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Within-Host Priority Effects Systematically Alter Pathogen Coexistence

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Within-Host Priority Effects Systematically Alter Pathogen Coexistence

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ABSTRACT: Coinfection of host populations alters pathogen prevalence, host mortality, and pathogen evolution. Because pathogens compete for limiting resources, whether multiple pathogens can coexist in a host population can depend on their within-host interactions, which, in turn, can depend on the order in which pathogens infect hosts (within-host priority effects). However, the consequences of within-host priority effects for pathogen coexistence have not been tested. Using laboratory studies with a coinfected zooplankton system, we found that pathogens had increased fitness in coinfected hosts when they were the second pathogen to infect a host, compared to when they were the first pathogen to infect a host. With these results, we parameterized a pathogen coexistence model with priority effects, finding that pathogen coexistence (1) decreased when priority effects increased the fitness of the first pathogen to arrive in coinfected hosts and (2) increased when priority effects increased the fitness of the second pathogen to arrive in coinfected hosts. We also identified the natural conditions under which we expect within-host priority effects to foster coexistence in our system. These outcomes were the result of positive or negative frequency dependence created by feedback loops between pathogen prevalence and infection order in coinfected hosts. This suggests that priority effects can systematically alter conditions for pathogen coexistence in host populations, thereby changing pathogen community structure and potentially altering host mortality and pathogen evolution via emergent processes.

Keywords: parasite interactions, coexistence, priority effects, coinfection.

Introduction

Multiple pathogen strains and species often coexist in host populations (Petney and Andrews 1998; Brogden et al. 2005; Balmer and Tanner 2011; Cox 2011). This has consequences for host health, as coinfection can increase or decrease host mortality rates and lead to the evolution of higher virulence (Levin and Bull 1994; Lawn 2004; Alizon et al. 2013). However, hosts are a limited resource for which pathogens have to compete, and it is not always possible for multiple pathogens to coexist. One mechanism that can alter pathogen coexistence is how pathogens interact within hosts (Mordecai et al. 2016*a*). Coexistence is fostered when pathogens facilitate one another, limited when pathogens have negative impacts on one another, and impossible when pathogens prevent coinfection via cross-protection, within-host resource depletion, or other mechanisms (Gupta et al. 1994; Vasco et al. 2007). These within-host interactions depend strongly on the order in which pathogens infect hosts (Goodman and Ross 1974; Hood 2003; de Roode et al. 2005; Jackson et al. 2006; Jager and Schorring 2006; Lohr et al. 2010; Leung and Poulin 2011; Hoverman et al. 2013; Devevey et al. 2015; Natsopoulou et al. 2015; Sandoval-Aguilar et al. 2015). However, to our knowledge, the impact of within-host priority effects on pathogen coexistence has not been explicitly tested.

Within-host priority effects exist across a range of coinfected taxa and have been demonstrated to alter infection patterns at the host population scale. Within-host priority effects have been found in mammal, plant, amphibian, insect, zooplankton, and fish hosts and emerge from diverse mechanisms. When multiple strains of the same pathogen infect one host, pathogens that arrive first in a host often have a competitive advantage over subsequent strains, possibly due to resource competition or apparent competition through the immune system (Hood 2003; de Roode et al. 2005; Jager and Schorring 2006; Devevey et al. 2015). However, when hosts are coinfected by two (or more) different pathogen species, pathogens may gain an advantage from first or second arrival, depending on the specific system (Goodman and Ross 1974; Al-Naimi et al. 2005; Jackson et al. 2006; Lohr et al. 2010; Leung and Poulin 2011; Hoverman et al. 2013; Natsopoulou et al. 2015; Sandoval-Aguilar

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et al. 2015; Wuerthner et al. 2017). For instance, early arriving pathogens may increase susceptibility in immune-compromised hosts and thereby facilitate infection of later-arriving pathogens (Rolff and Siva-Jothy 2003). Alternatively, vertebrate immune systems are characterized by Th1/Th2 trade-offs (Fenton et al. 2008), which could create positive or negative within-host priority effects between macroparasites and microparasites. Within-host priority effects can scale up to alter epidemic size (Halliday et al. 2017), host population density (Marchetto and Power 2017), average parasite load (Wuerthner et al. 2017), and parasite prevalence (Natsopoulou et al. 2015). However, we still lack a systematic understanding of how different types of within-host priority effects (e.g., late-arriver vs. early arriver benefit) impact coexistence of different pathogen species at the host population level.

We have abundant evidence from free-living communities, however, that priority effects alter community assembly and coexistence. The first organism to arrive in a patch can monopolize resources needed by its competitors, rapidly adapt to local conditions, or increase in size (and thus competitive ability) before competitors arrive (Urban and De Meester 2009; Rasmussen et al. 2014; Fukami 2015). Each of these processes increases the fitness difference between competing community members, reducing coexistence (Chesson 2000; Fukami et al. 2016). The first organism to arrive in a patch may also modify niches available to later-arriving organisms, either increasing or decreasing the fitness of particular community members and thus altering which community members may coexist (Fukami 2015). Priority effects at the patch scale can determine prevalence and coexistence at the metacommunity scale (Urban and De Meester 2009; Tucker and Fukami 2014). Thus, using the framework for priority effects developed in free-living communities, we expect that priority effects at the host (patch) scale should influence coexistence at the host population (metacommunity) scale.

In addition to gaining insights from the framework for free-living priority effects, our work also builds on earlier theoretical work on how infection order alters coexistence. In single-infection models, the first pathogen to infect a host prevents secondary infections. In this case, only the pathogen with the highest R_0 will persist within a host population (Gupta et al. 1994; though this may be disrupted by environmental feedbacks: see Lion and Metz 2018). In superinfection models, only one pathogen may infect a host at a time, but secondary pathogens may take over hosts that are first infected by pathogens with a lower virulence (within-host growth rate). In these models, coexistence between pathogens is possible as long as they differ enough in their level of virulence (May and Nowak 1994; Nowak and May 1994). Finally, in coinfection models, multiple pathogens can infect a host simultaneously, and this coexistence at the within-host scale facilitates coexistence at the host population scale as long as pathogen R_0 from coinfected hosts is greater than 1 (May and Nowak 1995). Further, if the first pathogen to infect a host permanently increases host susceptibility even after the infection is cleared, then pathogen coexistence will expand (Vasco et al. 2007). While these studies take infection order into account, none of them address within-host priority effects in which hosts can be infected with multiple pathogens at once and in which the fitness of both pathogens depends on the order of infection. Given the numerous examples of this type of within-host priority effect (Goodman and Ross 1974; Hood 2003; de Roode et al. 2005; Jackson et al. 2006; Jager and Schorring 2006; Lohr et al. 2010; Leung and Poulin 2011; Hoverman et al. 2013; Devevey et al. 2015; Natsopoulou et al. 2015; Sandoval-Aguilar et al. 2015; Wuerthner et al. 2017), it is important to understand its implications for pathogen coexistence.

