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Context Dependence, Cheating, and Long-term Conflict in Social Interactions

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by
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ABSTRACT OF THE DISSERTATION

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Professor David Queller, Chair

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Social interactions are widespread in nature and can have important ecological and evolutionary consequences. Interactions are often categorized by who is benefited or harmed by the symbiosis. For example, if both sides benefit, the interaction is cooperative. If one side benefits at the expense of the other, it is an antagonism. The kind of interaction can have important evolutionary implications, but interactions rarely fit neatly into these interaction categories. Instead, interactions often involve elements of cooperation and conflict.

My dissertation explores the consequences of interactions and how they can involve both cooperation and conflict using a range of experimental, genomic, and theoretical methods. Experimental work in this dissertation involves the symbiosis between *Dictyostelium discoideum* amoebae and *Paraburkholderia* bacteria. Previous work on this symbiosis showed that host *D. discoideum* can benefit or be harmed by symbiosis with *Paraburkholderia* depending on whether edible bacteria are present, but the effect of this context on the inedible *Paraburkholderia* symbionts is unknown.

In my first chapter, I show that two species of *Paraburkholderia* symbionts are also affected by the presence of bacteria that are edible for hosts. *Paraburkholderia* grow to higher densities when food bacteria are scarce. Moreover, on the host side, I use simulations to show that symbiosis may be an adaptation for living in harsh soils with variation in the number of edible bacteria. I follow up on this idea in my second chapter by using host-symbiont co-occurrence data to test whether the prevalence of symbiosis is associated with variable soil conditions. I found that the prevalence of two *Paraburkholderia* species is associated with variable rainfall. In my third chapter, I investigate how food bacteria are carried or left behind during the symbiosis between *D. discoideum* and *Paraburkholderia*. This chapter shows that *Paraburkholderia* causes hosts to leave food bacteria uneaten, but that hosts gain the ability to carry food bacteria more often in food-poor environments.

In my fourth chapter, I turn to interactions between members of the same species and test the hypothesis that pleiotropy can stabilize cooperation. Using genomic data from *Pseudomonas aeruginosa*, I found that quorum sensing genes involved in cooperation were more pleiotropic than genes involved in private functions. These results with *P. aeruginosa* support a role for pleiotropy in stabilizing cooperation.

In my last chapter, I use theoretical models to understand how evolutionary conflicts affect long-term evolution. I show that conflict results in arms race dynamics that affect fitness and the kinds of mutations that become fixed in populations. The results from this dissertation advance our knowledge of how context affects symbiotic interactions, the stability of cooperation, and the long-term consequences of conflict.

Chapter 1: Introduction

1.1 Cooperation and conflict in social interactions

Interactions between organisms, of the same or different species, are ubiquitous in nature (Janzen, 1985; McFall-Ngai et al., 2013; Thompson, 2013). These interactions have wide ranging consequences on ecosystems and humans. Some allow organisms, like ants, to dominate their environments (Wilson & Hölldobler, 2005). Others, like human pathogens or plants and their soil microbes, can affect human health and well-being (Duhamel & Vandenkoornhuyse, 2013; Morais et al., 2021).

Interactions between two partners are often categorized using an interaction grid (Table 1.1). These 2 by 2 grids break interactions into categories based on how partners affect each other (Bronstein, 1994). For example, if both receive benefits from the interaction, it is cooperative. If both are harmed by the interaction, it is competitive. When one partner benefits at the expense of another, it is antagonistic. Antagonistic interactions include things like predatory-prey, host-parasite interactions, and cheating. These antagonistic interactions can be thought of more broadly as examples of evolutionary conflicts, where both sides have divergent interests (Queller & Strassmann, 2018).

Table 1.1: Interaction grid. The effect on partner 1 is shown in the top columns. The effect on partner 2 is shown in the rows. Interactions are then categorized in the grid.

	Partner 1 benefits	Partner 1 is harmed
Partner 2 benefits	Cooperation	Antagonism/Conflict

Partner 2 is harmed	Antagonism/Conflict	Competition

Whether interactions involve cooperation, competition, or conflict has important implications for how an interaction evolves (Queller & Strassmann, 2018). In this dissertation, I will focus mostly on these implications for cooperation and conflict. One question for cooperative interactions is how cheaters are kept at bay (Gilbert et al., 2007; Jones et al., 2015; Bentley et al., 2022). Cheaters benefit from a cooperative interaction without performing a costly cooperative act. Because cheaters avoid the costs of cooperation while getting the benefits, they can take over in cooperative populations and make cooperation unstable.

When partners are in conflict, they should tend to evolve to minimize the costs of conflict. This can result in arms race dynamics, where partners adapt back and forth (Dawkins & Krebs, 1979; Daugherty & Malik, 2012; Brockhurst et al., 2014; Queller & Strassmann, 2018). Some of these arms race dynamics are thought to be responsible for exaggerated phenotypes and for genomic signals of strong selection (Paterson et al., 2010; Fumagalli et al., 2011).

While it can sometimes be useful to categorize interactions using an interaction grid, interactions rarely fit nicely in a single category. Interactions can move from one area of the grid to another due to evolution or a change in ecological conditions (Ewald, 1987; Bronstein, 1994; Herre et al., 1999; Chamberlain et al., 2014; Keeling & McCutcheon, 2017; Drew et al., 2021). For example, when large herbivores were excluded from sites with Acacia plants, plants increased antagonisms with ants that ordinarily protect plants from herbivores (Palmer et al., 2008). Similar context-dependence in interactions is common (Chamberlain et al., 2014) and ensures that interactions are a mix of cooperation and conflict.

This dissertation focuses on the consequences of interactions and how interactions can involve elements of both cooperation and conflict. In the first three chapters, I explore the consequences of context-dependence in the symbiosis between the social amoeba *Dictyostelium discoideum* and its *Paraburkholderia* symbionts. *D. discoideum* is well-known as a model organism for cooperation because of its social cycle (Medina et al., 2019; Jahan et al., 2021). During this social cycle individual amoebae aggregate together to form a fruiting body that disperses spores (Kessin, 2001). *Paraburkholderia* bacteria were discovered inside *D. discoideum* fruiting bodies and were found to allow hosts to carry edible bacteria through the social cycle (Brock et al., 2011, 2016; DiSalvo et al., 2015; Khojandi et al., 2019). However, having *Paraburkholderia* is also costly for hosts in some situations. In this dissertation, I investigated the how the environmental context affected cooperation and conflict between *D. discoideum* and *Paraburkholderia*.

In the fourth chapter, I investigate genetic mechanisms that may stabilize cooperation in the face of cheaters in *Pseudomonas aeruginosa*. One proposed mechanism for stabilizing cooperation is pleiotropy, the tendency for a single gene to affect multiple phenotypes. Cheater mutations that are pleiotropic should damage other important traits and make cheating maladaptive (Foster et al., 2004; Bentley et al., 2022). As a result pleiotropy may be higher in genes involved in cooperative traits. I tested this idea using genomic data in *Pseudomonas aeruginosa*.

In the fifth and final chapter, I use computer simulations to model the long-term consequences of evolutionary conflicts. Most modeling of conflict has focused on short-term changes in allele frequencies or quantitive genetic variation (Gavrilets, 1997; Gomulkiewicz et al., 2000; Nuismer et al., 2005; Nuismer, 2017). These models do not address the long-term consequences of

conflict across numerous fixations of new mutations. I combined Fisher's geometric model (Tenaillon, 2014) with joint phenotypes (Queller, 2014; Queller & Strassmann, 2018) to fill this gap and model the long-term consequences of evolutionary conflict.

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Chapter 2: Context-dependence in the symbiosis between *Dictyostelium discoideum* and *Paraburkholderia*

Trey J. Scott, David C. Queller, and Joan E. Strassmann

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2.1 Abstract

Symbiotic interactions change with environmental context. Measuring these context-dependent effects in hosts and symbionts is critical to determining the nature of symbiotic interactions. We investigated context-dependence in the symbiosis between social amoeba hosts and their inedible *Paraburkholderia* bacterial symbionts, where the context is the abundance of host food bacteria. *Paraburkholderia* have been shown to harm hosts dispersed to food-rich environments, but aid hosts dispersed to food-poor environments by allowing hosts to carry food bacteria. Through measuring symbiont density and host spore production, we show that this food context matters in three other ways. First, it matters for symbionts, who suffer a greater cost from competition with food bacteria in the food-rich context. Second, it matters for host-symbiont conflict, changing how symbiont density negatively impacts host spore production. Third, data-based simulations show that symbiosis often provides a long-term fitness advantage for hosts after rounds of growth and dispersal in variable food-contexts, especially when conditions are harsh with little

food. These results show how food context can have many consequences for the Dictyostelium-*Paraburkholderia* symbiosis and that both sides can frequently benefit.

2.2 Impact Statement

Many organisms form symbiotic relationships with other species. These symbioses often exhibit context-dependence, where the sign or magnitude of one partner's effect on the other will change in different environments. Context-dependent effects make it difficult to assign interactions to categories like mutualisms or antagonisms because they involve both benefits and costs depending on the environment. However, in some cases, accounting for context-dependence can clarify an interaction so that it more easily fits a mutualism or antagonism. We investigated context-dependence using the symbiosis between *Dictyostelium discoideum* and two symbiotic *Paraburkholderia* species. In this symbiosis, *Paraburkholderia* bacteria allow hosts to carry food bacteria to food-poor contexts, where hosts rarely survive without food, but reduce host fitness in the more hospitable food-rich contexts. The effect of food context on *Paraburkholderia* symbionts is unknown. We show that *Paraburkholderia* symbionts are also affected by this context, through facing reduced competition after being dispersed by hosts to food-poor contexts. We also identify a new way that symbionts affect hosts, where symbiont density reduces host fitness, but less so in food-poor contexts. Finally, we use simulations to show that infected hosts benefit in the long-term across variable food contexts, especially in the harshest environments with little food. These results show that context-dependence in symbiosis can have many consequences for hosts and symbionts, though in general for *D. discoideum* and *Paraburkholderia*, both are likely to benefit.

2.3 Introduction

Context-dependence, where the environment can change the sign or magnitude of one partner's effect on the other, is common in symbioses (Bronstein, 1994; Thompson, 1994; Chamberlain et al., 2014). These context-dependent effects on partners can be crucial to understanding the nature of symbiotic interactions (Keeling & McCutcheon, 2017; Iwai, 2019). For example, in the symbioses between Paramecium bursaria hosts and their Chlorella endosymbionts, hosts benefitted from symbiosis in light environments, but were harmed in the dark. For Chlorella, the effects of symbiosis were negative in co-culture, indicating that hosts exploited their endosymbionts for the benefits hosts receive in light conditions (Lowe et al., 2016). However, in the context of an environment with a Chlorella competitor, hosts benefited their symbionts by eating these competitors (Iwai, 2019). This example illustrates that understanding how partners affect each other across multiple contexts can change our view of the interaction, sometimes from one of exploitation to one of mutual benefit.

Context-dependence is important in the lifecycle of the social amoeba *Dictyostelium discoideum.* Amoebae need edible bacteria to grow and proliferate (Raper, 1937), but the abundance (Young, 2004; Vos et al., 2013) and quality (Kuserk, 1980; Brock et al., 2018) of food bacteria in the soil is known to vary. This results in a patchy environment where some patches are food-rich and other patches are food-poor. In response to starvation, amoebae aggregate and form a multicellular fruiting body to disperse resistant spores to new environments (smith et al., 2014). The patchy soil environment is considered an important selection pressure for this fruiting body structure (Bonner, 1982; Kessin, 2001).

D. discoideum interacts with three species of mostly inedible *Paraburkholderia* bacterial symbionts — *P. agricolaris*, *P. hayleyella*, and *P. bonniea* (Brock et al., 2020). Throughout this paper, we will use "*Paraburkholderia*" or "symbionts" as shorthand for the three symbiotic *Paraburkholderia* species. Hosts infected with *Paraburkholderia* have been isolated from multiple locations in the United States, with around 25% of screened hosts being infected by at least one species (Haselkorn et al., 2019). *Paraburkholderia* are able to enter and live inside *D. discoideum* cells and spores, but can also proliferate, albeit sometimes only slowly, without their hosts (DiSalvo et al., 2015; Shu et al., 2018a; Brock et al., 2020) unlike the obligate endosymbionts that are also found in *D. discoideum* (Haselkorn et al., 2021). There is some evidence consistent with coevolution between hosts and symbionts (Brock et al., 2016; Shu et al., 2018a; Garcia et al., 2019; Brock et al., 2020). For example, host clones naturally infected with *P. hayleyella* are harmed less by infection with this symbiont than host clones that were not infected in the wild (Shu et al., 2018a) indicating that *P. hayleyella* hosts have adaptations favoring symbiosis. Symbionts also have the ability to move towards hosts (Shu et al., 2018b), suggesting that being able to find hosts is beneficial.

The symbiosis with *Paraburkholderia* bacteria impacts the growth and proliferation of *D. discoideum*. Having symbionts allows hosts to carry food bacteria (and inedible *Paraburkholderia*) inside the spore-containing part of fruiting bodies called the sorus (DiSalvo et al., 2015). Whether this novel trait is advantageous or not depends on the presence of food bacteria after dispersal. When food is abundant, having symbionts can be costly, as shown by infected amoebae producing fewer spores than uninfected amoebae (Brock et al., 2011; DiSalvo et al., 2015). In food-poor environments, the cost of having *Paraburkholderia* is compensated by hosts gaining the ability to carry food bacteria in dispersing spores. This allows amoebae to

disperse and grow where they ordinarily could not (Brock et al., 2011; DiSalvo et al., 2015). These context-dependent effects on the host could be extremely important in the natural soil environment, where food-poor patches arise frequently (Kessin, 2001).

Less is known about how symbionts are affected across food contexts. Gaining the ability to disperse to new locations may be a major reason for symbionts to seek out social amoeba hosts (Garcia & Gerardo, 2014), but could also make the context of host food bacteria in the new environment important for symbionts. A benefit from being dispersed to patches with few bacteria could be that symbionts face reduced competition. If few bacteria are present, symbionts will mostly compete with food bacteria that were also carried in the sorus. This should be a relatively low competition situation because symbionts outnumber food bacteria in sori (Khojandi et al., 2019). Having few competitors should advantage symbionts while environments with plentiful bacteria could strongly limit symbiont growth because of their relatively slow growth rates, at least as measured in the lab (Brock et al., 2020). We will use "food-rich" and "food-poor" to describe newly colonized patches with many and few bacteria, respectively, of the sort edible by *D. discoideum*. These categories reflect the relationship to *D. discoideum* and could be called high and low competition in terms of their effect for *Paraburkholderia*.

It is unclear how the number of extracellular *Paraburkholderia* in the environment impacts hosts since previous studies have focused on intracellular *Paraburkholderia* (Shu et al., 2018b; Miller et al., 2020). When they are outside the amoebae, *Paraburkholderia* could affect *D. discoideum* fitness through interactions with food bacteria perhaps by reducing the amount of food for hosts through competition or by releasing diffusible toxins that affect amoebae. Thus host food context could also affect the relationship between symbiont density and host spore production.

The fitness effects of symbiosis for hosts have been tested only in food-poor and food-rich contexts individually. The benefits of symbiosis could pay out over the long-term across different food contexts in the soil. Growth rates in temporally variable contexts are best captured by geometric mean fitness rather than arithmetic mean fitness because only the geometric mean captures the lasting effects of periods of low fitness (Sæther & Engen, 2015). Ignoring geometric means can lead to incorrect assessments of the adaptive value of strategies in variable environments. One example of an adaptation that is only apparent from geometric mean fitness measures are bet-hedging phenotypes, where organisms adapt to uncertain environments by avoiding the worst effects of harsh contexts while being suboptimal in more favorable contexts (Slatkin, 1974; Philippi & Seger, 1989; Starrfelt & Kokko, 2012). This lowers the variance in fitness across time and results in higher geometric mean fitness at the expense of lower arithmetic mean fitness.

Bet-hedging is suspected to play a role in explaining observations of disadvantageous partnerships in plant-fungus mutualisms (Lekberg & Koide, 2014; Veresoglou et al., 2021). If bet-hedging occurs, short-term costs are acceptable if partnerships increase geometric mean fitness. Alternatively, symbiosis could increase both geometric and arithmetic mean fitness across contexts without the need for bet-hedging. In this case, the benefits of symbiosis simply outweigh the costs as in more traditional descriptions of symbioses (Douglas, 2010). However, these alternatives have not been tested in detail.

To understand context-dependence in the symbiosis between amoebae and *Paraburkholderia*, we used *D. discoideum* infected with either *P. agricolaris* or *P. hayleyella* — the two most common and best-studied species of *D. discoideum* symbionts. We investigate whether *Paraburkholderia* benefit from reduced interspecific competition when dispersed to

food-poor contexts, how symbiont density and food context impact host spore production, and whether symbiosis is beneficial for hosts when food conditions vary.

2.4 Methods

To understand the effects of symbiosis across food contexts, we used 4 naturally uninfected *D. discoideum* clones, 4 clones naturally infected with *P. agricolaris*, and 4 clones naturally infected with *P. hayleyella* (Figure 2.1). We cured the infected clones and re-infected them with their native symbionts to standardize infection density. Uninfected clones were left uninfected, but were otherwise treated the same as infected clones. This resulted in three host infection conditions: uninfected, infected with *P. agricolaris*, and infected with *P. hayleyella*. To mimic natural dispersal, we collected sori and transferred them to food-rich (with additional *K. pneumoniae* bacteria) or food-poor (KK2 buffer with no *K. pneumoniae*) nutrient plates. Bacteria appear on food-poor plates only if transferred sori contain bacteria, as expected for infected samples. We grew replicate experimental sets involving all conditions beginning on two separate dates, July 13 and 22, 2020, and followed up with additional experiments (see results) beginning on January 26 and April 23, 2021.

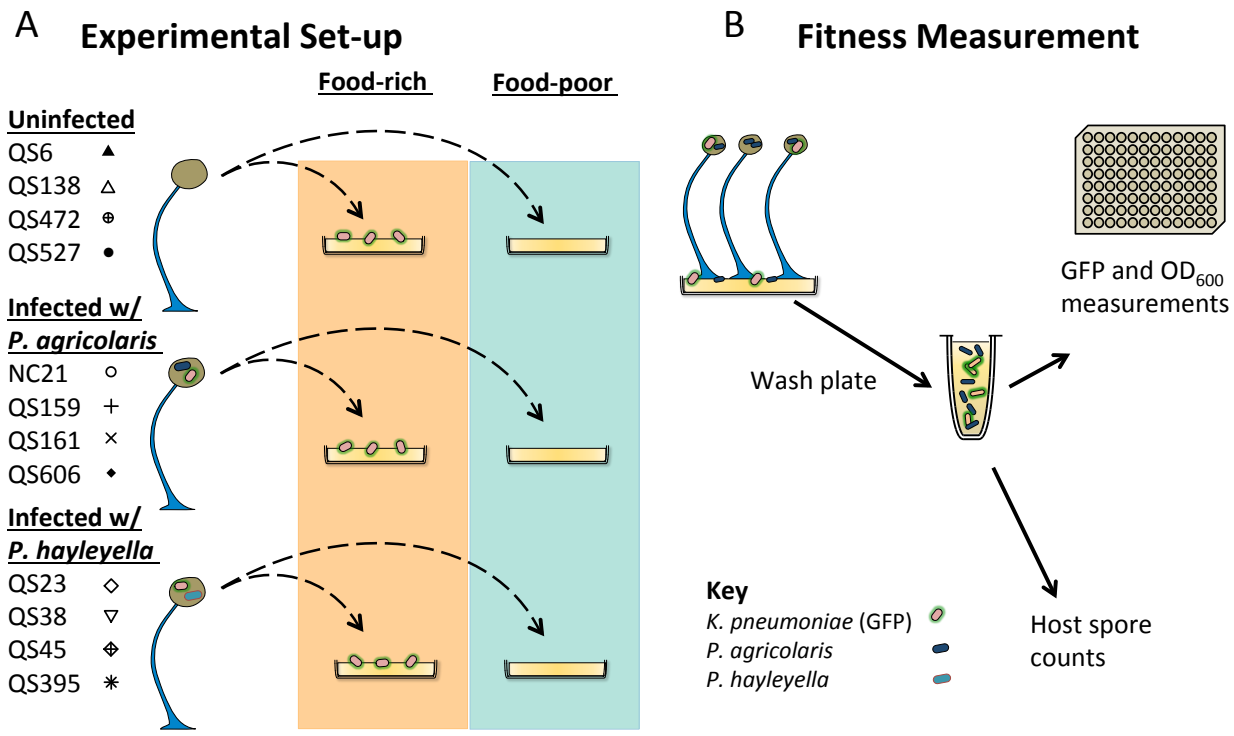

Figure 2.1: Schematic of experimental design. (A) Uninfected and infected *D. discoideum* fruiting bodies are collected and plated on food-rich and food-poor plates (after one passage on GFP-expressing *K. pneumoniae* food bacteria). These plates are grown for six days and then washed for bacterial measurement and spore counting (B). Bacteria are measured by calculating GFP fluorescence and optical density (see Methods). Host spore production is measured from washed plates.

Paraburkholderia isolation

To isolate *Paraburkholderia* from their hosts, we grew wild collected *D. discoideum* clones on SM/5 plates (2 g glucose (Fisher Scientific), 2 g Bacto Peptone (Oxoid), 2 g yeast extract (Oxoid), 0.2 g MgSO4 * 7H2O (Fisher Scientific), 1.9 g KH2PO4 (Sigma-Aldrich), 1 g K2HPO4 (Fisher Scientific), and 15 g agar (Fisher Scientific) per liter). Wild *D. discoideum* clones were grown with *K. pneumoniae* food bacteria that were suspended in KK2 buffer (2.25 g KH2PO4 (Sigma-Aldrich) and 0.67 g K2HPO4 (Fisher Scientific) per liter). After wild clones completed the social cycle (feeding, starvation, and fruiting body formation), we collected sori

with pipette tips and placed them on SM/5 plates. We allowed the bacteria and amoebae contained within to proliferate and then streaked out the resulting bacteria to get single colonies.

