Co-evolution of virulence and immunosuppression through multiple infections

Tsukushi Kamiya [1*], Nicole Mideo [1,2], Samuel Alizon [3,4]

[1] Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, ON M5S 3B2, Canada

[*] Corresponding author email: tsukushi.kamiya@mail.utoronto.ca

[2] Email: nicole.mideo@utoronto.ca

[3] MIVEGEC, CNRS, IRD, Université de Montpellier, France

[4] Email: samuel.alizon@cnrs.fr

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Abstract

Many components of the host-parasite interaction have been shown to affect the way virulence, that is parasite induced harm to the host, evolves. However, co-evolution of multiple traits is often neglected. We explore how an immunosuppressive mechanism of parasites affects and co-evolves with virulence through multiple infections. Applying the adaptive dynamics framework to epidemiological models with co-infection, we show that immunosuppression elevates the evolutionarily stable (ES) virulence through epidemiological feedbacks. We explore the co-evolution of the two parasite traits across different extrinsic mortality conditions, and find that the peak ES virulence occurs at an intermediate level of background host mortality when immunosuppression is considered. The highest co-ES virulence is achieved at the intermediate level of background mortality which we interpret by considering the abundances of each host types. In addition, we find that immunosuppression evolution is influenced considerably by the precise shape of the trade-offs determining the cost and benefit of immunosuppression. These results demonstrate that the ES virulence is shaped by immunosuppression, while highlighting that the evolution of immune evasion mechanisms deserves further research attention.

Introduction

The fundamental question of virulence evolution, that is, 'Why do some parasite strains harm their hosts more than others?' has been a central focus of evolutionary epidemiology for both its conceptual and applied significance (Ewald, 1994, Read, 1994, Schmid-Hempel, 2011, Méthot, 2012, Alizon and Michalakis, 2015). The adaptive explanation of virulence is typically centred around the understanding of trade-offs involving virulence and other parasite fitness components such as transmission and competitiveness in multiple infections (Anderson and May, 1982, Ewald, 1983, Alizon et al., 2009, 2013). While these trade-off theories explain the evolution of finite non-zero optimal virulence, exactly how much virulence a parasite should evolve depends on a variety of processes (Cressler et al., 2016). For example, host traits (e.g. host immune responses) and their interactions with co-evolving parasite adaptations (e.g. parasite immune evasion strategies; Frank and Schmid-Hempel, 2008, Alizon, 2008b, Cressler et al., 2016) are likely to influence the trade-offs. The present theoretical study explores how a parasite immunosuppression strategy, namely the ability of parasites to hinder host recovery, co-evolves with virulence.

The ability of parasites to suppress host immunity is ubiquitous in nature (Schmid-Hempel, 2009) and frequently help explain chronic infections (Virgin et al., 2009). In humans for instance, infections by human papillomaviruses (HPVs) and human immunodeficiency virus (HIV) offer two contrasting immune suppression strategies: while the former interferes with the cellular machinery to reduce the presentation of viral antigens or impede the interferon response (Doorbar et al., 2012), the latter infects and lyses T lymphocytes (Levy, 1998). Regardless of the specific mechanism involved, however, the adaptive benefit for the parasite is realised through prolonged infection duration (Schmid-Hempel, 2009). For the scope of our study, we generalise any parasite adaptation against host immunity that results in lowered host recovery rate as immunosuppression.

In the absence of constraints, it is in the parasite's best interest to evolve maximal immunosup-

pression, when immunity serves only to kill parasites. However, lowered host immunity is likely to impose at least one cost to the parasite: an immunocompromised host may be more vulnerable to further infection by conspecific and heterospecific parasites. A meta-analysis by Graham (2008) shows that lowered immune responses, due to the presence of an immunosuppressive helminth, increase microparasite population density within hosts. Furthermore, experimental evidence suggests that immunosuppression could lead to increased host mortality through additional infections by opportunistic parasites (Cornet and Sorci, 2010). Therefore, multiple infections — which are so prevalent that they could be argued to be the rule rather than the exception (Petney and Andrews, 1998, Cox, 2001, Read and Taylor, 2001, Juliano et al., 2010, Balmer and Tanner, 2011) — are likely a main driver of the co-evolution between virulence and immunosuppression.

If immunosuppression leads to more multiple infections, one might predict that this should lead to increased virulence. Many theoretical, and some empirical, studies support the notion that within-host competition leads to the evolution of higher virulence (reviewed in Mideo, 2009). Similarly, at the epidemiological level, as the density of co-infected hosts increases, so does the optimal level of virulence (van Baalen and Sabelis, 1995, Choisy and de Roode, 2010). However, given that the benefit of immunosuppression is assumed to be a longer duration of infection, increasing virulence would counteract this effect. Therefore, without a formal model, intuition fails to predict the direction in which virulence evolves when immunosuppression is considered.

