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Donnelly, Ruairi; White, Andrew Ronald; Boots, Mike

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Authors
Ruairi Donnelly[1,a]
Andy White[1,b]
Mike Boots[2,c]

Affiliations
[1]Department of Mathematics, Heriot-Watt University, UK.
[2]Biosciences, College of Life and Environmental Sciences, University of Exeter, UK.

Contact information
[a]rd501@cam.ac.uk
[b]A.R.White@hw.ac.uk
[c]M.Boots@exeter.ac.uk

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Corresponding author details
Ruairi Donnelly
Department of Plant Sciences
University of Cambridge
CB2 3EA
rd501@cam.ac.uk
00441223330228

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Host are typically challenged by multiple parasites, but to date theory on the evolution of resistance has mainly focused on single infections. We develop a series of models that examine the impact of multiple parasites on the evolution of resistance under the assumption that parasites coexist at the host population scale as a consequence of superinfection. In this way we are able to explicitly examine the impact of ecological dynamics on the evolutionary outcome. We use our models to address a key question of how host lifespan affects investment in resistance to multiple parasites. We show that investment in costly resistance depends on the specificity of the immune response and on whether or not the focal parasite leads to more acute infection than the co-circulating parasite. A key finding is that investment in resistance always increases as the immune response becomes more general independently of whether it is the focal or the co-circulating parasite that exploits the host most aggressively. Long-lived hosts always invest more than short-lived hosts in both general resistance and resistance that is specific to relatively acute focal parasites. However, for specific resistance to parasites that are less acute than co-circulating parasites it is the short-lived hosts that are predicted to invest most. We show that these results apply whatever the mode of defence i.e. whether it is through avoidance or through increased recovery, with or without acquired immunity, or through acquired immunity itself. As a whole, our results emphasise the importance of considering multiple parasites in determining optimal immune investment in eco-evolutionary systems.

Key words: epidemiology, ecology, host resistance, density dependence, superinfection, coexistence, lifespan.

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1. Introduction

In natural settings hosts are subject to attack from a multitude of parasites (Morand and Poulin, 2000; Nunn et al., 2003, 2005). The impact of multiple infection on the evolution of parasite virulence has been well studied (Bremermann and Pickering, 1983; Bonhoeffer and Nowak, 1994; Nowak and May, 1994; van Baalen and Sabelis, 1995; Frank, 1996; Mosquera and Adler, 1998; Gandon et al., 2001) with this theory suggesting that parasite diversity is associated with higher parasite virulence (though collective action between co-infecting parasites can alter this result, see Brown et al. (2002)). Furthermore, of particular interest is that the strength of this effect can decrease with the relatedness of the parasites (Frank, 1996; Gandon and Michalakis, 2002). The role of multiple infections in the evolution of host resistance, on the other hand, is less well studied (Poitrineau et al. (2003); Jokela et al. (2000); Kada and Lion (2015)) with all of the evolution of resistance theory that explicitly takes account of ecological feedbacks restricted to defence against a single parasite (or transient parasite diversity, see Kada and Lion (2015)). Parasites clearly interact directly through competition for susceptible hosts, but when the host evolves resistance to a focal parasite the extent to which the resistance also counters co-circulating parasites constitutes an additional, less obvious interaction between parasites. Therefore, the relationship between parasite diversity and the pattern of evolved resistance is likely to be complex. In particular there is considerable interest in the role that host lifespan plays in determining optimal investment in costly defence (van Boven and Weissing, 2004; Miller et al., 2007; Boots et al., 2013; Donnelly et al., 2015) but it is not yet understood how this will depend on the nature of co-circulating parasites.

There is a large body of work that examines the evolution of immunity in the context of ecological feedbacks and the presence of a single parasite strain (Bowers et al., 1994; Antonovics and Thrall, 1994; Boots and Haraguchi, 1999; van Baalen, 1998; van Boven and Weissing, 2004; Miller et al., 2007; Boots et al., 2013; Donnelly et al., 2015). In addition there are a few models that have considered parasite (or enemy) diversity in the study of defence (Jokela

et al., 2000; Poitrineau et al., 2003; Kada and Lion, 2015). Poitrineau et al. (2003) explored defence against two natural enemies and examined how cross resistance (synergy in resistance) influences optimal defence investment, while Jokela et al. (2000) focused on how the level of parasite diversity impacts on the optimal level of defence allocation. Both studies consider only evolutionary dynamics and do not incorporate ecological feedbacks so that the role of life-histories that influence evolutionary outcomes through population dynamics is not clear. In Kada and Lion (2015) which included a type of superinfection that did not involve stably coexisting parasites at the host population scale (rather, a rare invading parasite lineage superinfected a stably circulating parasite and vice versa), the co-evolutionary dynamics of recovery resistance and virulence were studied. They found that superinfection can lead to high virulence and high investment in defence but crucially, resistance developed to counter one parasite did not simultaneously feedback to the prevalence of the other in the form of superinfection modelled in Kada and Lion (2015). Here, we make a novel extension to these studies by applying an eco-evolutionary approach to the question of how stable co-circulating parasitic challenges determine natural selection for host resistance achieved through avoidance, recovery and acquired immunity. For the first time in a framework that allows multi-parasite coexistence at the host population scale and encompasses specific as well as non-specific immune response we account for the complex ecological feedbacks between the dynamics of multiple parasites and evolving resistance. In this way, we examine how traits such as host lifespan determine patterns of optimal investment in host defence.