Here we suggest that within-host priority effects may systematically alter pathogen coexistence by creating positive or negative frequency dependence. As a pathogen's prevalence increases, so does its probability of being the first pathogen to infect a host. Thus, if a pathogen receives a fitness benefit from first arrival, then its average per capita transmission rate from coinfected hosts should increase along with its prevalence (relative to the coinfecting pathogen's prevalence). This creates positive frequency dependence that should decrease the probability of pathogen coexistence. On the other hand, if a pathogen receives a benefit from second arrival, then its per capita transmission rate from coinfected hosts should increase when its prevalence decreases. This scenario can create negative frequency dependence that should increase coexistence by increasing pathogen fitness when rare.

To explore how within-host priority effects alter pathogen coexistence at the host population level, we measured within-host priority effects in zooplankton coinfected with bacterial and fungal pathogens and then used these measurements to parameterize a pathogen coexistence model that we used to explore the conditions under which within-host priority effects might foster pathogen coexistence or competitive exclusion. Using this system, we specifically asked the following questions: (1) How does the infection order of pathogens impact pathogen and host fitness in our system? (2) How do fitness advantages from first or second arrival alter pathogen coexistence? (3) Under what conditions do we expect within-host priority effects to make the difference between single and multipathogen persistence in our system? We found that within-host priority effects foster pathogen coexistence in our system by creating negative frequency dependence. This suggests that within-host priority effects can be important drivers of multipathogen communities in natural host populations.

Experimental Methods

Study System

Our focal host, *Daphnia dentifera*, is a cyclically parthenogenetic grazing zooplankton common in stratified lakes in the Midwestern United States (Tessier and Woodruff 2002). While filtering, *D. dentifera* incidentally ingests the two pathogens used in our study, the bacterium *Pasteuria ramosa* and the fungus *Metschnikowia bicuspidata*. Both pathogens are obligate killers. After being ingested, they replicate within *D. dentifera* until host death, at which point they are released into the water column once more until being ingested by a new host. The two pathogens differ in how they reduce host fitness. *Metschnikowia* replicates quickly and reduces host life span, while *Pasteuria* castrates hosts (Auld et al. 2012, 2014).

Experimental Setup

We used a clonal line of *D. dentifera* (Mid37) that was originally collected from Midland Lake, Indiana, and that has been maintained asexually in lab conditions for several years. In order to standardize effects of the environment, we reared individuals singly in beakers and isolated individuals from the third clutch. We then isolated individuals from the third clutch of this second generation of animals and used those individuals in our experiment. Throughout the experiment, hosts were kept singly in separate individual beakers filled with 30 mL of filtered lake water. For both pathogens, we used an isolate that had been grown in the lab for several years (Standard for *Metschnikowia* and G/18 for *Pasteuria*). This host clone and these pathogen isolates have been used successfully in previous work to study host-pathogen interactions (Auld et al. 2012, 2014).

We tested for within-host priority effects by exposing hosts to either *Pasteuria* or *Metschnikowia*, to both pathogens in different arrival orders, or to a control treatment with no pathogens, resulting in eight treatments (table 1). Each treatment was replicated with 30 individuals that were maintained singly in a beaker filled with 30 mL of filtered lake water. On day 7, treatments 2–6 (table 1) were exposed to either 1,000 spores/mL of *Pasteuria* and/or 500 spores/mL of *Metschnikowia*. Spore doses were chosen in an attempt to yield similar, high levels of infection, based on prior studies (M. A. Duffy, unpublished data). After 48 h, all individuals were placed in new water. On day 12, treatments 5–8 were exposed to either 1,000 spores/mL of *Pasteuria* or 500 spores/mL of *Metschnikowia*. Again, after 48 h, all individuals were placed in new water. The experiment ended on day 47, at which point all but four hosts had died.

Response Variables

Host Fitness. Host fitness was measured in terms of life span and number of offspring per day. Three times each week, we assessed whether individuals had died and counted the number of offspring produced per host. During inspection, hosts were transferred to clean beakers; offspring were not transferred.

Pathogen Fitness. We examined how within-host priority effects altered the number of infectious propagules created by an infection, or spore yield, which is a proxy for pathogen fitness. In our system, infectious spores are only released into the environment from a host after death. Thus, secondary infections are proportional to the number of spores found in a host at death. Dead hosts were preserved in 100 μL of nano-pure water. Preserved hosts were then ground up, and total *Pasteuria* and *Metschnikowia* spores per host were recorded.

Data Analysis

We tested for specific pairwise differences in spore yield among treatments to address three questions. First, we asked whether coinfection altered spore yield. We thus compared our single-infection treatments to our coinfection treatments, which shared pathogen infection timing (2 vs. 4, 2 vs. 5, and 6 vs. 7 for *Pasteuria* and 3 vs. 4, 3 vs. 6, and 5 vs. 8 for *Metschnikowia*). Second, we asked

Table 1: Experimental treatments

Treatment no.	Treatment name	Day 7	Day 12
1	Uninfected	None	None
2	Early *Pasteuria*	*Pasteuria*	None
3	Early *Metschnikowia*	*Metschnikowia*	None
4	Simultaneous infection	*Pasteuria* and *Metschnikowia*	None
5	*Pasteuria* first	*Pasteuria*	*Metschnikowia*
6	*Metschnikowia* first	*Metschnikowia*	*Pasteuria*
7	Late *Pasteuria*	None	*Pasteuria*
8	Late *Metschnikowia*	None	*Metschnikowia*

Note: Hosts were exposed to *Pasteuria* at 1,000 spores/mL and *Metschnikowia* at 500 spores/mL on days 7 and 12.

whether infection order altered spore yield from coinfected hosts, comparing our sequential coinfection treatments (5 vs. 6). Third, we asked whether differences in spore yield due to arrival order in coinfected hosts could be explained by differences in when each pathogen arrived. Thus, we compared our single-infection treatments where pathogens arrived on day 7 versus 12 (2 vs. 7 for *Pasteuria* and 3 vs. 8 for *Metschnikowia*). We calculated significance of pairwise differences using the glht function in the multcomp package in R, correcting for the false discovery rate due to multiple comparisons (Benjamini and Hochberg 1995). All response variables had a high level of heteroscedasticity, so variance was allowed to vary across treatments using the gls function in the nlme4 package in R. We used the same methods to test pairwise differences between life span and offspring, though for these variables, we tested all pairwise comparisons.