To elucidate the evolutionary outcome of the co-evolution of virulence and immunosuppression, we develop mathematical epidemiology models, in which we assume that the two infection traits are carried by the same parasite species (as in in van Baalen and Sabelis, 1995). Furthermore, we also investigate how the co-evolved optimal strategy is affected by the rate of host background mortality, a key epidemiological parameter that has been shown to alter evolutionary predictions (Sasaki and Iwasa, 1991, Day and Proulx, 2004, Cressler et al., 2016).

The model

We use an evolutionary epidemiology approach based on adaptive dynamics theory (Geritz et al., 1998, Dieckmann et al., 2002, Otto and Day, 2007). We first present the epidemiological model itself, then the evolutionary trade-offs that constrain evolution and finally we show how the (co-)evolutionary analyses are conducted.

Epidemiological dynamics

We employ a co-infection framework, which allows for coexistence of two parasite strains within a host (van Baalen and Sabelis, 1995). In this model, hosts are divided into three classes: susceptible, singly infected and doubly infected, occurring at densities S, I and D respectively. Following the notation of Table 1, we derive the following system of ordinary differential equations (ODEs) to describe the changes of the resident system over continuous time:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \rho - \mu\, S - \lambda_r\, S + \gamma\, I_r \tag{1a}$$

$$\frac{\mathrm{d}I_r}{\mathrm{d}t} = \lambda_r\, S - (\mu + \alpha)\, I_r - \sigma\, \lambda_r\, I_r - \gamma\, I_r + 2\,\gamma\, D_{rr} \tag{1b}$$

$$\frac{\mathrm{d}D_{rr}}{\mathrm{d}t} = \sigma\, \lambda_r\, I_r - (\mu + \alpha)\, D_{rr} - 2\,\gamma\, D_{rr} \tag{1c}$$

where the subscript r denotes the resident parasite strain. In this formulation, there is a constant input of susceptible hosts into the population at the rate ρ. Susceptible hosts exit the system through background mortality at the rate μ while infected hosts, both singly and doubly infected individuals, experience additional mortality caused by parasites (i.e., virulence α). Susceptible and singly infected hosts acquire infection according to the force of infection $\lambda_r = \beta I_r + \beta D_{rr}$, where β corresponds to the parasite transmission rate. The host class for double infection by the same strain, D_{rr} is included in the system for a technical motivation: it is necessary for an unbiased invasion analysis because the mutant strain would gain a frequency-dependent advantage in its absence (discussed in Alizon, 2008a,

Lipsitch et al., 2009). Within the existing epidemiological framework, the effect of host immunity can be implicitly accounted for as the rate of recovery (equivalent to the rate of parasite clearance). We assume that hosts recover from infection at a rate γ, in a stepwise fashion, i.e., doubly infected hosts (D) only lose one infection at a time). The key feature of our model is that we assume that singly infected hosts (I) suffer an increased risk of contracting a further infection at a rate proportional to a coefficient σ. We treat the host class D_{rr} similarly to singly infected hosts I_r, except for the fact that the doubly infected hosts cannot be infected any further. The resident equilibrium can be computed analytically.

Within-host processes and resulting trade-offs

When co-infection competitive advantage is linked to the extent of resource exploitation — which itself correlates with virulence — adaptive evolution of virulence is expected independently of the classic trade-off between virulence and transmission (van Baalen and Sabelis, 1995, Choisy and de Roode, 2010). Here, we assume that virulence (α) increases linearly with the level of resource exploitation by a parasite (x), such that $\alpha(x) = a\,x$, where a is a proportionality constant (we explore a transmission-virulence trade-off in the Supplementary Information 3). We then assume that finding themselves in a doubly infected host is inherently costly for parasites due to exploitation competition between co-infecting strains (Mideo, 2009, Schmid-Hempel, 2011), and that more virulence strains are more competitive in multiple infections (de Roode et al., 2005, Bell et al., 2006, Ben-Ami et al., 2008, Zwart et al., 2009):

$$\beta_{rm}(x_r, x_m) = \left(\frac{x_r}{x_r + x_m}\right)\beta \tag{2a}$$

$$\beta_{mr}(x_r, x_m) = \left(\frac{x_m}{x_r + x_m}\right)\beta. \tag{2b}$$

There is ample empirical evidence that immunosuppression benefits the parasites by prolonging

