doi:10.1111/evo.13843

How parasite interaction strategies alter virulence evolution in multi-parasite communities

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Received January 3, 2019
Accepted July 6, 2019

The majority of organisms host multiple parasite species, each of which can interact with hosts and competitors through a diverse range of direct and indirect mechanisms. These within-host interactions can directly alter the mortality rate of coinfected hosts and alter the evolution of virulence (parasite-induced host mortality). Yet we still know little about how within-host interactions affect the evolution of parasite virulence in multi-parasite communities. Here, we modeled the virulence evolution of two coinfecting parasites in a host population in which parasites interacted through cross immunity, immune suppression, immunopathology, or spite. We show (1) that these within-host interactions have different effects on virulence evolution when all parasites interact with each other in the same way versus when coinfecting parasites have unique interaction strategies, (2) that these interactions cause the evolution of lower virulence in some hosts, and higher virulence in other hosts, depending on the hosts infection status, and (3) that for cross immunity and spite, whether parasites increase or decrease the evolutionarily stable virulence in coinfected hosts depended on interaction strength. These results improve our understanding of virulence evolution in complex parasite communities, and show that virulence evolution must be understood at the community scale.

KEY WORDS: **Coinfection, multiple infection, parasite interactions, virulence evolution.**

The majority of infected hosts contain several parasite strains or species (Petney and Andrews 1998; Brogden et al. 2005; Telfer et al. 2008; Rigaud et al. 2010; Balmer and Tanner 2011; Cox 2011). These coinfections alter the impact of parasites on host fitness (i.e., parasite virulence, here defined as host mortality due to infection), and thus have implications for host population dynamics, parasite evolution, and host evolution (Lange et al. 2014; King et al. 2016). Whether coinfected hosts generally have lower fitness than singly infected hosts depends on how parasites interact within hosts (Alizon et al. 2013). For instance, hosts coinfected with parasites that directly attack one another can have lower mortality than singly infected hosts, whereas hosts coinfected with parasites that interact via immune suppression typically have a higher mortality than singly infected hosts (Inglis et al. 2009; Ezenwa and Jolles 2015). Similarly, within-host interactions determine the impact of coinfection on parasite fitness. For instance, immune mediated apparent competition lowers malaria fitness in coinfected hosts (Raberg et al. 2006). By changing parasite and host fitness, within-host interactions impose selection on parasites. Thus, to fully understand the impact that within-host interactions have on host mortality, we must understand how they drive the evolution of parasite virulence.

A parasite's fitness depends on the rate and duration of transmission, both of which can be altered by coinfection. In many host-parasite systems, increasing parasite reproduction not only increases transmission rate but also reduces host longevity and the infectious period (Salvaudon et al. 2005; de Roode et al. 2008). Thus, parasites must find a virulence strategy that balances host mortality and transmission rate. This trade-off is known as the virulence-transmission trade-off hypothesis (Anderson and May 1982; Alizon et al. 2009). If a parasite is too benign, its transmission rate will be too low to spread through a host population; if it is too virulent, then it will kill off its host before it can transmit. Given this trade-off, theory predicts that within a given

host-parasite system, parasites should evolve a single optimal level of virulence (Anderson and May 1982). However, when hosts are coinfected, all parasite species can influence within-host resources (Wale et al. 2017) and host mortality (Ezenwa and Jolles 2015), and directly or indirectly alter each other's growth rates (Inglis et al. 2009). Thus, coinfections have the potential to alter the optimal level of virulence for a given parasite by altering the relationship between virulence, transmission rates, and the length of an infection.

How coinfections alter the evolution of virulence depends on the mechanisms through which parasites interact within coinfected hosts. Coinfecting parasites can interact with each other through the immune system (e.g., immune-suppression or cross immunity; Brown and Grenfell 2001; Lawn 2004; Raberg et al. 2006; Ezenwa and Jolles 2011; Griffiths et al. 2015), through host mortality (e.g., immunopathology; Day et al. 2007), through direct interference (e.g., production of harmful chemicals; Gardner et al. 2004; Inglis et al. 2009), or through resource competition (Wale et al. 2017). These interaction mechanisms all have different impacts on the various components that determine the virulence-fitness relationship, and thus select for different levels of virulence in coinfections. For instance, coinfection selects for increased virulence in malaria parasites that compete for resources, whereas coinfection selects for decreased virulence among bacteria that produce shared resources (de Roode et al. 2005; Harrison et al. 2006). Ultimately, we cannot make prediction for virulence evolution in coinfected populations without understanding within-host interactions.

Hosts often contain heterogeneous parasite communities, either made up of multiple parasite species or phenotypically diverse strains of a single species (Harrison et al. 2006; Cox 2011). In heterogeneous parasite communities, parasite interactions may be asymmetric, meaning that not all parasites interact with their competitors in the same way. For instance, one parasite species may suppress the immune system, whereas the other does not, as in the case of many coinfections involving helminths or HIV (Lauer et al. 2002; Ezenwa et al. 2010). Alternatively, one parasite may directly interfere with a coinfecting parasite that does not return the interference, as in bacterial coinfections of *Caenorhabditis elegans* (Rafaluk-Mohr et al. 2018). In these cases, we must consider how an interaction mechanism alters the evolution of both the focal parasite (the one triggering the interaction) and the nonfocal parasite (the one receiving the interaction). However, the impact of within-host interactions on virulence evolution has mostly been explored under symmetric conditions (all parasites interact with one another in the same way) both in theory (Gardner et al. 2004, Day et al. 2007, Alizon and van Baalen 2008, Alizon et al. 2013, Kamiya et al. 2018; although interaction strength may be asymmetric) and empirically (de Roode et al. 2005; Bell et al. 2006; Inglis et al. 2009; Mideo 2009; Rumbaugh et al. 2009). Consequently, we know little about how asymmetric interactions influence parasite evolution in multi-species communities.

Using a general coinfection model that incorporates within-host and between-host dynamics, we ask how various symmetric and asymmetric within-host interactions impact the virulence coevolution of coinfecting parasites. Specifically, we examine cross immunity (when a parasite triggers an immune response that targets coinfecting parasites), immunopathology (when a parasite triggers an immune response that increases host mortality), immune suppression (when a parasite suppresses the immune system), and spite (when a parasites lowers its own growth rate to directly attack coinfecting parasites). Our results indicate that whether specific within-host interactions drive the evolution of increased or decreased virulence can switch depending on whether within-host interactions are symmetric or asymmetric.

Methods

Here we follow previous studies and model the evolution of parasite virulence using a "host exploitation strategy," ε, as the trait under selection. Following Alizon and van Baalen (2005), we model host exploitation strategy as the intrinsic growth rate of the within-host parasite population (offspring/parasite/time). Virulence (parasite-induced host mortality) is a positive function of ε. Parasite fitness is a function of both within-host dynamics and between-host dynamics. A parasite that outcompetes other parasites within hosts might still have low fitness if it transmits at a low rate or kills its hosts too quickly (Alizon et al. 2013). Therefore, we model disease dynamics at both the within-host and between-host organizational levels. Modeling disease dynamics at both scales allows us to examine how fine scale processes (within-host parasite interactions) impact large scale processes (host population level virulence evolution). These scales are connected by transmission and host mortality, both of which are functions of within-host parasite density.

We specifically model interactions between multiple parasite species, rather than interactions between multiple parasite strains. Previous models of within-host interactions and virulence evolution have not needed to specify whether they focus on interspecific or intraspecific coinfection, because interspecific coinfection models simplify to resemble intraspecific coinfection models if interactions are symmetric (Kamiya et al. 2018). We focus on interspecific coinfection because we are interested in the way that a parasite's interaction strategy alters its own virulence evolution, and the virulence evolution of those community members who do not share that interaction strategy.

