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Evolution of virulence: Interdependence, constraints, and selection using nested models

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Abstract

Natural selection acts on virus populations at two distinct but interrelated levels: within individual hosts and between them. Studies of the evolution of virulence typically focus on selection acting at the epidemiological or between-host level and demonstrate the importance of trade-offs between disease transmission and virulence rates. Within-host studies reach similar conclusions regarding trade-offs between transmission and virulence at the level of individual cells. Studies which examine selection at both scales assume that between- and within-host selection are necessarily in conflict. We explicitly examine these ideas and assumptions using a model of within-host viral dynamics nested within a model of between-host disease dynamics. Our approach allows us to evaluate the direction of selection at the within- and between-host levels and identify situations leading to conflict and accord between the two levels of selection.

Keywords: Host–parasite; Evolution of virulence; Viral dynamics; Within-host selection; Selection conflict

1. Introduction

A fundamental finding from theoretical studies of the evolution of virulence is that the optimum virulence rate for a pathogen is determined by the relationship between the rates of disease transmission and virulence (Bremermann and Pickering, 1983; May and Anderson, 1983; Sasaki and Iwasa, 1991; Lenski and May, 1994; Day, 2001; Williams and Day, 2001). These studies focus on how natural selection functions at the level of the host population, and essentially explore how pathogens compete for uninfected hosts.

Complementary studies at the within-host level use models of virus population dynamics within a single host to explore how pathogens compete for host resources such as uninfected cells (Antia et al., 1994; Antia and Lipsitch, 1997; Regoes et al., 1998; Almogy et al., 2002; Gilchrist et al., 2004). Here, the most successful pathogens are those that are able to balance trade-offs between intra-cellular virulence and inter-cellular transmission. However, success of a parasite within a single host does not necessarily lead to optimal exploitation of the population of uninfected hosts.

There are studies which indirectly deal with natural selection at the within-host level within the context of a between-host model. These studies use models in which hosts can be infected by different competing strains of parasites (Levin and Pimentel, 1981; Bonhoeffer and Nowak, 1994; Nowak and May, 1994; May and Nowak, 1995; van Baalen and Sabelis, 1995; Miralles et al., 2001; Gandon et al., 2002). These studies assume that the optimal within-host strategy differs from the optimal between-host strategy.

To date, no conceptually simple framework has been presented within which to explore the role of natural selection on pathogen virulence at both the between- and within-host levels. Indeed, most studies of the evolution of virulence at the between-host level view selection as acting on the virulence rate itself and do not take a mechanistic approach to the problem of linking disease transmission and virulence rates to within-host processes.

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Further, the contributing factors to the relationship between transmission and virulence rates, how it changes from system to system, and the limits to its values are not well understood.

Ideally, one should be able to derive the transmission-virulence relationship from knowledge of basic between- and within-host processes, rather than imposing a particular linkage. We propose that a useful framework for considering these questions is one of *nested* models where a within-host model of parasite population dynamics is linked to a population-level model for between-host infection dynamics (Sasaki and Iwasa, 1991; Antia et al., 1994; Ganusov et al., 2002; Gilchrist and Sasaki, 2002; Andre et al., 2003; Krakauer and Komarova, 2003; Alizon and van Baalen, 2005). This framework allows one to explicitly link parameters of the between-host model to the behaviour of the within-host model.

Our goal in this study is to develop this framework as a tool for studying the relationship between disease transmission and virulence rates and identifying potential conflicts between selection at the different levels. In addition we will show how limits to the behaviour of the within-host model lead to natural limits to the parameters of the between-host model. Specifically, we will use well-established between- and within-host models for pathogen dynamics to derive relationships between the transmission and virulence rates of an infection, infer physiological bounds for these values, and determine whether or not natural selection at the between- and within-host levels is in conflict or accord. The models we have chosen are at this stage are illustrative of our approach—different biological situations will lead in a mechanistic way to different relationships between transmission and virulence, and potentially, different trade-offs.

We begin by giving precise formulations of the two models and then illustrate how they may be linked using a series of examples. Our ideas are developed throughout with reference to viral infections, however, the framework can easily be modified to study other parasites. In the context of viral pathogens, this work builds upon our recent studies (Coombs et al., 2003; Gilchrist et al., 2004), where we explored how dependence of cellular virulence on virion production rate contributes to the within-host fitness of a viral strain and the equilibrium state of the virus–host system (Table 1).

