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E-Article

Emergence of a Convex Trade-Off between Transmission and Virulence

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abstract: Most models of virulence evolution assume that a parasite cannot raise its transmission rate without causing more harm to its host. However, the existence of such trade-off relationships has recently been challenged. Here, we study how a trade-off can emerge from a model that explicitly incorporates within-host dynamics. We find that the existence and the convexity of the trade-off are robust, which implies a definite level of evolutionarily stable virulence (ESV) for the parasite. However, we also show that the dependence of the ESV on parameter values may be very strong. One possible consequence of this sensitivity is that relationships between transmission and virulence observed across populations need not conform to the patterns expected on the basis of a common (fixed) trade-off. We discuss possible experiments and implications of our results for the development of virulence management strategies.

Keywords: host-parasite interactions, virulence evolution, immune system, constraints.

Anderson and May (1979) were the first to challenge the conventional wisdom that parasites will always evolve to eventually become completely avirulent. Arguing that a parasite cannot increase its transmission rate without inevitably shortening its infectious period by killing its host or provoking immune clearance, they showed that the parasite should adopt an optimum intermediate level of virulence. This trade-off approach, which has recently been challenged (Levin and Bull 1994; Ebert and Bull 2003), underlies much of the subsequent development of the theory of evolution of virulence.

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In the simplest case, the parasite's basic reproduction ratio is proportional to the product of its infectivity (i.e., the transmission rate) and the duration of a typical infection. If we denote the parasite's transmission rate by β, the natural host death rate by μ, the host recovery rate by ν, the host mortality rate induced by the parasite by α (this will be our definition of virulence), and the density of susceptible hosts by S, we obtain the standard expression for the basic reproduction ratio of a parasite:

$$R_0 = \frac{\beta}{\mu + \alpha + \nu} S \qquad (1)$$

(Anderson and May 1982). Equation (1) implies that the basic reproductive ratio always increases when α decreases. This conclusion seems to support the conventional wisdom, but as Anderson and May argued, it is impossible for parasites to maximize β (transmission) indefinitely without incurring some cost. Usually it is assumed that increasing β cannot be achieved without at the same time increasing α (virulence), as the parasite cannot get transmitted without causing at least some harm to its host (Anderson and May 1982; Ewald 1983). Anderson and May (1982) showed that with such a trade-off hypothesis, parasites will evolve toward a nonzero optimal virulence.

Some authors, such as Levin and Bull (1994) and Ebert and Bull (2003), have challenged the biological validity of the trade-off assumption. They claim there is very little experimental or theoretical evidence for relationships between virulence and transmission. However, even if they are scarce, there are experimental results by the following workers that support the trade-off theory for various host-parasite interactions: Day et al. (1993) for *Plasmodium falciparum* in humans, Ebert (1994) for *Daphnia magna* in a protozoa, Mackinnon and Read (1999*a*) for *Plasmodium chabaudi* in mice, and Messenger et al. (1999) for bacteriophage f1 in *Escherichia coli*. Various theoreticians have also explored how such trade-offs could emerge from the underlying interactions between pathogens and the immune system and transmission ecology of their hosts (Anderson and May 1982; Sasaki and Iwasa 1991; Antia

et al. 1994; Ganusov et al. 2002; Gilchrist and Sasaki 2002; André et al. 2003; Ganusov and Antia 2003).

In an attempt to take up the challenge posed by Ebert and Bull (2003), we extend these previous approaches to study under what conditions a trade-off emerges in a host-parasite system in which the hosts mount an immune response that contains (but does not eliminate) the parasite and, if one emerges, what shape it has. We then investigate the influence of the parameters on this shape and the resulting evolutionarily stable virulence (ESV). Finally, we explore how the ESV could be manipulated. We will discuss our findings in light of the consequences of imperfect vaccines predicted by Gandon et al. (2001) on the basis of a model that did not include explicit within-host dynamics.

Model Presentation

Description of System

Most of the previous studies of trade-off emergence assume that the immune system will eventually clear the infection (André et al. 2003; Ganusov and Antia 2003; but see Sasaki and Iwasa 1991). In many cases, however, the immune system is incapable of clearing the parasite, resulting in persistent infections. The severity of such an infection is determined to a large extent by the efficiency of the immune system (Sasaki and Iwasa 1991). Here, we suppose that the immune system cannot eliminate the parasite. Thus, our approach applies to long-lasting infections (herpes, HIV, tuberculosis, infections caused by *Neisseria*) rather than those that tend to be quickly eliminated (such as influenza). In terms of our model, the case of persistent infection translates to a recovery rate (ν) of 0.

As in previous studies (Antia et al. 1994; Bonhoeffer and Nowak 1994; Ganusov et al. 2002; André et al. 2003), we link the individual scale (within-host dynamics) to the host population scale (epidemiological dynamics), but our study contrasts with previous ones in a number of important ways. First, we do not introduce an arbitrary threshold above which hosts die (Antia et al. 1994; Ganusov et al. 2002; Ganusov and Antia 2003), because it causes the optimal parasite population size to be just below this threshold, which means, as noted by André et al. (2003), that under ESV conditions, no host will die from the parasite. Second, we do not suppose that the host population is heterogeneous (in contrast to Antia and Lipsitch 1997 or Ganusov et al. 2002), because fixed virulence parasites will be maladapted to some hosts (see André et al. 2003 for a more extensive discussion).

In this article, we suppose that every infected host is exploited by a single parasite strain. In reality, it is unlikely that a parasite can completely protect its host from superinfection, but by making this assumption, we ensure that virulence is not a consequence of parasite competition (Nowak and May 1994; van Baalen and Sabelis 1995). Our model can be modified to take into account interactions among multiple strains within hosts, as we will do in a sequel article.

Our approach is inspired by Sasaki and Iwasa (1991), who derived optimal parasite growth strategies from a model for within-host dynamics. We extend their approach by relaxing the assumption of arbitrary Lotka-Volterra-type, density-dependent, within-host growth and taking instead the immune system explicitly into account.

