Ecological and Evolutionary Drivers of Host Defenses and Pathogen Infectivity Shape Host-Parasite Interactions at Multiple Levels of Biological Organization

by

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DEDICATION

To the parents who go back to college.

To Bodhi and Rowan, with so much love.

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PREFACE

Ecological and evolutionary drivers of host defenses and pathogen infectivity shape host-parasite interactions at multiple levels of biological organization is first a product of the internet-free 1990s, which forced me to go outside and hunt for creepy crawlies. It is also a product of the WiFi-rich 2010s, which allowed me to search vast libraries of information to understand why and how those crawlies creep.

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ABSTRACT

To be classified as a parasite, a symbiont must reduce the fitness of its host (Zelmer 1998; Sorci and Garnier 2008; Méthot and Alizon 2014); as such, as a common and highly antagonistic interaction, parasitism plays an important role in shaping ecological communities and mediating host evolution. My dissertation considers various factors that modulate host and parasite fitness, including host resistance evolution, immune waning and immune escape, and ecological interactions among different parasite species. I employed two host-parasite study systems in my dissertation research: Chapters 2 - 4 use the *Daphnia*-microparasite study system (*Daphnia* is a genus of zooplankton commonly used in ecological and evolutionary research due to their experimental tractability and ecological importance in freshwater ecosystems). For Chapter 5 I use public health data on SARS-CoV-2 dynamics in the people of Southeastern Michigan.

In Chapter 2 I used the *Daphnia*-microparasite system to review how predation, competition, and abiotic factors impact rapid host-parasite evolution. Chapter 3 addresses the impacts of sexual recombination and gene flow on the maintenance of host resistance in a cyclical parthenogen. We sampled two wild populations of *Daphnia* before and after sex and gene flow, then used molecular methods to genotype each animal captured; we also found the resistance phenotype of each animal by raising maternal lines and conducting experimental parasite exposures. In one population, we found that genetic diversity and resistance to infection significantly increased after sexual recombination and remained high after temporal gene flow. The second population started with high genetic diversity and high resistance and maintained these levels through recombination and gene flow. This occurred despite resistance having a fitness cost, which implicates other selective forces at work which are maintaining both high resistance and high genetic diversity. In Chapter 4 I disentangle how the density of two co-existing environmentally transmitted parasites impacts disease dynamics within individual hosts and at the scale of host populations. I found evidence of density-dependent interspecific and intraspecific impacts on parasite infectivity. I additionally found that infection class had significant impacts on host lifespan and parasite transmission. Using this data for modeling epidemics revealed that parasite

dose determines the equilibrium condition of disease dynamics in this system, and that interspecific parasite-parasite interactions maintain a higher parasite burden overall. For Chapter 5 I used SARS-CoV-2 data collected from the people of Southeastern Michigan to model the joint impacts of waning host immunity and evolving SARS-CoV-2 immune escape on COVID-19 dynamics. This model has proven to be conceptually sound and will be used to predict Winter 2022-23 COVID-19 burden and explore counterfactuals around vaccine uptake and variant emergence. Overall, my research shows that host resistance (Ch 3) and immunity (Ch 5) to infection can change rapidly, and disease outbreaks can be difficult to predict as a result. This dissertation also shows that evolution of hosts (Ch 3) or parasites (Ch 5) can dampen or amplify disease prevalence. Finally, this work underscores how studying interactions between species--whether those are host-parasite or parasite-parasite interactions--requires careful consideration of parasite dose (Ch 4) and integration of fitness measures across multiple levels of biological organization (Ch 2, Ch 4).

CHAPTER 1:
Introduction

OVERVIEW

Approximately 40% of all life on Earth is parasitic (Dobson et al., 2008), which makes parasitism one of the strongest ecological and evolutionary forces on the planet. The foundation of the parasitic interaction occurs at the interface between a single host and a single parasite, and the likelihood of successful infection depends on a suite of factors driving host resistance and parasite infectivity. Just like all biological relationships, parasitism can be modulated by a wide range of biotic and abiotic factors (McLean & Duffy, 2020), and these factors can vary depending on the level of biological organization under study (Mideo et al., 2008; Sofonea et al., 2015). For example, one parasite species may outcompete a conspecific within a single host, yet support the persistence of that same conspecific at the level of host populations (Ch 4). Therefore, to understand disease ecology we must examine drivers of host resistance and parasite infectivity in individual hosts, but also consider how those drivers scale up to create epidemics in host populations.

Broadly speaking, my research fits into two fundamental frameworks. The first is understanding how ecological and evolutionary forces modulate parasite infectivity and/or host defenses (Fig 1.1). The second is studying these forces and their impacts at multiple levels of biological organization, from parasite species within hosts to full epidemics across multiple host populations (Fig 1.2). I specifically look at how host resistance evolution, immune waning and immune escape, and dose-dependent infectivity of competing parasite species can drive host-parasite dynamics using one of two study systems: in Chapters 2 - 4 I use a zooplankton-microparasite study system that is well-established for ecology and evolutionary research, and for Chapter 5 I use public health data on SARS-CoV-2 dynamics in Southeastern Michigan.

Hosts often develop ways to avoid or counteract harm caused by infectious disease (Miller et al., 2005). Host species without an adaptive immune system can still rapidly evolve increased resistance if they have 1) a short generation time relative to the duration of the parasite epidemic, and 2) sufficient standing variation in resistance phenotypes (Auld et al., 2012; Duffy et al., 2009). However, rapid evolution of resistance can be counteracted by diversity-maintaining factors like gene flow, fitness costs of resistance, and negative frequency-dependent selection (Antonovics & Thrall, 1994; Buckling & Rainey, 2002; Lively, 2001). Therefore, the resistance of a rapidly evolving host population is determined by a complex suite of competing forces that must be elucidated if one is to predict the selective effect of virulent epidemics. In contrast, hosts with both innate and adaptive immunity are able to mount specific immune responses against pathogenic infection (Iwasaki & Medzhitov, 2015). However, adaptive immunity can wane and eventually leave the host susceptible to infection (in cases of vaccination) or re-infection (in cases of natural immunity) (Goldberg et al., 2022; Heffernan & Keeling, 2009; Negi et al., 2022; Pérez-Alós et al., 2022). The rate of immune waning depends on the vaccine and/or pathogen that induced the immune response as well as the robustness of an individual's immune system. Shifts in host defenses--whether as the result of rapid evolution or adaptive immunity--will have significant impacts on host-parasite interactions and epidemic dynamics. By improving our understanding of how host resistance and adaptive immunity change over time, I advance the understanding of host-mediated drivers of host-parasite interactions.

Parasite infectivity varies across time and space (Boots & Mealor, 2007; Ferris et al., 2020). Infectivity is jointly determined by parasite infectivity and host immunity, and can be impacted by co-circulating parasites or pathogens (Auld et al., 2017; Rigaud et al., 2010; Woolhouse et al., 2001). While it is clear that multiparasite infections are common and important, most scientific understanding of multiparasite dynamics is either based on human clinical studies or laboratory experiments that over-saturate the study organisms with parasites to maximize the number of co-infected hosts; neither of these scenarios account for parasite density

in their analysis. However, there is an abundance of dose response studies for single parasite infections which find non-linear relationships between parasite dose (i.e., density) and infectivity, virulence, and transmission (Clay et al., 2021). It is therefore reasonable to assume that parasite spore density is a vital variable to explicitly incorporate into research on multiparasite systems and coinfections. Doing so will enable us to better understand how interactions among parasites influence infectivity and virulence in natural disease systems.

In addition to studying how environmental spore density intersects with parasite-parasite interactions, I built an epidemiological model to predict how the evolution of immune escape (in conjunction with immune waning in hosts) will form a complex landscape of pathogen infectivity within the state of Michigan (Goldberg et al., 2022; Pilz et al., 2022; Stich et al., 2022). Standard epidemiological models assume a simplistic immune landscape (i.e. hosts either have no immunity or full immunity to the pathogen of interest), but as pandemics transition into an endemic state with a wide range of immunity from various sources, we need to update models to account for the increasing heterogeneity of host immunity. By exploring the effects of parasite density, interspecific parasite-parasite interactions, and the joint effects of immune escape and immune waning on infectivity, I advance our understanding of how variation in parasite infectivity can have significant effects on large-scale disease dynamics.

STUDY SYSTEMS

Daphnia-microparasite

Ecologists and evolutionary biologists have long studied *Daphnia*, both because of their ecological importance and because of their tractability as a study organism (Duffy et al., 2021; Ebert, 2005; Lampert, 2006). *Daphnia* are dominant herbivores in many temperate aquatic ecosystems and serve as important links between primary producers (the phytoplankton they consume from the water column) and consumers (the small fish and predatory invertebrates that feed on *Daphnia*). In addition, their small size and rapid generation time make it possible to work with them in the laboratory and in field studies, allowing scientists to test possible

mechanisms underlying patterns observed in nature—an important bridge between the laboratory and the natural world that is not easily crossed in many study systems.

In the past few decades, *Daphnia* and their microparasites have emerged as a model system for understanding infectious diseases (Cáceres et al., 2014; Ebert, 2005, 2011; Lampert, 2011; Little & Ebert, 2004). A number of parasites including viruses, bacteria, fungi, oomycetes, microsporidians and protozoa regularly infect *Daphnia* (Ebert, 2005; Green, 1974; Toenshoff et al., 2018). These parasites have diverse infection dynamics (horizontal vs. vertical transmission, obligate killers vs. continuous transmission) and exert a wide range of effects on their hosts (including early death, castration, and even gigantism; (Ebert, 2005)).

SARS-CoV-2 in Michigan

Severe acute respiratory syndrome virus 2 (SARS-CoV-2) is a coronavirus that causes the disease COVID-19. This respiratory illness is responsible for the ongoing COVID-19 pandemic. SARS-CoV-2 is believed to have a zoonotic origin (V'kovski et al., 2021; Worobey et al., 2022) and, due to its relatively limited genetic diversity, it is estimated that the virus spilled over into humans sometime in late 2019 ((V'kovski et al., 2021; Worobey et al., 2022)). While the original strain of SARS-CoV-2 had an R_0 of approximately 2.4 – 3.4, some newer variants have evolved increased infectiousness (Billah et al., 2020).

The state of Michigan experienced four waves (each a distinct variant) of SARS-CoV-2 transmission during the COVID-19 pandemic through May 2022. Each variant wave has shown differing patterns of infection, disease severity, and mortality. Further research has shown evidence of increasing antigenic escape over time (Mykytyn et al., 2022).

SUMMARY OF CHAPTERS

Chapter 2: ***Ecological Context Influences Evolution in Host-Parasite Interactions: Insights from the Daphnia-Parasite Model System***

For this review, we zoomed out from infectivity and resistance to consider how ecological context (competition, predation, and abiotic factors) modulate evolution in host-parasite interactions. This is because host-parasite interactions are often considered in isolation, but the larger ecological context matters. Human activities are strongly impacting the ecological context in which host-parasite interactions are embedded. Humans are changing abiotic factors in terrestrial and aquatic habitats, including nutrient levels, precipitation regimes, temperature, and pH (Carpenter et al. 1998; Field et al. 2012; Weiss et al. 2018). Human activities are also strongly impacting species assemblages via environmental disturbance, climate change, and the introduction and extirpation of different species, including parasites and predators (Britton 2013; Doherty et al. 2016; Prugh et al. 2009; Sala et al. 2000; Urban 2015). Because ecological context influences the prevalence and severity of disease, human-driven changes in abiotic factors and species assemblages can have dramatic consequences for evolution in host-parasite systems. In this review, we highlight some of the ways in which ecological context, including human-driven changes to ecosystems, can influence evolution in host-parasite interactions. We also touch on some ways in which contemporary evolution may change ecological dynamics (i.e. eco-evolutionary feedbacks; Hendry 2016; Strauss et al. 2017). In doing so, we focus in on one particular study system that has yielded key insights: *Daphnia* and their microparasites. *Daphnia* are ecologically important and experimentally tractable, and have emerged as a model system for understanding the ecology and evolution of host-parasite interactions (Cáceres et al. 2014b; Ebert 2005). We first introduce this system, then review studies demonstrating the importance of predators, competitors, and the abiotic environment in altering evolution in host-parasite interactions.

Chapter 3: ***Sexual recombination and temporal gene flow maintain host resistance and genetic diversity***

Infectious disease can threaten host populations, and hosts can rapidly evolve resistance during

epidemic; this evolution is often modulated by fitness trade-offs (e.g., between resistance and fecundity). However, many organisms switch between asexual and sexual reproduction, and this shift in reproductive strategy can also alter how resistance in host populations persists through time. Recombination can shuffle alleles selected for during an asexual phase, uncoupling the combinations of alleles that facilitated resistance to parasites and altering the distribution of resistance phenotypes in populations. Furthermore, in host species that produce diapausing propagules (e.g., seeds, spores, or resting eggs) after sex, accumulation of propagules into and gene flow out of a germ bank introduce allele combinations from past populations. Thus, recombination and gene flow might shift populations away from the trait distribution reached after selection by parasites. To understand how recombination and gene flow alter host population resistance, we tracked the genotypic diversity and resistance distributions of two wild populations of cyclical parthenogens. In one population, resistance and genetic diversity increased after recombination whereas, in the other, recombination did not shift already high resistance and genetic diversity. In both lakes, resistance remained high after temporal gene flow. This observation surprised us: due to costs to resistance imposed by a fecundity-resistance trade-off, we expected that high population resistance would be a transient state that would be eroded through time by recombination and gene flow. Instead, low resistance was the transient state, while recombination and gene flow re-established or maintained high resistance to this virulent parasite. We propose this outcome may have been driven by the joint influence of fitness trade-offs, genetic slippage after recombination, and temporal gene flow via the egg bank.

Chapter 4: ***Exposure Dose Alters Within-Host Interactions Between Co-Infecting Parasites, with Consequences for Parasite Prevalence and Host Abundance***

The density of environmentally transmitted parasites varies wildly across time and space. Spore density (i.e. dose, inoculum) can have strong effects on likelihood of infection, transmission, and virulence, which makes it a vital consideration when thinking about disease dynamics. On a seemingly different note, parasites and pathogen regularly coexist and often co-infect shared

hosts. Multi-pathogen infection has become a popular area of study in the past two decades as a result. However, very little is known about how spore dose impacts multi-pathogen dynamics. I'm interested in understanding how interactions between parasite species at both within- and between-host scales impact the size, duration, and deadliness of epidemics. I use the host *Daphnia dentifera* and two of its common parasites, the fungus *Metschnikowia bicuspidata* and the bacterium *Pasteuria ramosa*, as a study system. I disentangled dose effects on parasite-parasite and parasite-host interactions by conducting a factorial dose experiment and using it to parameterize dose-dependent multiparasite epidemic models.

Epidemic models revealed that, while the bacterium suffered a fungus-dose-dependent reduction in its probability of infection, this negative effect was mitigated at the level of host populations. In fact, when this negative effect on the bacterium was removed from the model, total bacterial prevalence further declined and host populations increased. Overall, we found that multiparasite scenarios were detrimental to the bacterium (and had little effect on the fungus), but dose-dependent interspecific interactions mitigated that harm and resulted in higher parasite prevalence and lower host density than would have occurred otherwise. Ultimately, my work underscores how negative dose-dependent interactions between parasites can scale up to alter parasite burdens and host abundance in unexpected ways.

Chapter 5: ***Incorporating waning immunity and immune escape to better predict SARS-CoV-2 dynamics and explore counterfactuals around vaccine uptake and variant emergence***

A number of variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have emerged since the first reports of human infections in late 2019. These variants carry mutations that can confer increased transmissibility, disease severity, and/or antigenic escape from immune system antibodies, all of which are making pandemic management increasingly difficult and complex. Another source of complexity arises from how host immunity is conferred (i.e. through vaccination, infection, or reinfection, as well as combinations of all three) and how quickly that immunity wanes. As such, the simultaneous actions of vaccination regime change, infection or re-infection, immune waning, and a growing number of variants with differing levels of immune

escape make the dynamics of SARS-CoV-2 increasingly difficult to predict. Integrating such mechanisms of changing immunity into a transmission modeling framework will increase our ability to anticipate the dynamics of newly-arisen variants of SARS-CoV-2.

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FIGURES

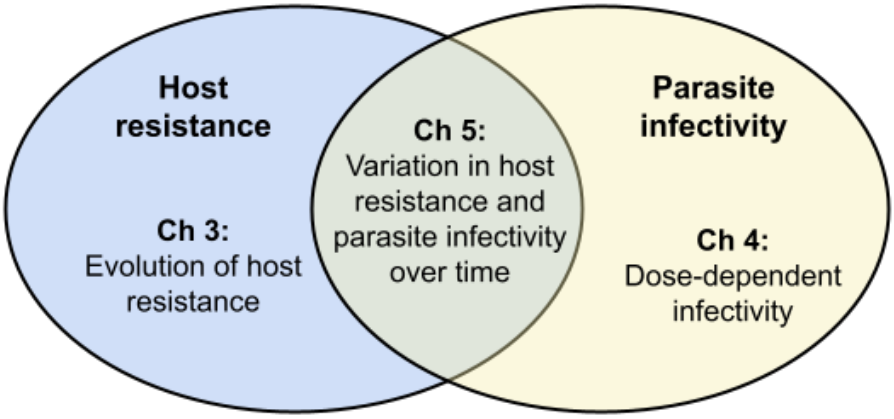

Fig 1.1. Venn diagram. A simple diagram showing how the research chapters in this dissertation are based around host resistance and/or parasite infectivity.

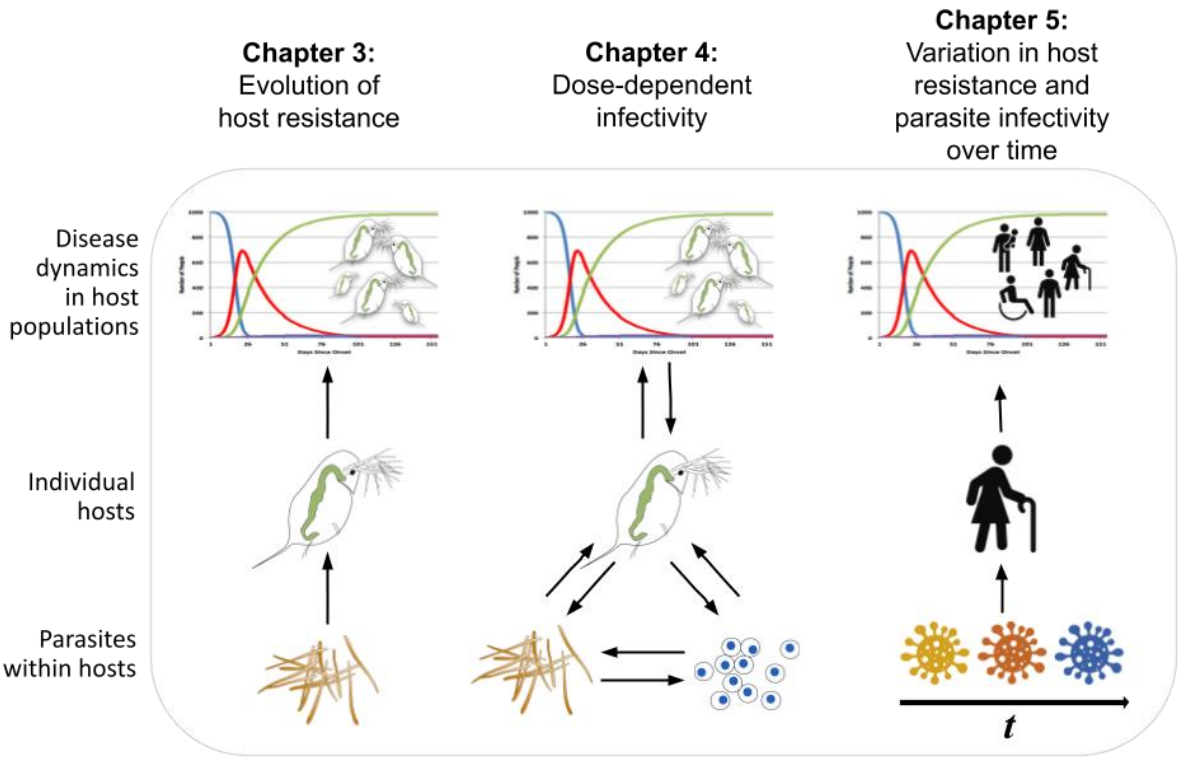

Fig 1.2. A visual summary of the unifying themes across the research chapters of this dissertation. i.e. all chapters measure aspects of infection biology which determine the likelihood that a host will become infected with a parasite, while exploring how those processes scale up to the level of epidemics.

CHAPTER 2:

Ecological context influences evolution in host-parasite interactions:

insights from the *Daphnia*-parasite model system[1,2]

ABSTRACT

Parasites exert strong selective pressure on their hosts, and many hosts can evolve rapidly in response. As such, host-parasite interactions have a special place in the study of contemporary evolution. However, these interactions are often considered in isolation from the ecological contexts in which they occur. Here we review different ways in which the ecological context of host-parasite interactions can modulate their evolutionary outcomes in important and sometimes unexpected ways. Specifically, we highlight how predation, competition, and abiotic factors change the outcome of contemporary evolution for both hosts and parasites. In doing so, we focus on insights gained from the Daphnia-microparasite system. This system has emerged as a model system for understanding the ecology and evolution of host-parasite interactions, and has provided important insights into how ecological context influences contemporary evolution.

INTRODUCTION

> "I want to suggest that the struggle against disease, and particularly infectious disease, has been a very important evolutionary agent, and that some of its results have been

[1] McLean, K.D., Duffy, M.A. (2020). Ecological Context Influences Evolution in Host-Parasite Interactions: Insights from the Daphnia-Parasite Model System*. In: Evolution in Action: Past, Present and Future. Genetic and Evolutionary Computation. Springer, Cham. https://doi.org/10.1007/978-3-030-39831-6_21

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rather unlike those of the struggle against natural forces, hunger, and predators, or with members of the same species." — JBS Haldane (1949)

Parasites are ubiquitous, and the outcomes of host-parasite interactions can often be measured in terms of life or death. Thus, it is not surprising that in the 70 years since Haldane postulated the importance of parasites as selective agents, studies of host-parasite interactions have provided striking examples of evolution in action (Allison 1954; Boots et al. 2004; Buckling and Rainey 2002; Buckling et al. 1997; Decaestecker et al. 2007; Deom et al. 1986; Dybdahl and Lively 1998; Epstein et al. 2016; Fenner and Fantini 1999; Gibson et al. 2018; Schiebelhut et al. 2018; Schild et al. 1983). Moreover, we now realize that the ecological context—the "natural forces, hunger, and predators" and "members of the same species" to which Haldane referred—modulates the evolutionary outcomes of infectious disease in important and sometimes unexpected ways. Here, we review recent studies that demonstrate that predators, competitors, and the abiotic environment strongly influence the evolutionary dynamics of host-parasite interactions.

Host-parasite interactions are often considered in isolation, but the larger ecological context matters, too. To give just two examples: excluding large vertebrate herbivores increased the prevalence of viruses in plants by increasing the abundance of highly competent hosts (Borer et al. 2009). Similarly, increasing nutrient inputs to ponds elevated levels of disease in frogs by increasing algal abundance which, in turn, increased the abundance of snails, who are intermediate hosts for the parasite (Johnson et al. 2007). These parasites strongly impact their hosts: the plant virus reduces plant longevity, growth, and seed production and the frog parasite causes severe limb deformities. Therefore, it does not require a large leap to imagine that these alterations to ecological context might alter parasite-mediated selection.

Human activities are strongly impacting the ecological context in which host-parasite interactions are embedded. Humans are changing abiotic factors in terrestrial and aquatic habitats, including nutrient levels, precipitation regimes, temperature, and pH (Carpenter et al. 1998; Field et al. 2012; Weiss et al. 2018). Human activities are also strongly impacting species assemblages via environmental disturbance, climate change, and the introduction and extirpation of different species, including parasites and predators (Britton 2013; Doherty et al. 2016; Prugh et al. 2009; Sala et al. 2000; Urban 2015). Because ecological context influences the prevalence and severity of disease, human-driven changes in abiotic factors and species assemblages can have dramatic consequences for evolution in host-parasite systems.

In this review, we highlight some of the ways in which ecological context, including human-driven changes to ecosystems, can influence evolution in host-parasite interactions. We also touch on some ways in which contemporary evolution may change ecological dynamics (i.e. eco-evolutionary feedbacks; Hendry 2016; Strauss et al. 2017). In doing so, we focus in on one particular study system that has yielded key insights: *Daphnia* and their microparasites. *Daphnia* are ecologically important and experimentally tractable, and have emerged as a model system for understanding the ecology and evolution of host-parasite interactions (Cáceres et al. 2014b; Ebert 2005). We first introduce this system, then review studies demonstrating the importance of predators, competitors, and the abiotic environment in altering evolution in host-parasite interactions.