Within-Host Dynamics

Following previous studies (Alizon and van Baalen 2005), we assume that the parasite titer (a measure of within-host parasite

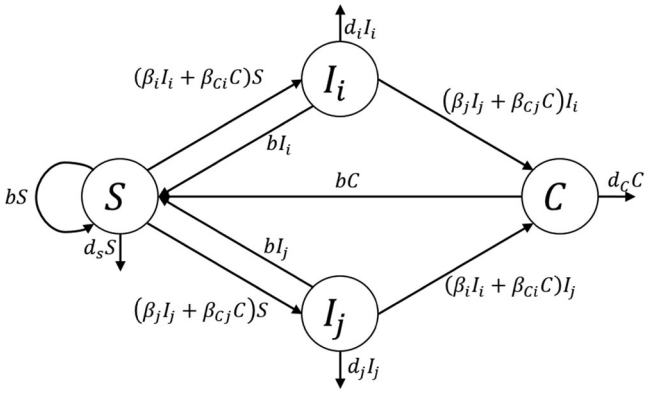

Figure 1. Birth, death, and infection dynamics for our coinfection model, as written in equations (3)–(6).

density, V) increases with the intrinsic parasite population growth rate (ε), and decreases as a function of the per capita killing rate (k) and density (L) of the host's lymphocytes. Given these conditions, the rate of change in titer for parasite i is

$$\frac{dV_i}{dt} = \overbrace{\varepsilon_i V_i}^{\text{growth}} - \overbrace{kL_iV_i}^{\text{mortality}}. \quad (1)$$

The rate of change of lymphocyte densities targeting i is given by

$$\frac{dL_i}{dt} = \overbrace{y + cV_i}^{\text{growth}} - \overbrace{mL_i}^{\text{mortality}}. \quad (2)$$

The change in lymphocyte density over time is determined by the baseline lymphocyte production (y), the increase in lymphocyte production (c) with parasite titer (V) in the case of infection, and the baseline mortality of lymphocytes (m). Replacing i subscripts with j in equations (1) and (2) describes parasite and immune dynamics in hosts singly infected by parasite j.

It is important to note that our model does not take into account that in some systems individual hosts can clear parasites from their system. We made the decision to only focus on the chronic case here for simplicity.

POPULATION LEVEL DYNAMICS

To determine how parasite virulence evolves, we need to account for population-level dynamics of hosts and parasites. Therefore, we model transmission between hosts, host mortality, and fecundity. Consider a host population infected with two parasites, denoted as parasite i and parasite j. Here, host individuals can fall into one of four infection classes: susceptible hosts without parasites (S), hosts infected with parasite i who are susceptible to parasite j (I_i), hosts infected with parasite j who are susceptible to parasite i (I_j), and hosts who are coinfected by both parasites (C). Dynamics of the system are given by the following set of equations, and shown in Figure 1:

$$S_{t+1} = S_t - \overbrace{\left(\beta_i I_{it} - \beta_j I_{jt} - \beta_{Cj}C_t - \beta_{Ci}C_t\right)S_t}^{\text{Infection}} + \overbrace{b(S_t + I_{it} + I_{jt} + C_t)}^{\text{births}} - \overbrace{d_sS_t}^{\text{death}}, \quad (3)$$

$$I_{it+1} = I_{it} + \overbrace{(\beta_i I_{it} + \beta_{Ci}C_t)S_t}^{\text{Infection}} - \overbrace{\left(\beta_j I_{jt} + \beta_{Cj}C_t\right)I_{it}}^{\text{coinfection}} - \overbrace{d_iI_{it}}^{\text{death}}, \quad (4)$$

$$I_{jt+1} = I_{jt} + \overbrace{\left(\beta_j I_{jt} + \beta_{Cj}C_t\right)S_t}^{\text{Infection}} - \overbrace{(\beta_i I_{it} + \beta_{Ci}C_t)I_{jt}}^{\text{coinfection}} - \overbrace{d_jI_{jt}}^{\text{death}}, \quad (5)$$

$$C_{t+1} = C_t + \overbrace{\left(\beta_j I_{jt} + \beta_{Cj}C_t\right)I_{it} + (\beta_i I_{it} + \beta_{Ci}C_t)I_{jt}}^{\text{coinfection}} - \overbrace{d_CC_t}^{\text{death}}. \quad (6)$$

All hosts (regardless of infection status) have the same birth rate (b). Death rate (d) depends on infection status and parasite identity. Transmission only occurs horizontally, and hosts can be infected by singly infected or coinfected hosts, with β_i is the transmission rate of i from a singly infected host, and β_{Ci} and β_{Cj} are the transmission rates from coinfected hosts of i and j, respectively. Note that hosts can only enter into a singly infected state from the susceptible state (because there is no recovery in this model), and coinfection occurs only sequentially, that is, hosts have to be infected first by one parasite before becoming infected by the second parasite. We originally ran our model allowing for simultaneous coinfections, but in all cases the number of hosts simultaneously infected was vanishingly small compared to the number of hosts sequentially infected. Thus, we removed simultaneous coinfection from our model. However, in many systems, shared transmission routes such as vectors may increase the likelihood of simultaneous coinfection. If we remove one of the parasites from the model, the model reduces to a simple one-parasite-one host SI model.

We do not include host classes that are doubly infected by a single parasite, and thus lose their susceptibility to the other parasites (i.e., coinfected classes would be C_{ii}, C_{jj}, or C_{ij}, and class C_{ii} could not be infected by parasite j) as suggested in Alizon (2013). Inclusion of host classes doubly infected by a single parasite prevents parasite fitness from increasing when rare, due to availability of susceptible hosts. This is important in multi-strain scenarios where one parasite is a rare invading

mutant. However, the two parasites in Figure 1 do not represent a resident and mutant strain. Further, double infection by a single parasite preventing further infection is not biologically realistic for many interspecific coinfections (e.g., multiple inoculations of HIV would not prevent a host from contracting a helminth infection).

Connecting within- and Between-Host Scales

Transmission rates and host mortality are typically a positive function of parasite titer (Brunner et al. 2005; Handel and Rohani 2015). To capture this connection, we set the transmission rate (β) equal to

$$\beta_i = \left(\frac{V_i}{V_i + \omega}\right) \beta_{\max}, \tag{7}$$

where $\beta_{\max}$ is the maximum possible transmission rate and ω is the half saturation constant. This means that as parasite titer (V) increases, the transmission rate increases in a saturating fashion, eventually reaching an asymptote at $\beta_{\max}$, a pattern seen in most parasite systems (Lange and Ferguson 2009). This saturating relationship is important for the virulence transmission trade-off —after a point, increasing host exploitation will continue to increase host mortality, while giving diminishing returns on transmission.

The death rate of a host is the sum of the intrinsic death rate (d_s) and parasite mediated mortality. Here, we assume that parasite-mediated mortality of parasite i is determined by the intrinsic growth rate of the parasite and the parasite titer ($\varepsilon_i * V_i$), which is equivalent to the amount of resources a parasite population takes from its host. The relative strength of baseline mortality versus parasite-induced mortality is determined by the mortality coefficient (α). Thus, the death rate of hosts infected by parasite i, j or both parasites are given, respectively, by

$$d_i = d_s + (\varepsilon_i V_i)\,\alpha, \tag{8}$$

$$d_j = d_s + \left(\varepsilon_j V_j\right)\alpha, \tag{9}$$

$$d_C = d_S + \left(\varepsilon_i V_i + \varepsilon_j V_j\right)\alpha. \tag{10}$$

The death rate depends on the product of titer by growth rate and not simply titer because mortality increases by the number of parasites in a host and the amount of resources that each of those parasites takes. As a baseline, parasites in coinfected host only interact through equation (10). Equations (1) and (2) remain unchanged in coinfected hosts, and within-host dynamics of parasites i and j occur simultaneously. We do not assume any interactions through the immune system or through host resources in our baseline equations because we want to isolate the effects of specific interaction mechanisms. However, competition for hosts does emerge via increased mortality in coinfected hosts even without including an explicit competition parameter. Note that our model assumes a virulence transmission trade-off, where increasing parasite exploitation increases both parasite transmission and host mortality. Thus, our results cannot be applied to systems where increasing parasite exploitation leads to reduction in host fecundity rather than host lifespan, or systems where parasite load is not related to transmission (Jensen et al. 2006; Cressler et al. 2014).