infections (reviewed in Schmid-Hempel, 2008), and lowered host immunity would increase the susceptibility to multiple infections (Palefsky and Holly, 2003, Rockstroh and Spengler, 2004, Cornet and Sorci, 2010). Thus, the key trade-off in our model is between infection duration and susceptibility to co-infections (both being mediated by immunosuppression). We, therefore, assume a trade-off between the rate of recovery, γ, and additional susceptibility of infected hosts to co-infection, σ, by making them both functions of immunosuppression intensity, θ. It is conceivable for the decline of recovery rate and the increase of additional susceptibility to either accelerate or decelerate with increasing immunosuppresion. Because the trade-off shape typically matters for evolutionary dynamics (Bowers et al., 2005, Kisdi, 2006) and little is known from empirical data, we explore the trade-offs involving recovery and susceptibility as both accelerating and decelerating functions of immunosuppression. The parameters δ_γ and δ_σ control the degree of concavity of the effect of immunosuppression on recovery and increased susceptibility, respectively (eq. 3; Fig. S1).

$$\gamma(\theta) = \gamma_{\max} \begin{cases} \left(1 - \frac{\theta}{\theta_{\max}}\right)^{\delta_\gamma}, & \text{if accelerating} \\ 1 - \left(\frac{\theta}{\theta_{\max}}\right)^{\delta_\gamma}, & \text{if decelerating} \end{cases} \tag{3a}$$

$$\sigma(\theta) = 1 + \sigma_{\text{range}} \begin{cases} 1 - \left(1 - \frac{\theta}{\theta_{\max}}\right)^{\delta_\sigma}, & \text{if accelerating} \\ \left(\frac{\theta}{\theta_{\max}}\right)^{\delta_\sigma}, & \text{if decelerating} \end{cases} \tag{3b}$$

With these functions, we assume that the realised recovery rate, $\gamma(\theta)$, decreases as a function of immunosuppression such that it equals the intensity of host immunity, $\gamma_{\max}$, in the absence of immunosuppression and approaches 0 as immunosuppression approaches $\theta_{\max}$. We also assume that the proportional gain in susceptibility to a further infection, $\sigma(\theta)$, elevates the force of infection experienced by an immunosuppressed singly infected host by up to $1 + \sigma_{\text{range}}$ fold at the upper limit of immunosuppression (when $\theta = \theta_{\max}$).

Table 1: **Parameter notation, description and default values.** Parameter values chosen to sustain non-zero and non-complex equilibria for the resident system and relevant evolutionarily singular strategies. Parameters that are functions of others, are indicated with the dependent parameters (or variables) inside parentheses. When we allow only immunosuppression to evolve virulence, α, is a constant; otherwise, α evolves as a function of a and x.

Symbol	Description	Value (or range)
ρ	Susceptible host birth rate	100
μ	Background mortality rate	$[0.001, 0.1]$
β	Transmission rate	0.001
λ	Force of infection	$\lambda(\beta, I, D)$
α	Virulence: parasite-induced mortality	$[0, 0.5]$ or $\alpha(a, x)$
γ	Realised recovery rate	$\gamma(\theta)$
σ	Increased susceptibility of infected hosts	$\sigma(\theta)$
θ	Immunosuppression	$[0, 100]$
θ_{max}	Maximum immunosuppression	100
γ_{max}	Maximum host recovery rate	0.5
$1 + \sigma_{\text{range}}$	Maximum susceptibility coefficient	$[1, 5]$
$\{\delta_\gamma, \delta_\sigma\}$	Recovery-co-infection susceptibility trade-off curve shape	$\{0.1, 0.5\}$
a	Virulence scaling parameter	0.1
x	Resource exploitation rate	$[0.001, 5]$

Evolutionary analyses

The mutant systems

We carry out an invasion analysis investigating perturbation of the resident state by adding a rare mutant strain, the densities and traits of which are denoted with subscript m. For the evolution of immunosuppression, the dynamics of the mutant strain are summarised in the following system of ODEs:

$$\frac{\mathrm{d}I_m}{\mathrm{d}t} = \lambda_m\, S - (\mu+\alpha)\, I_m - \sigma(\theta_m)\, \lambda_r\, I_m - \gamma(\theta_m)\, I_m + \gamma(\theta_{rm})\, D_{rm} \tag{4a}$$

$$\frac{\mathrm{d}D_{rm}}{\mathrm{d}t} = \sigma(\theta_r)\, \lambda_m\, I_r + \sigma(\theta_m)\, \lambda_r\, I_m - (\mu+\alpha)\, D_{rm} - 2\,\gamma(\theta_{rm})\, D_{rm} \tag{4b}$$

where $\lambda_r = \beta I_r + \beta D_{rr} + \beta_{rm} D_{rm}$ and $\lambda_m = \beta I_m + \beta_{mr} D_{rm}$. For simplicity we assume that the order of infection does not matter so that D_{rm} is identical to D_{mr}. We neglect hosts infected twice by the mutant strain (which would be D_{mm}) because it is unlikely that the same host gets infected twice by a rare mutant. Recovery from D_{rm} can be achieved through either clearing a resident or a mutant parasite. Other aspects of demographic changes of the mutant system are identical to the resident system described above.

