avery & You, 2012). Once in the intestine, *S. marcescens* will replicate until host death (Schulenburg *et al.*, 2004). We measured virulence as infection-induced host mortality rate, and imposed selection for increased virulence by collecting and passaging Sm2170 only from hosts that died after 24 hours of exposure (see Supp. Fig. 1 for detailed experimental design). Thus, parasite genotypes that facilitated rapid host killing were favored. We passaged Sm2170 populations through 6 different host heterogeneity treatments and a control, in which parasites were passaged in the absence of hosts (*in vitro*, 0-0) (Supp. Fig. 2).

For each new passage of experimental evolution, we plated 1,000 worms on a *Serratia* selection plate (Supp. Fig. 1) and allowed the worms to consume Sm2170 for 24 hours (Morran *et al.*, 2009; Penley & Morran, 2018). We then isolated 30 dead worms from the Sm2170 lawn. Dead worms were identified by a lack of movement in response to provocation with a platinum wire (Amrit *et al.*, 2014). Then, we extracted Sm2170 from the host carcasses, cultured them in standard lab conditions (28ºC shaker overnight), and inoculated an unseeded nematode growth media (US Biological, Salem, MA) plate to grow colony forming units (CFUs) for 48 hours at room temperature. From these plates, we randomly picked 40 CFUs per Sm2170 population, to inoculate the next passage. New naïve (non-evolved) hosts (from homozygous host lines kept at -80ºC) were then placed on the evolved bacteria and the process was repeated. For our *in vitro* control (0-0), 40 CFUs of Sm2170 were picked from the bacterial lawn. This treatment served as our control for genetic drift and adaptation to passage conditions other than host factors. The selection experiment concluded at the end of 10 passages (totaling hundreds of bacterial generations). At the end of each passage, a subset of the evolved bacteria was stored at -80ºC.

(b.) Mortality Assays

Mortality assays were used to determine virulence at the beginning and end of the selection experiment. Bacteria from passage 10 were used to infect homogeneous groups of either host genotype, and their mortality rates were compared to the ancestral bacteria. The steps outlined in the creation of the *Serratia* selection plates were identical to those of the mortality assays (see Supp. Info).

Two hundred worms of one genotype (either ewIR68 or CB4856) were placed on a mortality assay plate (Supp. Figure 2, step 1). After 48 hours at 20ºC, the number of dead worms on each plate were counted (Supp. Figure 2, step 2). Mortality rates were calculated as the proportion of dead hosts on the treatment plates divided by the number plated. When performing mortality assays, each replicate population had at least 3 technical replicates (¼ of the assays had 3 technical replicates per population and ¾ of the assays had 6 technical replicates per population) for a total of 360 mortality assay plates (Supp. Figure 2, step 3). Ancestral Sm2170 mortality assays on each host were performed both at the outset of the experiment and again when performing evolved Sm2170 mortality assays at the end of the experiment.

Statistical Analysis

To assess mean changes in mortality rate between ancestral and evolved parasite populations, we used JMP Pro 13 (SAS, Cary, NC) to perform a generalized linear model (GLM) with a link logit function and normal distribution. Factors in the model include treatment (e.g. homogeneous, heterogeneous, *in vitro*), host genotype (ewIR 68 or CB4856), and a treatment x host genotype interaction. We did not detect overdispersion using a Pearson test. Post-analysis Tukey contrast tests were used to determine significance of pair-wise comparisons. We report our values as chi-squared statistics and corresponding p-values. Multiple comparisons were corrected for using a Bonferroni correction of $p < 0.025$ ($p < a/k$, where $a = 0.05$, $k = 2$ comparisons) in Supp. Table 1 and $p < 0.0125$ (where $k = 4$ comparisons) in Supp. Tables 2-3.

Results

Ancestral populations of Sm2170 bacteria produced a mean mortality rate of 48.33% (SEM ± 0.03) in host strain ewIR 68 and 64.32% (SEM ± 0.04) in host strain CB4856. As predicted, we found that selection for virulence resulted in an increase in virulence. Parasites evolved in both homogeneous host populations were significantly more virulent than the *in vitro* controls (Figure 3.1a, CB4856: $X^2 = 30.32$, $p < 0.0001$; Figure 3.1b, ewIR 68: $X^2 = 48.01$, $p < 0.0001$). Parasites evolved in CB4856 hosts had a 64% increase in mortality rate in homogeneous populations compared to the ancestor, while parasites evolved in ewIR 68 had a 35% increase in mortality rate in homogeneous populations compared to the ancestral parasite.

There were no significant differences in mortality induced by parasites in either host between any of the pairs evolved on 75-25, 50-50, or 25-75 (Figure 3.1). When tested in CB4856 hosts, parasites evolved in heterogeneous host populations did not differ significantly in mortality rate from the *in vitro* parasites (Figure 3.1a, $X^2 = 0.30$, $p = 0.58$), indicating little to no adaptation to the CB4856 host genotype. However, the same parasites exhibited a significant increase in mortality rate compared to *in vitro* parasites when tested in ewIR 68 hosts (Figure 3.1b, $X^2 = 12.52$, $p = 0.0004$). Thus, parasites exposed to heterogenous hosts exhibited increased virulence against only one host genotype. However, the increase in virulence against ewIR 68 hosts exhibited by parasites evolved with heterogenous host populations was limited compared to parasites evolved with homogenous host populations. Parasites evolved in homogeneous ewIR 68 populations caused greater virulence in ewIR 68 than parasites evolved in heterogeneous populations (Figure 3.1b, $X^2 = 8.78$, $p = 0.0031$). Additionally, parasites evolved in homogeneous CB4856 populations caused greater virulence in CB4856 hosts than parasites evolved in

heterogeneous populations (Figure 3.1a, $X2 = 46.41$, $p < 0.0001$). Overall, these results demonstrate that host heterogeneity impedes parasite adaptation relative to host homogeneity.

Figure 3.1.

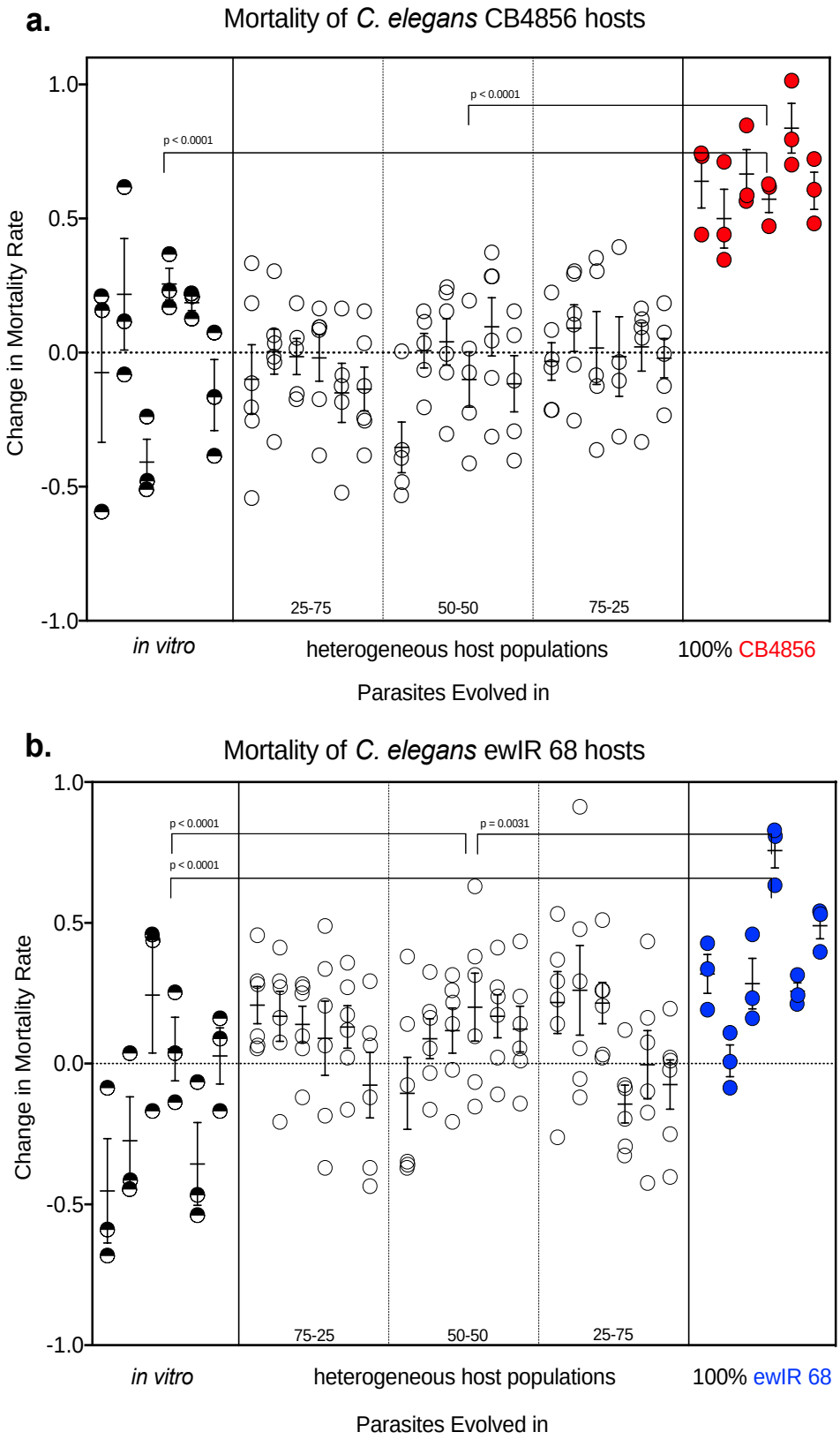

a.
Mortality of C. elegans CB4856 hosts
1.0
0.5
0.0
-0.5
-1.0
Change in Mortality Rate
p < 0.0001
p < 0.0001
25-75
50-50
75-25
in vitro
heterogeneous host populations
100% CB4856
Parasites Evolved in
b.
Mortality of C. elegans ewIR 68 hosts
1.0
0.5
0.0
-0.5
-1.0
Change in Mortality Rate
p < 0.0001
p < 0.0001
p = 0.0031
75-25
50-50
25-75
in vitro
heterogeneous host populations
100% ewIR 68
Parasites Evolved in

Figure 3.1. Mean change in host mortality rate relative to the ancestral parasites in *C. elegans* host strains CB4856 (panel a) and ewIR 68 (panel b). All experimental populations shared a common ancestor, and thus any change from the ancestral data is indicative of relative virulence. Parasites were evolved in either heterogeneous host populations, homogeneous host populations, or *in vitro* (no hosts), and then tested for changes in virulence. The heterogeneous populations, from left to right, are 75-25, 50-50, and 25-75. Circles represent the mean change within each technical replicate (18-36 each). Bars represent ± standard error of the mean (SEM).

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Next, we determined if parasites evolved on homogeneous host populations and then exposed to a novel host exhibited reduced virulence, and thus lower fitness, on the novel host as predicted by trade-off theory. In both cross-infections – ewIR 68 hosts infected with parasites that evolved with CB4856 hosts alone, and CB4856 hosts infected with parasites that evolved with ewIR 68 hosts alone - there was an increase in mortality rate relative to the ancestral strain and relative to the *in vitro* controls (Supp. Table 1). However, this increase in mortality was significantly lower than when parasites infected familiar hosts (Figure 3.2, $X_2 = 5.70$, $p = 0.01$, Supp. Table 1). This indicates that the bacteria evolved in homogeneous populations of either host did not cause the same level of virulence in novel hosts as bacteria did in their familiar hosts. This result is in accordance with the previous finding, that heterogeneous host populations limit the evolution of parasite virulence, and indicates a trade-off imposed by host heterogeneity.

Figure 3.2.

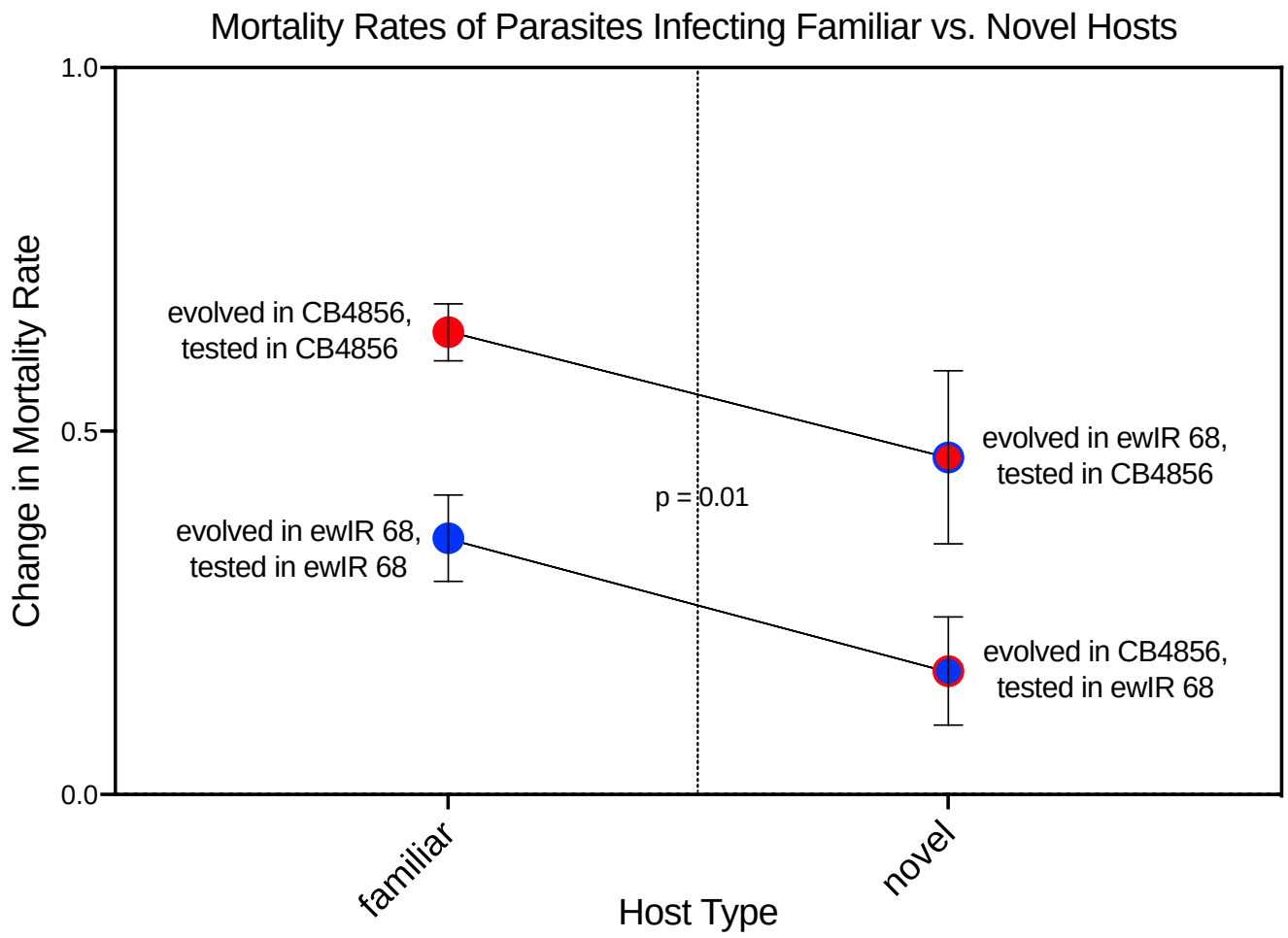

Figure 3.2. Each dot represents the treatment's average change in mortality rate of all populations and replicates relative to ancestral parasites. All experimental populations shared a common ancestor, and thus any change from the ancestral data is indicative of relative virulence. The x-axis shows the type of host infected: either familiar to the parasite or novel. The p-value is based on a post-GLM Tukey contrast test between all familiar hosts (left panel) and all novel hosts (right panel) ($X_2 = 5.70$, p = 0.01). In both cases, although all treatments had an increased mortality rate relative to the ancestor, novel hosts had a lower mortality rate than do the familiar hosts. Bars around mean represent SEM.