The *Daphnia*-microparasite study system

Ecologists and evolutionary biologists have long studied *Daphnia*, both because of their ecological importance and because of their tractability as a study organism (Ebert 2011; Lampert 2006). *Daphnia* are dominant herbivores in many temperate aquatic ecosystems and serve as important links between primary producers (the phytoplankton they consume from the water

column) and consumers (the small fish and predatory invertebrates that feed on *Daphnia*). In addition, their small size and rapid generation time make it possible to work with them in the laboratory and in field studies, allowing scientists to test possible mechanisms underlying patterns observed in nature—an important bridge between the laboratory and the natural world that is not easily crossed in many study systems.

The reproductive system of *Daphnia* also helps explain why they have emerged as an important study system. Most *Daphnia* are cyclical parthenogens, meaning they can reproduce sexually and asexually. Asexual reproduction makes it possible to propagate isofemale (i.e., clonal) lines under standardized laboratory conditions, allowing researchers to differentiate genetic and environmental effects on phenotypic traits. At the same time, the sexually produced offspring are enclosed in long-lived dormant eggs that accumulate in sediments, allowing studies that "resurrect" genotypes from earlier populations so that scientists may understand how populations have changed on scales from decades to centuries (Decaestecker et al. 2007; Frisch et al. 2014; Hairston et al. 1999; Rogalski 2017).

Another advantage of the *Daphnia* system comes from the ability to study multiple replicate lakes or ponds that have well-defined boundaries; this means it is possible to study multiple populations (essential for evolutionary studies, where population is the unit of replication) and to do so across ecological gradients (e.g., in predation or productivity).

In the past few decades, *Daphnia* and their microparasites have emerged as a model system for understanding infectious diseases (Cáceres et al. 2014b; Ebert 2005; Ebert 2011; Lampert 2011; Little and Ebert 2004). A number of parasites including viruses, bacteria, fungi, oomycetes, microsporidians and protozoa regularly infect *Daphnia* (Ebert 2005; Green 1974; Toenshoff et al. 2018). These parasites have diverse infection dynamics (horizontal vs. vertical transmission, obligate killers vs. continuous transmission) and exert a wide range of effects on their hosts (including early death, castration, and even gigantism; Ebert 2005).

As is true for all organisms, any particular *Daphnia*-parasite interaction is embedded within a much larger, richer ecological context (Miner et al. 2012). When thinking about this ecological context, we need to consider not only the types of interactions that have traditionally been the focus of ecological studies (such as resource levels and predation regimes), but also that pathogens are likely to be infecting multiple members of the food web, and that any one member of the food web is likely to be infected by multiple pathogens.

We begin by reviewing the impact of predation on evolution in host-parasite systems. Next, we consider the potential for species interactions within the same trophic level (especially the presence of multiple host species or multiple parasite species) to alter host-parasite evolution. Finally, we review some ways the abiotic environment can alter evolution of hosts and parasites. In each case, evidence from *Daphnia*-parasite interactions demonstrates that the ecological context impacts the evolution of both the host and the parasite.

Predation alters evolution in host-parasite systems

Predators should alter evolution in host-parasite systems in multiple ways, including by altering the amount of disease in a focal host population. Predation is often thought of as reducing infection prevalence in hosts, especially in cases where predators selectively remove infected hosts (Hudson et al. 1992; Ostfeld and Holt 2004; Packer et al. 2003). However, predators can also increase disease in their host populations via a variety of mechanisms (reviewed in Duffy et al. in press), including by changing prey community composition so that high-quality hosts dominate (Borer et al. 2009), inducing behavioral changes in the prey that increase the risk of infection (Orlofske et al. 2014), or by spreading transmission stages while feeding (Cáceres et al. 2009).

Predators can also alter evolution in host-parasite systems by introducing trade-offs that alter the selective landscape. As one example, hosts may face trade-offs between anti-predator defenses

and mounting an effective immune response (Navarro et al. 2004; Rigby and Jokela 2000; Stoks et al. 2006). Predators can also influence parasite evolution via impacts on trade-offs, most notably those related to virulence. Virulence is generally defined as the reduction in host fitness caused by infection, usually due to changes in fecundity or lifespan (Day 2002). Overall, much theory related to the evolution of parasite virulence has focused on the influence of parasites on the host's instantaneous mortality rate (Anderson and May 1982; Day 2002).

Under this framework, parasite fitness increases with transmission rate and also with the length of time that a host is infectious; it is generally assumed that higher replication rates of parasites increase transmission but reduce the length of time a host is infectious (e.g., by killing it or triggering an immune response), driving an intermediate optimal virulence (de Roode et al. 2008; Lenski and May 1994). Because of the nature of this trade-off, increases in mortality rates from sources other than infection (including predators) are expected to increase parasite virulence, since the cost the parasite pays for killing the host quickly is reduced (Lenski and May 1994), though other outcomes are also possible depending on the specifics of the interaction (Choo et al. 2003; Day 2002; Houwenhuyse et al. 2018).

The impacts of predation on host evolution have been the focus of several studies in the *Daphnia dentifera-Metschnikowia bicuspidata* system. In this system, predators influence the overall amount of disease from the common fungal parasite *Metschnikowia*: fish preferentially feed on infected *Daphnia*, strongly reducing infection prevalence (Duffy and Hall 2008; Duffy et al. 2005), whereas invertebrate predators are "predator spreaders" that increase infection prevalence (Cáceres et al. 2009). There are also important trade-offs between predation risk, resistance to disease, and fecundity. Larger bodied *Daphnia* are more susceptible to fish predation (Brooks and Dodson 1965) and to *Metschnikowia* (Hall et al. 2010), but less susceptible to predation by the common, voracious, gape-limited invertebrate predator *Chaoborus* (Pastorok 1981). In addition, there is also a trade-off between resistance to *Metschnikowia* and fecundity, with larger animals being more fecund but less resistant (Hall et al. 2010), though some populations contain animals with high fecundity and high resistance (Auld et al. 2013). These trade-offs—combined

with variation among lakes in vertebrate and invertebrate predation rates, resource levels, and host genetic variation—likely explain different evolutionary responses of populations to disease outbreaks, with some populations evolving increased resistance to disease, some evolving increased susceptibility, and some experiencing disruptive selection on resistance (Duffy et al. 2008; Duffy et al. 2012; Duffy and Sivars-Becker 2007).

Work in the *Daphnia dentifera-Metschnikowia bicuspidata* system has focused on evolution of the host but not the parasite because the parasite shows surprisingly little variation and limited evolutionary potential (Auld et al. 2014; Duffy and Sivars-Becker 2007; Searle et al. 2015). However, predators seem likely to drive evolution in other *Daphnia* parasites, including the common bacterial parasite *Pasteuria*. In an artificial selection experiment using *Daphnia dentifera* hosts and *Pasteuria,* parasites that were selected in an environment that simulated high predation evolved to produce more spores in that environment; however, that came at the cost of reduced performance in low predation environments (Figure 2.1; Auld et al. 2014). These results suggest that *Pasteuria* collected from lakes with high predation might be more virulent than those from low predation lakes.

Overall, studies on *Daphnia* and their microparasites demonstrate that predators can strongly alter parasite-mediated selection on host populations, trade-offs faced by hosts and by parasites, and selection on parasite traits.

Multihost, multiparasite interactions: influences of competition on evolution in host-parasite systems

Most parasites can infect multiple hosts, and most hosts are infected by multiple parasites (Fenton and Pedersen 2005; Lively et al. 2014). While this is the rule rather than the exception in nature, the majority of research on host-parasite evolution is based around a one-host one-parasite model (Read and Taylor 2001; Rigaud et al. 2010). However, there is an expanding field

of research uncovering the complex ways in which interspecific competition can change disease dynamics and evolutionary outcomes.

Multiple Hosts

High biodiversity in hosts can dilute (Johnson et al. 2013; Keesing et al. 2010; Keesing, Holt, and Ostfeld 2006; LoGiudice et al. 2003; Ostfeld and Keesing 2012) or amplify (Randolph and Dobson 2012; Searle et al. 2016; Strauss et al. 2015; Wood et al. 2014) the prevalence of disease. The dilution effect arises when species rich communities contain lower quality (that is, less competent) hosts that slow the spread of the parasite and therefore protect competent focal hosts from infection. While many studies have documented the dilution effect in wild systems (Civitello et al. 2015), there is still vigorous debate about how common dilution is (Randolph and Dobson 2012; Salkeld et al. 2013; Wood et al. 2014).

One major shortcoming of dilution effect theory is that it generally ignores competition between diluter and focal hosts, despite coexistence theory showing interspecific host competition has a strong impact on host-parasite dynamics (Bowers and Turner 1997; Greenman and Hudson 2000; Gyllenberg et al. 2012; Saenz and Hethcote 2006). Altering the number of host species changes not only the amount of disease in the system, but also the amount of interspecific competition a focal host experiences, with potentially complex effects on focal host density and disease prevalence (Cáceres et al. 2014a). If adding a host species increases total host density, it could potentially drive an increase in disease in a focal host (amplification), even if the additional host is less competent than the focal host (Searle et al. 2016).

Thus, when considering how multiple hosts might alter evolution in host-parasite systems, it is important to recognize that selection will occur both via changes in the amount of disease and via changes in host density, mediated by interspecific competition. A recent study tested the joint influence of infectious disease and competition on eco-evolutionary dynamics in the *Daphnia*-

Metschnikowia system (Strauss et al. 2017). The additional host species, *Ceriodaphnia,* is more resistant to *Metschnikowia* than the focal host, *Daphnia dentifera*, but also a competitor for resources. The expectation was that the combination of a virulent parasite and strong interspecific competition from *Ceriodaphnia* might drive populations of *Daphnia dentifera* to extinction (Strauss et al. 2017). Indeed, in populations where *Daphnia dentifera* had little genetic diversity (and thus low evolutionary potential), the combination of parasitism and interspecific competition resulted in very low densities of the focal host. However, in populations where *Daphnia dentifera* had high diversity (and thus high evolutionary potential), the populations thrived. Surprisingly, this rescue effect arose because hosts evolved increased competitive ability, but not increased resistance. Evolution rescued the focal host from the negative impacts of competition, but also drove larger disease outbreaks (as compared to populations with low evolutionary potential). This demonstrates that introducing a diluter host to curb an epidemic may have unexpected results if we ignore the potential for competition—and rapid evolution—between focal and diluter hosts.

At present, we know that interactions between host species can change transmission dynamics and drive evolution in unexpected ways, but the eco-evolutionary effects of parasitism and competition on a focal host remain difficult to predict. However, by integrating a mechanistic understanding of the types of host-host and host-parasite interactions that occur (Luis et al. 2018; Searle et al. 2016; Strauss et al. 2015), we can better understand how multihost systems can impact host fitness, change parasite transmission dynamics, and ultimately drive rapid evolution in hosts and parasites.

Multiple Parasites

When multiple parasites coexist within a host population, they have the potential to influence each other directly (via competition or facilitation within coinfected hosts) or indirectly (e.g., via

altering host lifespan or population density). As a result, the addition of a new parasite has the potential to alter selection on existing parasites in the system. Coinfections between helminths (including nematodes) and microparasites have been a particular focus of study, in part as a result of influences of helminths on host immune systems (Ezenwa 2016). Work on African buffalo, nematodes, and bovine tuberculosis has demonstrated how coinfecting parasites can influence one another, and also the importance of tests in real world situations. Nematodes suppress the response of the Th1 arm of the immune system in buffalo hosts; Th1 cells protect against microparasites, so the nematode-induced suppression of this part of the immune system should facilitate the invasion of tuberculosis in buffalo (Ezenwa et al. 2010). Those results suggest that removing helminths should decrease microparasite fitness. However, treating African buffalo with anthelminthics actually promoted the spread of bovine tuberculosis: anthelminthic treatment did not influence the likelihood of infection with tuberculosis, but did increase survival after infection, increasing transmission opportunities (Ezenwa and Jolles 2015). Such contrasting impacts of coinfection at the within-host scale vs. the host population scale is not unique to macroparasite-microparasite coinfections. As discussed more below, recent work motivated by the *Daphnia*-microparasite system found that priority effects (where the order of infection determines the impacts parasite species have on each other's fitness) can drive scenarios where parasite competition within a host can actually promote coexistence at the population scale (Clay et al. 2019b).

Host Mortality

One way in which multiparasite infections may alter the evolution of one or more of the coexisting parasites is by changing the lifespan of the host. As discussed in the predation section, shortening the lifespan of a host generally selects for the evolution of higher virulence, as the optimal virulence of a parasite is thought to reflect a trade-off between transmission rate and host mortality (Bull and Lauring 2014). If a single host individual is coinfected—that is, simultaneously infected by two or more parasite strains or species—that has the potential to alter evolutionary outcomes. In particular, if a coinfecting parasite is virulent (increasing mortality

rate on the host), that should select for higher virulence in the other parasite (May and Nowak 1995).

However, both in theory and in practice, coinfections often yield results that are more complicated than might initially be predicted (as reviewed in Alizon et al. 2013). For example, in a rodent malaria system, immunopathology leads to additional costs associated with parasite virulence, with the potential to drive negative virulence-transmission relationships (Long and Graham 2011). As a result, competition between genotypes coinfecting a single host individual can have major impacts on parasite evolution, increasing or decreasing virulence (Long and Graham 2011; Mideo 2009).

Work in the *Daphnia*-parasite system has also demonstrated that interactions between competing parasites can sometimes drive initially counterintuitive results. In an experiment using *Daphnia magna* and the gut microsporidian *Glugoides intestinalis*, treatments with *low* host mortality rates resulted in the evolution of *higher* virulence (Ebert and Mangin 1997). This pattern arose due to competition between coinfecting strains of the parasite; lower host mortality rates increased the amount of time parasites spent competing amongst themselves within hosts, driving the evolution of faster parasite growth and therefore higher virulence (Gandon et al. 2001). This underscores the need to understand the mechanisms of within-host interactions in order to predict parasite evolution.

Order of Infection

While much theory on the evolution of virulence focuses on the impacts of changes in host mortality rate, other factors can also influence virulence evolution. Increasingly, scientists are recognizing that the order in which parasites arrive in a host can influence both host and parasite fitness and that those impacts can vary between genotypes (Al-Naimi et al. 2005; de Roode et al. 2005; Marchetto and Power 2018; Pollitt et al. 2015).

In the *Daphnia-Pasteuria* system, a study found that virulence was influenced not only by infections consisting of multiple strains of a parasite, but also by the order of infection (Ben-Ami et al. 2008). In simultaneous coinfections or sequential infections where a more virulent parasite strain arrived first, virulence (host mortality rate) and parasite fitness (spore production) matched that of the more virulent strain. However, when the less virulent parasite infected first, virulence resembled an average between single infections of the two strains. Additionally, both parasites suffered lower fitness, likely due to interactions akin to scramble competition. Surprisingly, these mixed-strain infections also led to higher host fecundity than did single infections (*Pasteuria* has dramatic effects on fecundity; Ebert 2005), suggesting coinfections may be less harmful to hosts than single infections in the short term. Overall, the authors concluded that high rates of coinfection would select for virulent parasites, which outcompete less-virulent strains (Ben-Ami et al. 2008).

Studies of *Daphnia* infected with multiple parasite species (rather than multiple strains of the same species) also have found that the order of infection is important to host and parasite fitness. A study of *Daphnia galeata,* the fungus *Metschnikowia,* and the ichthyosporean *Caullerya mesnili* found that simultaneous coinfections were significantly more virulent (in terms of host lifespan and fecundity) than were single infections or sequential coinfections (Lohr et al. 2010). They found that *Caullerya* had higher fitness when it arrived first in sequential coinfections, whereas *Metschnikowia* had higher fitness if it arrived second. A new study on *Daphnia dentifera*, *Pasteuria*, and *Metschnikowia* also found *Metschnikowia* benefitted from second arrival (Clay et al. 2019b). However, in this case, *Pasteuria* fitness was highest in single infections and low in coinfections, regardless of whether it arrived first or second, likely due to the shortened host lifespan of coinfected hosts. Overall, priority effects can influence parasite prevalence and coexistence, changing pathogen community structure (Clay et al. 2019a; Clay et al. 2019b), which underscores the importance of linking within- and between-host processes to understand host-multiparasite dynamics.

In the case of the interactions between *Pasteuria* and *Metschnikowia*, it is interesting to note that the dominant driver of low fitness for *Pasteuria* in coinfections seems to be shortened host lifespans driven by *Metschnikowia* (Clay et al. 2019b). *Pasteuria* is a parasitic castrator, with a relatively slow life history compared to *Metschnikowia* (Auld et al. 2014). However, as discussed above in the predation section, experimental evolution studies have demonstrated that *Pasteuria* can evolve to increase its fitness in high mortality environments (Auld et al. 2014). In the future, it would be interesting to use experimental evolution to explore the potential of *Pasteuria* to evolve to better compete with *Metschnikowia* and other coinfecting parasites.

The influence of the abiotic environment on evolution in host-parasite systems

Humans are dramatically altering the abiotic environment in which host-parasite interactions take place. Perhaps most obviously, climate change is altering mean environmental temperature, as well as the duration and variation of temperature extremes (Field et al. 2012), which can strongly influence the outcome of host-parasite interactions (Lafferty 2009). However, climate change also alters precipitation regimes, with consequences for water clarity in aquatic systems (Williamson et al. 2015). Human activities also drastically alter nutrient levels in natural ecosystems (which drives changes in primary producer communities) and add pesticides and other novel chemicals to environments (Carpenter 2008; Stokstad and Grullón 2013). Our understanding of evolution in action developed from the *Daphnia*-parasite system makes it clear that these anthropogenic alterations to the abiotic environment should influence evolutionary dynamics of hosts and parasites (Figure 2.2).

Temperature

Climate change is altering mean temperatures as well as variability in temperature in ecosystems worldwide (Coumou and Rahmstorf 2012; Field et al. 2012). Temperature can influence the

likelihood of a host encountering and/or being infected by a pathogen (e.g., Elderd and Reilly 2014; Hall et al. 2006), parasite development rates and transmission stage production (e.g., Poulin 2006), host thermal stress (with impacts on things such as immune function; Dittmar et al. 2014), and the fitness impacts of infection on hosts (e.g., Vale et al. 2008). Thus, temperature should strongly influence evolution in host-parasite systems.

Research on the *Daphnia*-parasite system has helped us understand how altered temperatures might influence the amount of disease and how hosts evolve in response to disease outbreaks. Recent research on the *Daphnia-Metschnikowia* system suggests that a warmer world will be a sicker world (Shocket et al. 2018). A mesocosm experiment showed that warmer temperatures resulted in larger epidemics, primarily because of temperature dependence in transmission rates. Temperature-dependent transmission arose because the host encounters fungal spores while foraging, and foraging rate (and, therefore, parasite exposure rate) increased with temperature.

An experimental study of the *Daphnia-Pasteuria* system shows that these alterations in disease levels can alter evolutionary outcomes (Auld and Brand 2017b). The timing and magnitude of disease outbreaks depended on mean temperature and temperature variability, as did parasite-driven evolution of the host populations. An increase of 3°C drove much larger disease outbreaks that were associated with strong parasite-driven selection and associated reductions in host diversity. Interestingly, this study also looked at a second aspect of environmental variation—the impact of spatial structure on host-parasite populations. The study used physical mixing to homogenize populations, while no mixing allowed populations to form and retain spatial structure. As with temperature, the size of the epidemic and the tempo and mode of evolution were influenced by the mixing treatment. Furthermore, a follow up study found that mixing influenced patterns of adaptation and (co)evolution in the host and parasite (Auld and Brand 2017a). This has interesting potential links with climate change as well, as increasing severity of storms might change mixing regimes in aquatic habitats.

Water clarity

An underappreciated component of climate change is that increases in heavy precipitation bring more dissolved organic matter into aquatic systems, making surface waters darker and increasing turbidity and cloudiness in the water column (Williamson et al. 2017; Williamson et al. 2015). This means that climate change is leading to reduced light penetration in surface waters. This can reduce prey visibility, which will change the rate of predation and its impact on host-parasite dynamics. Notably, one study found that selective predation on infected *Daphnia* was eliminated in high dissolved organic matter conditions (Johnson et al. 2006), so the ability of fish predators to reduce disease in *Daphnia* hosts might be eliminated in darker waters.

Darker surface waters may also reduce the likelihood that waterborne parasites will be killed by sunlight. For example, *Metschnikowia* is highly sensitive to light, and darker lakes generally have smaller disease outbreaks (Overholt et al. 2012). Thus, changes in lake light environments should alter the size of disease outbreaks and the parasite-mediated selection associated with them. Moveover, it might drive selection on the parasite, if parasite genotypes vary in their sensitivity to light. An exciting potential avenue for future research would be to take advantage of spore banks (Decaestecker et al. 2007; Decaestecker et al. 2004) to look for evolutionary change over time in the parasite's ability to tolerate light associated with changes in light penetration.

Nutrient levels and primary production

Humans strongly alter nutrient levels, greatly increasing the amount of bioavailable nitrogen and phosphorus in the environment. This increases primary productivity, which can increase the amount of disease a focal host experiences, especially due to increases in host density (Johnson et al. 2007).

Work on several different *Daphnia*-parasite systems has explored links between nutrient levels, disease, and host fitness or evolution. In the *Daphnia-Metschnikowia* system, more productive lakes had larger disease outbreaks during which hosts evolved greater resistance to infection, whereas lakes with lower productivity had smaller disease outbreaks during which hosts evolved greater susceptibility to infection (Duffy et al. 2012). In the *Daphnia*-White Fat Cell Disease system, nutrient enrichment increased infection prevalence and intensity (Decaestecker et al. 2015) but also led to less efficient nutrient assimilation in *Daphnia*, resulting in lower disease tolerance (Reyserhove et al. 2017). A study on a natural lake population of *Daphnia longispina* found that the seasonal influx of environmental nutrients increased algal food quality, driving higher prevalence of two gut endoparasites; however, this also drove a decrease in the prevalence of an epibiont and overall parasite species richness (Aalto et al. 2014). These contradictory effects are likely due to species-specific stoichiometric demands of parasites and hosts (Aalto et al. 2014). Finally, a laboratory study using the *Daphnia-Pasteuria* system demonstrated that the nutrient content (C:P ratio) of the food a host consumes influences parasite virulence (Frost et al. 2008).

Increased nutrient levels can also strongly influence the community of primary producers; in lakes, high nutrient levels are typically associated with dominance by cyanobacteria. Work on *Daphnia* and their parasites suggests that cyanobacteria alter host susceptibility, though the specific effects vary across parasites (Coopman et al. 2014; Sanchez et al. in press; Tellenbach et al. 2016). An interesting avenue for future research will be understanding how human-driven changes in phytoplankton communities alter parasite-driven evolution of *Daphnia* populations.

Pesticides

Pesticides are widely used human-made chemicals, trailing only fertilizers in terms of the extent and amount of use (Stokstad and Grullón 2013). Work on other systems shows that pesticide use

can strongly influence host-parasite interactions. Sub-lethal pesticide exposure has been shown to increase susceptibility of the European honeybee *Apis mellifera* to a gut pathogen, the fungus *Nosema spp*. (Pettis et al. 2013; Wu et al. 2012), increase the within-host density of the fungus (Pettis et al. 2012), and even increase the mortality rate of bees already infected with the pathogen (Vidau et al. 2011).

Research on *Daphnia* also shows that pesticides can alter the virulence of their parasites. The virulence of *Pasteuria* on *Daphnia magna* increased with increasing concentrations of the pesticide carbaryl, including higher levels of early mortality and earlier castration of infected hosts (Coors et al. 2008), even with just short term exposure (Coors and De Meester 2011). Notably, increased virulence was also seen with a second parasite, the microsporidian *Flabelliforma magnivora* (Coors et al. 2008). Thus, the presence of pesticides in lakes and ponds could alter the virulence of parasites, which should alter the nature of the transmission-mortality trade-off (and, thus, the evolution of virulence), as well as alter selection on *Daphnia* populations. In future research, it would also be interesting to focus on the impact of other anthropogenic pollutants, including road salts (Cañedo-Argüelles et al. 2019), on *Daphnia*-parasite interactions.

Conclusions

In the 70 years since Haldane (1949) suggested that parasites might be especially important drivers of evolution, it has become abundantly clear that parasitism is, indeed, a major selective force. Haldane contrasted the impacts of parasites with those of other "natural forces, hunger, and predators, or with members of the same species". However, we now know that populations are not influenced by parasites *or* by other food web members—rather, they all interact. Thus, when studying evolution in host-parasite interactions, we need to consider that the amount of

disease and the nature and tempo of evolution will be modulated by the biotic and abiotic context in which the host-parasite interaction is embedded.