Paraburkholderia removal

To generate uninfected clones, we treated infected *D. discoideum* clones with antibiotics by plating on 30 μg/mL tetracycline SM/5 plates with 200 μL of 1.5 optical density (OD_{600}) tetracycline-resistant *K. pneumoniae* suspended in KK2 buffer. After passage on SM/5 plates without tetracycline to let the amoebae recover from any effects of the antibiotic, we collected single sori with a pipette tip and placed ten of them in different locations on SM/5 plates to confirm that we had successfully removed the bacteria. If bacteria are present, these spot tests will show bacterial growth and Dictyostelium proliferation as the spores hatch and eat the bacteria (Brock et al., 2011). Without bacteria, amoebae cannot proliferate and the spot will stay blank. We considered a clone to be cured if no bacteria showed up in spot tests. We similarly treated naturally uninfected hosts with tetracycline to control for any effect of curing on our results.

Paraburkholderia re-infection

We re-infected cured *D. discoideum* clones with their native *Paraburkholderia* isolates by plating 200 μL $2x10^5$ spores with 200 μL of 0.1% *Paraburkholderia* solution. This solution consisted of 1.5 OD_{600} *Paraburkholderia* and 1.5 OD_{600} *K. pneumoniae* in a 1:1000 ratio. To confirm re-infection (and also successful isolation), we performed spot tests as above, where

successful re-infection was inferred when bacteria grew on 8 or more spots out of the 10 we put down.

Artificial dispersal to food-rich and food-poor plates

To obtain sori to transfer to food-rich and food-poor plates, we started by growing *D. discoideum* clones from frozen stock, as described above, on 200 μL 1.5 OD *K. pneumoniae* expressing green fluorescent protein (GFP). We obtained GFP-expressing *K. pneumoniae* (strain ID DBS0349837) from the Dicty Stock Center at dictyBase (Fey et al., 2013). This initial growth period is to remove freezer effects and ensure that food bacteria that are carried to new plates are GFP-expressing since stocks were fed non-GFP bacteria before freezing. After six days of growth, we used pipette tips to collect sori from mature fruiting bodies. We counted spores using a hemocytometer and diluted spores to a concentration of $2x10^5$ per mL, and then plated them on plates with (food-rich) or without (food-poor) an additional 200 μL of the GFP-expressing food bacterium *K. pneumoniae* (Figure 2.1). In order to survive on food-poor plates, the host must carry food bacteria from the previous plate. We grew food-rich and food-poor plates for six days unless otherwise stated, enough time for mature fruiting bodies to form.

Measurement of bacteria density

To measure *Paraburkholderia* density, we measured the quantity of bacteria left on plates after *D. discoideum* formed fruiting bodies. We first collected plate contents by washing plates with 15 mL of KK2 buffer. To remove fruiting bodies and bacteria associated with fruiting

bodies, we centrifuged wash solutions for three minutes at 13000 rpm. We measured bacteria using optical density measured at 600 nm (OD_{600}), a frequency at which bacteria commonly scatter light. Because the OD_{600} is due to both *Paraburkholderia* and *K. pneumoniae*, we used GFP fluorescence measurements (with an excitation wavelength of 485 and emission wavelength of 515 nm) and a standard curve relating *K. pneumoniae* fluorescence to its OD_{600} to subtract out the component due to GFP-expressing *K. pneumoniae*. Both OD_{600} and fluorescence measures were performed in a 96 well plate with a Tecan Infinite 200 Pro microplate reader.

To validate our standard curve, we compared predicted OD_{600} of *P. agricolaris* and *K. pneumoniae* to colony forming unit (CFU) counts from the same samples. Linear regression revealed that predicted OD_{600} measurements explained most of the variation in CFUs, showing that our assay is reliable. We also checked our standard curve for significant quadratic terms, which can cause measurement errors when combining OD_{600} and fluorescence measures at high densities (Meyers et al., 2018), but our curve did not have a significant quadratic term.

Host spore production

Spore production is a standard fitness measure in *D. discoideum* (Buttery et al., 2009; Hall et al., 2013; Gruenheit et al., 2017). To measure host spore production, we estimated spore concentration in the supernatants from washed plates using a hemocytometer. We then calculated the total number of spores per plate by multiplying by the volume of wash solution.

Spore production simulations

To test whether infected hosts benefit across variable food contexts, we simulated rounds of growth and dispersal across soil patches with different probabilities of having food bacteria. We separately modeled three host phenotypes: (1) uninfected, (2) infected with *P. agricolaris*, and (3) infected with *P. hayleyella*. Co-infections are possible, but are rare in nature (Haselkorn et al., 2019) so we exclude them from our analysis.

We assumed that environments consisted of 100 discrete soil patches. Patches were either food-poor or food-rich (we investigated continuous amounts of food and found similar results; see Supplemental file 1). Food-poor patches at time t were drawn from a binomial distribution with probability pt. Food-rich patches were drawn with probabity 1-pt. To allow temporal variation, the value of pt in each generation was drawn from a beta distribution with mean p and variance vtemp. High values of vtemp resulted in more temporally variable environments. For low values, most of the variation was spatial.

Initially all patches were colonized. Each patch produced a number of spores, drawn from the distribution of our empirical spore production values, according to whether it was a food-rich or food-poor patch. To model costs, we penalized host spore production in food rich environments by reducing spore production by a percentage c. When c is 0, we modeled the scenario observed in this study, with no infection cost. We did not detect a cost of infection in food-rich contexts, but numerous other studies have documented this cost (Brock et al., 2011; DiSalvo et al., 2015; Miller et al., 2020). It is likely that we did not detect a cost because we infected hosts with fewer *Paraburkholderia*. Because these costs have been demonstrated repeatedly in other studies and because of the importance of costs to bet-hedging (Lekberg & Koide, 2014; Veresoglou et al., 2021), we included them as a variable. We summed cost-adjusted spore production values to get the total spore production across all patches. This is

divided by $2x10^5$, a rough estimate of the number of spores in a typical sorus, to get the total number of sori, which we are assuming to be the dispersal unit.

The global pool of sori is used to seed the next round. New patches are assumed to be empty and dispersal is assumed to be global such that sori from one patch can disperse to any other patch with equal probability. Dispersal is likely efficient in *D. discoideum* as sori can be dispersed long distances by arthropods (smith et al., 2014) and possibly even by birds (Suthers, 1985). Each sorus is randomly assigned to a patch and it successfully colonizes that patch g% of the time. Because the value of g for natural hosts is unknown, we investigated three values of g (50%, 5%, and %0.5) that range from cases where there are many more sori successfully establishing than available patches to cases where each patch produces around one sorus. We assumed that patches colonized by multiple sori were the same as singly colonized patches for the purposes of determining their subsequent spore production. Some patches may remain unfilled (though this is unlikely when g = 50%). We also assume that infection status is not associated with different rates of colonization as those differences are better captured by our empirical spore production values, which will include differences in growth efficiency or spore germination rate.

We vary the average probability of food-poor patches p from 0.1 to 0.9 and simulate four different cost regimes reflecting variation found in different *Paraburkholderia* isolates (Miller et al., 2020). We simulated dispersal to new patches for 100 rounds of growth and dispersal using 100 replicates for each combination of p, vtemp, and c for each phenotype. Within each replicate, all three phenotypes experience the same environment. At the end of the 100 rounds, we calculated the total spore production per round and calculated geometric and arithmetic mean spore production from these values across the 100 rounds. Within each replicate, we determined

whether infected hosts had higher geometric or arithmetic mean fitness for each individual simulation and whether any phenotype went extinct.

We assigned outcomes for each parameter combination by calculating the frequency that infected or uninfected hosts had higher geometric mean fitness or arithmetic mean fitness. Infected and uninfected hosts were assigned as winners if they had higher geometric mean fitness in 75% of replicates. We assigned an outcome as bet-hedging when infected hosts won and more than half of the winning replicates did so with lower arithmetic mean fitness. Extinctions occurred in some simulations and were treated as a distinct outcome. We assigned mixed outcomes when neither infected nor uninfected hosts were able to have higher geometric mean fitness in 75% of replicates. Some mixed outcomes involved individual replicates where infected hosts were found to bet-hedge.

Statistical Methods

We performed statistics in R version 3.6.3 (R Core Team, 2020). To compare bacteria density and spore production, we used linear mixed models (LMM) with the lme function in the nlme package (Pinheiro & Bates, 2006). To account for random variation from replicate clones and effects of dates when experiments were performed, we included clone and the date the experiment was performed — along with each variable on its own — as random effects. To select the best model of random effects, we used AICc, a sample-size-corrected measure of model fit that balances predictive ability and model complexity (Burnham & Anderson, 2004). Many of our models showed different variances between treatments. To account for these

differences in variance, we weighted models with the varIdent function in nlme (Pinheiro & Bates, 2006). We used the emmeans package (Lenth et al., 2018) to perform contrasts.

To understand how *Paraburkholderia* density affects host spore production across food conditions, we fit a LMM using only infected hosts that included symbiont density leftover on plates and whether the plate was food-rich or food-poor, along with the interaction between these variables. We included random effects for clone, date, and both crossed effects and selected the best random effect structure with AICc. We determined whether the interaction was important by comparing AICc of the model including the interaction with models including the other variables but lacking the interaction.

2.5 Results

Paraburkholderia dispersed by *Dictyostelium* sori have lower growth when host food bacteria are abundant

The context of a food-poor environment is known to be important for *D. discoideum* hosts. It is not known how *Paraburkholderia* are affected by this same context, but reduced competition with food bacteria seems likely. We tested this by growing infected sorus contents on food-poor and food-rich nutrient plates and measuring the density of *Paraburkholderia* after *D. discoideum* fruiting body formation (Figure 2.1). After infected hosts formed fruiting bodies, *Paraburkholderia* densities were lowest in food-rich conditions (Figure 2.2A), as expected if they compete with food bacteria. There was around five times more *P. agricolaris* on food-poor than food-rich plates (LMM, $p < 0.001$). *P. hayleyella* growth was higher in food-poor conditions

than food-rich, but this difference was not significant after 6 days (LMM, p = 0.416). Because *P. hayleyella* grows slowly, we performed two more experiments with *P. hayleyella* with 8 and 12-day growth periods (Figure 2.2B). Allowing for longer incubations did not result in significantly higher density of *P. hayleyella* (LMM, p = 0.633), suggesting that *P. hayleyella* reach their maximum density at or before 6 days, but including these additional experiments gave us enough power to find a significant increase in *P. hayleyella* density in food-poor conditions relative to food-rich (LMM, p = 0.027). These results show that symbiont density is context-dependent.

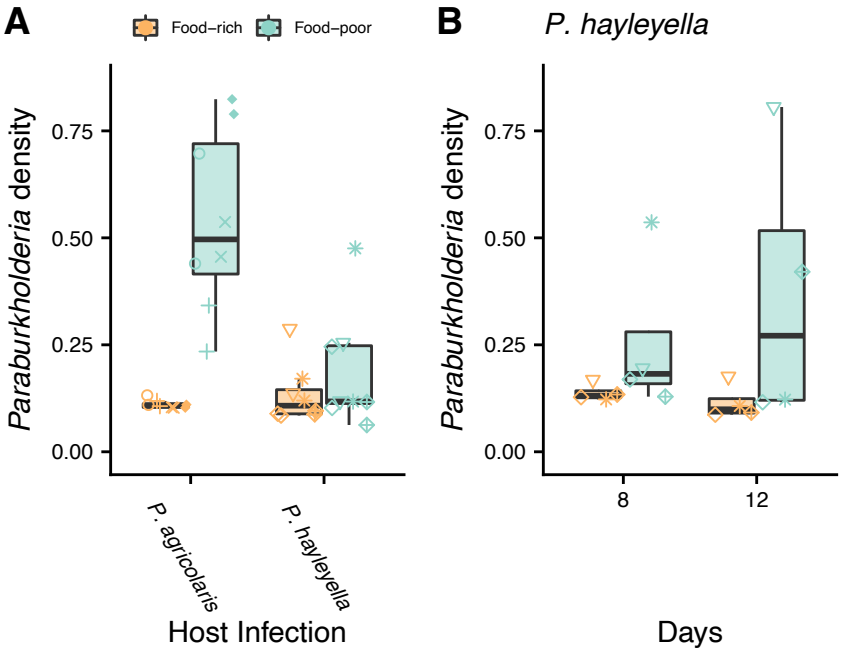

Figure 2.2: More *Paraburkholderia* were recovered from plates after fruiting body formation from food-poor plates (those that had not received additional *K. pneumoniae*). (A) *Paraburkholderia* density after 6 days. (B) *P. hayleyella* density after 8 and 12 days. Point shapes show individual clones (see Figure 2.1).

Higher symbiont density harms hosts, but less so in food-poor contexts

The host-food context may affect the relationship between symbiont density and host spore production and therefore the degree of conflict or cooperation between them. To investigate this, we also measured total host spore production from plates where we measured the growth of *Paraburkholderia* symbionts (Figure 2.1). We used uninfected hosts as a baseline for fitness without symbionts. We confirmed prior studies (Brock et al., 2011; DiSalvo et al., 2015) showing that infected hosts could carry food bacteria and proliferate on food-poor plates, while uninfected host could not (Figure 2.3A). Surprisingly, we did not observe a cost of being infected in food-rich conditions ($p > 0.5$ for both species) which has been seen in previous studies (Brock et al., 2011; DiSalvo et al., 2015; Shu et al., 2018a). This is likely a result of our lower infection dosage of 0.1%.

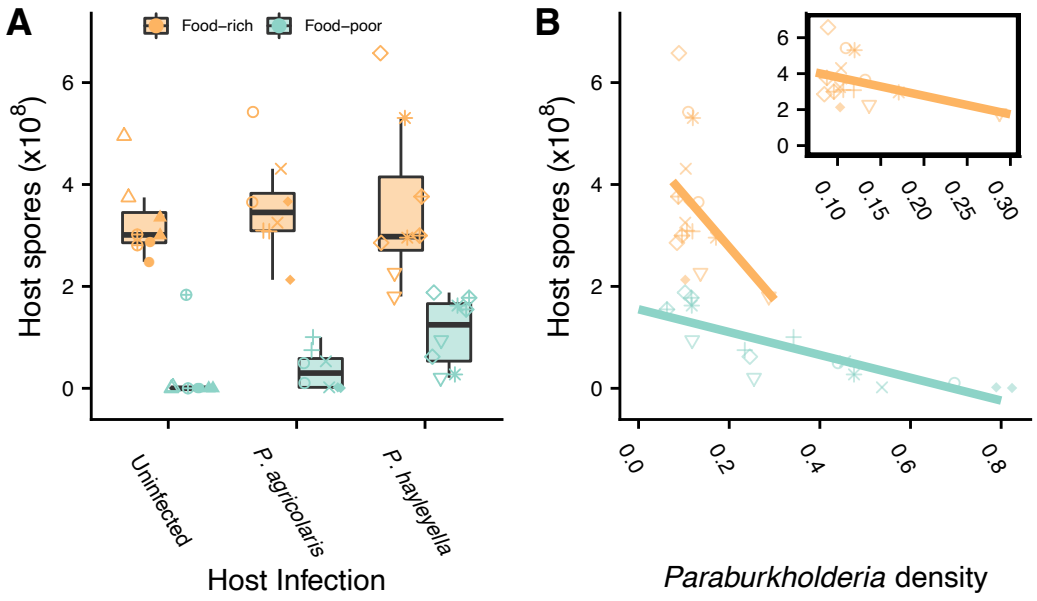

Figure 2.3: Effects of *Paraburkholderia* infection and density on host spore production. (A) Spore production of hosts from food-rich and food-poor plates for uninfected, *P. agricolaris* infected, and *P. hayleyella* infected hosts. (B) Interaction between measured *Paraburkholderia* density (OD_{600}) and food environment on host spore production. This interaction model explained 95% of the variance in spore production. Inset shows food-rich results on smaller scale. Point shapes show individual clones (see Figure 2.1).

While having some symbionts is essential for hosts to be able to carry food and survive in food-poor conditions, higher symbiont densities may nevertheless harm hosts, perhaps in ways that depend on food context. We found that larger populations of symbionts as measured by OD_{600} were associated with lower host spore production, but this harm was reduced in food-poor conditions. Lower host spore production was associated with being in a food-poor environment (βfood-poor = -3.283, se = 0.853) and symbiont density (βdensity = -10.317, se = 6.364), but the interaction between food scarcity and symbiont density showed that the harmful effect of higher symbiont densities was lessened on food-poor plates (βfood-poor*density = 8.078, se = 6.381; Figure 2.3B). These results indicate that symbiont density may come at the expense of host spore production, but that this cost decreases in food-poor environments.

Symbiosis is often beneficial for hosts across variable contexts

Because symbiosis helps hosts in food-poor contexts, we hypothesized that infected hosts would gain a long-term benefit across contexts compared to uninfected hosts. If infected hosts increased their geometric mean fitness at the expense of arithmetic mean fitness, infected hosts could even gain a bet-hedging advantage. We modeled this by using our empirical spore production values to simulate 100 rounds of growth and dispersal across environments where the number of food-poor patches was determined by the mean frequency (p) and the temporal variance (vtemp; more detail can be found in the methods). Because the natural conditions of this symbiosis are mostly unknown, we simulate a wide-range of parameter space to determine which conditions favor symbiosis. The supplement includes animations of representative simulations.

We first describe the results when dispersing sori successfully colonize new patches 5% of the time. When there was no cost of infection, we found that infected hosts were favored in every condition we tested (Figure 2.4; blue). We also simulated costs of infection because those have been found in other studies (Brock et al., 2011; DiSalvo et al., 2015; Miller et al., 2020). As the cost of infection in food-rich contexts increased, infected hosts were favored in the most food-poor environments while uninfected hosts were favored when food was abundant (Figure 2.4; orange). *P. hayleyella* was favored across more environments than *P. agricolaris*.

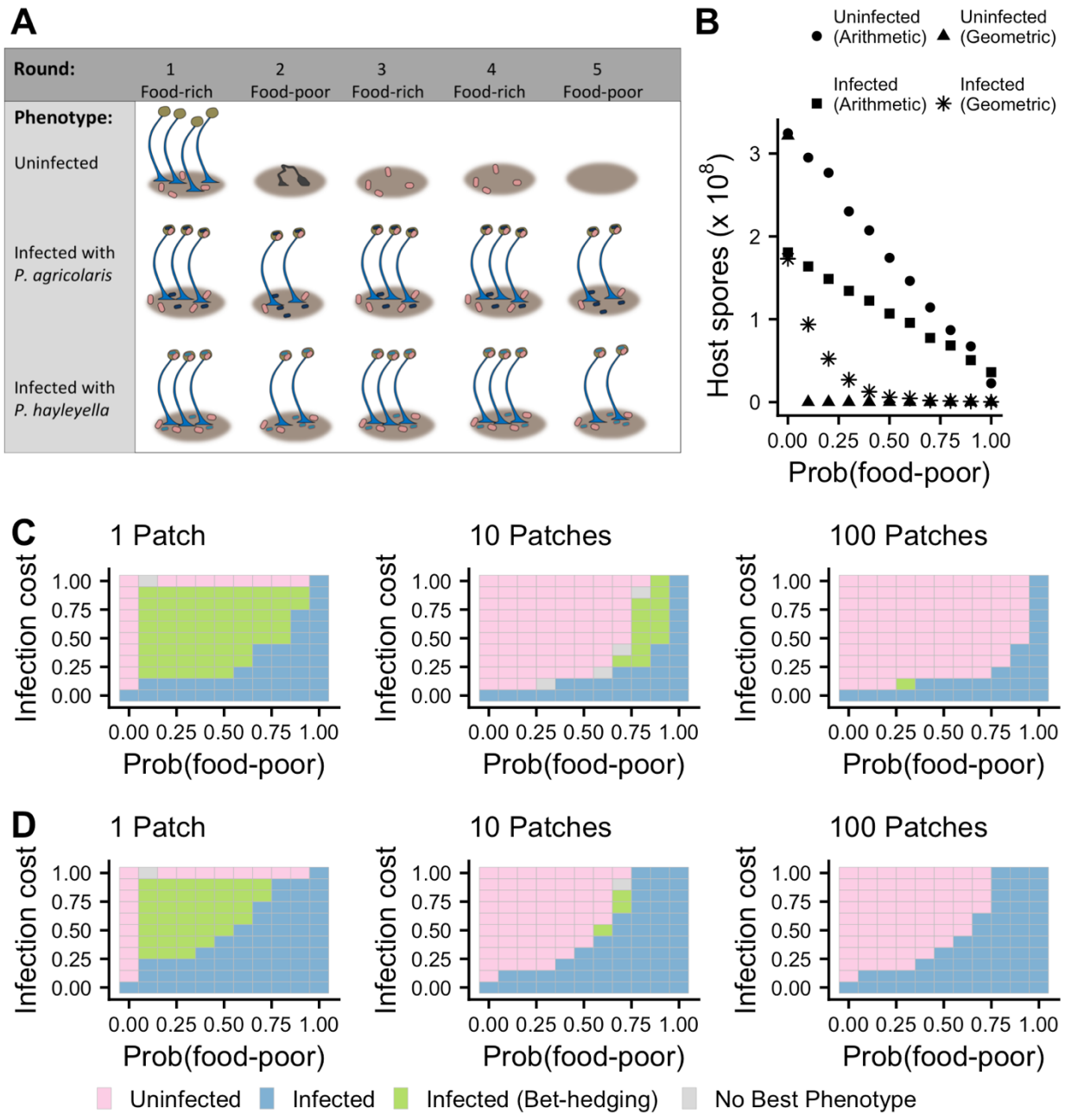

Figure 2.4: Benefits of symbiosis depends on variation in food availability and fitness costs. Winning phenotypes of *P. agricolaris* (top) and *P. hayleyella* (bottom) relative to uninfected for different costs of infection with a 5% probability of colonization. Orange shows when uninfected hosts have higher arithmetic and geometric mean spore production; blue shows when infected hosts have higher arithmetic and geometric mean spore production; green shows when arithmetic fitness is reduced for higher geometric mean fitness (bet-hedging); gray shows areas where both infection strategies can win; yellow shows where both strategies can win and where infected hosts bet-hedge.

Bet-hedging in this symbiosis appears to be rare (Figure 2.4; green and yellow). Infected hosts had a bet-hedging advantage when costs were added and food was intermediately rare. More temporally variable environments had a weak effect on increasing the likelihood of bet-hedging.

When dispersing sori successfully colonize new patches 50% of the time (each patch produces enough sori to completely fill the patches in the next generation), we found similar results. When only 0.5% were successful (each patch may only produce one or two sori for dispersal), we again found similar results except in the most food-poor conditions, where both uninfected and *P. agricolaris* infected hosts tended to go extinct. *P. hayleyella* infected hosts were able to survive in these food-poor contexts.