Interactions between Parasites and Lymphocytes

To take the immune system into account, we define a predator-prey-like relationship between lymphocytes and parasites similar to current models in theoretical immunology (Nowak et al. 1990; Nowak and May 1992; Bonhoeffer and Nowak 1994; De Boer and Perelson 1995). We make a number of assumptions to simplify the modeling of the immune system. First, we do not distinguish the humoral response (mediated by the B lymphocyte cells) from the cellular response (mediated by the T lymphocyte cells). We model the immune system in a way that is close to Nowak et al.'s (1990) approach in which each parasite strain is attacked by a specific lymphocyte clone and by a common nonspecific lymphocyte clone. However, as long as there is only one parasite strain in this model, it is not necessary to distinguish between the two lymphocyte populations, because they behave in the same way.

We suppose that the parasite strain (whose population size is denoted x) is specifically recognized by a single lymphocyte clone (y). Thus, we need two equations to describe the dynamics of the within-host system:

$$\frac{dx}{dt} = (\varphi - \sigma y)x,$$

$$\frac{dy}{dt} = b + cx^k - \delta y. \tag{2}$$

Here, φ represents the intrinsic within-host per capita growth rate of the parasite strain, σ the killing rate of the lymphocytes, c the increase of lymphocyte production due to the parasite, b the baseline production rate of the lymphocyte clone, and δ the lymphocyte mortality. Parameter k, which we call cooperativity, reflects the fact that immune response may be activated more easily by an abundant parasite. This phenomenon induces what is called in enzymology a cooperative effect, leading in turn to rates that depend on powers of densities.

The most important difference between our model and

that of Nowak et al. (1990) is in the activation term of the lymphocytes. Here, it only depends on the parasite's density (x), whereas in a classical Lotka-Volterra equation, it would depend on a product of the prey's and the predator's density (xy). We make this modification because lymphocytes can be activated without being in contact with their prey. The term involving the parasite densisty (cx) represents a complicated activation process (Bonhoeffer et al. 1997). Further development of this model could include an entity z that would link parasite identification and lymphocyte activation, for example, cytokine concentration or density of antigen-presenting cells.

In our system the parasite cannot be cleared, because the activation of the lymphocyte population drops when the parasite density diminishes. Our equation for lymphocyte dynamics is close to De Boer and Perelson's (1995) model. In contrast to other approaches (Antia et al. 1994; André et al. 2003), we choose our parameters such that lymphocyte and parasite populations eventually equilibrate. The parasite population is thus regulated through lymphocyte-induced mortality (Nowak et al. 1990; Ganusov et al. 2002; André et al. 2003), and the lymphocyte population is regulated through a density-dependent mortality term (Muller et al. 2001).

This model is conceptually simple but still rather difficult to analyze. To facilitate the calculations, we will assume for the moment that there is no cooperative effect of the lymphocytes (i.e., $k = 1$). We will study the influence of this parameter in a later section.

Parasite Transmission Rate and Induced Mortality

In the absence of parasites (i.e., if $x_0 = 0$), the immune system will equilibrate at a baseline level of lymphocytes ($y_0 = b/\delta$). If the parasite does not reproduce fast enough, that is, if $\varphi < \sigma y_0$, then it cannot establish in the host. In the remainder of this article, we will assume that such immediate clearance does not occur, that is, that the parasite reproduces fast enough. Thus, we are sure the parasite and the lymphocyte populations both reach an equilibrium (denoted $\tilde{x}$ for the parasite and $\tilde{y}$ for the lymphocyte) given by

$$\tilde{x}(\varphi) = \left(\frac{\delta}{c\sigma}\varphi - \frac{b}{c}\right)^{1/k},$$
$$\tilde{y}(\varphi) = \frac{\varphi}{\sigma}. \tag{3}$$

Parasite and lymphocyte densities may overshoot their equilibrium values during the establishment phase of the parasite. The size of the overshoot depends on the parameter values, and we will assume it has no influence on the results.

From these equilibrium values, we now define the two epidemiological parameters that characterize the hosts (β and α). For the parasite's transmission rate (β), we choose a linear function of the within-host parasite density ($\tilde{x}$). This kind of relation is often used for parasites that do not have a specialized dispersal form (Ganusov et al. 2002; Gilchrist and Sasaki 2002; André et al. 2003):

$$\beta(\varphi) = a\tilde{x}. \tag{4}$$

We assume disease-induced mortality (α) is given by

$$\alpha(\varphi) = u\varphi\tilde{x} + w\tilde{y}, \tag{5}$$

which incorporates several assumptions. Following André et al. (2003) and Gilchrist and Sasaki (2002), we assume that the negative effects of the parasite are not proportional to the equilibrium density of the parasites ($\tilde{x}$ alone) but rather to their overall rate of replication ($\varphi\tilde{x}$). Second, we suppose that the immune system itself has a cost that increases the mortality rate, thus a lymphocyte "detrimental effect" (or side effect) term $w\tilde{y}$, implying that the host cannot raise its defenses without paying a price. We will refer to the immediate negative effect of the parasites as their toxicity to distinguish it from their virulence, which represents their overall effect on mortality.

If we substitute the values obtained in equations (3) for $\tilde{x}$ and $\tilde{y}$, and if $k = 1$, we get

$$\alpha(\varphi) = \frac{u\delta}{c\sigma}\varphi^2 + \frac{cw - \sigma ub}{c\sigma}\varphi,$$
$$\beta(\varphi) = \frac{a\delta}{c\sigma}\varphi - \frac{ab}{c}, \tag{6}$$

which implies that the parasite's within-host growth rate (φ) is positively correlated both with the parasite's virulence (α) and with its transmission (β).