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Discussion

In our selection regime, higher host mortality equates to higher virulence, and thus greater parasite fitness. Our results show that parasites selected in homogeneous host populations evolved substantial increases in virulence when infecting those same hosts (for both ewIR 68 and CB4856) as compared to the ancestral parasite (Figure 3.1). However, parasites that were selected in mixed genotype host populations and then tested on homogeneous host genotypes exhibited limited increases in virulence (Figure 3.1), despite strong selection favoring increased virulence. We found no differences in the mortality rates of host infected by parasites evolved with any mixed host population on either host – neither comparing between each mixed treatment nor compared with the ancestor. Thus, exposure to heterogenous host populations impeded virulence evolution relative to exposure to homogenous hosts. Further, parasites evolved in homogeneous populations and then used to cross-infect the other (novel) host genotype exhibited smaller increases from the ancestral virulence than when infecting their familiar host (Figure 3.2). Therefore, we observed tradeoffs in virulence due to specialization on the parasites' familiar host genotype.

Tradeoffs in parasite virulence or infectivity due to specialization on a particular host genotype are often invoked as a reason heterogenous host populations may impede parasite adaptation. Here, we found that heterogenous host populations impeded the evolution of parasite virulence and we found evidence of tradeoffs in parasite virulence (Figure 3.1). However, the evolved tradeoffs in parasite virulence that we observed are not sufficient to explain the limited virulence evolution in parasites evolved with heterogenous host populations. Despite parasite

specialization (greater virulence) on familiar homogenous hosts, parasites evolved in homogenous host populations still exhibited increased virulence against novel hosts relative to the *in vitro* control parasites (Figure 3.2). Therefore, any potential cost of a tradeoff should have been mitigated in the heterogenous host populations, as adaptation to either host genotype still resulted in increased virulence against the other host genotype. Yet, we still observed a limited response to selection for increased virulence in parasites evolving in heterogenous host populations (Figure 3.1).

One possibility for the lack of a substantial tradeoff cost (i.e. a decline in parasite fitness) in the cross-infections may be that the *C. elegans* genotypes used, CB4856 and ewIR 68, share an identical region of chromosome V, which harbors many loci associated with innate immunity (Glater *et al.*, 2014). It is likely that parasites evolved in either genotype were under strong selection to evolve in response to that particular region of the genome. Despite the genetic similarity of the strains at many innate immune system loci, heterogenous host populations still impeded parasite adaptation relative to homogenous populations. While it is quite plausible that tradeoffs in virulence slowed parasite adaptation in the heterogenous host populations to some degree, tradeoffs alone are insufficient to explain the lack of increase in virulence exhibited by heterogenous-selected parasites when infecting CB4856 hosts (Figure 3.1). We hypothesize that this lack of response to selection was likely driven by a reduction in the efficacy of selection in the heterogenous host populations relative to the homogenous hosts. Selection imposed by different host genotypes can act on different groups of loci in the parasite genome (Croll & McDonald, 2017). As a result, the efficacy of selection on a particular set of loci in the parasites may be reduced in the heterogenous hosts as parasites encounter different host genotypes with

each infection (Bell, 2010). Although a portion of our host genomes were identical, the diverse genetic backgrounds of the CB4856 and ewIR68 strains may have imposed fluctuating selection on the parasite populations, resulting in limited parasite adaptation within heterogenous host populations.

Previous work has shown that heterogeneous host populations are common in nature (Zhan *et al.*, 2002; van Baalen & Beekman, 2006; Lively, 2010; Kubinak & Potts, 2013; Berngruber *et al.*, 2015) and our results demonstrate that host heterogeneity can alter the trajectory of parasite evolution. Importantly, parasites are capable of adapting to heterogenous host populations over time (Koskella & Lively, 2007). Nonetheless, our results indicate that parasite adaptation can be impeded by heterogenous hosts relative to homogenous host populations. While we observed little cost to host specialization in our experiment, costly tradeoffs to host specialization are likely to impede rates of parasite adaptation in heterogeneous host populations (Gibson *et al.*, 2019). We anticipate that changes in the efficacy of selection imposed heterogenous host populations may also contribute to reduce rates of parasite adaptation. Therefore, we believe it is critical to better understand the implications of host heterogeneity for disease evolution. The ability to manage parasite virulence in both human infectious diseases, agriculture, and in the conservation of wildlife has long been a goal of research on parasite evolution (Dieckmann *et al.*, 2002). Our results indicate that increasing host heterogeneity may not only be useful for decreasing disease prevalence and spread, but also for hindering parasite adaptation and virulence evolution.

Chapter III: Supplementary Materials

Whole Model Test

Model	-Log Likelihood	L-R Chi Square	DF	Prob > ChiSq
Difference	30.1270	60.2539	11	< 0.0001
Full	-16.3266			
Reduced	13.8004			

Supplementary Table 1
P-value considered significant if $p < 0.025$. Bold denotes significance based on adjusting for multiple comparisons (parasite treatment and host genotype) via Bonferroni corrections.

Results of contrast tests when combining the results of both host genotypes:

Combined Mortality Assays Results of Both Hosts	Evolved on 100% CB4856 → Tested on CB4856 AND Evolved on 100% ewIR 68 → Tested on ewIR 68	Evolved on 75-25, 50-50, & 25-75 → Tested on CB4856 AND Tested on ewIR 68	Evolved i*n vitro* (no hosts) → Tested on CB4856 AND ewIR 68
Evolved on 100% CB4856 → Tested on ewIR 68 AND Evolved on 100% ewIR 68 → Tested on CB4856	**$p = 0.0169$ $X_2 = 5.6999$**	**$p < 0.0001$ $X_2 = 20.6134$**	**$p < 0.0001$ $X_2 = 27.6466$**
Evolved on 100% CB4856 → Tested on CB4856 AND Evolved on 100% ewIR 68 → Tested on ewIR 68		**$p < 0.0001$ $X_2 = 44.6757$**	**$p < 0.0001$ $X_2 = 47.8344$**
Evolved on 75-25, 50-50, & 25-75 → Tested on CB4856 AND Tested on ewIR 68			$p = 0.0287$ $X_2 = 4.7875$

Supplementary Table 2
P-value considered significant if $p < 0.0125$. Bold text denotes significance.

Results of contrast tests with CB4856 hosts:

Mortality Assays on Host CB4856	Evolved on 100% CB4856 → Tested on CB4856	Evolved on 75%, 50%, and 25% CB4856 → Tested on CB4856	Evolved i*n vitro* (no hosts) → Tested on CB4856
Evolved on 100% CB4856 → Tested on ewIR 68	**p = 0.00002** **X_2 = 17.993**	p = 0.0131 X_2 = 6.1569	p = 0.1085 X_2 = 2.5754
Evolved on 100% CB4856 → Tested on CB4856		**p < 0.0001** **X_2 = 46.4054**	**p < 0.0001** **X_2 = 30.3206**
Evolved on 75%, 50%, and 25% CB4856 → Tested on CB4856			p = 0.5813 X_2 = 0.3042

Supplementary Table 3
P-value considered significant if p < 0.0125. Bold text denotes significance.

Results of contrast tests with ewIR 68 hosts:

Mortality Assays on Host ewIR 68	Evolved on 100% ewIR 68 → Tested on ewIR 68	Evolved on 75%, 50%, and 25% ewIR 68 → Tested on ewIR 68	Evolved i*n vitro* (no hosts) → Tested on ewIR 68
Evolved on 100% ewIR 68 → Tested on CB4856	p = 0.2835 X_2 = 1.1503	**p < 0.0001** **X_2 = 16.9641**	**p < 0.0001** **X_2 = 33.9912**
Evolved on 100% ewIR 68 → Tested on ewIR 68		**p = 0.0031** **X_2 = 8.7751**	**p < 0.0001** **X_2 = 25.3646**
Evolved on 75%, 50%, and 25% ewIR 68 → Tested on ewIR 68			**p = 0.0004** **X_2 = 12.5246**

C. elegans and *S. marcescens* strains

C. elegans strain ewIR 68 was acquired from Jan Kammenga (Wageningen University, 2017). Both *E. coli* OP50 and *C. elegans* CB4856 were acquired from the Caenorhabditis Genome Center (CGC) (University of Minnesota, 2010), and *S. marcescens* Sm2170 was acquired from Sue Katz (Rogers State University, 2006). All strains were frozen at -80ºC and thawed for each new experiment.

Experimental Evolution: supplemental methods

For the initial passage of the experiment, we streaked Sm2170 (stored at -80ºC frozen in glycerol) onto an NGM-Lite (US Biological, Salem, MA) plate using an inoculation loop. After 48 hours of growth at room temperature (RT), we inoculated one Sm2170 colony into 5 mL lysogeny broth (LB) in a lidded test tube and grown overnight in a 28ºC shaker incubator. We performed the same series of steps for *E. coli* OP50, the primary food source of *C. elegans*. After 24 hours of growth in 28ºC LB, we pipetted 35 μL of Sm2170 from the LB tube and spread on a NGM-Lite 10 cm petri dish on 1/3 of a plate, and we spread 35 μL of OP50 onto another 1/3 of a plate (Supp. Figure 1, step 1). The two bacterial lawns were separated by a strip of bare agar. These plates are hereafter referred to as *Serratia* selection plates (SSPs).

Once all plates were seeded with both bacteria, they were put in 28ºC to grow bacterial lawns overnight. The following day, approximately 1,000 larval stage 4 (L4) worms of differing mixtures of two *C. elegans* host genotypes were placed directly onto the lawn of *S. marcescens* Sm2170 on the SSPs (Supp. Figure 1, step 1). Each passage, we obtained synchronized populations of eggs using bleach and sodium hydroxide, which then developed into the L4 stage in around 44 hours [30]. Thus, we made sure to synchronize the worms two days before plating them and performed these assays at the same time as inoculating LB with each treatment bacteria.

The host genotype mixture treatments included the following: worms 1,000 CB4856 worms (100-0) (in other words, 100% CB4856 and no (0%) ewIR 68), 750 CB4856 worms plus 250 ewIR 68 worms (75-25), 500 CB4856 worms plus 500 ewIR 68 worms (50-50), 250 CB4856 worms plus 750 ewIR 68 worms (25-75), 1,000 ewIR 68 worms (0-100), and no worms as a 0-0 control (Supp. Figure 2). Each treatment included six replicate populations for a total of

36 treatment populations. After 24 hours, 30 dead worms were picked from the Sm2170 side of the plate into a 1.5 mL microcentrifuge tube filled with 1 mL of M9 buffer (Figure 1, step 2). Since many worms are still alive at the 24-hour time point, selecting for those killed early on likely selects for the most virulent parasites in the population. In the no-worm control (0-0) populations, the Sm2170 lawn was dabbed randomly 30 times by an inoculation loop and the selected bacteria were placed in a microcentrifuge tube with 1 mL of M9. All microcentrifuge tubes with dead worms were washed 6 times to rid any external bacteria. They were first spun at 3,000 rpm for 1 minute, supernatant was then pipetted off, and 900 μl of new M9 was added to the tube (Supp. Figure 1, step 3). Once washed, the internal bacteria were released from the worm bodies by using a cordless tissue grinder with autoclavable pellet mixers for 30 seconds (Supp. Figure 1, step 4). This was repeated for all 36 microcentrifuge tubes, including the 6 populations without worms (0-0).

Once homogenized, bacteria and worm bodies were streaked onto an NGM-Lite plate, then colonies of surviving bacteria were allowed to grow at room temperature for 48 hours and were stored at 4ºC until further use (Supp. Figure 1, step 5). OP50 colonies were also stored at 4ºC for use in subsequent passages and did not evolve in the course of this experiment. For the next passage, 40 Sm2170 colonies that had grown up on the plates stored in 4ºC were then used to inoculate a 5 mL vial of LB, then grown overnight at 28ºC, and used to inoculate the next round of SSPs (Supp. Figure 1, step 6-8). Each passage took approximately 1 week, and the entire process was repeated 10 times for a total of 10 weeks. After each passage of evolution, a subset of the evolved bacteria was frozen with glycerol and stored in -80ºC, and the other subset was used to seed the next round of SSPs. After ten passages of experimental evolution, we had a

chronological record of each generation's frozen bacteria. The experiment concluded at the end of 10 passages.