Table 1
Table of exact functions used to link between-host parameters to within-host variables and, in turn, the implicit relationship between β and α that results for each of the three examples

Example	Functions of intra-host model	Transformation into $\alpha(\beta)$
I	$\beta(V) = a_1 V$ $\alpha(T) = a_2(T_0 - T)$	$\beta(\alpha) = \frac{a_1 d}{k} \frac{d}{a_2 \lambda - \alpha d}$
II	$\beta(V) = a_1 V^z$ $\alpha(T) = a_2\left(\frac{1}{T} - \frac{1}{T_0}\right)$	$\beta(\alpha) = a_1\left(\frac{\lambda\alpha}{k a_2}\right)^z$
III	$\beta(V) = a_1 V + a_3 T^*$ $\alpha(T) = a_2\left(\frac{1}{T} - \frac{1}{T_0}\right)$	$\beta(\alpha, p) = a_1\left(\frac{\lambda\alpha}{k a_2}\right) + \frac{a_3}{p}\left(\lambda N(p) - \frac{cd}{k}\right)$

a_i are parameters of the functions.

Table 2
Between-host model parameters and definitions

Between-host model	
S	Density of susceptible hosts
I	Density of infectious hosts
$b(S, I)$	Population birth rate
β	Infection transmission rate
δ	Background death rate
α	Parasite virulence rate
α^*	Optimal within-host parasite virulence rate
$\alpha^\bullet$	Optimal between-host parasite virulence rate
$p^\bullet$	Between-host optimal cellular parasite production rate

1.1. Between-host model

One of the simplest epidemiological (i.e., between-host) models classifies hosts into two different groups: uninfected individuals who are susceptible to the infection and infected individuals who are also infectious. We assume that infections are chronic and transmitted directly between hosts, and we ignore any age structuring of the host. We also assume that the birth rate of uninfected hosts is a density dependent function of host density, but that the background death rate of hosts is density independent. Based on these assumptions we define the following model,

$$\frac{dS}{dt} = b(S, I) - \beta SI - \delta S, \tag{1}$$

$$\frac{dI}{dt} = \beta SI - (\alpha + \delta)I. \tag{2}$$

$S(t)$ and $I(t)$ represent the density of susceptible and infectious host classes. The parameters α, β and γ represent the parasite virulence rate (i.e., the increase in host death rate due to infection), the transmission rate of the infection, and the host background death rate, respectively. This model is an SI model in the language of mathematical epidemiology (Kermack and McKendrick, 1927). All parameters of this between-host model and their definitions can be found in Table 2.

Host births occur in a density dependent manner according to the function $b(S, I)$, which we take to be a monotonically decreasing function of the total host population density, $S + I$. In the absence of infection, the host population density will grow until it reaches its equilibrium population size, S_0, implicitly defined by $b(S_0, 0) = \delta$. The presence of the disease will drive the population below this equilibrium value.

We define the basic reproductive ratio of an infection, R to be equal to the expected number of new infections caused by each infected host per unit density of susceptible hosts. Because the actual number of new infections scales with the density of susceptible hosts, the infection will invade the population provided R exceeds $1/S_0$. R is proportional to the commonly used fitness term R_0, $R_0 = RS_0$.

For the SI model defined above, it can be shown that

$$R = \frac{\beta}{\alpha + \delta}. \tag{3}$$

If the system is at equilibrium, an invasion analysis shows that natural selection at the between-host level will favour the maximization of R. Consequently, R can be viewed as the fitness of an infection at the between-host level and in the absence of any interdependence or constraints concerning α, β and δ, natural selection at the between-host level will favour reductions in α and increases in β (Fig. 1). We will use this simple fitness landscape in our examination of the constraints on the evolution of α and β arising from within-host processes. We have chosen the SI model as the simplest possible model within which to study inter- and intra- host effects, leaving exploration of recovery of infected hosts, co- and super-infection, spatial effects, and host variability for future studies.