The natural environment of hosts is unlikely to involve food patches that are binary. Variation in the environment is also often auto-correlated, with the state of the environment at one time more often resembling the state of the environment in the near future (Ruokolainen et al., 2009). To determine whether our results were robust to variable environments with continuous food and temporal correlations, we ran additional simulations where the amount of food varied from 0 to 1 depending on a continuous resource that allowed us to tune autocorrelations. These additional simulations broadly supported our conclusions from the simpler simulations.

2.6 Discussion

Our results show how the context of host food abundance affects the Dictyostelium-*Paraburkholderia* symbiosis beyond the previously demonstrated advantage to hosts when food is rare (Brock et al., 2011). First, we found evidence that both *Paraburkholderia* species benefit from reduced competition when they are carried to food-poor environments (Figure 2.2). Second, symbiont density negatively affected host spore production, but symbionts harmed hosts less in food-poor conditions (Figure 2.3B). Third, infected hosts had an advantage over uninfected hosts in simulations when food conditions were harsh or when the cost of symbiosis was low (Figure 2.4).

Our finding that symbionts had higher growth when dispersed to food-poor contexts shows that *Paraburkholderia* symbionts experience parallel context-dependence as hosts. These results highlight the importance of context-dependence for both partners. *Paraburkholderia* may benefit from reduced competition when hosts bring them to food-poor environments because symbionts interact with fewer competitors or because hosts eat competitors. This, together with our finding that hosts can benefit across contexts, points to a relationship of mutual benefit in this symbiosis. Our results also fit with other findings of competitive benefits for symbionts (Iwai, 2019). Other benefits of symbiosis for *Paraburkholderia* remain to be tested.

Competition between symbionts and food bacteria may also be responsible for the context-dependent effects of symbiont density on host spore production. Our spore production results showed that higher symbiont densities resulted in lower host spore production, indicating that symbionts are harmful to hosts. However, higher symbiont densities are less harmful in food-poor conditions when competition is lower (Figure 2.3B). The reduced harm for hosts could

be the result of less antagonism between bacteria, which results in less collateral damage to amoebae through secreted toxins or other competitive interactions between food bacteria and symbionts. The generality of our results is limited somewhat by only using one species of food bacteria. While using a single food bacterium is more experimentally tractable, amoebae encounter multiple bacteria species in their natural environments (Brock et al., 2018). Different species, or combinations of species, could change competition with symbionts and affect host spore production in different ways.

Symbiosis benefits amoeba hosts by giving hosts the ability to carry food to food-poor contexts (Brock et al., 2011; DiSalvo et al., 2015). Using simulations, we showed that this ability resulted in higher fitness across variable contexts when costs were low and food was rare (Figure 2.4). Under conditions with plentiful food and high costs, being uninfected was advantageous. In nature, about 25% of clones are infected (Haselkorn et al., 2019), suggesting that symbiosis is not universally favored. This indicates that our finding of no cost to hosts in the symbiosis may be unrepresentative of many natural infections. On the other hand, a 25% infection rate is high if the symbiosis is generally harmful. This indicates that the prevalence of symbiosis could reflect a balance of forces where *D. discoideum* is not strongly selected to fight *Paraburkholderia* infection in a geographic mosaic of coevolution (Thompson, 1994). Unfortunately, the natural conditions of this symbiosis are the biggest unknowns in this system as it is difficult to study this symbiosis, and microbes more generally (Kraemer & Boynton, 2017), in nature.

Hosts could also benefit across contexts through bet-hedging, where geometric mean fitness trades off with arithmetic mean fitness (Seger & Brockmann, 1987). It is suspected that costly symbioses may be able to evolve because they are advantageous over the long-term even if they are not advantageous in the short term (Lekberg & Koide, 2014; Veresoglou et al., 2021).

We found that bet-hedging was rare in our simulations. Our finding that bet-hedging occurs between where conditions favor infected over uninfected hosts hints at the possibility that bet-hedging could facilitate the evolution of symbiosis where benign environments transition to harsh environments. However, as our simulations also reveal, symbiosis is more often favored without the need for bet-hedging even with costs. Our results thus weaken the case that costly symbiosis in some contexts are necessarily examples of bet-hedging since symbiosis was more often favored outright than by bet-hedging.

Symbiotic interactions may play a larger role in adaptation to variable environments than previously understood, even without bet-hedging. Symbioses are known to result in novel phenotypes that allow partners to survive in harsh conditions (Moran, 2007; Oliver et al., 2010). Rarely do studies incorporate environmental variation and long-term fitness. We investigated the long-term effects of context-dependence in the symbiosis between *D. discoideum* and *Paraburkholderia* and found that hosts frequently benefited from symbiosis in the harshest conditions. An understanding of the ecological contexts along with long-term measures of fitness will be important for understanding the evolutionary consequences of context-dependent symbioses.

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Authorship statement: TS, DQ, and JS designed the study and wrote the manuscript. TS performed the experiments and simulations and analyzed the data.

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Chapter 3: Uncertain soil conditions affect the prevalence of a microbial symbiosis

Trey J. Scott, Calum J. Stephenson, Sandeep Rao, David C. Queller, Joan E. Strassmann

3.1 Abstract

The evolution of symbiotic interactions may be affected by uncertain conditions. However, a link between prevalence of symbiosis and uncertain conditions has not been widely demonstrated. We test for this association using *Dictyostelium discoideum* social amoebae and its bacterial symbionts. *D. discoideum* are host to three kinds of endosymbiotic bacteria: *Paraburkholderia*, *Amoebophilus* and Chlamydiae. Three species of facultative *Paraburkholderia* symbionts are the best studied and give hosts the ability to carry food bacteria through the dispersal stage to new environments. *Amoebophilus* and Chlamydiae are obligate endosymbionts with no measurable impact on host fitness. We test whether the frequency of both single and coinfections of these symbionts is associated with the uncertainty of their soil environments by using symbiont presence-absence data from soil isolates from 21 locations across the eastern United States. We find that that *Amoebophilus* and Chlamydiae obligate endosymbionts and coinfections are not associated with uncertain soils, but that uncertain precipitation can promote or hinder symbiosis depending on the species of *Paraburkholderia* symbiont.

3.2 Main Text

The evolution of grouping often varies with ecological uncertainty. For example, the prevalence of cooperative breeding in birds is associated with uncertain environmental conditions [1, 2]. Grouping in these cases is thought to allow organisms to invade uncertain environments [3] or buffer against times when conditions are harsh [4]. So far studies on the relationship between ecological uncertainty and cooperation have mostly focused on interactions between members of the same species [1, 2, 9]. Grouping between a host and microbe in a symbiotic interaction may also have these effects [5–8], and may thus be associated with uncertain environments. Similar grouping-uncertainty relationships have not been tested in symbiotic partnerships.

We investigated whether the prevalence of symbiosis was associated with uncertain conditions in the microbiome of the social amoeba *Dictyostelium discoideum*. *D. discoideum* can host three species of facultatively endosymbiotic *Paraburkholderia* bacteria that allow hosts spores to carry other species of edible bacteria and seed out food bacteria populations after dispersal [10–13]. Two of these *Paraburkholderia* species, *P. hayleyella* and *P. bonniea*, may be more strongly host associated as shown by their reduced genomes, while *P. agricolaris* may be less host associated [14]. *D. discoideum* also harbors obligate endosymbionts: one from the genus *Amoebophilus* and different haplotypes from the phylum Chlamydiae. These obligate endosymbionts do not measurably affect host fitness [15]. We will refer to these obligate endosymbionts by their generic names as they have not been described at the species level.

Environmental sampling has found that *Paraburkholderia* prevalence is about 25% of sampled hosts but varies by sampling location [16]. Obligate endosymbionts are found in about

40% of sampled hosts [15]. *Paraburkholderia* and *Amoebophilus* coinfections are more common than expected, but do not impact host fitness when measured in the lab [15].

A key environmental condition for host *D. discoideum* is the presence of edible bacteria [5, 12, 17]. Bacterial density in soil is driven by factors like pH, temperature, nutrients, and soil moisture [18]. Unpredictable bouts of rain may have dramatic effects on soil bacteria. Rain can drastically shift the soil environment because of the complex structure and physical properties of the soil [19]. We thus suspect that uncertain precipitation, and possibly interactions with other soil factors, could be major drivers of food abundance for *D. discoideum* amoebae. These soil characteristics may also be associated with the prevalence of symbionts or coinfections in hosts.

To test for relationships between soil characteristics and symbiont prevalence, we used presence-absence data of symbionts that were collected from 22 collection trips to 21 locations (Figure 3.1) across the eastern United States [15, 16]. Because some coinfections are known to be more common than expected [15], we first tested all screened hosts for non-random coinfections that may also vary with the soil environment. *P. hayleyella* and *Amoebophilus* coinfections are more common than expected across our sampled sites (Figure 3.2). This extends prior findings that focused on a subset of locations [15]. Amoebophilus and Chlamydiae coinfections are less common than expected across our sampled sites. The rarity of *Amoebophilus* and Chamydiae coinfections may indicate competitive exclusion inside *D. discoideum* hosts. The association between *P. hayleyella* and Amoebophilus suggests that he abundance of both may be driven by the same environmental conditions.

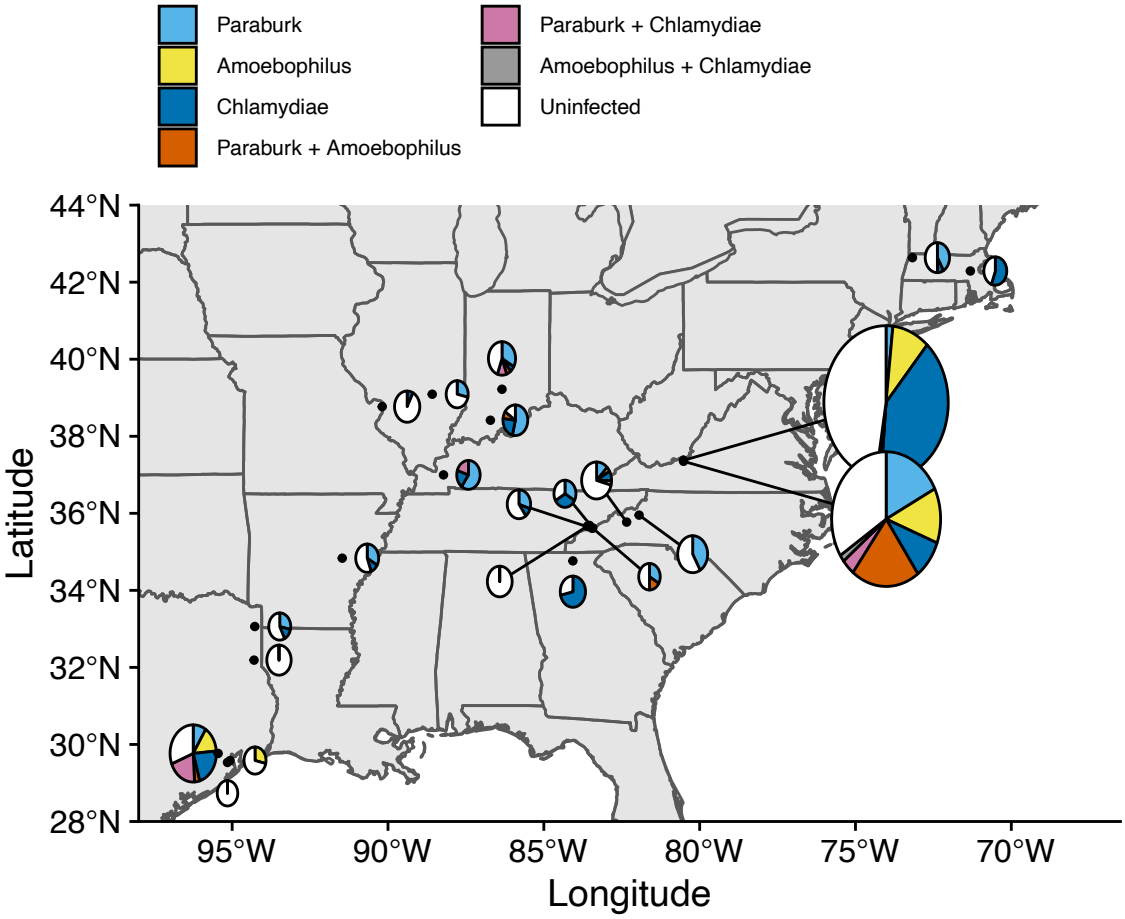

Figure 3.1: Map of *D. discoideum* sample locations and symbiont prevalence. Black points show locations. Pie charts show the frequencies of symbionts in screened hosts. Relative pie chart size indicates the number of sampled hosts at a location.

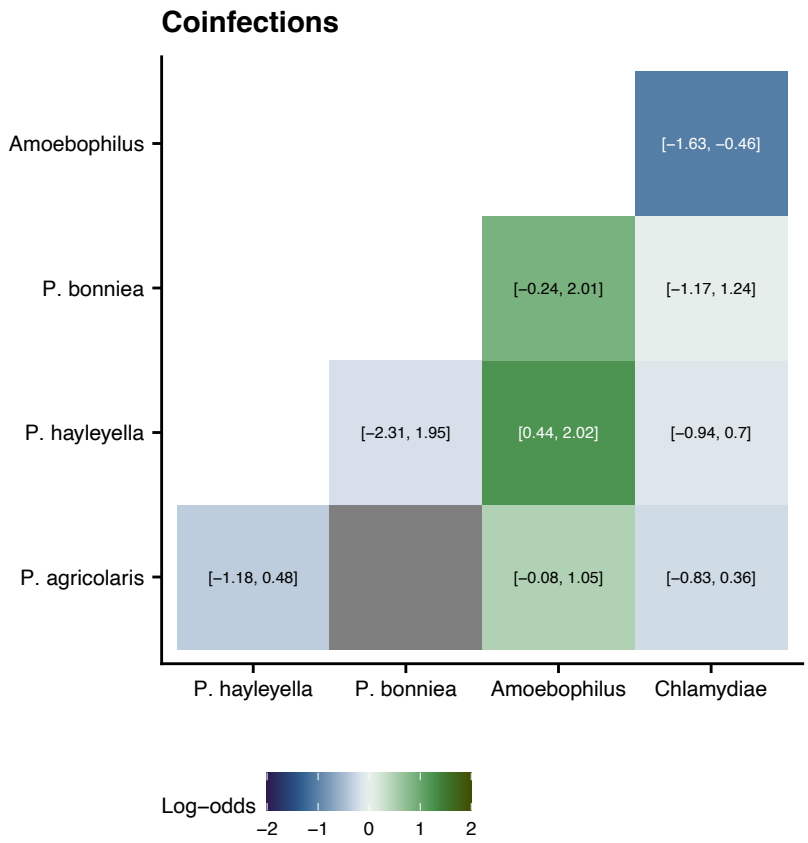

Figure 3.2: Patterns of coinfection between *D. discoideum* endosymbionts. Squares are colored according to their log-odds from logistic regression models. 95% confidence intervals are given inside squares. *P. agricolaris* and *P. bonniea* are never found together resulting in no variation for logistic regression.

To test for an association between symbiont or coinfection presence and environmental conditions, we acquired soil characteristics that have been associated with soil bacteria abundance (see SI Methods)[18]. As a measure of uncertain precipitation, we calculated Colwell's P (hereafter P_C) using monthly precipitation data for each location since 1901. P_C ranges from 0 to 1, with 0 being unpredictable and 1 being perfectly predictable [20]. We collected soil mean annual precipitation (MAP), mean annual temperature (MAT), soil carbon to nitrogen ratio (C/N), and soil pH data. We also included first order interactions between P_C and the other soil characters. To identify associations between symbiont prevalence and soil

conditions, we used logistic regression models (for details on model selection and testing for spatial autocorrelation see SI methods).

We found that the frequencies of the two *Paraburkholderia* species with reduced genomes, *P. hayleyella* and *P. bonniea*, were associated with PC (Figure 3.3A&B). Interestingly, *P. hayleyella* and *P. bonniea* prevalence responded differently to uncertain precipitation. *P. hayleyella* prevalence was higher in more uncertain (lower P_C) environments (Figure 3.3A; effect of P_C was negative for *P. hayleyella* frequency) while *P. bonniea* prevalence was higher in more predictable environments (Figure 3.3B). The prevalence of *P. agricolaris*, the obligate endosymbionts, and *P. hayleyella-Amoebophilus* coinfections were not associated with uncertain precipitation or the other soil measures we included in our models.

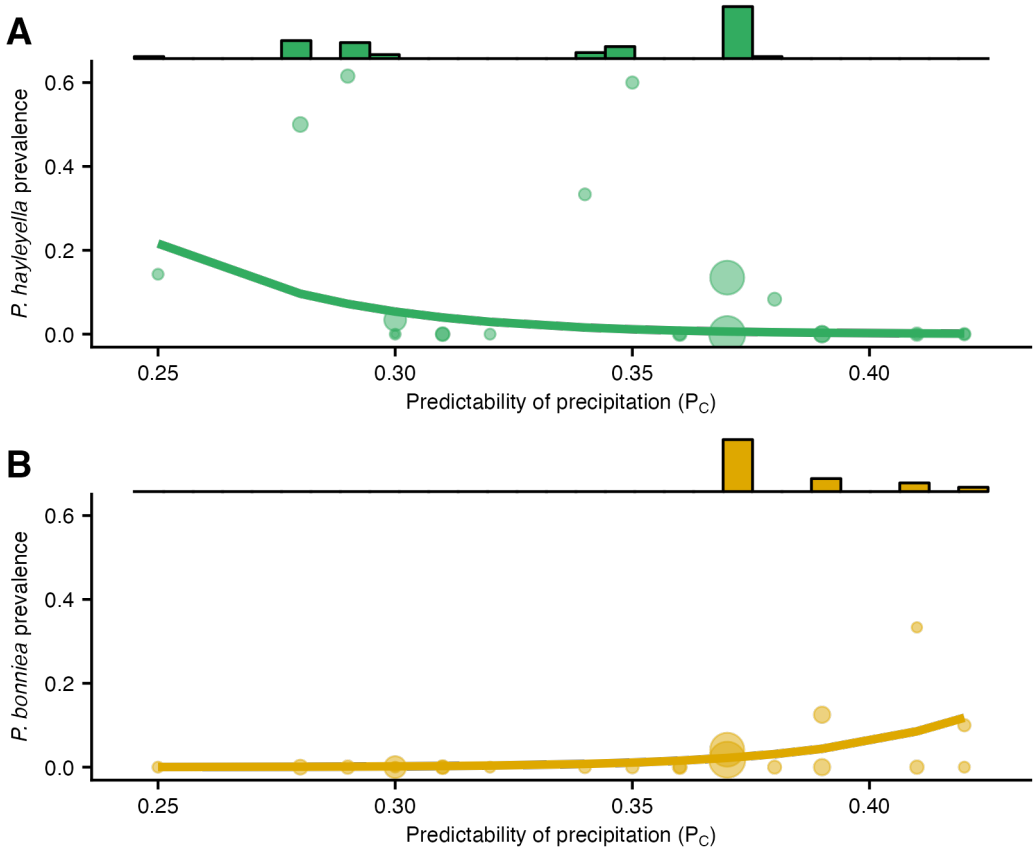

Figure 3.3: *P. hayleyella* and *P. bonniea* are differently affected by and inhabit different areas of precipitation predictability (P_C). Prevalence of *P. hayleyella* (A) and *P. bonniea* (B) for different value of predictability of precipitation (P_C) along with logistic regression fits. Prevalence values are shown for each location with the size of the shape being proportional to the number of screened hosts at a site. Histograms on top show the number of hosts with symbionts for a given value of P_C.

One explanation for why P_C affects *P. hayleyella* and *bonniea* prevalence in opposite directions is that these sister species [10] compete and are partitioning their niches based on P_C. Our data show that *P. hayleyella* is more prevalent in uncertain soils (low P_C) and *P. bonniea* is more prevalent in more certain soils (high P_C; Figure 3.3). However, both species already appear to inhabit different areas of soil uncertainty (Figure 3.3 histograms; Permutation test, $p < 0.001$) so selection for niche partitioning should be weak. Niche partitioning may also be unlikely because the prevalence of these symbionts is not high so that competition may be limited.

Another explanation for why P_C affects *P. hayleyella* and *bonniea* prevalence in opposite directions is that infection with these different symbionts impacts the survival of hosts differently. *P. hayleyella* may inhabit environments where the ability to carry food bacteria buffers hosts from uncertain conditions. Because of this buffering, infected hosts do better than uninfected in uncertain conditions and increase in frequency. In contrast, *P. bonniea* inhabit relatively certain soils where buffering is not advantageous. Instead, *P. bonniea* are more prevalent in more hospitable (certain) soils. We may thus have identified an inflection point: below a certain predictability, increasing uncertainty increases prevalence and above this value, increasing certainty increases prevalence.

Future laboratory work should test the possible roles of niche partitioning and buffering of hosts in this symbiosis. More broadly, we have demonstrated that the frequency of a microbial symbiosis is associated with uncertain environmental conditions.

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3.4 Supplemental Methods

Data Acquisition and Processing

To measure the frequency of symbiosis, we used data from prior environmental sampling [15, 16]. The first study [16] tested *D. discoideum* isolates from 21 locations (one location was sampled two separate times) for the presence of the three species of *Paraburkholderia* symbionts [10] using *Paraburkholderia* specific 16S sequencing. The second study [15] tested a similar set of *D. discoideum* isolates for Amoebophilus and Chlamydiae, but also included samples from a few additional countries. For this study, we focused only on the United States samples because sites from other countries were not well sampled and could skew the results. We used these data to construct a presence-absence variable for each *D. discoideum* clones for whether they were infected with any of the three species of *Paraburkholderia*, or *Amoebophilus*, or Chlamydiae.

To investigate the role of environmental variation on the *Dictyostelium-Paraburkholderia* symbiosis, we acquired data on long-term precipitation, soil pH, soil organic carbon, nitrogen, and temperature for each sample location from online databases. These variables are known to affect the abundance of bacteria in the soil [18]. For each location, we collected monthly precipitation data from 1901 to 2020 from the climate research unit database version 4.05 [21]. To measure the predictability of precipitation across these monthly measures, we calculated Colwell's P (hereafter PC) [20] using the Colwells function in the hydrostats package [22]. PC ranges from completely unpredictable (0) to completely predictable (1). We tested two PC measures meant to capture long-term and recent predictability: (1) calculated with precipitation data from 1901 to the year that a sample was collected and (2) calculated from precipitation data

from 5 years before the sample was taken. These measures were largely similar and did not change any of our results, so we include only the long-term measure in the main text.