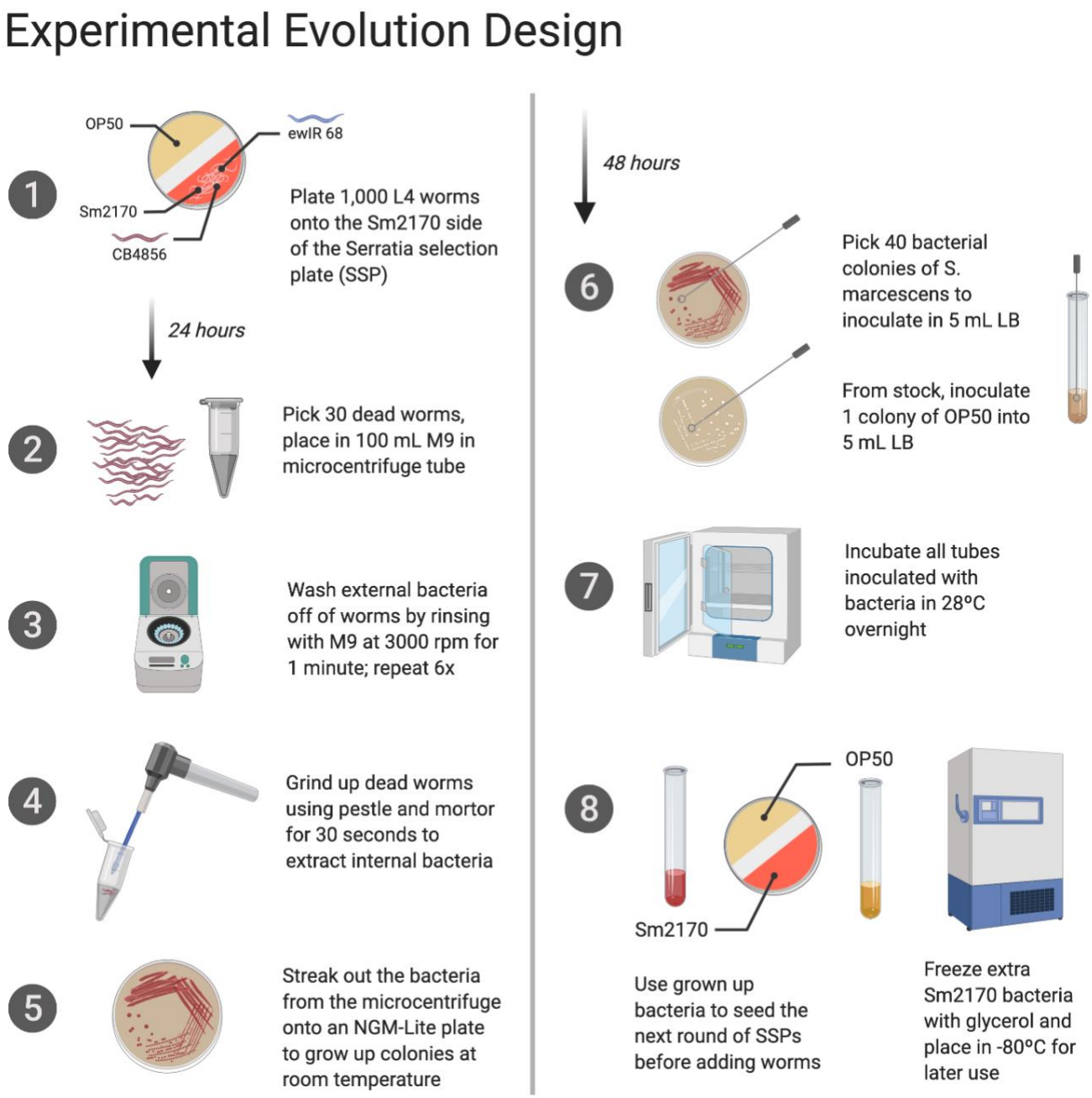
Mortality Assays: supplemental methods

For the mortality assays, we used bacteria frozen after passage 10 to compare with the ancestral bacteria. The steps outlined in the creation of the SSPs were identical to that of the mortality assay. Two-hundred worms of the same genotype were placed on a mortality assay plate (Supp. Figure 2, step 1). To calculate an average volume of 200 worms, a plate of healthy L4 worms was washed with M9 into a 1.5 microcentrifuge tube, 10 μl of the volume was pipetted into 3 glass wells, and the average number of worms per 10 μl was calculated to determine the volume for an average of 200 worms. We then pipetted that volume onto 3 unseeded plates each and averaged the number plated.

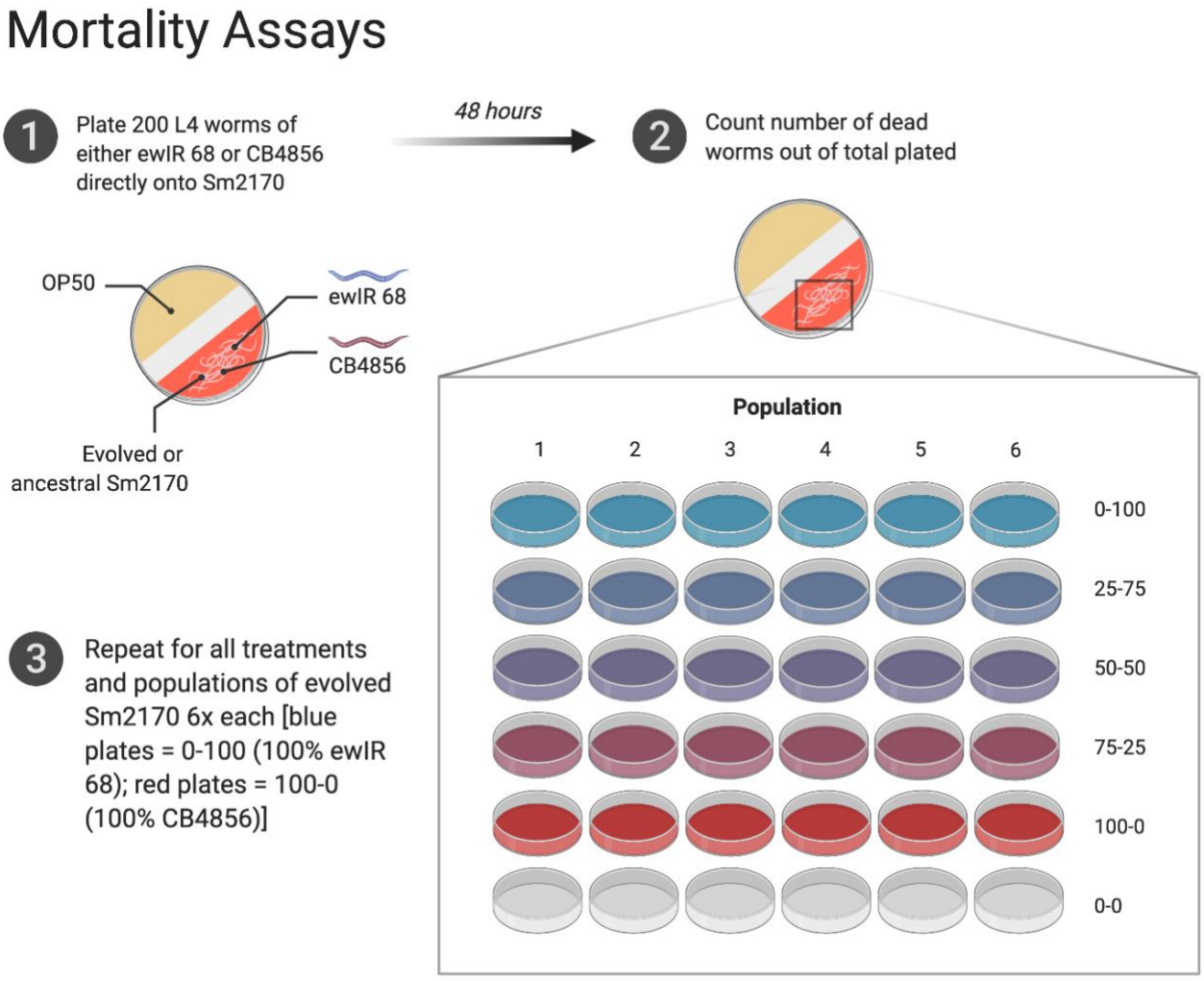
After 48 hours at 20ºC, the number of dead worms on each plate were counted (Supp. Figure 2, step 2). Mortality rates were calculated as the proportion of dead hosts on the treatment plates out of the averaged number plated on the unseeded plates. As stated previously, each treatment had a total of 6 replicate populations that were evolving against a particular mix of hosts. To determine whether the parasites evolved greater virulence over the course of the experiment, the evolved parasites were compared to the ancestral parasites in their ability to cause host mortality. Thus, both host strains were infected with the ancestral parasite in order to have a base mortality rate to compare with the evolved mortality rate at the end of ten passages. When performing mortality assays, we had 6 treatments x 6 population replicates x 6 (or 3) technical replicates = 360 total plates (Supp. Figure 2, step 3). To control for variation in

mortality assays, ancestral assays were performed alongside treatment assays in addition to those performed at the outset of the evolution experiment.

Supplementary Figure 1.



Supplementary Figure 2.



CHAPTER IV

Dauer life stage of *Caenorhabditis elegans* induces elevated levels of defense against the parasite *Serratia marcescens*

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Abstract

Host-parasite research often focuses on a single host life stage, yet different life stages may exhibit different defenses. The nematode *Caenorhabditis elegans* has an alternate dispersal life stage, dauer. Despite dauer's importance in nature, we know little of how it responds to parasites. Previous research indicates that non-dauer *C. elegans* prefer to consume the virulent bacterial parasite, *Serratia marcescens*, when given a choice between the parasite and benign *Escherichia coli*. Here, we compared the preferences of dauer individuals from six strains of *C. elegans* to the preferences of other life stages. We found that dauer individuals exhibited reduced preference for *S. marcescens*, and dauers from some strains preferred *E. coli* to *S. marcescens*. In addition to testing food preference, a mechanism of parasite avoidance, we also measured host mortality rates after direct parasite exposure to determine if life stage also altered host survival. Overall, dauer individuals exhibited reduced mortality rates. However, dauer versus non-dauer larvae mortality rates also varied significantly by host strain. Collectively, we found evidence of dauer-induced parasite avoidance and reduced mortality in the presence of a parasite, but these effects were strain-specific. These results demonstrate the importance of host life stage and genotype when assessing infection dynamics.

Introduction

Parasites and hosts impose strong selection on one another. Infections in nature are nearly ubiquitous and host defense against infection is critical in order to survive and reproduce. Hosts

primarily use avoidance, resistance, and tolerance as different means of defense. While researchers typically treat each as a separate response, all exist in an interconnected web that acts to prevent or mitigate parasite infection. Avoidance is typically defined as the detection of a parasite and a subsequent behavioral response to either move from an area where a parasite is located or temporarily pause ingestion of resources. Avoidance is often employed as a first line of defense, relative to either resistance or tolerance.

When a host retreats from an area with parasites, it can minimize the risk of infection. Hosts detect and avoid parasites in a variety of ways, including olfactory sensing of parasite toxins, altering food choice from those with potential toxins or high CO_2 levels, and avoiding areas previously known to harbor infection (Kiesecker *et al.*, 1999; Bretscher *et al.*, 2008; Meisel & Kim, 2014). Some avoidance behaviors may be dependent on the life stage of the host, and thus a host's chances of survival may differ throughout the host's life (Gentry & Dyer, 2002; Behringer *et al.*, 2006). For example, very young organisms may not be able to travel far due to small size or lack of mature phenotype to transport them from harm (e.g. flying) (Tate & Rudolf, 2012). Importantly, when employed successfully, many of these defense strategies prevent the host from becoming infected, so potentially costly immune responses are not always necessary to stave off infection.

Parasites infect hosts at multiple host life stages with varying effects, yet it is common for researchers to focus on a single host life stage when assessing infection dynamics. There are many examples of parasite infections varying in severity between different host life stages. For example, Balla *et al.* found that when larvae of the host *Caenorhabditis elegans* were infected with an intracellular microsporidia parasite at the first larval stage (L1), the larvae exhibited significantly reduced fecundity as compared with hosts that were infected at the fourth larval

stage (L4) (Balla *et al.*, 2015). The larvae of the Indian meal moth *Plodia interpunctella,* a commonly studied host organism in host-parasite interactions, suffers high mortality rates when infected by a granulosis virus, whereas adults do not appear to be infected at all (Boots, 1998). Alternatively, some diseases only affect adults, such as in the high mortality of adult female cassava mites (*Mononychelles tanajoa*) infected with a fungal pathogen, *Neozygites floridana,* which leaves other life stages unharmed (Elliot *et al.*, 2002). Host life stage may be an important variable when analyzing infection dynamics and host defense. In this study, we aimed to determine whether exposure to parasites at different host life stages may result in varying responses in host defense strategies in the nematode, *C. elegans.*

C. elegans is a model host organism that has been used to understand innate immunity and infection (Kurz & Ewbank, 2003; Schulenburg & Félix, 2017). In the wild, *C. elegans* live on a variety of decomposing organic materials, and thus come into contact with many types of microorganisms, whether pathogenic, beneficial, and benign (Zhang & Hou, 2013; Schulenburg & Félix, 2017). Because of the variety of microbes present in the worm's natural environment, *C. elegans* must be able to discriminate between food sources and potential pathogens or potentially incur reductions in fitness. Many different bacterial parasites are capable of infecting *C. elegans* (Hodgkin *et al.*, 2000, 2013; Félix & Duveau, 2012; Gibson & Morran, 2017). In previous studies, *C. elegans* have shown a surprising attraction to the pathogenic bacteria *Serratia marcescens* relative to the benign food source, *E. coli* OP50 (Zhang *et al.*, 2005; Pradel *et al.*, 2007). *S. marcescens* have been found in the same sampled natural substrates as *C. elegans* populations and thus is likely to be consumed by *C. elegans* in some environments (Pradel *et al.*, 2007; Schulenburg & Félix, 2017). It is possible that *C. elegans* has an evolutionary history with *S. marcescens* (Samuel *et al.*, 2016). Further, it is also possible that

isolated *C. elegans* populations have adapted to their local parasites (or vice versa), whereby we see variation in response to *S. marcescens* due to host or parasite genotypes (Schulenburg & Ewbank, 2004, 2007). The strain of *S. marcescens* used in Pradel et al. (2007) and Zhang et al. (2005) is a virulent parasite, which can significantly shorten the average life span of infected N2 L4 worms (Kurz *et al.*, 2003). It is likely that initial attraction to *S. marcescens* is elicited by the molecule lipodepsipentapeptide serrawettin W2, which is required for spreading growth in certain strains of *S. marcescens* (Pradel *et al.*, 2007). Despite this initial attraction to *S. marcescens, C. elegans* is able to exhibit learned avoidance behavior of the parasite after approximately 4 hours of exposure (Zhang *et al.*, 2005; Zhang & Hou, 2013). Nonetheless, several hours of feeding on a parasitic food source may be sufficient for infection (Vega & Gore, 2017). It would seem that innate repulsion from *S. marcescens* could be a more advantageous defense strategy than learned avoidance.