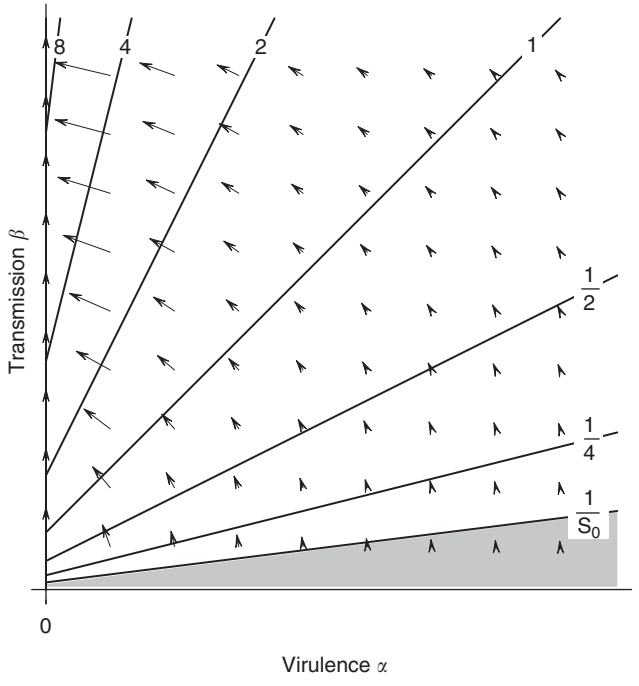

Fig. 1. The between-host fitness landscape for a parasite within an SI model. Fitness contours are labeled with their R values. Between-host fitness increases linearly with transmission, β, and decreases inversely with α. Arrows indicate the direction and slope of the selection gradients. In the absence of any interdependence or constraints between α and β, natural selection favours the evolution of ever increasing β and decreasing α towards zero. Note that in order for the infection to persist in the host population, $R \times S_0$ must be greater than 1. The region of (α, β) space in which the infection cannot persist (i.e., $R < 1/S_0$) is indicated by gray shading.

1.2. Within-host model

In order to study the within-host parasite dynamics, we present a model commonly used in the study of HIV (Perelson et al., 1996; Regoes et al., 1998) and Hepatitis C dynamics (Neumann et al., 1998). The model equations are

$$\frac{dT}{dt} = \lambda - kVT - dT, \tag{4}$$

$$\frac{dT^*}{dt} = kVT - (\mu(p) + d)T^*, \tag{5}$$

$$\frac{dV}{dt} = pT^* - cV, \tag{6}$$

where T is the density of uninfected host cells susceptible to infection, T^* is the density of productively infected host cells, and V is the density of free virions within a host. In studies of HIV, T and T^* are generally assumed to represent T cells. However, this model can be applied to pathogens which infect other types of host cells or tissue—this model treats cells as a replenished resource exploited by the virus, with no special reference to the biology of HIV. Thus we will refer to T and T^* as uninfected and infected target cells.

In this model uninfected target cells are created at constant rate λ and are infected by interactions with free virions according to a mass action law with rate constant k. Productively infected cells, T^*, produce new virions at a constant rate p. The background target cell death rate is d. Virion production uses host resources and infrastructure and may mark the cell for destruction by the immune system. Therefore, infected target cells experience an elevation in mortality that is related to production by the function $\mu(p)$. Finally, viral particles are cleared at rate c. We assume that the elevated mortality rate experienced by infected cells, $\mu(p)$, is an increasing function of p. $\mu(p)$ essentially describes the virulence of the virus at the cellular level, within a host. All parameters of this within-host model and their definitions can be found in Table 3.

The stability and equilibrium states of this within-host model are easily understood. Two equilibrium points exist, representing uninfected and infected hosts. At the uninfected equilibrium there are no virions or infected cells: $V = T^* = 0$ and $T = \lambda/d$. The second equilibrium point occurs at

$$\hat{T} = \frac{c}{kN}, \tag{7}$$

$$\hat{T}^* = \frac{1}{p}\left(\lambda N - \frac{cd}{k}\right), \tag{8}$$

$$\hat{V} = \frac{\lambda N}{c} - \frac{d}{k}, \tag{9}$$

where the burst size N is equal to the expected number of progeny virions produced over the lifetime of a single

Table 3
Within-host model parameters and definitions

Within-host model	
T	Density of target cells
T^*	Density of infected target cells
V	Density of virions
λ	Target cell production rate
k	Cellular infectivity of parasite
d	Background target cell death rate
p	Cellular parasite production rate
p^*	Within-host optimal cellular parasite production rate
$p_{\max}$	Maximum parasite production rate
N	Cellular burst size
$\mu(p)$	Additional infected target cell death rate
c	Parasite clearance rate
a_1, a_2, a_3, z	Transmission and virulence function parameters (see Table 1)

infected cell:

$$N = \int_0^\infty p \exp[-(\mu(p)+d)t]\,dt = \frac{p}{\mu(p)+d}. \tag{10}$$

Because we will consider the evolution of p under between- and within-host level selection, we note that $\hat{T}$, $\hat{T}^*$, $\hat{V}$ and N are all ultimately functions of p.

Persistence of the infection occurs when the first equilibrium point is unstable and the second point is stable (simply when $\hat{V}>0$). This condition can be written as

$$\frac{\lambda N}{c} > \frac{d}{k}. \tag{11}$$

For the purposes of this study we assume that the host dependent parameters, λ and d, are fixed, and that the viral parameters in the within-host model (p, k, and c) are independent of one another. Under these assumptions it can be shown that within-host level selection will favour the maximization of k and the minimization of c (Gilchrist et al., 2004). Thus we will assume here that k and c are fixed at their physiological maximum and minimum, respectively. In contrast, we suppose that p can vary between viral strains but that physiological constraints within the cell limit the size of p to some finite value: $p<p_{\max}$.

