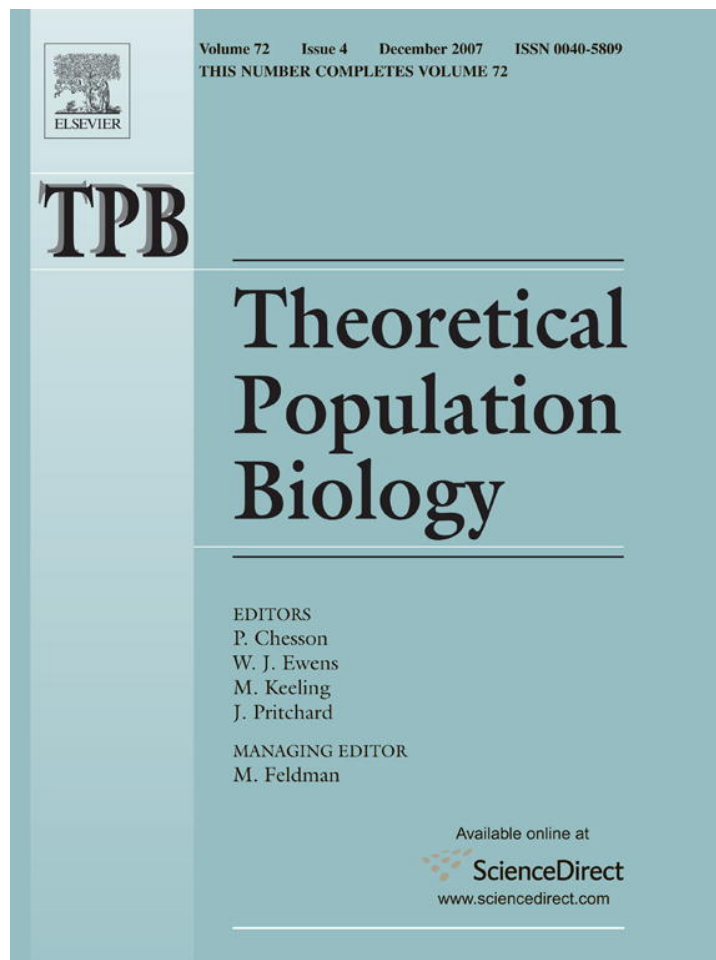


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Evaluating the importance of within- and between-host selection pressures on the evolution of chronic pathogens

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Abstract

Infectious pathogens compete and are subject to natural selection at multiple levels. For example, viral strains compete for access to host resources within an infected host and, at the same time, compete for access to susceptible hosts within the host population. Here we propose a novel approach to study the interplay between within- and between-host competition. This approach allows for a single host to be infected by and transmit two strains of the same pathogen. We do this by nesting a model for the host–pathogen dynamics within each infected host into an epidemiological model. The nesting of models allows the between-host infectivity and mortality rates suffered by infected hosts to be functions of the disease progression at the within-host level. We present a general method for computing the basic reproduction ratio of a pathogen in such a model. We then illustrate our method using a basic model for the within-host dynamics of viral infections, embedded within the simplest susceptible–infected (SI) epidemiological model. Within this nested framework, we show that the virion production rate at the level of the cell–virus interaction leads, via within-host competition, to the presence or absence of between-host level competitive exclusion. In particular, we find that in the absence of mutation the strain that maximizes between-host fitness can outcompete all other strains. In the presence of mutation we observe a complex invasion landscape showing the possibility of coexistence. Although we emphasize the application to human viral diseases, we expect this methodology to be applicable to be many host–parasite systems.

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Keywords: Evolution of virulence; Epidemiology; Short-sighted evolution; Parasite coexistence

1. Introduction

Infectious pathogens compete for resources at multiple levels. For example, within an infected host, viruses compete for appropriate host cells to infect while simultaneously, at the level of population of hosts, they compete for new hosts. We can imagine similar situations for other microparasites such as bacteria, protozoa, or fungi. At all such levels, natural selection operates on the pathogen, and such a *nesting* of selection pressures is a generic feature of host–parasite interactions. Indeed, the importance of selection at the epidemiological level, as opposed to the level of the individual virion or parasite, is one of the few generally accepted cases of group selection (Lewontin,

1970; Levin and Pimentel, 1981). (For completeness we note that viruses may compete for access to replication machinery and resources at the within-cell level; Krakauer and Komarova, 2003; Regoes et al., 2005. Such a competition adds another level of complexity which we do not consider here.)

The existence of selection at multiple levels raises the possibility of conflict in the direction of selection between levels. Just as importantly, if the selection pressures at different levels do conflict, how is the conflict resolved?

In simple epidemiological models, parasite fitness is described by the basic reproduction ratio of the parasite, defined along the lines of

$$R \sim \frac{\beta}{\delta + \alpha + \gamma}, \quad (1)$$

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where β , δ , α , and γ are constant rates of parasite transmission between hosts, uninfected host mortality, virulence (defined here as a pathogen-mediated increase in host mortality), and recovery from infection, respectively (Anderson and May, 1979, 1983; Levin and Pimentel, 1981; Getz and Pickering, 1983). This ratio can be multiplied by the density of susceptible hosts to find the basic reproduction number, R_0 . Eq. (1) clearly indicates how virulence α is detrimental to pathogen fitness. As a result, studies focused on the maximization of R generally assume that any observed non-zero virulence levels result from a tradeoff between virulence, α , and the transmission rate of the infection, β . Early work in this area explored this idea by examining explicit relationships between virulence and transmission (Anderson and May, 1983; Frank, 1992; Lenski and May, 1994). In these studies the relationship between virulence and transmission is assumed, rather than being derived from a description of the biological interaction between the host and the parasite.

A number of studies have worked to address this problem of how to link virulence and transmission. Beginning with Sasaki and Iwasa (1991), researchers began to conceptually link within-host processes to between-host processes. Such nested approaches are more mechanistic in nature and allow one to create an indirect relationship between transmission and virulence based on explicit biological assumptions about within- and between-host processes. Sasaki and Iwasa's (1991) work was followed by a number of studies of acute infections by Antia et al. (1994), Antia and Lipsitch (1997), and Ganusov et al. (2002) which included various biological aspects such as a host-immune response, host heterogeneity, and a threshold mortality function. Using an arguably more realistic mortality function, Gilchrist and Sasaki (2002) developed a similar framework of nested models which also considered the evolution of a host-immune response. Other important studies using nesting approaches to model acute infection include André et al. (2003) and André and Gandon (2006).

More recently, Alizon and van Baalen (2005) and Gilchrist and Coombs (2006) have independently extended these nested approaches to chronic infections. The two approaches differ primarily in terms of their within-host models. The within-host model used by Alizon and van Baalen includes an immune response but ignores resource competition. In contrast, the within-host model we use ignores any immune response and includes resource competition.

In contrast to the idea of between-host selection and the relationship of virulence and transmission driving the evolution of virulence, Levin and Bull (1994) argue that parasite virulence may be the by-product of competition (i.e. natural selection) at the within-host level. The idea that selection at the within- and between-host scales may conflict was an underlying theme in earlier theoretical work by Nowak and May (1994) and, more recently, has been examined in greater detail (Gilchrist and Coombs,

2006; Boldin and Diekmann, in press). Empirically, the importance of within-host evolution has been recognized in studies of chronic infections such as HIV (e.g. Levin et al., 1996; Li et al., 2004; Arien et al., 2005; Gao et al., 2005) and fitness on this level has many analogies to between-host fitness, R (Gilchrist et al., 2004).

In this paper we extend the framework we developed previously and propose a unified approach to modeling competition of parasites at both the within- and between-host levels. This approach is based on the identification and the linkage of submodels for four key aspects in the parasite life-cycle. We will explicitly consider the dynamics of parasite competition within individual hosts. At the between-host level, we allow more than one parasite strain to be transmitted during an infection event, but suppose that each host is infected only once. To simplify the analysis, we do not consider the effects of complex population structures such as host heterogeneity or interaction networks (Pfennig, 2001; Ganusov et al., 2002; Boots et al., 2004; Keeling and Eames, 2005). Further, we assume that the strains interact indirectly through competition for target cells and only consider competition between two different strains. We do not explicitly include the host-immune system in our model.

2. Methods

In developing the general formulation, we will consider the following four linked aspects (Fig. 1) of a within-host model, a description of the initial state of a new host, a model for the epidemic spread, and a way of linking disease transmissibility and virulence to the within-host state of a host. We now describe each in turn (Table 1).

1. *Within-host model*: We assume a within-host model that precisely describes the time evolution of the host–pathogen interaction. Such a model might include: mutation of the virus, the onset of host immunity, details of damage to the host, etc. For example, in the second part of this paper we focus on a chronic viral disease and employ a simple model of viral infection that can be written as three differential equations describing the evolution over age of

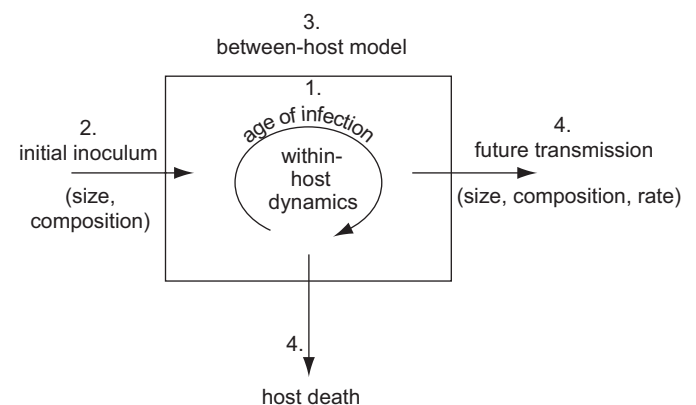


Fig. 1. The key aspects of a nested model.