We collected soil pH, nitrogen, and organic carbon data from the SoilGrids database version 2.0 [23]. SoilGrids are soil predictions based on empirical soil measurements and are generated at 250-meter scales. We collected soil temperature variables from Lembrechts et al. [24]. Temperature data were generated by calculating deviations of soil temperatures from air temperatures at 0 to 5 cm and 5-15 cm depths. We used 0-5 cm depths for soilGrids and soil temperature data because *D. discoideum* typically resides in the top layers of soil.

Statistical methods

To test for associations between different symbiont species across locations, we used mixed effect logistic regression from the lme4 package [25] in R version 4.1.2 [26]. To account for multiple observations at a location, we used location as a random effect. We treated the location that was sampled twice (Mountain Lake Biological Station) as two separate locations because soil samples were taken from different areas within Mountain Lake Biological Station and because samples were collected 14 years apart.

To test whether the occurrence of coinfections between the three *Paraburkholderia* species was different than expected in specific locations, we used Fisher's exact tests (Data S1). These tests were done in addition to our logistic regression models that tested for overall effects across locations. To perform Fisher's exact tests, we constructed a 2x2 contingency table for

each sampling location in which at least 2 of the investigated 3 symbionts were present. To correct for multiple comparisons, we adjusted p-values using Benjamini-Hochberg's correction.

To test for associations between soil characters and prevalence of symbionts, we fit a set of models derived from a full model that included the mean annual temperature (MAT), carbon to nitrogen ratio (C/N), mean annual precipitation (MAP), precipitation predictability (P_C), soil pH, and first order interactions between these variables. To identify top models, we used AICc values [27] and examined effect sizes of model estimates. We identify uninformative models if the model does not differ from an intercept only (null) model in terms of AICc and 95% confidence intervals of estimated effects overlap zero. We identify informative models if the model differs from the null model and at least one 95% confidence intervals does not overlap 0. To test for spatial autocorrelation in our models, we performed a Moran's I test on simulated residuals using the DHARMa package in R [13]. All models were free of spatial autocorrelation.

To test whether *P. hayleyella* and *P. bonniea* inhabit soils with different precipitation uncertainties, we used a permutation test with 10,000 samples. We investigated the differences between both the means and medians of the two species as sample statistics. Both mean and median difference statistics gave equivalent results. We report the median difference p-value in the main text.

3.5 SI Citations

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Chapter 4: Third-party effects in the *Dictyostelium-Paraburkholderia* symbiosis: food bacteria that are eaten, carried, or left behind

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This chapter is work that has been posted as a pre-print on bioRxiv (2022):

https://doi.org/10.1101/2022.11.06.513053

4.1 Abstract

Symbiotic interactions change depending on the abundance of third parties like predators, prey, or pathogens. Third-party interactions with food bacteria are central to the symbiosis between *Dictyostelium discoideum* social amoeba hosts and inedible *Paraburkholderia* bacterial symbionts. Symbiosis with *Paraburkholderia* allows host *D. discoideum* to carry food bacteria through the dispersal stage where host amoebae aggregate and develop into fruiting bodies that disperse spores. Carrying bacteria benefits hosts when food bacteria are scarce but harms hosts when food bacteria are plentiful. The nature of this cost is unknown, but hosts leave bacteria behind when they carry symbionts. If this left-behind bacteria includes uneaten food bacteria, infected hosts may lose potential growth. Thus, decisions about how many food bacteria to eat,

to carry, and to leave behind are crucial for understanding both benefits and costs in this symbiosis. We investigated how many food bacteria are uneaten and carried in this symbiosis by measuring fluorescently labeled food bacteria after fruiting body development. We found that *Paraburkholderia* infection makes hosts leave both symbionts and uneaten food bacteria but leaving food bacteria uneaten did not explain costs to hosts. Counts of food bacteria in fruiting bodies showed that hosts carry more food bacteria after developing in food-poor environments than in food-rich. This indicates that hosts, and possibly *Paraburkholderia* symbionts, actively modify how many food bacteria are carried to ensure hosts have food in the harshest conditions. Decisions about how many third-party bacteria to eat, carry, or leave may thus have important effects on this symbiosis.

4.2 Introduction

The fitness effects of symbiotic interactions can change depending on the environment (Bronstein, 1994; Chamberlain et al., 2014; Horas et al., 2022; Scott et al., 2022). One crucial component of the environment can be a third species that interacts with hosts and symbionts (Palmer et al., 2008; Wendling et al., 2017; Wood et al., 2018; Hafer-Hahmann & Vorburger, 2020; Cassidy et al., 2022). For example, in the symbiosis between ants and Acacia plants, ants benefit Acacia by fending off herbivores. However, when herbivores were prevented from accessing Acacias, ordinarily mutualistic interactions between ants and Acacias shifted towards antagonism because ants no longer provided a benefit to host Acacias (Palmer et al., 2008). Such shifts can influence whether host and symbiont fitness interests are aligned or in conflict (Keeling & McCutcheon, 2017; Iwai et al., 2019). However, the details of how third parties

affect symbioses are not well understood for many kinds of symbioses (Chamberlain et al., 2014).

The social amoeba *Dictyostelium discoideum* is an important model of microbial symbiosis. *D. discoideum* is a soil amoeba that feeds on bacteria (Raper, 1937). Upon starvation, amoebae aggregate to form a multicellular fruiting body that disperses spores to new soil patches (Kessin, 2001). Development of the fruiting body progresses through well-studied stages starting with aggregation of individual amoebae which then become motile slugs (Bozzaro, 2019). Slugs can move to locations with food or find a location to develop into a fruiting body. During fruiting body development, about 20% of the cells die and become stalk while the remaining cells develop into spores that sit atop the stalk in a structure called a sorus (Strassmann & Queller, 2011).

D. discoideum has symbioses with three species of facultatively intracellular *Paraburkholderia* bacteria (Brock et al., 2020). In natural isolates, around 25% of wild-collected *D. discoideum* are infected by one, and occasionally two, of the three species of symbionts (Haselkorn et al., 2019). When not referring specifically to one of the three species – *P. agricolaris*, *P. hayleyella*, or *P. bonniea* – we will refer to them collectively in this paper as either *Paraburkholderia* or symbionts. These symbionts are specialized for interacting with amoebae (Shu et al., 2018a; b; Brock et al., 2020). Symbionts move towards hosts (Shu et al., 2018b) and make host phagosomes less acidic so that the symbionts are not digested (Tian et al., 2022). One species of symbiont, *P. hayleyella*, appears to have led to host adaptation in response to the presence of symbionts (Shu et al., 2018a). *P. hayleyella* and *P. bonniea* also have reduced genomes relative to *P. agricolaris* and most other *Paraburkholderia* (Brock et al., 2020; Noh et

al., 2022), which indicates that *P. hayleyella* and *P. bonniea* have been associated closely with their hosts during a long evolutionary history (McCutcheon & Moran, 2012).

Fitness of *D. discoideum* hosts and *Paraburkholderia* symbionts is affected by interactions with a third set of organisms, the various food bacteria in the environment that are eaten by host amoebae (Brock et al., 2011; DiSalvo et al., 2015; Scott et al., 2022). *Paraburkholderia* symbionts are largely inedible by hosts, and are carried undigested inside the sorus along with additional edible bacteria (Brock et al., 2011; DiSalvo et al., 2015; Khojandi et al., 2019). When hosts disperse to food-poor environments where food bacteria are rare, hosts benefit from *Paraburkholderia* infection because hosts are able to carry food bacteria to seed new populations (Brock et al., 2011; DiSalvo et al., 2015; Scott et al., 2022). When infected hosts disperse to food-rich environments, hosts pay a cost of producing fewer spores relative to uninfected *D. discoideum* (Brock et al., 2011; DiSalvo et al., 2015). Presence of more *Paraburkholderia* is also associated with lower host spore production, but this effect depends on whether food bacteria are present (Scott et al., 2022). Thus, host fitness depends on the abundance of food bacteria in the environment prior to fruiting body formation.

Fitness of inedible *Paraburkholderia* symbionts also changes depending on the density of edible bacteria that are eaten by hosts, probably because they compete for resources. We could thus refer to food-rich and food-poor host environments as being high and low competition environments for *Paraburkholderia* symbionts. To be consistent between host and symbiont environments, we will use food-rich and food-poor but we note that this affects competition for symbionts. After symbionts are carried to food-poor environments, both *P. agricolaris* and *P. hayleyella* reach higher densities relative to food-rich environments where they face more competition (Scott et al., 2022).

The effect of food bacteria on hosts and symbionts highlights how food bacteria play an important role in this symbiosis. However, there are still many questions about how food bacteria affect this symbiosis throughout the development of *D. discoideum*. Of special interest is the period when fruiting bodies are formed and food bacteria are transferred into the sorus for carriage. This carriage period is important because the abundance of food bacteria impacts both parties (Brock et al., 2011; DiSalvo et al., 2015; Scott et al., 2022). It is important to note that carrying food bacteria may be a joint phenotype (Queller, 2014; Queller & Strassmann, 2018) that both hosts and symbionts affect together rather than being solely controlled by hosts. Joint phenotypes are interesting to study in symbioses because of the potential for cooperation and conflict over the value of the joint phenotype (Scott & Queller, 2019; Quides et al., 2021). Here, it is the disposition of food bacteria that is of interest – should they be eaten, carried, or left behind?

Leaving food bacteria behind would not seem to be adaptive, but this appears to happen when hosts are infected by *Paraburkholderia* but not when they are uninfected (Brock et al., 2011, 2016b). Initially, the key role of *Paraburkholderia* in causing hosts to leave behind bacteria was not known. It was thought that the bacteria left behind were all food bacteria (Brock et al., 2011). This was taken as a possible sign of prudent predation by host amoebae — if some bacteria were going to be saved for carriage, hosts cannot eat all the available food and may stop feeding and start developing earlier (Brock et al., 2011). It was hypothesized that hosts leaving food bacteria uneaten might explain why carriage is costly in some environments (Brock et al., 2011).

The essential role of *Paraburkholderia* in the symbiosis, including causing hosts to leave behind bacteria is now known (DiSalvo et al., 2015; Brock et al., 2016b). Moreover, at least

some of the bacteria left behind are not food bacteria but *Paraburkholderia* symbionts (Scott et al., 2022). Because of this new information, the prudent predation hypothesis that hosts are leaving behind bacteria in order to carry should be re-evaluated. Several questions need to be clarified. What fraction of the bacteria left behind are food bacteria – how much real food is left on the table? Does *Paraburkholderia* infection cause hosts to leave the food bacteria uneaten? Are food bacteria left because the hosts cease eating and start developing earlier? What costs are there for leaving food bacteria uneaten, or are costs instead due to the number of *Paraburkholderia* symbionts?

While infected hosts may leave some food bacteria uneaten, they also gain the ability to carry food bacteria along with dispersing spores in sori (Brock et al., 2011; DiSalvo et al., 2015; Khojandi et al., 2019). It is unknown whether the number of carried food bacteria changes in different environments. Changes in the number of carried food bacteria could depend on the abundance of food bacteria in the environment or on the fitness interests of *D. discoideum* hosts and *Paraburkholderia* symbionts.

The number of carried food bacteria could reflect a passive process that mirrors the densities of food bacteria in the environment. In this case, hosts that can carry (those that are infected with *Paraburkholderia*) would carry food bacteria proportional to the density of food bacteria in the environment. If this is the case, we expect hosts to carry more food after developing in a food-rich environment and fewer food bacteria after developing in a food-poor environment.

Alternatively, the number of carried food bacteria could reflect the evolutionary interests of hosts and symbionts. Since soil environments tend to be spatially and temporally structured

(Sun et al., 2003; Vos et al., 2013), developing in a food-poor environment may be associated with an increased probability that future environments will also tend to be food-poor. If this is the case, hosts may carry more food bacteria after developing in a food-poor environment than in a food-rich environment because hosts that seed the next food-poor environment with food bacteria will have an advantage. From the *Paraburkholderia* symbiont's perspective, allowing more carriage of food bacteria would keep their hosts alive and allow further dispersal of symbionts by hosts. Altering carriage in response to environmental conditions could therefore be adaptive for both hosts and symbionts.

We investigate three questions about the role of food bacteria in the symbiosis between *D. discoideum* and two commonly studied *Paraburkholderia* symbionts, *P. agricolaris* and *P. hayleyella* (Shu et al., 2018a; Garcia et al., 2019; Scott et al., 2022). We first re-evaluate some of the ideas behind the prudent predation hypothesis by measuring the density of any food bacteria that are left uneaten. We then test the fitness consequences of prudent predation on hosts and ask if hosts that leave more food bacteria uneaten pay a cost in spore production. Lastly, we turn to whether the number of food bacteria carried inside sori changes between food-poor and food-rich environments.

4.3 Methods

Clones and Culturing Methods

To compare between infected and uninfected hosts, we used 12 host *D. discoideum* clones, 4 clones uninfected, 4 infected with *P. agricolaris*, and 4 infected with *P. hayleyella*. We

refer to these infected clones as “reinfected” (Table 4.1) because they were cured with tetracycline and reinfected with 0.1% of the *Paraburkholderia* strain with which they were isolated. This reinfection scheme ensures that initial infection densities were the same across clones.

Table 4.1: List of clones.

Type of infection	Description	*Paraburkholderia* Treatment	Clones
Cured and reinfected	Clones that were cured of any native symbionts with tetracyline and reinfected in the lab with 0.1% of their native symbiont	*P. agricolaris* infected	QS159, QS161, QS606, NC21
		P. hayleyella infected	QS395, QS45, QS38, QS23
		Uninfected control	QS6, QS138, QS472, QS527
Uncured	Clones with or without natural infections that have not been treated with antibiotics	*P. agricolaris* infected	QS494, QS756, QS788, QS113, QS453, QS70, QS606
		P. hayleyella infected	QS45, QS101, QS46, QS2, QS23, QS529, QS38

		Uninfected control	QS4, QS6, QS14, QS18, QS9, QS8

We also used a set of uncured clones that have not been treated with antibiotics to modify their native infection levels. For these clones, we used 7 clones naturally infected with *P. agricolaris*, 7 clones naturally infected with *P. hayleyella*, and 6 clones that were not infected with any *Paraburkholderia* symbionts (Table 4.1). One naturally uninfected clone (QS1) was contaminated so we excluded it from the analysis leaving 6 clones in our last infection category. These uncured clones were useful for checking whether our results applied to unmanipulated infection densities.

To remove any effects of being in the freezer and to ensure that infected amoebae carried fluorescently labeled *Klebsiella pneumoniae* food bacteria, we grew amoebae through one round of feeding and fruiting body formation and then collected spores to initiate our experiments. This step may allow infection densities time to equilibrate (Miller et al., 2020). Amoebae were grown from frozen spores at room temperature on SM/5 plates (2 g glucose (Fisher Scientific), 2 g Bacto Peptone (Oxoid), 2 g yeast extract (Oxoid), 0.2 g MgSO4 * 7H2O (Fisher Scientific), 1.9 g KH2PO4 (Sigma-Aldrich), 1 g K2HPO4 (Fisher Scientific), and 15 g agar (Fisher Scientific) per liter) with 200 µL of 1.5 OD_{600} fluorescently labeled *K. pneumoniae* suspending in KK2 buffer (2.25 g KH2PO4 (Sigma-Aldrich) and 0.67 g K2HPO4 (Fisher Scientific) per liter). *K. pneumoniae* used in this study expressed green-fluorescent protein (GFP) and were provided by dictyBase (Fey et al. 2019). To mimic dispersal to food-poor and food-rich environments, we transferred 200uL of $2x10^5$/mL spore solution to plates with or without 200uL of 1.5 OD_{600} GFP-

expressing *K. pneumoniae*. We let bacteria and amoebae on plates proliferate at room temperature for six days, enough time for amoebae to form fruiting bodies.

Measurement of bacteria and host spore production

To measure the density of uneaten food bacteria after *D. discoideum* fruiting body formation, we first collected host spores and bacteria from plates after six days of growth by washing plates with 15 mL of KK2 buffer. We counted host spores from this washed solution using a hemocytometer. To measure bacterial density, we first removed host spores by centrifuging the wash solution for three minutes at 1300 rpm and collecting the supernatant. Removing host spores by manually removing sori with a pipette tip resulted in similar densities of uneaten food bacteria suggesting that the number of bacteria inside spores and sori is minimal relative to that left on the plate. After removing host material by centrifugation, we measured the optical density of the supernatant at 600 nm (OD_{600}) as well as fluorescence with an excitation wavelength of 485 and emission wavelength of 515 nm in a 96 well plate with a Tecan Infinite 200 Pro microplate reader.

Since the washed plate solution from infected clones contains GFP-expressing *K. pneumoniae* and unlabeled *Paraburkholderia*, the total OD_{600} is due to both kinds of bacteria but fluorescence only comes from the *K. pneumoniae*. To calculate the amount of OD_{600} due to fluorescing *K. pneumoniae*, we generated a standard curve relating fluorescence to OD_{600} using serial dilutions of GFP-expressing *K. pneumoniae* in KK2 and used this curve to predict the OD_{600} of *K. pneumoniae* in our samples. The remaining OD_{600} is the amount due to *Paraburkholderia* symbionts after subtracting off the background OD_{600} of the KK2 buffer. An

OD_{600} of 0.1 translates to around 5 x 10^7 *K. pneumoniae* cells and 1 x 10^8 *Paraburkholderia* cells according to the validation dataset in Scott et al. (2022).

To measure how many *K. pneumoniae* bacteria were carried in sori, we haphazardly sampled a single sorus from each of our experimental plates from re-infection experiments (n = 24). We suspended single sorus contents in KK2 buffer and plated out serial dilutions. Since *K. pneumoniae* are labeled with GFP, we could differentiate colonies of *K. pneumoniae* and *Paraburkholderia* and get counts of colony forming units (CFU). We then used these CFU counts to back-calculate the number of *K. pneumoniae* bacteria inside fruiting bodies.

Development assays for cured and reinfected hosts

To determine how symbionts affected host development, we took time-lapse images of cured and reinfected hosts growing in six well plates. We grew clones from the freezer on SM/5 plates for six days in case there were freezer effects as above. We then collected host spores and plated 30 uL of 2x10^5 spores per mL and 30 uL of 1.5 OD *K. pneumoniae* in each well. Photos were taken every hour until fruiting bodies developed using a Canon EOS Mark IV. We inspected photos to determine time points for when aggregates, slugs, and fruiting bodies first appeared in each well. We included three replicates for each species and performed each individual replicate on a separate date.

Calculating effect of uneaten food on host fitness

To estimate how much host proliferation was lost by leaving food bacteria uneaten, we used Kessin's (2001, p. 21) estimate that an amoeba is roughly 1,000 times larger than a bacteria and would thus require around 1,000 bacteria to divide. To estimate the number of food bacteria cells left uneaten, we used the validation data (Figure S1) in Scott et al. (2022) that predicted *K. pneumoniae* colony forming units from OD_{600}. The number of additional amoebae that would result from eating the uneaten food bacteria is then the estimated number of food bacteria divided by 1,000.

Statistical Methods

To compare the density of *K. pneumoniae* bacteria left uneaten on the plate for different infection categories (uninfected vs *P. agricolaris* infected vs *P. hayleyella* infected and cured vs infected), we fit linear models in R (version 3.6.3). For uncured infection comparisons that involved single measures of different clones, we used linear models fit by generalized least squares (GLS) in the nlme package (Pinheiro & Bates, 2006). For reinfection experiments, which were performed on two separate dates with the same clones, we fit linear mixed models (LMM) and included clone identity or date as a random effect depending on which random effect resulted in a better fit. To compare cured and infected hosts, we used ordinary linear regression with the lm function in R. We performed Tukey post-hoc tests for pairwise comparisons using the emmeans package (Lenth et al., 2019).

To determine whether the timing of development was affected by *Paraburkholderia* infection, we used generalized linear mixed models (GLMMs) with a Poisson link function. We

included the date that experiments were performed as a random effect to capture variation within plates.

To determine how leaving food bacteria uneaten and *Paraburkholderia* infection affect host spore production, we again used LMM and GLS for reinfections and uncured infections, respectively. If prudent predation results in a cost for hosts, we expected a decrease in fitness with increasing uneaten food bacteria. To test this, we fit linear models of host spore production for infections with each species. Uninfected hosts' spore production was included in each model to act as a baseline fitness when hosts are not affected by infection. Differences in host fitness could also be affected by the density of *Paraburkholderia* left on plates (Scott et al., 2022) or by infection category (uninfected vs infected) (Brock et al., 2011; DiSalvo et al., 2015; Haselkorn et al., 2019). To account for these possibilities, we also fit models with these variables along with a model that includes both uneaten food density and *Paraburkholderia* density. In total, we compared five models of spore production: (1) uneaten food density (Food model), (2) *Paraburkholderia* density (Para model), (3) uneaten food density + *Paraburkholderia* density (Food + Para model), (4) categorical infection status (Infection model), and (5) a null model fit with only the intercept. To more easily compare effects across the different models, we scaled all variables by subtracting the mean and dividing by the standard deviation. We selected the best models among these five using AICc, a measure of model fit that corrects for small sample sizes (Burnham & Anderson, 2004). Models that fit the data best have lower AICc values.

To compare the number of *K. pneumoniae* inside fruiting bodies in food-rich and food-poor environments, we used generalized mixed models with a logistic link function to test whether the food environment affected the probability of having food in a sorus. We transformed our response variable, the number of *K. pneumoniae* inside a fruiting body, to a presence absence

variable for the logistic regression. We used a logistic function in this case because of the number of zeros in our data, which could cause problems for linear mixed models and for generalized mixed models with Poisson fits. We fit our model to data from both species but determined that species identity was not a useful predictor using AICc.

4.4 Results

D. discoideum hosts infected with *Paraburkholderia* leave food bacteria uneaten

We investigated the food bacteria left behind after hosts formed fruiting bodies by measuring the density of leftover *K. pneumoniae* from cured and reinfected hosts and naturally uninfected controls (Table 4.1). These densities are estimated from fluorescence measurements since the *K. pneumoniae* used in this study expresses green fluorescent protein (GFP; see methods). First, we wanted to confirm that some of the bacteria hosts leave behind are food bacteria and not just *Paraburkholderia* symbionts. Leaving food bacteria uneaten so that hosts miss out on potential growth and proliferation was a key component of the prudent predation hypothesis, but previous studies did not differentiate what kind of bacteria were left behind (Brock et al. 2011, 2016b). Hosts that were cured and reinfected with *P. agricolaris* and hosts that were cured and reinfected with *P. hayleyella* leave more food bacteria on the plate (Figure 4.1A) than naturally uninfected *D. discoideum* (Tukey Post-Hoc test (TPH), $p \leq 0.001$ for both *Paraburkholderia* species).