PARASITE INTERACTION MECHANISMS

We examined the evolutionary impact of five common interaction mechanisms in our model (Table 1).

(1) *Baseline*: Parasites have no specific within-host interaction mechanism. Note that coinfected hosts still have a higher mortality than singly infected hosts here.

(2) *Cross Immunity*: Parasites can trigger an additional immune response that targets their competitors (Raberg et al. 2006). If parasite i triggers such cross immunity, then equation (2) becomes

$$\frac{dL_j}{dt} = y + cV_j + \boldsymbol{zcV_i} - mL_j. \tag{11}$$

Thus, lymphocytes targeting parasite j now increase as a function of both parasite j titer and parasite i titer. z is strength of cross immunity ($0 < z$).

(3) *Immune suppression*: Parasites can also suppress the host immune system (Ezenwa and Jolles 2011). If parasite i is immunosuppressive, then equation (2) becomes

$$\frac{dL_i}{dt} = (1 - \boldsymbol{p_i})y + (1 - \boldsymbol{p_i})cV_i - mL_i, \tag{12}$$

where p_i is immune suppression strength and increasing p_i reduces the immune response ($0 < p_i < 1$).

(4) *Immunopathology*: Parasites trigger an immune response that increases host mortality as a function of lymphocyte density (Day et al. 2007). If parasite i triggers such immunopathology, then equation (8) becomes

$$d_i = d + \varepsilon_i V_i \alpha + \boldsymbol{L_i \lambda}. \tag{13}$$

And equation (10) becomes

$$d_C = d_S + \left(\varepsilon_i V_i + \varepsilon_j V_j\right)\alpha + \boldsymbol{L_i \lambda}. \tag{14}$$

Table 1. The five parasite interaction mechanisms included in this model and their impacts on interaction parameters.

Parasite interaction type	Description	Effect in model
Baseline	Within-host dynamics described by equations (1) and (2)	N/A
Cross immunity	Parasite i triggers an immune response against parasite j	$L_j \uparrow$
Immunopathology	Parasite i triggers an immune response that increases host mortality	$d_i \uparrow d_c \uparrow$
Immune suppression	Parasite i reduces strength of immune system	$y \downarrow c \downarrow$
Spite	Parasite i attacks parasite j at cost of reduced growth of i	$V_j \downarrow \varepsilon_i \downarrow$

This means that the death rate of hosts infected with parasite i increases with lymphocyte response targeting i. λ represents strength of immunopathology ($0 < \lambda$).

(5) *Spite*: Parasites with "spite" reduce their own growth rate to directly attack competitors in coinfected hosts (Inglis et al. 2009). If parasite i employs spite, then equation (1) becomes (changes bolded for all interactions).

$$\frac{dV_i}{dt} = (\varepsilon_i (1-u) - kL_i) V_i, \tag{15a}$$

$$\frac{dV_j}{dt} = \left(\varepsilon_j - kL_j - \phi u V_i\right) V_j, \tag{15b}$$

where the decrease in titer of parasite j (V_j) is due to direct interference from parasite i, and the reduction of ε_i is determined by the strength of spite, u, which measures the redirection of parasite resources from growth to interference ($0 < u < 1$). ϕ determines how much parasite i can interfere with parasite per reduction in ε_i. High ϕ means a low cost of interference, whereas low ϕ means a high cost of interference ($\phi > 0$).

We examined the impact of parasite interaction strategies across a gradient of interaction strengths, for both symmetric and asymmetric interactions.

MODEL ANALYSIS

We used an adaptive dynamics approach to find coevolutionary stable virulence strategies of coinfecting parasites. Following Choisy and de Roode (2010), the fitness of a rare mutant of parasite j with exploitation strategy ε_{jm} in a host population coinfected by resident parasites i and j is

$$W_{jm}\left(\varepsilon_{jm}, \varepsilon_i, \varepsilon_j\right) = \frac{\beta_{jm}}{2\left(\beta_i \overline{I_i} + \beta_{Ci}\overline{C} + d_{jm}\right)}\overline{S} + \frac{\beta_{Cjm}}{2\left(d_C + d_{Cm}\right)}\overline{I_i} + \left\{ \frac{\beta_i \beta_{Cjm}\overline{SI_i} + \beta_{Ci}\beta_{Cjm}\overline{SC}}{\left(\beta_i \overline{I_i} + \beta_{Ci}\overline{C} + d_{jm}\right)\left(d_C + d_{Cm}\right)} + \left[\frac{\beta_{jm}}{2\left(\beta_i \overline{I_i} + \beta_{Ci}\overline{C} + d_{jm}\right)}\overline{S} + \frac{\beta_{Cjm}}{2\left(d_C + d_{Cm}\right)}\overline{I_i}\right]^2 \right\}^{1/2}. \tag{16}$$

$\overline{S}$ is the number of susceptible hosts at equilibrium in a population coinfected by two resident parasites with exploitation strategies ε_i and ε_j, and $\overline{I_i}$ and $\overline{C}$ are the number of hosts singly infected by parasite i and coinfected at equilibrium, respectively. β_{jm} and β_{Cjm} are the transmission rates of the mutant strain from singly infected hosts and hosts coinfected with parasite i, and d_{jm} and d_{Cm} are the mortality rates of the hosts singly infected by the mutant strain and coinfected by the mutant strain and parasite i. For a given value of ε_i, an evolutionary stable value of parasite j exploitation strategies requires that $\frac{\partial W_{jm}(\varepsilon_{jm}, \varepsilon_i, \varepsilon_j)}{\partial \varepsilon_{jm}}$ is 0 when $\varepsilon_{jm} = \varepsilon_j$, is positive for $\varepsilon_{jm} < \varepsilon_j$, and is negative for $\varepsilon_{jm} > \varepsilon_j$. This indicates that no mutant strains can invade the host population. Note that equation (16) can also be used to find the fitness of a rare mutant of parasite i if the i and j notations are switched. We find the coevolutionary stable strategies of ε_i and ε_j by finding the values of ε_i and ε_j that are uninvadable by mutant strains.

Our population level equations (eqs. 5–8) have no tractable analytic solution (Alizon et al. 2013). Therefore, to find $\overline{S}$, $\overline{I_i}$, and $\overline{C}$, we ran our model (eqs. 4–6) with two resident strains through numeric simulations. At every time step, we solve equation (1) (modified where needed for specific interaction types) to calculate viral titers within a host, and then use viral titers to calculate transmission and death rates via equations (8)–(12) as in Alizon and van Baalen (2005).We are interested in how various interaction mechanisms modulate virulence evolution within coinfected populations while controlling for the fact that host populations are coinfected. Therefore, we first find the ESS of ε (Evolutionary stable host exploitation rate) for a population coinfected with two "baseline" parasite species. Parasites are identical and thus should have the same ESS ε. Note, however, that the baseline ESS ε is different from that in single pathogen situation, as coinfected hosts have increased mortality.