Table 1
Symbols and parameters of the detailed model

Symbol	Description
<i>Within-host (virus dynamics) model</i>	
T, T^*, V	Host state variables: target cell density, infected target cell density, and free virion density.
λ, d	Birth rate and per-capita death rate of target cells.
k_1, k_2	Mass-action infectivity of free virions of strains 1 and 2 (both assumed equal to k).
p_1, p_2	Intracellular production rates of strains 1 and 2 (assumed not equal).
$\mu(p)$	Per-capita death rate of a cell infected with a p -producing virus. Assumed concave-up.
c_1, c_2	Clearance rates for free virions of strains 1 and 2 (both assumed equal to c).
p_{\max}, p^*	Maximum virus production rate and optimal virus production rate.
N_1, N_2	Burst sizes (expected numbers of new virions produced by a single infected cell).
ρ_1, ρ_2	Within-host reproductive numbers for each virus strain.
ξ	Initial virion concentration.
a	Age of infection.
<i>Between-host (epidemiological) model^a</i>	
$\beta(V)$	Mass-action infectivity of a host with virus density V . Assumed increasing.
$\alpha(T)$	Per-capita additional mortality of a host with target cell density T . Assumed decreasing.
b, δ	Host birth and per-capita death rates.

^aSee also Table 2.

infection a of the state variables:

$T(a)$ = density of uninfected target cells,

$V(a)$ = density of freely circulating virions and

$T^*(a)$ = density of productively infected target cells.

Although simple in structure, models of this form have been used to study many aspects of chronic viral infections such as HIV and can be fit to data from patients or animal models (Perelson et al., 1996; Neumann et al., 1998; Perelson and Nelson, 1999; Nowak and May, 2000; Stafford et al., 2000). Modifying such a model to allow for competition of more than one strain of the virus is easy to do (see the detailed example below) and allows us to examine pathogen competition at the within-host level and derive an expression for pathogen fitness at this level alone (Nowak and May, 2000; Coombs et al., 2003; Gilchrist et al., 2004). Such models do not explicitly include the host-immune system.

2. *Initial inoculum*: Infection begins with the introduction of a small mixture of pathogen strains into a host whose state variables represent the uninfected state. For example, in the example outlined above, we could specify initial conditions:

$$(T(0), T_1^*(0), T_2^*(0), V_1(0), V_2(0)) = (T^0, 0, 0, V_1^0, V_2^0) \quad (2)$$

representing the initial densities of uninfected target cells, infected target cells infected by strains 1 and 2, and free viruses of strains 1 and 2 (we suppose that new infections are caused by the transfer of free virus, not of infected cells). The initial densities of strains (V_1^0, V_2^0) are presumably functions of the nature and intensity of contact between the hosts and, crucially, the state variables of the transmitting host. At this point, we have not made any specific assumptions about the function mapping the state

of the transmitting host to the initial state of a new infection. We note, but do not address, that the evolution of rapidly mutating pathogens such as viruses may be strongly affected by the bottleneck implicit in transmission (modeled by Bergstrom et al., 1999).

3. *Between-host model*: The epidemiological literature contains many possible between-host models based on differential equations. To avoid unnecessary complications we will present here a modification of the simplest possible susceptible–infected (SI) differential equation model with identical hosts and a mass-action contact law. This model, detailed below, has disease-dependent parameters governing host mortality rate and infection transmissibility only. Despite our focus on this simple model, we emphasize that our approach can be modified to allow for more complex population structures (SIS, SIR, etc.), frequency- or vector-dependent transmission, or even non-differential equation-based models.

4. *Link epidemiological parameters to host state variables*: We now need to understand how the internal state of an infected individual affects the epidemiological parameters, and, as a result, the fitness of the pathogen at the between-host level. Our basic supposition is that the host state variables will govern the between-host parameters. It is reasonable to suppose, for example, that the viral load of the transmitting host should affect the rate at which it releases infectious inocula and, in turn, the probability of a successful transmission of an inoculum per host–host interaction. Conceptually, if we decompose the transmission rate of an infection into an interaction rate component and a probability of transmission per interaction, and assume that the infection does not affect the activity level of a host, the transmission rate of an infection should be an increasing function of viral load. Additionally, we assume that transmission is instantaneous and that the inocula which initiate a new infection are fixed in size and reflect

the strain mix of the infected host at the time at which it produces the inocula. Similarly, we suppose that the host mortality will depend on the state variables (for instance, as a function of the reduction in host resources).

3. Competition in an SI model

We now describe the simplest possible variant of an SI model that allows the internal state of infected hosts to vary over the course of an infection. We consider two viruses competing within each host and structure individuals according to their initial viral inoculum. We suppose that any initial inoculum can be described by the variable x_0 . In particular, we have in mind a model where $0 \leq x_0 \leq 1$ represents the fraction of viruses of strain 1 in a general inoculum composed of varying amounts of strains 1 and 2 (see Fig. 2 and detailed description below). Bearing this in mind, we will refer to x_0 as the initial strain mix of an infected individual. However, more general notions of the inoculum, including those with more than two strains, are compatible with the following analysis.

We let $S(t)$ represent the number of susceptible hosts in the population at time t and $I(t, a, x_0)$ represent the density of infected individuals that were initially infected by an inoculum of type x_0 at time $t - a$. We suppose that susceptible hosts join the system (through birth or immigration) at a fixed rate of b individuals/time and die at a fixed rate of δ per time. Infected individuals randomly contact susceptibles and new infections are generated at a

rate $\beta(a, x'_0, x_0)$. $\beta(a, x'_0, x_0)$ is the transmission rate from individuals that were initially infected with strain mix x_0 to new infections of strain mix x'_0 . While we structure the infected hosts by their initial strain mix, we emphasize that the transmission rate and mixture of strains being transmitted change with the age of the infection a . Although the notation we use obscures this fact somewhat, our approach does take these within-host changes into account. Finally, infected individuals are subject to natural mortality (at rate δ) and additional mortality due to the infection, at a time- and initial strain-mix-dependent rate $\alpha(a, x_0)$.

The dynamics are thus described by the integro-differential equation system

$$\frac{dS}{dt} = b - \delta S - S \int_0^\infty \int_0^1 \int_0^1 \beta(a, x'_0, x_0) I(t, a, x_0) dx_0 dx'_0 da, \quad (3)$$

$$\frac{\partial I(t, a, x_0)}{\partial t} + \frac{\partial I(t, a, x_0)}{\partial a} = -(\delta + \alpha(a, x_0)) I(t, a, x_0), \quad (4)$$

$$I(t, 0, x'_0) = S \int_0^\infty \int_0^1 \beta(a, x'_0, x_0) I(t, a, x_0) dx_0 da. \quad (5)$$

Note that in this study we assume that the transmission rate between hosts is dependent on the density of virions within a host. Because we focus on the equilibrium state of the whole system, changing this assumption to represent frequency-dependent transmission affects our conclusions only by a scaling factor.

Infected hosts die at a rate given by $\alpha(a, x_0)$, so we can simplify the governing equations by defining the survivorship probability for individuals infected by strain mix x_0 , a time units ago, $\sigma(a, x_0)$ as

$$\sigma(a, x_0) = \exp\left(-\delta a - \int_0^a \alpha(z, x_0) dz\right). \quad (6)$$

Further,

$$I(t, a, x_0) = I(t - a, 0, x_0) \sigma(a, x_0). \quad (7)$$

By performing this integration, we incorporate the partial differential equation (4) into Eqs. (3) and (5) to find an integro-differential equation and renewal condition:

$$\frac{dS}{dt} = b - \delta S - S \int_0^\infty \int_0^1 \int_0^1 \beta(a, x'_0, x_0) I(t - a, 0, x_0) \times \sigma(a, x_0) dx_0 dx'_0 da, \quad (8)$$

$$I(t, 0, x'_0) = S \int_0^\infty \int_0^1 \beta(a, x'_0, x_0) I(t - a, 0, x_0) \times \sigma(a, x_0) dx_0 da. \quad (9)$$

3.1. The basic reproduction number

At the beginning of an epidemic, the total number of new infections of strain type x'_0 caused by a single infected host

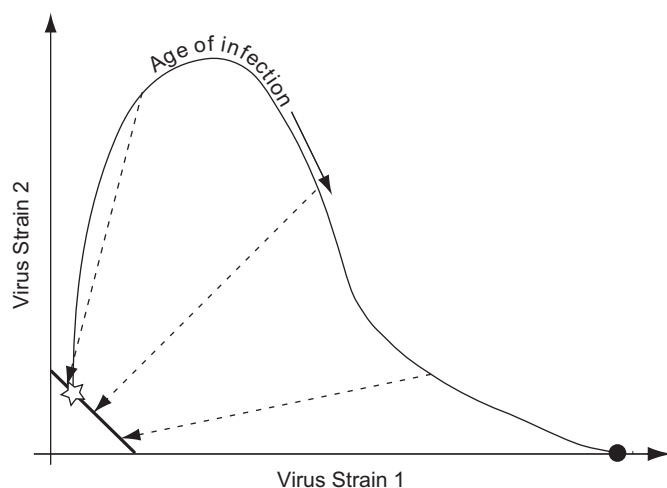


Fig. 2. A simple model for the initial inoculum. We consider two strains of a particular virus competing within an individual host and suppose that the initial inoculum of virus has a fixed size but that its make-up reflects the distribution of viruses within the transmitting host. Here, we plot the density of each virus within a particular host as a phase plane against age of infection. The individual is infected at the star with a particular strain mix. As time progresses and the within-host dynamics evolve, the individual transmits to new hosts (dashed lines). The within-host dynamics eventually reach an equilibrium where strain 2 is excluded (black dot). The initial states of new hosts lie along the (thick) line of fixed inoculum size. Their positions on that line depend on the state of the transmitting host via Eqs. (19)–(20).