The framework of evolutionary invasion analysis (Metz et al., 1996; Geritz et al., 1998) uses explicit ecological dynamics to derive fitness and provides tools for assessing the stability of evolutionary trajectories. Here we use these methods to examine resistance evolution in the presence of multiple parasites. This requires parasite coexistence, here referring to stable persistence of more than one parasite at the host population scale. To achieve this we assume a superinfection interaction, where individual hosts infected with a less virulent parasite are susceptible to infection, with displacement of the original parasite by a more virulent parasite (Nowak and May, 1994). Once co-existing multiple infections are incorporated in host parasite models the question of the specificity of resistance naturally arises, and in

this study we examine how the level of cross resistance impacts on the evolution of host resistance to infection. The ecological derivation of host fitness for a range of disease and host characteristics provides clear insight into the effect of co-circulating parasites on host resistance, and demonstrates consistent patterns of investment regardless of the type, or actual mechanism, of resistance.

There has been considerable interest in how host immune investment differs between populations of contrasting lifespans (van Boven and Weissing, 2004; Miller et al., 2007; Boots et al., 2013; Donnelly et al., 2015). In particular, a naive view of immune investment is that it will increase monotonically as lifespan and hence exposure increases. However, recent theory using evolutionary invasion analysis has shown that when ecological feedbacks are included the relationship between life-span and immune investment can be complex (van Boven and Weissing, 2004; Miller et al., 2007; Boots et al., 2013; Donnelly et al., 2015). However, as yet none of this theory has taken account of parasite diversity. Here, by incorporating ecological dynamics we achieve a key aim of our study: an examination of how investment in resistance varies with host lifespan when hosts are challenged by multiple parasites.

2. Methods

(*a*) *Epidemiological Model*

We assume a host structure based on susceptible, infected, and recovered/immune sub-populations (Kermack and McKendrick, 1927; Macdonald, 1957; Anderson and May, 1979). We extend the classical framework so that susceptible hosts, with density X, can be infected by either hosts with a focal infection, Y_1 or a co-circulating infection, Y_2. Hosts can recover and gain life-long immunity to the focal infection, Z_1 and related to this the host may be infected by the co-circulating parasite but immune to the focal parasite, Y_2^{Z1}. We allow therefore for a range of resistance mechanisms (to the focal parasite) in the presence of a co-circulating parasite but for simplicity there is no acquired immunity to the co-circulating parasite, see figure 1 for schematic depiction. Nevertheless immunity to the focal parasite and resistance in

general can carry over to the co-circulating parasite if it is non-specific. The epidemiological dynamics are governed by the following equations:

$$\frac{dX}{dt} = aH - qH^2 - bX - \beta_1 XY_1 - \beta_2 X(Y_2 + Y_2^{Z1}) + (1-\nu_1)\gamma_1 Y_1 + \gamma_2 Y_2 \quad (1)$$

$$\frac{dY_1}{dt} = \beta_1 XY_1 - (\alpha_1 + b + \gamma_1)Y_1 - s\beta_2 Y_1(Y_2 + Y_2^{Z1}) \quad (2)$$

$$\frac{dY_2}{dt} = \beta_2 X(Y_2 + Y_2^{Z1}) - (\alpha_2 + b + \gamma_2)Y_2 + s\beta_2 Y_1(Y_2 + Y_2^{Z1}) \quad (3)$$

$$\frac{dZ_1}{dt} = \nu_1\gamma_1 Y_1 - bZ_1 - \sigma\beta_2 Z_1(Y_2 + Y_2^{Z1}) + \gamma_2 Y_2^{Z1} \quad (4)$$

$$\frac{dY_2^{Z1}}{dt} = \sigma\beta_2 Z_1(Y_2 + Y_2^{Z1}) - (\alpha_2 + b + \gamma_2)Y_2^{Z1} \quad (5)$$

All parameters are non-negative and the total host density is given by $H = X + Y_1 + Y_2 + Y_2^{Z1} + Z_1$. All hosts produce susceptible offspring at rate a which is limited by intra-specific crowding, q. Hosts die at natural death rate b. In addition, infected hosts suffer additional disease induced mortality (virulence) at rate α_1 for the focal parasite and α_2 for the co-circulating parasite. The dynamics of transmission and recovery are shown in schematic form in Figure 1. In detail we assume that transmission of infection is a mass action process between susceptible and infected types, with transmission coefficient β_1 for the focal infection and β_2 for the co-circulating infection. Virulence is assumed to be correlated with the rate at which parasites exploit individual hosts. As a consequence, individuals infected with the less virulent parasite are susceptible to infection by the more virulent parasite (since the competitive advantage of high host exploitation leads to competitive replacement within the host i.e. *superinfection*, see e.g. Nowak and May (1994)). If $\alpha_2 > \alpha_1$ the more aggressive co-circulating parasite superinfects the focal parasite and this is the situation represented by equations $1-5$ and depicted as model 1 in figure 1. If $\alpha_1 < \alpha_2$ the focal parasite is more virulent and superinfects the co-circulating one and this is depicted as model 2 in figure 1 (for brevity the equations for this model are not shown but it is simply the above model with the

direction of superinfection reversed and a transmission coefficient for the superinfection term of β_1 rather than β_2). The superinfection coefficient s controls the strength of the interaction and for our purposes $0 \leq s \leq 1$. Infected hosts recover at rate γ_1 from the focal infection and γ_2 from the co-circulating infection, with a proportion of recoveries from the focal infection, $\nu_1 \in (0, 1)$, becoming immune to the focal parasite and the remaining individuals returning to a susceptible state. Immunity to the focal parasite can carry over to the co-circulating parasite if it is non-specific. This occurs if $\sigma < 1$ and implies that immunity to the focal parasite reduces the likelihood of infection to the co-circulating parasite, see figure 1 which shows that infection by the co-circulating parasite of class Z_1 occurs at σ the rate of that of X.