We assume that the level of immunosuppression in co-infection is the average between the resident and mutant strain, i.e. $\theta_{rm} = \frac{\theta_r+\theta_m}{2}$. For virulence evolution, we assume that the only within-host interaction between co-infecting parasites is competition for the shared host resources. Therefore, we also calculate the overall virulence of co-infection as the average of the two strains, i.e. $\alpha_{rm} = \frac{\alpha_r+\alpha_m}{2}$.

The mutant dynamics for virulence evolution are governed by

$$\frac{dI_m}{dt} = \lambda_m S - (\mu+\alpha(x_m))I_m - \lambda_r\sigma(\theta)I_m - \gamma(\theta)I_m + \gamma(\theta)D_{rm} \tag{5}$$

$$\frac{dD_{rm}}{dt} = \lambda_m\sigma(\theta)I_r + \lambda_r\sigma(\theta)I_m - (\mu+\alpha_{rm})D_{rm} - 2\gamma(\theta)D_{rm} \tag{6}$$

where λ_r and λ_m are the force of infection for the resident and mutant, respectively, defined here as $\beta I_r + \beta D_{rr} + \beta_{rm} D_{rm}$ and $\beta I_m + \beta_{mr} D_{rm}$. We again assume the trade-offs between recovery and co-infection susceptibility as functions of immunosuppression in this model.

Adaptive dynamics

The fate of a rare mutant strain is determined by its fitness function (here denoted R), that is, the ability to spread through a host population already infected with a resident parasite (Geritz et al., 1998, Dieckmann et al., 2002). In the continuous time scale, the mutant parasite invades and replaces the resident if R, calculated as the dominant eigenvalue of the Jacobian matrix of the mutant system, is positive (Otto and Day, 2007). Consequently, an evolutionarily singular strategy can be found where the change of R ceases with respect to the evolving trait. For example, an evolutionarily singular strategy of immunosuppression (denoted θ^*) can be found when θ^* is an extremum of R:

$$\left.\frac{\partial R}{\partial \theta_m}\right|_{\theta_m=\theta_r=\theta^*} = 0. \tag{7}$$

The properties of a singular strategy can then be assessed by the second derivatives of R. Following the notations used by Geritz et al. (1998), here we denote the second derivatives of R with respect to the resident and mutant strain with a and b:

$$a = \left.\frac{\partial^2 R}{\partial \theta_r^2}\right|_{\theta_m=\theta_r=\theta^*}, \; b = \left.\frac{\partial^2 R}{\partial \theta_m^2}\right|_{\theta_m=\theta_r=\theta^*} \tag{8}$$

The convergence stable ES (i.e. evolutionarily stable and convergent stable; the continuously stable strategy, CSS *sensu* Eshel (1983)) condition is satisfied when $b < 0$ and $a - b > 0$. The first condition states that R is at a local maximum and hence convergent stable and the second condition implies no mutant invasion is possible at the point, meaning evolutionarily stable (Geritz et al., 1998). Various other possible configurations of evolutionarily and convergence stability are discussed in

Geritz et al. (1998).

Co-evolution of virulence and immunosuppression

We graphically identified the convergence stable, co-evolutionarily stable strategy (co-ESS) as the intersection between the ESSs of immunosuppression and virulence (Choisy and de Roode, 2010, Alizon, 2013). This intersection can be interpreted game theoretically as the strategy for which no invasion of a mutant strain with respect to either immunosuppression or virulence is possible (Maynard Smith, 1982, Dieckmann et al., 2002). We then explore the co-evolution of the two traits across different extrinsic mortality conditions and immunosuppression trade-off concavity.

Results

Virulence evolution

We first assume that the level of immunosuppression is constant and infer the virulence level towards which the parasite population evolves, that is the evolutionarily stable virulence (ESV). We find that the higher the immunosuppression, the higher the ESV (grey curve in Figure 1a). Because immunosuppression renders infected hosts more susceptible to further infections, it consequently increases the relative abundance of doubly infected hosts. This favours more virulent parasites due to within-host competition assumption (see equation 2).

Immunosuppression evolution

We then set the virulence to a constant value and study whether parasite immunosuppression evolves towards an evolutionarily stable strategy (i.e. evolutionarily stable immunosuppression, or ESI; black curve in Figure 1a). We find that ESI decreases with virulence at first, but it increases again when virulence is high enough. The initial decrease can be attributed to two non-mutually exclusive processes.