with an initial strain mix x_0 is

$$S_0 \int_0^1 \Phi(x'_0, x_0) dx_0$$

where $\Phi(x'_0, x_0) = \int_0^\infty \beta(a, x'_0, x_0) \sigma(a, x_0) da$ (10)

is the transmission kernel and $S_0 = b/\delta$ is the initial number of susceptibles. $\Phi(x'_0, x_0)$ measures the total density of new infections of strain mix x'_0 (per available susceptible) produced by a single host who was infected by strain mix x_0 , over the lifetime of that host per unit density of susceptible hosts S . Using Φ , we can construct the next generation operator K where

$$K[i(x_0)] = \int_0^1 \Phi(x'_0, x_0) i(x_0) dx_0. \quad (11)$$

K acts on a function describing the density $i(x_0)$ of current infections with a given initial strain mix and gives the corresponding density of new x_0 infections in the next generation of infection.

The basic reproduction ratio, R_0 , is usually defined as the fold increase in the number of infected individuals caused by a small number of infectious individuals introduced into a wholly susceptible population. The term R_0 is a threshold quantity that determines whether the epidemic can possibly take hold or not (Diekmann and Heesterbeek, 2000; van den Driessche and Watmough, 2002; Heffernan et al., 2005). Since we structure infected individuals by a and x_0 , we must allow for the structure of the initial infective population in our definition of R_0 . In order to maintain the threshold nature of R_0 , we define it to be S_0 times the largest eigenvalue of the next generation operator, i.e. the largest solution Λ of the eigenvalue equation

$$K[i(x_0)] = \Lambda i(x_0). \quad (12)$$

Using this definition, R_0 represents the maximum rate of increase of the number of infectives over all possible structures of the initial infectious population (Diekmann et al., 1990). In Appendix A, we analyze competitive exclusion and coexistence of virus strains within this model, based on properties of Φ . We note here that our model inherits the simplicity of the basic SI model with respect to separating S_0 from the next generation operator (Dieckmann, 2002).

4. Detailed example: competition of two chronic viruses within an SI model

To demonstrate how the preceding approach may be applied, we now specifically examine competition of two chronic virus strains in an extremely simplified setting. We establish the four model elements introduced earlier in turn:

1. *Within-host model:* Chronic viral diseases (e.g. HIV, HCV) have been extensively studied using simple mechanistic models (Perelson et al., 1996; Neumann et al., 1998; Perelson and Nelson, 1999; Nowak and May, 2000;

Stafford et al., 2000). We will use a simple variant of such models that allows for within-host competition and inter-strain mutation between two strains: 1 and 2. We take as our state variables the density of uninfected target cells (T) as well as the free viruses and infected target cells T_i^* for each strain ($i = 1, 2$). We suppose the time dynamics of the numbers of target cells, infected target cells, and free viruses are modeled by the system of equations

$$\frac{dT}{dt} = \lambda - dT - (k_1 V_1 + k_2 V_2) T, \quad (13)$$

$$\frac{dT_1^*}{dt} = (1 - \varepsilon) k_1 V_1 T + \varepsilon k_2 V_2 T - \mu(p_1) T_1^*, \quad (14)$$

$$\frac{dT_2^*}{dt} = (1 - \varepsilon) k_2 V_2 T + \varepsilon k_1 V_1 T - \mu(p_2) T_2^*, \quad (15)$$

$$\frac{dV_1}{dt} = p_1 T_1^* - c_1 V_1, \quad (16)$$

$$\frac{dV_2}{dt} = p_2 T_2^* - c_2 V_2. \quad (17)$$

Uninfected target cells are produced at rate λ , die at rate d , and are infected by free virions according to the law of mass action with rates k_i . Infected target cells of two types are produced by this interaction. Infected target cells produce free virions at rates p_i and die with rates depending on this production rate according to a function $\mu(p)$. Free virions are cleared at rates c_i . In this model, cells may be infected only once and only by a single viral strain. The term $\mu(p)$ includes the background death rate of target cells (d) and we suppose that $0 < p \leq p_{\max}$ where p_{\max} is the maximum rate of virion production that allows a persistent infection to occur. The host parameters λ and d and the infected target cell death rate function $\mu(p)$ are assumed to be the same for all hosts. The small parameter ε governs the rate at which the viruses mutate from one strain to another and we suppose all mutations occur at the point of infection of a new target cell. This assumption is motivated by the fact that, following infection of a target cell by HIV, the unreliable machinery of reverse transcription copies the viral genetic information into the host. New copies of the viral RNA are then produced by the more-reliable host machinery. We also note that there is no direct competition between viral strains, except for host resources (target cells), in this model.

It is possible to show that the appropriate measure of within-host viral fitness for strain i is

$$\rho_i = \frac{N_i k_i (1 - \varepsilon) \lambda}{c_i d} \quad \text{where } N_i = \int_0^\infty p_i e^{-\mu(p_i) a} da = \frac{p_i}{\mu(p_i)} \quad (18)$$

is the *burst size*, the average number of progeny virions produced during the lifetime of a single infected cell (Gilchrist et al., 2004). ρ represents the net reproduction ratio of a virion within a single host and is analogous to the net reproduction ratio of an infection in a population,

R . ρ is a measure of within-host fitness while R is a measure of between-host fitness. In the absence of mutation ($\varepsilon = 0$), fitter (higher ρ) viruses competitively exclude weaker (lower ρ) viruses. We previously showed that supposing $\mu(p)$ is an increasing, strictly concave-up function (i.e. $\mu''(p)$ is strictly positive) there is a single optimal production rate p^* that maximizes the burst size (Coombs et al., 2003). Assuming that virulence α is a decreasing function of host resources co-opted by the infection (i.e. target cells T), it follows that the viral strains with the within-host optimal production rate p^* are also the most virulent (Gilchrist and Coombs, 2006). This is because such strains decrease the host target cell density by the greatest amount.

Using the form of $\mu(p)$ given in Table 2, p^* is relatively low compared to the maximum production rate allowing persistence in the host. This is conceptually similar to arguments at the epidemiological level for the evolution of disease virulence that are based on an increasing, concave-up relationship between disease transmissibility and host mortality. Here, we will consider viruses that differ only in their production rate, $p_1 \neq p_2$, and so we take $k_1 = k_2 = k$ and $c_1 = c_2 = c$. The within-host behavior of this model is analyzed extensively in Ball et al. (2007) and we draw upon these results when needed.

In the model that includes mutation, the uninfected steady state always exists at $T = \lambda/d, T_i = 0, V_i = 0$ for $i = 1, 2$ and there is a single infected steady state with both viruses present where the virus with higher ρ dominates provided ε is sufficiently small (Ball et al., 2007). In the model without mutation ($\varepsilon = 0$), at infected steady state, only the virus with higher within-host fitness ρ is present.

In all of the cases below, we will consider the within-host dynamics for two viruses, one with production rate $p_1 = p^*$ (optimal) and one with $p_2 \neq p^*$. We begin by examining the

within-host dynamics for the least complicated scenario where we suppose $p_2 < p^*$. Under this scenario the density of the within-host optimum p^* virions initially increases faster than the p_2 virions. Furthermore, because it is the superior within-host competitor, p^* quickly drives the density of target cells T below the level at which the p_2 virions can replace themselves. Thus, after a small initial spike, the density of p_2 virions declines exponentially towards zero while the density of p_2 virions asymptotically approaches its equilibrium value V_1 .

Next we examine the scenario where $p_1 = p^*$ and $p^* < p_2$. In contrast to the previous scenario, here we observe that V_2 may greatly exceed V_1 at early times, before eventually being outcompeted (Fig. 3). However, there is a threshold production rate p^\dagger above which V_2 is outcompeted by V_1 at all times, even if V_2 would, by itself, be able to establish a chronic infection.

Supposing that $p^* < p_2 < p^\dagger$, we see that the two strains have, in a sense, adopted distinct strategies (Fig. 3c, d). The optimal within-host strain eventually competitively excludes any other strain from the host and, as a result, inocula transmitted late in the infection are primarily composed of virions of the p^* strain. On the other hand, the competing p_2 strain initially grows faster than the p^* strain and, as a result, inocula transmitted early in the infection are primarily composed of virions of the p_2 strain.

Although this initial p_2 dominated phase may be short, it can have a disproportionate contribution to between-host fitness due to the fact that the transmission kernel Φ weights transmission by survivorship (cf. Eq. (10)). In other words, the potential for late-stage transmissions contributes less to the between-host fitness of the strain because the host may die before this stage is reached. It is in this range ($p^* < p_2 < p^\dagger$) that this initial domination by the p_2 strain can provide it a sufficient advantage at the epidemiological level such that it may be able to out-compete p^* when we consider the nested model.