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FIGURES

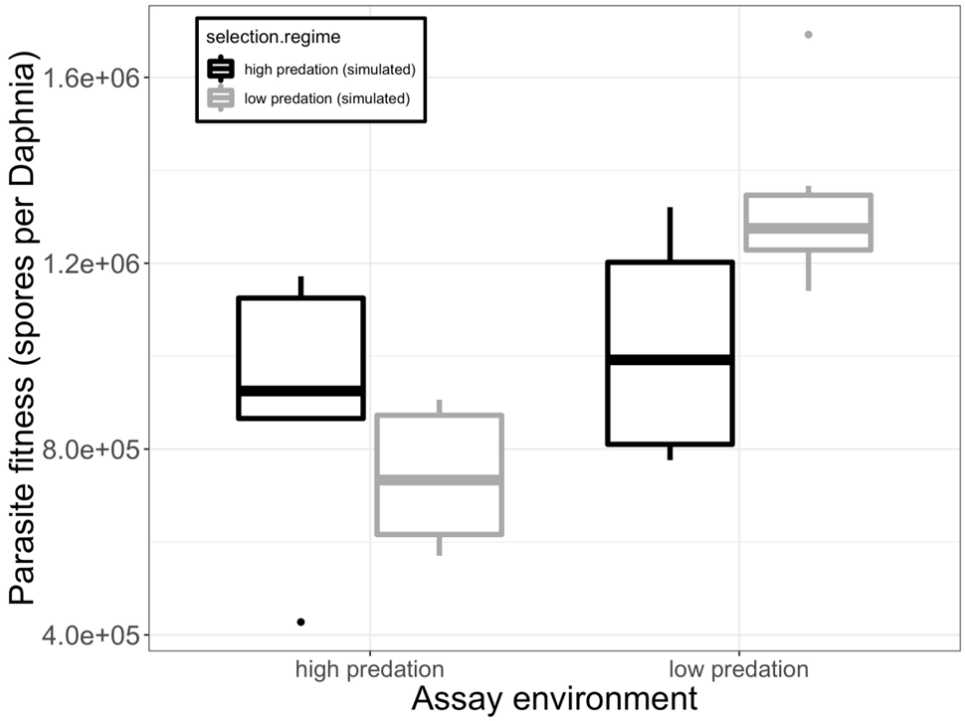

Figure 2.1. Parasite fitness in different predation environments. The bacterial parasite *Pasteuria ramosa* evolved higher fitness in high predation environments, but this came at the cost of lower fitness is low predation environments. *Pasteuria* was selected in environments that simulated high predation (shorter host life span; shown with black bars) or environments that simulated low predation (longer host life span; shown with gray bars). Parasite fitness was then assayed in two environments, one simulating high predation (shorter host lifespan) and one simulating low predation (longer host life span). High predation selection lines produced significantly more spores in high predation assay environments than did low predation selection lines when assayed in high predation environments (compare the gray and black bars on the left; planned contrast: $z = -3.07$, $p = 0.0021$). When assayed in low predation environments, however, low predation selection lines produced more spores (compare the gray and black bars on the right; planned contrast: $z = 2.70$, $p = 0.0070$). Data are replotted from Auld et al. (2014).

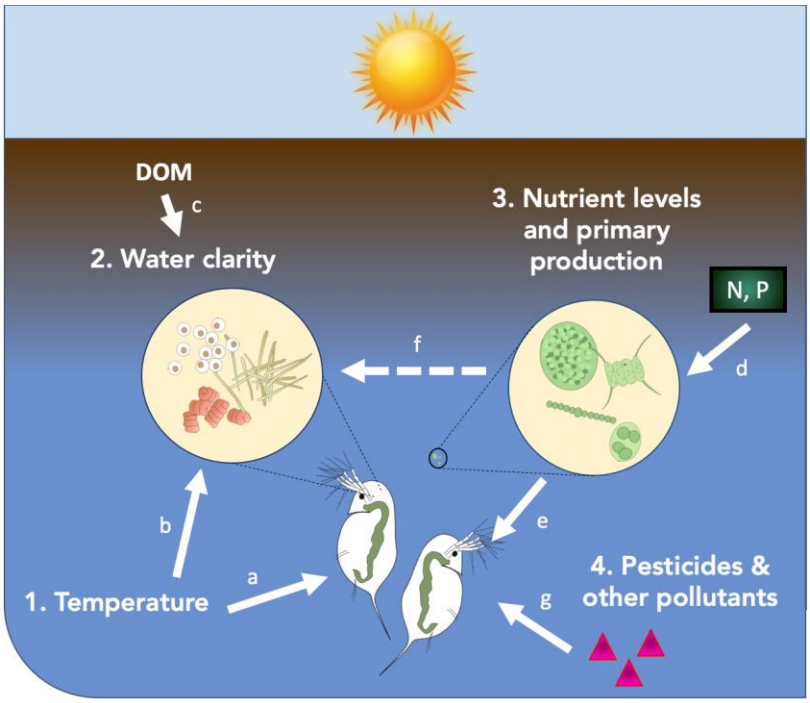

Figure 2.2. Visual abstract. Humans are dramatically altering the abiotic environment, with consequences for evolution of hosts and parasites. 1) Human activities are increasing average temperatures as well as the duration of temperature extremes; (a) this has direct effects on *Daphnia* (by impacting their feeding rate), and (b) parasites (by impacting their development rates). 2) Human activities are also altering precipitation regimes, which increases the amount of dissolved organic matter (DOM) arriving in lakes, making water darker and (c) potentially reducing degradation of parasites by sunlight. 3) Humans are also altering nutrient levels and therefore (d) primary production, which (e) changes *Daphnia* feeding rates (and therefore growth and infection rates). (f) The prevalence of primary producers indirectly affects parasites through changes to host feeding rates, plus the nutritional and medicinal quality of different phytoplankton mediate host resistance and tolerance. Finally, 4) agricultural practices and other human activities are adding pesticides and other novel chemicals to the environment, which (g)

can impact the wellbeing of hosts by reducing their tolerance to pathogens. All of those changes should have impacts on host-parasite interactions, as discussed in the main text.

CHAPTER 3:

Sexual recombination and temporal gene flow maintain host resistance and genetic diversity[3,4]

ABSTRACT

Infectious disease can threaten host populations. Hosts can rapidly evolve resistance during epidemics, with this evolution often modulated by fitness trade-offs (e.g., between resistance and fecundity). However, many organisms switch between asexual and sexual reproduction, and this shift in reproductive strategy can also alter how resistance in host populations persists through time. Recombination can shuffle alleles selected for during an asexual phase, uncoupling the combinations of alleles that facilitated resistance to parasites and altering the distribution of resistance phenotypes in populations. Furthermore, in host species that produce diapausing propagules (e.g., seeds, spores, or resting eggs) after sex, accumulation of propagules into and gene flow out of a germ bank introduce allele combinations from past populations. Thus, recombination and gene flow might shift populations away from the trait distribution reached after selection by parasites. To understand how recombination and gene flow alter host population resistance, we tracked the genotypic diversity and resistance distributions of two wild populations of cyclical parthenogens. In one population, resistance and genetic diversity increased after recombination whereas, in the other, recombination did not shift already high resistance and genetic diversity. In both lakes, resistance remained high after temporal gene flow. This observation surprised us: due to costs to resistance imposed by a fecundity-resistance trade-off, we expected that high population resistance would be a transient state that would be eroded through time by recombination and gene flow. Instead, low resistance was the transient state, while recombination and gene flow re-established or maintained high resistance to this virulent

[3] McLean, K.D., Gowler, C.D., Dziuba, M.K. *et al.* Sexual Recombination and Temporal Gene Flow Maintain Host Resistance and Genetic Diversity. *Evol Ecol* (2022). https://doi.org/10.1007/s10682-022-10193-6

[4] Coauthors are: Gowler, C.D., Dziuba, M.K., Zamani, H., Hall, S.R., Duffy, M.A.

parasite. We propose this outcome may have been driven by the joint influence of fitness trade-offs, genetic slippage after recombination, and temporal gene flow via the egg bank.

INTRODUCTION

Epidemics threaten many host populations — for organisms as different as blue whales and bacteria, virulent infectious disease can drive population decline and, in some cases, extirpation or extinction (Smith et al., 2006; Warner, 1968; Wyatt et al., 2008). Fortunately, most species can evolve resistance if their populations contain enough standing genetic variation (Betts et al., 2016; Bonneaud et al., 2011; Duffy & Hall, 2008; Duffy & Sivars-Becker, 2007; Laine, 2006). Resistance can evolve on ecological timescales, potentially protecting host populations from some of the harms of virulent parasites (Betts et al., 2016; Duncan & Little, 2007; Edeline et al., 2008; Lohse et al., 2006; Penczykowski et al., 2011; Strauss et al., 2017).

Rapid evolution of host resistance has drawn scientific interest in the past, but most perspectives focus on resistance evolution within a growing season (Betts et al., 2016; Duncan & Little, 2007; Edeline et al., 2008; Lohse et al., 2006; Penczykowski et al., 2011; Strauss et al., 2017) or across a span of multiple years to decades (Bonneaud et al., 2011; Dybdahl & Lively, 1998; Gandon et al., 2008). Relatively little attention has been paid to how population resistance translates across an annual cycle of dormancy and growth (though see Frickel et al., 2018). Yet, this scenario applies to organisms across the tree of life that engage in cyclical extinction-repopulation dynamics. Adaptation in these populations is modulated not only by evolution within a season, but also by annual genetic and phenotypic changes that occur during the (often sexual) production of dormant stages, and during temporal gene flow (i.e., recolonization) from the germ bank (Decaestecker et al., 2009; Gyllström & Hansson, 2004). We need to broaden our understanding of how germ banks alter rapid evolutionary dynamics if we want to predict if resistance evolution will provide lasting protection in seasonal host-parasite systems.

Recombination and gene flow are two mechanisms that can maintain the genetic variation necessary for natural selection, but they can also act in opposition to rapid local adaptation (Forde et al., 2007; García-Ramos & Kirkpatrick, 1997; Hendry et al., 2001; Lenormand, 2002). Recombination can lead to genetic slippage, wherein the population mean for a phenotype under selection will shift away from a new population optimum, and instead restore a previous phenotype distribution with lower mean fitness (Lynch & Deng, 1994). Furthermore, cyclical sexual reproduction (i.e., cyclical parthenogenesis) is frequently associated with the formation of diapausing propagules (Decaestecker et al., 2009; Gyllström & Hansson, 2004). These propagules can persist through harsh environmental conditions and, due to variable hatching rates, collect over time to create a genetic archive (or germ bank) of past populations (e.g., Cáceres, 1997; Cohen, 1966; Jones & Lennon, 2010; Locey et al., 2016; Warr et al., 1993). Gene flow out of the germ bank reintroduces allele combinations from past populations; this reintroduction can oppose recent adaptation. Therefore, a population that rapidly evolves resistance to a virulent parasite during an asexual phase may then experience a "reversal" to a more susceptible state due to sexual recombination and/or temporal gene flow from the germ bank.

Do these mechanisms – recombination and temporal gene flow – impact resistance in wild host populations? To explore this question, we sampled two populations of germ banking cyclical parthenogens at the end of one active season and the beginning of the next. We disentangled the effects of recombination and gene flow on host resistance in two phases. First, we compared the resistance phenotypes and the genotypes of asexually produced animals to their sexually produced offspring. Then, we compared resistance phenotypes and genotypes of animals hatched from sexually produced diapausing propagules (which entered the egg bank in the fall) to animals from the following spring (likely repopulated from the egg bank). Due to fitness (fecundity) costs associated with resistance in this system, we expected host populations to be susceptible to the pathogen unless a recent epidemic had selected for increased resistance. Then, if a population had high mean resistance in the fall, we expected this resistance to decrease after

sexual recombination due to genetic slippage toward the pre-epidemic (lower) mean resistance. As for the egg bank effect, if there was no temporal gene flow (i.e., no gene flow from resting eggs created in previous years), then all springtime animals will have hatched from resting eggs produced the previous fall. Therefore, the null expectation would be the resistance of the egg bank population would match that of the sexually produced offspring collected the previous fall. We expected our populations to deviate from this null hypothesis and show evidence of temporal gene flow. However, we acknowledge that these predictions are based on scenarios where only the listed factors operate; natural ecosystems are far more complex. A variety of factors, most notably predation regimes, have the potential to lead to more complicated dynamics, as we discuss below. Overall, this study helps us understand how two common phenomena — sexual recombination and gene flow from the egg bank — combine to impact host resistance across years in cyclically parthenogenetic germ banking species.

STUDY SYSTEM

The host, a facultatively sexual parthenogen: Daphnia is a genus of freshwater planktonic crustacean that hosts multiple parasites. Its short generation times and ability to reproduce asexually make it a particularly tractable study system (Ebert, 2005; Stollewerk, 2010), and allow for rapid evolution through clone competition. Our study focused on *Daphnia dentifera,* a daphniid commonly found in freshwater lakes across the midwestern United States (Tessier & Woodruff, 2002). *D. dentifera* is cyclically parthenogenetic; animals hatch from the resting egg bank in spring then reproduce asexually (clonally). During fall, female *D. dentifera* produce male offspring and switch to sexual production of resting eggs (Gowler et al., 2021). After the release of resting eggs, the active population dies off for winter.

The parasite and fitness components: This host species can suffer epidemics of *Metschnikowia bicuspidata* (hereafter: *Metschnikowia*), a virulent fungal pathogen of *D. dentifera* that reduces

host lifespan up to 50% (Clay et al., 2019) and reduces host fecundity by approximately 25% (Auld et al., 2012; Duffy & Hall, 2008). Fish also selectively prey on *D. dentifera* infected by *Metschnikowia*, further increasing their mortality rate (Duffy & Hall, 2008). Infection occurs when the host consumes fungal spores while filter-feeding in the water column. The spores pierce the host's gut wall and enter the hemolymph, where they enter a rapid growth phase and fill the host's body cavity with transmission stages (Metschnikoff, 1884; Stewart Merrill & Cáceres, 2018). Upon host death, spores enter the water column (Ebert et al., 2000).

Resistance and trade-offs: Resistance to *Metschnikowia* is highly variable in *D. dentifera* (Auld et al., 2013; Duffy & Sivars-Becker, 2007). Body size, feeding rate, and gut thickness in *D. dentifera* correlate with resistance to *Metschnikowia* (Hall et al., 2010; Stewart Merrill et al., 2021). Additionally, body size, feeding rate, and fecundity are positively correlated in *D. dentifera* (Burns, 1969; Hall et al., 2010, 2012). Hence, fecundity in *D. dentifera* trades off against resistance to *Metschnikowia*. This fitness trade-off may help explain complex evolutionary outcomes in this host-parasite system, such as disruptive selection for either very high or very low resistance (Duffy et al., 2008). It also raises the possibility that predation regimes might influence resistance, as visual predators such as fish select for smaller body sizes (Galbraith, 1967; Kitchell & Kitchell, 1980; Wells, 1970) while gape-limited *Chaoborus* larvae select for larger body sizes (Pastorok, 1981; Spitze, 1991). Finally, we know fungal epidemics can spur a rapid shift in host resistance within a single active (primarily asexual) season for *D. dentifera* (Duffy et al., 2008, 2009, 2012; Duffy & Hall, 2008; Duffy & Sivars-Becker, 2007). However, little is known about how such evolutionary shifts in host resistance translate from one active season to the next.

METHODS

Field Sampling

We collected *Daphnia* from two lakes, Midland and Hackberry Lakes, late in the year (December 2015) and in the following spring (April/May 2016). Midland and Hackberry Lakes are both dimictic lakes located in Greene County, Indiana, USA. These lakes were used because *Metschnikowia bicuspidata* is a common parasite of *D. dentifera* in Greene County, Indiana, with prevalence as high as 60% (Shaw et al., 2020). In 2015, Hackberry Lake had very few late-stage infections (annual maximum prevalence = 0.05%), while Midland Lake had a moderate epidemic (annual maximum prevalence = 17%; S.R. Hall, *unpublished data*).

In December 2015, we collected uninfected female *D. dentifera* bearing ephippia (i.e., sexually produced resting eggs) from the two study lakes. Each subsample was taken from a single whole-water column vertical net tow. Since parasitized animals do not spatially segregate (Hall et al., 2005), this tow should provide a representative sample of the resistance trait among sexually reproducing individuals. However, if clones vary in their propensity to reproduce sexually (as in *D. pulicaria;* Cáceres & Tessier, 2004a, Cáceres & Tessier, 2004b), our sample of sexually producing females might have captured genotypes that invest more in sexual reproduction. Ephippial females ('parents') released their ephippia in the laboratory. We hatched the ephippia, then maintained cultures of those offspring in standardized conditions (6 individuals/30 mL water, 20°C, 16:8 light/dark cycle, fed 10^6 cells/mL *Ankistrodesmus falcatus* 4 times weekly). Those conditions maintained isofemale (clonal) lines of each parent and their offspring asexually. The following spring (i.e., in April and May 2016), we sampled active populations (assumed to be newly hatched from the egg bank) with a single whole-water column tow. We then maintained clonal lines of these animals using the same methods. This yielded three sets of clones per lake — 'parents', 'offspring', and 'egg bank' — all maintained under standardized conditions for resistance (phenotypic) assays and genotyping.

Resistance assays

Infection assays followed standard protocols (Duffy & Sivars-Becker, 2007), with the experiment split into 4 blocks due to logistical constraints. Animals were maintained in

standardized conditions to standardize maternal effects. Additionally, the third (or later) clutches were used to propagate the next generation. We maintained this procedure for at least three generations until we accumulated 40 or more individuals of the same age for each clonal line.

For each clonal line, five *Daphnia* (6-8 days old) were distributed to each of eight 150 mL beakers (= 40 animals per clone) with 100 mL of filtered lake water. Each beaker received 1 mL of $1x10^5$ cells/mL of *Ankistrodesmus falcatus* phytoplankton as a food source. This ration encouraged spore uptake during exposure to 250 cells/mL of *Metschnikowia* for 24 hours. The *Metschnikowia* spores were from the "Standard" isolate from Baker Lake (Barry County, MI, USA). Resistance to *Metschnikowia* is a quantitative trait, and there is no evidence for host-parasite genotype specificity (Duffy & Sivars-Becker, 2007), so observations from this one fungal strain should generalize to the *Metschnikowia* species. Exposures were ended by transferring animals to new beakers with 100 mL of spore-free filtered lake water. Animals were fed 2 mL of $1x10^5$ cells/mL of *Ankistrodesmus falcatus* 4 times per week and kept at 20°C and on a 16:8 light/dark cycle throughout the experiment with weekly transfer to fresh water. We examined animals for visible terminal infection 11-12 days after exposure, a time point when infected hosts show symptoms but have not yet died from infection (Auld et al., 2012).

Genotyping

We also characterized the multilocus genotype of each clone to track shifts in genotypic diversity. We genotyped clones used in the assays for three reasons. First, since the host reproduces asexually through much of the year (Gowler et al., 2021), multiple isolated individuals might have belonged to the same genotype. Second, populations might shift genetically between the three periods without changes in the resistance phenotype. Third, genetic identities were needed to evaluate recombination, gene flow, and population genetic structure. Hence, we genotyped each clonal line collected.

We used six microsatellites from eight previously published sets of primers according to their map position, ease of scoring and allelic diversity (primers: Dgm105, Dgm106, Dgm107, Dgm109, Dgm112, Dgm113; Table S3.1; based on Fox, 2004). However, Dgm 107 did not provide us with any detectable peaks, leaving us with five microsatellite loci. Each locus was assigned one of four different fluorescent labels (6FAM; MAX; ATTO; ROX, Integrated DNA Technologies) in such a manner that no two markers with the same fluorescent dye had overlapping allele size ranges. We extracted DNA from a single uninfected animal from each clonal line using the standard protocol included in the DNeasy Blood & Tissue Kit (Qiagen).

Polymerase chain reaction (PCR) amplifications were performed in 96 well plates (one reaction per well) using QIAGEN® Multiplex PCR kit. PCR reactions were carried out in a final volume of 50 µL with 25 µL of 2x Qiagen multiplex mastermix (QIAGEN, Hilden, Germany), 0.2 µM of each forward and reverse primer pair (for a final volume of 1.2 µM), and <1 µg of DNA, with the remaining difference in volume made up by RNase-free water. Amplification conditions were: 95°C (15 min), then 35 cycles of 94°C (30 s) / 58°C (3 min) / 72°C (1:30 min), and a final extension at 72°C for 10 min. For genotyping, 1 µl of diluted (1:200) PCR products were added into capillary electrophoresis loading plates containing 11 µl Hi-Di formamide and a LIZ500 size standard. Fragment analysis was performed by the University of Michigan DNA sequencing core, and fragment lengths were read using GeneMapper (ThermoFisher Scientific).

We wanted to see if shifts in population genetics reflected the shifts in population resistance and also wished to match genotype and phenotype data for each clone. To quantify the impact of recombination on genetic variation, we compared the genotypic evenness and diversity of parents with their sexually recombinant offspring. Similarly, we quantified how temporal gene flow impacted genetic variation in a lake by comparing genotypic evenness and diversity of that lake's sexually recombinant offspring to the corresponding spring egg bank population. We used the *poppr* package (version 2.0.2) in R (version 4.0.0) to measure genotypic richness (number of multilocus genotypes (MLGs)) and genotypic diversity using three indices: Shannon-Wiener index (H), Stoddart and Taylors (G), and Simpson (λ). Using all three indices allowed us to look

for shifts in population diversity across multiple metrics (Fig 3.1). We also quantified MLG evenness to help detect dominant genotypes and used clone-corrected data to calculate the index of association (I_A, a measure of linkage disequilibrium; Table S3.2) for each group.

Due to logistical constraints, we could only assay a fraction of each population for the resistance trait. Moreover, due to a lab accident, we lost six isofemale lines that had been used in the resistance assays before genotyping. Therefore, we could not assign a resistance phenotype to every genotype.

Statistical analysis

For the main statistical analysis, resistance was calculated as the proportion of uninfected animals for each *Daphnia* clonal line. To measure the effect of sexual recombination on the resistance phenotype, we compared resistance to *Metschnikowia* between parents and their sexually produced offspring by modeling the number of uninfected hosts per beaker using a binomial GLMM fit by maximum likelihood (LaPlace approximation) with parent vs. offspring as a fixed effect using the lme4 package (v1.1-26; (Bates et al., 2014)). To incorporate the dependency among observations from the same clonal line and between parent-offspring pairs, we included 'clone' (i.e., whether individuals were the same multilocus genotype) and 'family' (i.e., whether the parent had produced the particular offspring) as random effects.

To measure the effect of temporal gene flow on the resistance phenotype, we analyzed the resistance of sexually produced offspring vs. egg bank individuals. If there was no temporal gene flow from resting eggs, then we expected that all springtime animals had hatched from resting eggs produced during the previous fall. Therefore, our null expectation was that the resistance of the egg bank population would match that of the sexually produced offspring collected the previous fall; we tested this statistically with a binomial GLMM with number of uninfected hosts per beaker as the response variable with time point ('offspring' vs. 'eggbank') as a fixed effect and 'clone' as a random effect.

Due to logistical constraints, we could not run all resistance assays simultaneously. Hence, we prioritized grouping based on the comparisons of interest. For example, since we were not interested in comparing between lakes, we ran clones from the different lakes in different blocks. Consequently, we only directly compared groups exposed within the same block. The one exception is for Hackberry, where we compared resistance in Hackberry offspring to Hackberry egg bank animals using data from two exposure blocks after confirming no block effects existed.

Finally, we calculated narrow-sense heritability for each lake population. To calculate it, we regressed mean offspring resistance vs. mean parent resistance, where h^2 is twice the slope of the regression (Falconer, 1981). If a parent had produced two offspring we used the mean resistance scores of both offspring. Because this analysis required that we have estimates of heritability for both the parent and at least one offspring, there are fewer parent (and offspring) individuals included in this analysis than in the other analyses.

RESULTS

Impact of sexual reproduction on mean resistance and genetic diversity

Mean resistance of the population increased after sexual recombination in Hackberry Lake (from 0.47 in parents to 0.62 in offspring; z = -2.0, p = 0.046; Fig 3.1A). Genotypic diversity also increased; the moderate genotypic diversity of parents (Simpson index = 0.72, 95% CI: 0.54 - 0.90; Fig 3.1E) increased significantly in their sexually produced offspring (Simpson index = 0.93, 95% CI: 0.89 - 0.96; Fig 3.1E; changes were qualitatively the same for the Shannon-Wiener and Stoddart & Taylors indices; Table S2). Notably, one relatively susceptible and dominant (53%) genotype drove both lower population-level resistance and genotypic diversity in Hackberry parents (MLG.50; mean resistance = 0.36; Figs 3.2 and S3.1). This same genotype was also found in sexually produced offspring, though at a lower frequency (14%). In most cases

(19 out of 26), offspring had a different multilocus genotype than their parent; in seven cases, a parent and its offspring shared identical MLGs. Narrow-sense heritability (h^2) of resistance was 0.52 in the Hackberry population (Fig 3.3).