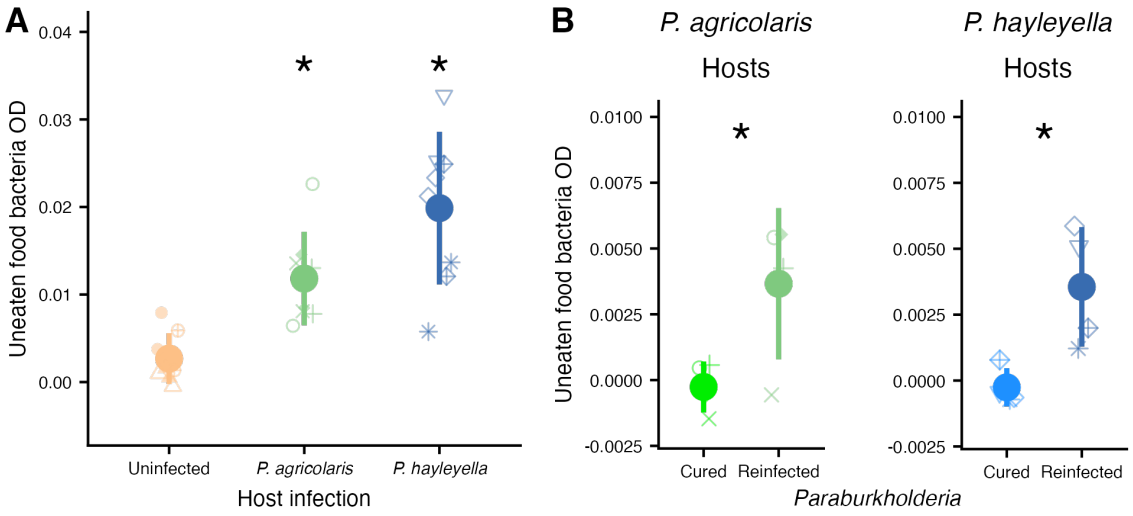

Figure 4.1: Symbionts cause hosts to leave food uneaten. (A) Density left on plate of uneaten *K. pneumoniae* food bacteria (measured by OD_{600}) for naturally uninfected, *P. agricolaris* cured and reinfected, and *P. hayleyella* cured and reinfected hosts. Reinfected hosts were reinfected with their natural symbiont partners. (B) Density left on plate of uneaten food bacteria for naturally infected *P. agricolaris* and *P. hayleyella* hosts that were either cured or reinfected. Shapes indicate clones (see Figure 1A in Scott et al. 2022). Dot and lines show mean and standard deviation, respectively. Asterisks indicate significant differences (in panel A comparisons are between infected and uninfected).

This comparison supports the view that symbionts cause food bacteria to be left uneaten but does not exclude the possibility that the differences are due to the different *D. discoideum* clones that we used. Therefore, we next compared cured and reinfected hosts to the same host genotypes that were cured of their native *Paraburkholderia* symbionts but not reinfected. Both *P. agricolaris* ($p = 0.0414$) and *hayleyella* ($p = 0.0185$) re-infections resulted in more food bacteria being left uneaten relative to cured but not reinfected *P. agricolaris* and *hayleyella* hosts (Figure 4.1B).

Host development is not affected by *Paraburkholderia* infection

We hypothesized that leaving food bacteria uneaten would be associated with faster development by infected hosts. To test this, we took time-lapse photos of amoebae aggregating into fruiting bodies that either did or did not also carry *Paraburkholderia*. We determined time courses for when they aggregated, formed slugs, and formed fruiting bodies. We found that development times were not affected by *Paraburkholderia* infection for either species (GLMMs; $p > 0.05$; Figure 4.2A&B).

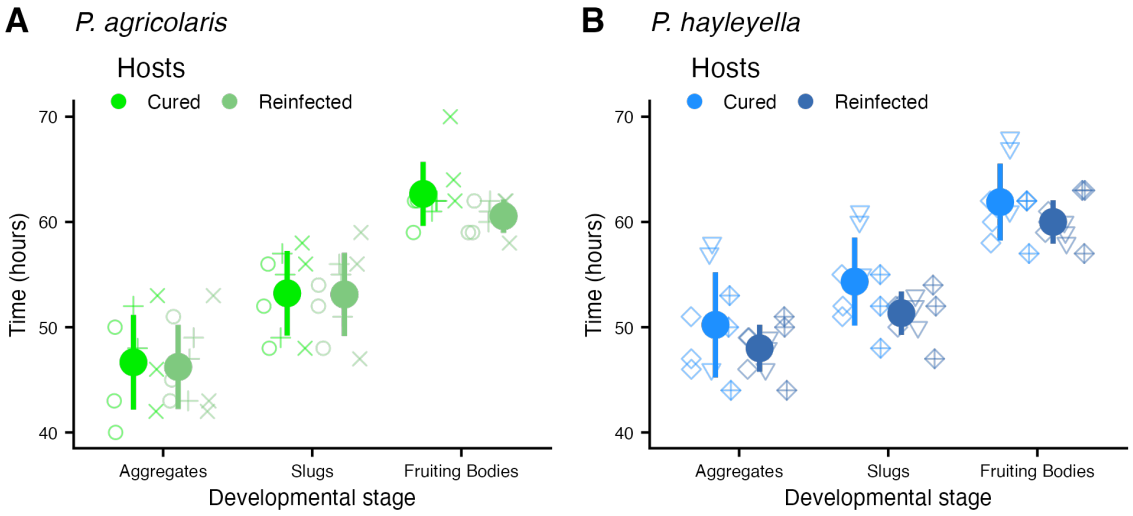

Figure 4.2: Symbionts do not affect development. Developmental time points for cured and reinfected (A) *P. agricolaris* and (B) *P. hayleyella* hosts at different stages of development. Shapes indicate clones (see Figure 1A in Scott et al. 2022). Dot and lines show mean and standard deviation, respectively.

Food bacteria left on plates is not associated with reduced spore production in hosts; *Paraburkholderia* infection is

Leaving more food uneaten is suspected to lower host fitness and could therefore explain the cost of *Paraburkholderia* infection relative to uninfected hosts (Brock et al., 2011). Alternately, the cost of infection may be due to *Paraburkholderia* — this could be measured as a

categorical variable (infected vs uninfected) or as a continuous variable (the density of *Paraburkholderia* symbionts on plates). Costs could also result from the density of both uneaten food bacteria and *Paraburkholderia* symbionts.

We first tested the role of uneaten food bacteria and *Paraburkholderia* on host spore production using our cured and reinfected hosts. For infections with both species of *Paraburkholderia*, null models fit to only the intercept fit the data best (Figure 4.3A; Supplemental File 1; the model that includes uneaten food bacteria and *Paraburkholderia* density for *P. agricolaris* infected hosts — Food + Para — did show confidence intervals that did not overlap zero, but this was the worst model in terms of AICc).

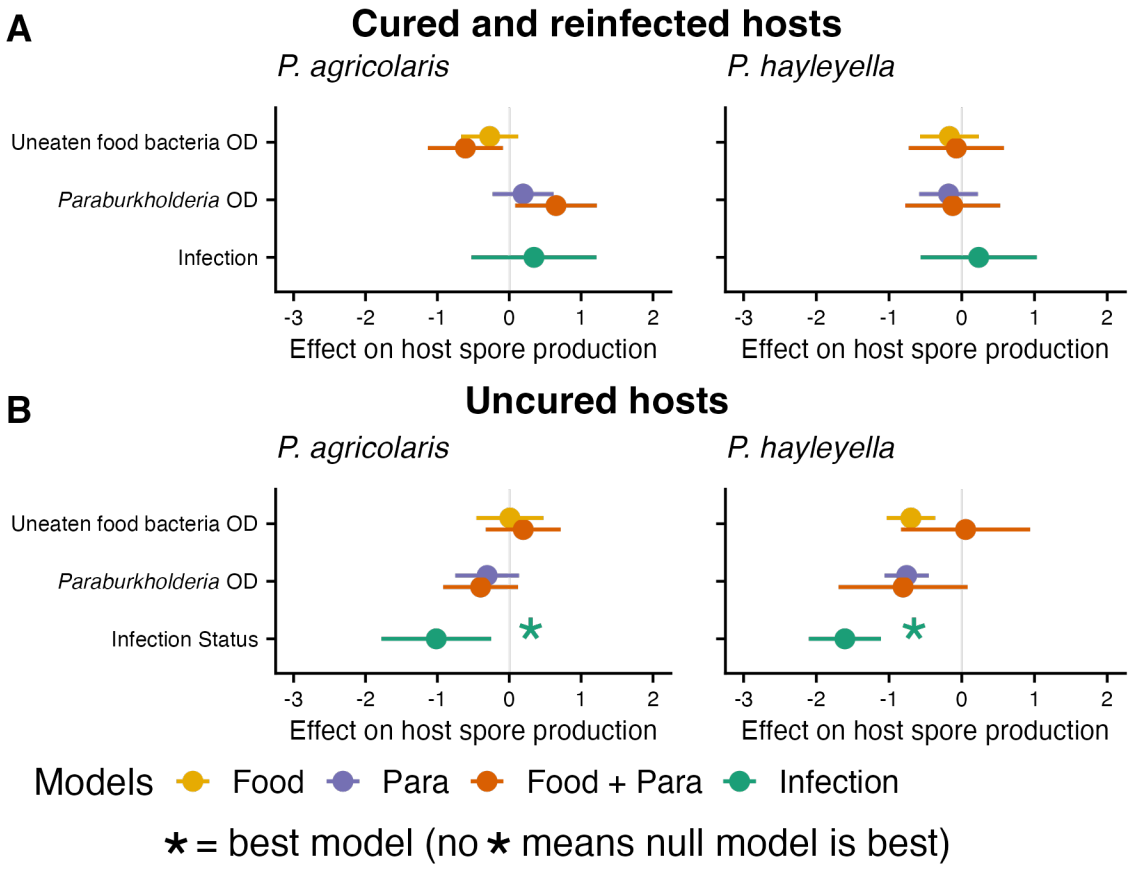

Figure 4.3: Estimated effects of bacteria left on plates and infection status on host spore production in (A) cured and reinfected (B) and uncured hosts (see Table 4.1). Hosts without *Paraburkholderia* are

included as baselines in all models. We compared models of host spore production (shown in different colors), predicted by *Paraburkholderia* density only (OD), uneaten food bacteria only (OD), both *Paraburkholderia* and uneaten food bacteria, or a categorical variable for infection status (infected with *Paraburkholderia* or not). Estimated effects are shown as points and 85% confidence intervals are shown as lines (null models with only the intercept are not shown). Asterisk color indicates the best model according to AICc (there is no asterisk in A because the null models fit with only the intercept are the best models).

Our experiment with cured and reinfected hosts showed no effects of any of these variables (Figure 4.3A) on host spore production (the null model without any variables was the best fit). This includes no significant cost of infection, contrary to earlier results (Brock et al., 2011; DiSalvo et al., 2015; Haselkorn et al., 2019). The lack of cost may be because hosts were infected with an extremely low density of *Paraburkholderia* symbionts (Scott et al., 2022). To test whether unmanipulated infection densities changed our results, we performed a separate experiment with uncured hosts that have not been treated with antibiotics (Table 4.1). Similar uncured clones have been shown to exhibit costs of infection (Brock et al., 2011).

Uncured hosts were afflicted by a cost of infection, but this cost was due to *Paraburkholderia* infection instead of lost growth and proliferation from leaving uneaten food bacteria. For uncured hosts with *P. agricolaris*, models of host spore production that included uneaten food (Food model in Figure 4.3B), *Paraburkholderia* density (Para model), and both variables together (Food + Para) were poor predictors of host spore production (Supplemental File 1). The model with infection status was the best predictor of host spore production for *P. agricolaris* uncured hosts (note that it is within 2 AICc units of the null model). These *P. agricolaris* results identify a cost of infection like that found in prior studies (Brock et al., 2011; DiSalvo et al., 2015; Haselkorn et al., 2019).

For uncured hosts with *P. hayleyella*, we also found that *Paraburkholderia* infection was responsible for costs. Uneaten food bacteria was associated with lower spore production only if uneaten food was the only variable included in the model (Figure 4.3B). Including *Paraburkholderia* density changed the effect of uneaten food to slightly positive with confidence intervals that overlap zero. *Paraburkholderia* density was a better predictor of hosts spore production than uneaten food and was highly correlated with uneaten food density (> 0.9). For uncured hosts infected with *P. hayleyella*, infection status was again the best predictor (Supplemental File 1). Our model comparisons for *P. hayleyella* also demonstrated a cost of infection. These models of host spore production in uncured hosts point to a more direct role for *Paraburkholderia* symbionts in reducing host spore production rather than a cost from prudent predation.

Uneaten food bacteria are a minority of left-behind bacteria

Food bacteria left behind did not have the predicted effects on either development time or spore production costs. One reason may be that the amount of left behind food is not as great as formerly thought. In fact, uneaten food bacteria make up a minority of left-behind bacteria, with the majority being *Paraburkholderia* (Figure 4.4). Using Kessin's (2001, p. 21) rough estimate that an amoebae needs to eat 1,000 bacteria to divide, we calculate that the number of uneaten food bacteria is only enough to produce 0.001-0.004% more spores than what we recovered from plates. It is thus unlikely that uneaten food bacteria can noticeably affect host fitness.

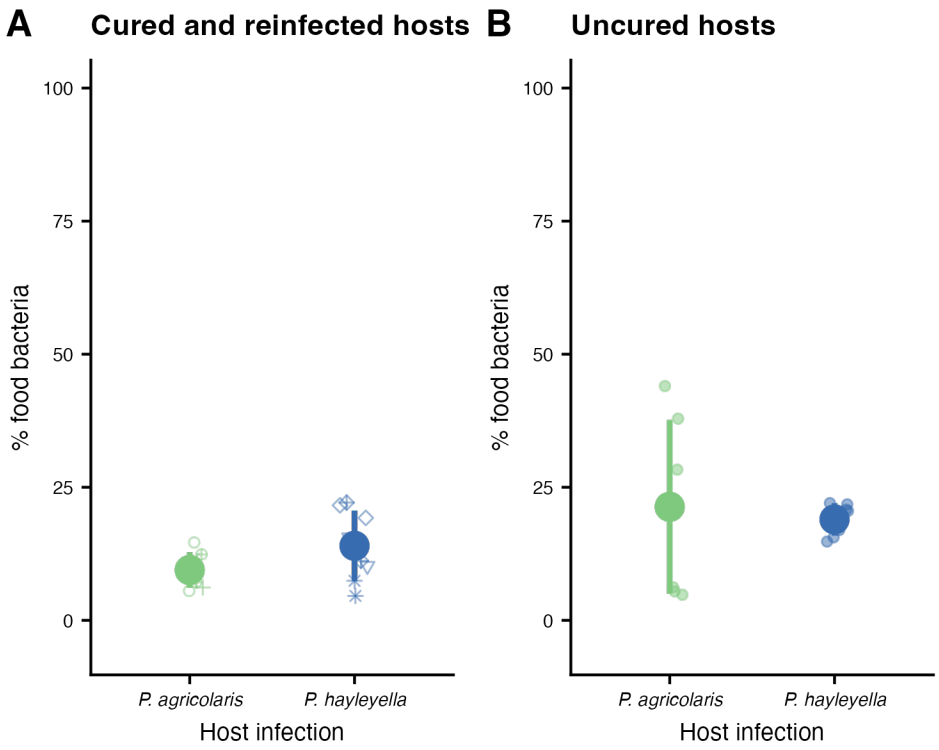

Figure 4.4: Most of the bacteria left behind by hosts were not food bacteria. Percent of the total left behind bacteria (includes food bacteria and *Paraburkholderia* symbionts) that was food bacteria from cured and reinfected (A) and uncured (B) hosts. Shapes in A show host clone replicates that were measured on two separate dates (see Figure 1A in Scott et al. 2022). Points in B are independent, so we do not show clone identity. Dot and lines show mean and standard deviation, respectively.

More food bacteria are carried after hosts develop in food poor conditions

To determine whether the number of food bacteria carried in sori simply reflects the number of food bacteria in the previous environment or the interests of hosts and symbionts, we measured the number of fluorescent food bacteria inside sori after growth on food-rich and food-poor plates (Figure 4.5A). Counter-intuitively, we found that for both *Paraburkholderia* species (Figure 4.5B&C), sori produced in food-rich environments had a lower proportion of containing food bacteria (GLMM, Log odds = -1.894, se = 0.942, p = 0.044). This shows that the number of carried food bacteria is affected by food context, but in the opposite direction to that expected if

food was carried in proportion to the density of food bacteria in the environment. Thus, the amount of carried food bacteria may depend on the fitness interests of hosts and symbionts that benefit from carrying more food bacteria when harsh conditions are expected.

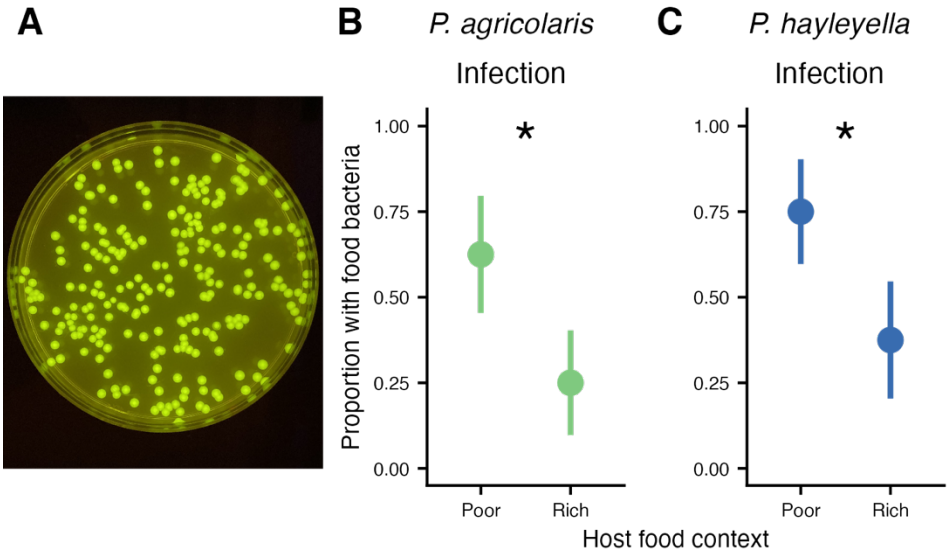

Figure 4.5: Hosts are less likely to carry food bacteria in sori after developing in food-rich environments. (A) Photo of fluorescent *K. pneumoniae* food bacteria colonies plated from an individual sorus. (B) Proportion of sori with carried food bacteria for reinfected *P. agricolaris* hosts from food-poor and food-rich contexts. (C) Proportion of sori with carried food bacteria for reinfected *P. hayleyella* hosts from food-poor and food-rich contexts. Points show proportions and lines show the standard deviation. Asterisks indicate significant differences (GLMM).

4.5 Discussion

Third parties that interact with symbiotic partners can affect the fitness effects of interactions (Palmer et al., 2008; Chamberlain et al., 2014; Wood et al., 2018; Hafer-Hahmann & Vorburger, 2020; Cassidy et al., 2022). Often the details of how third parties affect fitness effects are unknown. In the symbiosis between *D. discoideum* social amoebae and *Paraburkholderia* bacteria, the third-party food bacteria is normally eaten by the host but can also be carried or left

behind. We studied how many food bacteria are carried and left behind, and their impacts, by tracking fluorescently labeled food bacteria (*K. pneumoniae*) during fruiting body formation by hosts.

Our most surprising finding was that hosts were more likely to carry food bacteria after growing in food-poor environments (Figure 4.5). This result is surprising because it means that hosts actively change the number of carried food bacteria depending on environmental conditions. Since bacterial carriage is a joint phenotype affected by both hosts and symbionts, *Paraburkholderia* symbionts may also play a role in modifying carriage depending on the environment.

Our observation that food bacteria carriage depended on the environment (Figure 4.5) is possible evidence for mutual benefit between *D. discoideum* hosts and *Paraburkholderia* symbionts. Host and symbionts are likely to benefit from modifying carriage if future soil environments tend to resemble past soil environments Repeated food-poor environments are where hosts should most benefit from carrying extra food bacteria. *Paraburkholderia* symbionts could benefit from a stable association with hosts that allows for continued dispersal, though more work is needed on the costs and benefits of symbiosis for *Paraburkholderia* symbionts. We thus speculate that carrying more food in these contexts may represent cooperation between hosts and symbionts that allows the symbiosis to persist over repeated harsh environments. Such harsh conditions are potentially an important force shaping this symbiosis (Scott et al., 2022).

Questions remain about how hosts and symbionts affect the joint phenotype of carrying food bacteria. *Paraburkholderia* are more often carried inside spores while food bacteria are more often carried outside spores (Khojandi et al., 2019), but the mechanism for this is unknown.

Hosts are able to normally modify the contents of fruiting bodies through their immune cells that protect against toxins and bacteria by collecting potential threats and dropping off during the slug stage (Chen et al., 2007) but the role of these immune cells play in the symbiosis (Brock et al., 2016a) needs to be further explored. Manipulation of phagosomes could also play a role in determining the contents of fruiting bodies. *Paraburkholderia* symbionts increase the pH of phagosomes, presumably to prevent host digestion of symbionts (Tian et al., 2022). Similar modification of lysosomes is used by pathogens to evade human immune cells during infection (Isberg et al., 2009; Leseigneur et al., 2020). More work is needed to understand how symbionts and food bacteria get into fruiting bodies and how both hosts and symbionts contribute to bacterial carriage.

We also found that *Paraburkholderia* infection prevents hosts from eating all the food bacteria in an environment (Figure 4.1). We expected this result given the role of *Paraburkholderia* in causing hosts to carry food bacteria (DiSalvo et al., 2015) and leave behind bacteria (Brock et al., 2016b; Scott et al., 2022). Infection with *Paraburkholderia* likely interferes with host digestion because *Paraburkholderia* symbionts make phagosomes less acidic (Tian et al., 2022). As host amoebae eat food bacteria, *Paraburkholderia* symbionts make up a larger fraction of the ingested bacteria and eventually turn off the host feeding response because food bacteria are no longer being digested by hosts. This indigestion hypothesis is supported by our finding that *Paraburkholderia* infection did not affect development time (Figure 4.2). Development time should be faster for infected hosts since they appear to eat less food and should starve sooner. Indigestion by *Paraburkholderia* symbionts may be making hosts sick and delaying development enough to result in no difference between uninfected and infected hosts.