Results

Trade-Off between Transmission and Virulence

A parasite can maximize its basic reproduction ratio R_0 (eq. [1] with $\nu = 0$, as no recovery occurs) by optimizing the combination of transmission $\beta(\varphi)$ and virulence $\alpha(\varphi)$, which amounts to choosing the optimal within-host growth rate (denoted φ^*). By varying the parasite's growth rate (φ), we can use equations (6) to draw the trade-off curve in the $(\alpha[\varphi] + \mu, \beta[\varphi])$ plane (fig. 1). The parasite's optimum is then obtained at the point where the tangent

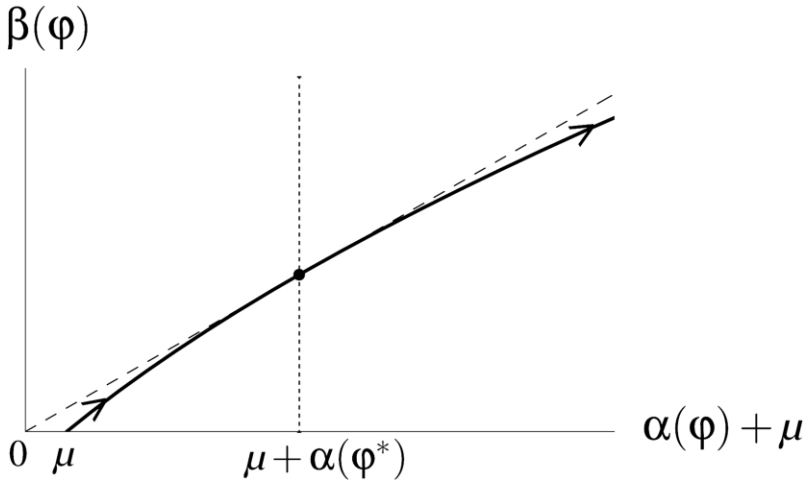

Figure 1: Trade-off between mortality and transmission. The parametric curve $(\alpha[\varphi] + \mu, \beta[\varphi])$ is plotted for $\varphi \in [0.01, 10]$ (arrows on the solid curve follow the increase of φ). The virulence optimizing the parasite's R_0 is given by the tangent of the parametric curve that passes through the origin (*dashed line*); it crosses the curve at the point where $\varphi = \varphi^*$ (*black dot*). The evolutionarily stable virulence is symbolized by a vertical dotted line.

of this curve passes through the origin (Anderson and May 1982; van Baalen and Sabelis 1995).

The key point in figure 1 is that the curve is convex: the parasite can always increase its infectivity $\beta(\varphi)$, but only at an accelerating cost in terms of its host's mortality $\alpha(\varphi)$. Whenever the emerging trade-off is convex, we are sure that an optimum growth rate φ^* exists. The virulence associated with this growth rate is called ESV because parasites with this virulence can resist invasion by mutants differing in virulence (Bremermann and Pickering 1983). Figure 1 also reveals that convexity is not very pronounced, meaning that the value of the ESV can vary easily with small parameter changes.

The Influence of Parameters on the Trade-Off

The example shown in figure 2 is based on parameters that are as realistic as possible. From within-host models (Nowak et al. 1990; Sasaki and Iwasa 1991; Antia et al. 1994; Bonhoeffer et al. 1997; André et al. 2003) or in vitro data (see Muller et al. 2001 for an example of parameter estimation in an HIV infection), we have some indications about general values for the baseline production rate of lymphocytes within a host ($b < 0.04$ lymphocytes activated/h), for the lymphocyte killing rate ($\sigma \leq 1$ parasites killed/lymphocyte/h), for the parasite growth rate ($\varphi \in [0.1, 10]$ parasites born/parasite/h), and for the lymphocyte mortality rate ($\delta \in [0.01, 1]$/h). Therefore, to be in a realistic range, we pick $\delta = 1$, $b = 0.01$, $\sigma = 1$, and $\varphi \in [0.02, 10]$. For the parameter k, we pick $k = 1$, as explained above. The parameter c (increase of lymphocyte production) is problematic because it represents a black box that accounts for parasite identification and lymphocyte multiplication. We arbitrarily fix $c = 10$ lymphocytes activated/parasite.

We also have to fix parameters for the epidemiological model. These parameters are defined with respect to a different timescale (the year) in contrast to within-host processes, which are defined with respect to a much faster timescale (the hour). For the transmission efficiency, we pick $a = 1$ host infected/parasite/year without loss of generality. For the toxicity constants, we pick a parasite toxicity (u) bigger than the lymphocyte side effect coefficient (w): $u = 0.5$ and $w = 0.25$. Finally, for the natural death rate (μ), authors pick many different values ($\mu \in [0, 2]$) because it depends strongly on the host type. We know the mean lifetime of a host is μ^{-1} units of time. We pick $\mu = 0.02$, which gives a life expectancy of the host of 50 years.

Because we have to make some arbitrary choices to set our parameter values, we need to study the consequences of parameter variations so as to assess the robustness of our trade-off relation. We obtain an idea of the parameters' influence by changing them one by one while keeping the others constant. In figure 2 (for changes in epidemiological parameters) and figure 3 (for changes in immunological parameters), we plot trade-off curves for different parameter values (*solid curves*). For each of these curves, we indicate the optimal growth combination for the parasite (*black dot*). By linking these optima, we can thus predict the consequences of parameter variation on the optimum toward which the within-host parasite population will