Four individuals lived till the end of the experiment (one in treatment 1, one in treatment 2, and two in treatment 6). These individuals were not included in calculations of host life spans. Including these individuals in our analysis assuming slightly extended life spans (2 days longer than the experiment) does not change statistical results. Individuals were only included in analyses if they were successfully infected by all pathogens to which they were exposed. *Pasteuria* spores were found in four individuals that were not exposed to *Pasteuria* (three in treatment 3 and one in treatment 8). These individuals were removed from all analyses. In addition, hosts that had partially decomposed between death and collection were not included in mean spore counts (22 out of 240 hosts), as hosts begin to lose spores with decomposition.

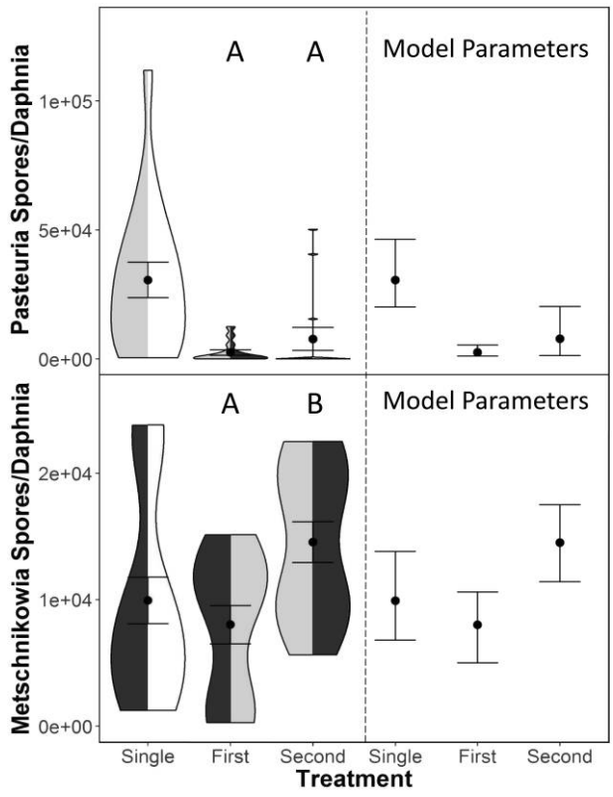

Figure 1: Number of spores found in hosts infected with *Pasteuria* (*top*) and *Metschnikowia* (*bottom*). *Left*, violin plots show the distribution of spore yield from singly infected hosts ("single"), coinfected hosts infected first by the focal pathogen ("first"; the focal pathogen is *Pasteuria* on the top and *Metschnikowia* on the bottom), and coinfected hosts infected second by the focal pathogen ("second"), with points representing average spore yield and bars representing standard error. The color of the left half of each violin plot indicates the infection treatment on day 7 (white = no pathogen, light gray = *Pasteuria*, dark gray = *Metschnikowia*), while the color on the right half of each violin plot indicates the infection treatment on day 12. Spore yield from sequential coinfection treatments with different letters were significantly different, indicating the presence of priority effects. *Right*, shown are bootstrapped means and 95% confidence intervals of spore yield used to parameterize the coexistence model. Plots including individual data points from all treatments are included in the appendix (figs. B1, B2). Data underlying all figures are deposited in the Dryad Digital Repository: https://dx.doi.org/10.5061/dryad.v118180 (Clay et al. 2019).

Experimental Results

In our figures, we show only results from treatments that were used to parameterize our coexistence model. Full results including all treatments can be found in the appendix (figs. B1–B4; figs. B1–B9 are available online).

Pathogen Fitness

We found that within-host priority effects determined *Metschnikowia* spore yield, whereas *Pasteuria* spore yield was only determined by whether it was in a singly infected or coinfected host. We found that coinfection significantly reduced *Pasteuria* spore yield when comparing treatments 2 and 5 (early *Pasteuria* vs. *Pasteuria* first; $p = .022$; "single" vs. "first" in the top panel of fig. 1) and 6 and 7 (*Metschnikowia* first vs. late *Pasteuria*; $p = .0016$). Within coinfections, arrival order did not significantly impact *Pasteuria* spore yield ($p = .93$ for comparing sequential coinfection treatments; "first" vs. "second" in the top panel of fig. 1). *Metschnikowia*, on the other hand, had a significantly higher spore yield from coinfected hosts in which it arrived second than from coinfected hosts in which it arrived first ($p = .042$; "first" vs. "second" in the bottom panel of fig. 1). However, spore yield from neither coinfection treatment was significantly different than that from singly infected hosts when comparing treatments 3 and

6 (early *Metschnikowia* vs. *Metschnikowia* first; $p = .51$; "single" vs. "first" in the bottom panel of fig. 1) or 5 and 8 (*Pasteuria* first vs. late *Metschnikowia*; $p = .74$). Thus, *Pasteuria* spore yield was determined by whether the host was coinfected and displayed no significant within-host priority effects, whereas *Metschnikowia* spore yield was driven by within-host priority effects in coinfections more so than by whether the host was coinfected.

Host Fitness

The key driver of host life span was *Metschnikowia* infection: *Daphnia* that were infected with *Metschnikowia* (treatments 3, 5, and 6) lived on average 13 ± 1.1 days after day 7 (day of first possible infection), whereas *Daphnia* that were not exposed to *Metschnikowia* (treatments 1 and 2) lived on average 27 ± 1.0 days after day 7 (fig. 2; error estimates are standard error of the mean). Based on pairwise comparisons corrected for false discovery rate, there was a significant difference in life span ($p < .001$ for all comparisons) between all *Metschnikowia*-infected and *Metschnikowia*-uninfected treatments. There was no significant difference in life span in pairwise comparisons among treatments infected by *Metschnikowia*, nor was there a significant difference in pairwise comparisons among treatments that did not include *Metschnikowia*. Thus, host life span was determined by whether individuals were infected by *Metschnikowia*, while *Pasteuria* infection had no significant effect on host life span.

All infections reduced host fecundity (fig. 3). Pairwise comparisons corrected for false discovery rate showed that unexposed hosts had significantly greater fecundity than all infected hosts ($p < .001$ for all comparisons). There were no significant differences in fecundity between *Metschnikowia* infected, *Pasteuria* infected, and coinfected hosts. Uninfected hosts had, on average, 4.1 ± 0.14 offspring per day, while infected hosts had 1.6 ± 0.16 offspring per day.