We define p^* as the virion production rate which maximizes viral burst size, N. From the perspective of natural selection within a host, p^* is the optimal virion production rate (Gilchrist et al., 2004). Because N is a function of both p and the cellular virulence function, $\mu(p)$, the value of p^* will change with the exact form of μ. Whether or not p^* is equal to or less than the physiological maximum virion production rate, $p_{\max}$, depends on the relationship between cellular virulence and production, $\mu(p)$ (Sasaki and Iwasa, 1991; Coombs et al., 2003). In either case, we define the maximum burst size of an infected cell, $N_{\max}$, occurring when $p=p^*$.

The fact that $N_{\max}$ exists implies that $\hat{T}$, $\hat{T}^*$ and $\hat{V}$ are bounded by the interaction between the virus and the target cells. $\hat{T}$ cannot drop below $c/(kN_{\max})$ and $\hat{V}$ cannot rise above $\lambda N_{\max}/c - d/k$. Similarly, because $\hat{T}^*$ is a function of both N and p, it also has an upper bound. As we shall show in the next section the physiological bounds on $\hat{T}$, $\hat{T}^*$, and $\hat{V}$ will constrain the range of possible values of the between-host parameters β and α. We conclude by noting that because the steady state densities $\hat{T}$ and $\hat{V}$ are functions of burst size, N, it follows that

$$\frac{d\hat{T}}{dp} = \frac{d\hat{T}}{dN}\frac{dN}{dp} \quad \text{and} \quad \frac{d\hat{V}}{dp} = \frac{d\hat{V}}{dN}\frac{dN}{dp}. \tag{12}$$

Eqs. (12) imply that if p^* is an internal optimum, then

$$\left.\frac{d\hat{T}}{dp}\right|_{p=p^*} = 0, \quad \left.\frac{d\hat{V}}{dp}\right|_{p=p^*} = 0 \tag{13}$$

and

$$\left.\frac{d\hat{T}^*}{dp}\right|_{p=p^*} = \frac{1}{p^2}\left(\frac{cd}{k} - \lambda N_{\max}\right) < 0. \tag{14}$$

These results will be important to our analysis of the potential conflict between between- and within-host selection.

1.3. Linking the between- and within-host models

We assume that the internal state of the host will affect the rates of disease transmission and virulence. For example, the infectiousness of a host is likely to increase with viral load and host survivorship is likely to decrease as more resources (such as the target cells) are co-opted by the infection. Therefore, we propose that α and β can be written as functions of the state variables of the within-host model: virion density V, host resource density T and infected resource density T^*.

We have assumed in the formulations of the between- and within-host models that infections are chronic. For simplicity, therefore, we ignore the transient dynamics of the within-host model and instead assume that the within-host system is effectively at equilibrium throughout the course of the infection (i.e., $T=\hat{T}$, $T^*=\hat{T}^*$, and $V=\hat{V}$ for all infected hosts). As a result we will view the host level virulence and transmission rates as functions of the equilibrium behaviour of the within-host model, $\alpha = \alpha(\hat{V},\hat{T},\hat{T}^*)$ and $\beta=\beta(\hat{V},\hat{T},\hat{T}^*)$. This is equivalent to assuming that within-host dynamics are fast compared to those of transmission and host mortality.

Because the equilibrium state of the within-host system is a function of the virion production rate, it follows that β and α can be written as functions of p. We are considering $0<p\leqslant p_{\max}$ to be the single variable trait of the virus. As p varies over its range, $(\alpha(p),\beta(p))$ traces out a path on the fitness landscape of $R(\alpha,\beta)$ as shown in Fig. 2. The point on this path that maximizes $R(\alpha,\beta)$ corresponds to the value of p that is optimal from the point of view of natural selection at the between-host level. We define $p^\bullet$ to be this optimal