We then repeated this process while either parasite i (the focal parasite) or both parasites evolved one of four different interaction mechanisms: cross immunity, immune suppression, immunopathology, or spite. If only one parasite evolves an interaction strategy, then interactions are asymmetric. If both parasites adopt an interaction strategy, then interactions are symmetric. In this way, we could see the impact of each interaction mechanism on virulence evolution while controlling for basic effects of coinfection. We repeated this process across ranges of parasite interaction strengths. All other parameter values are given in Table 2.

Table 2. Variables within our model. We explored model results over ranges of all parameters, where variables have a single parameter value, changes in that parameter did not yield qualitative differences in our results, and we report the parameter value used to produce Figures 2–4.

Symbol	Parameter	Value
ε_i	Host exploitation by parasite i	Evolves par/par/time
V_i	Within-host density of parasite i	State variable
L_i	Lymphocytes targeting parasite i	State variable
S	Susceptible hosts	State variable
I_i	Hosts singly infected by parasite i	State variable
C	Coinfected hosts	State variable
k	Lymphocyte killing rate	1/(lymph × time)
y	Baseline lymphocyte production	1 lymphocyte/time
c	Parasite-induced lymphocyte production	1 lymph/para./time
m	Lymphocyte mortality rate	1 lymph/lymph/time
b	Host birth rate	17 hosts/host/time
β_{max}	Maximum transmission rate	0.02 1/(host × time)
ω	Half saturation transmission constant	5 parasites/host
d_s	Baseline host mortality	0.03 hosts/host/time
α	Mortality Coefficient	0.10
z	Cross immunity strength	0.0–0.6
λ	Immunopathology strength	0.0–0.05
p	Immune suppression strength	0.0–0.3
u	Spite strength	0.0–0.25
ϕ	Interference cost	0.5–10.0

Results

EVOLUTION OF HOST EXPLOITATION RATES

In our model, parasites with symmetric interactions (both parasites evolve same interaction strategy) evolve to identical host exploitation rates (Figs. 2, 3). Parasites with cross immunity, immunopathology, and spite all evolved increased host exploitation rates, whereas parasites with immune suppression evolved decreased host exploitation rates. Asymmetric parasite interactions (only focal parasite evolves an interaction strategy, nonfocal parasite has baseline strategy) caused host exploitation rates of coinfecting parasite to diverge in some scenarios. A parasite that triggers asymmetric cross immunity temporarily only drives the evolution of a higher exploitation rate in the nonfocal parasite. Similarly, a parasite that triggers asymmetric immunopathology evolves a higher host exploitation rate, while driving the evolution of a decreased host exploitation rate in the nonfocal parasite. Spite always increases the exploitation rate of the focal parasite, but whether spite increases or decreases the exploitation rate of the nonfocal parasite depends on the cost of spite. When the cost of attacking competing parasites is high, spite decreases the exploitation rate of the nonfocal parasite, but increases the exploitation rate of the nonfocal parasite when cost is low (Fig. 3). Thus, in our model, immunopathology and spite can cause the evolution of decreased host exploitation rates in nonfocal parasites, and thus the divergence of exploitation rates, if interaction strategies are asymmetric. Triggering asymmetric immune suppression or spite causes the evolution of increased exploitation rates in all parasites. However, coinfecting parasites do not reach identical host exploitation rates (Figs. 2, 3).

MECHANISMS OF EVOLUTION

The observed evolution of host exploitation rates can be explained by several factors. One potential factor is coevolution (differences in how parasites evolve if only one parasite is allowed to evolve at a time versus if both parasites evolve at once). Parasites in coinfected populations evolve higher host exploitation rates in our model because coinfection increases host mortality, and increases in host mortality increase ESS exploitation rates. Thus, as a parasite evolves higher host exploitation rates, the mortality of coinfected hosts will increase, thereby forcing competing parasites to also evolve a higher host exploitation rate. On the other hand, as a parasite evolves lower host exploitation rates, the mortality of coinfected hosts will decrease, causing competing parasites to also evolve a lower host exploitation rate. Thus, if a within-host interaction shifts the ESS exploitation strategy of both parasites in a system in the same direction, their magnitude of evolution will be greater if they coevolve. However, coinfecting parasites that evolve in different directions have smaller shifts in host exploitation rates due to coevolution. Overall, coevolution influences the magnitude of change in ESS

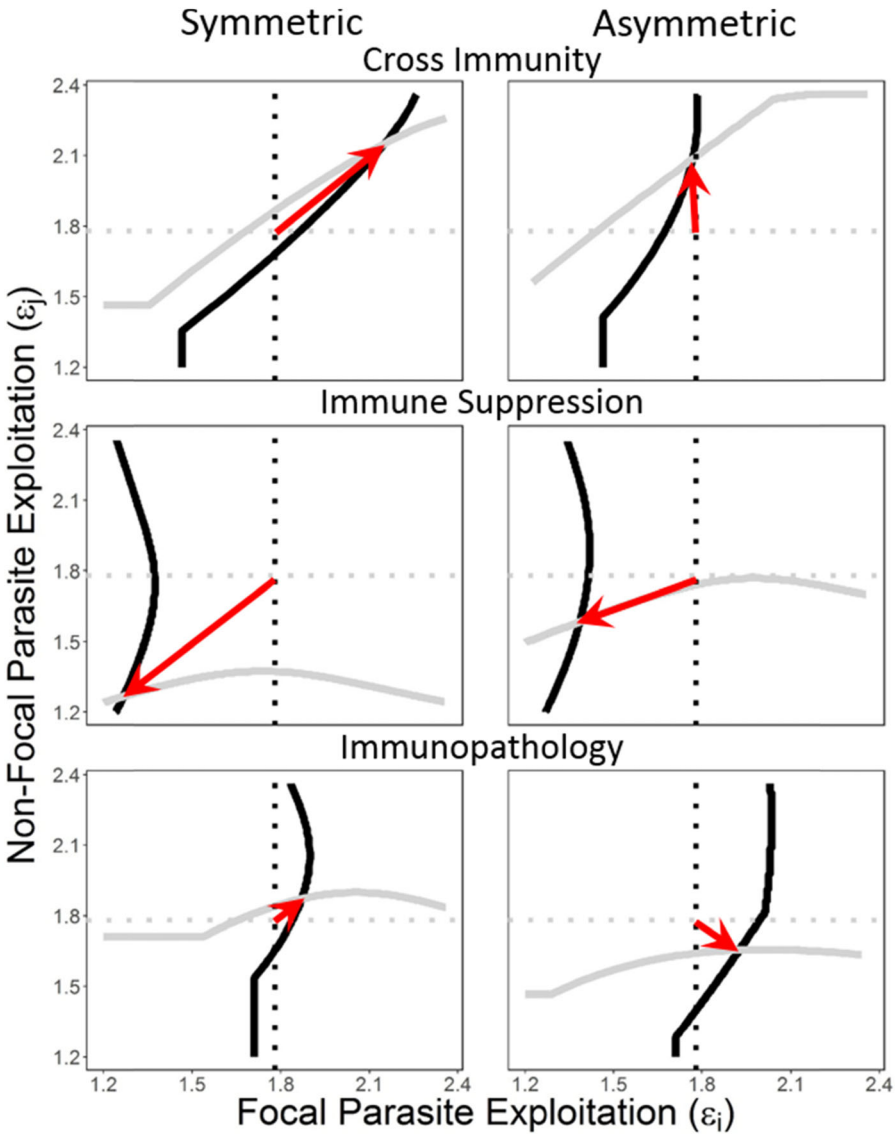

Figure 2. Evolutionary and coevolutionary stable strategies of parasites under cross immunity ($z = 0.2$), immune suppression ($p = 0.1$), and immunopathology ($\lambda = 0.05$). For symmetric interactions, both parasites employ the interaction mechanism. For asymmetric interactions, only the focal parasite employs the interaction mechanism. Black solid lines show the ESS exploitation rate of the focal parasite over changing exploitation rates of the nonfocal parasite. Gray solid lines show ESS exploitation rate of the nonfocal parasite over changing exploitation rates of the focal parasite. Coevolutionary stable strategies occur where solid lines intersect. Dashed lines indicate coevolutionary stable strategies for two baseline parasites. Red arrows show change in coevolutionary stable exploitation strategies for both parasites.

exploitation strategy due to a parasite interaction, but not whether the interaction causes parasites to evolve higher or lower levels of exploitation.