In contrast, sexual recombination had no effect on the mean resistance in Midland Lake. This population had relatively high resistance in the fall 'parent' population (0.74) and this did not change for 'offspring' (i.e., after sexual recombination; 0.72; z = 0.26, p = 0.79; Fig 3.1C). Similarly, genotypic diversity of this population was already high in parents and did not significantly change after sexual recombination (Simpson index: parents = 0.96, 95% CI: 0.93 - 0.98; offspring = 0.96, 95% CI: 0.94 - 0.98; Fig 3.1F; again, all three diversity metrics had qualitatively consistent results). Furthermore, in Midland Lake, no genotype obviously dominated after sexual recombination, as 24 out of 30 offspring had unique MLGs (Fig. S3.1). Narrow-sense heritability (h^2) of resistance was 0.33; this lower heritability in Midland relative to Hackberry was likely due to less variation in resistance in Midland (Fig 3.3).

Mean resistance and genetic diversity after hatching from the egg bank

The Hackberry Lake population maintained high resistance after gene flow from the egg bank (Fig 3.1B): mean resistance in fall offspring (0.63) did not differ from that of spring egg bank clones (0.71; z = -0.02, p = 0.98). However, genotypic diversity increased between fall offspring and the spring egg bank clones (Fig 3.1E). While this increase was not statistically significant for the Simpson index (fall offspring = 0.93, 95% CI: 0.89 - 0.96; spring egg bank = 0.96, 95% CI: 0.94 - 0.98), this index was near its upper bound, and the increase was significant for the other two diversity indices (Shannon-Wiener: fall offspring = 2.81, 95% CI: 2.56 - 3.06; spring egg bank = 3.41, 95% CI: 3.21 - 3.60; Stoddart and Taylors: fall offspring = 13.76, 95% CI: 10.48 - 17.05; spring egg bank = 23.56, 95% CI: 18.41 - 28.78). The Hackberry parent population also stood out for having unusually high linkage disequilibrium, as quantified by the index of association (I_A = 0.54 for Hackberry parents *vs.* <0.16 for all other lake-time point combinations;

Table S3.2); however, we note that the relatively small number of loci in our study means I_A should be interpreted with caution.

In Midland Lake, the population also maintained high resistance after gene flow from the egg bank (Fig 3.1D), similar to Hackberry Lake. However, in contrast, genotypic diversity in Midland Lake did not increase for egg bank clones (Fig 3.1F); instead, the genotypic diversity of the egg bank clones was the same as (Simpson: fall offspring = 0.96, 95% CI: 0.94 - 0.98; spring egg bank = 0.91, 95% CI: 0.85 - 0.96; Shannon-Wiener: fall offspring = 3.26, 95% CI: 3.05 - 3.48; spring egg bank = 2.87, 95% CI: 2.56 - 3.19) or lower than (Stoddart and Taylors: fall offspring = 25.00, 95% CI: 21.37 - 28.63; spring egg bank = 10.77, 95% CI: 6.25 - 15.28) that of the fall offspring.

DISCUSSION

How do recombination and gene flow impact population resistance across a seasonal cycle of extinction and recolonization? In our system, resistance and fecundity trade off as a result of their joint relationships with host feeding rate (Hall et al., 2010; though this does not happen in all populations: Auld et al., 2013). Given this trade-off, we expected populations would evolve toward higher susceptibility (due to its fecundity advantages) unless an epidemic had recently selected for resistance. If an epidemic did occur, we expected resistance to increase temporarily. Then, due to sexual recombination and temporal gene flow, we expected the population to shift back towards the recent susceptible state. Contrary to our expectations, susceptibility was the transient state, with recombination and gene flow restoring and/or maintaining high resistance. Moreover, we expected that fall offspring would show greater genotypic diversity than their parents due to the effects of sexual recombination; this was observed in Hackberry Lake but not in Midland, where genotypic diversity of parents was already very high. We further expected that the eggbank clones would have higher diversity than the fall offspring, since we anticipated

hatching of individuals produced across multiple years; again, this was observed in Hackberry but not in Midland. Given that logistical constraints rendered it impossible to quantify selection (due to parasitism and/or other selective forces) throughout the season, we cannot tell whether the differences in Hackberry vs. Midland are driven by the difference in infection prevalence in those two lakes. However, our cross-season study confirms that sexual recombination and temporal gene flow are both important players in determining inter-annual variation in host resistance in this study system, and that this area of inquiry warrants further study.

Our data might indicate stronger selection in Hackberry Lake (which did not have an epidemic) than in Midland. *D. dentifera* collected during the fall in Hackberry had the lowest resistance, lowest genotypic diversity, and the highest index of association of either population at any sampling time point in the study. Together, these results suggest recent selection favoring increased susceptibility in this population. In contrast, Midland Lake, which had an epidemic with an annual maximum prevalence of 17%, had high genetic diversity and low linkage disequilibrium (Table S2). Low linkage disequilibrium suggests random genetic shuffling, while high linkage disequilibrium can be a sign of strong selection, very high clonal reproduction, and/or genetic drift (Slatkin, 2008). Genetic drift seems unlikely in these very large populations, leaving high clonal reproduction and strong selection as possible explanations. Both are possible.

Both Midland and Hackberry had high clonal reproduction during summer and into fall (S.R. Hall, unpubl. data). When *Daphnia* shift from asexual to sexual reproduction, males first appear in the population, followed by ephippial (sexual) females. In 2015, males were not observed in these populations until October, and sexual females were not observed until the end of October in Hackberry and beginning of November in Midland (S.R. Hall, unpubl. data). Midland Lake invested more heavily in sexual reproduction, with 40% of the population being males or ephippial females in November *vs*. 21% in Hackberry. Together, this suggests that both populations had high levels of clonal reproduction, but that the impact of this in Midland may have been somewhat mitigated by a greater shift to sexual reproduction. However, given that the first ephippial females appeared in the population right around when we collected ephippial

females for this study, it is highly unlikely that any of the 'parents' that we collected for this study were the result of sexual recombination during 2015, as there would not have been enough time for those ephippia to be produced, released, hatch, and for the individual to reach adulthood prior to us collecting our samples. Thus, it is likely that the strength of selection differed between these two lakes.

Why would selection be stronger in the lake that did *not* experience an epidemic of a highly virulent parasite? While prior work has focused particularly on a resistance-fecundity trade-off, myriad other factors could influence the selective environment. Indeed, the genotype data suggests this was the case. In Hackberry, one highly susceptible genotype, MLG.50, dominated in the fall population while other susceptible genotypes that were present remained rare. Perhaps, then, not all susceptible genotypes enjoyed fitness advantages in this population. Overall, multiple relationships link traits such as resistance, fecundity, predation, and resource acquisition with body size, with selective pressures shifting throughout the active season. For example, if faster-feeding genotypes are more susceptible, then they are at a disadvantage when food quality is low. This scenario could arise because *D. dentifera* experience trade-offs in their ability to exploit high- versus low-quality food (Hall et al., 2012), and food quality changes throughout the summer and fall (Hall et al., 2009). Another possible mechanism is predation by invertebrate and vertebrate predators, which also correlates with body size of *D. dentifera* (Strauss et al., 2016) and can therefore indirectly select on host resistance. These mechanisms are at play in other host-parasite systems, as well. Resistance generally comes with a cost to fitness (Roy & Kirchner, 2000; Simms & Triplett, 1994) and trade-offs between resistance and fecundity, longevity, and rate of maturation are found across a diversity of hosts (Buckling & Brockhurst, 2012; Gwynn et al., 2005; Kraaijeveld et al., 2002; Langand et al., 1998). Additionally, ecological interactions such as predation and mate selection mediate the strength of these trade-offs in these other systems (Clayton et al., 2015; Møller, 2008; Toor & Best, 2015), similar to *Daphnia*. Ultimately, understanding the drivers of resistance evolution in any host-parasite system will require understanding the impacts of multiple selective agents and ecological processes.

Alternatively, it's possible that the pattern actually reflects parasite mediated selection in the lake that experienced an epidemic, Midland. Prior to the parasite outbreak, the selection pressures in Midland may have been similar to those in Hackberry, which would have favored a relatively susceptible genotype such as MLG.50. Once the epidemic began, we would expect selection against susceptible genotypes, which would lead to a 'parent' population with high mean resistance but that still had relatively high diversity (that is, a scenario that looks like the Hackberry parents in Figure S3.1, except missing the one highly dominant, susceptible genotype). Distinguishing between these two scenarios (stronger selection in Hackberry *vs*. Midland) will require future studies that monitor changes in genetic composition more frequently while also tracking ecological dynamics.

If mating was random, why did sexual recombination increase resistance in Hackberry? One possible explanation involves genetic slippage. Depending on the mode of gene action and the selection function, the action of segregation and recombination can cause the mean phenotypic value of a population to move in a direction contrary to selection (Ameline et al., 2021; Lynch & Deng, 1994). This has been seen in *Daphnia pulicaria* as well as facultatively sexual rotifers (Becks & Agrawal, 2012), *Chlamydomonas* (Kaltz & Bell, 2002), and yeast (Goddard et al., 2005). Non-random mating may also play a role, as noted in past work (Duffy et al., 2008), though no studies have directly detected assortative or otherwise non-random mating in *Daphnia*. Chemical signals or differences in habitat use could increase the likelihood of non-random mating, which could shift the trait distribution of the population (in a direction dependent on whether similar or dissimilar animals mate more frequently). Such non-random mating has been described in a wide range of taxa and has significant ecological and evolutionary consequences (Crespi, 1989; Janicke et al., 2019; Jiang et al., 2013).

Temporal gene flow out of the diapausing egg bank increased genetic diversity in Hackberry but, if anything, decreased it in Midland. In both cases, we hypothesize that this was due to the hatching of genotypes that had been produced in previous years. Two lines of evidence support this claim. First, in Hackberry Lake, the susceptible genotype MLG.50 hatched out of the egg

bank at a different frequency than it was deposited into it (Fig S3.1), resulting in a more diverse (if equally resistant) population. We hypothesize that the novel resistant clones that hatched from the egg bank may have been produced during previous years with large epidemics; unfortunately, no long-term monitoring data exists for Hackberry Lake so we cannot test this hypothesis with existing data. Second, during the 'egg bank' phase a new clone became common in Midland (MLG.57, which made up 12 out of 48 (25%) of Midland egg bank clones; Figs 3.2 and S3.1). This clone was not present in either the 'parent' or 'offspring' samples, suggesting it emerged from the egg bank after having been deposited in years past. This clone had moderate resistance. An additional possibility for the Midland Lake result, however, is that the dominance of MLG.57 in the spring 'egg bank' clones may have resulted not from it being dominant in the egg bank but, rather, from it *not* investing in sexual reproduction. In other species of *Daphnia*, some genotypes invest less in sexual reproduction (Spaak 1995; Zeis et al. 2010; Tessier and Caceres 2004), instead maintaining populations in the water column even through unfavorable conditions – this strategy is similar to a plant that invests in vegetative growth rather than producing seeds. While we have not found *D. dentifera* under the ice, our winter sampling has been quite limited. It would be interesting to better assess whether some *D. dentifera* individuals persist in the water column through winter and, if so, if genotypes vary in their propensity to do so.

In conclusion, our findings highlight the importance of recombination and germ banks in maintaining genetic diversity in asexual or cyclically parthenogenetic organisms. Furthermore, we found that both factors likely underpin interannual dynamics of resistance in germ banking organisms. Future studies monitoring the genotypic and phenotypic values of populations across multiple sequential (seasonal) extinction-recolonization events, while also tracking epidemiological dynamics, would help determine the generality of our findings while better connecting rapid interannual selection dynamics with longer-term evolution.

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FIGURES

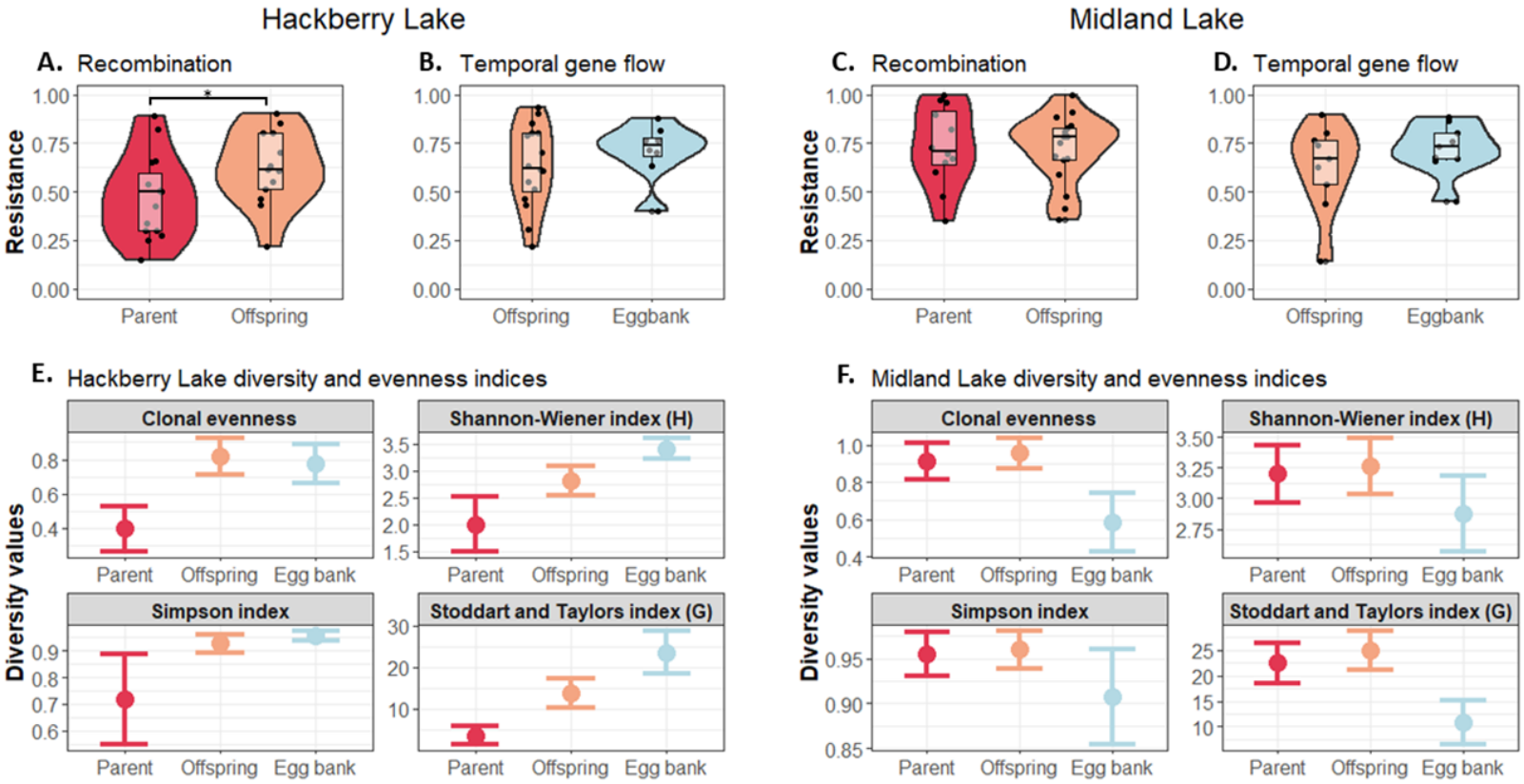
Hackberry Lake
Midland Lake
A. Recombination
B. Temporal gene flow
C. Recombination
D. Temporal gene flow
Resistance
1.00
0.75
0.50
0.25
0.00
*
Parent
Offspring
Eggbank
E. Hackberry Lake diversity and evenness indices
F. Midland Lake diversity and evenness indices
Diversity values
Clonal evenness
Shannon-Wiener index (H)
Simpson index
Stoddart and Taylors index (G)
Egg bank

Figure 3.1. Resistance and genotypic diversity measures. Resistance and genotypic diversity significantly increased after recombination occurred in Hackberry Lake, but remained constant in Midland Lake. Fig 3.1A and 3.1C show mean resistance of isofemale lines collected at two time points: ephippial females in December 2015 ('Parent') and offspring hatched from those ephippia ('Offspring'). Fig 3.1B and 3.1D show mean resistance of offspring hatched from ephippia produced in December 2015 ('Offspring') and individuals collected in Spring 2016 after the active population was refounded from the egg bank ('Egg bank'). Phenotype comparisons are paired by experimental blocks (one block shown in A, another in B, etc.), so resistance in parents and egg bank animals cannot be directly compared. The violin plot outlines illustrate kernel probability density, i.e., the width of the shaded area represents the proportion of the data located there. Fig 3.1E and 3.1F show the observed diversity measures and bootstrapped 95% confidence intervals. The bootstrapped estimates often skew from the observed measures, and confidence intervals were centered around the observed diversity measures as recommended by Grünwald et al., 2017.

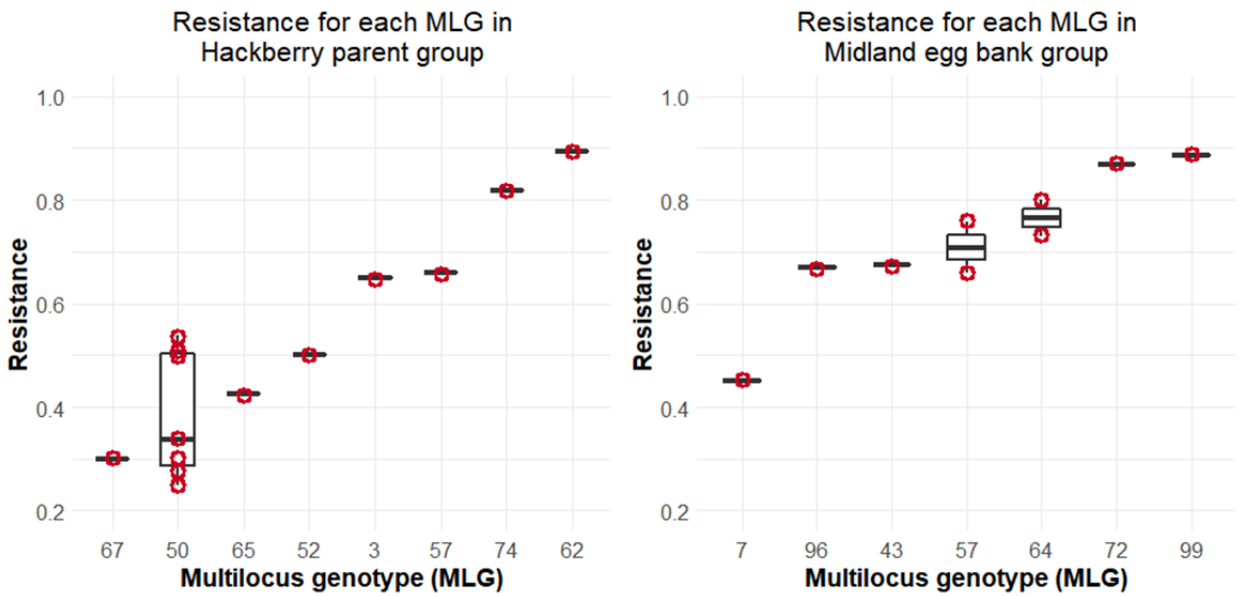

Figure 3.2. Resistance phenotypes of multiple multilocus genotypes. Multilocus genotype 50 (i.e., MLG.50, left plot) was the most prevalent genotype in the Hackberry parent lake-group. It was also on average less resistant to infection by *Metschnikowia bicuspidata* compared to the majority of other, co-existing genotypes. Multilocus genotype 57 (i.e., MLG.57, right plot) was the most prevalent genotype in the Midland egg bank lake-group even though it was not detected in the fall prior. This is evidence of temporal gene flow, i.e., that resting eggs produced during earlier years help recolonize lakes in the spring. MLG.57 does not have an extremely susceptible or resistant phenotype compared to other, coexisting genotypes.

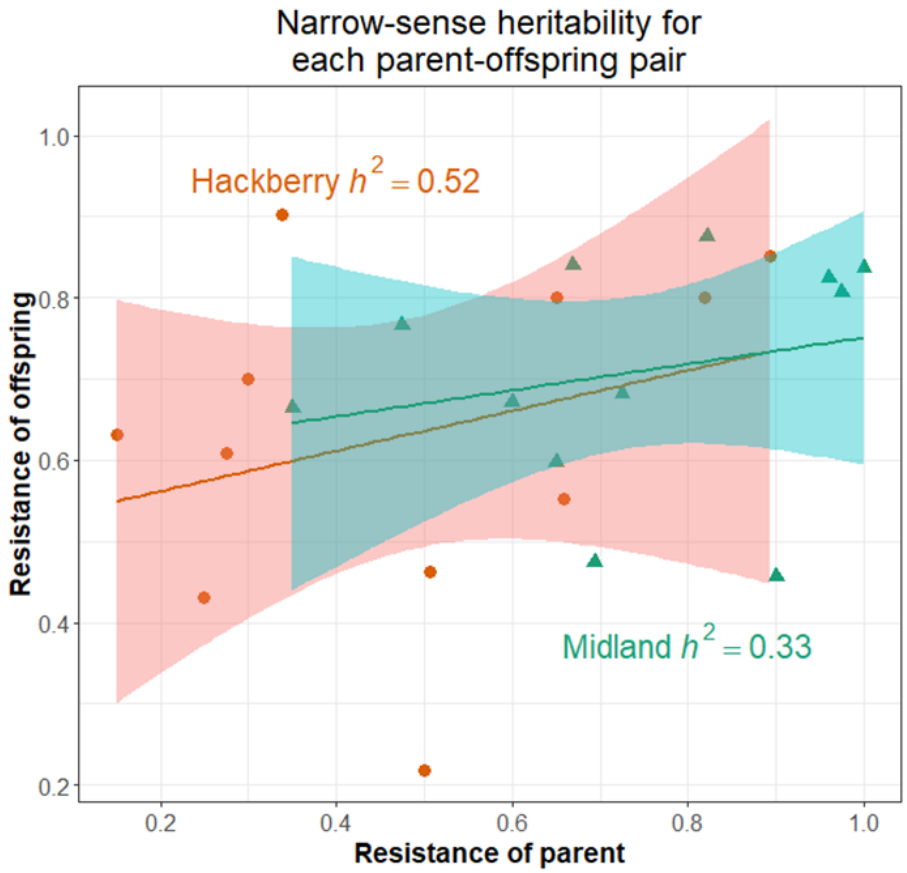

Figure 3.3. Heritability of resistance in two populations. Both populations showed moderate heritability of resistance (h^2); the Hackberry Lake population (orange) had an h^2 of approximately 0.52, while the Midland Lake population (green) had h^2 of approximately 0.33. Midland scored lower due to low variance (i.e., high similarity) in resistance for both parents and offspring of the population. Narrow-sense heritability for both lake populations was found by doubling the slope of the linear regression of parent vs. offspring resistance.

SUPPLEMENTAL TABLES AND FIGURES

Table S3.1. Multi-locus genotype (MLG) scores and corrections. Variable peak readings were corrected by rounding to the nearest likely value based on relative peak placement combined with the number of nucleotides in and expected length of the microsatellites.

Locus	Original peak(s)	Final peak
Dgm 105	182	181
	184	184
	186, 187, 188	187
	190	190
	124	124

Dgm 106	**127**	**127**
	130	**130**
	132, 133	**133**
	136, 137	**136**
Dgm 109	**243**	**243**
	248, 249	**249**
	251, 252	**252**
	256	**255**
	259	**258**

Dgm 112	**109,110**	**109**
	111,112	**112**
Dgm 113	**146, 147, 148**	**147**
	152, 153, 154	**153**
	156	**156**

Table S3.2. Genotypic data supports the hypothesis that the Hackberry parents group underwent a recent selection event. This group has the lowest genotypic diversity and evenness scores out of all Lake-groups, as well as a high index of association (I_a; a measure of linkage disequilibrium). Lake/group denotes the sampled group. N is the number of individual animals genotyped. MLG is the number of multilocus genotypes (MLGs) observed. H is the Shannon-Wiener Index of MLG diversity (Shannon, 2001). G is Stoddart and Taylors Index of MLG diversity (Stoddart & Taylor, 1988). λ is the Simpson Index (Simpson, 1949). C.λ is the Corrected Simpson Index (Grünwald et al., 2017; Hamrová et al., 2011). E.5 is a measure of evenness (Pielou, 1975; Ludwig & Reynolds, 1988; Grünwald et al., 2003). Ia is the index of association (Brown, Feldman & Nevo, 1980; Smith et al., 1993). r_D is the standardized index of association. See Figure 3.1 for more information about these metrics.