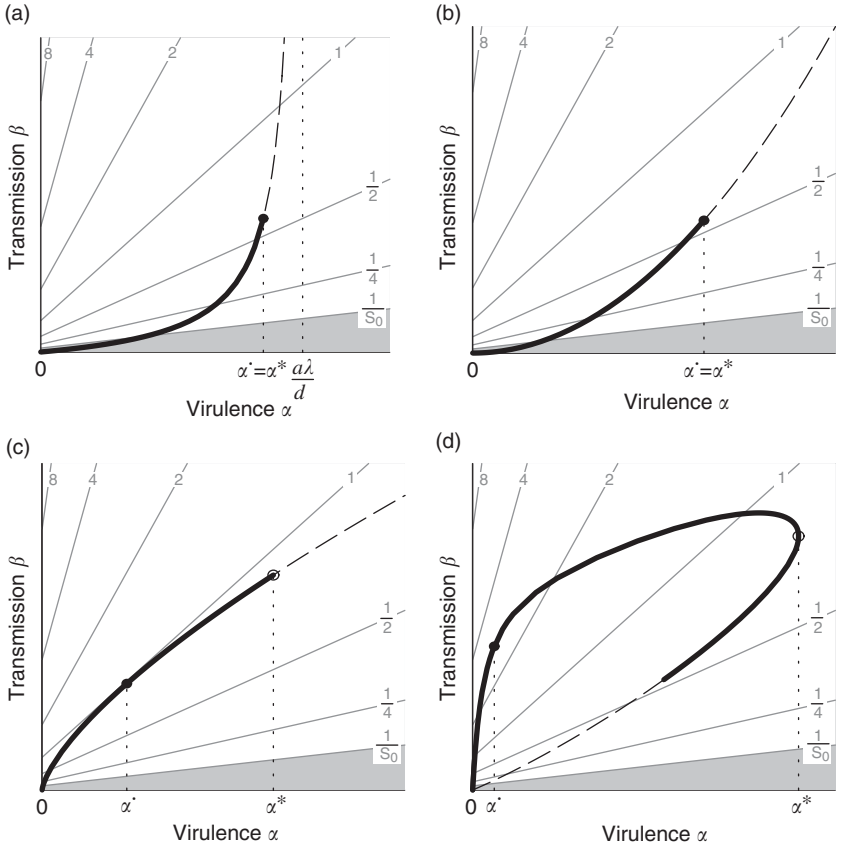

Fig. 2. Illustrations of the behaviour of transmission, β as a function of host level virulence, α, the three different examples superimposed on a between-host fitness landscape (Fig. 1). Between-host fitness landscape contours are labeled with representative values of R. Curves represent the relationship between β and α for each specific example (Table 1). Solid curves show physiologically possible parameter values ($0 \leqslant p \leqslant p_{\max}$); dashed extensions are physiologically impossible ($p > p_{\max}$). $\alpha^\bullet$ and α^* indicate the α values between 0 and $\alpha_{\max}$ which maximizes between-host fitness, R, and within host fitness, Nk/c, respectively. (a) Example I, (b) Example II with $z \geqslant 1$, (c) Example II with $z < 1$, (d) Example III.

between-host level production rate. Because natural selection occurs at both the between- and within-host levels, we ask whether or not $p^\bullet$ is the same as the within-host optimal virion production rate, p^*. If they are the same, then there is no conflict between selection at the two different levels. Otherwise, a conflict exists between natural selection at the within- and between-host levels.

1.4. Examples

We now introduce three examples to illustrate our approach. Each example is defined by two functions α and β presented in the first column of Table 1. Our justification for the assumptions we make in each of the examples is as follows. One would expect that the rate of transmission will increase with increasing viral load, and potentially with the density of infected target cells. Consequently, in the first two examples, I and II, we will assume that the between-host infection transmission rate β is an increasing function of the viral density in the host, V. We represent this dependence of transmission on viral density as $\beta(V)$. Observational data supports assumptions of this kind. For example, HIV transmission is known to be dependent on viral load (Quinn et al., 2000; Chakraborty et al., 2001; Gray et al., 2004). Similarly, vertical and horizontal transmission of hepatitis C virus (Molin et al., 2002; Hisada et al., 2000) and vertical transmission of human papillomavirus (Kaye et al., 1994) correlate with viral load (although the evidence is not strong for horizontal transmission of hepatitis C (Hisada et al., 2000)). In Example II we will consider the case where transmission is a concave down (but still monotonically increasing) function of viral load ($z < 1$) as well as the concave up case ($z > 1$). If $z < 1$ then the marginal gain from increased viral load decreases as the viral load increases. This could be used to model a situation where viruses are transmitted in inocula containing approximately fixed numbers of virions. As the number of free viruses increases within the host, it is possible that the inocula become saturated by virus and so the marginal transmission benefit of additional free virus is reduced.

In Example III, we assume that transmission is a linear increasing function of both viral and infected target cell densities, i.e. $\beta(V, T^*)$. For HIV transmission, there is considerable evidence that infection via transfer of infected cells is possible (Carreno et al., 2002; Moore et al., 2003) although the relative importance of this means of transmission relative to cell-free virus is unclear (Miller

and Shattock, 2003 and references therein). Cell-mediated transmission of feline immunodeficiency virus (FIV) is well established (Burkhard and Dean, 2004).