Previous assessments of *C. elegans* bacterial preference have been performed on adults and L4 larvae, which may experience weak selection for bacterial preference because these life stages are not known to be associated with dispersal in nature. Interestingly, the vast majority of natural samples of *C. elegans* have been isolated in dauer, a stage of developmental arrest induced by resource scarcity and crowding (Schulenburg *et al.*, 2004; Kiontke & Sudhaus, 2005). The dauer life stage takes the place of a molting stage between larval stage 3 (L3) and larval stage 4 (L4) (Karp, 2016). Importantly, the dauer life stage is non-feeding, and thus relies on chemotaxis to discern environmental cues. Previous work has shown that dauer individuals have a greater attraction towards CO_2 than do adult individuals, which may signal to the dauer worms a nearby bacterial food source (Hallem *et al.*, 2011). Conversely, a study by Albert and Riddle (1983) found that dauer worms are unresponsive to sodium ions (Na +) compared with non-dauer

life stages (Albert & Riddle, 1983). Thus, multiple sensory avenues have been found to differ between various *C. elegans* life stages.

Physiological processes which induce development into dauer are elicited by pheromones and environmental cues, particularly crowding and food limitation (Hu, 2007). Dauer individuals can survive exposure to starvation, heat, and desiccation stress for much longer periods of time than all other life stages of *C. elegans* (Hu, 2007). Dauer is phenotypically unique compared to other larval stages, and dauer induction is known to induce specific changes in gene expression throughout the genome relative to the other *C. elegans* life stages (Jones *et al.*, 2001). In nature, the dauer life stage is thought to facilitate the colonization of new food sources and thus selection may favor dauer individuals that can successfully discriminate between benign and pathogenic bacteria (Samuel *et al.*, 2016). Therefore, parasite avoidance could be a function of the dauer life stage, while other life stages may exhibit more indiscriminate feeding.

Here, we tested the effects of *C. elegans* life stage on food preference during interactions between *C. elegans* hosts and the bacterial parasite, *Serratia marcescens*. Specifically, we were interested in the effects of *C. elegans* dauer stage, relative to other host life stages, on its ability to discriminate between benign and pathogenic food sources. We assayed food preference in the hosts by comparing behavioral responses of each host life stage to a choice between pathogenic *S. marcescens* (Sm2170) and benign *Escherichia coli* (OP50). We next compared individuals, including L4 and adult worms, which had emerged from dauer and resumed development as compared with those that did not transition to dauer during development. Then, we assessed the effects of direct exposure to Sm2170 on dauer mortality as a means of measuring the effects of host life stage on host defense overall. All of these experiments were performed in CB4856 and N2, both of which are lab-adapted strains and the subject of previous studies on *C. elegans*-

pathogen interactions. Additionally, we utilized 4 natural isolates, all of which had been collected in dauer and reared in the lab for fewer than 15 generations, to determine the effects of dauer on host defense within isolates from different types of substrates and geographic areas.

Methods

Bacterial Strains

The primary food source of *C. elegans*, Escherichia *coli* OP50, was acquired from the Caenorhabditis Genomics Center (CGC, University of Minnesota) in 2010 by L.T.M, frozen at -80°C, and periodically thawed for each new experiment. The strain of *S. marcescens* used, Sm2170, was acquired from Curt Lively at Indiana University in 2010, also frozen at -80°C, and then thawed for each new experiment. The experimental strain of *Escherichia coli* HB101 was acquired from Steve L'Hernault at Emory University in 2018.

C. elegans Strains

N2 and CB4856 are both common laboratory strains of *C. elegans* that L.T.M originally received from the CGC in 2010. The four natural strains used in our experiments were all isolated in the dauer life stage and collected by either Marie-Anne Felix or Matt Rockman and then immediately frozen at -80ºC for later use. JU543 was isolated in 2004 by M.A. Felix from a woodlouse, *Oniscus asellus,* in a rural garden in Primel Tregastel, France. JU2140 was isolated in 2011 by M.A. Felix on a slug in rotting acorns in a forest in La Blanc, France. JU2816 was isolated in 2014 by M.A. Felix on vertebrate feces containing plum remains in an orchard in

Orsay, France. QX1233 was isolated in 2007 by M. Rockman from a compost heap in an urban garden in Berkeley, CA.

Choice Assays

The dauer life stage replaces the L3 life stage of non-starved individual worms, thus most of our comparisons are between L3 and dauer individuals. Since previous work has focused mostly on the preference of adult individuals, we also rely heavily on comparisons between adult and dauer.

Choice assays were modeled after Zhang *et al.,* 2005 and Glater *et al.,* 2014. For each worm strain gravid adults were bleached using standard laboratory bleach and NaOH methods to isolate eggs (Stiernagle, 2005). Half of the eggs were then placed on a 10 cm NGM-Lite plate seeded with *Escherichia coli* OP50, then placed in a 20°C incubator and allowed to grow to the desired developmental stage (Cassada & Russell, 1975). The other half of the eggs were also placed on a 10 cm OP50-seeded NGM-Lite at 20°C and allowed to go into dauer arrest. After 2 weeks of starvation at 20ºC, nearly all surviving individuals developed into dauer. This process repeated for each *C. elegans* strain. Using a time to maturity chart and visual cues, we waited the amount of time it takes on average for eggs to hatch then go through each subsequent developmental stage (Penley & Morran, 2017). We visually checked to see if a substantial majority of individuals (> 85%) were in the appropriate life stage at the corresponding time. The L3 larvae have not yet formed a vulva, which differentiates them from L4. Dauer individuals have a unique phenotype permitting easy identification. No worms had prior exposure to Sm2170. The choice assay plate was the first encounter the nematodes had with *S. marcescens*. Finally, to acquire post-dauer L4 and post-dauer adult individuals, we placed dauer naïve to *S.*

marcescens on an *E. coli* OP50 plate to resume development (development into L4 resumes approximately 16 hours after feeding (Karp, 2016), and development into adult occurs after 36 hours). To prepare the bacteria for the choice assay, *E. coli* OP50 and *S. marcescens* 2170 were grown overnight in liquid LB at 28°C. Overnight growth in OP50 and Sm2170 results in a mean OD600 of 1.5 (55 x 10^8 CFUs) for OP50 and a mean OD600 of 1.0 (7.8 x 10^8 CFUs) for Sm2170. 25µl of each bacteria was placed on the opposite sides of an unseeded 10 cm NGM-Lite plate (Supplementary Figure 1). For each treatment, the worms were washed 3x in M9 buffer to remove external OP50, then approximately 100 worms were placed directly in the center of each plate. After two hours, the worms were counted under a dissecting microscope to determine how many individuals were on Sm2170, OP50, or elsewhere on the plate. The recorded counts were used to calculate the choice index (CI):

$$\frac{\#\ on\ Sm2170 - \#\ on\ OP50}{\#\ total\ number\ plated}$$

Previous choice indices have used the total number of worms that chose one bacterium or the other, excluding or including those that did not choose (Zhang *et al.*, 2005; Glater *et al.*, 2014). Glater *et al.* found < 5% of the worms did not make a decision for either bacteria, thus excluding them from analysis. However, because we found > 5% make neither decision, we included all individuals plated in our calculations (Supplementary Figure S1).

This was repeated for all 6 strains and for 6 life stages: L3, dauer, L4, post-dauer L4, adult, and post-dauer adult – for a total of 36 treatments. Each treatment was repeated 10 times for a total of 360 replicates. The same choice assay methodology was repeated for assessing preference of N2 and CB4856 between *Escherichia coli* HB101 vs OP50. In this assay, the same number of worms was used to analyze preference as in our previous assays: 100 individuals of

either worm genotype and either life stage (L3 or dauer), for a total of 4 treatments with 10 replicates (40 replicates total).

Statistical analysis

JMP Pro13 (SAS Institute, Cary, North Carolina) was used to perform statistical analyses on the data. The bacterial choice data were transformed into binomial data (yes = chose Sm2170, no = chose either OP50 or nothing) using the OFFSET function in Excel (Microsoft, Redmond, Washington). In addition, we transformed binomial data on whether or not worms chose any bacteria (yes = chose Sm2170 or OP50, no = chose neither) to determine if there were differences between strains and life stages in whether or not any choice was being made. In both analyses, we used binomial data as opposed to performing analyses on ratio means. Using this binomial data, we then used JMP Pro13 to perform a success/failure generalized linear model (GLM) with a binomial distribution and logit link function (strain, life stage, and strain by life stage effects on bacterial choice). We tested for overdispersion using the Pearson chi-squared test but did not detect significant overdispersion. Contrast tests were run between each strain, life stage, strain by life stage, and replicate population effects for both binomial datasets. We present the data as choice indices below (Fig. 1) and as the proportion that chose either or neither bacteria (Supplementary Figure 1). Standard linear models may be inappropriate in the case of our choice index data as they are discrete data with upper and lower bounds of 1 and -1. However, because many of our response variable values are not close to the bounds, an ANOVA can still perform well. Thus, we ran a two-way ANOVA in JMP Pro13 for all CI data and performed Tukey's multiple comparisons tests. The GLM and the ANOVA models yielded results that were qualitatively in agreement. Here, we present the ANOVA results because our

figures present CI data. In addition, we ran an ANOVA for preference between the benign strains of *Escherichia coli* HB101 and OP50 in L3 and dauer worms. The nematodes had been raised for many generations on the OP50 strain, so we tested to see if preference was due to familiarity with the OP50 strain by comparing their attraction to it with the HB101 strain. These data were also analyzed in JMP Pro13 as described above.

Mortality Assays

In addition to behavioral avoidance, we wanted to test whether dauer was better able than non-dauer L3 individuals at surviving *Serratia marcescens*. In other words, does an increase in avoidance of the parasite also confer an increase in resistance? In these assays, we grew a lawn of *S. marcescens* from each treatment, one treatment per plate, and then allowed worms to become infected by placing them on the treatment bacteria. By placing the worms directly on the *S. marcescens* lawn, but maintaining the possibility of escape from the lawn, we allow for survival via avoidance mechanisms. Further, individuals that ingest the parasite have the opportunity to also resist or tolerate infection. Mortality assays were performed using all abovementioned *C. elegans* strains but only in the dauer and L3 life stages. To prepare the assay, one-third of a 10 cm petri dish containing NGM-Lite (US Biological, Salem, Massachusetts) was seeded with 35µl OP50 grown up overnight in liquid LB at 28°C. The opposite one-third was seeded with 35µl of Sm2170 or heat-killed Sm2170 (the control) as described in Penley and Morran (Penley & Morran, 2017). The middle one-third was kept unseeded. These plates were then grown up in 28°C overnight. Before worms were plated, 20µl of 100mg/ml Ampicillin antibiotic was placed in the middle of the plate to prevent the spread of Sm2170. To achieve the desired life stage, egg hatches were synchronized as done for the choice assays above. Worms at

the desired life stage were washed 3x with M9 buffer to remove external OP50, counted, then placed directly on the Sm2170 (or heat-killed Sm2170). Plates were then incubated for 48 hours at 20ºC. Subsequently, numbers of living worms were then counted by assessing movement and prodding with a platinum wire (Vassilieva *et al.*, 2000; Gill *et al.*, 2003; Troemel *et al.*, 2006; Ford *et al.*, 2016). We then repeated this procedure with heat-killed Sm2170 as a control. Mortality assays directly assess the host's ability to defend against Sm2170 infection by capturing a total measure of host defense, whether by avoidance, resistance and/or tolerance. These mortality assays were repeated 10 times for all six strains in both L3 and dauer individuals, for a total of 600 individuals tested for mortality per strain per life stage.

We calculated mortality rates as the proportion of hosts dead on the treatment plates out of the average total number alive on the control plates to account for any host mortality that did not occur due to the parasite.

Statistical analysis

JMP Pro13 was again used to perform statistical analyses on the data. Nonparametric Wilcoxon tests were performed to determine significance between all treatment groups (each strain: CB4856, N2, JU543, JU2816, JU2140, JU543, and life stage: dauer and L3), and one-way chi square values are reported. Similar to the choice indices, the parameter in this model is a single outcome, whether the worms either died (=yes) or survived (=no). Here, we corrected for multiple statistical tests (two tests: either treatment group or life stage) using a Bonferroni-corrected p-value of 0.025 (0.05/2).

Results

Choice Indices (CI)

We tested the effect of *C. elegans* life stage on bacterial food preference using the relatively benign food source, *E. coli* OP50, and the virulent parasite, *S. marcescens* Sm2170. Food preference was quantitatively measured using a choice index (CI). A mean positive CI indicates a preference for the parasite, whereas a mean negative CI indicates a preference for the benign food source. A CI nearing zero indicates there is no preference for either.

Life Stage Effects

By comparing *C. elegans* dauer individuals with L3 individuals across all six strains, we found a significant difference in preference between the two life stages ($F_{1,502}$ = 76.0173, $p < 0.0001$) (Table S1). Specifically, L3 individuals exhibited a strong preference for *S. marcescens* Sm2170 (CI = 0.278). However, dauer individuals exhibited a very slight mean preference for *E. coli* OP50, albeit essentially a lack of preference between the bacteria (CI = -0.008). Nonetheless, dauer individuals exhibited a significant decrease in their attraction to the parasite relative to L3 individuals (Fig. 4.1, Table S2).