This general model form can be used to capture a wide range of classical infection scenarios. For example, if $\nu_1 = 0$ the model represents a Susceptible−Infected−Susceptible (SIS) framework, where there is no immune memory and recovered individuals are completely susceptible to both infections. On the other hand if $\nu_1 = 1$ we have the Susceptible−Infected−Recovered (SIR) model with specific ($\sigma = 1$) or non-specific ($\sigma < 1$) life-long immunity (though, for simplicity, the structure due to the co-circulating parasite remains SIS). In this SIR example specificity (of acquired immunity) is denoted by σ i.e. if σ is high then specificity is high. In all the other forms of resistance, specificity is denoted by c, and is defined as a parameter in the host trait that resists the co-circulating infection (i.e. $\beta_2 = \beta_2(c)$ for avoidance and $\gamma_2 = \gamma_2(c)$ in the case of recovery) and here high values of c correspond to low specificity (see later for more details). The fundamental forms of host defence can be defined as follows (Boots et al., 2013): (i) avoidance reduces the probability of becoming infected and resistant hosts therefore have a lower transmission rate (β_1), (ii) recovery increases the rate of clearance of infection (γ_1) and (iii) acquired immunity increases the probability of recovering to a life-long immune state (ν_1). We first consider routes of innate resistance, i.e. avoidance and recovery (i and ii above) in an SIS setting, then in an SIR setting with specific life-long immunity and later evolution of acquired immunity itself.

(*b*) *Population Dynamics*

A key measure of the ability of a parasite to spread in a host population is R_0, the basic reproduction number, given here by

$$R_0^i = \beta_i X^0/(\alpha_i + b + \gamma_i) \tag{6}$$

for parasite i in the absence of the alternative parasite. In equation 6 X^0 represents the equilibrated density of susceptible hosts in the absence of any infection (i.e. the host carrying capacity, $X^0 = (a-b)/q$). A second key measure is endemic disease prevalence, the frequency of infected individuals in the equilibrium host population. In single infection models of this type, whether the population structure is SI, SIS, SIR (i.e. our model with $s=0$) or $SIRS$, prevalence at the endemic equilibrium satisfies,

$$\alpha\frac{Y}{H} = a - qH - b \tag{7}$$

i.e. prevalence scaled by virulence equals per capita host population turnover (i.e. density dependent net reproduction). However, when there are two infections in the population, as per the model represented by equations $1-5$, per capita turnover at equilibrium equals the sum of the prevalences of the two infections weighted by their respective rates of virulence,

$$\alpha_1\frac{Y_1}{H} + \alpha_2\frac{Y_2}{H} = a - qH - b \tag{8}$$

Therefore, as per single infection models, equilibrium infection in the host population is determined by the supply of susceptible individuals (i.e. turnover) but with the key difference that host turnover is shared amongst the multiple infections. One consequence of equation 8 is that coexistence of parasites means that equilibrium prevalence of any one parasite is always less than it would be if it were circulating in the host population alone. A condition for the

stable coexistence of parasites at the host population scale can be found in Nowak and May (1994) for a similar model.

(*c*) *Trade-off*

There is strong empirical evidence for the association of resistance with physiological costs through the diversion of resources to the development and maintenance of the resistance. For example, in Fuxa and Richter (1989) the percentage of eggs that hatch as well as the number produced per female were all lower in fall armyworm lines selected for resistance to NPV. Longer development time, reduction in egg viability as well as an increase in pupal weight were a consequence of selection for resistance to a granulosis virus in *Plodia interpunctella* (Boots and Begon, 1993). There is also evidence of reduced larval competitive ability in immune-selected *Drosophila melanogaster* (Kraaijeveld and Godfray, 1997). Taken together these studies represent a sound basis for assuming that costs to resistance can be manifested in reduced host reproduction or reduced competitive ability. In this study we assume an association between level of resistance and reproduction rate such that recovery, avoidance and acquired immunity are all positive decreasing functions of host reproduction rate. This is consistent with the majority of previous studies that examine the evolution of resistance to parasites (see Boots et al. (2009)).

(*d*) *Specificity of Immune Response*

We begin by considering an SIS framework where the focal parasite is less virulent than the co-circulating parasite (i.e. $\alpha_1 < \alpha_2$). Hosts invest in costly resistance, $0 \leq \theta(a) \leq 1$, through avoidance of the focal infection (i.e. $\hat{\beta}_1 = \beta_1(1-\theta(a))$) and resistance may carry over to the co-circulating infection depending on the specificity of resistance ($0 \leq c \leq 1$, when $c = 0$ the resistance is specific to the focal infection), i.e. $\hat{\beta}_2 = \beta_2(1-c\theta(a))$. As c increases the resistance becomes more general. Alternatively resistance can be through recovery (i.e. $\hat{\gamma}_1 = \gamma_1(1+\theta(a))$ and $\hat{\gamma}_2 = \gamma_2(1+c\theta(a))$). Similarly the focal infection can be more virulent than the co-circulating parasite for each of the above cases (i.e. $\alpha_1 > \alpha_2$). When it comes to an SIR framework we consider all of the above cases but, for brevity, only present results for

cases where the focal parasite is less virulent than the co-circulating parasite. Finally in an SIR framework resistance may be through acquired immunity, corresponding to $\hat{\nu}_1 = \theta(a)\nu$. For convenience we view specificity of acquired immunity not in terms of the probability of clearance of the co-circulating infection to an immune state, but rather as the decrease in transmission of the co-circulating infection to individuals who are immune to the focal infection. For this reason, specificity in acquired immunity is a fixed coefficient, σ, in equations 4-5 with $\sigma = 1$ when resistance is specific or $\sigma < 1$ when it is not specific. For simplicity, we do not allow the less intuitive case where σ exceeds 1 (i.e. resistance developed to counter a focal parasite is more effective against a co-circulating parasite). See table 1 for a summary of the cases studied.