Whether a parasite's ESS host exploitation rate increases or decreases can be explained by shifts in the proportion of hosts infected by that parasite that are coinfected ($\frac{C}{C+I_i}$, hereafter referred to as *coinfection pressure*). In response to an interaction mechanism, the prevalence of both singly infected and coinfected hosts increases or decreases in all cases, shifting the coinfection pressure. In all asymmetric cases and most symmetric cases, if a within-host interaction causes a parasite's coinfection pressure to increase, then it will evolve an increased host exploitation rate, and vice versa (Fig. 4). This pattern emerges because within-host competition leads to the evolution of increased host exploitation rates, and the coinfection pressure represents the amount of within-host competition a parasite faces at the host-population scale.

When interaction strategies cause the coinfection pressure of coinfecting parasites to shift in the same direction, their host exploitation rates evolve in the same direction. However, when

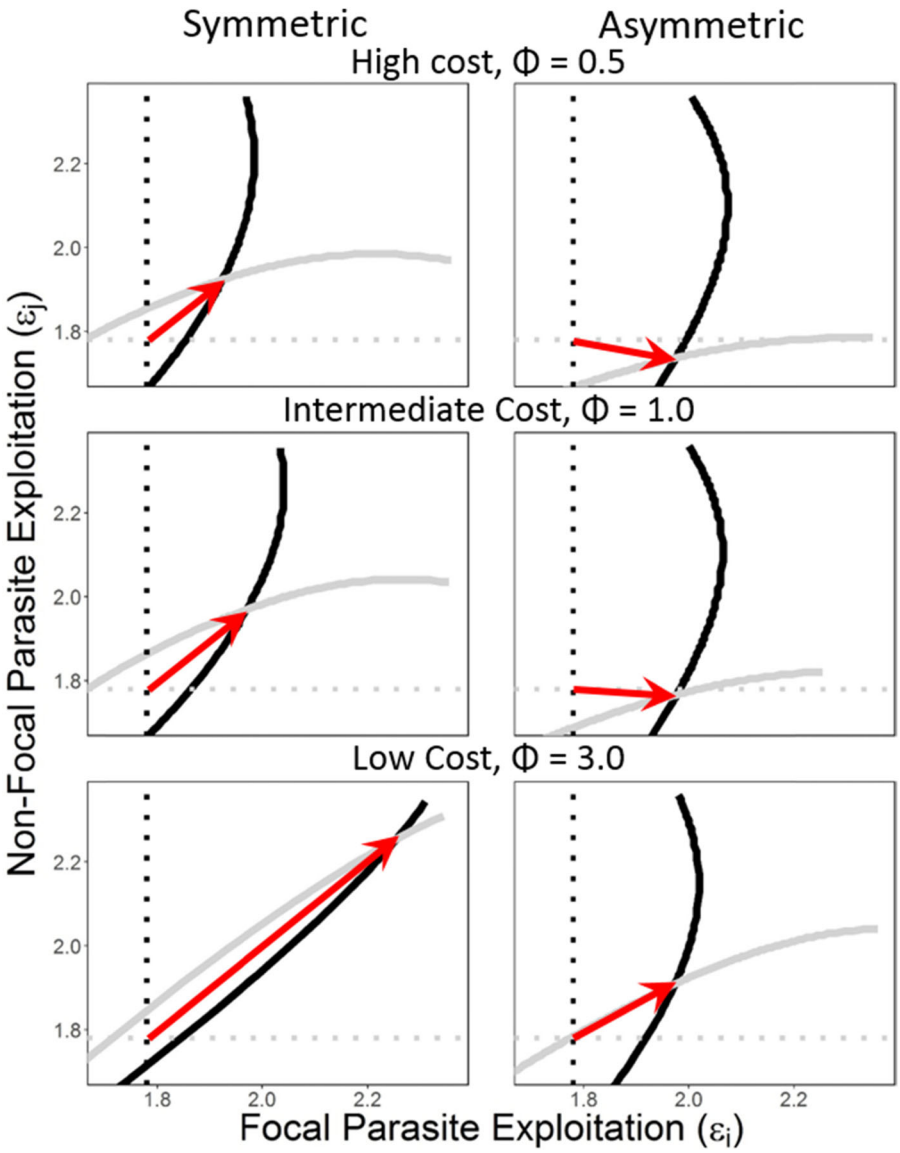

Figure 3. Evolutionary and coevolutionary stable strategies of parasites with spite ($u = 0.2$), and varying costs of spiteful behavior. Figure 3 can be read the same way as Figure 2.

interaction strategies cause the coinfection pressure of coinfecting parasites to shift in opposite directions (asymmetric immunopathology and asymmetric low-cost spite), then they will evolve divergent host exploitation rates (Fig. 3). Divergent shifts in coinfection pressure result from asymmetric within-host interactions that increase the fitness of one parasite while decreasing the fitness of the second parasite. For instance, when a focal parasite triggers asymmetric cross immunity, it lowers the within-host density of the nonfocal parasite, reducing its rate of transmission, and thus fitness. This reduced within-host density lengthens the host's lifespan, increasing the total transmission, and thus fitness, of the focal parasite. These changes in fitness increase the prevalence of the focal parasite, and decrease the prevalence of the nonfocal parasite. As the prevalence of a parasite increases, so does the likelihood that a host will become infected by that parasite over a given length of time. Thus, as the prevalence of the nonfocal parasite increases, so does the likelihood that hosts singly infected by the focal parasite will become coinfected, increasing coinfection pressure. Therefore, asymmetric interactions that benefit one parasite while harming the other parasite lead to the evolution of divergent host exploitation rates.

In only one case do parasites evolve increased host exploitation rates in the face of decreased coinfection pressure—under symmetric immunopathology (Fig. 3). This disruption of the usual pattern is because parasites facing symmetric immunopathology face very high host mortality in all infection classes, which we expect to lead to the evolution of increased host exploitation rates.