As we described earlier, the instantaneous host mortality rate is proportional to the host's number of target cells which varies throughout the infection. Since the rapidly growing strains ($p > p^*$) are more successful at colonizing the host during the initial stage of infection, it might be thought that hosts die because of the rapid reproduction of the rapidly growing strain during that initial stage. However, numerical experiments (not shown) using the parameters from Table 2 indicate that there is very little difference in the minimum target cell density over the range of production rates $p^* < p < p^\dagger$. Furthermore, the rate of recovery from this minimum level to the steady state is an increasing function of p on this range. Overall, the virulence during the initial stage of infection is very similar for viral strains with production rates in this range, so this is not an important effect.

2. Initial inoculum: For viral diseases, the initial inoculum is very small compared to the eventual peak viral load and the initial growth of the virus is thought to be approximately exponential. Therefore, except for small variations

Table 2
Parameter estimates and functions used in the detailed model

Within-host model parameter	Estimate
λ (cells/ μ l/day)	0.1
d (/day)	0.01
k (cell/day)	6.5×10^{-4}
$\mu(p)$	$d \exp(0.0043p)$
c (/day)	3
p_{\max}	1300
p_{\min} (/day)	4
p^* (/day)	230
ξ (virions/ μ l)	10^{-5}
Functions of between-host model	
Transmissibility	$\beta(V_1, V_2) = b_1 (V_1 + V_2)$
Virulence	$\alpha(T) = a_1 \delta(T_0 - T)$
Between-host model parameter	Estimate
a_1	Variable
δ (/day)	10^{-4}
b_1	Scaled out

See Appendix B for details.

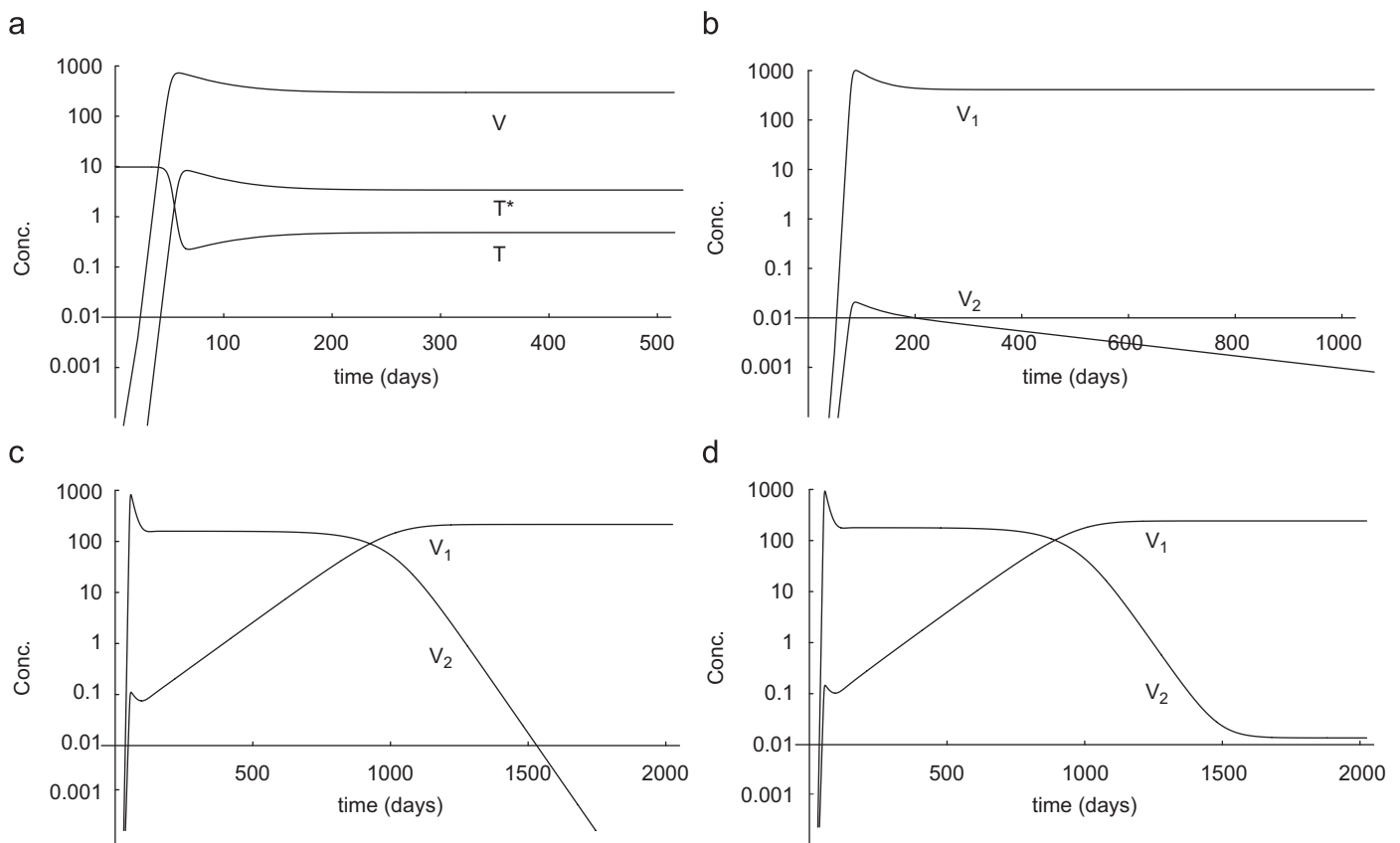


Fig. 3. A simple virus dynamics model. (a) We plot T , T^* , and V for the one-strain analogue of our particular within-host model. The initial inoculum size is supposed to be sufficient to achieve an initial viral titer of 10^{-5} virions/ μl . (b–d) We plot V_1 , V_2 using the full model with the following parameters: (b) $p_1 = p^*$, $p_2 = 0.5p^*$ (second virus slow producer; equal numbers of each virus initially; no mutation). (c) $p_1 = p^*$, $p_2 = 2p^*$ (second virus fast producer; equal numbers of each virus initially; no mutation). (d) $p_1 = p^*$, $p_2 = 2p^*$ (second virus fast producer; equal numbers of each virus initially; mutation parameter $\epsilon = 10^{-5}$).

in the time to the viral peak (Fig. 2), the exact size of the initial inoculum is not important so long as the transmission leads to onset of a full infection (Perelson and Nelson, 1999; Nelson et al., 2004). Thus, for simplicity, we assume that the inoculum size is independent of the state of the transmitting host, but the proportions of the viral strains in the new infection are equal to that of the transmitting host at the time of transmission. This is consistent with a model where infected hosts produce infection packets at a rate dependent on their total viral load, but the probability of infection by multiple packets is small enough to be neglected.

As a specific transmission model, we define the strain mix at the time of infection as $x_0 = V_1/(V_1 + V_2)$ to be the fraction of viruses of type 1 within the transmitting individual and write

$$V_1^0 = \xi x_0 \tag{19}$$

and

$$V_2^0 = \xi(1 - x_0), \tag{20}$$

where ξ represents a fixed initial virion concentration introduced by each inoculum. On the V_1 – V_2 phase plane, new infections have an initial condition along the line

$V_1 + V_2 = \xi$. The point on the line is chosen by taking the position of the infected individual, and drawing a straight line to the origin. The intersection of the two lines is the new initial condition (Fig. 2). While different x_0 values lead to different trajectories in the V_1 – V_2 phase plane, all trajectories converge asymptotically upon the same final state where the superior within-host competitor, strain 1, has excluded the inferior competitor, strain 2.

In summary, we have the initial conditions

$$(T(0), T_1^*(0), T_2^*(0), V_1(0), V_2(0)) = (\lambda/d, 0, 0, \xi x_0, \xi(1 - x_0)). \tag{21}$$

ξ is the inoculum concentration and x_0 is the strain mix $V_1/(V_1 + V_2)$ of the transmitting host at the time of infection.

3 and 4. *Between-host model and model linkage:* At the between-host level we will use the SI model described above with the functional forms for β and α given in Table 2. Here, we use the simplest linear forms for β and α that reflect the assumption that, as the target cell density decreases, the host mortality increases, and that transmissibility is proportional to total viral load (Ragni et al., 1998; Quinn et al., 2000; Chakraborty et al., 2001; Gray et al.,

2004). We also assume that hosts are infected at most once (see the Discussion).

In general we write the transmission rate *from* individuals that were initially infected with strain mix x_0 to new infections of strain mix x'_0 as $\beta(a, x'_0, x_0)$. This notation is useful for the formation and analysis of the nested model. However, in order to clearly highlight the assumption that the total transmission rate caused by a host depends only on that host's total viral load, in Table 2 we write the transmission rate as $\beta(V_1(a), V_2(a))$. This latter notation also provides a clearer link to that of Gilchrist and Coombs (2006).

We solve the equations numerically by converting the integral representations to matrices. This is equivalent to allowing only a discrete number of possible initial infection states in the model (see Appendix B for details). We specify parameter values in Table 2. Throughout, we will consider two strains of the virus, strain 1 with production rate $p_1 = p^*$, the within-host optimal strain, and strain 2 with an arbitrary p_2 . For each case, we plot the steady-state fraction of transmissions of strain 1 over a range of values of p_2 .

5. Results

The model we presented extends the earlier model of Gilchrist and Coombs (2006) by incorporating within-host dynamics and pathogen mutation. Within the earlier model, we found that a conflict between the within-host optimal competitor and the between-host optimal competitor could occur under certain circumstances. We first analyze the scenario where there are just two competitive strains. We use these results to extrapolate to a more general framework of more than two competing strains.