Model Methods

Model Equations

To test for the conditions under which we find coexistence in our system, we used a susceptible-infected model with environmental transmission to model a *Daphnia* population infected by *Pasteuria* and *Metschnikowia* with spore densities P and M, respectively. Hosts can be susceptible (S), singly infected by either pathogen (I_P, I_M), or coinfected. To keep track of within-host priority effects, coinfected hosts are divided into two groups: those where *Pasteuria* arrived first (C_{PM}) and those where *Metschnikowia*

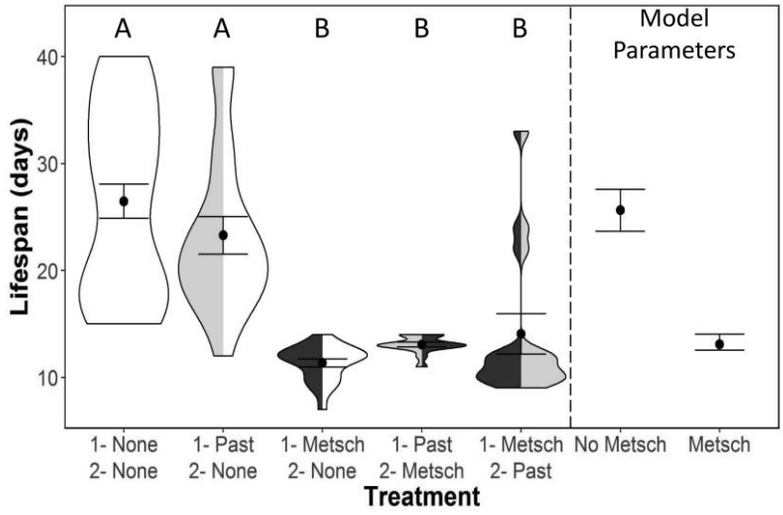

Figure 2: Number of days past initial infection date (day 7) that individuals survived. *Left*, violin plots show the distribution of life spans within each treatment, with points representing average life span and bars representing standard error. The color of the left half of each violin plot indicates the infection treatment on day 7 (white = no pathogen, light gray = *Pasteuria*, dark gray = *Metschnikowia*, also indicated in the top row of the *X*-axis), while the color on the right half of each bar plot indicates the infection treatment on day 12 (also indicated in the bottom row of the *X*-axis). Treatments with shared letters were not significantly different. *Right*, shown are bootstrapped means and 95% confidence intervals of postinfection life spans of individuals uninfected and infected by *Metschnikowia* used to parameterize the coexistence model. A plot including individual data points from all treatments (including the simultaneous coinfection treatment) is included in the appendix (fig. B3).

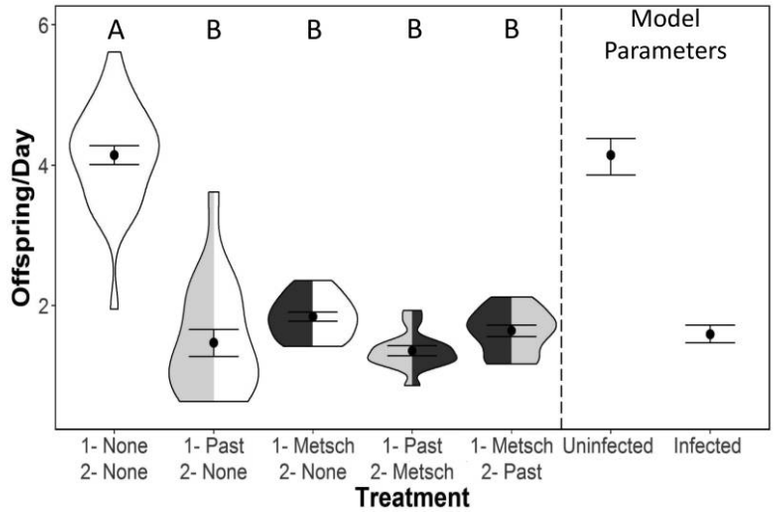

Figure 3: Average offspring per day. *Left*, violin plots show the distribution of offspring/day within each treatment, with points representing average offspring/day and bars representing standard error. The color of the left half of each violin plot indicates the infection treatment on day 7 (white = no pathogen, light gray = *Pasteuria*, dark gray = *Metschnikowia*, also indicated in the top row of the *X*-axis), while the color on the right half of each bar plot indicates the infection treatment on day 12 (also indicated in the bottom row of the *X*-axis). Treatments with shared letters were not significantly different. *Right*, shown are bootstrapped means and 95% confidence intervals of offspring/day of uninfected and infected individuals used to parameterize the coexistence model. A plot including individual data points and all treatments is included in the appendix (fig. B4)

arrived first (C_{MP}). We modeled chronic infections with no recovery as hosts cannot clear infections in our system. We initially incorporated a simultaneously coinfected class in our model. However, when we derived an analogous discrete time model for this system, with each time step representing 1 day, the proportion of coinfected hosts that were infected by both pathogens in a single day was <0.01 for all parameter values explored, so we removed the simultaneous coinfection class for simplicity.

Susceptible hosts are born into the population via all infection classes (no vertical transmission) and become singly infected by consuming spores in the environment, $(f\mu_P P + f\mu_M M)S$. Dynamics of the susceptible host class are given by

$$\frac{dS}{dt} = \overbrace{b(S, I_P, I_M, C_{PM}, C_{MP})\left(1 - \frac{N}{K}\right)}^{\text{births}} - \overbrace{(f\mu_P P + f\mu_M M)S}^{\text{infection}} - \overbrace{d_S S}^{\text{deaths}}, \tag{1}$$

where μ_P and μ_M are the per spore infectivities of *Pasteuria* and *Metschnikowia*, respectively; f is the host feeding rate; K is host carrying capacity; N is total population size; $b(S, I_P, I_M, C_{PM}, C_{MP})$ is the birthrate; and d_S is the death rate. Here $b(S, I_P, I_M, C_{PM}, C_{MP})$ is the summed birthrate of each infection class, given by

$$b(S, I_P, I_M, C_{PM}, C_{MP}) = \overbrace{b_S S}^{\text{offspring from } S} + \overbrace{b_I(I_P + I_M + C_{PM} + C_{MP})}^{\text{offspring from infected hosts}}. \tag{2}$$

Since most infected individuals have similarly decreased fecundity compared to uninfected individuals (fig. 3), infected hosts have a birthrate b_I and susceptible hosts have a birthrate b_S.

Singly infected hosts can transition to the coinfected host class by consuming spores of the pathogen they are not infected by ($f\mu_M I_P M$ or $f\mu_P I_M P$). Since *Metschnikowia* infection reduces host life span (fig. 2), all hosts infected by *Metschnikowia* die as a function of *Metschnikowia*-induced mortality (d_M). Changes in the numbers of infected and coinfected hosts are then given by

$$\frac{dI_P}{dt} = \overbrace{f\mu_P SP}^{\text{infection}} - \overbrace{f\mu_M I_P M}^{\text{coinfection}} - \overbrace{d_S I_P}^{\text{deaths}}, \tag{3}$$

$$\frac{dI_M}{dt} = \overbrace{f\mu_M SM}^{\text{infection}} - \overbrace{f\mu_P I_M P}^{\text{coinfection}} - \overbrace{d_M I_M}^{\text{deaths}}, \tag{4}$$

$$\frac{dC_{PM}}{dt} = \overbrace{f\mu_M I_P M}^{\text{coinfection}} - \overbrace{d_M C_{PM}}^{\text{deaths}}, \tag{5}$$

$$\frac{dC_{MP}}{dt} = \overbrace{f\mu_P I_M P}^{\text{coinfection}} - \overbrace{d_M C_{MP}}^{\text{deaths}}. \tag{6}$$

Increasing host population density does not increase host death rate in our model, as this would decrease the available growth time—and thus spore yield—for each pathogen. As we did not measure this relationship, we did not include it in our model, but see Auld et al. (2014) for discussion of how earlier mortality of infected hosts could alter spore yield—and thus host persistence—in this system.