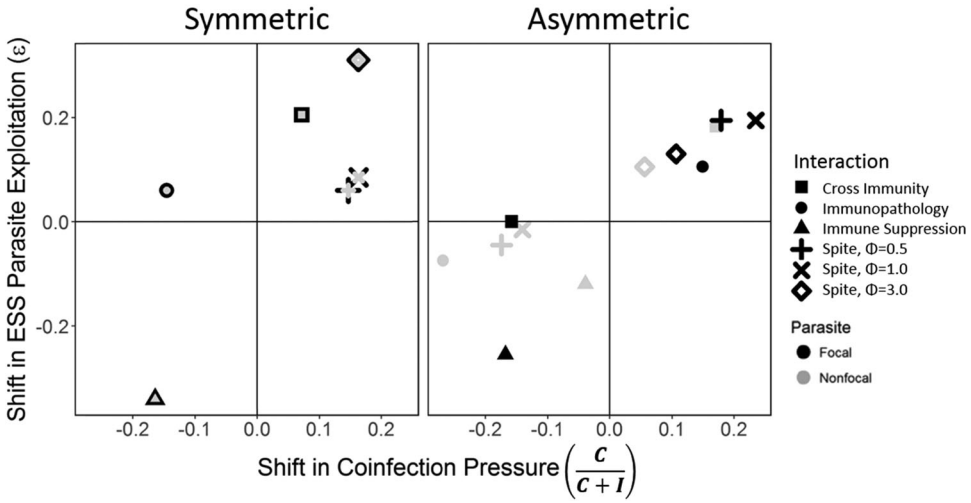

Figure 4. **The relationship between shifts in coinfection pressure due to within-host interactions, and the evolution of host exploitation rates. The x-axis represents coinfection pressure at equilibrium directly after a parasites evolves within-host interactions, minus the coinfection pressure at equilibrium for baseline parasites. Note that this shift is calculated before host exploitation rates evolve to ESS levels in response to within-host interactions. The y-axes show the resulting shift in ESS host exploitation rates. Note that for symmetric interactions, the points for focal and nonfocal interactions are identical. Focal, asymmetric spite points for $\phi = 0.5$ and $\phi = 1.0$ have been jittered to avoid overlap.**

EVOLUTION OF PARASITE INDUCED HOST MORTALITY

Increasing the strength of an interaction strategy often increases the mortality of singly infected hosts at ESS virulence, while decreasing the mortality of coinfected hosts, or vice versa (Figs. 5, 6). For instance, as we increase the strength of asymmetric spite at intermediate cost (Fig. 6), the mortality of hosts singly infected by both focal and nonfocal hosts increases at ESS virulence, whereas the mortality of coinfected hosts decreases. This difference is because spite and the resulting evolution of host exploitation rates have opposite effects on host mortality, and are partitioned differently across different infection classes. Spite lowers within-host density, and thus host mortality. However, both focal and nonfocal parasites evolve increased host exploitation rates in response to spite, increasing host mortality. In coinfected hosts, decreased mortality from the spiteful interaction outweighs increased mortality from evolving exploitation rates. In singly infected hosts however, parasites do not engage in spiteful behavior. Thus, host mortality is only increased. We see these qualitative differences in how the mortality of singly infected versus coinfected hosts responds to within-host interactions in every interaction strategy within our model.

In several cases, we see nonmonotonic relationships between interaction strength and the mortality of coinfected hosts at ESS virulence (Figs. 5, 6). For instance, at low interaction strength, symmetric cross immunity lowers coinfected host mortality. But at higher interaction strength, symmetric cross immunity increases coinfected host mortality. Symmetric cross immunity reduces within-host parasite density, thus decreasing parasite transmission while increasing the infectious lifespan of hosts. Cumulatively, symmetric cross immunity increases total parasite transmission over a host's lifetime, increasing parasite fitness, and thus prevalence. As we increase the prevalence of two parasites in a system, we expect more overlap in the hosts they infect, and thus higher coinfection pressure. The nonlinear impact of cross immunity on host mortality arises because increasing the strength of cross immunity increases the evolution of host exploitation at an accelerating rate, but decreases within-host parasite density, and thus host mortality, at a linear rate. Thus, at low interaction strength, the increase in host mortality from the evolution of host exploitation is lower than the decrease in host mortality received from cross immunity, but the opposite is true at high interaction strengths. A similar process underlies the nonlinear relationship between the strength of spite and the ultimate impact on coinfected host mortality at low cost of interference (Fig. 6).

Discussion

Coinfections are ubiquitous in nature and drive the evolution of virulence, defined here as parasite-induced host mortality (Levin and Bull 1994; Read and Taylor 2001; Pedersen and Fenton 2007). However, the impact that coinfection has on virulence evolution depends on how parasites interact within hosts (Alizon et al. 2013). Here we demonstrate that the impact of specific within-host interactions on virulence evolution depends heavily on whether parasites are embedded in communities that share symmetric

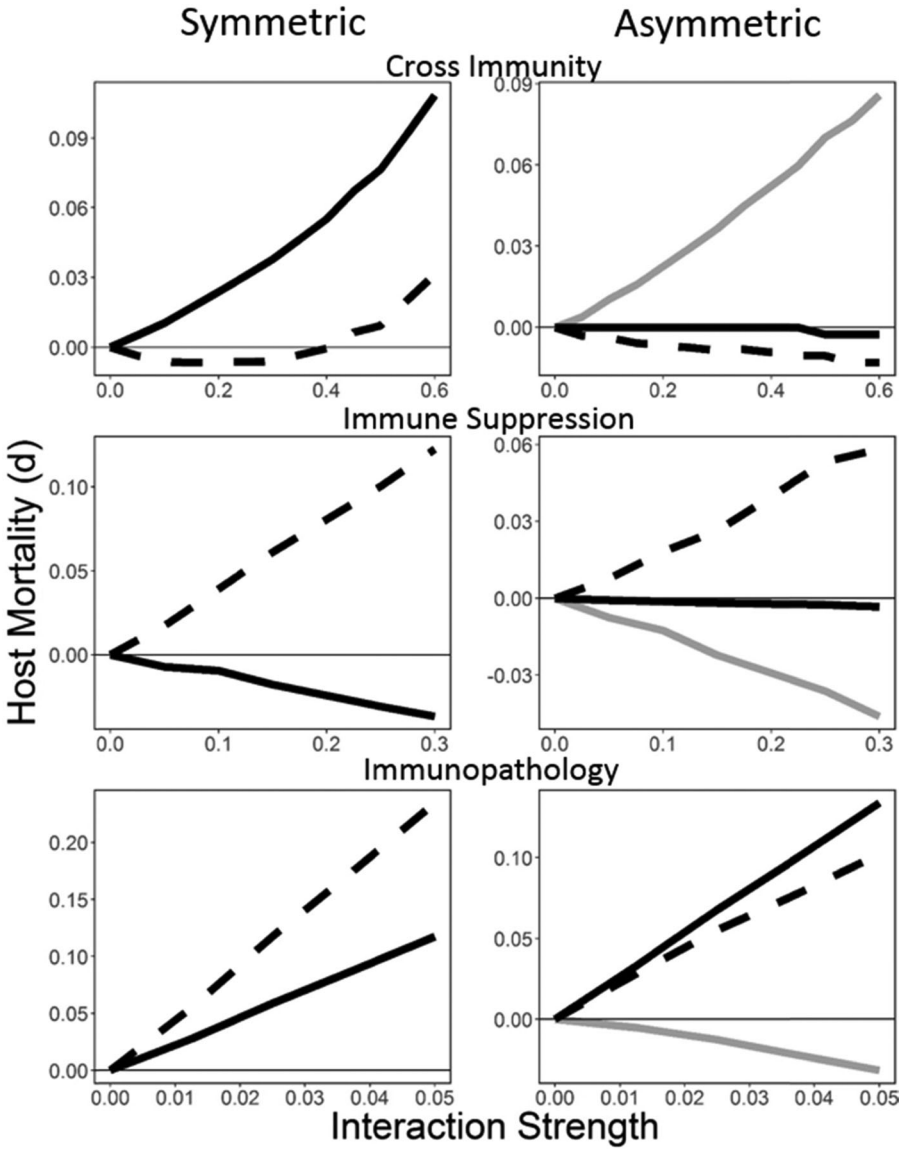

Figure 5. The ESS parasite-induced mortality (virulence) in coinfected hosts (dashed line), hosts singly infected by the focal parasite (solid black), and singly infected by the nonfocal parasite (solid gray over increasing strength of cross immunity (*z*), immune suppression (*p*), and immunopathology (λ) (See Table 2, eqs. 11–15). At interaction strength of zero, parasites are equivalent to two coinfecting baseline parasites with no within-host interactions. Y-axis shows ESS host mortality of an infection class minus the mortality of that infection class in a population coinfected by two baseline parasites. Note that for symmetric interactions, virulence of hosts singly infected by focal and nonfocal parasites is identical. Y-axis is scaled differently is each panel, as we are interested in qualitative, not absolute patterns.

interaction phenotypes, or communities connected by asymmetric interactions. Further, we find that no within-host interaction causes virulence to purely increase or decrease. Rather, all within-host interactions cause the evolution of lower virulence in some hosts, and higher virulence in other hosts, depending on which parasites coinfect the host in question. Finally, our results indicate that whether some within-host interactions increase or decrease the mortality of coinfected hosts depends on the strength of those interactions. Overall, these results provide predictions for how common within-host interactions will alter virulence evolution in multi-parasite settings, and indicate that virulence evolution must be understood at the community scale.