3. Results

Using the next generation method (Diekmann et al., 1990; van den Driessche and Watmough, 2002; Hurford et al., 2010), see *supporting information S1*, we derive a proxy for invasion fitness, denoted $s_r(m)$, for the set of models outlined in the *methods* section for each of the cases detailed in table 1. Under the assumptions of adaptive dynamics (Metz et al., 1996; Geritz et al., 1998) a population will evolve through small, rare mutations in the direction of the gradient of the invasion fitness until an evolutionary singularity, where the mutant derivative of invasion fitness is zero, is reached (alternatively the evolving population may reach the limit of the phenotypic range). Evolutionary singularities can be classified according to their evolutionary and convergence stability properties (Metz et al., 1996). If a singularity is both evolutionary and convergence stable it is an uninvadable evolutionary attractor and an end point of evolution (Eshel, 1983). We wish to examine how the position of such singularities, which is determined by selection pressures, change when model parameters, in particular host lifespan, are varied. The results presented throughout are obtained using mathematical software for symbolic computation (Maple). They are additionally supported with simulations of the adaptive dynamics process whereby population dynamics of interacting resident and mutant host sub-populations are numerically solved with mutants, of similar effect to residents,

randomly introduced on a time scale slower than that of the ecological dynamics (for further detail on adaptive dynamics simulations see Donnelly et al. (2015)).

For *SIS* innate resistance we use the invasion fitness proxy, $s_r(m)$, for hosts bearing a mutant investment phenotype, $\theta^m(a^m)$, to locate evolutionary attractors and to show how the evolved level of the resistance phenotype varies with host lifespan, see figure $2a-d$. This can be shown when resistance is specific (black curves, figure $2a-d$) and also when resistance is non-specific (grey curves, figure $2a-d$). It can also be shown when resistance is through avoidance (figure $2a$ & b, i.e. cases $1-4$ table 1) and when resistance is through recovery (figure $2c$ & d, i.e. cases $5-8$ table 1), when the resistance is developed primarily to counter a relatively avirulent focal infection (figure $2a$ & c) or to counter a relatively virulent focal infection (figure $2b$ & d). The resulting graphs indicate that regardless of the route of innate resistance, investment increases with host lifespan except when it is specific to an avirulent infection.

Focusing on the case where resistance evolves to counter an avirulent focal infection we show that these results extend to an SIR framework, arising through the presence of acquired immunity specific to the avirulent focal infection (i.e. $\sigma=1$), see figure $3a$ & b for avoidance, i.e. cases $9-10$ table 1, and see figure $3c$ & d for recovery, i.e. cases $11-12$ in table 1. As the proportion of immune individuals in the population increases (due to changing the value of ν, i.e. the probability of inducing acquired immunity upon recovery, from $\nu=0$ represented by a black curve to $\nu=1$ represented by a light grey curve) there is no qualitative change, though the overall magnitude of investment tends to decrease (because recovery to immunity decreases prevalence, reducing the need for resistance). Finally, we analyse optimal acquired immunity developed to counter the less virulent parasite. Here, the mutant investment phenotype is $\nu_1^m(a^m)$ and immunity extends to the virulent infection if $\sigma<1$. When immunity is non-specific, investment increases with increasing lifespan, when immunity is specific investment decreases with increasing lifespan, see figure $4a$, i.e. cases $13-14$ in table 1.

As a whole, the results show that resistance to a relatively avirulent focal infection in the presence of a co-circulating virulent infection varies with host lifespan in a manner that is dependent on the specificity, but significantly, is not dependent on the route of resistance.

In general, investment increases as the level of specificity in resistance decreases. We provide a further illustration of this in figure $4b - d$ where curves are given for different lifespans for evolving avoidance, $4b$, recovery, $4c$ and acquired immunity, $4d$, respectively. Investment is greater at low lifespans when resistance is specific (c is low) but investment is greater at long lifespans when resistance is relatively general (c is high). Therefore, there is a level of specificity for each form of resistance below which investment decreases with increasing host lifespan and above which investment increases with increasing host lifespan. This transition occurs for relatively small values of specificity for the innate forms of resistance (i.e. avoidance and recovery) compared to the relatively high value of specificity at which it occurs for acquired immunity.

4. Discussion

Hosts are typically challenged by multiple parasites, but to date theory on the evolution of resistance has mostly focused on single infections. We have developed a series of models that have examined the impact of multiple parasites on the evolution of resistance with explicit feedbacks between the ecological and evolutionary dynamics. Our key assumption is that parasites coexist as a consequence of superinfection which assumes that a more virulent parasite can replace a less virulent parasite within an individual host. Our results show that co-circulating parasitism dramatically impacts on the evolution of resistance to a focal parasite. In particular, the specificity of the resistance with respect to co-circulating parasites is critical to the outcome. A key, intuitive, result is that investment in resistance increases as the immune response becomes more general. This finding is related to those of previous studies that considered the impact of multiple enemies on resistance evolution in the absence of ecological dynamics. Jokela et al. (2000) considered the evolution of resistance for different levels of parasite diversity. They showed that specific host resistance is less effective when faced with a diverse range of parasites and therefore that host resistance increases as parasite diversity decreases. Poitrineau et al. (2003) examined the evolution of defence to two separate enemies and considered scenarios of synergy or interference in defence response, showing that investment increases as the level of synergy increases. Our finding that resistance increases as immune investment becomes more general is related to these results, and extends them to systems including explicit feedbacks between the ecological and evolutionary dynamics.

Risk of infection by pathogens and parasites has led hosts to evolve a wide range of defence mechanisms from behavioural strategies (Joop et al., 2014) to the bio-chemical cascades of the complement system and the memory B and T cells of acquired vertebrate immunity (Schmid-Hempel, 2002; Frank, 2002). Intuition suggests that the longer a host lives the more it is likely to benefit from immunity. This observation has been used to explain macro-evolutionary patterns of investment such as the lack of acquired immunity in invertebrates (Ricklefs and Wikelski, 2002; Tieleman et al., 2005) and is supported by a number of empirical studies. For example, a positive correlation between immunity and lifespan in avian hosts has been

demonstrated for humoral, cell mediated, and constitutive immune responses (Ardia, 2005; Tella et al., 2002; Versteegh et al., 2012; Lee et al., 2008). Theoretical models that have examined the evolution of resistance in the face of a single parasite make the assumptions underlying this intuition explicit. They have provided some support for this pattern but also deviate from it in important ways (van Boven and Weissing, 2004; Miller et al., 2007; Boots et al., 2013; Donnelly et al., 2015). For example, in contradiction to the intuition, optimal resistance in hosts capable of permanent acquired immunity can be maximal at intermediate lifespan (Boots et al., 2013; Donnelly et al., 2015) and in the case of innate resistance this can be true even in the absence of acquired immunity (Miller et al., 2007; Donnelly et al., 2015). However, a key aspect of these studies is that host populations are burdened by only one infection. Here we address the key question of how optimal investment changes with lifespan in the face of co-circulating parasites.