Infected hosts transmit spores into environmental spore pools, whose dynamics are given by

$$\frac{dP}{dt} = \overbrace{d_S\beta_{P(P)}I_P + d_M\beta_{P(PM)}C_{PM} + d_M\beta_{P(MP)}C_{MP}}^{\text{spore release}} - \overbrace{\alpha_P P}^{\text{loss}}, \tag{7}$$

$$\frac{dM}{dt} = \overbrace{d_M\beta_{M(M)}I_M + d_M\beta_{M(PM)}C_{PM} + d_M\beta_{M(MP)}C_{MP}}^{\text{spore release}} - \overbrace{\alpha_M M}^{\text{loss}} - \overbrace{fMN}^{\text{uptake}}, \tag{8}$$

where $\beta_{i(j)}$ represents the number of spores i from host class j, corresponding to spore yields in figure 1. Thus, all hosts that are infected by a given pathogen add spores of that pathogen to the environment on death. Spores have a loss rate (α_i), which represents spore degradation and spores moving out of the system (e.g., due to settling). *Metschnikowia* spores are removed from the environment by host feeding (f), an important driver of disease dynamics in this system (Civitello et al. 2013). *Pasteuria* spores, alternatively, can survive passage through the host gut and thus are not removed by host feeding (King et al. 2013).

Model Parameterization

To parameterize our model, we used bootstrapping methods with 5,000 replicate draws from the boot and boot.ci functions in R to estimate the mean and 95% confidence intervals of spore yield, host fecundity, and host life span from our empirical results. Treatments were grouped for this analysis as described above and shown in figures 1–3. Derivation of host birthrate, death rate, and all other parameters are described in appendix B (apps. A, B are available online), along with a table of all parameter values (table A1; tables A1, B1 are available online). Our model was deterministic and used single mean parameter values for our main results. However, we additionally ran our model across the parameter space given by the 95% confidence intervals of our parameters to check model sensitivity.

Incorporating Priority Effects

We ran our model under three scenarios to compare coexistence predictions: (1) pathogens maximize their fitness in coinfected hosts by being the first pathogen to arrive (first-arrival advantage scenario), (2) arrival order does not alter pathogen fitness (no-advantage scenario), and (3) pathogens maximize their fitness in coinfected hosts by being the second pathogen to arrive (second-arrival advantage scenario). Both pathogens had higher spore yield from coinfected hosts when they arrived second (though this was only significant for *Metschnikowia*; fig. 1). Thus, for our second-arrival advantage scenario, we set spore yield from coinfected hosts equal to that found in our experiments. For our first-arrival advantage scenario, we switched the spore yields of both pathogens from coinfected hosts where *Pasteuria* arrived first and coinfected hosts where *Metschnikowia* arrived first. For our no-advantage scenario, we averaged the spore yields of both pathogens across both sequentially infected host classes by pooling the spore yields from both our sequential infection treatments and bootstrapping new mean spore yield estimates. Exact parameter values can be found in the appendix (table A1).

Testing Coexistence

To test coexistence of *Pasteuria* and *Metschnikowia*, we initialized our model with a susceptible *Daphnia* population at carrying capacity with one individual in each singly infected class and recorded whether neither, one, or both pathogens were circulating in the host population at equilibrium conditions. We ran our model using the lsoda function in the deSolve package in R. To test whether initial conditions altered equilibrium values, we independently varied the initial prevalence of each pathogen from 1% to 100%. For the ranges of parameter values we considered, we found that stable states were not dependent on initial prevalence. If the sum of all infection classes for a pathogen was reduced to less than 0.01, that pathogen was considered to be extinct. We also conducted a formal invasion analysis, which confirmed our numerical results (fig. B7).

We tested how pathogen coexistence varied along a fitness gradient of both hosts by varying spore loss rates of both pathogens (α_P and α_M). Decreasing the spore loss rate of a pathogen increases pathogen R_0. Varying the spore loss rate of pathogens is a good proxy for how pathogen fitness changes over space and time in natural settings, as there is evidence that environmental variables influence spore loss (Overholt et al. 2012). We examined pathogen coexistence for spore loss rates ranging from approximately one-seventieth to 1.9 times (for *Metschnikowia*) and one-

ninetieth to 1.6 times (for *Pasteuria*) the loss rates measured under laboratory conditions (app. A).

Model—Coexistence Results

The Impact of First- versus Second-Arrival Advantage on Coexistence

Coexistence of both pathogen species in the host population varied depending on the presence and type of priority effect. Compared to systems without within-host priority effects, pathogens were more likely to coexist in a host population if they had second-arrival advantage but less likely to coexist if they had first-arrival advantage (fig. 4). The parameter space in which *Pasteuria* could exist was not sensitive to whether pathogens gained an advantage from first or second arrival. Instead, the likelihood of coexistence was driven by the parameter space in which *Metschnikowia* could maintain itself in a host population. *Metschnikowia* had a much larger parameter space in which it could exist with second-arrival advantage than with first-arrival advantage.

These coexistence patterns can be explained by (*a*) the prevalence of coinfections and (*b*) the frequency dependence created by within-host priority effects. As we decrease the spore loss rate of a pathogen (and thus increase its fitness), the prevalence of that pathogen increases. Further, as the prevalence of one pathogen increases, more of the infections by the other pathogen are in coinfected hosts. For example, as we decrease *Metschnikowia*'s loss rate, the proportion of hosts infected by *Pasteuria* that are also coinfected by *Metschnikowia* increases. *Pasteuria* spore yield is significantly reduced by coinfection (fig. 1). Thus, decreasing *Metschnikowia* spore loss also decreases the average spore yield of *Pasteuria* (fig. 5*A*–5*C*). On the other hand, *Metschnikowia*'s average spore yields in sequentially coinfected hosts and in singly infected hosts are approximately the same (fig. 1). However, *Metschnikowia* spore yield is dependent on the order in which pathogens arrive in coinfected hosts. As we decrease *Metschnikowia* prevalence, *Metschnikowia* is more likely to arrive second in coinfected hosts. If *Metschnikowia* spore yield was decreased when arriving second in coinfected hosts, then it would have low per capita spore yield at low prevalence, preventing coexistence (fig. 5*D*). However, since *Metschnikowia* has an increase in spore yield from arriving second in coinfected hosts (fig. 4), it will have high per capita spore yield at low prevalence (fig. 5*F*). This creates negative frequency dependence, which facilitates coexistence.