DIFFERENCES BETWEEN SYMMETRIC AND ASYMMETRIC INTERACTIONS

Hosts contain complex communities of parasites that interact in a variety of ways (Pedersen and Fenton 2007), but we know surprisingly little about how virulence evolves in these multi-parasite

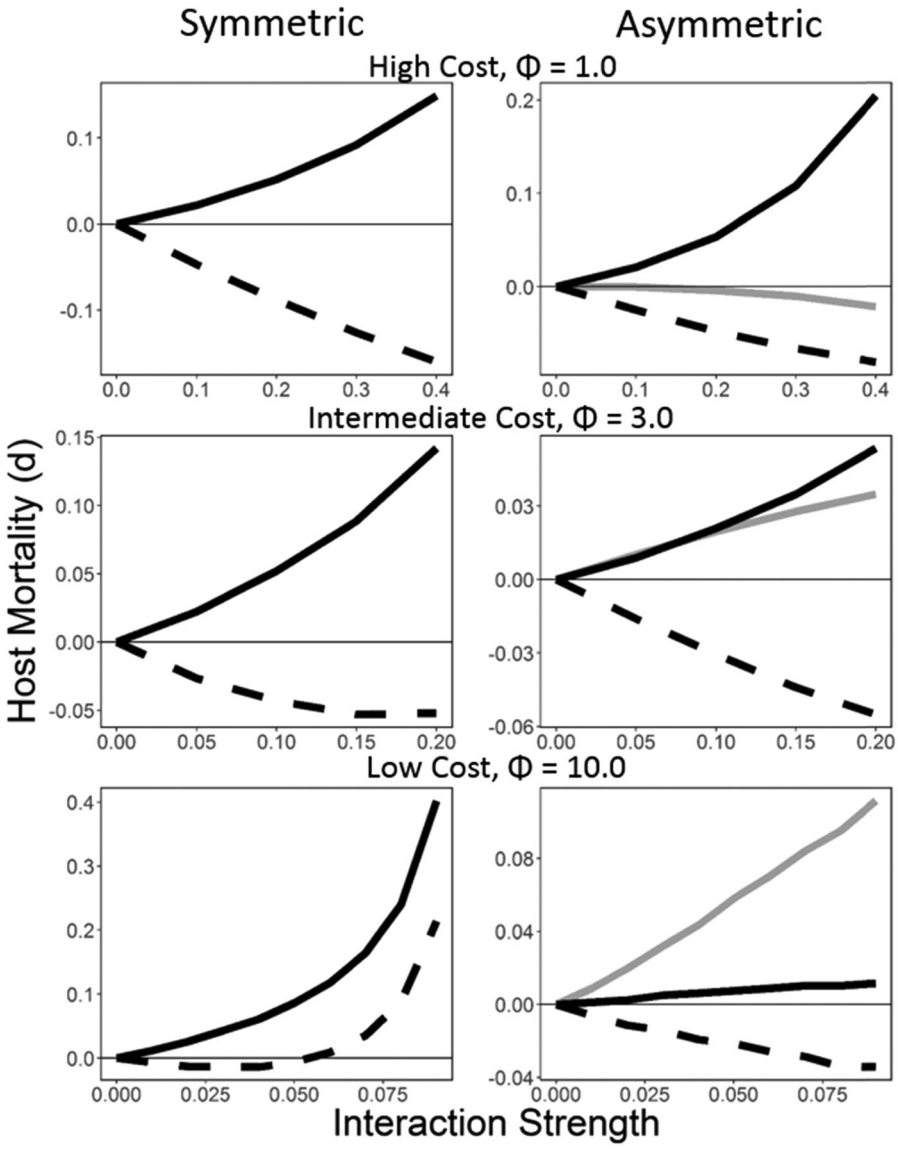

Figure 6. **The ESS parasite-induced mortality (virulence) in coinfected hosts (dashed line) hosts singly infected by the focal parasite (solid black) and singly infected by the nonfocal parasite (solid gray) over increasing strength of spite (*u*), for varying costs of interference (ϕ) (See Table 2, eqs. 11–15). At interaction strength of zero, parasites are equivalent to two coinfecting baseline parasites with no within-host interactions. Y-axis shows ESS host mortality of an infection class minus the mortality of that infection class in a population coinfected by two baseline parasites. Note that for symmetric interactions, virulence of hosts singly infected by focal and nonfocal parasites is identical. Y-axis is scaled differently is each panel, as we are interested in qualitative, not absolute patterns.**

communities (Betts et al. 2016). Previous theoretical and empirical work has largely focused on how parasites evolve in multi-strain populations where parasites all interact with each other in the same way (Alizon et al. 2013). Our results show that the impact of within-host interactions on virulence evolution changes between symmetric (same interaction type for all parasites) and asymmetric (different interaction types for coinfecting parasites) scenarios. This finding is important because it indicates that much previous work on how within-host interactions alter virulence evolution does not generalize to asymmetric multi-parasite scenarios.

Symmetric and asymmetric interaction models differ in that symmetric models cannot distinguish between a within-host interaction's impact on the focal parasite's virulence evolution and the nonfocal parasite's virulence evolution. For instance, from our symmetric model, we would conclude that cross immunity leads to the evolution of increased host exploitation (Fig. 4). However, this obscures that the evolution of increased host exploitation in our symmetric model is the sum of two drivers that become apparent in our asymmetric model: attacking coinfecting parasites through the immune system leads to the evolution of decreased

host exploitation, whereas being attacked by coinfecting parasites via the immune system leads to the evolution of increased host exploitation. Ultimately, cross immunity, immunopathology, and spite can all lead to reductions in host exploitation rates, but this potential is only revealed in our asymmetric models. All of the interactions analyzed in this study have been found to take place in multi-parasite, asymmetric scenarios (Graham et al. 2005; Ezenwa and Jolles 2011; Shrestha et al. 2013; Rafaluk-Mohr et al. 2018). Thus, analyzing them in biologically relevant scenarios is a step forward toward understanding virulence evolution in the field.

EVOLUTION OF INCREASED OR DECREASED VIRULENCE

Ultimately, no interaction purely increases or decreases host mortality in our model. Rather, all interactions except symmetric immunopathology lead to increases in the mortality of some host classes, and decreases in the mortality of other host classes (Figs. 5,6). This is due to three mechanisms. First, almost every asymmetric interaction causes an increase in the host exploitation rate of one parasite, and a decrease in the host exploitation rate of the coinfecting parasite (Figs. 2–4). Second, increases (or decreases) in host mortality due to evolving host exploitation rates often accompany opposite impacts on host mortality due to the direct impact of within-host interactions. Third, the direct impact of within-host interactions on host mortality does not apply to all host classes. For instance, consider asymmetric cross immunity. The evolution of the focal parasite's exploitation rate decreases both the mortality of hosts it singly infects and the mortality of coinfected hosts. The evolution of the nonfocal parasite's exploitation rate increases both the mortality of hosts it singly infects and the mortality of coinfected hosts. Finally, cross immunity itself reduces the density of parasites in coinfected hosts, decreasing coinfected host mortality. As a result, cross immunity increases the mortality of hosts singly infected by the nonfocal parasite, slightly decreases the mortality of hosts singly infected by the focal parasite, and greatly reduces the mortality of coinfected hosts. Thus, whether a within-host interaction increases or decreases virulence cannot be determined by measuring aspects of a single parasite/host combination. Rather, virulence evolution occurs at the community scale, and we must consider how within-host interactions alter the mortality of hosts infected by every iteration of the parasite community.