We begin our analysis of our new model by considering the special case where there is no mutation between strains. This assumption will afford us semi-analytical insights into the role of mutation in the general case.

5.1. The case with no mutation

In order to determine whether strain coexistence is possible in the mutation-free model we begin by noting that even hosts infected with a mixture of strains those that survive long enough will eventually transmit essentially pure-strain inocula. This is important because, in the absence of mutation, hosts infected by only one strain produce only new infections of that strain. In our example, given that strain 1 eventually outcompetes strain 2 within a host, there must exist a sub-population of hosts which is infected solely by strain 1. This sub-population is an absorbing state which grows monotonically over time. Simultaneously, the set of all multi-strain infections declines monotonically over time. Noting that at the SI model equilibrium (a) each infected host generates one new infected host over its infectious lifetime and (b) multi-strain infections eventually convert to pure-strain infections and,

thus, contribute to the pure-strain-1 sub-population, it follows that mixed strain infections cannot exist at equilibrium. Thus, in the absence of mutation, the equilibrium population can only consist of hosts infected purely by a single strain.

Thus, in the absence of mutation, the equilibrium population can only consist of pure-strain infected hosts. While any mixed strain infections will ultimately give rise to pure-strain-1 infections, it is still possible that before an equilibrium is reached the number of pure-strain-2 infections in the host population will grow at a faster rate than the pure-strain-1 infections. Thus, whether this population consists solely of strain 1 or 2 depends on the fitness of the two pure strains.

To determine which strain will dominate the system, we note that our multi-strain, co-infection model can now be replaced by a much simpler two pure-strain model which has been examined extensively (Levin and Pimentel, 1981; Anderson and May, 1983; Getz and Pickering, 1983). In such a model, the reproduction ratio for each strain can be shown to be

$$R_i = \int_0^{\infty} \beta(V_i(a))\sigma(a) da, \quad i = \{1, 2\}, \quad (22)$$

where V_i and σ are calculated by integrating the within-host model. In this simpler system, the strain with higher R_i value will competitively exclude the other strain.

Using the functional forms from Table 2 with the mutation rate $\varepsilon = 0$, we examine viral strain competition and numerically verify the argument given above. We plot the results in Figs. and 5a. We find that, at equilibrium in the population, only a single virus strain persists. This strain is the strain with the higher individual value of R (illustrated in Fig. 5b for one case).

Eq. (22) gives a precise criterion for dominance, but we can also interpret our results verbally. We find that the within-host optimal strain competitively excludes any virus for which $p < p^*$ (Fig. 5). In this case, strain 1 expands more quickly upon initial infection, and is not outnumbered by the second strain at any future time during the infection. As a result all new infections are primarily composed of strain 1. Second, we see that strains with *slightly* higher p values competitively exclude strain 1. This is explained by noting that a slightly higher p value leads to initial dominance (through the early phase of the infection), contributing to a high rate of new strain 2-dominated infections during this phase (because the individual is probably still alive, and because the virus load is at its peak during this phase). Further, the time taken for strain 1 to overtake the strain 2 within the host is long if $p^* \simeq p_2$. For p_2 greater than a threshold that depends on the host sensitivity to resource loss parameter a_1 , we see that strain 1 is again dominant. We also observe that the width of the window in which strain 2 is dominant becomes wider as the host sensitivity parameter a_1 increases. This is as we would expect: strain 2 is mostly transmissible at early stages of infection, which are weighted increasingly highly as the sensitivity increases.

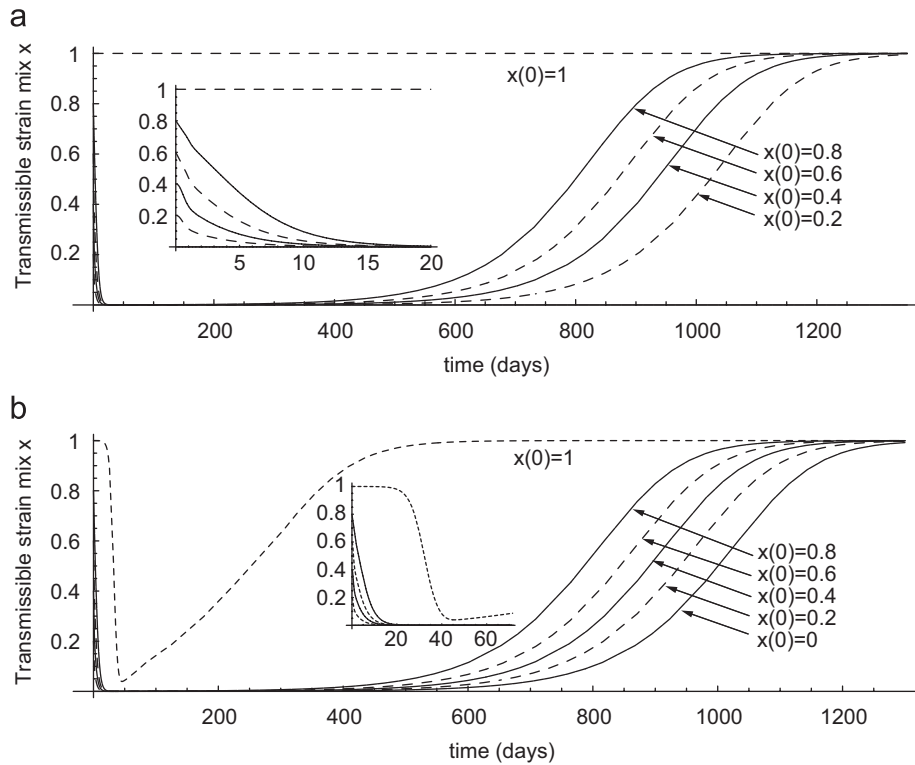


Fig. 4. Dynamics of transmissible strain mix. We plot the transmitted strain mix from an individual of age of infection a for a variety of initial infection strain mixes of that individual. The initial inoculum size is supposed sufficient to achieve an initial viral titer of 10^{-5} virions/ml and we suppose that strain 1 reproduces at the optimal rate $p_1 = p^*$ and competes with a faster-reproducing strain 2, $p_2 = 2p^*$. (a) Evolution of strain mix $x = (V_1/(V_1 + V_2))$ for different initial ratios of virus (no mutation). Inset shows short time dynamics. (b) Evolution of strain mix $x = (V_1/(V_1 + V_2))$ for different initial ratios of virus (with mutation: $\varepsilon = 10^{-5}$). Inset shows short time dynamics. Comparing inset of (a) and inset of (b) we see that a low level of mutation prevents strain 2 from entirely dominating the within-host competition over the first few hundred days of infection.

After a few generations of genuine within-host competition, the two viruses compete only at the between-host level, in the search for uninfected hosts. In terms of resolving potential conflicts between within- and between-host dominant strains, we observe that it is the individual reproduction ratio R that determines the dominant strain, rather than the within-host measure of fitness, ρ . R as defined in Eq. (22) is the age-of-infection-structured analogue of the basic reproduction ratio (Eq. (1)) and, as such, indicates the importance of between-host selection.

5.2. The case with mutation

Using mutation rate parameters of $\varepsilon = 10^{-7}$ and 10^{-5} , a more complex picture emerges (Fig. 6). First, we observe that, for $p_2 > p^*$ or $p_2 < p^*$ (both of which conditions imply p_2 does not expand in the host if p^* is also present; see above), p^* dominates the two systems completely.

For $\varepsilon = 10^{-5}$ and 10^{-7} , in the presence of mutation and at low host sensitivity to target cell death, we find that no strain outcompetes p^* completely. Consider the extreme case of zero host sensitivity (i.e. $a_1 = 0$) and moderate to low background host death rate. Under this scenario, a long-lived host always yields dominance by p^* (not shown) since late transmissions will not be substantially discounted

by the risk of prior host death. As a_1 increases from zero, a range of competitors which can stably coexist with the p^* strain emerges. This indicates that, if host sensitivity is low, the within-host selection is important in determining viral fitness overall. Co-infection occurs essentially because even a pure transmission of strain 1 generates, via mutation and expansion of strain 2 at the within-host level, some rare new infections that initially contain a large fraction of strain 2 (see Fig. 4).

Surprisingly, two bands of successful competitors to p^* appear for intermediate levels of host sensitivity ($a_1 = 5$). First, a band of competitors with slightly higher production rates succeeds because they expand because the time to replacement by p^* in the host increases as p_2 approaches p^* , and therefore they are rarely replaced by p^* during the host lifetime. The second band of competitors with intermediate p_2 succeeds because they expand rapidly early on in the infection process, before either host death or their competitive exclusion within the host.

At high single, wide levels of host sensitivity ($a_1 = 20$), p_2 dominates p^* over a single, wide range of p_2 -values. In this region between-host competition leads to exclusion of the p^* strain from inocula causing new infections, due to the pressure to infect new hosts before the death of the current host. The rare host that survives for a long period of time will primarily be infected by the p^* strain.