Lake	Group	N	MLG	Evenness (E.5)	Shannon-Wiener (H)	Simpson (λ)	Stoddart & Taylors (G)	Corrected Simpson (C.λ)	Index of Assoc. (I_A)	Std. Index of Assoc. ($\underline{r}D$)
				observed (95% CI)	**observed** (95% CI)	**observed** (95% CI)	**observed** (95% CI)			
Hackberry	parents	31	16	**0.40** (0.26, 0.53)	**2.00** (1.47, 2.53)	**0.72** (0.54, 0.90)	**3.55** (1.00, 5.65)	0.74	0.54	0.14
Hackberry	offspring	35	20	**0.82** (0.70, 0.93)	**2.81** (2.56, 3.06)	**0.93** (0.89, 0.96**)**	**13.76** (10.48, 17.05)	0.95	-0.30	-0.08
Hackberry	egg bank	68	37	**0.78** (0.66, 0.89)	**3.41** (3.21, 3.60)	**0.96** (0.94, 0.98)	**23.56** (18.41, 28.78)	0.97	0.16	0.05
Midland	parents	30	26	**0.91** (0.82, 1.01)	**3.20** (2.97, 3.43)	**0.96** (0.93, 0.98)	**22.50** (18.72, 26.28)	0.99	-0.14	-0.04
Midland	offspring	30	27	**0.96** (0.88, 1.03)	**3.26** (3.05, 3.48)	**0.96** (0.94, 0.98)	**25.00** (21.37, 28.63)	0.99	-0.04	-0.01
Midland	egg bank	48	26	**0.58** (0.42, 0.75)	**2.87** (2.56, 3.19)	**0.91** (0.85, 0.96)	**10.77** (6.25, 15.28)	0.93	-0.03	-0.01

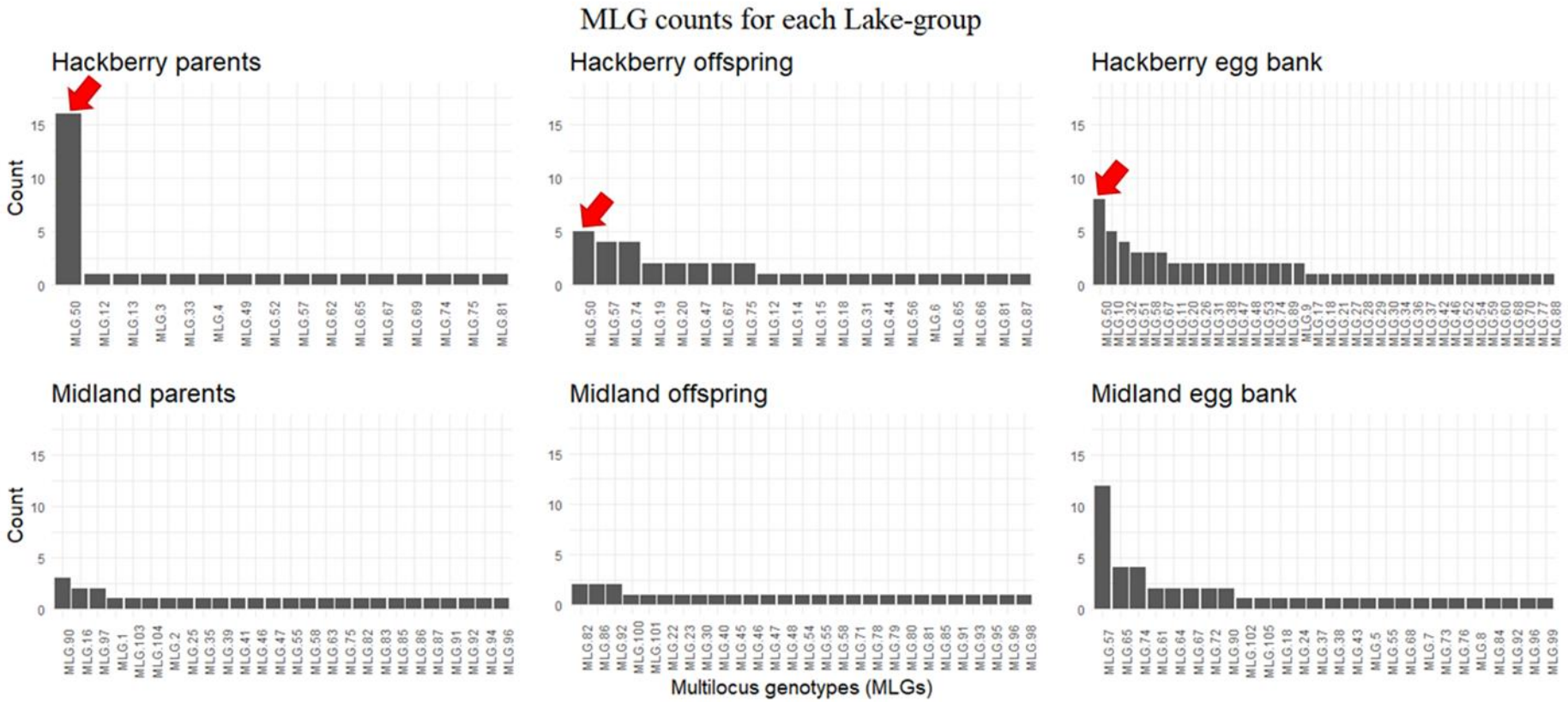

Figure S3.1. Histogram of the number of each unique multi-locus genotype (MLG) detected within each Lake-group. MLG.50 (indicated with red arrows) was the dominant genotype in Hackberry parents and remained the dominant genotype after sexual recombination and recruitment from the egg bank.

CHAPTER 4:

Exposure Dose Alters Within-Host Interactions Between Co-Infecting Parasites, with Consequences for Parasite Prevalence and Host Abundance[5]

ABSTRACT

Parasite dose and the presence of multiple parasite species both have important consequences for infection dynamics, but it is not clear how these aspects of infection biology interact — though they likely do. We disentangled these effects and their interactions by conducting a factorial dose experiment and using it to parameterize dose-dependent multiparasite epidemic models.

The parasites in the study system (one bacterium and one fungus) form environmental spores that degrade at variable rates; this creates ranges of parasite doses over time and space. We used factorial exposures to quantify how combinations of different spore doses from the two parasites impacted likelihood of infection, host lifespan, and transmission potential. We used this data to parameterize epidemic models to understand how these relationships within hosts scale up to host population epidemics. Additionally, we created a base model in which interspecific parasite-parasite interaction effects were removed. Comparisons between the fully parameterized and base simulations allowed us to detect emergent dose-dependent properties and interspecific interaction effects.

We found bacterial infection was strongly influenced by both bacterial dose (which positively related to infection) and fungal dose (with higher fungal doses reducing bacterial infections). In contrast, fungal likelihood of infection was determined by fungal dose alone. Impacts on host lifespan and parasite fitness were strong, though dose independent: infection

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with the fungus significantly shortened host lifespans and, when co-infecting with the bacterial parasite, drastically reduced bacterial fitness.

Epidemic models revealed that, while the bacterium suffered a fungus-dose-dependent reduction in its probability of infection, this negative effect was mitigated at the level of host populations. In fact, when this negative effect on the bacterium was removed from the model, total bacterial prevalence further declined and host populations increased. Overall, we found that multiparasite scenarios were detrimental to the bacterium (and had little effect on the fungus), but dose-dependent interspecific interactions mitigated that harm to the bacterium and resulted in higher parasite prevalence and lower host density than would have occurred otherwise. Ultimately, our work underscores how negative dose-dependent interactions between parasites can scale up to alter parasite burdens and host abundance in unexpected ways.

INTRODUCTION

Multiple-parasite infections are the rule rather than the exception in nature (Cox 2001; Petney and Andrews 1998; Webster 2002; Poulin 2011), meaning the prevalence and transmission rate of a parasite is not only determined by its host and environment, but also by its interactions with other parasite species. As such, a multiparasite framework has gained increasing visibility in the scientific literature within the past two decades (Rynkiewicz et al. 2015; Pedersen & Fenton 2007; Rigaud et al. 2010). There is now strong evidence that parasite-parasite interactions impact parasite transmission, host health, and the success of disease control strategies (Telfer et al. 2010; Lello et al. 2004; Johnson et al. 2013).

While it is clear that multiparasite infections are common and important, most scientific understanding of multiparasite dynamics is either based on human clinical studies or laboratory experiments that over-saturate the study organisms with parasites to maximize the number of co-

infected hosts; neither of these scenarios account for parasite density in their analysis. This is surprising for multiple reasons. First, density is a foundational variable when studying the community ecology of free-living (i.e. non-parasitic) organisms, where it plays an important role in modulating the outcome of interspecific interactions between such organisms (Mittelbach and McGill 2019). Community ecology is an area of research that multiparasite research heavily pulls from, so incorporating parasite density into a multiparasite framework will make it that much easier to share theory and methodologies between the two areas of study. Second, there is an abundance of dose response studies for single parasite infections which find non-linear relationships between parasite dose (i.e. density) and infectivity, virulence, and transmission (Clay et al. 2021). It is reasonable to assume these same dose relationships exist in multiparasite systems, which could impact parasite-parasite interactions and therefore significantly change disease dynamics in a dose-dependent way. Finally, parasite density varies widely in nature, both within hosts (due to heterogeneity in host quality, parasite-parasite interactions, or effects of medication) and at the level of host populations (due to variation in degradation rates of transmission stages, which is of particular importance for environmentally transmitted parasites). It is therefore reasonable to assume that parasite spore density is a vital variable to explicitly incorporate into research on multiparasite systems and coinfections. Doing so will enable us to better understand how interactions among parasites influence infectivity and virulence in natural disease systems.

Interactions between parasite species occur at two biological levels of organization: at the within-host level and the between-host (i.e. host population) level (Alizon et al. 2013). It is important to incorporate both biological levels of organizations into multiparasite studies, particularly in disease scenarios where there is a reciprocal relationship between environmental parasite density and within-host disease processes (Mideo et al. 2008). This is because it is common for interactions between parasites within coinfected hosts to impact transmission, recovery rate, and virulence, which all scale up to influence higher-level host population dynamics--but it is less common for population- and community-level dynamics to reciprocally

influence within-host dynamics. However, this reciprocity can arise in environmentally transmitted parasites if environmental spore density (driven by the density of infected hosts) impacts transmission, recovery rate, and/or virulence at the level of individual hosts. Therefore, it is important to study relationships between parasite density and disease processes at both levels of biological organization; it is through this multi-level lens that we can begin to capture more realistic complexities of multiparasite communities.

How does parasite dose impact within- and between-host dynamics of two environmentally transmitted parasites? We used factorial exposures to quantify how different combinations of spore doses from two parasites impacted likelihood of infection, host lifespan, and transmission potential. We found that spore density had direct impacts on infectivity of both parasites, and indirect effects on host lifespan and parasite transmission. These relationships created reciprocal effects between environmental spore density and within-host interactions (summarized in Fig 4.1), which can scale up to host populations in complex ways. Therefore, in order to understand how such interactions impact epidemic dynamics in host populations, we used this data to parameterize epidemic models with and without the measured dose-dependent effects, and in one- versus two-parasite scenarios. Comparisons between these simulations allow us to detect emergent dose-dependent properties and/or impacts of intra- and inter-specific interactions. Overall, this combination of experimentation and simulation allow us to answer the question, “Does what matters within hosts matter for the host population?” For digestibility, this paper’s methods and results will be separated into Experimental and Simulation sections; this will hopefully enable a more intuitive and accessible review of the complex relationships uncovered within individual hosts (via empirical work) as well as in host populations (via simulation).

EXPERIMENTAL METHODS

Study System

The focal host, *Daphnia dentifera*, is a cyclically parthenogenetic zooplankton. It is a dominant species in ponds and lakes in the Midwestern United States and host to the fungus *Metschnikowia bicuspidata* and the bacterium *Pasteuria ramosa*. *D. dentifera* incidentally ingests transmission stages (spores) of both pathogens while filter feeding; these spores must pass through the host's gut wall and enter the hemocoel to replicate (Green 1974; Metchnikoff 1888; Ebert 2005). Parasites replicate within the hemocoel until host death, at which point the spores are released from the cadaver. The two pathogens differ in how they impact the host: the bacterium castrates the host but does not substantially impact lifespan (Ebert et al. 2004; Auld et al. 2012), while the fungus significantly shortens the host lifespan and has more modest negative effects on reproduction (Clay et al. 2019).

Experimental Methods

We used a single clonal lineage of *D. dentifera* ('Mid37') that was isolated from Midland Lake in Indiana multiple years prior then maintained asexually in the lab. Both pathogen isolates ('Standard' for the fungus, 'G/18' for the bacterium) had been cultured for multiple years in the laboratory as well. Spores were harvested for the experimental exposures by grinding up heavily infected hosts collected from laboratory cultures of the parasite in question. Each parasite dose was achieved by assessing the spore density in the resulting slurry, then exposing experimental hosts to the appropriate volume to achieve the desired experimental spore dose.

We tested for density-dependent effects by creating exposure groups using seven environmental spore densities for each parasite: for the fungus we used 0, 5, 50, 250, 500, 1000, and 2000 spores/mL, and for the bacterium we used 0, 20, 200, 1000, 2000, 4000, and 8000 spores/mL. Spore doses were chosen to replicate a range of realistic environmental spore densities that can occur throughout an epidemic; the bacterium *Pasteuria ramosa* produces higher per-host spore yields than the fungus *Metschnikowia bicuspidata* (Auld et al. 2017), so our experimental spore dose range for the bacterium is higher than the experimental range for the fungus. We tested for interactions between the two parasite species by creating simultaneous

exposure groups via a response surface experimental design, which allows us to disentangle the contribution of intra- and interspecific interactions on parasite community structure (Inouye 2001; Vandermeer and Goldberg 2013). These spore densities were combined in a factorial manner to create a total of 48 co-exposure treatments (though we did not have an uninfected treatment, i.e., 0 spores/mL fungus + 0 spores/mL bacterium). There were ten replicates for each exposure group.

Hosts were exposed to the spore treatments at 6 days old (+/- 24 hours) for 24 hours (+/- 3 hours) and fed 0.5 mL of $1x10^5$ *Ankistrodesmus falcatus* (half of the normal food density, which encourages uptake of spores). Each host was individually kept in a 50mL beaker with 25mL filtered lake water throughout the experiment. Hosts were fed 1 mL of $1x10^5$ *Ankistrodesmus falcatus* 4 times per week and kept on a 16:8 light-dark cycle at 20°C. Individuals were checked for symptoms of infection beginning 11 days after treatment using a dissection microscope; examination of symptoms is possible in this manner because *D. dentifera* have transparent bodies and infected individuals are visibly filled with pathogen spores by day 11. Animals that died before day 11 (9 out of 480 experimental animals) could not be reliably diagnosed and were therefore excluded from the study. All uninfected animals were discarded at day 11 while infected animals were retained and checked daily for mortality. These infected animals were transferred to clean beakers once per week. Offspring (produced asexually) were not transferred. Upon death, cadavers were stored in 2mL tubes in 100uL of milliQ water at -20°C so that spore densities could later be quantified. For both parasites, spores are released into the environment upon host death, so the spores contained in hosts at death is indicative of the potential for transmission to new hosts. Spore counts were completed by grinding up thawed cadavers with a pestle then counting spores using a hemocytometer. We were interested in whether within-host interactions could impact spore size as well as spore density, therefore we measured across the longest visible spore subsections of a haphazard subset of 10 spores per infected cadaver (using Olympus CellSens software).

Analyzing data

Likelihood of infection: Likelihood of infection for the bacterium and fungus, as well as likelihood of coinfection, was measured using generalized additive models (GAMs) with a logit link function. GAMs were used because they are similar to Generalized Linear Models (GLMs) but they relax the assumption of linearity by using smoothing functions to measure arbitrarily non-parametric relationships (Wood 2017). This allows GAMs to reveal linear, monotonic, or even more complex relationships among variables. We assumed a binomial distribution because infection status is a binary outcome (infected/uninfected). Optimal smoothing parameters were selected with REML. For each infection scenario, the probability of infection by the parasite(s) in question was modeled as a function of the natural log of bacterial dose, the natural log of the fungal dose, and an interaction term between the natural logs of the two doses. The computations were implemented using the 'mgcv' package (Wood and Wood 2015) in R (version 4.1.1).

We tested for an interaction effect on the probability of coinfection by comparing a series of GLMs of decreasing complexity. The proportion of coinfected animals over the product of the proportion singly infected with the fungus and singly infected with the bacterium (i.e. coinfected / (bacterium-only x fungus-only)) was modeled as a function of a constant, and the intercept was taken. This intercept provides an approximation for the proportion of coinfected animals that were observed to be coinfected versus what one would expect when a mass-action principle with no interaction effect determined the proportion of coinfected hosts.

Host lifespan: To analyze the effect of spore dose on lifespan, we modeled host lifespan as a function of fungal dose, bacterial dose, and infection class (i.e., singly infected with the bacterium, singly infected with the fungus, or coinfected) using a Poisson GLM. Because a significant interaction effect was observed, we also subset the data by infection class and used linear regression (lm in base R version 4.1.1) to evaluate the relationship between spore dose and lifespan within each infection class. Additionally, we ran post-hoc analyses (Kruskall-Wallis) to compare host lifespan of the three infection classes.

Within-host spore yield: We used generalized linear models (GLMs) with the base glm function in R version 4.1.1. to find relationships between within-host spore yield and parasite dose, host lifespan, and infection class. Model selection was conducted with a stepwise regression approach based on the Akaike information criterion by removing non-significant interactions (Crawley 2012). We analyzed the effect of bacterial and fungal doses on the within-host spore yield of each parasite, plus accounted for possible infection class effects by including each infection class as a constant. When it was found that there were strong class effects on within-host spore yield, we subset the data and analyzed the impact of spore dose on within-host spore yield within each infection class. For the bacterium, we analyzed the effects of bacterial and fungal doses on log-transformed bacterial spore yield data using a Gaussian GLM. For the fungus, we analyzed the effects of bacterial and fungal doses on fungal spore yield data using a Poisson distribution (to account for data skew). We assessed the impact of host lifespan on within-host spore yield in a similar manner. We subset the data by infection class, then analyzed any relationships between host lifespan and within-host spore yield using linear regression (lm function in base R version 4.1.1). Kruskall-Wallis post-hoc comparisons were made to assess differences in mean within-host spore density for each parasite among infection classes. Finally, we used linear regression to assess the relationship between the within-host spore yields of both parasites as they co-occurred in coinfected hosts.

Spore size: We computed a Pearson correlation coefficient to assess the relationships between spore size and spore dose. For thoroughness, we also ran an ANOVA in which we treated spore dose as a categorical variable. Then, to assess the relationship between spore size and host lifespan, we subset the data by whether or not the host was infected by the parasite of interest before computing a Pearson correlation coefficient. We also used the same data subset to assess how spore size was impacted by the within-host spore yield of the conspecific parasite. ANOVA was used to detect any differences in average spore size between different infection classes (i.e., single infections versus coinfections).

EXPERIMENTAL RESULTS

Likelihood of infection

Likelihood of infection by the bacterium was impacted by both bacterial and fungal spore densities (Fig 4.2A; $\chi^2 = 22.046$, $p < 0.001$, and $\chi^2 = 5.272$, $p < 0.001$, respectively). Bacterial infections were highest when bacterial dose was high and fungal dose was low (as shown by the highest infection prevalence in the lower right of Fig 4.2A). In contrast, likelihood of infection by the fungus was determined primarily by fungal spore density, with no statistically significant interaction between fungal and bacterial spore density (Fig 4.2B; bacterial density: $\chi^2 = 6.5$, $p = 0.13$, fungal density: $\chi^2 = 107.10$, $p < 0.001$, bacterial * fungal density: $\chi^2 = 1.9$, $p = 0.09$): fungal infection peaked at moderately high fungal doses, then slightly decreased at the highest doses tested (as shown by the low infection levels in the bottom half of Fig 4.2B and highest levels near – but not at – the top of the panel). Likelihood of coinfection was driven most strongly by fungal spore density with bacterial spore density having a weaker, but still significant, effect ($F_{1,2} = 15.4$, $p < 0.0001$ and $F_{2,1} = 3.5$, $p = 0.03$, respectively). As a result, coinfection was highest when both parasite doses were high (that is, in the upper right corner of Fig 4.2C). Finally, when compared to the mass action probability of coinfection, the observed level of coinfection was approximately half of the expected value (intercept = 0.48; $p < 0.0001$), indicating interference between the two parasites.

Host lifespan

Host lifespan was influenced by infection class but not spore dose at exposure (infection class: $\chi^2 = 473.2$, $p < 0.001$, fungus dose: $\chi^2 = 1.0$, $p = 0.32$, bacterium dose: $\chi^2 = 0.07$, $p = 0.79$). Infection with the fungus was the strongest driver of host lifespan; individuals with single fungal infections or coinfections lived approximately half as long as hosts infected by the bacterium alone (Fig 4.3). Additionally, coinfection with the bacterium had a modest positive impact on

host lifespan as compared to single infections by the fungus, with coinfected hosts living approximately 2 days longer than fungus-only infected hosts (Fig 4.3).

Spore yield

Infection class was the main predictor of spore yield for both parasites (Fig 4.4). Coinfection significantly reduced bacterial spore yield by 79% (Fig 4.4A, Wilcoxon: $W = 565.5$, $p < 0.0001$); conversely, coinfection resulted in a modest increase in fungal spore yield by 28% (Fig 4.4B, Wilcoxon: $W = 3687$, $p = 0.0002$).

Spore yield was also associated with host lifespan for both parasites, but in opposite directions. For the bacterium, spore yield was significantly impacted by host lifespan (Fig 4.4C, $t = 2.05$, $p = 0.04$). More specifically, post-hoc analyses found that bacterial spore yield in singly-infected hosts decreased as host lifespan increased ($F_{1,165} = 24.9$, $p < 0.0001$), but lifespan did not impact bacterial spore yield in coinfected hosts ($F_{1,29} = 0.2$, $p = 0.69$). In contrast, fungal spore yield increased with increasing host lifespan in both singly-infected and coinfected hosts (Fig 4.4D, $t = 5.13$, $p < 0.0001$). Finally, there was no significant relationship between spore yield of the two parasite species when they co-infected the same host (Fig 4.S1, $t = 0.77$, $p = 0.45$).

Spore size

Spore size of the bacterium and the fungus did not vary with spore dose (bacterium: Pearson correlation coefficient = 0.20, $p = 0.11$, fungus: Pearson correlation coefficient = 0.01, $p = 0.87$) or spore yield of the heterospecific parasite (Fig 4.S2, Pearson correlation coefficient = -0.02, $p = 0.96$). Bacterial spores were smaller in coinfected hosts versus singly infected hosts (Welch two-sample t-test; bacterium only vs. coinfected, $t(14.9) = 2.97$, $p = 0.01$), but fungal spores did not differ in size between infection classes (Welch two-sample t-test; fungus only vs. coinfected, $t(14.5) = 0.35$, $p = 0.73$). Finally, host lifespan did not have a significant impact on spore size for the bacterium (Pearson correlation coefficient = 0.19, p-value = 0.12) or the fungus (Pearson correlation coefficient = 0.05, p-value = 0.52).

SIMULATION METHODS

In addition to the individual-level analyses above, we were interested in how parasite-parasite interspecific interactions scaled up to influence disease prevalence and host population size. We used two simulations to isolate and measure the impact(s) of interspecific interactions on disease dynamics: a base model with no interspecific interactions, then a more complex model with interspecific interactions parameterized with our full experimental data. We ran each model to equilibrium and compared bacterial prevalence, fungal prevalence, and host density over a range of spore densities; variation in environmental spore density was achieved by varying spore degradation rate, with higher degradation rates leading to lower densities in the environment, all else being equal. This allowed us to quantify the impacts of interspecific interactions among parasites on the three populations in this system across a range of environmental spore densities.

This model has six state variables: Uninfected (susceptible) hosts, S, hosts infected by the bacterium, I_B, hosts infected by the fungus, I_F, coinfected hosts, I_C, density of bacterial spores in the environment, B, and density of fungal spores in the environment, F.

Dynamics of the system are given by:

$$dS/dt = (bS + b/2 * (I_F + I_B + I_C)) * (1 - N/K) - S(a_S + \sigma fB + \mu fF - seg * \sigma fB * \mu fF) \quad \text{(eq. 1a)}$$

$$dI_B/dt = \sigma fBS - a_B I_B - \sigma fBI_F \quad \text{(eq. 1b)}$$

$$dI_F/dt = \mu fFS - a_F I_F - \mu fFI_B \quad \text{(eq. 1c)}$$

$$dI_C/dt = \mu fFI_B + \sigma fBI_F + seg * \sigma fBS * \mu fFS - a_C I_C \quad \text{(eq. 1d)}$$

$$dB/dt = \delta(a_B I_B + a_C I_C) - \varrho B - fN/2 \quad \text{(eq. 1e)}$$

$$dF/dt = \omega(a_F I_F + a_C I_C) - \kappa F - fN \quad \text{(eq. 1f)}$$

Daphnia are born into the population at a rate b per uninfected host, and this rate is roughly halved for all infected parents (b_i; Clay et al 2019). Births decrease as the population density (N) reaches carrying capacity (K), where $N = S + I_B + I_F + I_C$. *Daphnia* that are not infected can become infected by one or both of the parasites, with infection rate dependent on the rate at which the hosts feed on the spores, f, the per-spore infectivity of the parasite (σ for the bacterium and μ for the fungus; see more below), and the density of spores of a given parasite in the environment. Hosts that are singly infected can become coinfected by a similar process (the last terms in eq. 1b and 1c). Uninfected hosts die at rate a_S, bacterium infected hosts die at rate a_B, fungus infected hosts die at rate a_F , and coinfected hosts die at rate a_C (however, mortality rate is the same for all hosts infected with the fungus so $a_F = a_C$). Upon death, hosts infected by the bacterium release δ bacterial spores into the environment, and hosts infected by the fungus release ω fungal spores into the environment. Bacterial spores degrade in the environment at a rate ϱ, and fungal spores degrade in the environment at a rate κ. Spores are also removed from the environment when fed upon by hosts (fN). This removal rate is halved for the bacterium, as it sometimes survives consumption and remains infective when released into the environment in *Daphnia* feces (King et al. 2013).