First, the benefits gained by increasing immunosuppression (i.e., slower host recovery) are reduced as virulence increases since the duration of infection decreases. In a similar way, ESI decreases as host mortality increases (Figure 2a). Second, the decreasing pattern may originate from demographic feedbacks: increasing virulence reduces the number of doubly infected hosts. In doubly infected hosts, parasites no longer pay the cost of contracting further infections but can still gain benefits from higher levels of immunosuppression. For low levels of virulence, most infections are double infections (Figure 1d) and ESI is high. As virulence increases, the proportion of doubly infected hosts goes down, and so does ESI as a consequence.

We also find that the ESI increases with virulence when virulence is high enough. As the host lifespan of an infected host decreases due to high parasite-induced morality, it becomes unlikely for a host to survive a single infection long enough to get infected again. At this point, co-infections are sufficiently rare (Figure 1d) that a parasite with a high level of immunosuppression would rarely suffer the cost associated with that trait. Taken together, focusing on the prevalence of co-infections alone is not enough to predict how ESI will evolve.

The co-ESS is found at the intersection between the two curves in Figure 1. For our default parameters, this occurs at intermediate values of immunosuppression and virulence. We now investigate how changes in host mortality and trade-off shape affect this co-ESS.

Co-evolution of virulence and immunosuppression

We first explore how the co-ESS varies with respect to the rate of host background mortality. We find that co-ES immunosuppression (co-ESI) always decreases with host background mortality (black line in Figure 2a). This result is in agreement with the intuition that immunosuppression represents a lost investment if the host dies too rapidly.

For co-ES virulence (co-ESV), we find that it peaks at an intermediate value of background mortality (gray line in Figure 2a). Based on earlier models (van Baalen and Sabelis, 1995, Gandon et al.,

2001), we expected increasing background mortality to select for reduced parasite virulence through a reduction in multiple infections (purple line in Figure 2b), where more virulent strains were assumed to have a competitive advantage.

However, the availability of singly infected hosts, or rather lack thereof, adds another layer of complexity to the problem. As shown in Figure 2b, when the force of infection and immunosuppression are too high, most resident hosts are co-infected and hence most resident parasites are 'locked up' in co-infections, creating a shortage of hosts singly infected with the resident parasite, I_r. In this case, when a rare mutant is introduced to the system, it only has access to uninfected (S) hosts. This 'protection effect' may hinder the evolution of a parasite trait such as virulence that is assumed advantageous only in doubly infected hosts (D_{rm}). Increasing host mortality diminishes this protection effect by increasing the relative density of I_r, thereby favouring more virulent strains (see Figure 2b and the Supporting Information 2 for details on how the input into I_r and the duration of infection is greater where there is immunosuppression).

Little is known about how immunosuppression impacts host recovery and susceptibility to further infection. Therefore, we also explored the sensitivity of our co-ESS results to the qualitative shape of the immunosuppression trade-off and the extent of its concavity using parameters, δ_σ and δ_γ. For immunosuppression, we find that the singular strategy is evolutionarily unstable when the recovery concavity is accelerating (Fig. 3a) meaning that in this case immunosuppression is either maximised or minimised depending on the initial conditions. Furthermore, we find that immunosuppression is maximised for a large area of the linear and decelerating recovery trade-off space, δ_γ. Intermediate ESI levels are observed for decelerating recovery, δ_γ, and accelerating susceptibility, δ_σ. Overall, this suggests that there is a tendency for parasites to specialise in immunosuppressing their host or to completely avoid doing so.

For virulence, we find that the evolutionary dynamics are qualitatively less variable and that the singular strategies are always convergence and evolutionarily stable (Figure 3b). Regarding the ES

virulence value itself, the concavity of the susceptibility function (δ_σ) has the strongest effect, with decelerating trade-offs leading to higher co-ESV. As in the rest of this model, since the only benefit associated with virulence is increased competitiveness in co-infected host, the co-ESV is a marker of the relative prevalence of each type of host (susceptible, infected and co-infected), which itself is shaped by immunosuppression.

Discussion

Host immune responses present a major challenge for parasites, and hence establishing a successful infection often depends upon a parasite's ability to evade host immunity (Schmid-Hempel and Frank, 2007). Despite its ubiquity among all major groups of parasitic organisms (Schmid-Hempel, 2009), the effect of immunosuppression on virulence evolution has largely been overlooked (but see Hurford and Day, 2013). We modelled immunosuppression through its joint effect on host recovery and susceptibility to co-infection in an attempt to understand epidemiological forces driving the co-evolution of virulence and immunosuppression.