A prior study suggested that leaving food bacteria uneaten because of prudent predation may explain the cost of infection relative to uninfected hosts (Brock et al., 2011). We instead found that these costs to hosts were better explained by *Paraburkholderia* infection than by the amount of food bacteria left behind (Figure 4.3). The quantity of left behind food bacteria may be too small to noticeably affect host spore production since we observed that only a minority of left-behind bacteria was food bacteria, with the majority being indigestible *Paraburkholderia* (Figure 4.4). A rough calculation of lost growth also showed that uneaten food bacteria was not enough to substantially increase host fitness. We thus conclude that there is little support that prudent predation explains the cost of infection in this symbiosis.

Instead of hosts paying a cost in potential growth because they leave food bacteria uneaten, we suspect that *Paraburkholderia* infection causes both observations: that hosts leave food uneaten and infected hosts pay a cost. Support for this idea comes from our findings that *Paraburkholderia* density was correlated with uneaten food bacteria and *Paraburkholderia* density was a better predictor of host fitness in *P. hayleyella* uncured hosts (Figure 4.3). The role of *Paraburkholderia* in reducing host spore production is more evidence in support of there being some conflict in this symbiosis (DiSalvo et al., 2015; Scott et al., 2022).

Conflict may be more pronounced between *P. hayleyella* and its hosts than between *P. agricolaris* and its hosts. We found that *P. hayleyella* density could explain some of the decrease in uncured host spore production but did not find the same for *P. agricolaris* density (Figure 4.3; Supplemental File 1). This difference between *P. agricolaris* and *P. hayleyella* may result from *P. hayleyella* being more toxic than *P. agricolaris* (Shu et al., 2018a; Haselkorn et al., 2019; Khojandi et al., 2019). In addition to being more toxic, *P. hayleyella* also has a reduced genome relative to *P. agricolaris* (Brock et al., 2020; Noh et al., 2022). Reduced genomes are a common

result of persistent host association in beneficial symbionts and pathogens (McCutcheon & Moran, 2012). Since *P. hayleyella* maintains toxicity, it likely retains some pathogenic ability against hosts.

An interesting remaining question in the *D. discoideum-Paraburkholderia* symbiosis and in other symbioses is the mechanisms controlling conflict and causing symbiont infection to reduce fitness. One potential explanation is that *Paraburkholderia* symbionts directly feed on host cells or otherwise extract nutrients from hosts. Measures of *Paraburkholderia* density inside sori have so far not been found to be correlated with host fitness within species (Miller et al., 2020), though the more toxic *P. hayleyella* does appear to infect a higher percentage of spores than less toxic *P. agricolaris* (Shu et al., 2018a; Khojandi et al., 2019). Another promising hypothesis for the *D. discoideum-Paraburkholderia* symbiosis that deserves further study is that hosts have lower spore production because of "collateral damage" from competitive interactions between *Paraburkholderia* and food bacteria (Scott et al., 2022). Competition between bacteria is often mediated by chemical warfare (Granato et al., 2019) that could reduce host *D. discoideum* fitness as a side-effect.

Whether a specific symbiosis involves fitness alignment or conflict may depend on a third party that affects the costs and benefits of symbiosis. Our results show that third parties can have complex effects on symbioses; the symbiosis between *D. discoideum* and *Paraburkholderia* appears to involve elements of conflict and cooperation that are affected in multiple ways by food bacteria.

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Chapter 5: Cooperation loci are more pleiotropic than private loci in bacterium *Pseudomonas aeruginosa*

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5.1 Abstract

Pleiotropy may affect the maintenance of cooperation by limiting cheater mutants if such mutants lose other important traits. If pleiotropy limits cheaters, selection may favor cooperation loci that are more pleiotropic. However, the same should not be true for private loci with functions unrelated to cooperation. Pleiotropy in cooperative loci has mostly been studied with single loci and has not been measured on a wide scale or compared to a suitable set of control loci with private functions. I remedy this gap by comparing genomic measures of pleiotropy in previously identified cooperative and private loci in *Pseudomonas aeruginosa*. I found that cooperative loci in *P. aeruginosa* tended to be more pleiotropic than private loci according to the number of protein-protein interactions, the number of gene ontology terms, and gene expression specificity. These results show that pleiotropy may be a general way to limit cheating and that cooperation may shape pleiotropy in the genome.

5.2 Introduction

Many loci are pleiotropic, where a pleiotropic locus is defined as one that affects multiple traits. Pleiotropy constrains evolution because mutations with beneficial effects on one trait can have deleterious effects on other traits. Several measures of pleiotropy have been used and shown to be associated with evolutionary constraint. Three examples are the number of protein interactions (1), the number of functional annotations (2), and how widely genes are expressed across tissues (3).

Pleiotropy is thought to limit cheaters and stabilize cooperation (4–7; see 8 for a view on synergistic pleiotrpoy and 9 for a dissenting view). Explaining how cheater evolution can be limited is a central question in the study of cooperation (10). Cheaters benefit from cooperation without paying the costs and are expected to outcompete cooperators. This cheater advantage can lead to the breakdown of cooperation unless cheaters are constrained (10).

Pleiotropy can limit cheaters when mutations at a locus underpinning a cooperative trait (cooperative locus) cause cheater phenotypes that come with harmful effects on other traits. One example of this comes from the social amoeba *Dictyostelium discoideum*. *D. discoideum* has a cooperative stage where 20% of cells sacrifice themselves to become stalk. The remaining cells become spores that are held up for dispersal by the stalk (10). This act of cooperation can be exploited by cheaters that contribute less to the stalk and increase their abundance in spores (10). Amoebae with dimA mutations are potential cheaters because they ignore the signal to become stalk (5). This should increase dimA mutant representation as spores. Instead, dimA mutants are excluded from spores when mixed with wildtype cells as a pleiotropic effect (5). This trade-off between becoming a stalk cell and entering spores limits the cheating ability of dimA mutants.

If pleiotropy at a cooperative locus limits cheaters and stabilizes cooperation, selection on cooperative groups may favor higher pleiotropy at cooperative loci relative to private (non-cooperative) loci (4, 6). It is unknown whether cooperative loci are generally more pleiotropic than private loci, though this pattern has been observed in silico (4, 6). Prior studies of pleiotropy and cooperation in living organisms mostly focused on individual loci (5) or on gene co-regulation (7). Such studies do not explicitly quantify pleiotropy or compare between cooperative and private loci. Private loci are an important control because they reflect the background pleiotropy and selection does not favor higher pleiotropy in these loci (4, 6).

Here, I take a genomic approach to compare loci involved in cooperative or private traits as categorized by Belcher et al. (11) in four *P. aeruginosa* gene sets. To identify cooperation loci, Belcher et al. (11) combined functional annotations and experimental data to identify gene products that act as a public good and can be cheated (often those that are secreted). These sets of loci have been constructed so that cooperative and private loci in a set are expressed under similar conditions and are similarly exposed to selection, all else being equal within a set (11). Belcher et al. (11) found evidence for relaxed selection in cooperation loci relative to private loci, a pattern that is consistent with kin selection. This is additional support that the cooperation and private categories capture something about the social effect of these loci. These sets of cooperative and private loci are therefore ideal for testing whether cooperative loci are more pleiotropic.

4.3 Methods and Results

To test whether pleiotropy is higher in cooperation loci compared to private loci, I first used 315 quorum sensing (QS) loci (12) that were previously categorized as cooperative (N = 41) or private (N = 274; Dataset S1) based on gene annotations and experimental data (11). As measures of pleiotropy, I used the number of protein interactions contained in the STRING database (13), the number of biological process Gene Ontology (GO) terms (14), and gene expression pleiotropy for each locus with available data (Figure 5.1; Dataset S2). More detailed methods can be found in the SI Extended Methods. These pleiotropy measures were not highly correlated and thus represent independent measures of pleiotropy (see SI Extended Methods).

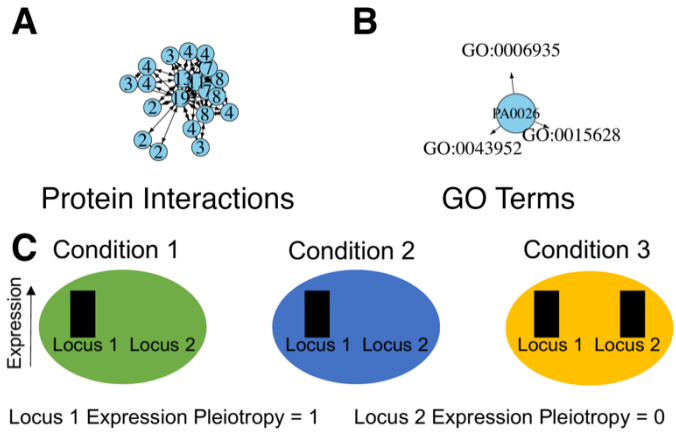

Figure 5.1: Examples of pleiotropy measures used in this study. (A) Number of protein interactions (STRING database). Arrows show predicted interactions that are summed to measure pleiotropy (the numbers on the nodes). (B) Number of biological process Gene Ontology (GO) terms from the *P. aeruginosa* genome database. Arrows show annotations for an example locus with three GO terms. (C) Gene expression pleiotropy is calculated from gene expression data across multiple conditions. Locus 1 is expressed in every condition and is maximally pleiotropic. Locus 2 is specialized for a single condition and is minimally pleiotropic.

I found that cooperative loci in the QS pathway were more pleiotropic across all three measures of pleiotropy (Figure 5.2A). Cooperative loci had about 65 more STRING interactions on average than private loci (generalized linear model (GLM); $p = 0.016$). Cooperative loci had 2

GO terms while private loci tended to have only 1 (GLM; $p = 0.009$). Gene expression pleiotropy was around 18% higher in cooperative loci than private loci (beta regression; $p < 0.001$).

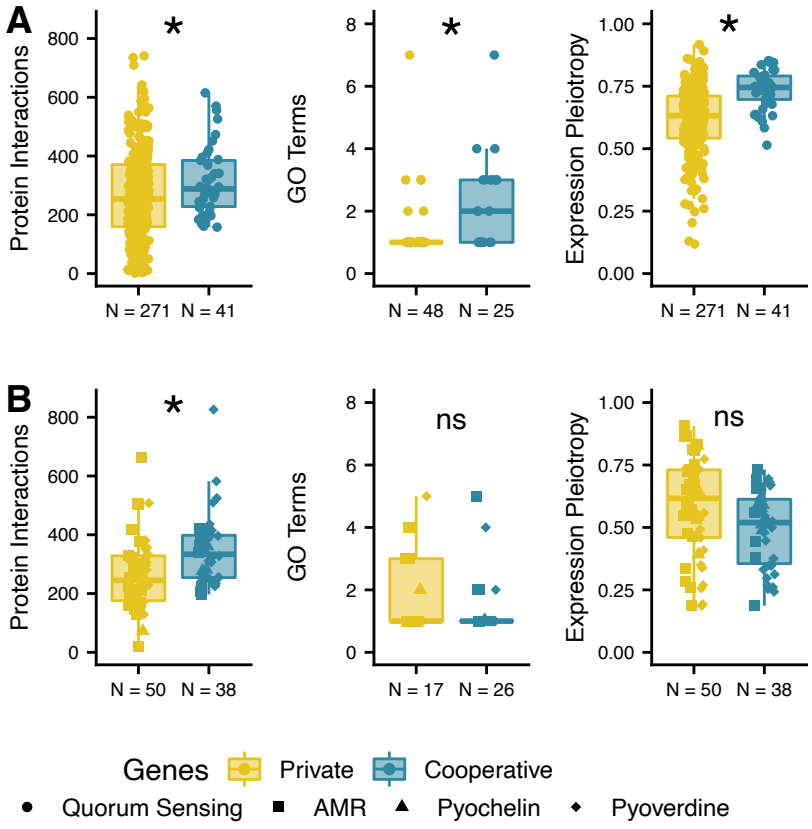

Figure 5.2: Cooperative loci tend to be more pleiotropic than private loci in the (A) quorum sensing and (B) additional sets of loci in *P. aeruginosa* (AMR = antibiotic resistance). Panels show the number of protein-protein interactions in the STRING database (left), the number of gene ontology (GO) terms (middle), and the gene expression pleiotropy (right). Number of loci with measures of pleiotropy are shown on the x-axis. Colors are the same as in (11) for easy comparison. *'s indicate $p \leq 0.05$.

To test whether these results apply beyond the QS pathway, I tested additional sets of cooperative and private loci (Dataset S3) in *P. aeruginosa* that were included in Belcher et al. (11). These sets consisted of antibiotic resistance genes (AMR) and pyochelin and pyoverdine genes that are involved in binding iron. To increase statistical power, I included set as a covariate (Figure 5.2B; see SI Extended Methods). Cooperative loci tended to have more STRING protein

interactions than private loci (GLM; $p = 0.008$). However, cooperative and private loci were not different in terms of GO terms (GLM; $p = 0.578$) and expression pleiotropy (beta regression; $p = 0.301$).

5.4 Discussion

Cooperation can break down because of the evolution of cheaters that benefit from cooperation without helping (10). The advantage of a cheater mutant at a cooperative locus can be limited if the cheater has pleiotropic effects on other important traits (4–7). Selection for cooperative groups may result in high pleiotropy at cooperative loci to limit cheaters (4, 6). Prior studies have focused on single loci (5), coregulation (7), and in silico analysis (4, 6) instead of measuring pleiotropy across many loci.

Using three independent measures of pleiotropy in *P. aeruginosa*, I found that pleiotropy tended to be higher in cooperative loci than in private loci regulated by QS (Figure 5.2A). Only one comparison of the additional sets (Figure 5.2B) resulted in more pleiotropy in cooperative loci. Pleiotropy may thus be higher only in QS loci or the effect of pleiotropy in the additional sets is detectable only as measured by STRING interactions. The additional sets also consist of fewer loci which could have limited my ability to detect an effect.

My finding of increased pleiotropy in cooperation loci (Figure 5.2) may strengthen the conclusions of Belcher et al. (11). This study found relaxed selection in cooperation loci relative to private loci consistent with predictions from kin selection theory. Belcher et al. (11) assumed

that expression would be similar between cooperation and private loci because they are part of the same pathway. This would rule out relaxed selection due to cooperation loci being conditionally expressed in only some conditions (15). My expression pleiotropy measure shows that conditional expression does not explain the signals of relaxed selection in cooperative QS loci since these were expressed across a wider set of conditions (Figure 5.2A). Pleiotropy also affects patterns of selection, but it should be in the opposite direction of kin selection. Pleiotropy is associated with signals of conservation (2) and stabilizing selection (16), which should decrease genetic diversity and divergence. My results thus mean that the signal of kin selection in (11) may be an underestimate since increased pleiotropy in cooperation loci should dampen the signal of relaxed selection.

Theoretical studies have disagreed about the direction of causality between cooperation and pleiotropy and whether pleiotropy is able to stabilize cooperation if pleiotropy itself can evolve (4, 6, 8, 9). An interesting possibility that deserves more study is that cooperation creates the conditions for increased pleiotropy (6, 9), possibly through the evolution of more complex regulatory architectures underlying cooperative traits. While the data in this study cannot resolve these theoretical questions, they show that pleiotropy and cooperation are linked *in P. aeruginosa*. Cooperation may thus shape patterns of pleiotropy in the genomes of other cooperative organisms through its link with high pleiotropy.

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Data Availability

Data for this analysis is included in the supporting information. Data and R code for analysis can also be found at www.gitlab.com/treyjscott/kin_selection_pleiotropy.

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5.6 SI Extended Methods

Gene Sets

I gathered 315 quorum sensing genes from Schuster et al. (1) and categorized 41 of these genes as cooperative from Belcher et al. (2). The remaining genes were classified as private genes that did not have a social function (Dataset S1). Additional pyochelin, pyoverdine, and AMR cooperative and private genes were also gathered from Belcher et al. (2) and are provided in Dataset S2.

Pleiotropy

I investigated three measures of pleiotropy (Dataset S3). First, I used predicted protein-protein interactions for *P. aeruginosa* PAO1 downloaded from the STRING database version 11.5 on May 6th, 2022 (3). STRING contains predicted protein interactions collected from high-throughput experiments, text mining, and other resources. These interactions may not necessarily

involve physical interactions between proteins but should convey functional relationships. STRING entries have confidence scores that provide a measure of quality for interaction predictions. I incorporated this measure in statistical models by weighting according to the average score for a protein's combined interactions.

My second measure of pleiotropy was the number of non-redundant biological process gene ontology annotations for *P. aeruginosa* PAO1. These annotations convey information about the functions that a gene has or is predicted to have (4). These data were downloaded on April 4th 2022 from the *P. aeruginosa* genome database version 20.2 (5), which updates annotations based on new results published on *P. aeruginosa*.

My final measure was gene expression pleiotropy, a measure of how widely genes are expressed across conditions. This measure is useful in addition to the above measures from databases because it should be free from any biases associated with how loci are annotated. I calculated gene expression pleiotropy as 1 - τ, where τ is a common measure of gene expression specificity (6). τ ranges from 0, when a gene is expressed in all conditions tested, to 1, where the gene is expressed in only 1 condition and is calculated for each gene as

$$\tau = \frac{\sum_i \left(\left(1 - ln(x_i)\right)/ln(x_{max})\right)}{N-1},$$

where N is the number of conditions, xi is the expression level in conditions i, and xmax is the maximum expression across all conditions (7). τ is usually calculated across different kinds of tissues. Since *P. aeruginosa* does not have conventional tissues, I instead used gene expression data (GSE55197) from strain PA14 grown in 14 different conditions (8). I normalized raw

transcripts using DESeq2 (9). To ensure that log expression was positive, I manually changed the minimum expression to 1.

Statistics

To determine whether the three pleiotropy measures were independent, I checked for correlations using Spearman's ρ. Correlations were weak ranging from -0.015 between GO terms and expression pleiotropy to -0.346 between protein interactions and expression pleiotropy. The correlation between GO terms and protein interactions was 0.039. These pleiotropy measures were thus relatively independent.

To test whether cooperative genes were more pleiotropic than private genes for the quorum sensing pathway, I used generalized linear models (GLMs). For protein interactions and GO terms, I fit models with Poisson errors. If I detected overdispersion, I fit quasi-Poisson and negative binomial models for the final analysis. I conservatively reported the highest p-value between quasi-Poisson and negative binomial models if more than one model was fit. To include STRING confidence scores (see above), I weighted protein interaction models by the average score of its interactions. For gene expression pleiotropy, I used beta regression (10) to account for this measure being bounded from 0 to 1. To calculate means and p-values from statistical models, I used the emmeans package (11). I performed statistical tests in R (12) (version 4.1.2).

To test for differences between cooperative and private genes for the additional gene sets, I again used GLMs as above. I included the pathway (pyoverdine, pyochelin, or AMR) as a covariate in models, but compared means only between cooperative and private genes.

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Chapter 6: Long-term evolutionary conflict, Sisyphean arms races, and power in Fisher's geometric model

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6.1 Abstract

Evolutionary conflict and arms races are important drivers of evolution in nature. During arms races, new abilities in one party select for counter-abilities in the second party. This process can repeat and lead to successive fixations of novel mutations, without a long-term increase in fitness. Models of co-evolution rarely address successive fixations and one of the main models that uses successive fixations – Fisher's geometric model – does not address co-evolution. We address this gap by expanding Fisher's geometric model to the evolution of joint phenotypes that are affected by two parties, such as probability of infection of a host by a pathogen. The model confirms important intuitions and offers some new insights. Conflict can lead to long-term Sisyphean arms races, where parties continue to climb towards their fitness peaks, but are dragged back down by their opponents. This results in far more adaptive evolution compared to the standard geometric model. It also results in fixation of mutations of larger effect, with the important implication that the common modeling assumption of small mutations will apply less

often under conflict. Even in comparison with random abiotic change, evolution under conflict results in greater distances from the optimum, lower fitnesses, and more fixations, but surprisingly, not larger fixed mutations. We also show how asymmetries in selection strength, mutation size, and mutation input allow one party to win over another. However, winning abilities come with diminishing returns, helping to keep weaker parties in the game.

6.2 Introduction

Through natural selection, species may become adapted to their environments. Environments include interactions with other organisms, both within and between species. When there is evolutionary conflict, that is when each party can increase its fitness at the expense of the other party, this process of adaptation can drive antagonistic co-evolution and arms races, leading to maladaptation of one or both parties (Van Valen, 1973; Dawkins & Krebs, 1979; Brockhurst et al., 2014; Queller & Strassmann, 2018). These interactions may be important drivers of evolution in nature (Thompson, 2013; Queller & Strassmann, 2018). Evidence that this is so includes rapid adaptation in response to new biotic foes (Reznick & Ghalambor, 2001), molecular evolution in response to pathogens (Endo et al., 1996; Tiffin, 2004; Enard et al., 2016), and the many examples of arms races found in nature (Berenbaum & Zangerl, 1998; Benkman et al., 2003; Decaestecker et al., 2007; Hanifin et al., 2008; Toju, 2008; Edger et al., 2015) and evolved in the lab (Pal et al., 2007; Paterson et al., 2010). This evidence supports the idea (Van Valen, 1973; Dawkins & Krebs, 1979) that conflicts may drive a great deal of adaptive evolution, though without necessarily leading to higher fitness.

Conflict has been analyzed in various models of co-evolution (Kokko et al., 2006; Nuismer, 2017). However, Fisher's (1930) geometric model is conspicuously absent from studies of co-evolution. This is surprising because of the geometric model's success as a general model of adaptation and because of its focus on successive fixations (Tenaillon, 2014), an essential element of many arms races (Woolhouse et al., 2002; Daugherty & Malik, 2012; Marston et al., 2012; Edger et al., 2015) that is usually left out of co-evolutionary models (unless changes are assumed to be very small (e.g. Dieckmann & Law, 1996; Nuismer, 2017)). The ability to model successive fixations should make the geometric model a potentially powerful tool to investigate how conflict can lead to arms races.