Ecological context may determine whether specific within-host interactions lead to increased or decreased mortality in coinfected hosts. Our results show that for symmetric cross immunity and spite, whether a within-host interaction increases or decreases the mortality of coinfected hosts at evolutionary stability depends on interaction strength (Fig. 5 cross immunity, Fig. 6, low cost spite). Interaction strength may vary in natural host populations due to both evolutionary constraints, and ecological factors such as dispersal limitation (Gardner et al. 2004). Thus, in some populations, these interactions will lead to an overall increase in coinfected host mortality, whereas in other populations they will lead to an overall decrease in coinfected host mortality. Previous studies have analyzed how ecological factors determine the optimal strength of spite and cross immunity (Gardner et al. 2004; Ashby and King 2017). Thus, following Kamiya et al. (2018) future studies may simultaneously model both the evolution of the strength of these interactions, and within-host exploitation rates, in order to ascertain the conditions under which cross immunity and spite will ultimately increase or decrease the mortality of coinfected hosts.

COMPARISON TO PREVIOUS THEORY

Our results appear to contradict previous theory on how within-host interactions should impact virulence evolution. For instance, Day et al. (2007) predicts that immunopathology will select against high virulence if virulence and immune-induced mortality are linked, but select for higher virulence if they are independent. Given these expectations, in our model a parasite that triggers immunopathology should decrease its own ESS virulence (virulence linked to immunopathology) while increasing competitor ESS virulence (virulence independent of immunopathology). We find the exact opposite results in our model (Figs. 2, 4), although our results replicate those found by Day et al. (2007) if we reduce our model to a single parasite species. This difference arises because the scope of Day et al. (2007) does not include how immunopathology interacts with coinfection pressure. Thus, their model results apply best to cases where coinfection by multiple parasite strains or species is rare, whereas ours applies best to cases where coinfection is common. In the case of immune suppression, previous theory suggests that as the strength of symmetric immune suppression increases, host exploitation should increase as well (Choisy and de Roode 2010; Kamiya et al. 2018), whereas our results predict the opposite. This difference arises from how immune suppression within hosts is parameterized. Kamiya et al. (2018) parameterized immune suppression to increase host susceptibility and reduce host clearance, whereas our model parameterizes immune suppression to increase parasite load, and thus host mortality and parasite transmission. Both models are appropriate in different contexts; the Kamiya et al. (2018) model is appropriate in circumstances where immune suppression extends the length of infection, and thus the probability of coinfection (Schmid-Hempel 2008), whereas ours is most applicable to circumstances where immune suppression reduces the length of infections due to increased host mortality (Ezenwa and Jolles 2015). Overall, comparing our results to previous theory indicates that we must predict the impact that within-host interactions have on virulence

evolution while considering the specific biology of particular systems.

COMPARISON TO PREVIOUS EMPIRICAL WORK

Empirical work linking within-host interactions to virulence evolution rarely expands beyond the single-host scale. Thus, empirical studies must be paired with theoretical models to predict evolution at the host population scale. Before we can use our model to make predictions at the host population scale based on empirical data at the single host scale; however, we must make sure that empirical evidence at the single host scale matches with our model assumptions. There is mixed empirical evidence that cross immunity increases the relative fitness of more virulent parasites at the within-host scale (Raberg et al. 2006; Barclay et al. 2014). If this is true, then it provides support for the within-host portion of our cross immunity model. Similarly, spite lowers host mortality at the single host scale (Garbutt et al. 2011), once again in line with the assumptions of our model. Although we cannot currently match our model results to tests of how within-host interactions drive the evolution of virulence at the host-population scale, we can look to empirical evidence to validate portions of our model.

MECHANISMS DRIVING VIRULENCE EVOLUTION

Although virulence evolution may seem idiosyncratic for each within-host interaction in this study, it can be predicted for each interaction via a common framework. Parasite interaction pathways mainly shift optimal host exploitation strategies in our model by changing coinfection pressure (i.e., $\frac{C}{C+I_i}$). Parasites generally evolve higher host exploitation in coinfected hosts because they must use up host resources before their competitors (Levin and Bull 1994), so higher coinfection pressure creates a steeper selection gradient toward high host exploitation (Nowak and May 1994; Kamiya et al. 2018). Our work shows how several common within-host interactions alter coinfection pressure, and thus drive the evolution of host exploitation. Understanding that the evolution of host exploitation depends on coinfection pressure highlights future steps for empirical research. Primarily, we must experimentally establish the direction a within-host interaction pushes coinfection pressure for all parasites in a host population. A within-host interaction should decrease coinfection pressure for a parasite if it decreases the fitness of other parasites in the host population. For instance, if a parasite interferes with conspecifics, (e.g., via cross immunity), they will transmit at a lower rate, and thus have a lower chance of infecting hosts already infected by the focal parasite. An interaction pathway may also lower coinfection pressure by lowering the susceptibility of singly infected hosts to coinfection (Kamiya et al. 2018). If we can predict how a within-host interaction changes coinfection pressure, then we can predict the impact of any within-host interaction on virulence evolution.

GENERALIZING RESULTS

To maximize the usefulness of our model, we need to delineate the systems our model most applies to. Our model includes several standard assumptions. First, to follow the virulence trade-off hypothesis, we assume that transmission increases in a saturating manner, and that host mortality increases in a linear manner as parasite density inside hosts increases. This results in the saturating relationship between host mortality and transmission central to the virulence trade-off. Supporting evidence for these assumptions has been found in pathogens important to both human health such as HIV and malaria, and pathogens important to wildlife population dynamics such the monarch butterfly pathogen *Ophryocytis elektroscirrha* (Fraser et al. 2007; de Roode et al. 2008; Mackinnon et al. 2008; Alizon et al. 2009). However, our model does not apply to systems where virulence is expressed via castration (Jensen et al. 2006; Cressler et al. 2014), or where parasite density within hosts is largely made up of nontransmitting stages, and thus is disconnected from transmission (Garnham 1966). Further, our model can best be used to understand virulence evolution in directly transmitted parasites, rather than trophically transmitted parasites (Hoverman et al. 2013). Thus, future work should refine extensions of our model to that we can understand how within-host interactions drive virulence evolution for various expressions of virulence and various transmission modes.

AUTHOR CONTRIBUTIONS

PAC conceptualized this project, wrote the code, and was the primary author on the manuscript. VHWR helped develop project concepts, reviewed code, and edited manuscript.

ACKNOWLEDGMENTS

This research was made possible by the National Science Foundations Graduate Research Fellowship Program.

DATA ARCHIVING

Code for this project has been deposited in a git repository: https://github.com/patrickaclay/virulence_evolution_within_host_interactions. The doi for this article is https://doi.org/10.5061/dryad.dbrv15dwn.

ETHICS STATEMENT

No humans or vertebrate animals were used in this study.

CONFLICT OF INTEREST

The authors have no competing interests.

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Associate Editor: K. Koelle
Handling Editor: D. W. Hall