When a host population is challenged by multiple parasites the investment in immunity is critically dependent on the specificity of the defence. When the resistance is relatively general, then investment increases with host lifespan. In contrast, when immunity is specific the pattern of investment relative to host lifespan depends on the nature of the co-circulating parasite. If the co-circulating parasite is less aggressive in exploiting the host than the focal infection, then investment increases with lifespan. However, if the co-circulating parasite is more aggressive, then specific immune investment decreases as host lifespan increases because the ratio of infected individuals with the co-circulating parasite to individuals with the focal parasite increases (since there is a higher incidence of superinfection at high host lifespans). These patterns are true in our model when the evolving resistance is innate in a host incapable of immune memory, is innate in a host responding additionally with immune memory or when the evolving resistance is itself acquired. This is an important insight since it shows that the life-history patterns will depend on the nature of the co-circulating parasite, and the specificity of the response, but not the mode of resistance itself, which is in stark contrast to single infection models where patterns fundamentally depend on the type of resistance (i.e. innate vs acquired) but not the exact mode (for example avoidance vs recovery within the innate type) (van Boven and Weissing, 2004; Miller et al., 2007; Boots et al., 2013; Donnelly

et al., 2015). Therefore, a key implication of our work is that, in contrast to single infection models, the classic idea that more investment should occur in longer-lived hosts is generally supported when there are multiple parasites.

What are the underlying processes that lead to these different findings (i.e. in the effect of host lifespan on optimal immune defence) when host are faced by multiple rather than single parasites? Single infection models deviate from the intuition that investment increases with lifespan because of two important effects that are undermined by the presence of co-circulating infections (see detailed analysis in Donnelly et al. (2015)). In single infection models, optimal investment that is maximal at intermediate lifespans (Miller et al., 2007) is a hallmark of innate resistance because it is characterised by the return or maintenance of individuals to a susceptible state as opposed to the conversion of them into an immune state (Donnelly et al., 2015). Since susceptible individuals are vulnerable to reinfection which is likely at high levels of prevalence, the benefit of innate resistance is low at high prevalence and therefore low at high lifespans (in SIS systems prevalence increases with increasing host lifespan). With multiple parasites and superinfection, more virulent parasites take over hosts infected with less virulent parasites. When hosts live longer, the period during which these conversions occur is longer and this favours the virulent parasite. However, the higher virulence of these parasites also acts to reduce the infectious period and as a consequence, prevalence does not rise to the high levels that are seen in equivalent single infection models. As such, optimal investment increases with lifespan in the face of multiple infections and superinfection in models where it would be maximal at intermediate lifespans without the co-circulating infection because the prevalence of the focal parasite is strongly limited due to the share of susceptible hosts taken by the co-circulating parasite, see equation 8.

There is a second process that comes into play once there is permanent immunity to the parasite. In single infection models where the host is long-lived, permanent immunity leads to high host density. When host density approaches the carrying capacity there is little host turnover and prevalence levels are low (see equation 7). Therefore long-lived host populations with permanent immunity have a relatively small risk of infection and will evolve weaker resistance (Miller et al., 2007; Boots et al., 2013; Donnelly et al., 2015). For this reason a

long-lived immune class can decrease the need for immunity in general at high lifespans. However, crucially, when there are multiple infections the impact of an especially long-living class arising from recovery to a permanent immune state will be substantially less because the immune individuals will be susceptible (at least to some degree) to infection by co-circulating parasites. Therefore, when acquired immunity evolves in the face of multiple parasites and superinfection, just as for innate immunity, optimal investment is higher in long-lived host populations in models where it would be maximal at intermediate lifespans without the co-circulating infection.

There is one important exception to our general prediction that investment in immunity rises with host lifespan. When the co-circulating parasite is more virulent and the evolving response is specific to the less virulent focal parasite, then investment decreases with increasing lifespan. Two simple interactions are responsible for this result: *1)* if the co-circulating parasite is more virulent then it is the superinfector and it is favoured at high lifespans. Therefore the benefit of specific resistance to the focal parasite, which by definition is not effective against the co-circulating parasite, diminishes as lifespan increases. *2)* Responding through resistance to the less virulent focal parasite can actually increase the risk of infection with the more virulent co-circulating parasite (since there is an increase in the availability of susceptible individuals for the co-circulating infection). Therefore, taken together, there is little fitness benefit to investing resources into fighting the lesser of your enemies and specific resistance to the less aggressive parasite is not favoured at high host lifespans under an assumption of superinfection. We note that several of these interactions are a consequence of the interplay between strain prevalence, their relative virulence and virulence associated superinfection. For this reason it is important to acknowledge that alternative mechanisms of coexistence may lead to different results.