Dose effects on infection: The base model does not incorporate interspecific dose effects, but it does incorporate intraspecific dose effects. Also, because exposure dose did not affect spore yield or host lifespan, those terms are not influenced by the density of spores in the environment at the time of infection and are therefore unchanged between the base and interspecific models.

Births: We did not collect birth data from our experiment. Thus reproduction occurs at a rate of $(b) * (1 - N/K)$ if the host is uninfected or $(b/2) * (1 - N/K)$ if the host is infected with either or both parasites, based on prior studies (Clay et al., 2019). All offspring are added to the uninfected compartment.

Deaths: Time until death was recorded for all hosts in our experiment. Lifespan differed between infection classes, and the death rate for hosts in each infection compartment was calculated as the inverse of the mean infection class host lifespan.

Spore release: Spore release upon death, δ and ω , were the average spore yield at time of host death. Each infection class had a different mean spore release density (which was calculated via the spore dose experiment).

Spore host feeding rate is the same for all hosts and is taken from Clay *et al.* 2019.

Spore degradation rate: The range of environmental spore degradation rates for the fungus (κ = 0.02, 0.22, 0.42, 0.62, 0.82, 1.02, 1.22, 1.42, 1.62, 1.82) and the bacterium (ϱ = 0.004, 0.044, 0.084, 0.124, 0.164, 0.204, 0.244, 0.284, 0.324, 0.364) were set so the simulation modeled scenarios ranging from local extinction to high established prevalence for each parasite.

Interspecific effects model

The interspecific effects model incorporates interspecific and intraspecific dose effects. Specifically, the interspecific effects model was parameterized using the full set of empirical data on parasite dose and probability of infection, allowing fungal spore density to impact the probability of infection of both the fungus and the bacterium. Our experiments showed simultaneous coinfection happened at a lower prevalence than would occur at random (a sign of infection segregation), so a correction term for simultaneous coinfection "seg" was included in the model ($-seg * \sigma fB * \mu fF$). As in the base model, hosts that become infected transition to one of three categories: fungus-infected, bacterium-infected, or coinfected.

Analyzing model

We initialized our model with the host population density at equilibrium ($N = K$), with one host singly infected by the bacterium with a randomized dose at infection, and one host singly

infected with the fungus with a randomized dose at infection. All other hosts were initially uninfected (results are not sensitive to initial conditions). We ran our model until equilibrium over a range of spore degradation rates of both the bacterium and the fungus. The higher a spore degradation rate is, the less time that a spore will be able to survive in the environment, and the lower spore density/dose will be.

SIMULATION RESULTS

Comparisons between the base and interspecific models suggest that interspecific impacts on probability of infection mitigate the antagonistic effects seen in the experimental data. First, the impact of interspecific spore density on infection mitigated fungus-driven reduction in bacterial prevalence (Fig 4.5C), with the proportion of hosts infected with the bacterium in the interspecific interaction model up to 70% greater than that seen in the base model, particularly at low to intermediate bacterial spore densities and low to intermediate fungal spore densities. This increase in prevalence occurred both by allowing the bacterium to coexist with the fungus over a larger range of parameter space (additional regions of > 0 proportion infected in Fig 4.5B vs. 4.5A) and by increasing prevalence in regions where it already was able to coexist. Second, the interspecific interaction effects reduced fungal prevalence (Fig 4.5F) by up to 50%. When interspecific impacts on infection probability were included in the model, the fungus was excluded at some intermediate doses where it had been able to persist in the absence of interspecific interaction effects (compare Fig 4.5D&E). These changes in parasite prevalence had little impact on host density, though host density did decrease when fungal density, and therefore fungal infection prevalence, was at its highest (Fig 4.5C).

DISCUSSION

Overlapping epidemics of multiple parasites are common in natural systems. Such overlapping epidemics have complex dynamics dependent on the ways in which parasites interact both within and among hosts. Simultaneously, parasite density or "dose" impacts infectivity, virulence, and transmission (Clay et al. 2021) -- but such dose effects have almost exclusively been studied in single parasite scenarios. As a result, little is known about how these realities overlap; i.e. how parasite dose impacts the dynamics of multiparasite epidemics. Our study used ranges of experimental dose exposures to disentangle intra- and interspecific parasite-parasite interspecific interaction effects on infectivity, spore yield, and host lifespan. We uncovered dose-dependent interspecific effects on infectivity, yet also found that host lifespan and parasite spore yield were primarily determined by the species of the infecting parasite(s). We used this data to parameterize epidemic simulations and explore how these interspecific parasite-parasite effects on infectivity scale up to have dramatic dose-dependent impacts on parasite prevalence and host density.

Our experimental trials showed that probability of infection varied with spore density. This contrasts with standard compartment models of environmental transmission, which implicitly assume that the infectivity of each spore is constant. If per-spore infectivity varies with spore density, this relationship must be included in transmission models if they are to produce accurate epidemiological predictions.

Intraspecific density-dependence of infectivity were detected in the fungus but not the bacterium, i.e. the bacterium showed a constant infectivity across the full range of environmental bacterial spore densities tested, but the infectivity of the fungus decreased at the highest densities of environmental fungal spore density. (Conversely, interspecific effects were detected in the bacterium but not the fungus; i.e. infectivity of the bacterium decreased linearly with the environmental spore density of the fungus, but the infectivity of the fungus was not significantly impacted by environmental density of the bacterium--though a weak positive relationship showed borderline statistical significance). Ultimately, this suggests the environmental spore

density of the fungus has complex impacts on the infectivity of both parasites. These findings explicitly show us that the density of environmentally transmitted parasites can have important impacts in both single and multiparasite disease scenarios, and the strength and nature (neutral or negative) of these effects change depending on parasite density.

The mechanism(s) of these impacts of fungal density on fungal and bacterial infectivity are unclear. For the fungus, the intraspecific effects only occur at the highest spore densities tested; this could be due to indirect competition via the host immune response in the earliest stages of infection. The fungus is an ascomycete that pierces the gut wall of its host, and multiple spores can infect an individual host (Stewart Merrill, et al. 2018). Infectivity may be reduced at high environmental spore densities because, after initial infection, the host mounts an immune response that can sometimes prevent infections from becoming fully established (Stewart Merrill et al., 2021). Alternatively, infectivity of the fungus may falsely appear to decrease at the highest fungal doses if hosts do not survive the process of infection. This is particularly important to consider if the process of infection is damaging and carries the possibility of host mortality. If this is the case, an increased proportion of hosts exposed to the highest doses in this study would die before they could be diagnosed and would then be removed from the experimental sample. However, out of the 70 hosts that were exposed to the highest fungal dose (2000 spores/mL), only 3 died before they could be diagnosed while 16 survived exposure and were confirmed to be uninfected, so this seems unlikely.

While intraspecific effects were only seen at the highest fungal doses, the interspecific effect on bacterial infectivity occurred across the entire range of doses we explored. More specifically, the bacterial infectivity decreased at a constant rate as fungal dose increased. The mechanism underlying this is unknown, though direct competition with the fungus for physical access to the gut wall or growth nutrients is possible. Additionally, indirect competition through a generalized immune response triggered by the fungal spores could explain the negative relationship. Finally, the bacterium's infectivity could also decrease as fungal dose increases due

to behavioral changes in *Daphnia*; studies show these daphniids can slow their feeding rate when exposed to high densities of the fungus.

While infectivity was the only disease process shown to be impacted in a dose-dependent manner, spore yield of both parasites and host lifespan were strongly impacted by the infection class of the host (therefore, spore density has indirect effects on life span and spore yield). Coinfection reduced bacterial spore yield by 79% (from a mean bacterial spore yield of approximately 100,000 spores/host to 21,000 spores/host). This could be due to the fact that coinfected hosts lived a mean of 24 days while hosts only infected with the bacterium lived for a mean of 39 days. However, resource and immune-mediated indirect competition are also possible explanations. Additionally, host lifespan was positively correlated with bacterial spore yield in bacterium-only infected hosts but negatively correlated in coinfected hosts. However, there is no overlap in lifespan between bacterium-only and coinfected hosts, so it is impossible to differentiate which factor (lifespan or infection class) is the main driver of spore yield. It is also possible that these factors are both at play in determining the final spore yield of the bacterium. Finally, spore yield of the fungus was 28% higher in coinfections (increasing from a mean spore yield of 82,400 spores/host in fungus-only infections to a mean spore yield of 105,370 spores/host in coinfected hosts), which further suggests that the fungus derives a benefit from co-occurring with the bacterium.

The life-shortening virulence of the fungus is well-documented, so it was unsurprising that infection with the fungus was the strongest determinant of host lifespan, i.e. of the three infection classes (fungus-only, bacterium-only, and coinfected), hosts singly infected with the fungus had the shortest average lifespan while host singly infected with the bacterium had the longest lifespan. Interestingly, hosts coinfected with the fungus and the bacterium had a slightly longer lifespan than those infected with the fungus alone. Coinfected hosts also had significantly more fungal spores and much fewer bacterial spores. In this way, coinfection benefited both the host (relative to fungus-only infected hosts) and the fungus while harming the bacterium.

Overall, experimental results showed that, at the level of individual hosts, co-occurrence and co-infection benefits the fungus and damages the bacterium. Additionally, the strength of these impacts was determined by the infection class, which were driven by environmental spore density. This is where the reciprocal link between within- and between-host dynamics arose: changes in spore yield changes environmental spore density, which in turn impacts infectivity for the next round of host infections. Thus, environmental spore density will drive epidemic dynamics in complex and difficult-to-predict ways. We used simulations to understand how such complex relationships played out across ranges of environmental spore densities for single and co-occurring epidemics in host populations.

Our simulation shows that interspecific impacts on probability of infection mitigate the strength of these relationships--they decrease the prevalence of the fungus, increase the prevalence of bacterium, and have weak impacts on host density unless the fungus is at peak prevalence, in which case host density decreases slightly.

Coinfection at the within-host level benefited the fungus, harmed the bacterium, and harmed the host (i.e. infection with the fungus was worse for the host compared to infection without the fungus). However, interspecific interaction effects on infectivity scaled up to mitigate this harm at the level of host populations. We show that interspecific interactions among parasites can have a range of positive to negative effects on the organisms involved, and the nature and directionality of these effects depends on the environmental spore density of the parasites as well as the scale of biological organization under study. Our findings underscore how studying disease processes across a range of parasite doses provides vital insight into the strength, directionality, and nature of parasite-parasite and parasite-host interactions. Researchers can incorporate a factorial exposure design into experimental exposures, which will allow us to more realistically predict long-term dynamics of many environmentally transmitted parasites and pathogens.

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FIGURES

Table 4.1. Parameters and their values

Parameter	**Symbol**	**Units**	**Value**
Birth rate	b	births/day	1.6
Proportion of births per infected host	inf.fec	NA	0.59
Carrying capacity	K	*Daphnia*/10 L	10
Host feeding rate	f	1/host/day	0.004
Lifespan of uninfected host	Sls	days	*calculated from this study*
Lifespan of bacterium-only infected host	IPls	days	*calculated from this study*
Lifespan of fungus-only infected host	IMls	days	*calculated from this study*
Lifespan of coinfected host	Cls	days	*calculated from this study*
Mean bacterial spore yield per bacterium-only infected host	BIP	No. spores/5 L	*calculated from this study*
Mean fungal spore yield per fungus-only infected host	BIM	No. spores/5 L	*calculated from this study*

Mean bacterial spore yield per 1 coinfected host	BCP	No. spores/5 L	*calculated from this study*
Mean fungal spore yield from 1 coinfected host	BCM	No. spores/5 L	*calculated from this study*
Bacterial degradation rate	ϱ	proportion	0.004, 0.044, 0.084, 0.124, 0.164, 0.204, 0.244, 0.284, 0.324, 0.364
Fungal degradation rate	κ	proportion	0.02, 0.22, 0.42, 0.62, 0.82, 1.02, 1.22, 1.42, 1.62, 1.82
Reduction in probability of coinfection due to interspecific effects*	seg*	proportion	-0.48

**seg* was excluded from the base model.

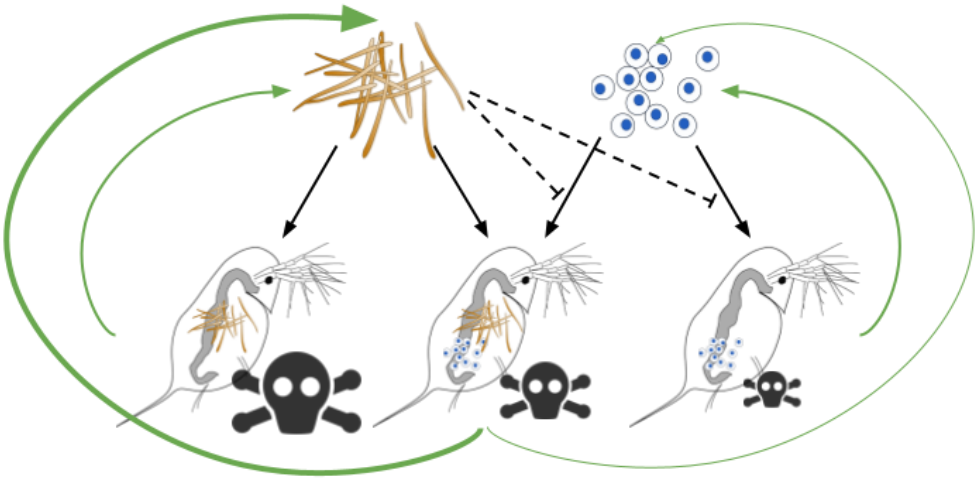

Fig 4.1. Visual abstract of empirical results. Black lines (arrows or dashes) indicate relationships driven by spore density. Environmental fungal spore density (represented by orange needle-like spores) had a largely positive correlation with probability of infection by the fungus; conversely, it had a negative correlation with probability of bacterial infection (dashed lines). Environmental bacterial spore density (represented by blue round spores) had a positive relationship with probability of bacterial infection. Green arrows indicate relationships driven by infection class. Fungus-only infected hosts produced a standard fungal spore yield. Comparatively, co-infected hosts produced a slightly larger fungal spore yield. Bacterium-only infected hosts produced a standard bacterial spore yield. Comparatively, co-infected hosts produced a drastically smaller bacterial spore yield. Finally, host mortality (indicated with skull and crossbones) was greatest for fungus-only infections, slightly less for coinfections, and much lower for bacterium-only infections.

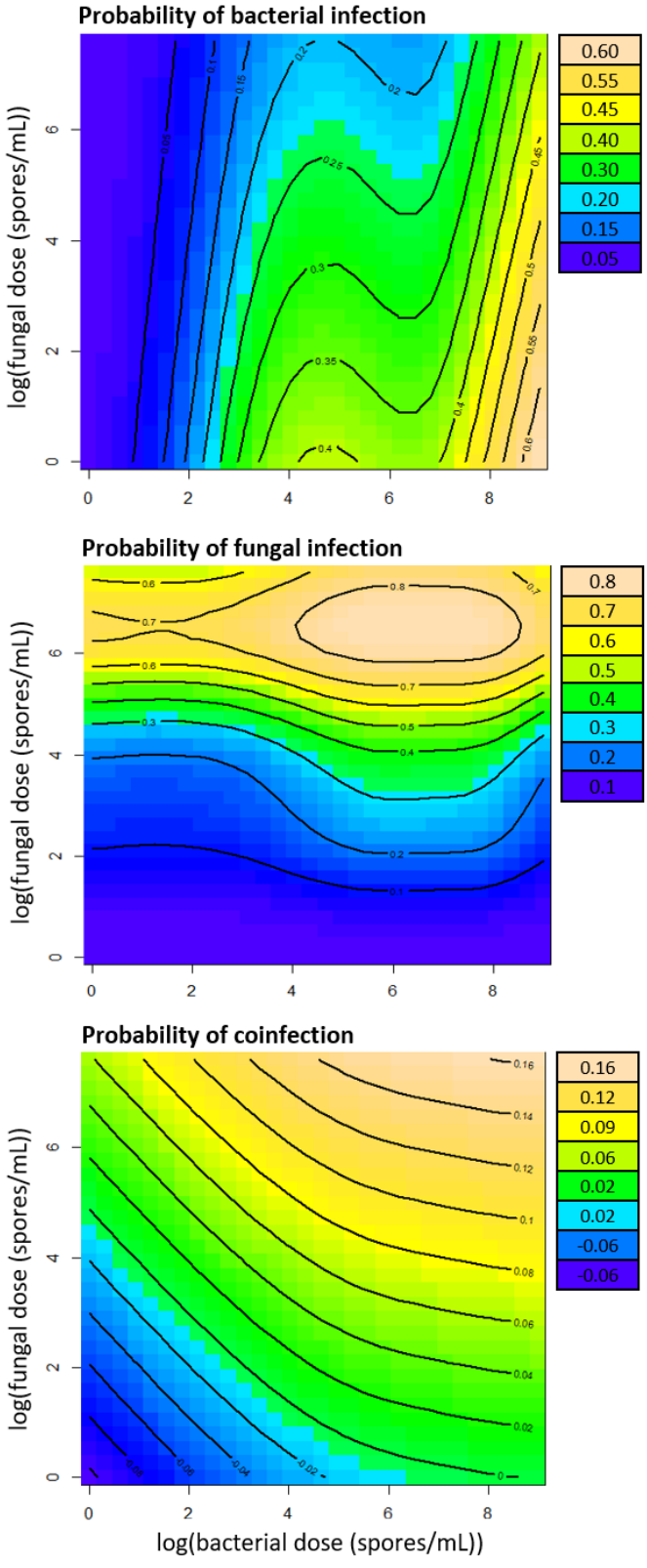
Probability of bacterial infection
log(fungal dose (spores/mL))
0.60
0.55
0.45
0.40
0.30
0.20
0.15
0.05
Probability of fungal infection
log(fungal dose (spores/mL))
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Probability of coinfection
log(fungal dose (spores/mL))
0.16
0.12
0.09
0.06
0.02
0.02
-0.06
-0.06
log(bacterial dose (spores/mL))

Figure 4.2. Infection probabilities for three infection scenarios across a spore density landscape. Probability of infection increased with spore dose for both parasites, though the fungus showed reduced probability of infection at its highest doses**. A. Probability of bacterial infection:** The highest probability of infection occurs at the highest dose of the bacterium. The bacterium appears to be harmed by the presence of the fungus, with probability of infection by the bacterium decreasing as fungal dose increases. **B. Probability of fungal infection:** The highest probability of infection occurs at 1000 fungal spores/mL (y≈6) at which there is an inflection point and probability begins to decrease. The fungus appears to benefit from the presence of the bacterium, with the greatest probability of infection occurring at moderately high, but not the highest, doses of both parasites (see peak at 1000 fungal spores/mL (y≈6) and 4000 bacterial spores/mL (x≈6)). **C. Probability of coinfection:** The highest probability of coinfection occurs at the highest spore doses for both parasites (upper righthand corner).

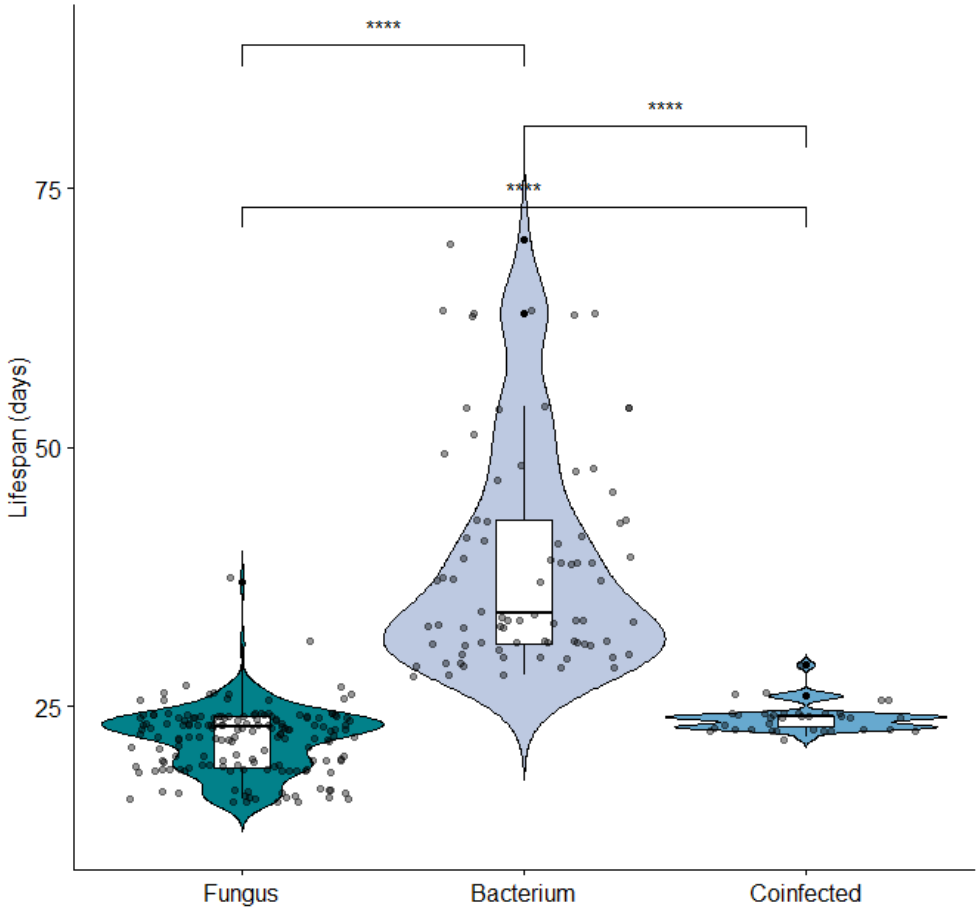

Figure 4.3. Mean host lifespan of different infection classes. Mean host lifespan was shortest for hosts infected by the fungus alone and longest for hosts infected with the bacterium alone. Hosts coinfected with both parasites had a lifespan similar to, but still significantly longer than, fungus-only infected hosts. This suggests that, while the fungus has a strong negative impact on host lifespan, co-infection with the bacterium may slightly ameliorate this effect. Contrasts: fungus-infected vs. coinfected: (Wilcoxon: $W = 3768$, $p < 0.0001$); fungus-infected vs. bacterium-infected: (Wilcoxon: $W = 65$, $p << 0.0001$); bacterium-infected vs. coinfected: (Wilcoxon: $W = 0$, $p << 0.0001$).