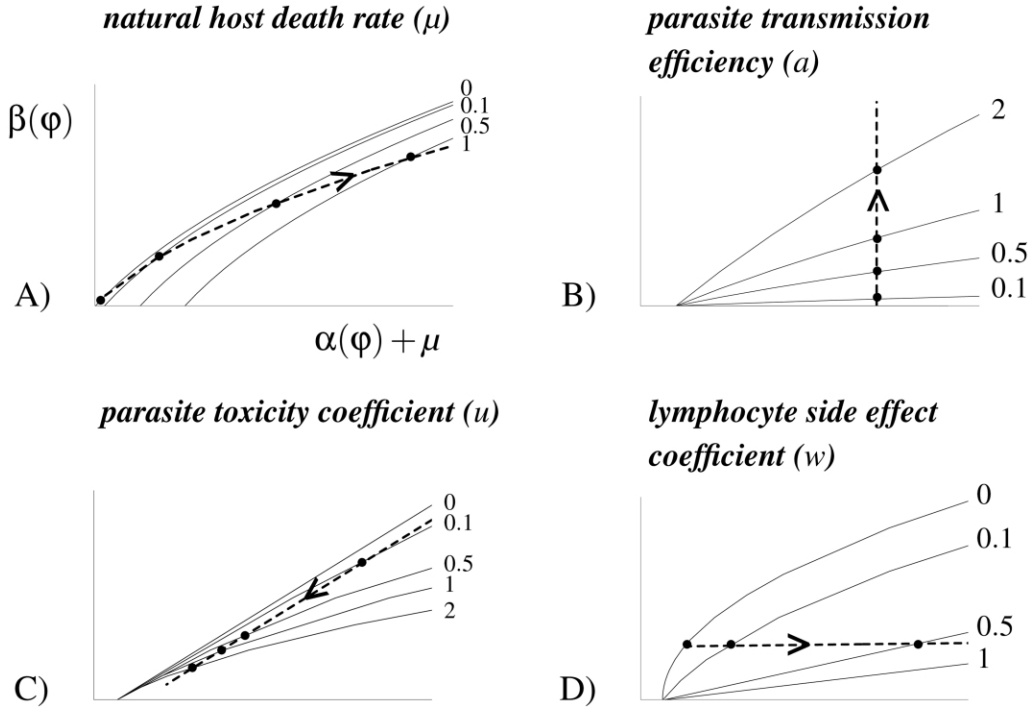

Figure 2: Influence of epidemiological parameters on the trade-off curve. Solid curves are the trade-off curves for a given parameter value. Dots show the evolutionary equilibrium value of a curve. The dashed curve reflects the consequences of an increase (the direction is given by the arrow) of the parameter's value. In all panels, the horizontal axis represents total mortality rate ($\alpha[\varphi] + \mu$), and the vertical axis represents transmission ($\beta[\varphi]$).

evolve (*dashed bold curve*). These results are summarized in table 1.

Robustness of the Convexity of the Trade-Off Curves

Figures 2 and 3 illustrate the robustness of the convexity of the trade-off curve for wide ranges of parameter values. In our model, both virulence and transmission are functions of the parasite's within-host growth rate (φ). The convexity comes from the fact that when φ changes, the increase in virulence ($\alpha(\varphi)$) accelerates faster than the increase in transmission ($\beta(\varphi)$). Mathematically, the trade-off function is convex if

$$\frac{d^2\beta}{d\alpha^2} < 0,$$

which, if $d\alpha/d\varphi \neq 0$ and $d\alpha^2/d\varphi^2 > 0$, leads to

$$\frac{d^2\alpha(\varphi)}{d\varphi^2} > \frac{d^2\beta(\varphi)}{d\varphi^2} \tag{7}$$

(see app. B). The derivative $d\alpha/d\varphi$ is nonzero as virulence depends explicitly on the within-host growth rate, but that $d\alpha^2/d\varphi^2 > 0$ is less obvious; here, it holds because virulence depends on the overall rate of replication (φx).

If we substitute the expressions for α and β in equation (7), the condition for convexity becomes, after a bit of manipulation,

$$2u\frac{d\tilde{x}}{d\varphi} + (u\varphi - a)\frac{d^2\tilde{x}}{d\varphi^2} + w\frac{d^2\tilde{y}}{d\varphi^2} > 0, \tag{8}$$

which shows that even without making explicit assumptions about the relationship between $\tilde{x}$ and $\tilde{y}$ and φ, the curvature of the trade-off curve depends on the slope and curvature of $\tilde{x}$ with respect to φ and on the curvature of $\tilde{y}$ with respect to φ.

For the specific model that we consider, we know from equations (3) that if $k = 1$, then $d^2\tilde{y}/d\varphi^2 = 0$, $d^2\tilde{x}/d\varphi^2 = 0$, and $d\tilde{x}/d\varphi \geq 0$. In this case, the condition for convexity always holds provided that $d\tilde{x}/d\varphi \neq 0$.

Thus, convexity is robust and does not depend on parameter values. The only parameter values for which the trade-off is not convex are the ones we excluded because $\tilde{x}$ and $\tilde{y}$ are not defined (if $\varphi < b\sigma/\delta$, or if $c = 0$, or if