In conclusion, there are multiple factors that determine the relationship between optimal investment in immunity and host lifespan. This results in a variety of patterns for single infection models (Miller et al., 2007; Boots et al., 2013; Donnelly et al., 2015; van Boven and Weissing, 2004) but here we have shown that this intricacy can be lost when diversity in the

parasite burden of the host population is considered. Instead it is the classic idea that long-lived hosts invest more in immunity that is generally supported when this key aspect of natural populations is included. Our main focus has been on how multiple parasites impact on the relationship of host lifespan to resistance, but more generally our inclusion of realistic ecological feedbacks in evolutionary models of resistance extends results of previous multi-enemy models that assumed constant rather than dynamic populations (Poitrineau et al. (2003); Jokela et al. (2000)). Future work should relax the assumption that superinfection occurs and may therefore involve different population feedbacks whose effects should be assessed. Such co-infection models would be more challenging theoretically, but the importance of including ecological feedbacks is emphasized by our work. Furthermore, there is a need for a variety of defence interactions against a range of enemies beyond resistance to two parasites to be examined in this broader eco-evolutionary context.

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Case	Resistance type under evolution	Infection hierarchy	Specificity	Figure
1	SIS avoidance ($\beta_1(a), \nu = 0$)	acute co-circulating ($\alpha_1 < \alpha_2$)	specific, $c = 0$	see figure 2*a*
2	SIS avoidance ($\beta_1(a), \nu = 0$)	acute co-circulating ($\alpha_1 < \alpha_2$)	general, $c > 0$	see figure 2*a*
3	SIS avoidance ($\beta_1(a), \nu = 0$)	acute focal ($\alpha_1 > \alpha_2$)	specific, $c = 0$	see figure 2*b*
4	SIS avoidance ($\beta_1(a), \nu = 0$)	acute focal ($\alpha_1 > \alpha_2$)	general, $c > 0$	see figure 2*b*
5	SIS recovery ($\gamma_1(a), \nu = 0$)	acute co-circulating ($\alpha_1 < \alpha_2$)	specific, $c = 0$	see figure 2*c*
6	SIS recovery ($\gamma_1(a), \nu = 0$)	acute co-circulating ($\alpha_1 < \alpha_2$)	general, $c > 0$	see figure 2*c*
7	SIS recovery ($\gamma_1(a), \nu = 0$)	acute focal ($\alpha_1 > \alpha_2$)	specific, $c = 0$	see figure 2*d*
8	SIS recovery ($\gamma_1(a), \nu = 0$)	acute focal ($\alpha_1 > \alpha_2$)	general, $c > 0$	see figure 2*d*
9	SIR avoidance ($\beta_1(a), \nu > 0$)	acute co-circulating ($\alpha_1 < \alpha_2$)	general, $c > 0$	see figure 3*a*
10	SIR avoidance ($\beta_1(a), \nu > 0$)	acute co-circulating ($\alpha_1 < \alpha_2$)	specific, $c = 0$	see figure 3*b*
11	SIR recovery ($\gamma_1(a), \nu > 0$)	acute co-circulating ($\alpha_1 < \alpha_2$)	general, $c > 0$	see figure 3*c*
12	SIR recovery ($\gamma_1(a), \nu > 0$)	acute co-circulating ($\alpha_1 < \alpha_2$)	specific, $c = 0$	see figure 3*d*
13	SIR acquired immunity ($\nu_1(a)$)	acute co-circulating ($\alpha_1 < \alpha_2$)	general, $\sigma < 1$	see figure 4*a*
14	SIR acquired immunity ($\nu_1(a)$)	acute co-circulating ($\alpha_1 < \alpha_2$)	specific, $\sigma = 1$	see figure 4*a*

Table 1. Table of evolving resistance scenarios detailing the infection framework and type of resistance to the focal parasite that can evolve.