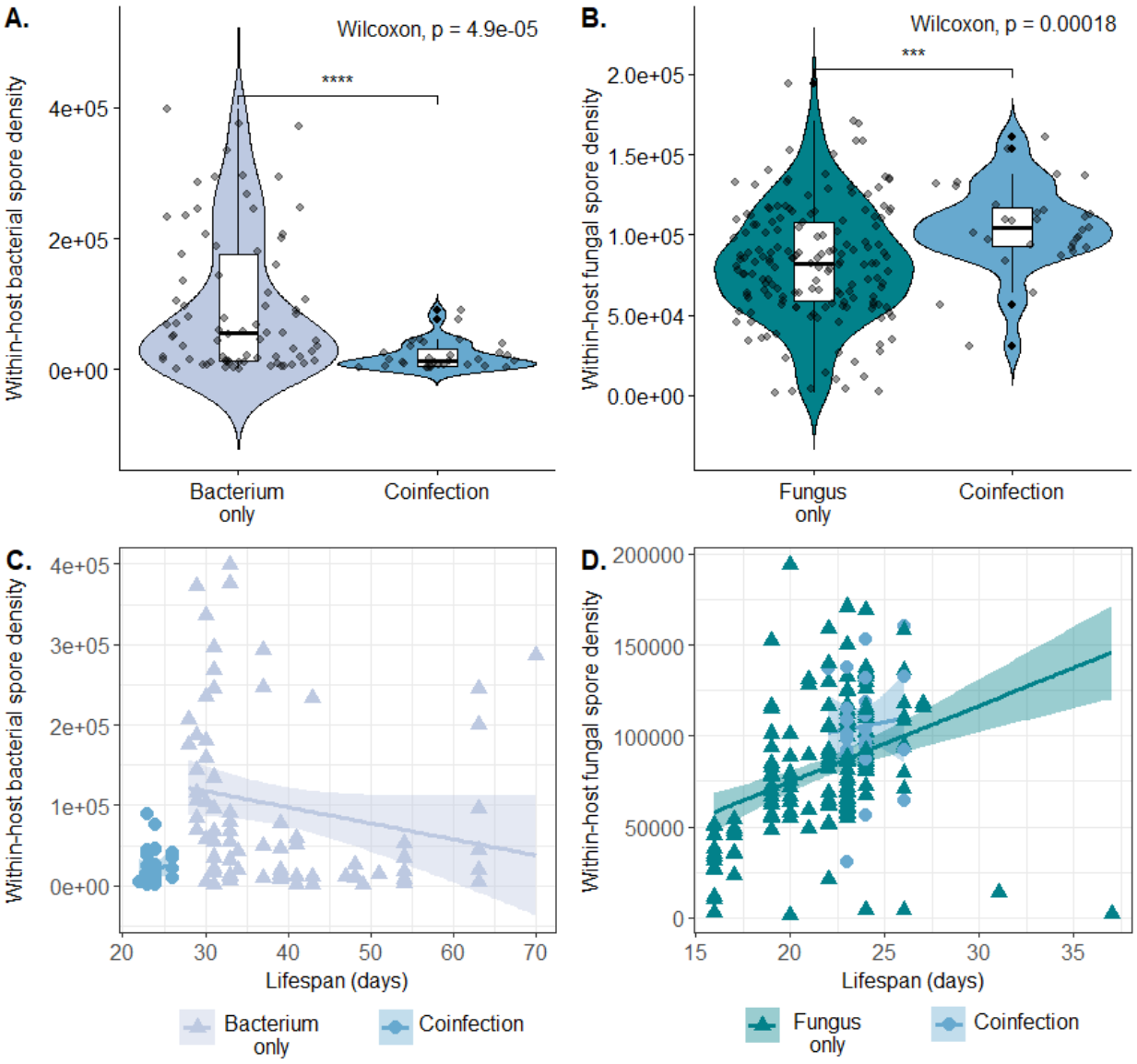

Figure 4.4 Spore yield in each infection class and across host lifespan. A. Bacterial spore yield was significantly lower in coinfected hosts (Wilcoxon: W = 595.5, p << 0.0001). **B.** Fungal spore yield was significantly higher in coinfected hosts (Wilcoxon: W = 3687, p = 0.0002). **C.** Bacterial spore yield was negatively (though weakly) associated with host lifespan in singly infected hosts (t = 2.05, p = 0.04) but had no relationship in coinfected hosts ($F_{1,29}$ = 0.2, p = 0.69) due to host lifespan being too short to draw comparisons. **D.** Fungal spore yield was

positively associated with host lifespan in singly infected hosts ($F_{1,165} = 24.9$, $p < 0.0001$) but had no relationship in coinfected hosts ($F_{1,29} = 0.16$, $p < 0.0001$).

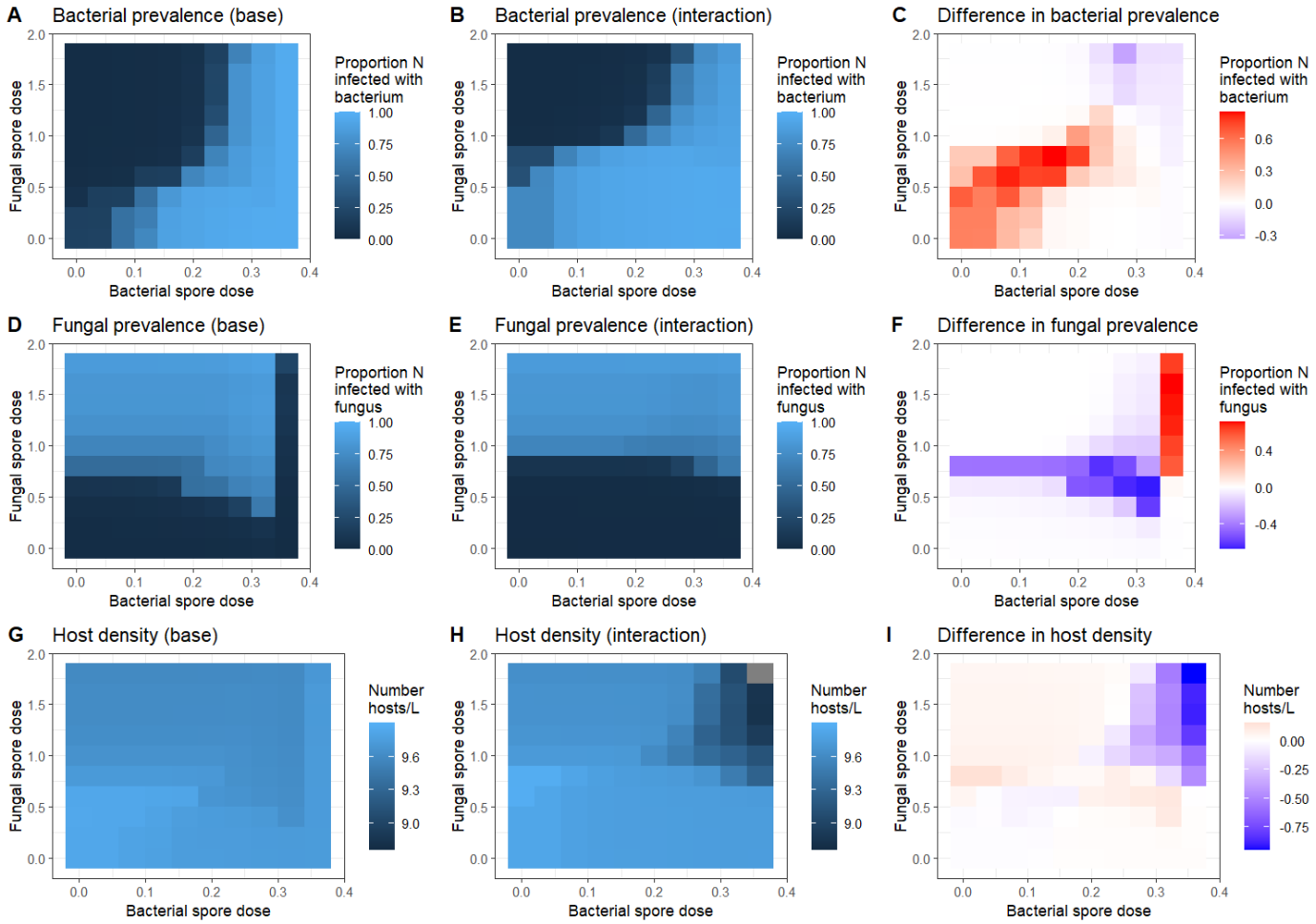

Figure 4.5. Parasite and host density across an environmental spore density landscape. Interspecific impacts were quantified as the difference between the variable of interest in the experimental model (with parasite-parasite interspecific interactions) and the base model (no parasite-parasite interspecific interactions). **A. Bacterial prevalence (base)** increased as bacterial spore dose increased, but decreased as fungal dose increased. **B. Bacterial prevalence (interspecific interaction)** showed a similar pattern, though bacterial prevalence was greater

than in the base model. This is displayed in panel **C. Difference in bacterial prevalence**, which shows that interspecific interactions benefited the bacterium, especially at high bacterial spore densities and moderate fungal spore densities. **D. Fungal prevalence (base)** increased with increasing environmental fungal spore density. **E. Fungal prevalence (interspecific interaction)** had a similar pattern, but **F. Difference in fungal prevalence** shows that interspecific interactions suppressed fungal prevalence, particularly at moderate fungal doses. **G. Host density (base)** decreased as fungal dose increased. It also decreased at the highest bacterial doses. **H. Host density (interspecific interaction)** had a similar pattern, but with important differences as shown in panel I. **I. Difference in host density** shows interspecific interactions impact host density via its effects on parasite prevalence. Host density was higher for dose combinations where the fungus was suppressed. Additionally, host density was slightly lower for dose combinations that resulted in increased bacterial prevalence.

SUPPLEMENTAL FIGURES

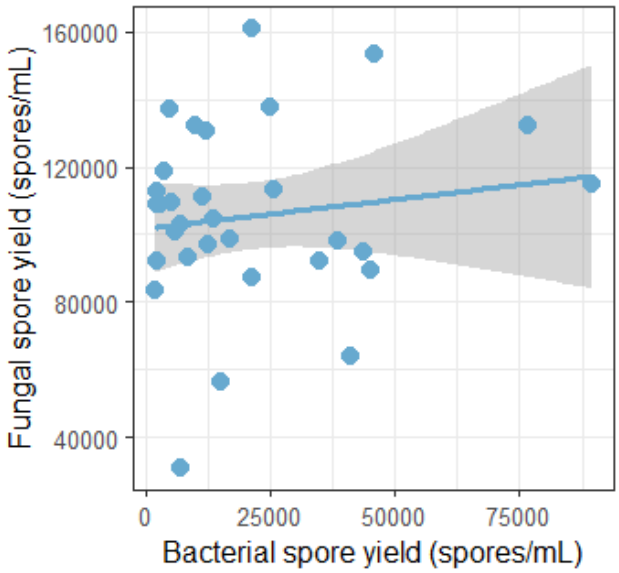

Figure S4.1. Relationship between fungal and bacterial spore yield in shared hosts. There was no significant relationship between the spore yield of the two parasites when they coinfected the same host ($t = 0.77$, $p = 0.45$).

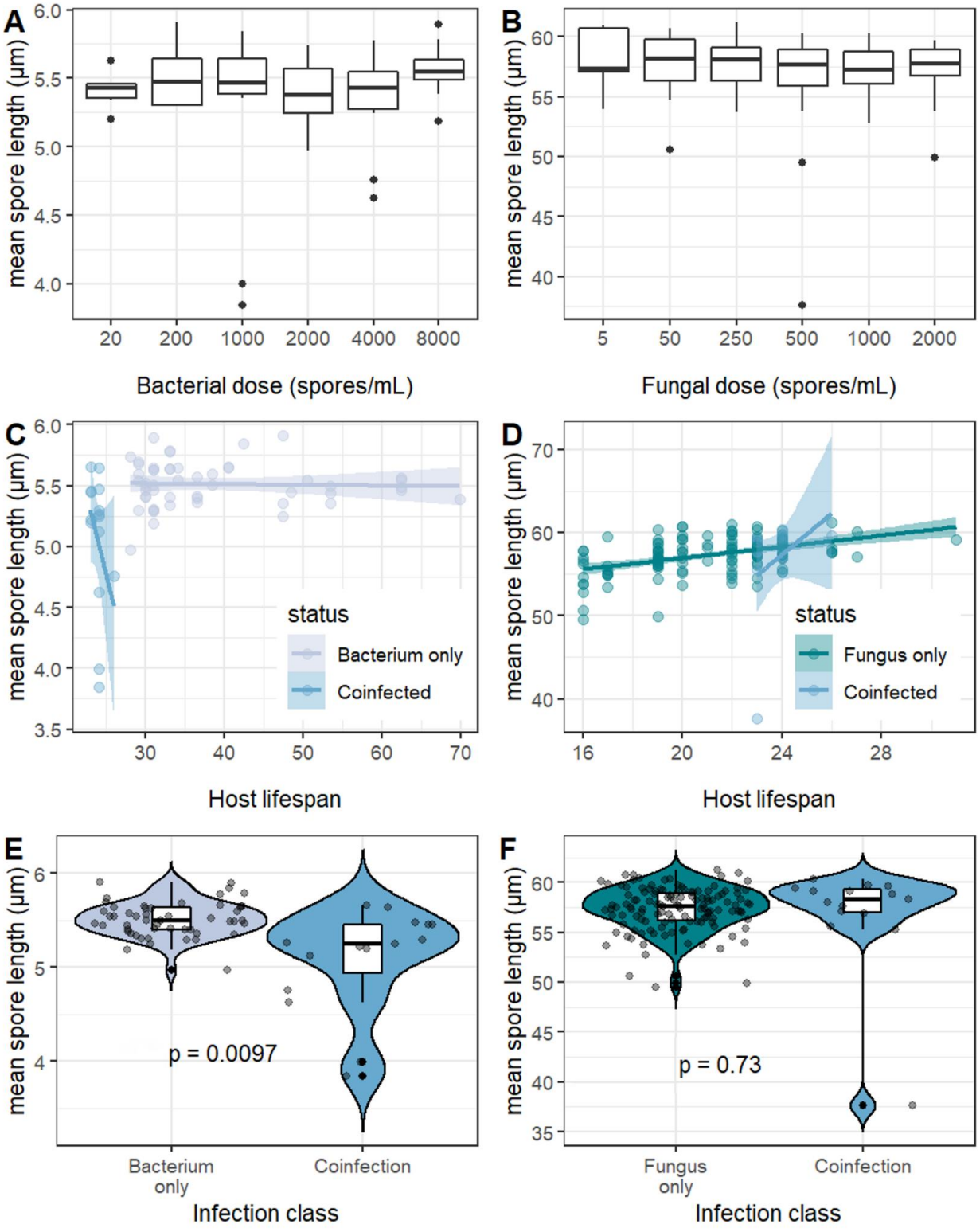
A
mean spore length (µm)
6.0
5.5
5.0
4.5
4.0
20
200
1000
2000
4000
8000
Bacterial dose (spores/mL)
B
mean spore length (µm)
60
55
50
45
40
5
50
250
500
1000
2000
Fungal dose (spores/mL)
C
mean spore length (µm)
6.0
5.5
5.0
4.5
4.0
3.5
30
40
50
60
70
Host lifespan
status
Bacterium only
Coinfected
D
mean spore length (µm)
70
60
50
40
16
20
24
28
Host lifespan
status
Fungus only
Coinfected
E
mean spore length (µm)
6
5
4
p = 0.0097
Bacterium only
Coinfection
Infection class
F
mean spore length (µm)
60
55
50
45
40
35
p = 0.73
Fungus only
Coinfection
Infection class

Fig S4.2. Relationships between spore size and spore dose, host lifespan, and infection class. Spore size of either parasite did not significantly correlate with spore dose or host lifespan. Infection class had an effect on bacterial spore size; they were slightly smaller in coinfected hosts. In contrast, infection class had no impact on fungal spore size. **A.** There was no significant difference of spore size between parasite doses(bacterium: Pearson correlation coefficient = 0.20, p = 0.11, fungus: Pearson correlation coefficient = 0.01, p = 0.87). **B.** Host lifespan was not a strong predictor of spore size when spore size data from coinfected and singly infected hosts was pooled by parasite species (bacterium: Pearson correlation coefficient = 0.19, p = 0.12; fungus: Pearson correlation coefficient = 0.05, p = 0.52). However, there did appear to be differentiation in the size of the bacterium in coinfected versus singly infected hosts. **C.** The bacterium was slightly smaller in coinfected hosts compared to singly infected hosts (Welch two-sample t-test; bacterium only vs. coinfected, $t(14.9) = 2.97$, $p = 0.01$; fungus only vs. coinfected, $t(14.5) = 0.35$, $p = 0.73$), however, our data don't allow us to conclusively differentiate between the effects of coinfection and the effects of host lifespan since all coinfected host lifespans were shorter than all singly infected host lifespans.

CHAPTER 5:

Incorporating waning immunity and immune escape to better predict SARS-CoV-2 dynamics and explore counterfactuals around vaccine uptake and variant emergence[6]

ABSTRACT

Existing models of SARS-CoV-2 dynamics assume a simple immune landscape; i.e., that hosts have either no immunity or full immunity against infection by the virus. This assumption becomes increasingly inaccurate as the pandemic wears on, particularly as the virus continues to evolve and people gain immunity from multiple different sources. As such, transmission models that incorporate immune waning rate as well as variant-specific immunity are necessary to predict SAR-CoV-2 dynamics in the future. Furthermore, this would also allow researchers to turn an eye to past COVID-19 outbreaks and develop a more mechanistic understanding of past dynamics, plus enables the exploration of counterfactuals (i.e. what if primary vaccination occurred at different points in time or at different rates?). This study uses a Susceptible-Resistant model with four categories of decreasing resistance and varying levels of immune protection. This proof-of-concept model sufficiently partitions Michigan's population into the set resistance categories and progresses the population through those categories in a way that accurately encompasses the state population size, total SARS-CoV-2 prevalence, and vaccination rates set by the modelers.

INTRODUCTION

A number of variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have emerged since the first reports of human infections in late 2019 (Covariants, 2022). These

[6] Coauthors: Duffy, M.A. and Eisenberg, M.C.

variants carry mutations that can confer increased transmissibility, disease severity, and/or antigenic escape from immune system antibodies, all of which are making pandemic management increasingly difficult and complex (Aleem et al., 2022; Fernandes et al., 2022; Salimi-Jeda et al., 2021). Another source of complexity arises from how host immunity is conferred (i.e. through vaccination, infection, or reinfection, as well as combinations of all three) and how quickly that immunity wanes (Calcoen et al., 2022; Mazzoni et al., 2022; Negi et al., 2022; Pilz et al., 2022). As such, the simultaneous actions of vaccination regime change, infection or re-infection, immune waning, and a growing number of variants with differing levels of immune escape make the dynamics of SARS-CoV-2 increasingly difficult to predict. Integrating such mechanisms of changing immunity into a transmission modeling framework will increase our ability to anticipate the dynamics of newly-arisen variants of SARS-CoV-2.

New types of transmission models are needed. Existing transmission models assume a simplistic immune landscape (i.e. people are either fully immune or completely susceptible), but these assumptions are becoming increasingly inaccurate (Goldberg et al., 2022; Pérez-Alós et al., 2022; Stich et al., 2022). As COVID-19 transitions into an endemic state and populations develop wide ranging immunity to emerging variants, we need to incorporate such complexity into public health models to increase our ability to predict SARS-CoV-2 dynamics in coming months. Furthermore, this model can increase understanding of past disease scenarios; by incorporating the mechanisms of waning immunity and variant-specific immune evasion, this model will allow researchers to reassess early dynamics of SARS-CoV-2 with greater accuracy and explore counterfactual scenarios of past vaccination strategies.

The state of Michigan experienced five waves of SARS-CoV-2 transmission during the COVID-19 pandemic through May 2022. Each wave was dominated by a different variant and had differing effects on public health and hospital systems, suggesting differing infectivity for each variant. Michigan is also one of few states with significant peaks of Alpha (B.1.1.7 lineage) and Delta (B.1.617.2 lineage) variants, though the highest infection rates yet detected were due to the

Omicron variant; over 2,000 new cases per million people per day were detected at the height of the Omicron wave in January 2021. As is true worldwide, there is state-wide concern about reduced efficacy of vaccination and reduced ability to anticipate SARS-CoV-2 dynamics. The purpose of this study is to build a model that will allow the quantification of the resistance landscape in Washtenaw County so that we can eventually predict Winter 2022-23 COVID-19 burden and explore counterfactuals around vaccine uptake and variant emergence.

METHODS

Proof-of-Concept Model

This study is in the proof-of-concept stage. As such, the overall model structure is finalized, but the parameters presented in this chapter are toy parameters for confirming the functionality of the model. More details about parameters can be found in Table 5.1. How the toy values will be replaced in the final working model is discussed in Future Directions. All modeling and analyses were completed in R version 4.1.1. All data was obtained from the Michigan Disease Surveillance System (MDSS), excluding those reported in the Michigan Department of Corrections system. MDSS data were accessed via a data use agreement between the University of Michigan and the Michigan Department of Health and Human Services.

S-R Model Structure

This study uses a Susceptible-Resistant model with four categories of decreasing resistance (Fig 5.1). For this version of the model, resistance levels have been set such that hosts in R = 100% resistant (relative probability of infection vs. susceptible individuals = 0.0), R_1 = 75% resistant (relative probability of infection = 0.25), R_2 = 50% resistant (relative probability of infection = 0.50), and R_3 = 25% resistant (relative probability of infection = 0.75). Hosts move between

resistance categories at rate τ_i and from R_3 to S at rate τ_4. Hosts in S, R_1, R_2, and R_3 can become infected and thus move back to the R category at rate $\beta_i(t)$. Rate of vaccination is $\sigma(t)$, which also moves hosts into the R category. For this proof of concept, we do not distinguish between boosters and primary series vaccinations, and simply assume that any vaccination event moves individuals from S, R_1, R_2, or R_3 into R.

$$dS/dt = \tau_4 R_3 - \sigma(t)S - \beta(t) \quad \text{(eq. 1a)}$$

$$dR/dt = f_R(\beta(t) + \beta_1(t) + \beta_2(t) + \beta_3(t)) + \sigma(t)S + \sigma_1(t)R_1 + \sigma_2(t)R_2 + \sigma_3(t)R_3 - \tau \quad \text{(eq. 1b)}$$

$$dR_1/dt = \tau_1 R + f_{R1}(\beta(t) + \beta_1(t) + \beta_2(t) + \beta_3(t)) - \tau_2 R_1 - \sigma_1(t)R_1 - \beta_1(t) \quad \text{(eq. 1c)}$$

$$dR_2/dt = \tau_2 R_1 + f_{R2}(\beta(t) + \beta_1(t) + \beta_2(t) + \beta_3(t)) - \tau_3 R_2 - \sigma_2(t)R_2 - \beta_2(t) \quad \text{(eq. 1d)}$$

$$dR_3/dt = \tau_3 R_2 + (1 - f_R - f_{R1} - f_{R2})(\beta(t) + \beta_1(t) + \beta_2(t) + \beta_3(t)) - \tau_4 R_3 - \sigma_3(t)R_3 - \beta_3(t) \quad \text{(eq. 1e)}$$

Where the β_i terms are time varying as a function of cases per day, and defined as:

$$\beta = cases(t) \times S / (S + p_1R_1 + p_2R_2 + p_3R_3) \quad \text{(eq. 2a)}$$

$$\beta_1 = cases(t) \times p_1R_1 / (S + p_1R_1 + p_2R_2 + p_3R_3) \quad \text{(eq. 2b)}$$

$$\beta_2 = cases(t) \times p_2R_2 / (S + p_1R_1 + p_2R_2 + p_3R_3) \quad \text{(eq. 2c)}$$

$$\beta_3 = cases(t) \times p_3R_3 / (S + p_1R_1 + p_2R_2 + p_3R_3) \quad \text{(eq. 2d}$$

Confirmed COVID-19 cases were those reported to the Michigan Disease Surveillance System (MDSS), excluding those reported in the Michigan Department of Corrections system. Linear interpolation was used to convert the number of new COVID-19 cases detected each day to an instantaneous rate of cases(t). Finally, the $S/(S + p_1R_1 + \ldots)$ and $p_iR_i/(S + p_1R_1 + \ldots)$ terms are used to capture the fraction of resistant cases that come from each class at each timepoint.

Progressing the S-R model through SARS-CoV-2 variants

While it is true that variants co-exist, this coexistence is commonly short-lived (as illustrated in Fig. 5.2), thus this model runs through one variant at a time. The model is switched from the

parameter space of one variant to the parameter space of another on the date at which <50% of detected sequences are the old variant and >50% are the new variant of interest. In the model, this shift between variants instantaneously changes the distribution of hosts present in all model categories, which is achieved by multiplying the number of hosts in each category at the end of the old variant epidemic by the relative resistance to the new variant (Fig 5.3); this will be determined using data on cross-reactivity antibody titers to approximate hosts' resistance to the new variant/the amount of immune escape the new variant has evolved.

This method allows the model to reflect how population-level resistance via vaccination, boosters, infection, and re-infection is impacted by naturally waning immunity in hosts as well as the level of immune escape unique to each SARS-CoV-2 variant. In future work, the model will be parameterized differently for each variant using best available data (i.e. parameters τ_i, p_i, and fR_i will be specific to each variant; see Future Directions for more details).

Testing model using prevalence data from the original and Alpha variant waves

To test the functionality of the model, prevalence data from the original or "non-variant" variant of SARS-CoV-2 was used. The model was then switched to reflect the arrival of the Alpha variant (B.1.1.7 lineage); this switch can be seen as the instantaneous reduction in the R category in Fig 5.2 on day 371. **Original "non-variant" variant:** The total population was set equal to 10,077,331 (total Michigan population as of April 1, 2020 according to Michigan state census data). Vaccination rate ($\sigma_i(t)$) = 0 from initiation of the model to day 306, which equates to January 5 2021, the first date of recorded public vaccinations in Michigan. We only consider primary series vaccinations since this simulation only runs through the Alpha wave. Also, most individuals who initiate do complete their primary series, so for simplicity in this proof of concept we used initiation of vaccination as proof of full vaccination. Publicly available data indicates 17.6% of the Michigan population had initiated vaccines and 10.1% had completed the

primary vaccine series by March 7 2021, which is 61 days from January 5 2021. Vaccination rate was calculated using the same method we used to calculate transmission rate; i.e. linear interpolation was used to convert the number of new initiated vaccination series each day to an instantaneous rate of cases(t). **Alpha variant:** The Alpha variant was the dominant variant in Michigan SARS-CoV-2 transmission events from March 7, 2021 to June 27, 2021. As of June 27 2021 48% of the population had initiated the primary vaccination series and 41.8% had completed it. Vaccination rate calculations were completed using the same linear interpolation method as used for the Original variant vaccination regime.