In addition, when comparing dauer individuals with adult individuals across all strains, dauers exhibited significantly reduced preference for Sm2170 relative to adults ($F_{1,502}$ = 34.4982, $p < 0.0001$) (Table S1). Adults had an overall CI = 0.133 compared with the aforementioned CI = -0.008 for dauers (Table S2). Overall, we found a greater mean CI value for all other life stages (including L4, post-dauer L4, and post-dauer adult) compared with dauer (Table S2), suggesting that dauer is unique. Further, we calculated the proportion of individuals that made a choice

(chose either bacterium) versus those that made no choice (remained on the unseeded areas of the plate). Across all strains, only 18% of dauer worms did not choose a bacterium, whereas 44.3% of L3 individuals did not choose ($p < 0.0001$). Dauer chose one or the other bacterium at a much higher rate than did all the non-dauer life stages (Fig. S1; $p < 0.0001$). Thus, the lack of a strong preference exhibited by dauer individuals is not the result of few individuals making a choice.

C. elegans Strain Effects

In addition to comparisons across life stages, we also assessed whether different strains of *C. elegans* differed in their preference for *S. marcescens* Sm2170 and *E. coli* OP50. We found significant effects of worm strain on bacterial choice ($F_{1,502} = 146.3386$, $p < 0.0001$) (Table S1). Averaged across all life stages, CB4856 had the lowest CI, while JU543 had the highest CI (Figs. 4.1b, 4.2e; Table S2). Additionally, we observed significant strain by life stage effects ($F_{1,25} = 4.4523$, $p < 0.0001$) (Table S1, S3). The dauer life stage of the N2, CB4856, and JU2816 strains showed an overall preference for *E. coli* (CIs = -0.274, -0.181, and -0.015, respectively), while the other strains (QX1233, JU543, and JU2140) had a preference for *S. marcescens* Sm2170 while in dauer (CIs = 0.038, 0.122, and 0.138, respectively) (Fig. 4.1a-f, Table S2).

Figure 4.1

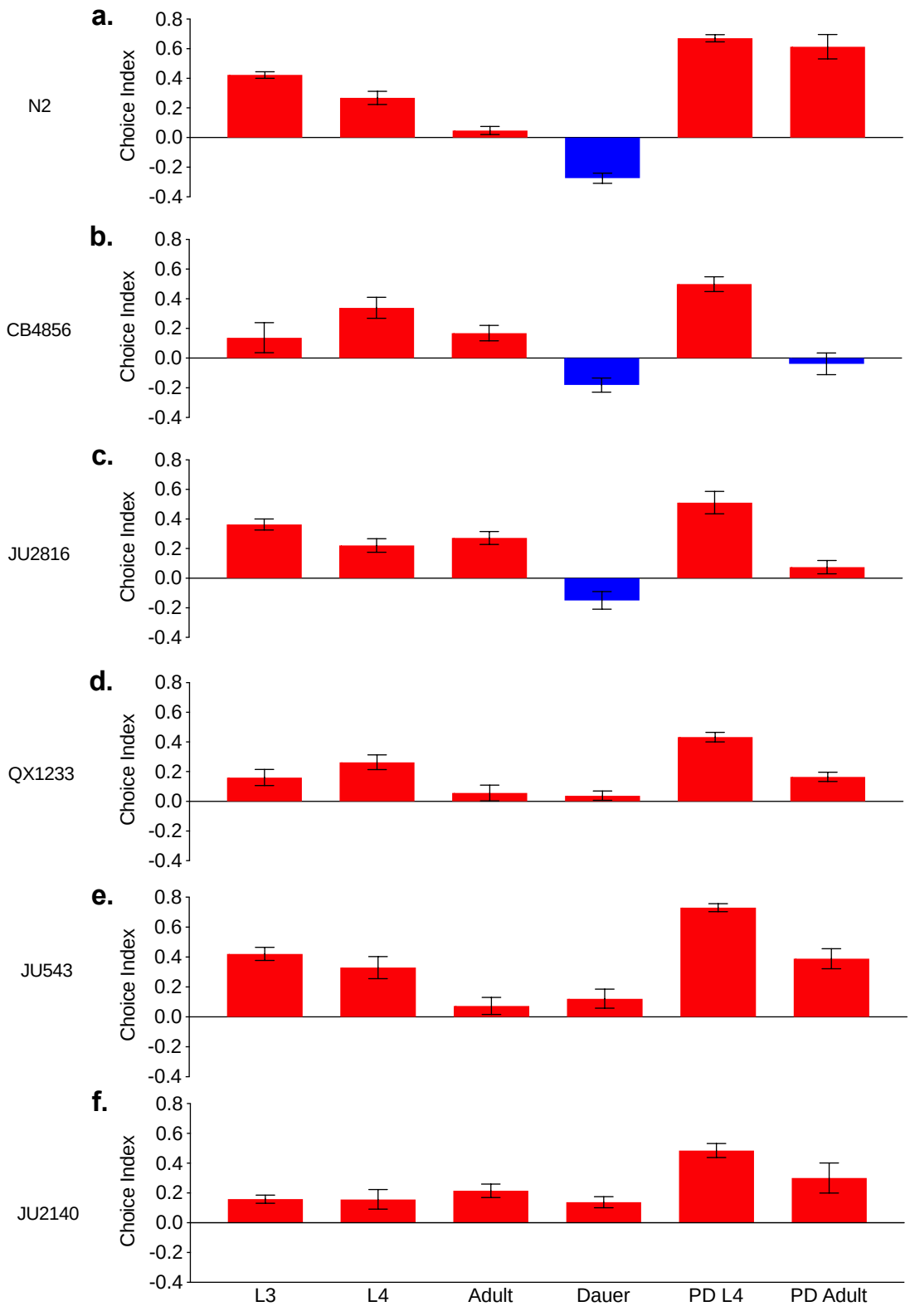
a.
b.
c.
d.
e.
f.
N2
CB4856
JU2816
QX1233
JU543
JU2140
Choice Index
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
L3
L4
Adult
Dauer
PD L4
PD Adult

Figure 4.1a-f. Sm2170 and OP50 choice assays in all strains and life stages. CI < 0 (blue) are found in higher proportions on OP50, CI > 0 (red) are found in higher proportions on Sm2170, whereas a score of CI = 0 indicates no preference. Each bar represents an average of 1000 worms (100 worms per plate x 10 replicates). The vertical lines within each bar depict the standard error of the mean (SEM). PD = post dauer.

OP50 vs. HB101

One potential caveat to our results is that rather than an aversion to Sm2170, we may have detected an attraction of dauer toward OP50, particularly in our N2 and CB4856 strains. Such attraction could be a result of lab adaptation, as OP50 serves as the standard laboratory food source and N2 and CB4856 have been maintained on OP50 for many generations. To distinguish between Sm2170 avoidance and OP50 preference, we exposed CB4856 and N2 dauer worms to both *E. coli* OP50 and *E. coli* HB101, a similarly benign strain of bacteria. We compared the choices of dauer individuals with that of L3 individuals in both CB4856 and N2 strains to determine if dauers consistently preferred OP50. We found no significant difference in preference between life stages or strains ($F_{1,35} = 1.906$, $p = 0.1762$, power = 75.2% to detect a CI difference of 0.1), nor any strong preference for OP50 (Fig. 4.2). Thus, the CI differences exhibited by N2 and CB4856 dauer individuals in the presence of *S. marcescens* (Fig. 4.1), relative to other life stages, are driven by parasite avoidance.

Figure 4.2.

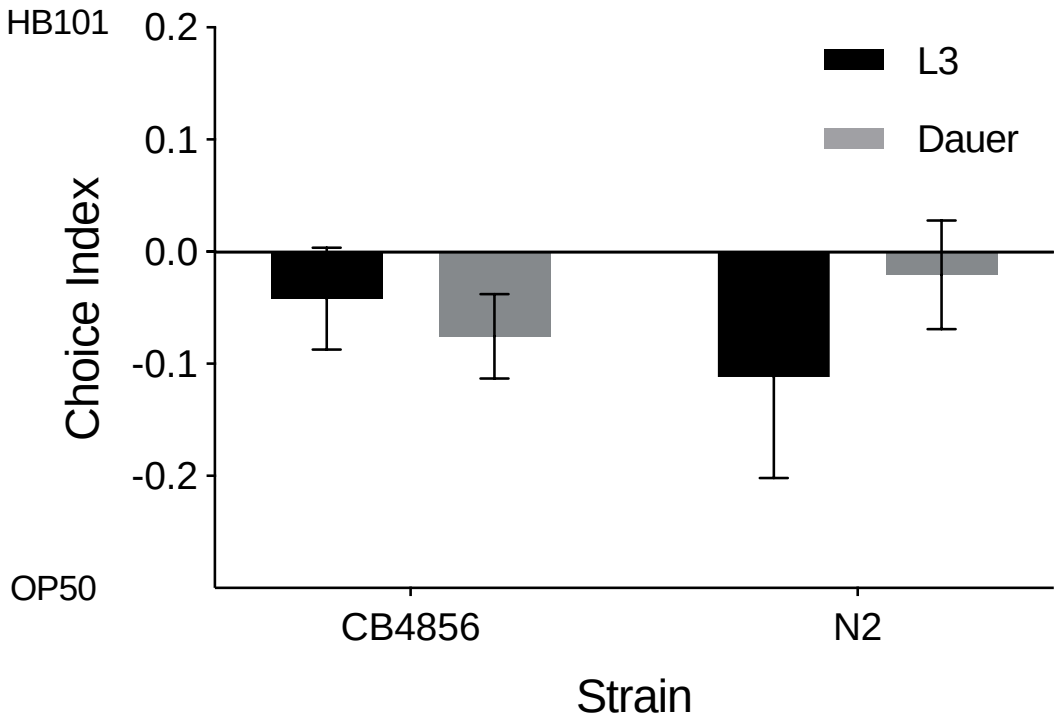

Figure 4.2. Choice assays between OP50 and another benign *E. coli* strain, HB101 tested with *C. elegans* strains CB4856 and N2 in the L3 and dauer life stages. A two-way ANOVA found no significant difference across either strain ($p = 0.8989$), life stage ($p = 0.6278$), or the interaction between the two ($p = 0.6278$) in preference for OP50 over HB101. Error bars represent SEM.

Mortality Assays

While we observed diminished attraction to Sm2170 in most dauer individuals across strains, we next determined if dauer individuals were better able to survive when directly exposed to Sm2170. To test this, we performed mortality assays on both L3 and dauer individuals and compared mortality rates across all *C. elegans* strains. We found that dauer

individuals exhibited significantly lower mean mortality rates when exposed to *S. marcescens* Sm2170 than did L3 individuals when averaged across all strains ($X_1^2 = 9.1513, p = 0.0025$). There were also significant effects of strain on mortality rates ($X_5^2 = 34.1737, p = 0.0001$). N2 and CB4856 both exhibited reduced mortality in dauer (N2 had a 0.255 reduction in mortality when in dauer, CB4856 had a 0.334 reduction); whereas the natural isolates varied in their responses (Fig. 4.3, Table S5).

Figure 4.3.

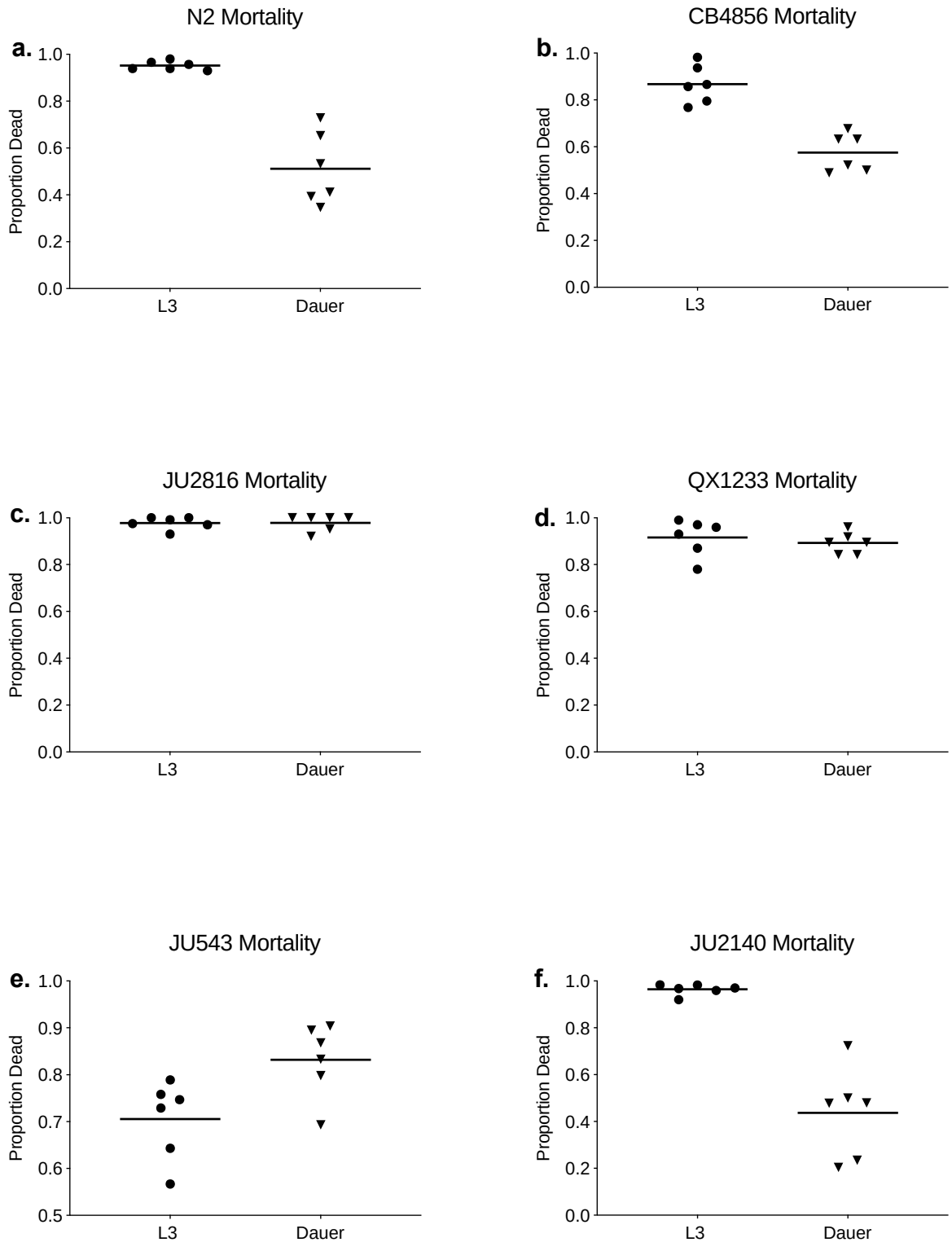
a.
N2 Mortality
Proportion Dead
1.0
0.8
0.6
0.4
0.2
0.0
L3
Dauer
b.
CB4856 Mortality
Proportion Dead
1.0
0.8
0.6
0.4
0.2
0.0
L3
Dauer
c.
JU2816 Mortality
Proportion Dead
1.0
0.8
0.6
0.4
0.2
0.0
L3
Dauer
d.
QX1233 Mortality
Proportion Dead
1.0
0.8
0.6
0.4
0.2
0.0
L3
Dauer
e.
JU543 Mortality
Proportion Dead
1.0
0.9
0.8
0.7
0.6
0.5
L3
Dauer
f.
JU2140 Mortality
Proportion Dead
1.0
0.8
0.6
0.4
0.2
0.0
L3
Dauer

Figure 4.3a-f. Mortality of L3 and dauer individuals 48 hours after placement of individuals directly on Sm2170. Horizontal bar shows the mean proportion dead across all replicates. Significant differences in survival between dauer and L3 while on the pathogen were observed for (a) N2 ($X_1^2 = 8.3368, p = 0.0039$), (b) CB4856 ($X_1^2 = 8.3368, p = 0.0038$), and (f) JU2140 ($X_1^2 = 7.4103, p = 0.0065$).