We found that immunosuppression increases the optimal parasite exploitation by creating more co-infections, in which more competitive (and hence more virulent) strains are favoured. On the other hand, the evolution of immunosuppression is driven by the balance between the benefit conferred by immunosuppression to evade clearance from the host and the associated cost of contracting further infections, which introduce a competitor for limited host resources. Because virulence simultaneously decreases both the benefit (by killing hosts faster) and the cost (by reducing the risk of co-infection), its effect on the optimal immunosuppression is nuanced — increasing virulence can both increase or decrease the optimal immunosuppression depending on the baseline virulence of the parasite. In addition, immunosuppression evolution is influenced considerably by the precise shape of the trade-offs determining the cost and benefit of immunosuppression.

We then investigated the change in co-evolutionarily optimal strategies of the both traits over host background mortality. We find that mortality decreases the co-evolutionarily stable level of immunosuppression, which is a lost investment when hosts die too fast anyway. In the absence of immunosuppression, we expect the optimal virulence to consistently decrease with host background mortality because, again, investing in the competitive ability (with which virulence correlates) is a wasted investment when co-infections are rare (van Baalen and Sabelis, 1995, Gandon et al., 2001). When co-evolving with immunosuppression, however, we find that evolutionarily stable virulence peaks for an intermediate level of host mortality. This stems from the fact that for low host mortality, co-infections are very prevalent and because we put a limit to the maximum number of strains a host can be co-infected by, rare mutants can only infect uninfected hosts. Biologically, such a scenario may arise from a priority advantage for space and resources for the resident, or apparent competition mediated through the immune system (Mideo, 2009, Hoverman et al., 2013).

In light of our theoretical model, we can formulate testable predictions. In *Daphnia*, for example, the rate of host background mortality can be experimentally manipulated and its effect on virulence evolution of microsporidian parasites can be quantified (Ebert and Mangin, 1997). Microsporidians are common eukaryotic parasites of many animals including *Daphnia*, which often harbour multiple infections (Ebert, 2005). In their mosquito host, microsporidians have been suggested to suppress host immunity by manipulating the production pathway of a host immune defence molecule (nitric-oxide, NO), which is part of the innate immune system conserved in all animals (Biron et al., 2005). Conveniently, the production of NO can also be experimentally enhanced and blocked, making it possible to investigate the effects of manipulating host immune intensity (Rivero, 2006). Therefore investigation of the NO pathway in the *Daphnia* system may be useful for understanding how immunosuppression interacts with the effect of host background mortality and host immunity on virulence evolution.

A natural extension to the model of co-infection by the same species (van Baalen and Sabelis, 1995) is the model that accommodates two distinct resident parasite species, each of which can be

challenged by a mutant (Choisy and de Roode, 2010). Under the different species model, two co-evolving traits (e.g. immunosuppression and virulence) could be carried by two separate parasite species, which better reflect the reality for some immunosuppressing parasites, e.g, the immunosuppressing capabilities of HIV render the host susceptible to the virulence induced by opportunistic infections. Similarly, in an amphipod system, Cornet and Sorci (2010) show that immunosuppressive parasites elevate host mortality by promoting opportunistic pathogen infections. Furthermore, there is evidence that pathological severity of malaria infection can by amplified through immunosuppression caused by helminths, which are common parasites in malaria prevalent tropical regions (Graham et al., 2005). Given the positive link between host mortality and virulence evolution predicted by the virulence-transmission trade-off (which we did not consider in the present study), immunosuppression may also elevate the evolutionarily stable virulence by increasing mortality of co-infected individuals. That being said, considering multiple species would force us to revisit our assumption that more virulent mutants are more competitive than their resident at the within-host level. Indeed, this assumption has recently been shown to hold for a variety of within-host processes but only if the mutant traits are close to that of the resident (Sofonea et al. in prep). Therefore, adding more details about the within-host interactions, e.g. via a nested model (Mideo et al., 2008), seems necessary to study co-infection by different species.

In the present model, we assumed no direct link between immunosuppression and virulence. However, immune evasion strategies of bacteria and viruses have been empirically linked to a range of pathological effects (Casadevall and Pirofski, 2003, Monack et al., 2004, Stanford et al., 2007). On the other hand, immunosuppression may decrease immunopathology which can therefore reduce host mortality, as shown experimentally using rodent malaria infections (Long et al., 2008, Long and Graham, 2011). In fact, helminth therapy, which involves deliberate ingestion of parasitic worms, takes advantage of the parasite's ability to mediate host immunity and has been successful in countering inflammations caused by immune-mediated diseases (Day et al., 2007, Elliott and Weinstock, 2009,

Summers et al., 2003).