1

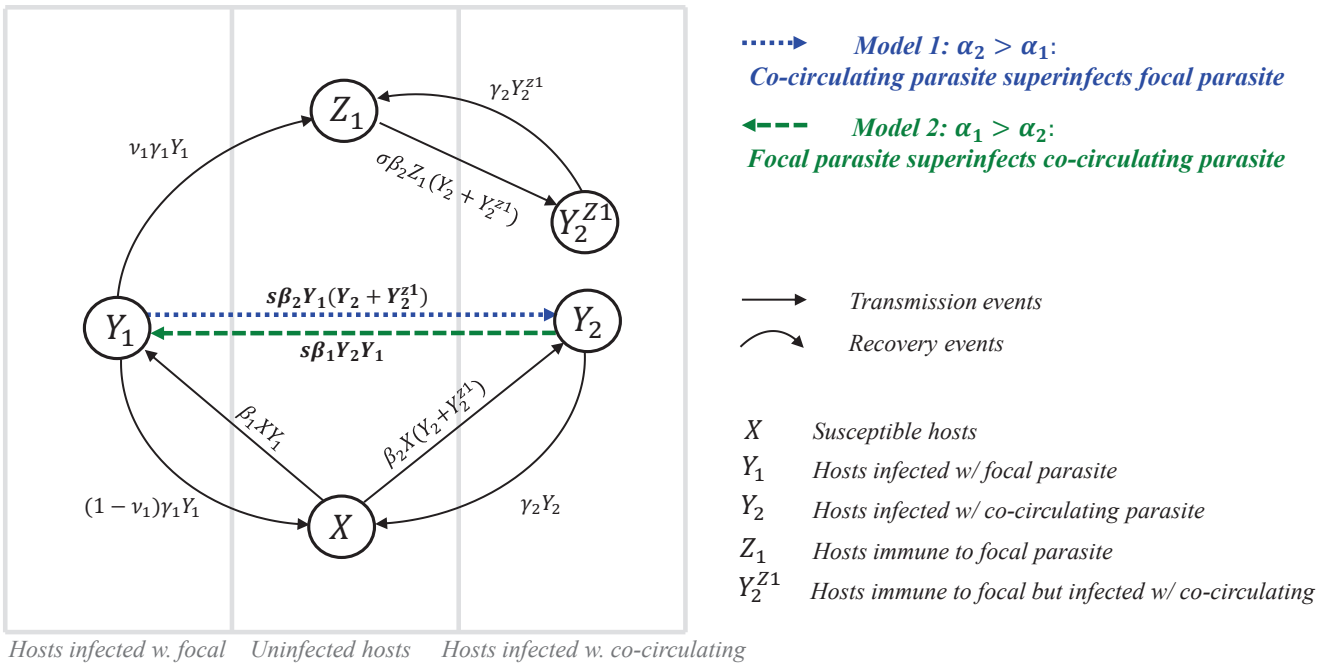

Figure 1. Flow chart showing epidemiological transitions for a situation where a host can recover to immunity against a focal parasite but where there is in addition a second parsite co-circulating in the host population (for simplicity there is no immunity to the co-circulating parasite). Parasite coexistence in the host population (and not within individual hosts) is facilitated by virulence associated superinfection. In *model 1* the co-circulating parasite (represented by the density of hosts infected with that parasite, Y_2) is more virulent than the focal parasite (represented by Y_1) and therefore individuals move from the focal infection class Y_1 to the co-circulating infection class Y_2 when the co-circulating infection is transmitted to an individual infected with the focal infection. In *model 2* the focal parasite is more virulent than the co-circulating parasite and therefore individuals move from the co-circulating infected class Y_2 to the focal infected class Y_1 when the focal infection is transmitted to an individual already infected with the co-circulating infection. Birth and death of hosts also occur but are omitted here for simplicity.

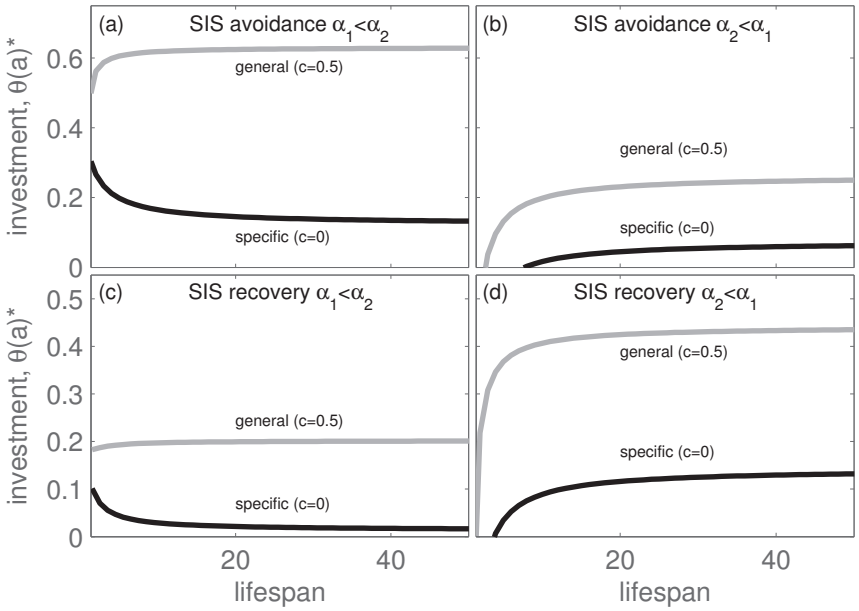

Figure 2. Optimal investment in specific and non-specific resistance in an SIS structured host population. In (a) and (b) the function of the resistance is avoidance. In (c) and (d) the function of the resistance is recovery (i.e. increased rate of disease clearance). In (a) and (c) resistance is evolved to counter the relatively avirulent infection while in (b) and (d) resistance is evolved to counter the relatively virulent infection. In all cases both infections will be equally countered when resistance is completely general ($c = 1$). Parameters were: $q = 0.1$ $\beta_1 = 2$ $\beta_2 = 4$ $\alpha_1 = 2$ $\alpha_2 = 8$ with $s = 0.45$, in the case of evolving avoidance $\hat{\beta}_i = \beta_i(1 - 0.5\theta)$ with $\gamma_1 = \gamma_2 = 0.35$ and in the case of evolving recovery $\hat{\gamma}_i = \gamma_i(1 + 2.5\theta)$ with $\beta_1 = \beta_2 = 1$. In all cases investment in resistance relates to reproduction according to $\theta(a) = 1 - (a^{\mu})/(a^{\mu}_{max})$ with $a_{max} = 1.9$ and $\mu = 12$.

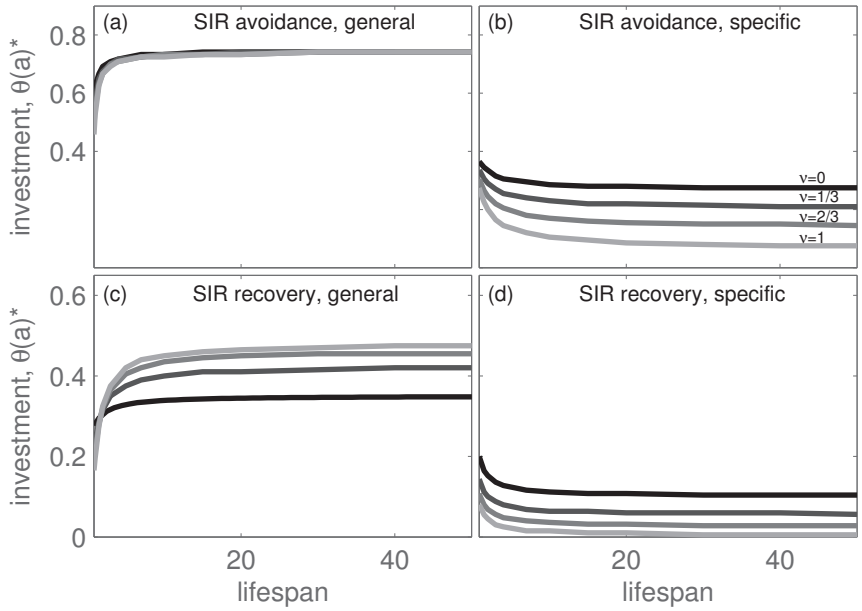

Figure 3. Optimal investment in specific and non-specific resistance in an SIR structured population developed to counter the relatively avirulent infection. In (a) and (b) resistance is through avoidance while in (c) and (d) resistance is through increased recovery. The proportion of recovered individuals entering the immune class is ν while the proportion returning to a susceptible state is $1-\nu$. As ν increases above 0 towards 1 the population becomes SIR (dark grey through to light grey curves). In (a) and (c) $c=0.5$ while in (b) and (c) $c=0$. In (a) and (b) the trade-off exponent is $\mu=18$, in (c) and (d) $\mu=24$. Note, $\alpha_1<\alpha_2$ and $\sigma=1$ throughout, for other parameter values see caption of figure 2.