We will also assume that the virulence of the viral infection increases with decreasing values of uninfected target cells, T. In the case of HIV, the mechanism is clear—host mortality from opportunistic infections is known to increase substantially at low T cell densities (Phillips et al., 1991; Taha et al., 2000). Similarly, the destruction of liver cells in hepatitis C patients leads to mortality. In Example I we assume that α decreases linearly with T, while in Examples II and III α scales with $1/T$.

1.5. Interdependence and constraints on β and α

Using the given functional dependences for α and β, we determined how α and β are related in each example (Table 1). The forms of $\beta(\alpha)$ in the first two examples reveal the interdependence of α and β which results from linking these parameters to the within-host model, in that β can be viewed as only being dependent on α. As a result in these examples we find a one to one correspondence between β and α (Figs. 2(a)–(c)). In Example III, which assumes that β changes with both V and $\hat{T}^*$, we find that β is dependent on both α and p. As a result we no longer find a one to one correspondence between β and α (Fig. 2(d)).

The fact that both N and p are bounded (see above) implies that α and β are always subject to physiological constraints at the within-host level. In all examples the greatest physiologically possible value of α, which we designate as $\alpha_{\max}$, occurs when $\hat{T}$ is at its physiological minimum. $\hat{T}$ is minimized when $N = N_{\max}$ which corresponds to p being equal to p^*. Thus, under the assumptions made here, within-host selection always selects for the maximum between-host virulence rate, α^*, i.e. $\alpha^* = \alpha_{\max}$. In Examples I and II, the greatest possible value of β is also obtained when $N = N_{\max}$ (Fig. 2(a)–(c)). However, in Example III, the maximum value of β depends on both the value of $\hat{V}$ and $\hat{T}^*$. From Eqs. (13) and (14) it can be shown that in Example III, β is always less than its maximum possible value at p^* (Fig. 2(d)).

1.6. Natural selection at the between-host and within-host levels

As we just demonstrated, linking the between- and within-host models introduces constraints on the possible relationship between disease transmission, β, and virulence, α, as well as limits to their possible values. Natural selection at the between-host level favours the evolution of the virion production rate, p, to the value which maximizes R under the above constraints and limits. In contrast, natural selection at the within-host level favours the p value which maximizes burst size, N. In this section we examine each of the three examples and identify when selection at the between- and within-host levels is in accord and when it is in conflict.

Example I. Because we can rewrite β solely as a function of α (Table 1) it is possible to view R as a direct function of α, i.e.,

$$R(\alpha) = \frac{\beta(\alpha)}{\alpha + \delta}. \tag{15}$$

Differentiating $R(\alpha)$ with respect to α indicates that R is an increasing function of α in the range $[0, a\lambda/d]$ (where $a\lambda/d$ represents an intrinsic upper bound of α due to the assumptions that α is proportional to T and $T>0$). Therefore, R is maximized at $\alpha^* = \alpha_{\max}$ (Fig. 2a) and between-host selection will favour the same virion production rate as within-host selection, $p^\bullet = p^*$. In this example there is no conflict between selection acting at the different levels.

Example II. Taking a similar approach to that of Example I, we can rewrite β and consequently R as a function of α. We find that if the power $z \geqslant 1$ then $dR/d\alpha > 0$ for all values of α. Thus, if $z \geqslant 1$, then $\alpha^\bullet = \alpha_{\max}$ and, correspondingly, $p^\bullet = p^*$ (Fig. 2b). However, if z is less than 1, then R becomes a peaked function of α, maximized when $\alpha = z\delta/(z-1)$. In the instances where $\alpha_{\max} < z\delta/(z-1)$, the physiological constraints of the within-host model prevent α from reaching theoretical between-host optimum. As a result we again find that $\alpha^\bullet = \alpha_{\max}$ (not shown). If $\alpha_{\max} > z\delta/(z-1)$ then $\alpha^\bullet < \alpha_{\max}$ indicating that between-host selection favours an intermediate level of virulence (Fig. 2(c)). Because $\alpha^\bullet < \alpha_{\max}$, it follows that $p^\bullet \neq p^*$. In other words, if $z<1$ and $\alpha_{\max}$ is sufficiently large then between- and within-host selection on p will be in conflict. However, if the physiological maximum production rate $p_{\max}$ is sufficiently small, $\alpha_{\max}$ may be so small that $\alpha^\bullet = \alpha_{\max}$, removing the conflict. This situation shows the potential importance of physiological limits on viral production at the cell level.