The only cost of immunosuppression we assumed is indirect (co-infection facilitation), however the production of immunosuppressive compounds could impose a direct fitness cost to individual pathogens. At the within-host level, immunosuppression would therefore be seen as a public good since parasites that do not invest in it can still reap the benefits (Diard et al., 2013, Rundell et al., 2016). In fact, our model predicts that invasive repellers are common for immunosuppression (Fig. 3a) while coexistence of two strains with extreme immunosuppression strategies (i.e., zero and maximum immunosuppression) is always possible regardless of trade-off concavity (figure not shown). These findings suggest that it may be common for some strains to specialise in immunosuppressing and others in exploiting these immunosuppressed hosts.

Understanding how host immunity and the corresponding parasite immune evasion strategies affect virulence evolution is a key challenge for contemporary evolutionary epidemiology (Frank and Schmid-Hempel, 2008). Our results demonstrate that immune evasion mechanisms are among the major forces shaping virulence evolution at the between-host level. Future theoretical studies may focus on multi-species epidemiological dynamics, direct trade-offs between immunosuppression and virulence and life-history perspectives.

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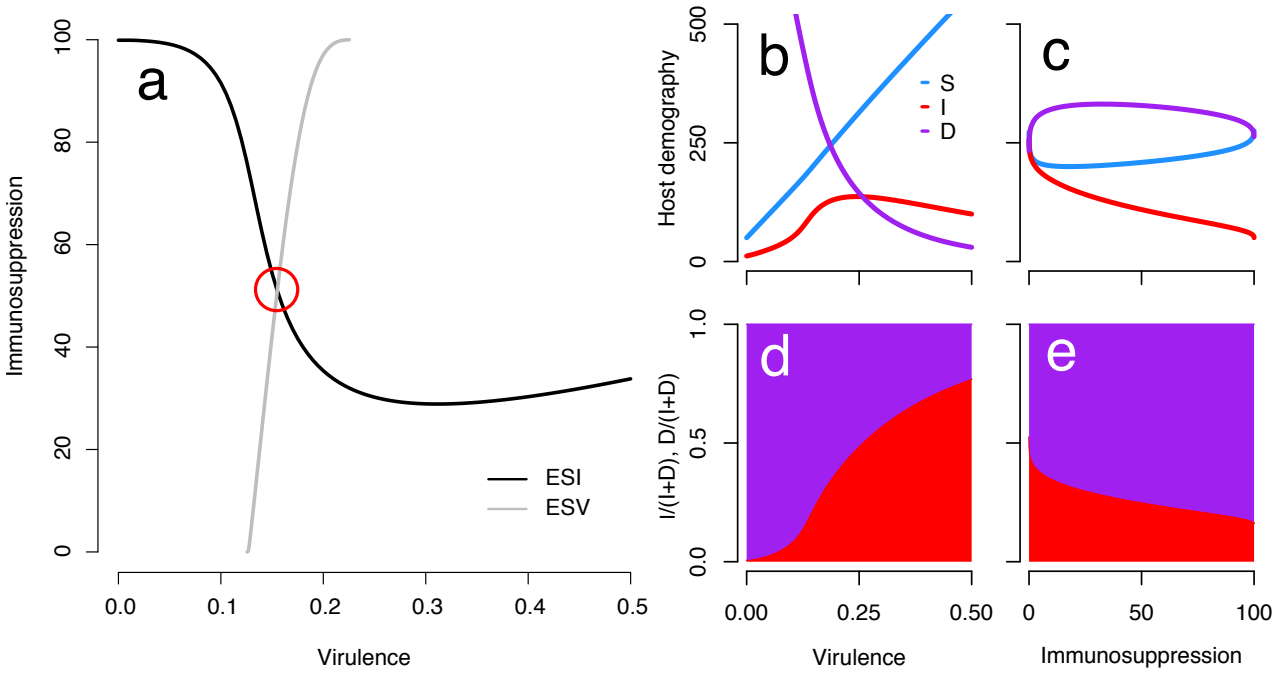

Fig. 1: (a) Evolutionarily stable immunosuppression (ESI; black) and virulence (ESV; grey) against fixed values of the other trait. The co-evolutionarily stable strategy (co-ESS) of the two traits occurs at the intersection of the two lines, indicated by the red circle. The immunosuppression trade-offs for the recovery rate and additional susceptibility were decelerating and accelerating, respectively with shape parameters $\delta_\gamma = 0.05$ and $\delta_\sigma = 0.25$. The population size of the three host classes — susceptible (S; blue), singly infected (I; red) and doubly infected (D; purple) — underlying the ESI for a given level of virulence and the ESV for a given level of immunosuppression is presented in (b) and (c). The relative abundances of singly (red) and doubly (purple) infected host are plotted in (d) and (e).