Discussion

It is possible to miss important insights about an organism if its life history and ecology are not taken into consideration in laboratory experiments, particularly in the case of novel host-parasite interactions. Despite *C. elegans* fundamental use as a model to advance the biological sciences, the ecology of *C. elegans* has rarely been taken into account when designing and interpreting experiments. Our work aims to highlight the importance of incorporating observations from nature and applying more ecological perspective to future host-parasite research. Here, we show the life stage of a host upon encountering a parasite may determine both the host's mechanism of defense and the host's overall level of defense. Previous studies have found that late larval (L4) and early adult *C. elegans* are preferentially attracted to the parasite *S. marcescens* when given a choice between the parasite and their benign food source, *E. coli* (Zhang *et al.*, 2005; Glater *et al.*, 2014). Some have posited that this attraction may be due to behavioral manipulation of *C. elegans* by *S. marcescens* as a type of "trojan horse" mechanism known to occur in other bacterial pathogens (Niu *et al.*, 2010). However, these studies did not assess individuals in the alternative dauer life stage, which is critical for dispersal in *C. elegans* (Schulenburg & Félix, 2017). We hypothesized that dauer individuals would exhibit a decreased preference for the parasite, *S. marcescens*, and increased preference for the benign food source,

E. coli, due to dauer's role in the colonization of new habitats. Here, we found that dauer individuals overall exhibited significantly reduced preference for *S. marcescens* relative to other life stages of *C. elegans* (Fig. 4.1). Further, several strains exhibited a mean preference for *E. coli* relative to *S. marcescens*, whereas other strains showed no preference (a CI close to 0), or still slightly preferred *S. marcescens*. (Fig. 4.2). Importantly, increased dauer preference for *E. coli*, relative to L3 individuals, was conditional on the presence of *S. marcescens* (Figs. 4.1, 4.2), therefore the altered food preference exhibited by dauer individuals functions as a form of parasite avoidance. Our results are also consistent with Glater *et al.,* 2014, in which CB4856 adults were found to have a low CI. N2 and CB4856 had an overall lower preference for *S. marcescens* Sm2170 compared with natural isolates (lab strains had an average CI = 0.181, natural isolates had an average CI = 0.245) (Table S2). We then addressed whether dauer individuals exhibited greater levels of host defense, relative to L3 larvae. We found that, overall, the mean mortality rates exhibited by dauer individuals were reduced. However, this effect of dauer was largely strain specific. Thus, *C. elegans* life stage can alter the mechanism of defense employed by the host via parasite avoidance. Further, dauer individuals can also exhibit increased levels of host defense.

We observed both increased parasite avoidance at the dauer life stage, as well as increased levels of host defense. Did parasite avoidance reduce levels of host mortality? The most substantial decreases in preference between L3 and dauer individuals were in both of the laboratory-adapted strains, CB4856 and N2 (Fig. 4.1a-b). Interestingly, both CB4856 and N2 also exhibited greater levels of host defense (Fig. 4.3a-b). However, JU2816, had a significantly increased preference for OP50 when in dauer (Fig. 4.1c), but both L3 and dauer individuals died at high rates when directly exposed to the parasite (Fig. 4.3c). Conversely, strain JU2140

exhibited high levels of host defense (Fig. 4.3f) without parasite avoidance (Fig. 4.1f). Importantly, the mortality assays were constructed to permit parasite avoidance after initial exposure. However, the nematodes were directly exposed to the parasite to begin the assay, therefore the hosts cannot avoid the parasite altogether which may reduce the efficacy of avoidance. Regardless, taken together, these results suggest that dauer can impact both avoidance and overall defense. Specifically, avoidance may contribute to the substantially greater levels of host defense in CB4856 and N2 dauer individuals by facilitating avoidance of the parasite. However, increased host defense in the dauer stage is unlikely to be solely driven by avoidance because the JU2140 strain exhibits increased defense in the absence of avoidance.

In nature, dauer individuals likely discriminate between benign and pathogenic food sources. Therefore, parasite avoidance behavior at the dauer stage may be under strong selection. Although L4 larvae generally exhibit a preference for *S. marcescens*, previous work has shown that L4 individuals can evolve elevated levels of parasite avoidance when experimentally evolved in the presence of *S. marcescens* (Penley & Morran, 2017). Thus, L4 individuals do not necessarily lack the ability to detect a parasite, but it is plausible that selection primarily acts on the dauer stage rather than the L4 stage in nature. Importantly, post-dauer L4's and adults generally do not exhibit parasite avoidance (Fig. 4.1, but see Fig. 4.1b), indicating that dauer induction is not sufficient for parasite avoidance compared with worms that develop normally (i.e. go through the L3 life stage instead). Curiously, post-dauer L4 individuals, in particular, have a much greater preference for the parasite than do dauer individuals (Fig. 4.1 & Fig. S1). Clearly, food preference is substantially different in the dauer life stage, but only transiently, and it appears that avoidance of Sm2170 is constrained to the dauer stage. Yet, our data also indicate that dauer induction may alter food choice in subsequent life stages.

An important caveat to note about our experimental design is the way in which we induced dauer development. As opposed to many previous dauer studies that induced dauer formation via chemical cues (Golden & Riddle, 1984; Butcher *et al.*, 2007; McGrath *et al.*, 2011; Green *et al.*, 2014), here we allowed populations to exhaust their lawn of *E. coli*, experience overcrowding, and starve for a period of two weeks. Importantly, *C. elegans* maternal effects can induce parasite avoidance via the induction of diapause in the offspring of mothers that were exposed to the parasite (Palominos *et al.*, 2017). It is plausible that starved mothers may produce dauer offspring with greater levels of parasite avoidance relative to dauer individuals that were induced chemically. Therefore, our results may depend on dauer induction via overcrowding and starvation. Nonetheless, dauer induction via crowding and starvation is likely more relevant to the boom-and-bust conditions *C. elegans* encounter in nature than exposure to concentrated dauer pheromone (Schulenburg & Félix, 2017).

While our data supports dauer individuals' ability to discriminate between a benign versus a harmful bacterium, it may be the case that local genotype by genotype (GxG) or genotype by environment (GxE) interactions are responsible for the variation between strains in both parasite avoidance and defense (Schulenburg & Ewbank, 2007). *C. elegans* in nature may be locally adapted to its parasites, and thus recognize local parasites more effectively than foreign parasites (Richaud *et al.*, 2018). While it is plausible that natural isolates of *C. elegans* encounter *S. marcescens* and other *Serratia* species in nature, they likely have not encountered the Sm2170 strain used in this study (Samuel *et al.*, 2016). Rather, some host strains may have responded to serrawettin W2 produced by Sm2170 more than other host strains due to their specific evolutionary histories with parasites. Conversely, the strain differences we observed may largely be due to genetic drift, rather than signatures of local adaptation. To assess the

contributions of selection versus drift, it would be ideal to compare the substrates in which the *C. elegans* isolates were collected to determine the natural variation between strains of bacterial parasites and their associated *C. elegans* hosts. Nonetheless, our results show the importance of extending analysis beyond the standard lab strains to incorporate natural isolates, without which we would not have observed substantial variation in response to the parasite.

This study demonstrates the importance of considering both the natural history and ecology of *C. elegans* as well as its facultative life stage in understanding laboratory studies of host-parasite interaction dynamics. Without testing dauer, researchers would continue to assume an overall attraction of *C. elegans* to *S. marcescens*. This work provides a step in better elucidating the unique differences of *C. elegans* dauer life stage and the role that *C. elegans* life history may play in nature. Future work could assess the effects of dauer on long-term population growth and the evolution of host defense by passaging both wildtype and dauer-deficient mutant strains in the presence of parasites. In addition, while our work focused solely on *S. marcescens*, there is evidence to suggest that many types of parasites naturally infect worm populations. Therefore, it is critical to determine if parasite avoidance is a general response in *C. elegans* dauers, or if avoidance is highly specialized. Furthermore, how do olfactory responses to parasites change throughout a worm's lifetime, and what ramifications do these various responses have in natural populations?

Beyond *C. elegans*, each life stage of a particular host may hold differing strategies of ridding itself of parasites or avoiding them altogether. Particular life stages may play specific roles in nature (Boots, 1998; Elliot *et al.*, 2002; Balla *et al.*, 2015) that could shape the evolution of defense mechanisms that they employ. When only a single host life stage is measured, important dynamics of the host-parasite interaction may be missed. Our work shows how a

developmental life stage that is essential in nature, but not in the lab, is able to avoid parasites and contribute substantially to host defense. The effects of life stage may be more common than we know in a wide array of species and may be worth examining more rigorously. This approach may be of particular importance to species with dispersal life stages in which fitness strongly depends upon avoiding infections in newly colonized habitats.

Data Availability

Data is available on Dryad: https://doi.org/10.5061/dryad.8v98d03

Acknowledgements

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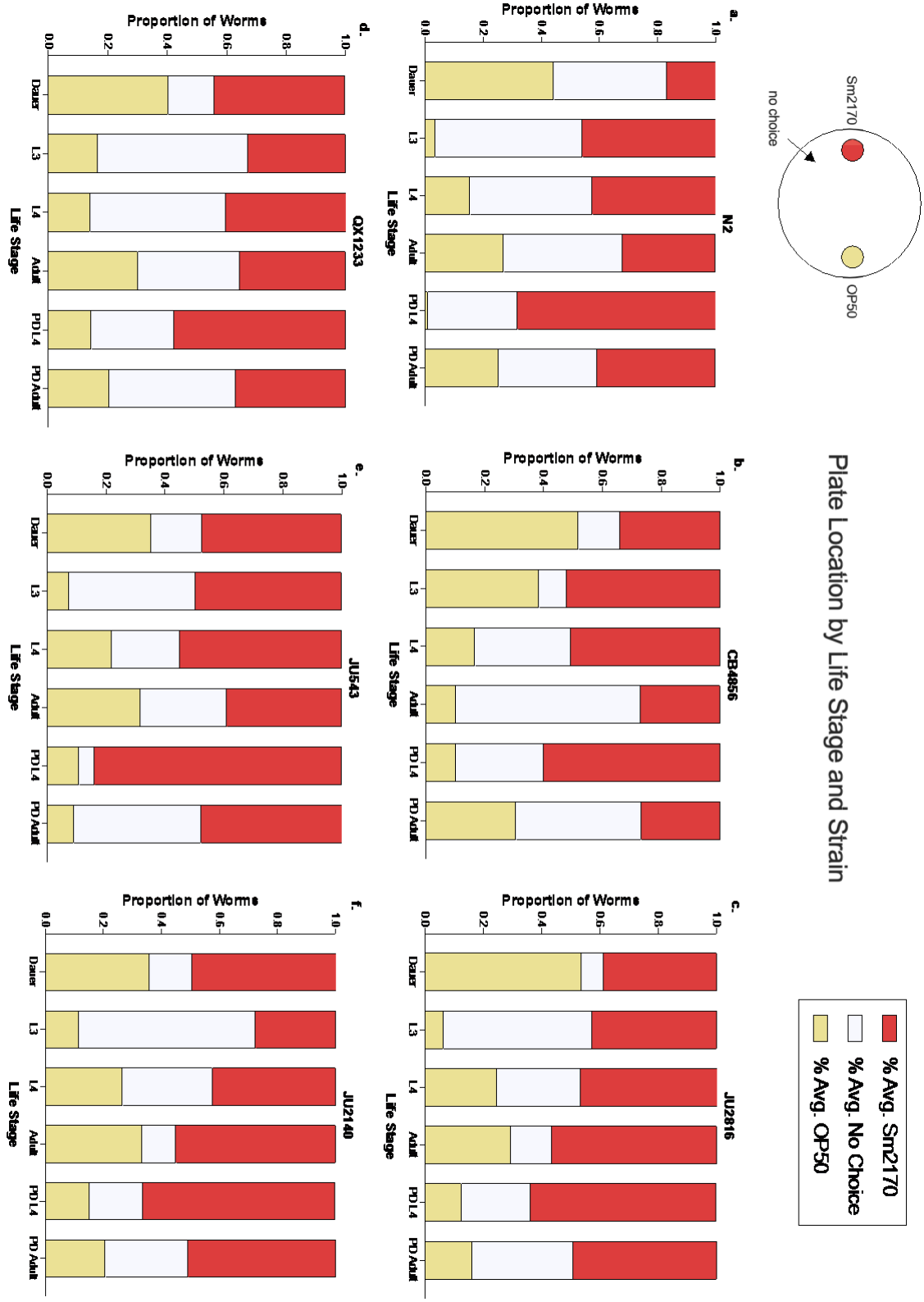
Author Contributions

LTM and DMS conceived of and performed the initial experiments.
LTM helped with the statistical analysis and manuscript writing.
APT performed the initial mortality assay experiments.
MJP helped design the protocols.
PSW performed all experiments and wrote the manuscript.