Fisher's geometric model treats an adapting population as an n-dimensional vector of trait values. Somewhere in n-dimensional space is an optimum where the population is most fit for all n traits. In an initially monomorphic population, a random mutation is introduced, typically from a Gaussian distribution, causing an additive shift in the trait space. This mutation can either fix or be lost, making the population monomorphic again. Selection favors mutations that move the population closer to the optimum (Figure 6.1A shows a single-trait version of this process). By this process of successively fixing mutations, a population goes on an "adaptive walk", usually from lower fitness to higher fitness closer to the optimum (Tenaillon, 2014). Large populations in Fisher's geometric model move relatively rapidly to the optimum and stay there. With small population sizes, fixations of small deleterious mutations due to drift keep a population at a variable, but usually small, distance from the optimum (Poon & Otto, 2000). An important result from studies of these adaptive walks is that mutations are less likely to fix when a population is well adapted, and this is especially true for mutations of large effect (we will call these large mutations) because they can overshoot the optimum (Fisher, 1930; Orr, 1998, 2005).

To capture evolution under biotic conflict with Fisher's geometric model we need to consider two evolving parties instead of one. In order to link them, we use the concept of a joint phenotype, a trait that is the shared outcome of the actions of two different parties (Queller, 2014; Queller & Strassmann, 2018). For example, we could think of the probability of infection as a joint phenotype; various traits of the pathogen and host may interact to make the joint phenotype and evolution in either party can change the value of this phenotype. Other examples of joint phenotypes are the probability that a predator catches a prey in a given encounter and the amount of food a cuckoo chick gets from its host. More formally, joint phenotypes are a general way to model interactions between different parties, where the interaction between two traits x and y is reduced to a single measure $z = f(x,y)$. Thus joint phenotypes include more commonly used interaction models, such as those that are determined by the difference between traits $z = x - y$ (Nuismer, 2017).

Joint phenotypes are often high-level phenotypes in the sense that they can be affected by many lower-level private phenotypes or traits, that is, traits that belong to one of the two parties. The joint phenotype emerges from the net effect of interactions between lower-level private traits. For the predator and prey these private traits might include speed, agility, stealth, sensory capabilities, weapons, and defenses (with still lower-level phenotypes contributing to each of those). The joint phenotype is the net effect - the probability a prey is caught by a predator. This kind of multi-dimensionality must be important in many arms races but is only beginning to be studied (Gilman et al., 2012; Débarre et al., 2014).

Two parties experience potential conflict (Ratnieks & Reeve, 1992) when selection is expected to pull the joint phenotype in opposite directions, that is, whenever the trait value lies between the optimal trait values for the two parties (Queller, 2014; Queller & Strassmann, 2018).

In this region, a change in the trait in one direction usually benefits one party but harms the other. The evolution of joint phenotypes has been explored in a quantitative-genetic context, including derivation of a conflict version of Fisher's fundamental theorem of natural selection (Queller, 2014). Adding joint phenotypes into Fisher's geometric model offers a straightforward way to model conflict evolution via fixation of successive novel mutations. Here, we examine the simplest case, a one-dimensional (single trait) model of conflict in which the two parties have different optima. This case corresponds to the evolution of a joint trait that is uncorrelated with other traits.

Based on what is known about adaptive walks in the standard geometric model, we can make some predictions about how the model might behave with conflict over a joint phenotype (Queller & Strassmann, 2018). Both parties should fix mutations that move them closer to their respective optima. However, a novel feature is that when one party fixes a mutation, it should usually pull the second party away from its optimum. On average, the joint phenotype should therefore lie somewhere between the optimal values of the two parties. In agreement with the Red Queen hypothesis (Van Valen, 1973), each party should run more-or-less in place just to maintain its fitness. However, they should not stay exactly in place; each party in an arms race should push the trait in a direction that increases its own fitness, but always be dragged back down by the other party. We call these Sisyphean dynamics, after Sisyphus who, in Greek mythology, was condemned to forever push a boulder up a hill, only to have Zeus roll it back down.

Because parties with conflict should be farther from their optima, we expect two standard outcomes of Fisher's original model under that condition: higher fixation probability and larger fixed mutations. More mutations should fix because being far from the optimum enlarges the

space of beneficial mutations. When the population is close to the optimum, some large mutations in the right direction should be disfavored because they overshoot the optimum, but this should happen less when the population is far from the optimum.

We also expect that conflict may be more pernicious than a changing abiotic environment (Kopp & Hermisson, 2009a; b; Connallon & Clark, 2015). Both kinds of changes can cause a mismatch between trait value and optimum value, but once sufficiently far away from the optimum, the two kinds of changes may have different effects. A random abiotic change may often alter the optimum in a beneficial direction, up to about half the time when the change is small, but a change due to a conflicting party should usually be non-random in the direction away from the optimum.

Extending previous modeling (Gandon & Michalakis, 2002), we also explore how parties can gain an advantage during coevolution because of various asymmetries, such as population size, relative input and effect size of mutations, and the strength of selection (the dinner-life principle (Dawkins & Krebs, 1979)).

6.3 Methods

In order to illustrate the most fundamental properties of the geometric model with conflict, we study the simplest possible version of the model, with a single trait and with two parties that differ in their optima for that trait. We begin with a single axis corresponding to a phenotypic trait, shared or influenced by two parties, whose value is represented as z. The two

parties could be either different species or different roles within species, such as males and females.

Fitness is assumed to be a Gaussian function of a party's distance from its optimum, o_i, where i indexes parties to the conflict and takes the values 1 or 2 for a two-party conflict:

$$w_i = e^{-\omega_i(o_i - z)^2}.$$

At o_i, fitness is equal to 1 for party i, but falls off in both directions. The shape-parameter ω_i determines how quickly fitness falls off for each party.

Figure 6.1B shows fitness functions for two parties, in this case having the same ω. The point of intersection is interesting because it is the value of z at which the two parties have equal fitnesses, w_0, and therefore equal reductions in fitness or lag loads, defined as $1 - w_0$ (Maynard Smith, 1976). We will use this shared value of lag load as a convenient summary measure of degree of conflict (though other measures of conflict are possible). It describes how much each party stands to gain by moving from this point to its optimum (Figure 6.1B). By varying the lag load in the model, we adjust the intensity of conflict and the distance between the optima. For convenience, we define the point where the fitness functions intersect as the origin. Solving the fitness function for z = 0 gives two optima, $o_i = \pm\sqrt{-\ln(w_0)/\omega_i}$ showing that, for a given shape-parameter value, increasing the shared lag load $1\text{-}w_0$ increases the distance between the optima.

As in the standard geometric model, we assume selection is strong relative to mutation, such that selection acts on a single mutation at a time. The model consists of steps that consist of drawing a mutation and then determining whether the mutation will be fixed by selection. If so, it

becomes a fixation and changes the value of the trait, affecting the fitness of both parties. The effects of mutations in the phenotypic space are assumed to be unbiased and normally distributed, $m_i \sim \mathcal{N}(0, \sigma_i)$, where σ_i is the standard deviation (but see Figure 6.4C). Since mutation sizes are always positive, σ_i determines the average size of mutations $\bar{m}_i = \sigma_i\sqrt{2}/\sqrt{\pi}$. We will vary this parameter to compare scenarios where mutations are very small relative to the distance to the optimum with cases where a party could reach its optimum with a single fixation.

In order to focus on the most basic features of conflict, we first examine the simplest possible case where the two partners are identical in every respect except their fitness optima o1 and o2 before moving on to models with asymmetries. Thus we initially let the shapes of their fitness curves be the same ($\omega_1 = \omega_2 = 2$) and also let the mutation distributions be the same ($\sigma_1 = \sigma_2$). We drop subscripts for σ and ω when both parties have the same values. The number of new mutations will also be assumed to be the same for each party, in that each iteration of a simulation consists of a round of mutation and possible fixation (adaptation) for each party. A randomly chosen party mutates first each iteration, followed by the second party.

We later relax these assumptions to test whether asymmetries in evolutionary potential, for example in selection strength or mutational input, allow one party to win over the other. To change the strength of selection, we define a relative selection strength parameter f, that increases selection on party 1 relative to party 2 ($\omega_1 = f\omega_2$). We manipulate the relative mutation sizes by giving party 1 κ-fold larger average mutation sizes than party 2 ($\bar{m}_1 = \kappa\bar{m}_2$). Lastly, we define a relative mutational input parameter r, where party 1 will generate (and potentially fix) r mutations for every 1 mutation of party 2 ($\mu_1 = r\mu_2$).

To measure "winning" during conflict, we use the idea of fitness power (Queller & Strassmann, 2018). Fitness power for party 1 is defined as $P_{w1} = 1 - L_1/(L_1 + L_2)$, where L_i is the lag load for party i. This power value ranges from 0 to 1, where values closer to 1 mean that party 1 is winning the conflict in terms of being closer to its fitness maximum, a value of 0.5 means that both parties have equal lag loads, and power less than 0.5 means that party 2 is winning.

Unless stated otherwise, we assume that both parties are in very large populations where the effect of drift is negligible, so the probability of fixation is $\Pi_i = 1 - e^{-2s_i}$ for positive values of si ($\Pi_i = 0$ for si < 0), where s_i is the selection coefficient for a new mutation (Kimura, 1983). For smaller population sizes, Ni, the probability of fixation is $\Pi_i = (1 - e^{-2s_i}) / (1 - e^{-4N_i s_i})$ for both positive and negative values of s_i (Kimura, 1983). The selection coefficient is $s_i = w_m/w_i - 1$, where w_m is the fitness of a new mutation and w_i is the population's current fitness.

For comparison, we examine a single population with one trait adapting to its optimum without conflict – the standard geometric model (Figure 6.1A). Our non-conflict control populations are always assigned the same parameter values as party 1 in the corresponding conflict scenario.

Each simulation consists of many iterations of the mutation and selection process to model an adaptive walk. When our interest is in equilibrium conditions, we first eliminate data from 250/ω iterations, which simulations showed to be long enough for the simulated parties without conflict to be well adapted and fix very few new mutations. After this initial adaptation period, we record average results over the next 5,000 iterations. We repeat these simulated

adaptive walks for each parameter combination 1,000 times (for a total of 5,000,000 iterations for each condition simulated). We expect that conflict will result in populations that are often away from their optima, resulting in more fixations of larger phenotypic effect (which we will simply call larger fixations).

We also expect conflict to be more detrimental to fitness than simulations involving a randomly changing abiotic environment, primarily because more changes due to a conflicting party should be away from the optimum. To test this, we need a non-conflict control simulation with non-directional changes of the same magnitude as changes in the conflict simulation. This is complicated by the fact that the changes are of different types in the two scenarios. In our conflict model, what changes is the joint phenotype, while the optimum remains the same (the pathogen's joint phenotype changes when hosts evolve more resistance, but its optimum is still to have a high probability of infection). In models of abiotic change (Gordo & Campos, 2012; Matuszewski et al., 2014; Connallon & Clark, 2015), the reverse is typically assumed; the environmental change does not alter the phenotype but does alter the optimum. But common currency can be found because both shift the distance between trait and optimum along the z-axis. Therefore, for each conflict simulation, we simulated a parallel single-population moving-optimum model, where the environment changes the optimum in every iteration by exactly the same amount (including no change) that party 1's opponent changes the joint phenotype in that same iteration of the conflict simulation, but in a random direction (Figure 6.1C). Thus both conflict and abiotic change simulations involve the same magnitude of change, but we expect that the effect on the joint phenotype may usually be deleterious for party 1 while the effect on the abiotic optimum may be deleterious or beneficial.

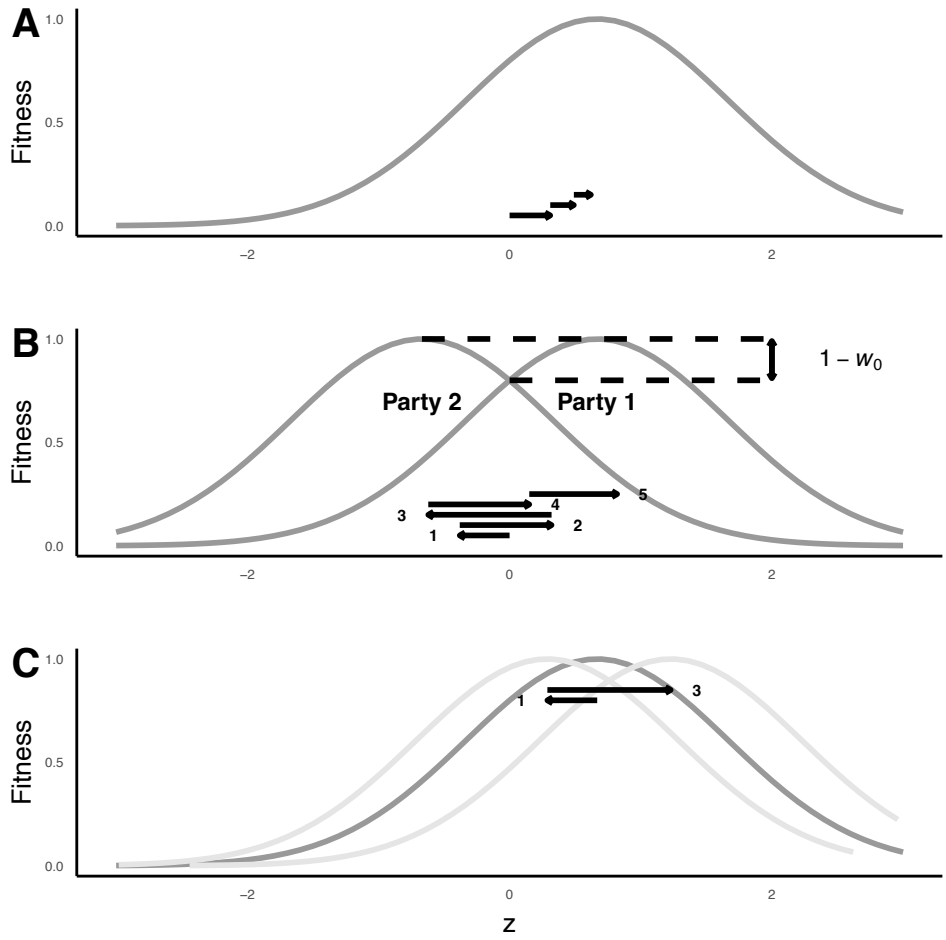

Figure 6.1: Schematic diagrams of the three versions of Fisher's geometric model studied: standard adaptation, conflict, and a moving optimum. Each panel shows one or more fitness curves as a function of a trait value z and some fixations (arrows). (A) In a standard geometric model, parties adapt a trait z to a single stable optimum by fixing beneficial mutations. (B) In models with conflict, z represents values of a joint phenotype, and there are two fitness functions, corresponding to Party 1 and Party 2 (we arbitrarily assign Party 1 the positive optimum value). Conflict is measured by the lag load, $1-w_0$, at the point of intersection for both fitness functions. Parties can fix beneficial mutations back and forth (arrows), with Party 1 fixing 2, 4, and 5 and Party 2 fixing 1 and 3 in this example. (C) Abiotic environmental change is modeled by shifting a single party's optimum in a random direction. In order to compare changes of equal size to the biotic conflict scenario, in each iteration we shift the optimal value of trait z, in a random direction, by the same amount that Party 1 experiences biotic environmental change in the conflict simulation – that is, by the amount that antagonistic Party 2 changes z (fixations 1 and 3).

6.4 Results

A sample simulation shows, in accord with prior results (Tenaillon, 2014), that in the standard geometric model, a single party fixed many mutations initially and then stabilized near the optimum (Figure 6.2A). But with conflict, regardless of whether the simulation started at the origin or elsewhere, there was no straightforward walk to a stable point. Instead, populations contested the value of the joint phenotype, resulting in back-and-forth Sisyphean movement of the joint phenotypic value (Figure 6.2A). Conflict parties constantly fixed adaptive mutations but the improvement was cancelled out by changes due to the other party, as proposed by the Red Queen hypothesis (Van Valen, 1973).

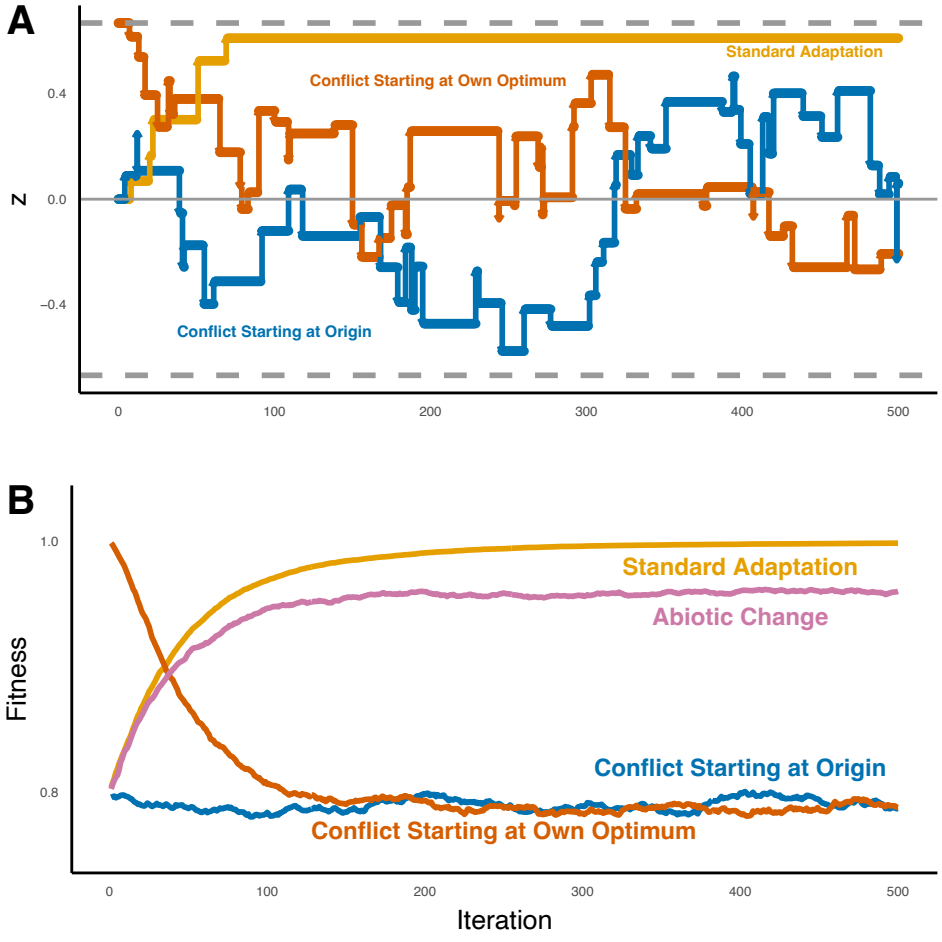

Figure 6.2: The effect of conflict on adaptive walks and fitness trajectories. (A) Three adaptive walks of mutations fixed with and without conflict. We include a standard model that adapts to a single optimum, and models with conflict that begin at either the origin or at Party 1's optimum. Horizontal dashed lines indicate the optimal phenotypes of the two parties. (B) Fitness trajectories based on averaged fitness from 1,000 simulated adaptive walks of each type. For all simulations, both parties had conflict intensity values of 0.2, average mutation sizes of 0.1, a fitness function shape-parameter of 1/2, and infinite populations.

We investigated how these dynamics affected fitness when averaged over 1,000 adaptive walks (Figure 6.2B). In the standard model, parties increased fitness over time and then stayed near the maximum fitness of 1. In contrast, parties with conflict were far from the maximum fitness. We also show the fitness trajectory for a party adapting to random abiotic change that is equal to the magnitude of change due to fixations from an antagonistic partner. This example showed more rapid adaptation and maintenance of higher fitness under abiotic change than under conflict.

To better understand how conflict affects long-term evolution, we compared the equilibrium properties, after the initial period of rapid adaptation, of replicate conflict and no-conflict simulations for different combinations of parameters.

In contrast to parties under the standard model, which essentially went to their optimum values (Figure 6.3A, yellow), parties with conflict (blue) and abiotic change (pink) were generally away from their optima though, as predicted, parties adapting to abiotic change approached their optima more than conflicting parties. Fitness is essentially maximized for standard adaptation parties without conflict after the period of rapid adaptation but, as expected, parties with conflict have decreased fitness relative to standard adaptation (Figure 6.3B). Parties with conflict were largely stuck near their intersection fitness values defined by the lag load. Abiotic change also resulted in reduced average fitness but, as we predicted, to a lesser intensity

than conflict. This difference was greatest when movement away from the optimum is strongest (high conflict) and movement towards it is weakest (low mutation size).