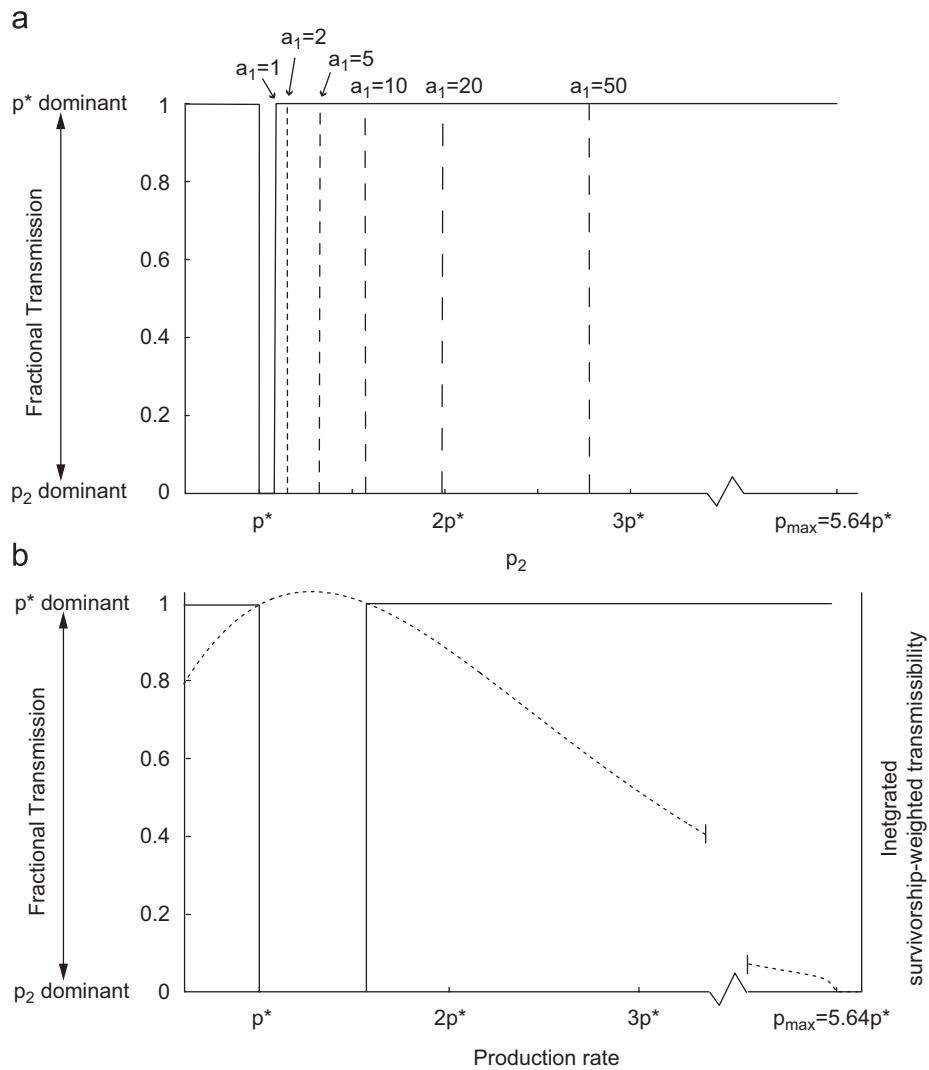


Fig. 5. Dominant strain with no mutation. (a) We plot the overall fraction of strain 1 virions transmitted across the whole population, at steady state of a two-strain competition, for a variety of levels of host sensitivity. For each level of host sensitivity, the fraction of strain 1 transmitted is either 0 or 1. Strain 1 is the within-host dominant strain. Host sensitivity to disease is controlled by a_1 as given for the functional form (I) in Table 2. For all values of a_1 , strain 1 is completely dominant for $p_2 < p^*$, followed by a window where strain 2 will outcompete strain 1. For high values of p_2 , $p_2 \approx p_{\max}$, strain 1 is always dominant. (p_{\max} is the maximum value of p that allows a single-strain infection to establish itself within a host.) Coexistence of strains is not found. (b) For $a_1 = 10$, we replot panel (a) (solid line) superimposing the reproduction ratio $R = \int_0^\infty \beta(V_1(a) + V_2(a))\sigma(a) da$ as a function of p , for a single infection (dashed line). This shows dominance of the strain with higher R .

5.3. Strain replacement

In the case with no mutation, Eq. (22) tells us exactly which of all possible strains will dominate: the strain with the greatest between-host fitness R in the absence of any within-host competition. On the other hand, if mutation is allowed pure-strain infections are impossible and the situation becomes more complex. In Fig. 7 we show the results of general two-strain competition scenarios. We find, as before, pairs of strains that lead to single-strain dominance (black indicates strain 1, white indicates strain 2) and pairs that coexist.

We can use these plots to understand certain kinds of invasions (Dieckmann, 2004). Suppose that a single strain is present in the population, corresponding to a starting point on the diagonal, and further suppose

that only nearby mutants will arise. Then if the diagonal cell has a white cell below it and a black cell above then the single strain can be invaded and replaced by the next lower-valued strain. In the diagram, this corresponds to a move diagonally downwards and to the left. Conversely, a strain whose diagonal cell has a black cell below it and a white cell above it can be replaced by the next higher-valued strain. Therefore, a single strain will be stable to the introduction of nearby strains if its diagonal cell has black cells above and below it with white cells to its left and right (indicated by S in the figure).

However, this locally stable strain may still be invaded by substantially different strains that can achieve coexistence. The single strain is only stable to invasion by distant (non-stepwise) strains if all of the cells below and

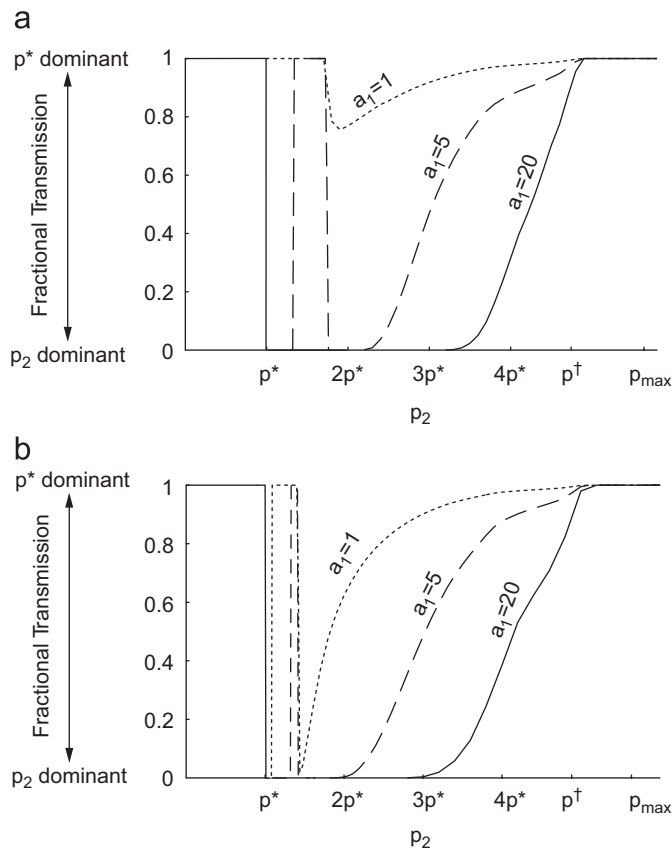


Fig. 6. Dominant strain with mutation. (a) We plot the overall fraction of strain 1 virions transmitted across the whole population, at steady state of a two-strain competition, for a variety of levels of host sensitivity to loss of target cells and with the mutation parameter $\epsilon = 10^{-5}$. Strain 1 is the within-host dominant strain with cellular production rate p^* . Host sensitivity is controlled by a_1 as given for the functional form in Table 2. We observe a substantial range of p_2 values for which coexistence of the two strains is predicted. (b) As panel (a) except lower mutation rate: $\epsilon = 10^{-7}$.

above its diagonal cell are black and all the cells to the left and right are white.

6. Discussion

We have presented a general scheme to study the competition of parasites such as viruses that incorporates selection at both the within- and between-host levels. At the within-host level, virus strains compete for host cells, while at the between-host level, infections must compete for susceptible hosts. We then presented a specific example where we applied our method to the competition of two strains varying in intracellular production rate, finding situations in which the optimal within-host strain could be excluded from the host population by a superior between-host competitor. We also found (provided a low level of mutation from one strain to the other was permitted) situations in which both the strains were able to persist in the population of infected hosts.

Our results complement and extend recent studies of nested models in which the transitory dynamics of the within-host system were ignored (Day and Proulx, 2004; Alizon and van Baalen, 2005; Gilchrist and Coombs, 2006) and provide a approach within which to understand when short-sighted evolution to a more virulent state can be predicted (Levin and Bull, 1994). In particular, Day and Proulx (2004) used a quantitative genetics method to highlight interesting transitory dynamics of virulence in epidemic models, allowing for different modes of transmission, recovery, and within-host mutation. Here, we have explicitly modeled the within-host dynamics while ignoring the dynamics at the epidemiological level and, instead, focusing on the equilibrium state of the entire nested model. Fully resolving the dynamics at both the levels is a

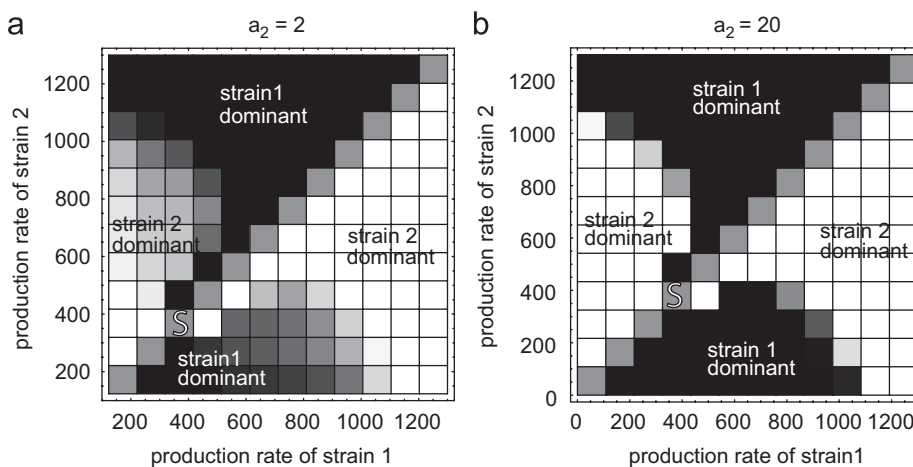


Fig. 7. Pairwise competition and coexistence. Plots indicate the equilibrium strain mixture achieved by two competing strains. Darker/lighter values indicate greater representation of strain 1/2, respectively. Cells along the diagonal $p_1 = p_2$ are 50% gray, representing a single-strain equilibrium. Symbol S indicates a locally stable single-strain equilibrium. $p^* = 230$ /day. (a) Low sensitivity of host mortality to resource loss ($a_1 = 2$). (b) High sensitivity of host mortality to resource loss ($a_1 = 20$).

challenging prospect that may be best approached by simulation (Ball, 2006).