Supplementary Information



Supplementary Figure S1. Mean proportion of individuals exhibiting each potential outcome during choice assays for each strain. These graphs depict the average proportion of individuals in each life stage that went to either OP50 (yellow bar), Sm2170 (red bar), or did not choose either OP50 or Sm2170 (white bar). "No choice" worms were found on the unseeded parts of the plate. Note that dauer is the first bar of each graph. Across all strains, significantly more dauer worms chose one or the other bacteria than did all other life stages (p = 1.476607e-62).

Supplementary Table S1
Choice Assay Data – ANOVA

Whole Model
Two-way ANOVA,
standard least-squares Alpha: 0.05

Analysis of Variance

Source	**DF**	**SS**	**Mean Square**	**F Ratio**	
Model	35	20.658067	0.590230	12.7114	
Error	502	23.309356	0.045433	**Prob > F**	
C. Total	537	43.967423		<0.0001	
Effect Tests	**Nparm**	**DF**	**SS**	**F Ratio**	**P value**
Interaction	25	25	5.168361	4.4523	P<0.0001
Life Stage	5	5	15.512685	66.8175	P<0.0001
Strain	5	5	1.766846	7.6103	P<0.0001

Contrast Tests: Tukey's Multiple Comparisons

Life Stage	**SS**	**NumDF**	**DenDF**	**F Ratio**	**Prob > F**
Dauer vs. L3	3.53	1	502	76.0173	<0.0001
Dauer vs. Adult	1.602	1	502	34.4982	<0.0001
Dauer vs. all other life stages	6.795	1	502	146.3368	<0.0001

Supplementary Table S2
Choice Indices

Strain	L3	Dauer	L4	PD L4	Adult	PD Adult	Averages
JU543	0.421	0.122	0.330	0.730	0.074	0.389	**0.284**
JU2816	0.363	-0.150	0.222	0.511	0.272	0.099	**0.241**
QX1233	0.161	0.038	0.263	0.433	0.056	0.165	**0.207**
JU2140	0.159	0.138	0.157	0.485	0.215	0.301	**0.248**
CB4856	0.138	-0.181	0.339	0.499	0.169	-0.038	**0.171**
N2	0.423	-0.274	0.268	0.671	0.048	0.154	**0.190**
Averages	**0.278**	**-0.008**	**0.263**	**0.529**	**0.133**	**0.191**	**0.227**

Dark orange denotes natural isolates. Green text denotes lab strains.

Supplementary Table S3
Choice Assay Data – GLM Binomial

Life Stages: L3, L4, Adult, Dauer, PD L4, and PD Adult
Strains: CB4856, N2, JU543, JU2816, JU2140, QX1233

Whole Model Test

Model	-Log Likelihood	L-R ChiSquare	DF	Prob > ChiSq
Difference	2414.9995	4829.999	60	<0.0001*
Full	33136.7724			
Reduced	35551.7719			
Goodness of Fit Statistic	**ChiSquare**	**DF**	**Prob > ChiSq**	
Pearson	52359.85	51863	0.0618	
Deviance	66273.54	51863	<0.0001*	

Effect Tests

Source	DF	L-R ChiSquare	Prob > ChiSq
Strain	5	439.7864	<0.0001*
Life Stage	5	2280.6587	<0.0001*
Replicate	25	789.01358	<0.0001*
Strain*Life Stage	25	1814.0432	<0.0001*

Contrast Test All Strains: Dauer vs. Non-Dauer

Life Stage Overall	-Log Likelihood	DF	L-R ChiSquare	Prob > ChiSq
L3 vs. Dauer	33493.604713	1	713.66463197	3.1936e-157

Supplementary Table S4

Choice Assay Data (chose either bacteria or chose neither) – GLM Binomial

Life Stages: L3, L4, Adult, Dauer, PD L4, and PD Adult
Strains: CB4856, N2, JU543, JU2816, JU2140, QX1233

Whole Model Test

Model	-Log Likelihood	L-R ChiSquare	DF	Prob > ChiSq
Difference	3236.52246	6527.045	35	<0.001*
Full	30586.2972			
Reduced	33849.8196			
Goodness of Fit Statistic	**ChiSquare**	**DF**	**Prob > ChiSq**	
Pearson	52354.00	52318	0.4549	
Deviance	61172.59	52318	<0.001*	

Effects Tests

Source	DF	L-R ChiSquare	Prob > ChiSq
Life Stage	5	377.56523	<0.0001*
Strain	5	550.72470	<0.0001*
Life Stage*Strain	25	5554.67900	<0.0001*
Replicate	1	44.472647	<0.0001*

Contrast Tests

Life Stage Overall	-Log Likelihood	DF	L-R ChiSquare	Prob > ChiSq
All Life Stages vs. Dauer	30725.623388	1	278.65239522	1.476607e-62
L3 vs. Dauer	30701.904974	1	275.67378993	6.582637e-62
L4 vs. PD L4	30566.817157	1	5.4980566642	0.019
Adult vs. PD Adult	30573.892661	1	19.649063525	9.3048903e-6

Supplementary Table S5
Mortality Assay Data – GLM Binomial

Life Stages: L3, Dauer
Strains: CB4856, N2, JU543, JU2816, JU2140, QX1233

strain
life stage
replicate [strain * life stage] strain * life stage

Whole Model Test

Model	-Log Likelihood	L-R ChiSquare	DF	Prob > ChiSq
Difference	860.190156	1720.380	71	<0.0001*
Full	2960.76507			
Reduced	3820.95522			
Goodness of Fit Statistic	**ChiSquare**	**DF**	**Prob > ChiSq**	
Pearson	6885.000	7265	0.9993	
Deviance	5921.530	7265	1.0000	

Effects Tests

Source	DF	L-R ChiSquare	Prob > ChiSq
Strain	5	404.05623	<0.0001*
Life Stage	1	1.5237e-5	0.9969
Replicate	60	339.59913	<0.0001*
Strain*Life Stage	5	321.34359	<0.0001*

Contrast Tests

Life Stage Overall	-Log Likelihood	DF	L-R ChiSquare	Prob > ChiSq
L3 vs. Dauer	2960.7650764	1	0.000015237	0.9968854994

CHAPTER V

Phoresy: a quick guide

Reprinted material from: White, P.S., Morran, L.T., and de Roode, J.C. (2017). Phoresy. Current Biology *27*, R578–R580.

What is phoresy? Phoresy is an interaction in which a phoretic animal (or phoront) latches itself onto a host animal for the purpose of dispersal. The word phoresy derives from the Greek *phorein*, which means 'to carry'. Typically, the phoront is an animal, such as a nematode or mite, with limited ability to travel great distances on its own, and thus requires aided dispersal by a highly mobile host, such as a fly or bee. Phoresy has been defined in many different ways. A widely used definition is the one proposed by Houck and O'Connor in 1991, who refer to phoresy as "a phenomenon in which one organism (the phoretic) receives an ecological or evolutionary advantage by migrating from the natal habitat while superficially attached to a selected interspecific host for some portion of the individual phoretic's lifetime." The benefit provided to the phoront is thus measured in terms of dispersal, as opposed to direct nutritional or developmental benefits from parasitizing the host. It is also important to realize that phoresy involves temporary interactions. Consequently, long-lasting associations, such as that between barnacles and whales, are not phoretic.

So, phoresy is not a form of parasitism? Correct. Strictly speaking, phoresy is considered a commensal interaction, in which the phoront obtains a fitness benefit, while the host gains neither a fitness benefit nor carries cost. However, it is important to realize that the lack of parasitism is restricted to the actual transportation phase of the species interaction. In some cases,

phoronts hitch a ride on their hosts without causing direct harm, but for the purpose of future parasitization. For example, the egg parasitoid wasp *Trichogramma brassicae* latches onto female cabbage white butterflies. This allows the wasp to parasitize the newly laid eggs of its butterfly host. Because the adult wasps do not directly harm the host while being transported, this interaction is considered a form of phoresy. In contrast, while many other parasitic species, such as fleas and intestinal worms, obtain dispersal through their hosts, they are not considered phoretic because they directly harm their hosts during dispersal.

Can phoresy turn into parasitism over time? Yes, they can. Despite the strict definition of phoresy, transportation of phoronts may carry mild costs. Depending on how many phoronts are on the hosts, and the distance that hosts travel with their phoronts, phoresy may have some costs. In addition, once phoretic relationships are established, parasitism can evolve. For example, necromantic nematodes hitch rides on their millipede hosts and consume their host upon its death. While there is no current evidence that they speed up mortality, selection for mortality-inducing nematodes may occur. Phoretic interactions may also become mutualistic. For example, the mite *Ensliniella parasitica* hitches rides on the wasp *Allodynerus delphinalis*. Both host and phoront are attacked by a parasitoid wasp, but the mite is able to fight off this wasp, thereby protecting itself and its host. Thus, phoretic interactions are likely to be preludes to the evolution of parasitism and mutualism.

How common is phoresy? Phoretic interactions span the entire spectrum of the animal kingdom and are extremely diverse. African black fly larvae obtain dispersal through crabs, some beetles are phoretic on bees, nematodes are hitchhikers on flies and slugs, and ostracod crustaceans have

been found to hitch rides on lizards and frogs. There are some 35 phoretic species of parasitoid wasps that parasitize their hosts' eggs, and these utilize host species ranging from moths and butterflies to grasshoppers, pentatomid bugs and mantids. There is also variation in the number of phoronts per host: single beetles hitch rides on bees, but hundreds of mites can assemble on flies. While these examples represent a wide diversity of species, phoresy is much more common in some groups than in others. While there are approximately 35 phoretic species of egg parasitoid wasps, this represents less than one percent of egg parasitoid species. In contrast, phoresy appears to be much more common in mites and nematodes. Studies have found phoretic mites on the fossilized remains of 49 million-year-old spiders and 320 million-year-old insects, demonstrating the long evolutionary history of phoresy.

Figure 5.1. Examples of phoresy. Top left: SEM of the mite *Macrocheles muscaedomesticae* attached to the fly *Drosophila hydei* (photo by Heather Proctor). Top right: the wasp *Trichogramma evanescens* on the eye of a large cabbage white butterfly, *Pieris brassicae* (photo by Nina E. Fatouros). Bottom left: A recently emerged blowfly, *Calliphora vicina*, covered in *Poecilochirus austroasiaticus* mites. Typically, the mites are phoretic on carrion beetles, thus showing a potential loss of host-specificity (photo by M. Alejandra Perotti). Bottom right: A male solitary bee, *Habropoda pallida*, with phoretic beetle larvae (*Meloe franciscanus*). The mass of larvae looks like a female bee and latches onto the male bee as it attempts to mate with the mass (photo by Leslie S. Saul-Gershenz, published with permission from Proc. Natl. Acad. Sci. USA (2006), *103*, 14039-14044).

Are phoronts specialists or generalists? It depends. The mite *Histiogaster arborsignis* is a generalist phoront, utilizing more than 40 host species from three insect orders. In contrast, the nematode *Caenorhabditis drosophilae* is only phoretic on the fly *Drosophila nigrospiracula*, with both species utilizing the rotting flesh of saguaro cacti. Many phoronts have evolved specific traits that increase their phoretic success and fitness. For example, the deutonymph stage in mites and *dauer* stage in nematodes are specialized life stages that facilitate phoresy. These stages provide resistance to changes in temperature and humidity. They also have structures that facilitate clinging on to their hosts, such as anal suckers that allow mites to attach to their fly hosts. In addition, *Trichogramma* wasps recognize the aphrodisiacs transferred by male cabbage whites to their partners during mating, allowing these egg parasitoids to specifically hitch rides on gravid female butterflies. Phoronts may also trick their hosts into giving them a ride. The bee *Habropoda pallida* is attracted to a swarming mass of blister beetle larvae (*Meloe*

frascincanus) due to its resemblance of a female bee. When the male attempts to mate with the mass, the larvae latch on to the bee.

Why is phoresy important? Phoresy allows animals with low mobility to disperse to new habitats. Such dispersal provides the same benefits that highly mobile species obtain from their own dispersal, including reduction of competition for food and mates. Phoresy allows the colonization of new food patches and the escape from parasitism and predation in the natal habitat. Many phoronts inhabit ephemeral environments, such as carrion, manure and host nest sites. They thus depend on the colonization of new sites to avoid population extinction. Phoresy-aided dispersal also increases gene flow and thereby may reduce inbreeding depression and the accumulation of deleterious mutations in local populations. For example, while the soil nematode *Caenorhabditis remanei* has a limited dispersal ability on its own, its ability to hitch rides on slugs, snails and isopods has likely aided in its maintenance of local genetic diversity. Phoresy may lead to parasitism and mutualism over time. Thus, the study of phoresy could provide insights into the initial stages of the evolution of these symbiotic relationships.

How should we study phoresy? Phoresy remains understudied, and most studies have been observational. While we know many examples of phoresy, we still don't understand the effects of phoresy on host and phoront ecology and evolution. Why do some taxa have more phoronts than others, what are the initial conditions that lead to phoretic relationships, and how often do phoretic interactions become parasitic or mutualistic? There remains much to be learned about this intriguing symbiosis.

Where Can I Learn More?

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CHAPTER VI

DISCUSSION

Summary of results

Multiple aspects of a host population can affect host-parasite interactions, thereby altering the evolutionary trajectories of both partners. In this dissertation, I investigated 1) the degree to which the evolution of host resistance and the maintenance of resistance is dependent on the host population size, 2) whether genetic heterogeneity can mitigate the evolution of infection-induced host mortality and whether that mitigation is due to a cost of specialization, and 3) how different host life stages may employ different mechanisms to resist infection.

First, host population size is predicted to influence the evolutionary trajectory of host-parasite interactions. Specifically, drift may act to prevent adaptation in a small population due to the removal of beneficial alleles from the population by random chance. To test this prediction, I infected both large and small host populations with parasites and imposed strong selection pressure for the hosts to survive infection. In small populations, most evolved hosts were no better at surviving the parasite than they were at the outset of the experiment. Large populations, however, were indeed able consistently to evolve increased resistance against the parasite. I then performed the same experiment beginning with a more resistant host genotype, again selecting for host survival against parasite infection in large and small host populations. I saw that most small host populations exhibited significant increases in parasite-induced host mortality, whereas large host populations consistently maintained resistance. These results show strong evidence that population size can alter the evolutionary trajectories of hosts, even when alleles conferring elevated parasite resistance are prevalent in the population.