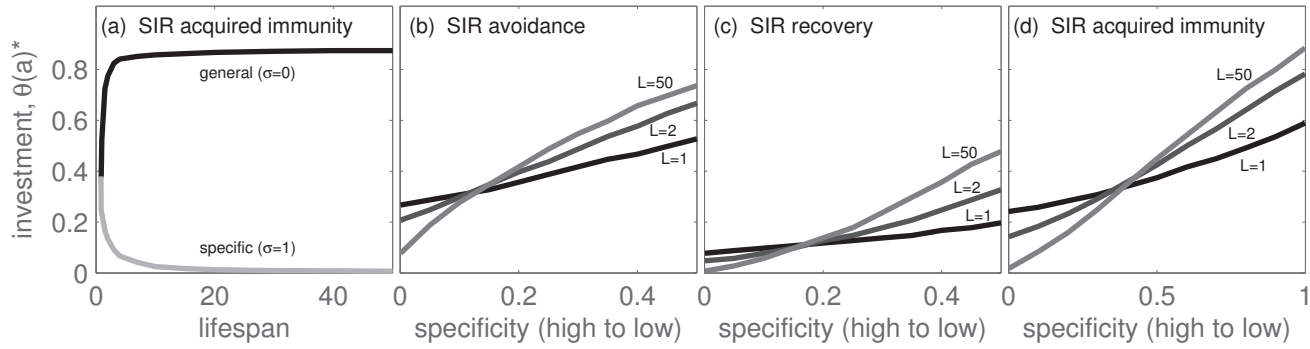

Figure 4. Optimal investment in specific (grey curve) and non-specific (black curve) acquired immunity developed to counter the relatively avirulent infection is given in (a). In (b), (c) and (d) optimal investment for a range of values of specificity is given for avoidance, recovery and acquired immunity respectively in an SIR structured population. In each case three separate curves are displayed for the following values of host lifespan, $1/b = 1$ (black curve), $1/b = 2$ (dark grey curve) and $1/b = 50$ (grey curve). (b), (c) and (d) indicate that there is a critical value of specificity below which, where resistance is general, high lifespans are associated with higher investment than low lifespans. On the other hand, beyond this critical value, where resistance is specific to the relatively avirulent infection, low lifespans are associated with higher investment than high lifespans. In (a), (c) and (d) the trade-off exponent is $\mu = 24$ and in (b) $\mu = 18$. In (b) and (c) $\sigma = 1$ and $\nu = 1$. Note, $\alpha_1 < \alpha_2$ and for other parameter values see caption of figure 2.

Supporting Information S1 Next Generation Matrix

Invasion fitness (Metz et al., 1996; Geritz et al., 1998) can be derived through a linear stability analysis of a mutant ecological model in a population consisting of residents at their population attractor (usually a stable point equilibrium). If the steady state corresponding to no mutants but positive residents is unstable then the mutant can invade. Hence, eigenvalues (of the coefficient matrix, A, of the linearised system, $\dot{x} = Ax$) determine the invasion potential of the mutant and in particular the dominant eigenvalue is a measure of invasion fitness. When a mutant host invades a resident population that is challenged by multiple infections, high dimensionality prevents direct derivation of invasion fitness. Instead, following the next generation method (Diekmann et al., 1990), the linearised system can be decomposed into two matrices, $A = F - V$. If the largest absolute value of the eigenvalues of the matrix FV^{-1} is greater (smaller) than 1, then by the next generation theorem (van den Driessche and Watmough, 2002; Hurford et al., 2010) the invasion fitness is positive (negative), but note that conditions on the matrices F and V apply, see van den Driessche and Watmough (2002).

For general resistance as described in the main text, i.e. allowing for the possibility of evolving avoidance $(\beta_1(a^m), \beta_2(a^m))$, or evolving recovery $(\gamma_1(a^m), \gamma_2(a^m))$ or evolving acquired immunity $(\nu(a^m))$, the corresponding birth and death matrices are:

$$\mathrm{F}= \begin{bmatrix} a^m - qH^r & a^m - qH^r & a^m - qH^r & a^m - qH^r & a^m - qH^r \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix},$$

$$\mathrm{V}= \begin{bmatrix} \beta_1(a^m)Y_1^r + \beta_2(a^m)(Y_2^r + Y_{21}^r) + b & -(1-\nu_1(a^m))\gamma_1(a^m) & -\gamma_2(a^m) & 0 & 0 \\ -\beta_1(a^m)Y_1^r & \alpha_1 + b + \gamma_1(a^m) + s\beta_2(a^m)(Y_2^r + Y_{21}^r) & 0 & 0 & 0 \\ -\beta_2(a^m)(Y_2^r + Y_{21}^r) & -s\beta_2(a^m)(Y_2^r + Y_{21}^r) & \alpha_2 + b + \gamma_2(a^m) & 0 & 0 \\ 0 & -\nu_1(a^m)\gamma_1(a^m) & 0 & b + \sigma\beta_2(a^m)(Y_2^r + Y_{21}^r) & -\gamma_2(a^m) \\ 0 & 0 & 0 & -\sigma\beta_2(a^m)(Y_2^r + Y_{21}^r) & \alpha_2 + b + \gamma_2(a^m) \end{bmatrix}$$

2

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