We also provide a more mechanistic alternative to arbitrarily assumed rates of strain switching within host in multi-strain epidemiological models (Lipsitch and Nowak, 1995; Li et al., 2004). The nested structure of our framework is similar to a recent metapopulation study of two competitors with explicit local dynamics (Feng et al., 2004). Here, however, unlike most metapopulation models, because infections are chronic, the within-host viral populations are inherently stable rather than unstable (Hanski, 1998) and only go extinct as a result of the death of the host as opposed to because of clearance within the host. Interestingly, recent empirical evidence suggests that hosts themselves may represent metapopulations of viral populations isolated to different cell types or locations within a host (Frost et al., 2001; Jridi et al., 2006).

In our specific example, we consider only two strains of a particular virus, and suppose that switching between strains of different characteristics occurs due to mutation, but in a continuous way (i.e. not as a stochastic event). Therefore, our model bears hallmarks of the phenomenon of HIV coreceptor switching, where early-stage disease is often marked by a predominance of virions that bind the CCR5 coreceptor on host cells. In about half of all patients, a strain of virus binding CXCR4 on target cells (or both CCR5 and CXCR4) dominates later on (Regoes and Bonhoeffer, 2005). This switch is also known to impact *in vitro* competitive fitness (Troyer et al., 2005; Arien et al., 2006). We emphasize that the mechanism whereby we achieve an early/late switch (via a change in viral production rate in our model) is not thought to underlie the switch in HIV.

In the within-host model we use here, the competing strains interact indirectly through their competition for uninfected cells. Therefore, it is perhaps not surprising that, as a result, in the absence of mutation, there is competitive exclusion. Alizon and van Baalen (2005) use an alternative within-host model in their nested framework which does not include such competition but, instead, includes an immune response. Using the methods developed here for incorporating selection at both the within- and between-host scales, it would be interesting to see how the addition of an immune response affects the ability for strains to coexist. By increasing the number of resources for which the viruses compete to include 'immune response free' space (similar to the enemy-free space discussed in predator-prey studies (Holt, 1977; Jeffries and Lawton, 1984; Holt and Lawton, 1994)), we hypothesize that the range of parameter space that allows strain coexistence would increase.

6.1. Single vs. multiple infection events

In this study we supposed that hosts are infected at most once, although potentially by more than one virus strain. We did not consider the possibility of subsequent infection

events (superinfections). Our specific model does (in a sense) capture the within-host effects of superinfection, in that (provided mutation is present) there is a constant small influx of both viral strains in all patients. Because inocula are so small relative to the size of the viral population soon after the infection is established, it makes no difference whether that influx is from mutation, or from superinfection events. Superinfection would provide a way for the superior within-host competitor to take over hosts infected with the other strain even in the absence of mutation. The likelihood of this happening would depend on the infection structure of the population, providing an interesting feedback from the epidemiological level to the within-host level (cf. Nowak and May, 1994; van Baalen and Sabelis, 1995; Mosquera and Adler, 1998), and would also tend to favor more transmissible and less virulent viral strains (André and Godelle, 2006).

A new approach to superinfection has recently been presented by Boldin and Diekmann (in press). In their model, the possibility of invasion of the resident strain by a superinfecting strain is derived from an explicit within-host model. The within-host dynamics are supposed to be fast compared to the time between infection events, and so the resident infection is taken to be at steady state. Depending on the details of the superinfection process, the relative importance of within- and between-host fitness is then determined. Within a framework that explicitly includes the within-host dynamics, the age of infection of the host at the moment of superinfection would be important since it would change the lag time before the superinfecting strain could take over the host, as well as determining the subsequent transmission rates from superinfected hosts. We plan to consider these points in future work.

6.2. Competitive exclusion of within-host optimal strain

Using our detailed model of viral dynamics coupled to an SI epidemic model, we found that the best (long-term) within-host competitor could be competitively excluded at the between-host level by another within-host competitor which was transmitted more effectively during the early stages of infection. We note that our approach naturally incorporates the within-host dominance of one strain over another along with the need to find new hosts, allowing us to evaluate the importance of within- and between-host selection in a given situation. Generally speaking, we find that as the sensitivity of the host's mortality rate (i.e. virulence) to resources co-opted by the infection increases, the range of within-host competitors which can ultimately prevail over the best long-term within-host competitor increases. This is due to the fact that future transmission by a late-dominating within-host strain is greatly discounted due to the large risk of host death early in an infection. This finding suggests that virus coexistence may be dependent on the relative 'importance' to the host of the type of cell it infects.

When mutation is not present in the system, competitive exclusion is strict: the strain with the larger R will ultimately prevail. However, when mutation is present, exclusion is curiously different: the overwhelming majority of transmissions are exclusively of the weaker within-host strain but the disease mutates to the best within-host strain and this is the eventual winner within any host that survives for a sufficient period of time. Hosts initially infected by the best within-host strain generate the weaker strain through mutation, which then expands within the host temporarily leading to it being preferentially transmitted. In our model, virus strains found in long-term carriers are more aggressive (they use the host resources better and so depress the level of target cells further), but, depending on the circumstances, may only be responsible for a small fraction of new infections. Therefore, in a situation where strains 1 and 2 coexist in a population (e.g. see Fig. 6), a vaccine against strain 2 would result in quicker dominance of strain 1, increasing the mortality rate of the infected population. However, from a public health perspective the increase in mortality might be partially offset by a decrease in the rate of infections (Gandon et al., 2001, 2003; André and Gandon, 2006).

6.3. Understanding nested models

Within-host models for infectious diseases of clinical significance have progressed rapidly in recent years. More complex models than that given here can incorporate a great deal of experimental and clinical details and paint a more accurate picture of specific diseases. We believe that this detailed understanding should be leveraged to improve our understanding of large-scale pathogen evolution, epidemic spread over time, and emergence of new pathogens. Given the great diversity of host–parasite systems, it is clear that no single model will explain every aspect of all the host–parasite interactions. However, we hope that the method presented here can be used to incorporate detailed cell-level biology into our understanding of the evolution of parasite virulence, appropriately evaluating the importance of selection pressure at multiple levels.

Acknowledgments

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Appendix A. Competitive exclusion and coexistence

Without making any assumptions about Φ we can look at conditions for existence of an endemic equilibrium. The

mathematical framework for a similar (but simpler) question is explained in detail in e.g. Chapter 8 of Brauer and Castillo-Chavez (2000) using an example where individuals are structured by age. Here, individuals are structured by age of infection a and by their initial state x_0 . We write the steady-state (i.e. time-independent) distribution of infected individuals of age 0 as $I(t, 0, x_0) = \hat{i}(x_0)$ and the steady-state density of susceptibles as \hat{s} . Upon substitution of $\hat{i}(x_0)$ into (8)–(9), we find that

$$\frac{1}{\hat{s}} \hat{i}(x_0) = \int_0^1 \Phi(x'_0, x_0) \hat{i}(x'_0) dx'_0 = K[\hat{i}(x_0)], \quad (23)$$

$$\hat{s} = \frac{b}{\delta + \int_0^1 K[\hat{i}(x_0)] dx_0}. \quad (24)$$

The equality $K[\hat{i}(x_0)] = (1/\hat{s})\hat{i}(x_0)$ found in Eq. (23) is equivalent to the eigenvalue equation (12). Therefore, any given equilibrium density of new infections $\hat{i}(x_0)$ is an eigenfunction of K with a specific eigenvalue $1/\hat{s}$.

We write such an equilibrium $\hat{i}(x_0) = z f_j(x_0)$ where $f_j(x_0)$ is the j th eigenfunction of K with corresponding eigenvalue Λ_j . Dropping the j subscript for notational simplicity, we find that

$$\frac{1}{\hat{s}} z f(x_0) = z \Lambda f(x_0). \quad (25)$$

Using (24) and (25), we find that the eigenvalue corresponding to this equilibrium is

$$\Lambda = \frac{\delta + \Lambda z \int_0^1 i(x_0) dx_0}{b} \quad (26)$$

with

$$z = \frac{\Lambda b - \delta}{\Lambda \int_0^1 i(x_0) dx_0}. \quad (27)$$

In order for a solution to be biologically plausible, z must be positive. Thus, Eq. (26) implies that for an endemic equilibrium to exist, the dominant eigenvalue Λ must be greater than the equilibrium density of hosts in the absence of the infection δ/b . Equivalently, $R_0 > 1$ for endemicity. This is equivalent to the result that the largest eigenvalue of the next generation matrix must be positive.