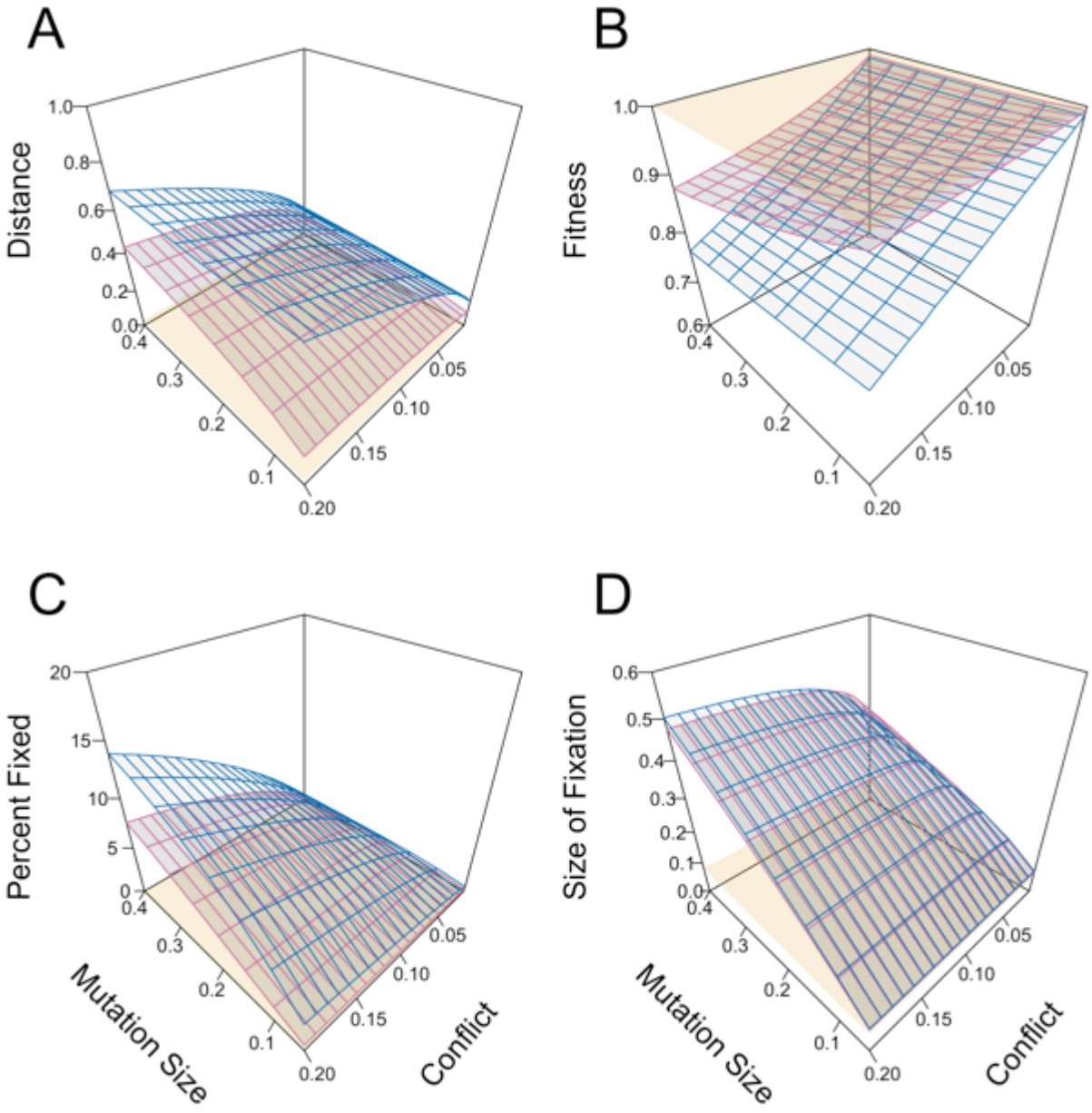

Figure 6.3: Equilibrium properties of the geometric model under varying average mutation sizes (normally distributed) and conflict intensities (measured as lag load at the origin where the fitness functions intersect ($1-w_0$) from fitness functions with a shape parameter of 1/2). Colors indicate the version of the geometric model: standard adaptation (yellow), conflict (blue), and abiotic change (pink). (A) Mean distance to the optimum. (B) Mean fitness during equilibrium. (C) Percent of mutations that are

fixed during equilibrium. (D) Effect size of fixed mutations. Means are calculated based on data collected from iteration 500 to 5,500 from 1,000 replicate simulations with infinite populations.

In standard adaptation simulations, few mutations fixed (after the period of initial adaptation) because the party was close to, or at, the optimum value. Conflicting parties fixed more mutations, especially with larger mutation sizes (Figure 3C). Similarly, increased conflict resulted in more fixations. Abiotic change simulations showed a similar pattern to conflict simulations, but with fewer overall fixations.

The average phenotypic effect of fixation (fixation size for short) was larger under conflict and abiotic change compared to fixations from standard adaptation (Figure 6.3D) an effect previously shown for abiotic change (Kopp & Hermisson, 2009b; a). This is expected because being away from the optimum decreases the likelihood that large mutations will overshoot the optimum. However, under this logic one would expect that the conflict case would fix larger mutations than the abiotic one and, interestingly, this is not the case (Figure 6.3D).

Up to this point, we have assumed that mutations are normally distributed and that the shape-parameter, ω, is 1/2. We investigated whether our results were robust to changes in these assumptions. Setting the mutation distribution to be either uniform or exponential did not notably impact our results. Similarly, normal fitness functions that are fourfold narrower ($\omega = 2$) or wider ($\omega = 1/8$), respectively, gave qualitatively similar results to those when ω is 1/2.

We also investigated whether asymmetries in factors affecting adaptive potential allow one party to win the conflict using the measure of fitness power defined earlier (see Methods). One factor that considerably affected power was the relative selection strength, f. When party 1 is under stronger selection ($f > 1$), it also had greater power though with diminishing returns for

larger values of f (Figure 6.4A). There was an interaction with mutation size, where power tended to decrease with larger average mutation size, especially when selection strength (ω) was high (Figure 6.4A). Large mutations in the party close to its optimum often overshoot, whereas for the losing party they offer a chance to get close to its optimum quickly.

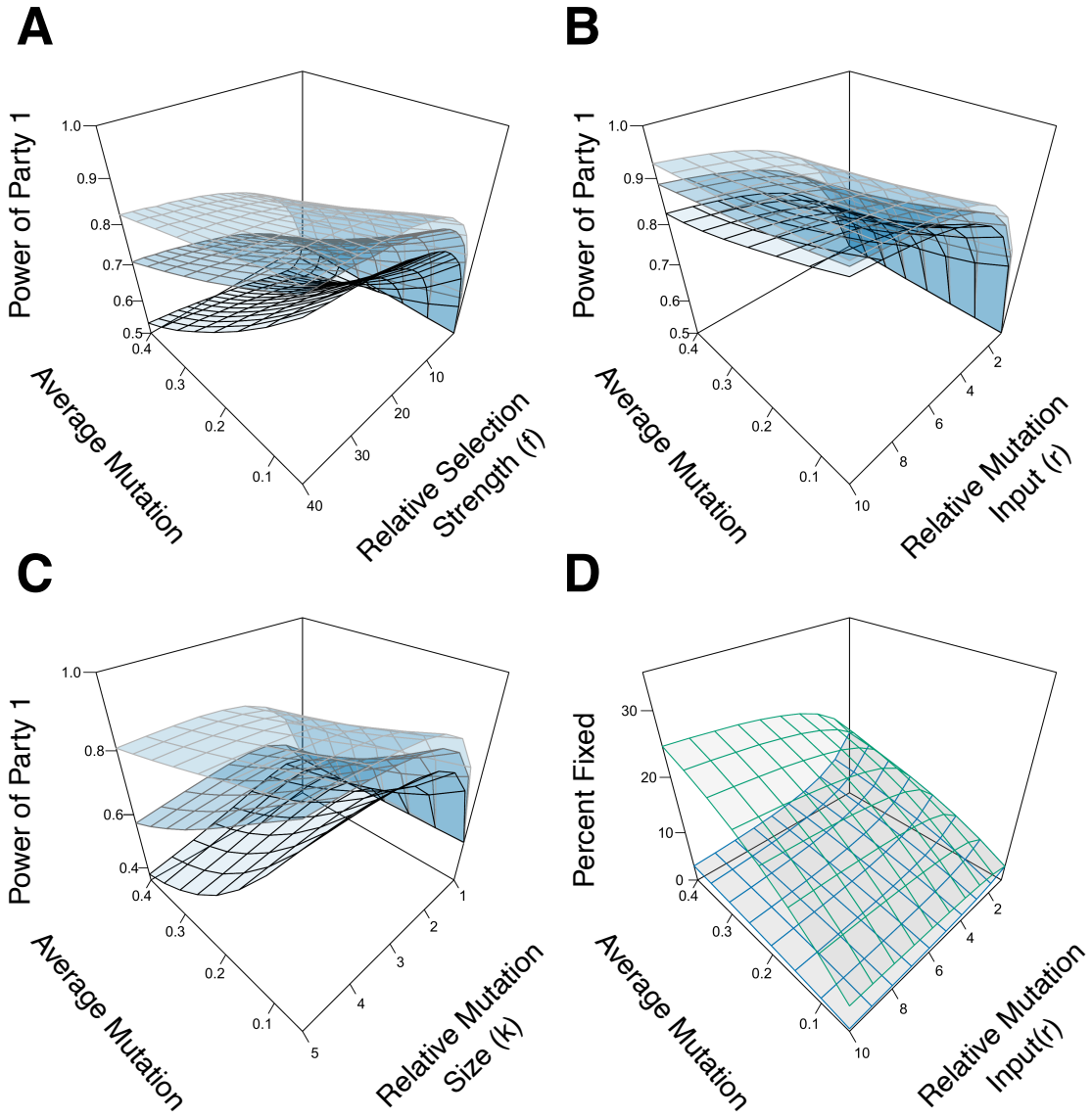

Figure 6.4: Fitness power is usually greater for the party with higher selection strength, mutational input, or mutation size. Fitness power was calculated according to $P_{w1} = 1 - L_1/(L_1 + L_2)$, where L_i is the average lag load for party i. Intensity of conflict was 0.2 and population sizes were infinite for all simulations. (A) Fitness power for party 1 when $\omega_1 = 2$ (black), 1/2 (gray), or 1/8 (light gray) and $\omega_2 = \omega 1/f$, where f is the relative selection strength. Each party has one mutation per iteration. (B) Fitness power for party 1, where

party 1 generates r times more mutations than party 2. Colors correspond to the same ω_1 values as shown in A, but ω is the same for both populations. (C) Fitness power for party 1 when $\overline{m_1} = \kappa\overline{m_2}$, where κ is the relative mutation-size. Each party has one mutation per iteration. Results are shown for different values of ω. The average mutation axis shows the average mutation size for party 2 ($\overline{m_2}$). (D) Percent of mutations that are fixed for party 1 (blue) and party 2 (green) with varying relative mutation inputs and mutation sizes. Average fitness is calculated from 5,000 iterations as outlined in the text.

Power was greater when a party had a higher mutational input. When party 1 generated more mutations for every mutation of party 2, the fitness power for party 1 was higher (Figure 6.4B). This effect leveled off with increasing mutation rate and was largely independent of mutation size and ω. In our model the mutational input was controlled by a single parameter, but in real populations it could differ either through a difference in mutation rates or in population sizes so our result indicates that either of these can increase power.

A distinct effect of population size, the effect of drift on fixation, was tested separately in our model. Given the same total mutational input, larger populations should fix more beneficial mutations and fewer deleterious ones. However, this resulted in only a slight increase in power even when one population is very small.

Higher relative selection strength and higher relative mutational input always gave party 1 higher power (>0.5) under the parameter values we explored. So did higher relative mutation size in most, but not all, parameter combinations. Increasing the relative mutation size for party 1 when party 2 generated small mutations resulted in higher power for party 1 (Figure 6.4C). However, increasing party 1's relative mutation-size when party 2 generated large mutations resulted in lower power because of the increased tendency to overshoot the optimum. At the most extreme values, this reduction in power actually results in party 2 winning the conflict as shown by power values below 0.5.

Winning and losing parties tended to fix different amounts and sizes of mutations as a result of being closer or farther from the optimum (Figure 6.4D). Winning parties tend to fix fewer and smaller mutations, moving close to the non-conflict case, while losing parties tend to fix more and larger mutations.

Finally, we note that asymmetries did not alter our qualitative conclusions (Figure 6.3) about differences from abiotic change or the standard model. When party 1 is given higher or lower selection strength, mutation size, or mutational input, it still has larger distances to the optimum, lower fitness, more fixations, and larger fixations than parties with the same parameters under abiotic change and standard adaptation.

6.5 Discussion

One cannot fully understand adaptation without also understanding maladaptation. Previous modifications of the geometric model allowed it to model maladaptation due to factors like genetic drift and environmental changes to optima (Poon & Otto, 2000; Kopp & Hermisson, 2009a). But it remained largely silent about what may be the major source of maladaptation, evolutionary conflict between organisms (Queller & Strassmann, 2018). By introducing joint phenotypes into Fisher's geometric model, we have expanded a powerful model of long-term adaptation by successive fixations to the study of conflict and arms races, where such successive fixations are likely to be especially important. This approach erases a major shortcoming of the geometric model and brings the power of the geometric model to bear on co-evolution.

Our results confirm the longstanding view that conflict and a succession of de novo mutations can engender long-term arms races (Dawkins & Krebs, 1979). Whereas the standard model rapidly approaches its optimum and largely stops evolving, both parties under conflict are held off their optima (Figure 6.3A), suffer decreased fitness (Figure 6.3B), and consequently continue to fix numerous mutations (Figure 6.3C). This running rapidly to stay in the same place is what is expected under the Red Queen hypothesis (Van Valen, 1973) with the qualification that the "same place" is a long-term average of Sisyphean advances and rollbacks.

Another interesting result from this work is that conflict has more severe effects on average fitness than does a randomly changing abiotic environment, when the changes are forced to be of the same magnitude (Figure 6.3B). This is largely because abiotic changes are modeled as changing the optimum in a random direction, whereas environmental change in the form of evolution of an opposing party naturally tends to change the joint trait in a malevolent direction. Our model allows abiotic change to extend indefinitely. However, in nature, abiotic changes may often vary around, and tend to return to, some central value. This would tend to reduce abiotic maladaptation, making the difference from biotic factors even starker than in our simulations. This supports the intuitive idea that conflict is a distinctly detrimental type of interaction that plays a powerful selective role, although the model cannot address whether abiotic or biotic changes in nature are larger in magnitude.

Some of these results are intuitive extensions of the standard model with the modification that the population is kept off its optimum (Queller & Strassmann, 2018). However, most of the results also show less-than-obvious non-linear interactions among the variables. The most surprising concerns the size of fixations (Figure 6.3D). Being farther from the optimum should allow fixation of larger mutations to be fixed and we see that is true for both the conflict and

abiotic models relative to the standard model. But parties in conflict and parties adapting to abiotic change fix roughly the same sizes of mutations, despite parties with conflict being farther from the optimum. The reason appears to lie in the relationship between mutation size, conflict, and distance to the optimum (Figure 6.3A). When conflict is low, distance to the optimum is always low in both biotic and abiotic simulations, so there is no major difference in fixation size. If conflict is high (back right face of Figure 6.3A) distances to the optimum are larger, but in an interesting way. When mutation sizes are small then a conflict party stays at substantially greater distances from the optimum than a party adapting to abiotic change. That would appear to open the door for larger fixations for the conflict case, but it does not because mutation sizes are too small - there are very few mutations large enough to fix in the conflict case but not in the abiotic. On the other hand, if mutation sizes are large relative to conflict, then the distances from the optimum are not so different for the conflict and abiotic cases and so fixation sizes are also not too different.

We also found both familiar and novel results when investigating how asymmetries during conflict can allow one party to win. First, by varying the fitness functions between parties, we were able to investigate whether stronger selection can favor one party over another. This could include the life-dinner principle, in which one party pays more dearly for losing (Dawkins & Krebs, 1979) and the rare-enemy effect, in which individuals of one party experience the other less often (Dawkins, 1982). We find that a party with stronger selection does have higher fitness power, but that this effect is diminished with larger mutation sizes (Figure 6.4A). Stronger selection means a more narrow fitness function, which increases the probability that large mutations will overshoot. Thus when mutations are large, stronger selection can be a

disadvantage. However, our model does not include the possibility that strong selection could drive a population extinct.

Mutational input, which could increase either with the mutation rate or the population size, is an important parameter for winning an arms race (Figure 6.4B). Mutation rates have been shown to be important for winning in matching alleles models (Gandon & Michalakis, 2002) and in experiments with bacteria (Pal et al., 2007). Our results broaden that conclusion to the case where all evolutionary change is due to de novo mutations, which is more likely in bacteria.

Larger populations have also been shown to be advantageous (Gandon & Michalakis, 2002). Our results show that this is true to the extent they increase mutational input, but the other potential advantage – of weaker drift – is generally very small.

Mutations play another role in determining the winner of an arms race through the sizes of mutations available to a party. We found that larger relative mutations increase power as long as mutations are not so large that they consistently overshoot the optimum. This result suggests that larger mutational neighborhoods and increased "evolvability" may be advantageous during conflict provided they do not increase the chances of overshooting the optimum.

Interestingly, none of our results show one party winning absolutely. Instead, increases in power tend to saturate with increases in selection, mutational input, drift, and mutation size (Figure 6.4A-C). This appears to be a result of the adaptive process described by the geometric model. The more power a party has, the more it will approach its optimum and decrease its pool of beneficial mutations. Because the second party is farther from its optimum, its pool of mutations will increase, leading to larger and more frequent fixations (Figures 6.4D). This means that adaptation saturates for the winning party.

A similar dynamic works when two factors increase power; if Party 1 has a greater evolutionary potential from one factor, it usually gets less added benefit from another factor. For example, when Party 1 has greater potential in terms of selection strength (Figure 6.4A, right axis) and then mutation size is increased equally for both parties (Figure 6.4A, left axis) Party 1's advantage is diminished (it still wins, but not by as much). Because Party 1 is closer to its optimum owing to greater selection strength, it has less room to improve and is less able than its partner to take advantage of the equal increase in mutation sizes.

This saturation of power for the stronger party reflects a force that tends to keep weaker parties in the game. But this is not absolute. Fisher's model does not include population dynamics and the possibility that a strong partner will drive its antagonist to extinction. More explicit eco-evolutionary models would be needed to address this question.

Arms races and Red Queen evolution have been classified into three types: fluctuating, escalatory, and chase (Brockhurst et al., 2014). The first three columns of Table 6.1 list some of their characteristics, modified from Brockhurst et al. (2014) and the fourth column lists the characteristics of the kind of arms race we have modeled. We call the new arms race Sisyphean, to emphasize the constant pushing of the trait uphill only to have it roll back.

Table 6.1: Types of arms races.

Adapted and expanded from Brockhurst et al. 2014. *Same as joint phenotype in this paper, "trait" is used here for consistency with other entries. 1Decaestecker et al. (2007) 2Hanifin et al. (2008) 3Benkman et al. (2003) 4For more examples see Queller and Strassmann (2018)

trait	*fluctuating*	*escalatory*	*chase*	*Sisyphean*

dynamics				
possible example	Daphnia and their pathogens1	Garter snakes and toxic newts2	Crossbills and lodgepole pine3	Cuckoos and their hosts4
genetic architecture of traits	few major loci	polygenic or quantitative trait	polygenic or quantitative trait	successive single-gene fixations
basis of trait interaction	matching to partner's trait	excess over partner's trait	matching to partner's trait	tug of war over joint trait
selection mode	fluctuating	directional (unidimensional)	directional (multidimensional)	directional (multidimensional)
allele frequency dynamics	oscillations	selective sweeps	selective sweeps	selective sweeps
adaptive landscape	multiple fitness optima	fixed fitness optimum	shifting fitness optimum	one fixed fitness optimum for each party

The key differences in Sisyphean arms races are in the kind of trait evolving and in the timescale on which evolution is followed. Sisyphean arms races are best understood through joint phenotypes where two parties have different optimal values, as opposed to the separate private traits each with a single optimum in more traditional coevolutionary models. This joint phenotype is a general way to conceptualize conflict that does not require specification of the private traits (Queller & Strassmann, 2018). The trait interaction is based on a tug of war over the joint phenotype. The tug-of-war metaphor has often been used for more specific cases, for example over the joint phenotype of offspring provisioning (Moore & Haig, 1991; Haig, 1993) or use of group resources for reproduction (Reeve et al., 1998; Shen & Kern Reeve, 2010), The joint phenotype is the object of the tug of war and in Sisyphean arms races, the tug of war occurs over long timescales via successive fixations. We can thus differentiate arms races on the short end of the continuum, like fluctuating arms races with recurring changes in frequencies of the same set of genes, from Sisyphean arms races, where change happens on longer timescales and is mediated by successive fixations of different genes resulting in fluctuating joint phenotype values.

The boundaries among the types of arms race in Table 6.1 are not always clear-cut. In fact, sometimes other arms races, which consider only private traits, could rescale into Sisyphean arms races when we consider the joint phenotype over long periods of time. For example, an escalatory arms race between seed hardness and beak strength of a bird is also a Sisyphean arms race over the probability that the seed gets eaten. Likewise, the individual color and pattern traits of a butterfly mimic may evolutionarily chase those of its model, but this is also a Sisyphean arms race over the abstract joint trait of degree of similarity, with the mimic having an optimum

at high similarity and the model having an optimum at very low similarity (although our particular model may need to be adjusted because it assumes fitness fall-offs on both sides of the optimum).

Biologically, complex Sisyphean arms races are more likely to entail long-term persistent antagonistic evolution. Strong selection on a single trait, like cheetah speed, might ultimately deplete it of possible beneficial mutations, but this is less likely for a joint trait with many subtraits. Moreover, the interactions of these subtraits might lead to reversals in individual traits. If gazelles evolve greater agility, cheetahs might have to respond with more agility at the expense of speed, enriching the potential for more speed evolution in the future. Such trait interactions may lead to complex paths through phenotype space as in evolutionary chase arms races (Brockhurst et al., 2014). Our geometric model does not currently capture all of these processes and other kinds of models might be required to address them explicitly, but it does at least point to their importance. Mathematically joining or reconciling joint-phenotype and separate-phenotype models is an interesting topic for the future.

There are also other questions that could be explored by combining Fisher's geometric model with the joint phenotype concept. An obvious extension is to include correlated non-conflict traits to understand how conflict influences pleiotropic effects on other traits and the cost of complexity (Orr, 2000). We might expect evolution due to conflict to keep non-conflict traits from their optima because of pleiotropy, and as a result increase the rate of evolution of non-conflict traits.

The results here are therefore just a first step towards using the geometric model to understand conflict and arms races. But they show that Fisher's geometric model is capable of

incorporating conflict and describing some of the major features long thought to be important in arms races and also generating more novel insights.

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Conflict of Interest

We have no conflicts of interest to disclose.

Data Accessibility

Python code for simulations will be uploaded to GitHub (https://github.com) upon acceptance.

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Chapter 7: Conclusions

Interactions between organisms involve cooperation and conflict. This dissertation explored some special consequences of cooperation and conflict using a range of methods. The results of this dissertation have important implications for the evolution of cooperation and the genetics of conflict.

Chapter two through four were focused on the role of context dependence in the interaction between *Dictyostelium discoideum* amoebae and their *Paraburkholderia* symbionts. While there are numerous conclusions to be drawn about this symbiosis from the results of this dissertation, the most important and general is that symbiotic interactions may play an underappreciated role in host adaptation to harsh environments. Symbioses, even with costs, may buffer hosts against the harshest contexts and favor the evolution of symbiotic interactions. Future research should investigate the buttering role of symbioses in other systems. Such research may be especially important for hosts to weather changes in the environment due to climate change.

The fifth chapter was focused on how pleiotropy could thwart conflict due to selection for cheaters. My result, that pleiotropy is higher in cooperation genes, shows that pleiotropy likely stabilizes cooperation in *Pseudomonas aeruginosa.* Future work on this topic can employ similar methods on a wider set of organisms to test the generality of this conclusion.

My last chapter focused on the genetic consequences of evolutionary conflict by modifying a popular model of adaptation to incorporate conflict. This chapter showed that

adaptation involving conflict with another party is different than other kinds of adaptation. This important insight explains many of our intuitions about arms races and evolutionary conflict.