Example III. Understanding how between-host level selection acts in Example III is more complicated than Examples I and II because we cannot write β solely as a function of α. Consequently, we take a slightly different approach of trying to identify the necessary criteria for $p^\bullet$ to equal p^*. We begin by writing α as a function of $\hat{T}$ and β as a function of $\hat{V}$ and $\hat{T}^*$. In turn, we note that $\hat{T}$, $\hat{V}$ are functions of N which is a function of p. Similarly, $\hat{T}^*$ is a function of both N and p. Thus we can write R ultimately as a function of p:

$$R(p) = \frac{\beta(V(p), \hat{T}^*(p))}{\alpha(p) + \delta}. \tag{16}$$

Differentiating R with respect to p we find

$$\frac{dR}{dp} = \frac{1}{\alpha(T(p)) + \delta}\left(\frac{\partial\beta}{\partial\hat{V}}\frac{d\hat{V}}{dp} + \frac{\partial\beta}{\partial\hat{T}^*}\frac{d\hat{T}^*}{dp} - \frac{R(p)}{\alpha(\hat{T}(p)) + \delta}\frac{d\alpha}{d\hat{T}}\frac{d\hat{T}}{dp}\right). \tag{17}$$

If p^* is an internal optimum then $dN/dp|_{p^*}=0$ and $d\hat{T}^*/dp|_{p^*}<0$ (Eq. (13)). Hence, from Eq. (14),

$$\left.\frac{dR}{dp}\right|_{p^*}=\frac{1}{\alpha(T(p))+\delta}\left(\frac{\partial\beta}{\partial\hat{T}^*}\frac{d\hat{T}^*}{dp}\right)\Bigg|_{p^*}<0$$

$$\text{provided } p^*<p_{\max}, \tag{18}$$

and thus $p^\bullet<p^*$ and there is a conflict between inter- and intra-host selection. If $p_{\max}$ is sufficiently small that p^* is a boundary optimum ($p^*=p_{\max}$) then the direction of selection at p^* will be determined by the sign of the term in parentheses in Eq. (17). If the sign is negative then $p^\bullet<p^*$ and a conflict in selection exists; otherwise $p^\bullet=p^*$ and there is no conflict.

1.7. Summary of results

In Examples I and II we assumed transmission was solely a function of virion density within the host, finding a one to one correspondence between β and α. In the more complex example where we assumed that transmission was a function of both virion and infected target cell density, we found that there was no longer a simple one to one mapping. In addition to determining the relationship between β and α, we also found that the equilibrium behaviour of the within-host model leads to physiological limits on the range of possible values of β and α. This is an inherent property of using a nested framework and is an advantage of this approach over a less mechanistic approach to determining the relationship between transmission and virulence.

In all three examples we found that within-host selection favoured virion production rates, p, which maximized α. This is a consequence of our choice of within-host model. In our model, parasites compete for target cells and maximize their fitness by driving the target cell density as low as possible. Because $\alpha(T)$ was a strictly decreasing function in each example, it is clear that α should be maximized by within-host selection. In regards to between-host selection, in Example I we found that between-host fitness was maximized at $\alpha_{\max}$. In Example II we found that if the disease transmission rate was a linear or concave up function of within-host virion density ($z\geqslant 1$) then between-host fitness was maximized at $\alpha_{\max}$ as well. However, if the disease transmission rate was a concave down function of within-host virion density ($z<1$), then between-host fitness was maximized at a virulence rate less than $\alpha_{\max}$. Finally, because in Example III the disease transmission rate is a function of both virion and infected target cell densities, between-host selection will always favour a virulence rate less than $\alpha_{\max}$, provided $p_{\max}$ is sufficiently large.

2. Discussion

Using a framework of nested between- and within-host models we were able derive a mechanistic relationship between disease transmission and virulence rates, β and α. Our approach links these parameters together in a sensible manner, and also results in natural limits to the possible range of parameter values of the between-host model. By examining the behaviour of this nested system of models we were able to identify some general sets of assumptions that lead to either conflict or accord in natural selection at the between- and within-host levels. Our results indicate that the general assumptions that both virulence and transmission increase with parasite load do not necessarily lead to a conflict in selection at the between- and within-host levels. For what seem to be the most biologically plausible cases where transmission is a concave down function of parasite load or when transmission can also occur through infected cells, within-host selection favours a higher virion production rate than between-host selection. In both of these cases we find that the overall relationship between transmission and virulence is, at least in part, concave down. A recent study by Alizon and van Baalen (2005) used a similar approach to the one developed here and also found such a relationship.

Nonetheless, it is clear that a wide range of biologically plausible conditions, lead to violation of the assumption that between- and within-host selection are in conflict. Given the diversity of host–parasite systems and the fact that we have focused here on a small subset of possible models and assumptions, it seems likely that such violation of these assumptions do occur in nature. In addition, even in cases where our models predict no conflict in selection, results from spatially structured models suggest that such conflict may arise due to the fact that in these scenarios between-host selection favours lower transmission rates, even when transmission is decoupled from virulence (Rand et al., 1995; Haraguchi and Sasaki, 2000; Read and Keeling, 2003; Boots et al., 2004).