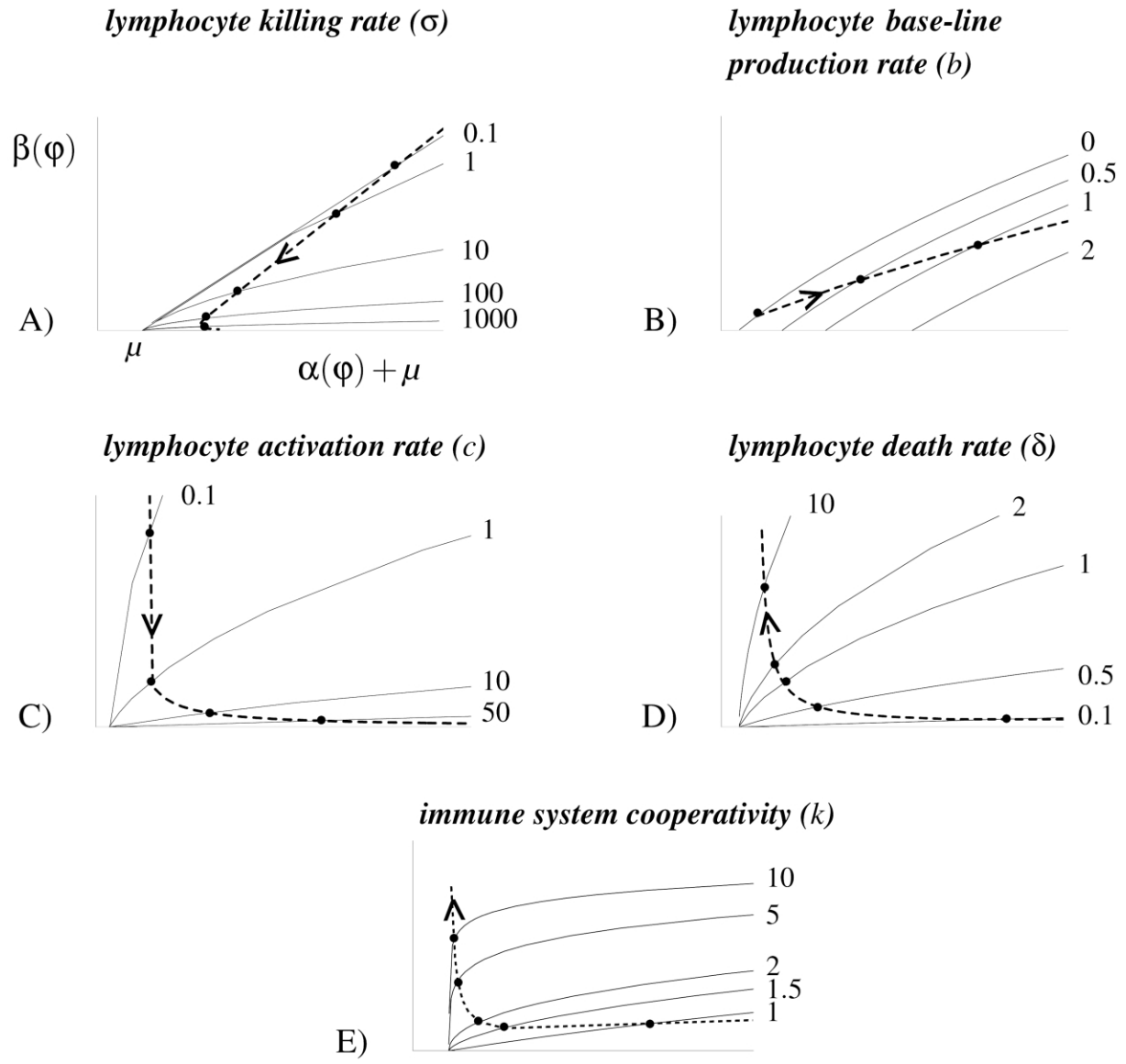

Figure 3: Influence of immunological parameters on the trade-off curve; for further details, see the legend to figure 2

$\sigma = 0$) or because R_0 cannot be derived (if $u = 0$, or if $\delta = 0$). Parameter k could influence the convexity of the trade-off (eqq. [3]), but with the definitions we choose (eqq. [2], [4], [5]), the trade-off is still convex if $k > 1$. The only problem with such a cooperative effect of the lymphocytes is that the immune system may clear the parasite infection more easily, which puts this case outside the scope of our article (which is about persistent infections). This is why if $k > 1$ the convexity depends on the immune system's parameter values (b and σ must not be too strong, and δ must not be too small).

To obtain a linear or a concave trade-off, we would need to fundamentally change the definition of model components. Modifications that suggest themselves are the equation for lymphocyte dynamics (eqq. [2]), for transmission (eq. [4]), and the definitions for virulence (eq. [5]).

Condition 8 suggests that if $\tilde{y}$ were an accelerating function of φ, different results would be obtained. For example, fast-replicating viruses do more damage to the cells they exploit and thus are easier to identify by the immune system. This would imply that the killing rate of the lymphocytes (σ) is a function of φ instead of a constant. In our model, however, $\tilde{y}$ is always linear.

Table 1: Parameters' effects on the evolutionary equilibrium

Parameter increasing	α	β	Figure
Lymphocyte killing rate (σ)	↘ then ↗	↘ then 0	3*A*
Lymphocyte basic growth rate (b)	↗	↗	3*B*
Lymphocyte activation (c)	↗	↘	3*C*
Lymphocyte mortality (δ)	↘	↗	3*D*
Immune system cooperativity (k)	↘	↗	3*E*
Natural host death rate (μ)	↗	↗	2*A*
Parasite transmission efficiency (a)	0	↗	2*B*
Parasite toxicity (u)	↘	↘	2*C*
Lymphocyte side effect coefficient (w)	↗	≈0	2*D*
Quantity of antibiotics taken by the hosts (m)	↗	0	None

Note: Consequences of an increase of a parameter's value on the virulence and on the transmission at equilibrium. ↗ indicates an increase, ↘ a decrease, and 0 an absence of effect.

We also assumed that β is a linear function of $\tilde{x}$. With a more complex mechanism of transmission (Day 2002), for instance, one that was dose dependent, we might obtain accelerating forms of $\tilde{x}$ that would weaken the trade-off's convexity (eq. [8]).

Finally, if virulence (α) were to depend only on the density of parasites ($\tilde{x}$) instead of the overall rate of replication ($\varphi\tilde{x}$), then we would get a linear trade-off if the cooperative effect of lymphocytes k were not too strong ($k \leq 1$). Such a dependence on parasite density (instead of the overall rate of replication) might correspond to host-parasite interactions where the parasite does not reproduce within the host and where virulence depends on the initial dose of parasite at infection. This seems to be the case for mosquitoes *Anopheles gambiae* infected by *Plasmodium falciparum* (Drakeley et al. 1999).

Variability of the Optimal Level of Virulence

Our results suggest that the trade-off retains its convex shape when parameters change but that virulence and transmission values at the evolutionary equilibrium can vary in any direction when parameters change. In other words, the convex trade-off relationship is robust, but the optimum value is variable. This is important, because most of the immune system's parameters are likely to vary among species and even among individuals. Mackinnon and Read (1999*a*) observed a significant effect of the host on the parasites' transmission values and on the timing of the disease. As the hosts they used were genetically identical, their result stresses the importance of individual (random) variations in immune system functioning on the ESV value. Such variations would mean the optimum strategy would differ for different hosts, blurring the relationship between virulence and transmission. Only if variations are sufficiently small will a single ESV that represents the optimum compromise exist (Gandon et al. 2002).

Even if there is variation among individuals, we can use our results to make inferences about how the hosts can defend themselves against parasites. A host's only aim is to minimize the parasite's optimal level of virulence, whatever the transmission rate. A simple reading of table 1 allows us to see which parameter the host should maximize (if α ↘) or minimize (if α ↗).

Figure 2*A* indicates that the more unstable the parasite's environment (high natural host death rate), the higher the ESV is. This is a classical result in virulence evolution (Sasaki and Iwasa 1991; van Baalen 1998; Gandon and Michalakis 2000). We will consider the parameter μ separately because it does not influence the shape of the trade-off curve, and we suppose we cannot reduce natural host mortality. What we are interested in is which parameter changes will be followed by an evolutionary reduction in virulence.

An increase of the lymphocyte killing rate (σ) first reduces host mortality (α) and parasite transmission rate (β), but above a given threshold ($\sigma \approx 1{,}000$ for default parameter values), host mortality again increases (fig. 3*A*). Hosts with overly large lymphocyte killing rates select for higher parasite virulence.

Varying parameters c (lymphocyte activation rate) and δ (lymphocyte mortality rate) reveals similar patterns (fig. 3*C*, 3*D*); once above a given threshold, host mortality increases strongly ($c > 1$ or if $\delta < 1$, with our default parameters). In these cases, the effect is mainly due to the cost of the immune system, because if we remove the lymphocyte side effect (i.e., if $w = 0$), the pattern disappears (except when $\delta \approx 0$). This means that the detrimental effect of the lymphocytes, more than the effect of the parasites themselves, is responsible for this increase of host mortality. Lymphocytes accumulate in the host, which then dies early from the consequences of its own immune response. Even if it is clear that small values of δ or large values of c are strongly selected against, this is still an

interesting case of conflict between immune cells and the organism.