Conditions under Which Within-Host Priority Effects Determine Coexistence

We expect within-host priority effects to foster coexistence between *Metschnikowia* and *Pasteuria* when the *Metschnikowia* loss rate is high. Under loss rates found in lab conditions ($\alpha_P = 0.31$, $\alpha_M = 1.2$; see app. B for estimation), our model indicates pathogen coexistence regardless of within-host priority effects (fig. 6*A*). However, in field conditions, we expect loss to be higher as infectious spores are exposed to ultraviolet light, extreme temperatures, and settling out of the water column (Overholt et al. 2012; Civitello et al. 2013; Shocket et al. 2018). Thus, we examined whether

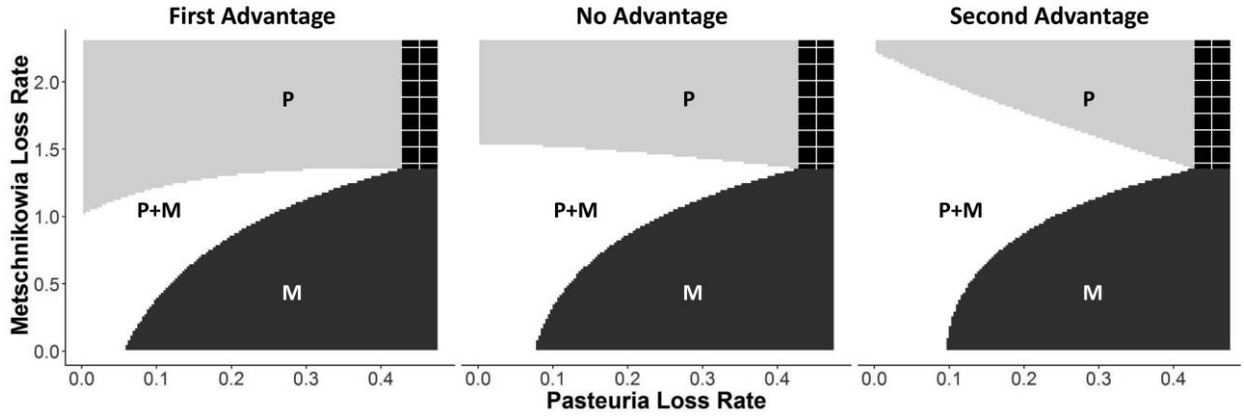

Figure 4: Coexistence phase planes of *Metschnikowia* and *Pasteuria* in a shared population across a gradient of spore loss rates of *Metschnikowia* (*X*-axis) and *Pasteuria* (*Y*-axis): when the first pathogen to arrive has a fitness advantage, when the arrival order does not matter, and when the second pathogen to arrive has a fitness advantage. Pathogen fitness increases along both axes. Plots show whether only *Pasteuria* (light gray), only *Metschnikowia* (dark gray), both pathogens (white), or neither pathogen (black with grid marks) can maintain themselves across a parameter space of *Metschnikowia* and *Pasteuria* loss rates.

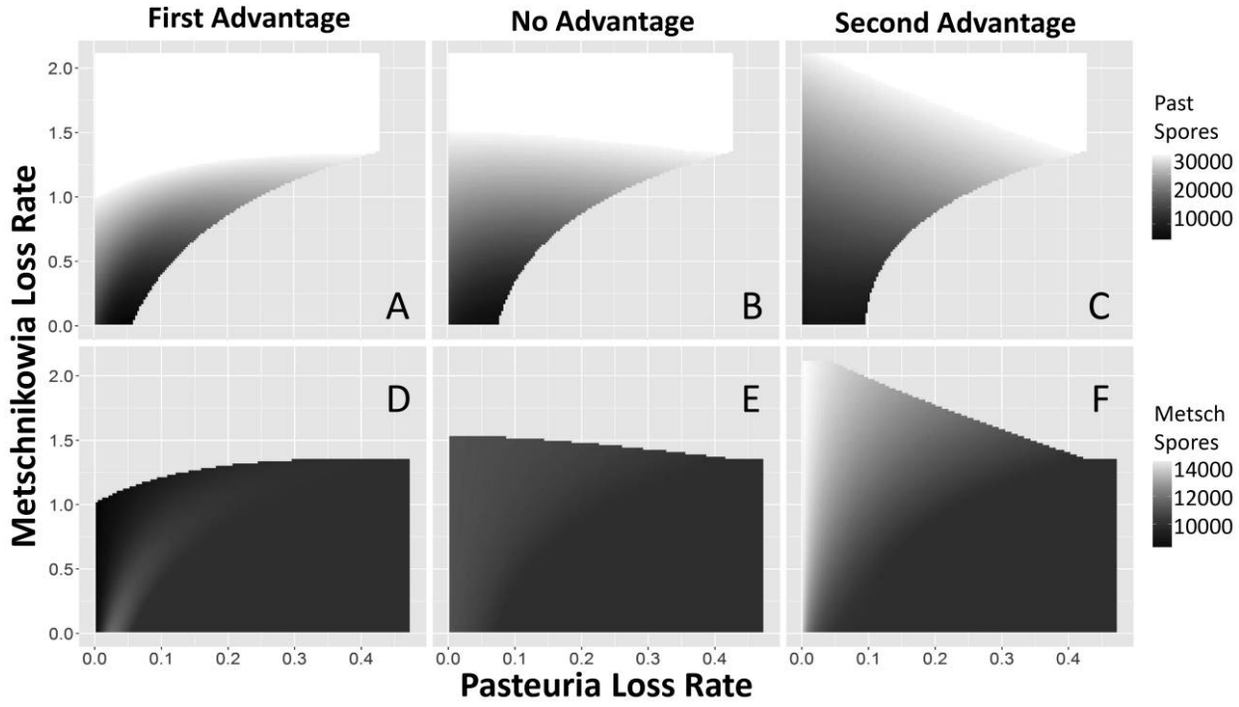

Figure 5: Mean spore yield of *Pasteuria* (*A*–*C*) and *Metschnikowia* (*D*–*F*) from infected hosts when pathogens have first-arrival advantage (*A*, *D*), no arrival advantage (*B*, *E*), and second-arrival advantage (*C*, *F*). Scale bars represent the spore yield gradient for each row, from black representing low spore yield to white representing high spore yield. Gridded parts of the phase plane show the parameter space in which the given pathogen cannot maintain itself within the host population.