RESULTS

The model sufficiently partitions Michigan's population into the set resistance categories and progresses the population through those categories in a way that accurately encompasses the state population size, total SARS-CoV-2 prevalence, and vaccination rates set by the modelers (Table 5.2). The model also has the capacity for future expansion to allow for exploration of counterfactuals as well as predictions of near-future SARS-CoV-2 dynamics.

DISCUSSION & FUTURE DIRECTIONS

Parameter estimates

Each variant of SARS-CoV-2 will be assigned its own parameter values based on the best available data. A burden analysis modified from Petrie *et al.* 2022 will allow us to determine the fraction of confirmed cases that are due to each variant during phases where two variants are co-circulating. Level of immune protection (p_i) will be determined by correlating the probability of reinfection with existing data on the cross-reactivity of neutralizing antibodies elicited by infection and/or vaccination (Fig 5.5 gives an example for how this correlation is found; Bekliz et al., 2022; Gilbert et al., 2022; McLean et al., 2022; Suryawanshi & Ott, 2022). Latent (L) and

Infected (I) categories will be added to the model. Infection rate will be found using exponential interpolation to convert daily infection rates to an instantaneous infection rate. Rate of immune waning will be determined by data on the rate at which cross-reactivity of neutralizing antibodies wanes over time. Vaccination and booster rates will be calculated using exponential interpolation to convert daily rates into instantaneous rates of vaccination.

Future analyses

The updated model will be run through the periods of time where the Original, Alpha, and Delta strains were dominant. It will be parameterized for the Omicron variant using data that was available early in the outbreak (to reflect how this model would be used to predict newly-arisen variants). The model will then be run and its predictions will be measured against real data on the Omicron outbreak to assess the accuracy of its prediction capacities. After this point, we can explore answers to the question "What if a new variant with *x*, *y*, or *z* properties emerges?" In an extension of this, this model can be used to contribute to the COVID-19 Scenario Modeling Hub, a global modeling effort which sets forth a specified set of scenarios and target outcomes to allow alignment of model projections for collective insights. This model will also be used to run counterfactual scenarios, such as different vaccination uptake in 2021. Many of these counterfactuals can be achieved through sampling the matrix space of *newvariantmatrix* values, which will allow us to explore a range of possible disease dynamics across a landscape of realistic combinations of immune waning and immune escape.

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FIGURES

Table 5.1. COVID-19 model parameter values.

Parameter	Symbol	Value (OS pre-vaccination)	Value (OS post-vaccination)	Value (Alpha)
Level of immune protection for R_1	p_1	0.75	0.75	0.75
Level of immune protection for R_2	p_2	0.5	0.5	0.5
Level of immune protection for R_3	p_3	0.25	0.25	0.25
Rate at which people are infected from S	β	caserate(t)*S/(S + p1*R1+ p2*R2 + p3*R3)	caserate(t)*S/(S + p1*R1+ p2*R2 + p3*R3)	caserate(t)*S/(S + p1*R1+ p2*R2 + p3*R3)
Rate at which people are infected from R_1	β_1	caserate(t)*p1*R1/(S + p1*R1+ p2*R2 + p3*R3)	caserate(t)*p1*R1/(S + p1*R1+ p2*R2 + p3*R3)	caserate(t)*p1*R1/(S + p1*R1+ p2*R2 + p3*R3)
Rate at which people are infected from R_2	β_2	caserate(t)*p2*R2/(S + p1*R1+ p2*R2 + p3*R3)	caserate(t)*p2*R2/(S + p1*R1+ p2*R2 + p3*R3)	caserate(t)*p2*R2/(S + p1*R1+ p2*R2 + p3*R3)
Rate at which people are infected from R_3	β_3	caserate(t)*p3*R3/(S + p1*R1+ p2*R2 + p3*R3)	caserate(t)*p3*R3/(S + p1*R1+ p2*R2 + p3*R3)	caserate(t)*p3*R3/(S + p1*R1+ p2*R2 + p3*R3)
Fraction of infections that end up in R	fR	0.5	0.5	0.5

Fraction of infections that end up in R_1	fR_1	0.25	0.25	0.25
Fraction of infections that end up in R_2	fR_2	0.15	0.15	0.15
Fraction of infections that end up in R_3	fR_3	(1 - fR - fR1 - fR2)	(1 - fR - fR1 - fR2)	(1 - fR - fR1 - fR2)
Rate of immune waning ($R \rightarrow R_1$)	τ_1	1/(30.437)	1/(30.437)	1/(30.437)
Rate of immune waning ($R_1 \rightarrow R_2$)	τ_2	1/(3*30.437)	1/(3*30.437)	1/(3*30.437)
Rate of immune waning ($R_2 \rightarrow R_3$)	τ_3	1/(3*30.437)	1/(3*30.437)	1/(3*30.437)
Rate of immune waning ($R_3 \rightarrow S$)	τ_4	1/(3*30.437)	1/(3*30.437)	1/(3*30.437)
Vaccination rate for S	$\sigma(t)$	0	vaxrate(t)	vaxrate(t)
Booster rate for R_1	$\sigma_1(t)$	0	vaxrate(t)	vaxrate(t)
Booster rate for R_2	$\sigma_2(t)$	0	vaxrate(t)	vaxrate(t)
Booster rate for R_3	$\sigma_3(t)$	0	vaxrate(t)	vaxrate(t)

Table 5.2. Cumulative confirmed cases vs. model output. Cumulative confirmed cases and vaccinations as found by the model match closely with real-world total cases and vaccinations.

	Model	**Data**
Cumulative confirmed cases (through Original strain)	817,737	818,455
Cumulative confirmed cases (through Alpha variant)	1,210,089	1,209,809
Cumulative vaccination	47.8%	48%

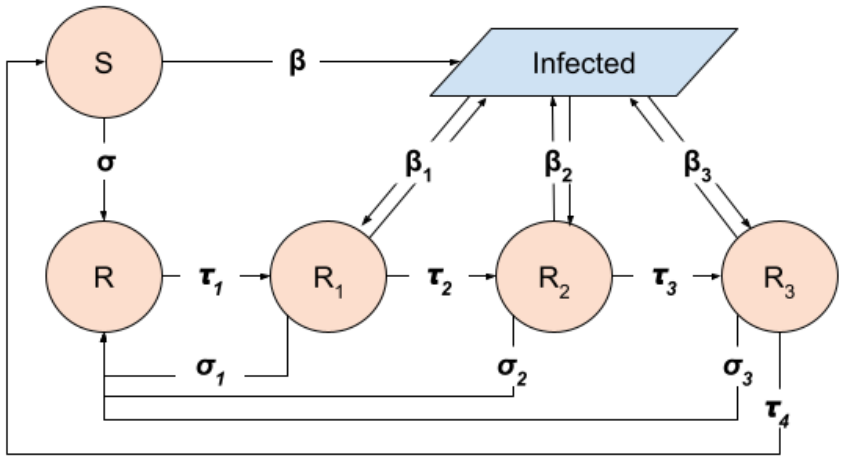

Figure 5.1. S-R model schematic.

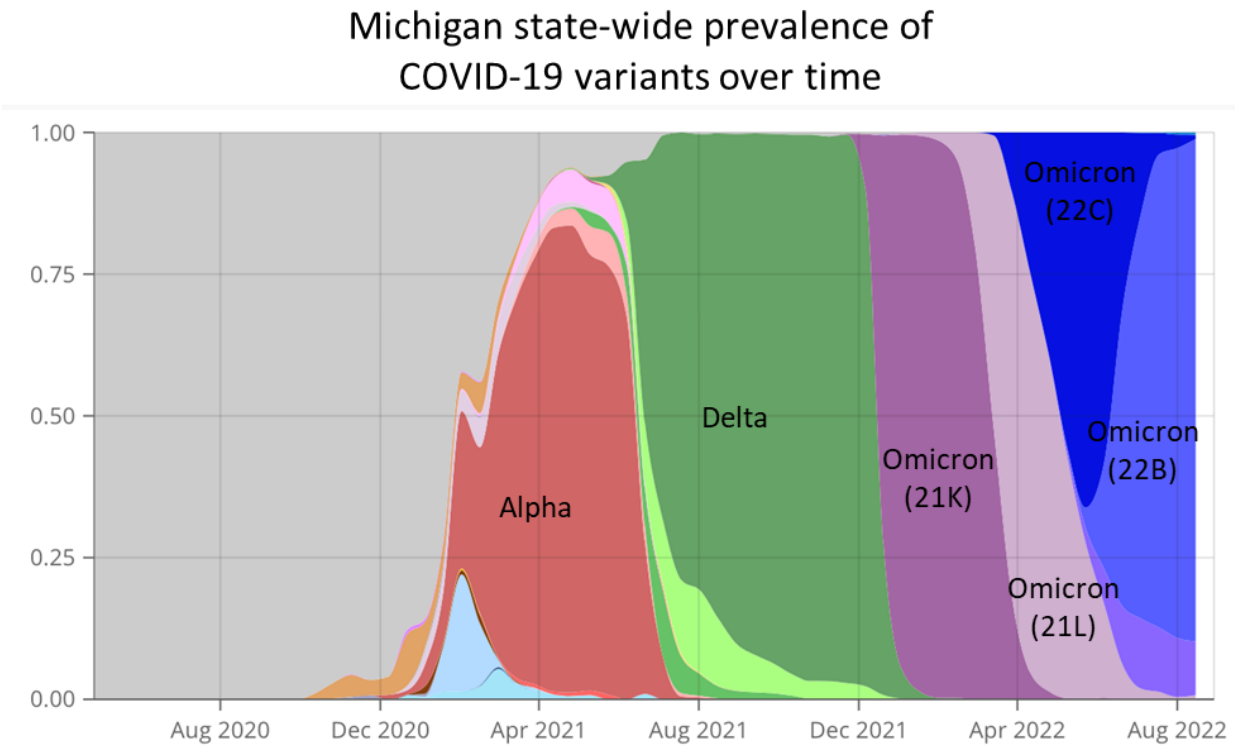

Figure 5.2. SARS-CoV-2 variant distribution over time from Michigan sequence data. New variants tend to invade rapidly and take over as the dominant variant. Data from covariants.org.

$$\begin{bmatrix} 1 & 0.00 & 0.10 & 0.25 & 0.50 \\ 0 & 0.50 & 0 & 0 & 0 \\ 0 & 0.25 & 0.50 & 0 & 0 \\ 0 & 0.15 & 0.25 & 0 & 0 \\ 0 & 0.10 & 0.15 & 0 & 0 \end{bmatrix} \times \begin{bmatrix} S \\ R \\ R_1 \\ R_2 \\ R_3 \end{bmatrix}$$

Figure 5.3. Example matrix. Matrix math used to transition the population so that R, R_1, R_2, and R_3 proportions change when a new variant becomes dominant.

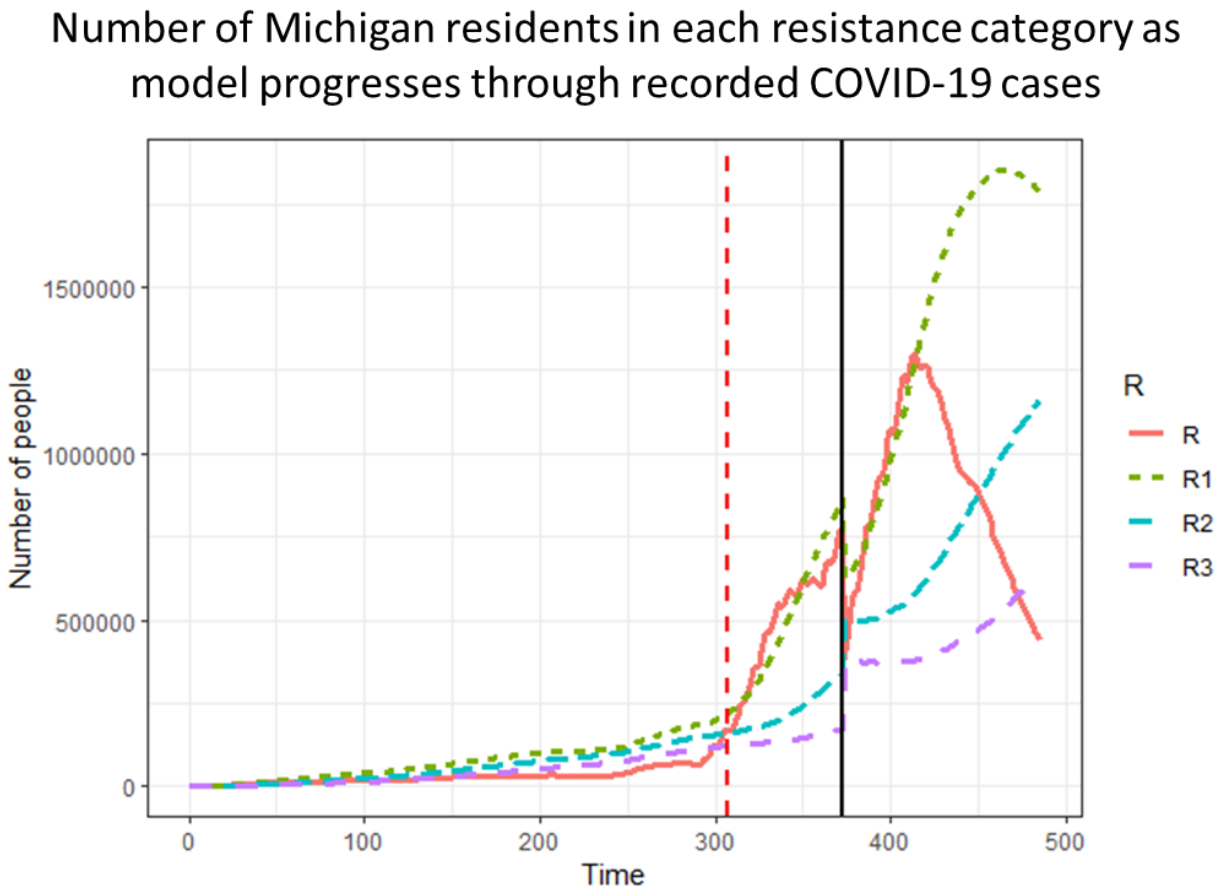

Figure 5.4. Number of Michigan residents in each resistance category over time. Changes in the number of people distributed across the different resistance categories R, R_1, R_2, and R_3 (i.e. proof-of-concept model outcomes) are displayed above. Public vaccination began on the date indicated by the dashed red line. The dominant variant switched from the Original Strain (OS) to the Alpha variant on the date indicated by the solid black line.

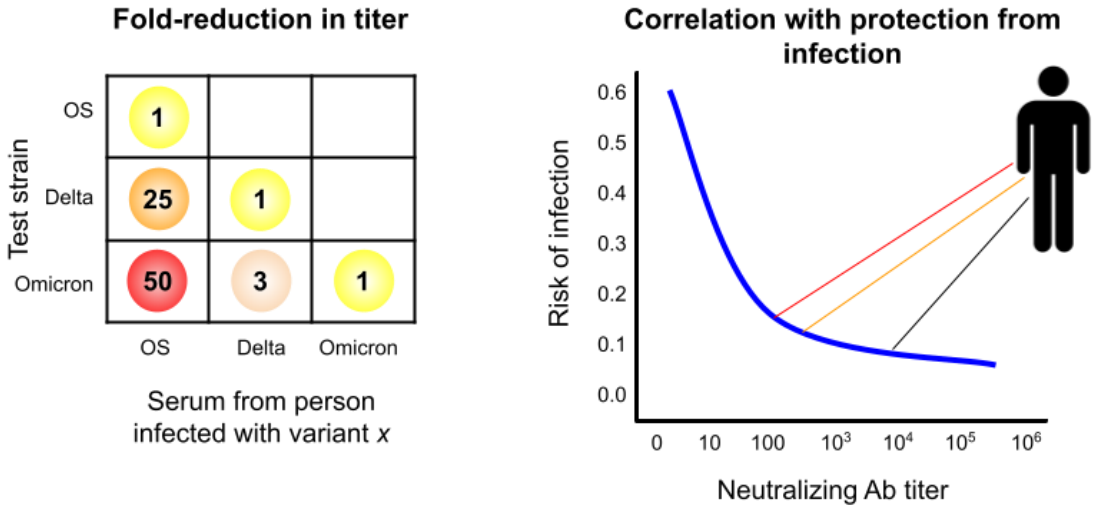

Figure 5.5. How to correlate neutralizing antibody titers and level of immune protection: an example of how neutralizing antibody titers can be correlated with protection from infection. The person above is infected with the original variant of SARS-CoV-2 (OS) and has 10k titer after infection. Based on the heat map to the right, they should have 25-fold lower ability to neutralize Alpha (titer = 400), and 50-fold lower ability to neutralize Omicron (titer = 200).

CHAPTER 6:
Conclusions

SUMMARY

The tension between parasite infectivity and host resistance to infection is foundational to host-parasite ecology and evolution. In this dissertation, I studied how ecological and evolutionary forces drive changes to parasite infectivity and host defenses (McLean et al. 2022, McLean et al. 2022), and how these changes scale up to impact disease dynamics in two different host-parasite systems (Chapters 4 and 5). The first was the *Daphnia dentifera*-microparasite system, which has been established as a model system for the study of ecology and evolution of infectious disease due to its experimental tractability as well as the significant ecological importance of *Daphnia* to freshwater ecosystems. The second was the SARS-CoV-2 outbreak in the state of Michigan (USA), from March 2020 to May 2022. This local snapshot of the COVID-19 pandemic provided important insights into ongoing drivers of this disease as it transitions from pandemic to endemic disease.

First, I reviewed how host-parasite evolution is modulated by the broader ecological context in which host-parasite interactions (including infectivity and resistance) are embedded. Next, I tested how sexual recombination and gene flow impacted the evolution of host resistance in wild invertebrate hosts. Third, I used empirical and modeling methods to investigate density-dependent and -independent interspecific interaction effects on the infectivity of two co-existing parasites. Finally, I built an epidemiological model of SARS-CoV-2 dynamics which incorporated waning immunity (i.e. reduction in host immunity over time) and immune escape (i.e. the evolution of immune evasion/increased infectivity in the virus) to allow for more accurate prediction of COVID-19 dynamics as the virus become endemic and the immune landscope become increasingly complex.

I used a combination of approaches to conduct the research outlined above. Each research chapter was based on epidemics observed in nature and combined observational data, experimental methods, and/or disease simulation modeling. To understand the effects of recombination and gene flow on host resistance, I used field observations, laboratory experiments, and molecular methods. Specifically, I took advantage of Daphnia dentifera's parthenogenetic lifecycle and sampled the two lake populations before and after sexual reproduction and before and after temporal gene flow from the egg bank. I used laboratory infection experiments to assign resistance phenotypes to each individual host, plus used molecular methods to find the genotype of each individual host. For my next study in the *Daphnia*-microparasite system I used parasite exposures to measure density-dependent effects on infectivity, transmission, and virulence. I used this experimental data to parameterize a model, which allowed me to see how the phenomena observed in the individual hosts could scale up in real life epidemics. Finally, for research on SARS-CoV-2 dynamics, I used a combination of observational (i.e. public health) data and epidemiological transmission models. These models are built to incorporate data on immune waning and immune evasion to predict SARS-CoV-2 dynamics in an age of complex immune landscape and endemic COVID-19.

Taken together, my work indicates that a complex suite of biotic and abiotic factors determine how parasite infectivity and host resistance vary over time and space. In turn, the dance between infectivity and resistance shapes epidemic dynamics and disease distributions. Below I summarize and connect my studies and point to future directions.

Chapter 2. *Ecological Context Influences Evolution in Host-Parasite Interactions: Insights from the Daphnia-Parasite Model System.*

Parasites exert strong selective pressure on their hosts, and many hosts can evolve rapidly in response. As such, host-parasite interactions have a special place in the study of contemporary evolution. However, these interactions are often considered in isolation from the ecological contexts in which they occur. I reviewed different ways in which the ecological context of host-

parasite interactions can modulate their evolutionary outcomes in important and sometimes unexpected ways. Specifically, predation, competition, and abiotic factors can change the outcome of contemporary evolution for both hosts and parasites.

Chapter 3. *Sexual Recombination and Temporal Gene Flow Maintain Host Resistance and Genetic Diversity.*

Hosts can rapidly evolve resistance during epidemics, and this evolution is usually modulated by fitness trade-offs (e.g., between resistance and fecundity). However, many organisms switch between asexual and sexual reproduction, and this shift in reproductive strategy can also alter how resistance in host populations persists through time. *Daphnia dentifera* experience strong selective pressure to evolve resistance to a virulent fungal parasite *Metschnikowia bicuspidata*. In fact, *D. dentifera* has been shown to evolve increased resistance over the course of a single large epidemic. Despite the fact that we can detect this rapid evolution, host populations in the wild show a wide range in resistance; i.e. some *Daphnia* remain very susceptible to the fungus while other *Daphnia* remain totally resistant. If there is relatively consistent selection for resistance, how is such variety maintained? I explored two factors that we expected to work against selection for host resistance, and therefore maintain variation in the trait: sexual reproduction and temporal gene flow. My findings highlight the importance of recombination and germ banks in maintaining genetic diversity in asexual or cyclically parthenogenetic organisms. Furthermore, we found that both factors likely underpin interannual dynamics of resistance in germ banking organisms.

Future studies monitoring the genotypic and phenotypic values of populations across multiple sequential (seasonal) extinction-recolonization events, while also tracking epidemiological dynamics, would help determine the generality of my findings while better connecting rapid interannual selection dynamics with longer-term evolution.

Chapter 4. *Exposure Dose Alters Within-Host Interactions Between Co-Infecting Parasites, with Consequences for Parasite Prevalence and Host Abundance.*

Parasite dose and the presence of multiple parasite species both have important consequences for infection dynamics, but it is not clear how these aspects of infection biology interact — though they likely do. We disentangled these effects and their interactions by conducting a factorial dose experiment and using it to parameterize dose-dependent multiparasite epidemic models.

The parasites in my study system (one bacteria and one fungus) form environmental spores which degrade at variable rates; this creates ranges of parasite doses over time and space. We used factorial exposures to quantify how different combinations of spore doses from the two parasites impacted likelihood of infection, host lifespan, and transmission potential. We found bacterial infection was strongly influenced by both bacterial dose (which positively related to infection) and fungal dose (with higher fungal doses reducing bacterial infections). In contrast, fungal likelihood of infection was determined by fungal dose alone. Impacts on host lifespan and parasite fitness were strong, though dose independent: infection with the fungus significantly shortened host lifespans and, when co-infecting with the bacterial parasite, drastically reduced bacterial fitness.

Epidemic models revealed that, while the bacterium suffered a fungus-dose-dependent reduction in its probability of infection, this negative effect was mitigated at the level of host populations. In fact, when this negative effect on the bacterium was removed from the model, total bacterial prevalence further declined and host populations increased. Ultimately, my work underscores how negative dose-dependent interactions between parasites can scale up to alter parasite burdens and host abundance in unexpected ways.

Future work could incorporate observational data on overlapping epidemics of environmentally transmitted parasites to link my experimental understanding to real-world scenarios. This work would be challenging--it would require the ability to accurately measure environmental spore density in the field, and a multitude of overlapping epidemics would need to be sampled (since one instance of simultaneous epidemics would be a single unit of replication). Alternatively, a meta-analysis of pre-existing research on spore density and overlapping

epidemics of environmentally transmitted parasites could provide some insight into the generalizability of this study.

Chapter 5. *A Model for Incorporating Waning Immunity and Immune Escape to Better Predict Winter 2022-23 COVID-19 Burden and Explore Counterfactuals Around Vaccine Uptake and Variant Emergence.*

Existing models of SARS-CoV-2 dynamics assume a simple immune landscape; i.e., that hosts have no immunity or full immunity. This assumption becomes increasingly inaccurate as the pandemic wears on, particularly as the virus continues to evolve and people gain immunity from multiple different sources. Transmission models that incorporate immune waning rate as well as variant-specific immunity are necessary to predict SAR-CoV-2 dynamics in the future. This would also allow for a more mechanistic understanding of past COVID-19 outbreaks and enables the exploration of counterfactuals (i.e. what if primary vaccination occurred at different points in time or at different rates).

This study uses a Susceptible-Resistant model with four categories of decreasing resistance and varying levels of immune protection. This proof-of-concept model sufficiently partitions Michigan's population into the set resistance categories and progresses the population through those categories in a way that accurately encompasses the state population size, total SARS-CoV-2 prevalence, and vaccination rates set by the modelers.

In the future, resistance and immune protection will be parameterized using the antigenic relationship between variants (via the correlation between neutralization titer measurements and risk of re-infection). Latent and Infected state variables will be added to the model and prevalence of SARS-CoV-2 variants will be calculated using a burden analysis approach (Petrie et al., 2022). The model will be trained using data from past strain dynamics (original, alpha, and delta variants) and tested against current data on the ongoing omicron outbreaks. This model will be used to both explore counterfactual scenarios of past vaccination strategies as well as predict SARS-CoV-2 dynamics in Winter 2022 into Spring 2023.