An increase of the lymphocyte baseline production rate (b) induces an increase of host mortality. Contrary to the results for the two parameters, this increase is not suppressed at all if we remove lymphocytes' side effects (results not shown). This means that virulence comes from the parasites' toxic effect and that increasing parameter b will select for higher within-host growth rates. Finally, note that lymphocyte detrimental effect is not essential for the trade-off to emerge: if $w = 0$, an optimal level of virulence still exists (fig. 2*D*).

All of these variations of immune-system parameters reveal that the more the immune response harms the parasite, the higher the ESV value is. This high value of virulence can depend on the side effects of the immune system (as in the case of the efficiency of parasite detection c, of the lymphocyte mortality rate δ, and of the killing rate σ) or on the parasites' toxic effect (for the baseline lymphocyte production rate b).

Consequences of Imperfect Vaccines for Virulence Evolution

Gandon et al. (2001) studied the expected consequences of vaccines with imperfect efficiency and imperfect coverage. They developed an epidemiological approach (with four classes of individuals: vaccinated or not and infected or not) and supposed an a priori trade-off relationship between virulence and transmission. They incorporate a superinfection process by which one parasite can oust another. With this model, they studied the evolutionary effects of several vaccines. To compare our results with theirs, it is necessary to consider the same case. Here, we study vaccines acting on different forms of immunity (antiparasite growth rate, antitransmission, anti-infection). All of the vaccines have full coverage (all the hosts are vaccinated) and variable efficiency (targeting various life-history traits, the vaccine slows parasite growth but does not kill it altogether).

Without superinfection and with full coverage of the vaccine, Gandon et al.'s (2001) model predicts that anti-infection and antitransmission vaccines have no effect on virulence evolution and that antiparasite growth rate and antiparasite toxicity vaccines both select for higher virulence. In our model, a decrease of the value of the parasite toxicity parameter (u) corresponds quite well to an antiparasite toxicity vaccine. Like Gandon et al. (2001), we find that such a vaccine selects for higher levels of virulence.

In our model, an antiparasite growth rate vaccine alters equations (2) and (5), as it leads to a reduction in parameter φ. Two kinds of effects ensue: the vaccine decreases the within-host growth rate because it prevents the parasites from reproducing, but it also causes parasites to respond by evolving higher "intrinsic" growth rates. This second effect will only be apparent if some hosts of the population are not vaccinated and are thus susceptible to infection by strains expressing higher growth rates than before the vaccination campaign.

Antiparasite growth rate vaccines are similar to the use of antibiotic treatments because they both cause a decrease of parasite density. In our model, such treatments can be modeled by adding an extra mortality term (mx) in equations (2). If we plot a trade-off graph as we did for the other parameters, we find a result similar to the one in figure 2*D* (result not shown): increasing the amount of antibiotics per host (m) selects for higher levels of virulence and has no consequences on the evolution of the transmission rate. Thus, in accordance with Gandon et al. (2001), we found that both antiparasite growth rate vaccines and antibiotic treatments select for higher levels of virulence.

Finally, a decrease of parasite transmission efficiency (a) corresponds to an antitransmission vaccine. Again, our model leads to the same conclusion as Gandon et al. (2001): such a vaccine does not affect virulence (in their article, they find that antitransmission vaccine selects for lower virulence, but this is due to their allowance of superinfection).

Our model can help to predict which evolutionary responses may follow when medical practices become common. An increase in lymphocyte production rate b (fig. 3*B*) can mimic the consequences of a large-scale vaccination campaign: in every host the baseline level of defense is boosted. We find that increasing b leads to higher virulence, suggesting that such campaigns should be reserved for truly dangerous parasites. Other forms of vaccination may increase b less, instead speeding up the immune response after infection. This effect can be modeled by increasing the cooperative effect parameter k (fig. 3*E*), which probably increases with increased immune memory. Assuming no cooperativity ($k = 1$) clearly underestimates the capacity of the immune system to trigger a chain reaction to activate many lymphocytes. If parameter k is higher, the trade-off curve is more convex due to the cost of the immune response. Thus, taking the cooperative effect into account (i.e., choosing $k > 1$) produces ESVs that are less sensitive to changes in other parameters.

Very high values of lymphocyte side effect (w) can be related to autoimmune diseases where the immune system directly attacks the organism's own cells. One possible example is SARS (Sudden Acute Respiratory Syndrome), where it seems that some of the damage is due to immune response attacking the host's own tissues (Nicholls et al. 2003). Interestingly, increasing the cost of the immune

system also slightly increases the parasite's optimal transmission rate, β (and hence its virulence), because if its host dies early, it has to reproduce faster. This is not obvious in figure 2*D*, where the parasite transmission seems to be constant with respect to w, but it appears clearly in the expression that gives $\beta(\varphi^*)$ (eq. [A7]).

We find that host defenses select for more virulent parasites, which reveals a conflict for the hosts. The best strategy for the population is to avoid an arms race with the parasite (cooperation between hosts), whereas for an individual, the best strategy is to protect itself as much as possible (selfishness). Thus, the population and the individual need not share the same optimum, as noticed by van Baalen (1998). For example, the depression of within-host parasite growth rate by antibiotics (m) has to be small for the global virulence to decrease, but for a given host the best strategy is to choose a very high value of m. Our theoretical result suggests that a generalization of these treatments may lead not only to bacterial resistance but also to higher virulence, as supposed by several authors (Ewald 1994; van Baalen 1998; Wilkinson 1999).

A Blurred Trade-Off Relationship

We distinguish two effects that might blur the trade-off relationship, and they occur on different timescales. The first effect is instantaneous and is due to the fact that parameters describing the host-parasite interaction may vary among hosts (immune recognition, efficiency of destruction) even if they have the same genotype. This means that parasites with the same within-host growth rate can express different virulence or transmission values for each host they infect. Antigenic variation of the host and of the parasite will be very important here, an aspect that we will investigate in a sequel article. In addition, experimental setups cannot exclude variation between hosts (Mackinnon and Read 1999*a*). Our results suggest that such small variations may blur the trade-off curve as parasites with the same within-host growth rate will have different values of virulence and transmission depending on the host they find themselves in.