To summarize, we have thus found criteria for the existence of endemic equilibria, each one of which corresponds to an eigenfunction of K whose eigenvalue satisfies the inequality $\Lambda > \delta/b$. The next question we must address is ‘How many such eigenfunctions and eigenvalues are there?’ The answer to this question is provided by the spectral theory of linear integral operators that is analogous to the theory of the same name for matrices (Diekmann and Heesterbeek, 2000; van den Driessche and Watmough, 2002).

As mentioned earlier, the operator K acts on a function $i(x_0)$ defined on $0 \leq x_0 \leq 1$ (densities of infected hosts structured by their initial strain mix). Suppose we start with an arbitrary distribution of infected individuals structured by initial condition, $\phi_0(x)$, and operate on it

repeatedly with K (each operation is analogous to one generation of disease spread). The simplest possibility is that, irrespective of $\phi_0(x_0)$, all possible strain mixes are eventually found in the population. That is, for every strain mix y , there is a generation n such that $\phi_n(y) = K^n[\phi_0(x_0)]$ ($y > 0$). This property is known as ergodicity (Reed and Simon, 1978) and such an operator is the analogue of an irreducible, positive matrix. In this case, it can be shown that there is a single dominant eigenvalue (R_0) and it is the only eigenvalue with a non-negative eigenfunction, indicating that there is only one stable distribution of strain mixes $\hat{i}(x_0)$. (Jentzsch, 1912).

However, it should be noted that the operator K need not be ergodic. Under certain scenarios when that is the case, the form of K may result in isolated collections of initial infection states. In that case, the final distribution of infected individuals over x_0 depends on the initial distribution $\phi_0(x_0)$. We illustrate certain distinctions between ergodic and non-ergodic operators in Fig. 8.

Appendix B. Numerical methods

Numerically, it is not convenient to work with the integral representations given above, so we will approximate the interval of initial strain mixes $0 \leq x \leq 1$ by $n + 1$ initial strain mixes taken to be at $\{0, 1/n, 2/n, \dots, (n - 1)/n, 1\}$. Analogous to the continuous transmission operator K given above, we generate the discrete transmission matrix M . The transmission matrix, M , is a $n \times n$ matrix where each element m_{ij} is defined to be the number of new transmissions with strain mix $x_0 = i$ caused by a host with initial strain mix $x'_0 = j$, over the lifetime of the infection per unit density of susceptible hosts.

Practically, the transmission matrix M is generated by numerically integrating the within-host dynamics (Eqs. (13)–(17)) to find time courses for the host state variables, using the initial condition corresponding to a particular strain mix. We then use the functions β and α to calculate the rate at which this individual causes new

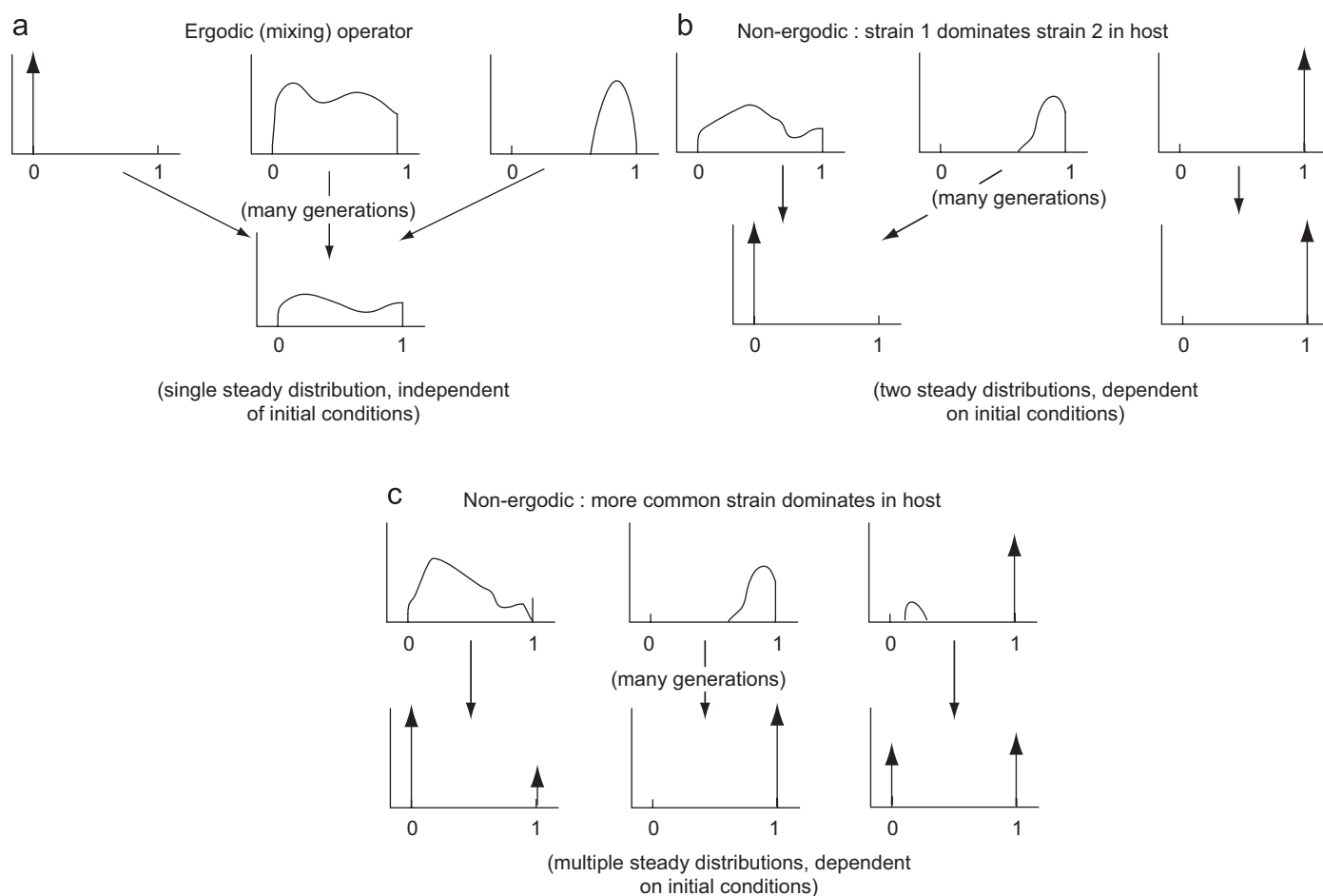


Fig. 8. Contrasting next generation operators K . We plot three examples of distributions of initial strain mixes across initially infected individuals, for three example cases. Arrows in graphs are Dirac delta functions. (a) Any ergodic (mixing) operator acts on initial densities of strain mix leading after many generations to a stable distribution of transmitted strain mixes that is independent of the initial conditions. (b) A non-ergodic example. Suppose strain 1 quickly dominates strain 2 within the host, so all new transmissions are of strain 1 alone if any viruses of strain 1 were initially present. (We assume no mutation between strains.) All initial distributions of strains lead to a pure-strain-1 state, except if strain 1 is not at all present. (c) A second non-ergodic example. Suppose the strain initially present in greater numbers dominates the within-host competition so that, as the chain of infections occurs, the strain mix tends to a pure strain. Then the final distribution of strain mixes in the population depends on the initial strain-mix distribution. (Again, no mutation.)

infections. These new infections are then partitioned according to the rule that the new infection has the strain mix closest to the transmitting host's current strain mix. We repeat this procedure to calculate all entries of the transmission matrix $M = (m_{ij})$. We then numerically find the eigenvectors and eigenvalues of the transmission matrix M . Positive real eigenvalues with positive eigenvectors correspond to equilibria of the whole system. The entries of the eigenvectors correspond to the equilibrium rate of new infection by each strain mix in our model.

This procedure is appropriate to find stable steady states of the model, and also gives us the fastest-growing initial strain-mix distribution during the initial epidemic dynamics. It is true, however, that we have collapsed the age since infection here and this assumption would not be appropriate if we were concerned with tracking dynamics of strain populations during the approach to steady state.

We specify parameter values in Table 2. Throughout, we consider two strains of the virus, strain 1 with production rate $p_1 = p^*$, the within-host optimal strain, and strain 2 with an arbitrary p_2 . For each case, we plot the steady-state fraction of transmissions of strain 1 over a range of values of p_2 . In producing results, we took $n = 10$ and the virus dynamics equations were integrated until the probability of survival (σ) fell below 10^{-2} . Numerical experiments were performed to check that this cut-off did not substantially affect our results.

Parameter estimates for the within-host model are taken from studies of HIV (Stafford et al., 2000). $\mu(p)$ is a simple concave-up function chosen so that the observed rate of infected T cell death is 40 times that of uninfected T cells (Stafford et al., 2000). p_{\max}, p_{\min} are the limiting rates of virus production beyond which the virus cannot persist in the host in this model. p^* is the computed optimal viral production rate for this model (Coombs et al., 2003). Transmission is rescaled by the between-host parameter b_1 (so no estimate is required at steady state, other than $R_0 > 1$ so that the disease persists in the population) and our results are plotted for a range of the host sensitivity parameter a_1 .

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