within-host priority effects foster coexistence if we increased *Metschnikowia* loss rate, *Pasteuria* loss rate, or the loss rates of both. Note that it is biologically reasonable to independently vary *Pasteuria* and *Metschnikowia* loss rates because the pathogens respond differently to environmental variables; for example, *Metschnikowia* is more sensitive to ambient radiation in lakes than *Pasteuria* (C. L. Shaw, S. R. Hall, E. P. Overholt, C. E. Cáceres, C. E. Williamson, and M. A. Duffy, unpublished data). We increased the *Metschnikowia* loss rate to values that approximate conditions where 78% of all *Metschnikowia* spores are lost from the water column each day ($\alpha_M = 1.5$) and increased the *Pasteuria* loss rate to values that approximate conditions where 28% of all *Pasteuria* spores are lost from the water column each day ($\alpha_P = 0.33$). We found that with a high *Metschnikowia* loss rate, within-host priority effects (second-arrival advantage in accordance with our empirical results) allow for both pathogens to coexist, whereas only *Pasteuria* may persist if arrival order does not alter pathogen spore yield (fig. 6*B*). Increasing the *Pasteuria* loss rate as well provides a similar pattern, though it is less likely that within-host priority effects will create coexistence (fig. 6*C*). Finally, if we only increase the *Pasteuria* loss rate, only *Metschnikowia* may persist regardless of priority effects. When *Metschnikowia* loss rates were high, within-host priority effects that gave advantage to first arrivers limit pathogen coexistence (fig. 6).

Discussion

In natural systems, hosts can harbor a diverse community of pathogen species, but it is still unclear what factors structure the composition of these pathogen communities. Our study shows that within-host priority effects influence pathogen fitness and scale up to alter pathogen coexistence patterns in host populations. Specifically, we found that infection order played an important role in determining spore yield (and, hence, transmission) of the fungal pathogen *Metschnikowia*: when *Metschnikowia* arrived second, it had a higher spore yield than when it arrived first in a coinfected host. This benefit of arriving second within a coinfected individual scales up to influence population-level patterns, increasing the likelihood of pathogen coexistence in our system. In addition, we found that if *Metschnikowia* has a higher spore loss rate than coinfecting pathogens (as we have previously measured in natural environments), within-host priority effects should alter coexistence outcomes. These results demonstrate that with-

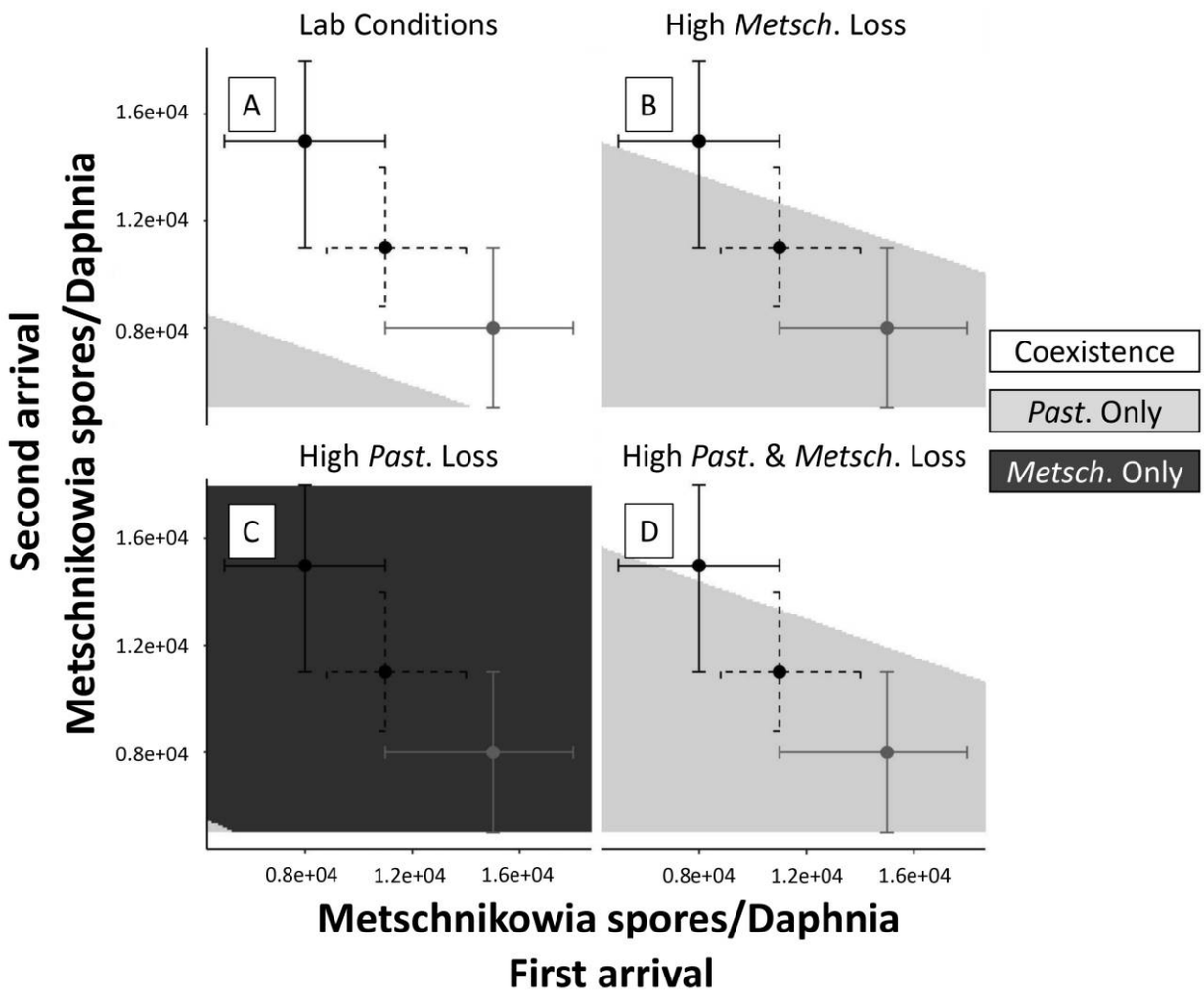

Figure 6: Within-host priority effects are most likely to drive coexistence in scenarios with high *Metschnikowia* spore loss rates. The axes represent the number of *Metschnikowia* spores released from coinfected hosts in which *Metschnikowia* is the first to arrive (*X*-axis) or second to arrive (*Y*-axis). Points surrounded by black, solid bars correspond to bootstrapped estimates of the mean and 95% confidence intervals found for our empirical spore yield data (fig. 3). This is our scenario for within-host priority effects. For points surrounded by dashed bars, we repeated the same bootstrapping procedure but lumped together the *Metschnikowia* spore yields from all hosts that were coinfected sequentially. Thus, this is our scenario for no within-host priority effects, where the number of spores released from hosts where *Metschnikowia* arrives first is the same as from hosts where it arrives second. Points surrounded by gray confidence intervals represent spore yield if pathogens gain first-arrival advantage. We did not focus on *Pasteuria* spore yield because altering the order of pathogen arrival did not alter *Pasteuria* spore yield.

in-host priority effects can have systematic impacts on disease patterns at the host population scale in natural populations, potentially affecting host mortality, pathogen evolution, and pathogen prevalence (Abu-Raddad et al. 2006; Alizon et al. 2013).