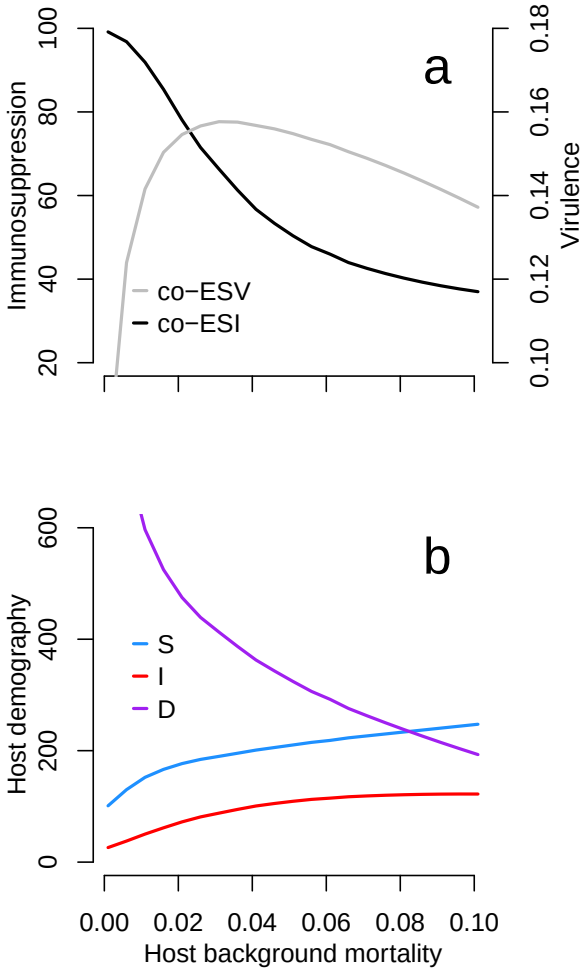

Fig. 2: Co-evolutionarily stable immunosuppression (co-ESI; black) and virulence (co-ESV; grey) strategies against host background mortality (a) and the equilibrium population size of the three host classes — susceptible (S; blue), singly infected (I; red) and doubly infected (D; purple) that result from the co-ES trait combination.

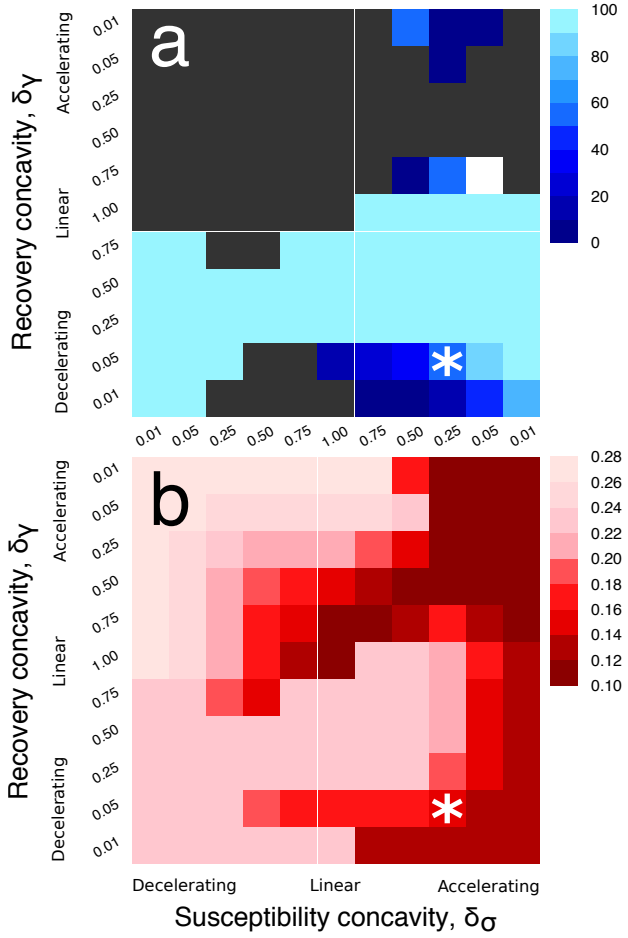

Fig. 3: The trade-off concavity affects the evolutionarily outcome of (a) immunosuppression and (b) virulence at the co-evolutionarily singular strategies. The asterisk (*) indicates the default set of trade-off parameters explored in Figure 1 and 2. The dark grey squares in (a) indicate that the immunosuppression strategy is evolutionarily and convergent unstable at the co-evolutionarily singular strategy, i.e. invasive repeller. The white square in (a) indicates that the immunosuppression strategy is convergence stable, but evolutionarily unstable, i.e., an evolutionary branching point.