The second effect requires evolution of the parasite. When comparing virulence and transmission values among evolved host-parasite systems (with different genotypes or of different species), it is possible that host populations differ in their immune-system response parameters, which may lead to a pattern in ESV values that is completely opposite to the pattern one would expect from within-host models. For example, studying the influence of the parasites' efficiency (w) of transmission on two groups of hosts having different lymphocyte baseline production rates (b) might give counterintuitive results, as the host with the lowest rate will select for less virulent parasites (fig. 3*B*). Thus, failure to reconstruct trade-off curves by comparing different host-parasite combinations does not imply that trade-offs do not exist in every particular case. Our results suggest that even with such variability, convex trade-offs may underlie the evolution of virulence. What remains to be investigated is exactly now trade-off heterogeneity will affect the evolution of parasite strategies.

Discussion

Recently, Ebert and Bull (2003) have drawn attention to the unsatisfactory state of affairs in which many theories for the evolution of virulence assume a trade-off between transmission and virulence for which, they argue, little experimental evidence exists. A number of studies have suggested that trade-offs emerge quite naturally from underlying principles, such as the parasite's interaction with the immune system (Gilchrist and Sasaki 2002; André et al. 2003). So how do we explain the apparent lack of evidence of such trade-offs?

In this article, we studied a model for the interaction between a parasite and its host's immune system that permitted us to assess the consequences of parameter variations on the evolutionary outcome. Our analysis suggests that the convexity of the trade-off relationship, which is instrumental in determining the location of the optimum strategy for the parasite, depends on the relative costs of parasite reproduction and the activation of the immune system. With our definitions, a convex trade-off emerges, implying that a definite ESV exists (van Baalen and Sabelis 1995), for a very wide range of parameters. However, we also found that the shape of the trade-off, and consequently the value of the ESV, is sensitively dependent on a number of parameters. If these parameters are variable, as some of them are very likely to be (at least among but also within species), plotting virulence-transmission combinations measured in different host-parasite combinations will therefore not expose a common underlying trade-off.

This result has important consequences for our ability to assess these constraints. Consider a particular host-parasite system and think of the kinds of experiments that we could conduct to infer the shape of the immune system. Ideally, we would want to modify the parasite's replication rate and measure the effects on the host. However, practical and ethical considerations usually preclude such experiments, so we are limited to using preexisting variation.

Implications for Treatment at the Host and Population Levels

In our analysis, we cannot infer the impact of the immune system by simply removing it from the model because we do not consider other mechanisms that regulate within-host parasite density. Altering the immune system in a more restricted sense, we find that its efficiency imposes selection on virulence (through parasite toxicity or lymphocytes' side effect). This corroborates experimental (Mackinnon and Read 2003) and theoretical results (Gandon et al. 2001; André et al. 2003).

We also corroborate some of the results on imperfect vaccination obtained by Gandon et al. (2001) in that vaccines (or antibiotic treatments) decreasing parasite growth rate or toxicity favor higher virulence. Our analysis suggests that decreasing the parasite transmission efficiency might not always be followed by a decrease in virulence. Using an explicit model for within-host dynamics allows us to obtain a more realistic trade-off relation. Moreover, we can study more precisely the evolution of the ESV, which is a step forward toward virulence management. We suggest that it might be interesting to study possible evolutionary consequences of vaccines reducing parasite growth rate or parasite toxicity because they might select for higher virulence.

In this study, we supposed all hosts to be identical so that we are comparing different homogeneous host populations. A next step would be to incorporate heterogeneity in the host population and eventually to allow the host population to coevolve. This addition would be equivalent to adding within-host dynamics to van Baalen's (1998) study.

We find that selfish host strategies (a high level of immune defenses) favor higher virulence, whereas cooperation between hosts would allow a decrease of global virulence: there is conflict between the optima of the individual and of the population. This result poses the question of whether hosts can avoid strategies that trigger an arms race with the parasite even if the strategy is optimal at an individual level (van Baalen 1998). Interestingly, herd immunity may complicate this dilemma. It is known that if enough hosts in the population are vaccinated against a parasite, it will not be able to maintain itself (Anderson and May 1991; van Baalen 1998). If the vaccine has a cost for the individual, then cheaters may emerge: they do not pay the cost of vaccination, but they benefit from the parasite's eradication. Thus, optimizing the individual immune response is complicated and may have important epidemiological consequences (Medley 2002).

A concrete application of our study would require appropriate use of antibiotic treatments in public health policies. We show that increasing such treatments (which are selfish host strategies) leads to higher virulence, as suggested by Ewald (1994) and by Wilkinson (1999), and may thus trigger an arms race. In a virulence management perspective, this result stresses the importance of using antibiotics sensibly, which would imply not prescribing antibiotics for benign infections and particularly not for prophylactic use in cattle (Perreten et al. 1997).

Consequences for Experimental Studies

We show that it may take only a small level of parameter variation (e.g., in lymphocyte production rate or in the immune response's efficiency) to blur the value of the ESV significantly. This result could thus help to explain Mackinnon and Read's observation of significant variations of transmission rates between their different replicates (Mackinnon and Read 1999*a*).

The most convincing experimental trade-off relationships have been shown in hosts without an immune system (Messenger et al. 1999 showed it using viral parasites of bacteria; see Lipsitch and Moxon 1997 for other examples). Trade-offs in hosts with an immune system may be difficult to detect, because experiments with such hosts rarely use parasite-induced host mortality as the measure of virulence (Mackinnon and Read 1999*a*, 1999*b*; 2003). This limitation is due both to practical reasons (host without an immune system are generally much smaller than the ones with an immune system) and biological reasons (if the host has an immune system, it might easily get rid of the parasite). One solution is to work only with sublethal parasites (O'Keefe and Antonovics 2002; Schjørring and Koella 2003), but our work stresses the need for experimental studies in which parasites face real dilemmas, such as choosing between intensity and duration of infectivity.

Perspectives

The result that ESV depends sensitively on parameter values suggests that it is important to take individual variation into account. Such variation could be incorporated by using different parameter values for every host in the system (Gandon 2004) or by associating cells with their antigenic determinants (as we will do in a sequel article). Such host heterogeneity can have important consequences for virulence evolution (Gandon and Michalakis 2000; Gandon et al. 2002).