When multiple pathogens can infect the same host, they also compete for resources, both at the within-host and between-host scale, which can lead to competitive exclusion of pathogens in host populations. Within hosts, pathogens may compete for resources such as nutrients, may directly interfere with one another, or may trigger apparent competition via the immune system (Gardner et al. 2004; Pedersen and Fenton 2007; Wale et al. 2017). At the between-host scale, pathogens compete for susceptible hosts. In the absence of coexistence mechanisms, pathogens that compete for these limiting resources cannot coexist, and barring complex environmental feedbacks, only the pathogen with the highest fitness (R_0) will persist in a host population (Gupta et al. 1994; Lion and Metz 2018). Stabilizing coexistence mechanisms can promote coexis-

tence of competing pathogens by increasing the fitness of an organism when it is rare (Chesson 2000). This may be achieved via niche differentiation, where pathogens use different within-host resources, age classes of hosts, or areas within the host's body (Power 1996; Fitt et al. 2006). Additional stabilizing mechanisms include competition colonization trade-offs and specialization to different vectors (Ojosnegros et al. 2010; Mordecai et al. 2016*a*, 2016*b*). Such stabilizing mechanisms can help explain the rich pathogen and parasite communities that have been observed in an abundance of host populations (Petney and Andrews 1998; Brogden et al. 2005; Cox 2011).

Our work expands the current framework of pathogen coexistence by demonstrating that feedbacks between pathogen prevalence and within-host pathogen interactions can have stabilizing or destabilizing impacts on pathogen coexistence. Within-host priority effects that give advantage to the first pathogen to infect a host can decrease the likelihood of pathogen coexistence by decreasing the fitness of pathogens at low prevalence (figs. 4, 5). On the other hand, within-host priority effects that give advantage to the second pathogen to infect a host increase the likelihood of pathogen coexistence by increasing the fitness of pathogens at low prevalence (figs. 4, 5). These findings integrate within-host priority effects into the modern coexistence framework of frequency dependence (Chesson 2000; Adler et al. 2007). Our study also identifies destabilizing mechanisms in previous studies where pathogens could not coinfect a single host (Gupta et al. 1994). In these systems, the first pathogen to infect a host blocked infection from any other pathogens. This blocking can be interpreted as an extreme case of within-host priority effects that benefit the first pathogen to arrive in a host, which our results indicate should strongly reduce coexistence. Given the ubiquity of within-host priority effects in naturally coinfected systems (Hoverman et al. 2013; Natsopoulou et al. 2015; Wuerthner et al. 2017), the ability to make systematic predictions about their consequences for disease dynamics is valuable. For example, malaria strains benefit from first arrival in hosts (de Roode et al. 2005), thus within-host priority effects may decrease strain diversity in malaria, decreasing host mortality and the evolution of virulence. As new and reemerging infectious diseases spread through coinfected human and wildlife populations, better understanding the consequences of within-host priority effects might allow us to better understand which new diseases will reach endemic states and which will eventually be outcompeted.

Our results also expand the understanding of priority effects and coexistence that has been established in free-living systems. Fukami et al. (2016) point out that late-arriving species in a patch will initially have a low relative abundance compared to early arriving species. If an organism's relative fitness increases with its relative abundance, then this acts as a destabilizing mechanism (Chesson 2000), resulting in competitive exclusion of late-arriving species. However, the role of priority effects as a stabilizing coexistence mechanism due to second-arriver advantage has been overlooked. This is not because there are no examples of second-arriver advantage in free-living organisms (Fukami 2015). However, priority effects occur as a stabilizing mechanism when second-arriver advantage at the patch (or host) scale alters coexistence at the metacommunity (or host population) scale. Studies explicitly linking priority effects at the patch scale to coexistence at the metacommunity scale are rare and have focused on first-arriver advantage scenarios (Urban and De Meester 2009; Tucker and Fukami 2014). Given the many examples of second-arriver advantage in free-living organisms (Fukami 2015), we should consider how priority effects may act as a stabilizing coexistence mechanism across both free-living and within-host communities.

Our results provide a framework to understand when within-host priority effects should alter pathogen coexistence in natural populations. First, an invading or rare pathogen must encounter heterospecific pathogens within hosts at an appreciable rate. In our system, we find up to 35% prevalence of *Pasteuria* in *Daphnia dentifera* (M. A. Duffy, unpublished data). Thus, when rare, *Metschnikowia* infections have up to a 1 in 3 chance of infecting hosts previously infected by *Pasteuria*, thus boosting spore yield and increasing *Metschnikowia* invasion ability. Second, within-host priority effects will be important for determining coexistence when there are large fitness differences between pathogens (fig. 6*B*). This is likely in our system, as *Metschnikowia* and *Pasteuria* have different responses to environmental factors such as light and predation (Auld et al. 2014; C. L. Shaw, S. R. Hall, E. P. Overholt, C. E. Cáceres, C. E. Williamson, and M. A. Duffy, unpublished data). Third, our study demonstrates that the impact of within-host priority effects on pathogen fitness must be strong compared to the average impact of coinfection on pathogen fitness to alter coexistence. The average fitness of a pathogen is determined by (*a*) the proportion of infected hosts that are coinfected and (*b*) the proportion of coinfected hosts in which the pathogen arrives first or second. For *Pasteuria*, fitness is determined almost entirely by *a*, which is not determined by *Pasteuria* prevalence (fig. 5). Thus, including within-host priority effects does not change *Pasteuria* fitness when rare. For *Metschnikowia*, fitness is determined more by the order of arrival in coinfections than the coinfection proportion (fig. 1). Whether *Metschnikowia* can persist in a population therefore changes when we include within-host priority effects. Adding within-host priority effects to models of coinfected populations inevitably increases their complexity. Examining the above factors will allow us to determine when this added complexity is necessary to understand infectious disease patterns.

Links between within-host and between-host processes create feedbacks that drive host-pathogen dynamics. Our work illustrates how between-host processes (prevalence and transmission) alter within-host dynamics via within-host priority effects. To fully apply this framework to pathogen communities in other natural systems, we must understand how within-host priority effects function in species-rich pathogen and parasite assemblages (Budischak et al. 2016). Further, we need to study whether within-host priority effects function in a discreet or continuous manner. Our study examines the order of parasite arrival while ignoring variation in the time between infection events, as do most previous experimental studies. Future work should quantify how changing the time lag between the arrival of two pathogens influences pathogen fitness. Overall, an improved understanding of how within-host priority effects influence pathogen fitness will enable us to better predict patterns of pathogen coexistence in multipathogen communities.

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