Second, host genetic heterogeneity is predicted to mitigate the evolution of parasite-induced host mortality. Genetic heterogeneity is biologically relevant, yet very few studies have explicitly tested the evolutionary trajectories of parasites on differing host genetic backgrounds. To test this prediction, I infected multiple host populations comprised of varying ratios of genotypes (including homogeneity). I found that after several hundred parasite generations, parasites adapted to homogeneous populations, but rates of adaptation were greatly reduced when parasites evolved in heterogeneous host populations. Next, I wanted to determine if there was a fitness cost due to specializing on the homogeneous populations. I exposed novel hosts to parasites evolved in the homogeneous treatments. Surprisingly, I found that parasites evolved in homogeneous populations also caused a significant increase in parasite-induced host mortality in novel hosts, signifying that in this system, there was a limited cost of specialization. Here, I show that genetic diversity in the host population can indeed mitigate the evolution of parasite-induced host mortality, but I cannot conclude that this result is due to the mechanism of specialization. Instead, it is likely that multiple selection pressures (i.e., applied by two different host genotypes) cause a reduced efficacy of selection, impeding parasite adaptation to heterogeneous populations.

Third, different life stages of the host may be differentially able to resist parasite infection (Barrett *et al.*, 2008). In particular, dispersal-specific life stages are likely to face strong selection pressure from parasites in in new locations or environments. I predicted that this particular life stage would avoid exposure to parasites at a higher rate than non-dispersal life stages. To test this, I exposed dispersal-stage hosts and non-dispersal hosts to a highly virulent parasite (i.e., causing high host infection-induced mortality) and a benign food source and allowed individuals to preferentially choose between the two. I found that indeed, dispersal-stage hosts avoided the parasite at a much higher rate that non-dispersal stage hosts. Many organisms

in nature must disperse to new habitats to ensure survival and reproduction, and thus strategies for successful dispersal are likely key for the persistence of these species.

Lastly, I gave an overview of a particular dispersal strategy, phoresy, and its ubiquity in nature. Phoresy is a phenomenon in which an animal (the phoront) hitches a ride on a host animal, which then transports it to a new habitat (White *et al.*, 2017). We know very little about how phoresy impacts the population dynamics of species (and how it may aid in parasite avoidance), but I predict that it is essential for a more holistic understanding of metapopulation dynamics and population genetics for all species involved (phoront, host, and parasite).

Future work

The results of this dissertation open multiple avenues for further exploration. First, genetic drift is likely to play an important role in the ability of a host population to adapt to its environment (e.g., (Paland & Schmid, 2003)). Small populations of hosts in these experiments were unable to maintain or evolve resistance in the face of a parasite. How may this result translate to a natural population of hosts? There is evidence that natural host populations undergo bottlenecks each time dispersal occurs (Papkou *et al.*, 2016). The parasite used here to infect hosts was extremely virulent (initial host population mortality of 80%). It may be the case that parasites in nature are rarely that extremely virulent. Thus, it would be beneficial to rerun the experiment using natural parasites, as they are likely to better represent virulence levels more commonly seen in nature. determine the average virulence of natural parasites can inform experiments (Samuel *et al.*, 2016).

I found that heterogeneous host populations are capable of lessening the severity of virulence. However, I ran this experiment for 10 host passages. Would an additional 10 or 20

passages have begun to differentiate the treatments? From the parasitic perspective, the use of more extreme host ratios as experimental treatments may allow us to pinpoint the threshold ratio needed to outweigh the negative effects of heterogeneity. For example, in addition to 75-25, 50-50, and 25-75 (where I saw heterogeneity as detrimental to the parasite), the use of 90-10, 10-90 or even 99-1 and 1-99 may allow us to understand the ratio at which parasites gain a fitness benefit. I also found that while parasites adapted quickly, resulting in high mortality in homogeneous host populations, I did not see a cost of infecting a novel host. A wealth of studies in agriculture and in Hymenoptera have shown that heterogeneity decreases parasite load and prevalence. However, these studies do not impose experimental evolution on either host or parasite for the purpose of understanding long-term dynamics. In my dissertation work, I aimed to determine how parasite infection of varied host environments may influence the evolutionary trajectory of parasites. Some studies on parasite adaptation assume that adaptation to a particular host genotype incurs a cost of infecting a different host genotype (Fry, 1996; Liersch & Schmid-Hempel, 1998; Agudelo-Romero *et al.*, 2008; Fleming-Davies *et al.*, 2015). Neither did these studies perform experimental evolution nor did they perform cross-infection experiments, and yet they assume a tradeoff of specialization. In contrast, I highlight a case in which specialization does not confer a fitness cost. Had I not performed cross-infections, I may have assumed that the effects of heterogeneity were due to a tradeoff, and a valuable insight would have been lost. For example, work performed by Kröner *et al.* (2017) found that when cross-inoculating mold spores from potatoes into potato plants (familiar), mold fitness was high, but when the same spores were inoculated into tomatoes (novel), mold fitness was low (Kröner *et al.*, 2017). The authors concluded that this result stemmed from a specialization trade-off, but no experimental evolution was performed to properly assess this claim (Kröner *et al.*, 2017).

Further, host genetic heterogeneity may differ in natural populations (Barrière & Félix, 2005a). Many host populations, such as those that reproduce by sexual reproduction, have a much higher degree of genetic heterogeneity than those I tested (Hamilton, 1980). In particular, many host populations are comprised of more than two genotypes, which may dramatically alter the results presented here (Allendorf & Leary, 1986). For example, King and Lively (2012) developed a mathematical model in which multiple genotypes were gradually introduced into a host population from an initial size of 10, where transmission rate was highest, up to a population of 40, in which the parasites were unable to transmit effectively (King & Lively, 2012). A study using the host *Daphnia magna* and its microsporidian parasites found that in a *Daphnia* population of 10 genotypes, parasite prevalence was reduced (Ganz & Ebert, 2010). In contrast with the initially clonal parasite used here, most parasite populations in nature have some standing genetic variation, which may allow for the parasite to more quickly adapt to heterogeneous host backgrounds (Barrett & Schluter, 2008). A similar experiment to the one performed here may be repeated but with strains of hosts and parasites that have a relevant evolutionary or ecological history with one another. This could shed light on dynamics of adaptation to heterogeneous host populations. In addition to experimental evolution, it is necessary to understand the specific genes underlying host resistance or parasite virulence. If we can understand the precise traits selected for in each partner, we can then design experiments with factors that directly impact these traits, which may lead to a more precise understanding of host-parasite evolutionary dynamics.

Lastly, the work performed here on life stage dynamics only scratched the surface of our understanding of its importance in parasite avoidance. In particular, I did not see a universal avoidance response across all host genotypes while in the dispersal life stage. Interestingly, I saw

a much stronger response in lab-adapted hosts than I did in wild isolates. What accounts for this variation? Further, there was no correlation between those that had a strong response to infection and the ability to survive infection when avoidance was impossible. As in the previous two major experiments, I believe that in addition to using host isolates, it will be important to use parasites that hosts are found to be in association with (Samuel *et al.*, 2016). It may be that familiar parasites elicit stronger avoidance responses or increased survival of hosts while in the dispersal life stage. While there is some evidence that dispersal is used to escape parasites and cull infected individuals in transit, dispersing organisms may also bring their parasites in tow when arriving to a new resource or habitat patch (Altizer *et al.*, 2011). Thus, in addition to the population dynamics of the dispersing host, it may also be important to understand how dispersal of hosts actually aids in the dispersal of the parasite as well.

All experiments in this dissertation were performed using multiple genotypes of the nematode roundworm *Caenorhabditis elegans* and one genotype of the bacterial parasite, *Serratia marcescens* (Sm2170). Many of the questions posed in this dissertation would not be possible (or much more difficult) to investigate without this powerful model system. *C. elegans* allows for population manipulation on an unprecedented scale (Teotónio *et al.*, 2017). Many genetic tools have been developed to test fundamental questions about biological processes, such as organismal development, the structure of DNA, programmed cell death, and much more. *C. elegans* populations are easily cultured in laboratory conditions, grown to massive quantities, and their time to reproduction spans only 3.5 days (Stiernagle, 2005). They have a simple body plan comprised largely of many nerves connected to a gut. Further, they are transparent, allowing for easy identification of infection (at least in the case of the bright red *S. marcescens*). This system allows us to understand the many possibilities of what *can* happen in nature, though we know

less about what frequently *does* happen. However, rapid advances in techniques used in natural studies have been developed over the past decade, largely due to the work of Marie-Anne Félix and colleagues (isolation procedures, sequencing of isolates, observations of other organisms associated with *C. elegans*, studies of other species of *Caenorhabditis* (Frézal & Félix, 2015)). The work presented in this dissertation demonstrates important aspects of the host population that should be considered when assessing or predicting host-parasite interactions. However, there are limitations to what a well-established model organism can tell us. In future work, ecological studies of *C. elegans* will be critical in deciding when to manipulate variables to test fundamental questions of host-parasite interactions. Indeed, it is important to consider the evolutionary history and ecology of natural populations in order to make the best-informed conclusions regarding species interactions.

As mentioned previously, phoresy (hitchhiking) has been shown to be a widely employed dispersal mechanism in nature but is systematically understudied. As resource patches are exhausted, organisms must migrate to new patches to ensure survival. Many animals that would otherwise be unable to migrate long distances to new food patches, mates, or other resources, use phoretic hosts to maximize dispersal success. Nematodes and mites are the two largest phoretic groups currently known, and many species of both have evolved to specialize on phoretic hosts, increasing the likelihood of successfully dispersing to a new habitat (Binns, 1982; Krishnan *et al.*, 2010). Despite phoresy's prevalence, we know very little regarding specific mechanisms of phoresy. Experiments and phylogenetic analyses, of phoronts and phoretic hosts, can help elucidate the coevolutionary history of the partners. Studies on host specificity or generalism, driven by environmental pressures, may help uncover strategies of phoronts for ensuring dispersal. As shown previously, the *C. elegans* dispersal stage, dauer, has been found to be

phoretically associated with a number of animals such as molluscs, arachnids, and insects (Petersen *et al.*, 2015). Dispersal is likely to cause genetic bottlenecks of migrating species (Lenormand, 2002). What role does genetic drift play in the population dynamics of phoronts? Do phoronts inadvertently bring their associated parasites with them to new habitats? Is dispersal via hosts necessary, or do many dispersing species access new habitats by random dispersal via water or wind? How likely is it that genetically different hosts, or hosts from different natal environments, will arrive at the same habitat patch? Does mating strategy influence success of phoronts in a new patch? Further, are most phoronts able to self-fertilize, as a cursory glance of phoront diversity suggests? As so little is currently known about phoresy, an entire new subfield of species interactions is open for exploration. A better understanding of phoresy in the context of *C. elegans* dispersal stage, dauer, may help explain many of the effects seen in the experiments outlined in my dissertation, such as strain-specific results of parasite avoidance.

Once all of the above factors are better understood (population size, host genetic heterogeneity, life stage differences, and the impact of phoresy), then we can begin the work of combining these factors to understand general principles of host-parasite evolutionary dynamics. For example, how do the impacts of varying population sizes *and* genetically heterogeneous hosts combine to influence host and parasite trajectories? Each of the chapters outlined in this dissertation provides an important contribution to various aspects of host-parasite evolution. Hosts and parasites are influenced by a multitude of dynamics, including aspects of the host population, which is the focus of this dissertation. Host heterogeneity is likely to be important in nearly every host-parasite system. Many studies have shown a reduced parasite prevalence in the presence of multiple host genotypes (Pilet *et al.*, 2006). The underlying assumption of this reduction has been due to the intrinsic tradeoff of simultaneously infecting and causing mortality

in multiple host genotypes, due to a cost of specialization (Altermatt & Ebert, 2008; Poullain *et al.*, 2008). However, I did not observe a substantial cost of specialization in my experiments, suggesting that it is more likely the reduced efficacy of selection that is impeding rates of parasite adaptation in heterogeneous host populations. It may be that many other systems in which we see a reduction in parasite-induced host mortality are caused not by specialization trade-offs, but by other factors. This work illuminates the need to directly test specialization, especially if future work relies on the presence of trade-offs. In addition to testing the interaction of multiple host factors at once, experiments need to be performed with ecologically relevant strains. Only then can we get a better understanding of not just what *may* be possible in nature, but what is actually occurring.

In addition to understanding how host dynamics influence host-parasite evolutionary dynamics, we must also turn to the parasite population. What happens when aspects of the parasite population are put under scrutiny? For example, parasite populations go through continuous bottlenecks during their life cycle: transmission from host to host typically allows only a subset of genotypes to be transferred; those that are successfully transmitted are then subjected to the immune system of the host, which causes a subsequent bottleneck; and even when surviving the immune system, not all parasites reproduce an equal number of propagules (Papkou *et al.*, 2016). How do parasite bottlenecks, particularly in concert with host bottlenecks (e.g., in dispersal) influence one another? Further, all the experiments here are performed with an initially clonal parasite. How might the results change if the population of the parasite is genetically diverse? Would adaptation to multiple host genotypes occur more rapidly? How might things like population size, standing genetic variation, and dispersal of the parasite population impact host-parasite interactions? Lastly, parasites also go through varying life stages

(Thomas & Rudolf, 2010). For example, some parasites need multiple hosts to complete their life cycle (Wilson *et al.*, 2005). Parasites may display life stage-dependent avoidance behaviors, preventing them from infecting the "wrong" host, such as hosts that parasites have not evolved to infect.

Altogether, this dissertation adds important evidence to our growing, but nascent, understanding of host-parasite dynamics. In particular, it adds to our understanding of multiple factors of host populations, such as population size, genetic heterogeneity, and life stage differences, and how these factors influence host-parasite evolutionary trajectories. In addition, I show further evidence that using a tractable host and parasite system, such as *C. elegans* and *Serratia marcescens*, is essential for isolating important factors of each partner and illuminating their reciprocal adaptation on one another. This work also contributes to a broader understanding of the complexities of hosts and parasites in general, and an analysis of the future avenues of research makes clear that a lot is left to be discovered. Further, this work reinforces the idea that a comprehensive understanding is only possible when considering the evolutionary history and ecology of the organisms studied, as an eye toward nature will help us better interpret results we find in experimental biology in the laboratory.

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