One major assumption in this study was that the within-host dynamics reach equilibrium quickly. This assumption clearly ignores the transitory evolutionary dynamics that may occur before the equilibrium is reached. Such early within-host competition may be important for transmission during the early phase of the infection, but will not affect the long term competitive outcome within a single host.

Alizon and van Baalen (2005) make a similar equilibrium assumption in their study of the trade-off between transmission and virulence. As in that study, the equilibrium assumption allows us to investigate the system analytically rather than through simulation. The equilibrium internal state of the host (the state variables for the within-host model: T, V and T^*) was found in terms of the viral reproduction rate p. As p varies, a path is traced out on the fitness landscape at the between-host level, and thus the optimal between-host value of p may be determined. We could then compare this optimum to that predicted by the within-host model, and identify whether a conflict arose between natural selection at the different levels. A further comment is that permitting variation of a second viral parameter (for instance, the cellular infectivity k) would

lead to an optimization problem over a two-dimensional region of the between-host fitness landscape.

The within-host equilibrium assumption is justifiable for some chronic diseases in which the time of infection is long and so the majority of transmission events occur during the chronic phase of the disease. Making this assumption allowed us to transparently illustrate the utility of using nested between- and within-host models. The key aspect of the nested approach is that the epidemiological parameters, α and β, are linked to the state of the infection within a host, whether or not the equilibrium assumption is made.

Besides looking at chronic infections, the nested model approach could also be applied to transitory infections by explicitly incorporating host immune response dynamics (cf. Gilchrist and Sasaki, 2002) within a structured SIR model. Because in transitory infections the within-host equilibrium values of the virions and infected cells are zero, studies in this area would have to consider the dynamics of the infection within the host rather than just the equilibrium values. Such a model should give insight into the question of why some diseases are chronic while others are transitory.

Similarly, relaxing the equilibrium assumption may be of great importance in modelling certain chronic diseases such as HIV. The viral dynamics are important in this case because data suggests that many new infections occur during the early (acute) stage of the infection (Jacquez et al., 1994; Levin et al., 1996; Pilcher et al., 2004). Interestingly, a theoretical study suggests that population structure effects (casual or steady sexual partners) may be important in determining the relative importance of early and chronic infection in HIV (Xiridou et al., 2004). More generally, chronic-stage transmission is likely to be a major source of new infections in diseases such as hepatitis B and C (Atkins and Nolan, 2005; Kim, 2002).

It is important to recognize that knowing the conditions which lead to conflict of between- and within-host selection says nothing about how such a conflict plays out in the evolutionary arena. This shortcoming is not an inherent limitation of using nested models, but results from the fact that in this study we do not explicitly model evolutionary dynamics. From the perspective of an individual host, within-host selection should constitute the more powerful evolutionary pressure. Furthermore, it is generally assumed that between-host selection and that the replacement of one strain with another within a host will occur rapidly (e.g. Levin and Pimentel, 1981; Bonhoeffer and Nowak, 1994; Nowak and May, 1994). Nonetheless, if the transmission advantage of a viral strain close to the between-host optimum is large and the rate at which the viral population within a host evolves towards the within-host optimum is slow, it seems likely that multiple strains could coexist within a host. Alternatively, if mutation is sufficiently common, then viral strains at the within-host optimum could act as continual sources for viral strains at the between-host optimum, leading to viral strain coexistence. We are currently pursuing a more general analysis of the nested models presented here in which hosts are structured by the density of the different viral strains. This will be necessary to study conditions for viral coexistence (M.A. Gilchrist and D. Coombs, in progress). Ideally such work will also be extended to include heterogeneity between hosts (e.g. Ganusov et al., 2002).

Whether looking at chronic or transitory infections, an advantage of the nested model approach over the 'black box' approach taken by most studies is that it allows us to link the behaviour of important biological parameters at the between-host level to their likely underlying causes at the within-host level. Researchers can then explore how variation in the underlying biology at the within-host level or in the link between within-host processes and between-host parameters can affect how natural selection acts on a pathogen (Ganusov and Antia, 2003). Alternatively, nested models can provide more specific criteria or hypotheses about the underlying biology necessary for particular evolutionary behaviour. Our understanding of the within-host dynamics of viral infections is incomplete but improving, with extensive collaborations between experimental and theoretical teams (e.g. Dixit et al., 2004; Dahari et al., 2005). We propose that understanding the within-host dynamics and how they are linked to the epidemiological level will be necessary steps towards a general theory of the evolution of parasite virulence.

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