Another extension to our model would be a nonnull recovery rate (ν). Anderson and May (1982) propose that ν is negatively correlated with the parasite's virulence (α), as do André et al. (2003) and Gilchrist and Sasaki (2002). But parasite clearance implies the existence of immune memory, because a host that has recovered from an in-

fection will mount a much more efficient immune response if it faces this infection again. It also raises technical problems, because recovery (which is equivalent to host death from the parasite's point of view) may occur before the populations have reached their equilibria. Often, within-host and between-host dynamics are overlapping, as stressed by Day and Proulx (2004). Still, this extension would allow us to study virulence evolution in response to efficient treatments and thus might bring interesting insights for virulence management.

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APPENDIX A

Calculation of the Evolutionarily Stable Strategy

We can easily calculate the parasite's optimal growth rate (φ^*) in a case with no cooperative effect of lymphocytes ($k = 1$). Optimal growth rate φ^* is defined by the following relationship:

$$\left.\frac{dR_0}{d\varphi}\right|_{\varphi=\varphi^*} = 0. \tag{A1}$$

By replacing R_0 by its value (cf. eq. [1]), we get

$$\left.\frac{d}{d\varphi}\left[\frac{\beta(\varphi)}{\mu + \alpha(\varphi)}\right]\right|_{\varphi=\varphi^*} = 0. \tag{A2}$$

By replacing $\alpha(\varphi)$ and $\beta(\varphi)$ by their expressions (cf. eqq. [4], [5]), we obtain

$$\left.\frac{d}{d\varphi}\left(\frac{a\tilde{x}}{\mu + u\varphi\tilde{x} + w\tilde{y}}\right)\right|_{\varphi=\varphi^*} = 0. \tag{A3}$$

Finally, by replacing $\tilde{x}$ and $\tilde{y}$ by their values (eqq. [3]) and after some simplifications, we get the condition

$$\left.\frac{d}{d\varphi}\left[\frac{\delta\varphi - ab\sigma}{c\mu\sigma + (cw - ub\sigma)\varphi + \delta u\varphi^2}\right]\right|_{\varphi=\varphi^*} = 0. \tag{A4}$$

We know that $c\mu\sigma + (cw - ub\sigma)\varphi + u\delta\varphi^2 \neq 0$, because $\mu + \alpha(\varphi) > 0$, and we get the following equation:

$$\delta u\delta\varphi^2 - 2u\delta b\sigma\varphi + b^2u\sigma^2 - b\sigma cw - \delta c\mu\sigma = 0. \tag{A5}$$

By solving this equation, we find that

$$\varphi^* = \frac{\sqrt{c\sigma u}\sqrt{bw + \delta\mu} + bu\sigma}{u\delta}. \tag{A6}$$

The values of parasite virulence and transmission at equilibrium are

$$\begin{aligned}\alpha(\varphi^*) &= \frac{2bw}{\delta} + \mu + \frac{w}{\delta}\sqrt{\frac{c(bw + \delta\mu)}{u\sigma}} \\ &\quad + \frac{b}{\delta}\sqrt{\frac{u(bw + \delta\mu)\sigma}{c}}, \\ \beta(\varphi^*) &= a\sqrt{\frac{bw + \delta\mu}{cu\sigma}}.\end{aligned} \tag{A7}$$

APPENDIX B

Simplifying the Condition for Convexity

The condition for convexity is

$$\frac{d^2\beta(\varphi)}{d\alpha(\varphi)^2} < 0.$$

If we denote by a prime ($'$) a derivative with respect to φ, we have

$$\frac{d\beta(\varphi)}{d\alpha(\varphi)} = \frac{\beta'(\varphi)d\varphi}{\alpha'(\varphi)d\varphi} \tag{B1}$$

$$= \frac{\beta'(\varphi)}{\alpha'(\varphi)}, \tag{B2}$$

which leads to

$$\begin{aligned}
\frac{d^2\beta(\varphi)}{d\alpha(\varphi)^2} &= \frac{d}{d\alpha(\varphi)}\left[\frac{d\beta(\varphi)}{d\alpha(\varphi)}\right] \\
&= \frac{d}{d\alpha(\varphi)}\left[\frac{\beta'(\varphi)}{\alpha'(\varphi)}\right] \\
&= \frac{\frac{d\beta'(\varphi)}{d\alpha(\varphi)}\alpha'(\varphi) - \frac{d\alpha'(\varphi)}{d\alpha(\varphi)}\beta'(\varphi)}{\alpha'(\varphi)^2} \\
&= \frac{\left[\frac{d}{\alpha'(\varphi)d\varphi}\beta'(\varphi)\right]\alpha'(\varphi) - \left[\frac{d}{\alpha'(\varphi)d\varphi}\alpha'(\varphi)\right]\beta'(\varphi)}{\alpha'(\varphi)^2} \\
&= \frac{\beta''(\varphi) - \frac{\beta'(\varphi)}{\alpha'(\varphi)}\alpha''(\varphi)}{\alpha'(\varphi)^2} \qquad \text{(B3)} \\
&= \left[\frac{\beta''(\varphi)}{\alpha''(\varphi)} - \frac{\beta'(\varphi)}{\alpha'(\varphi)}\right]\frac{\alpha''(\varphi)}{\alpha'(\varphi)^2}.
\end{aligned}$$

If $d\alpha/d\varphi \neq 0$ and $d\alpha^2/d\varphi^2 > 0$, then equation (B3) implies that

$$\frac{d^2\beta(\varphi)}{d\alpha(\varphi)^2} < 0 \iff \frac{\beta''(\varphi)}{\alpha''(\varphi)} < \frac{\beta'(\varphi)}{\alpha'(\varphi)}. \qquad \text{(B4)}$$

We are interested in the convexity when the system is at an equilibrium. This means that conditions [A1] and [A2] are true, which implies that

$$\begin{aligned}
\frac{\beta'(\varphi)}{\alpha'(\varphi)} &= \frac{\beta(\varphi)}{\mu + \alpha(\varphi)}, \\
\frac{\beta'(\varphi)}{\alpha'(\varphi)} &= R_0^{\text{eq}}. \qquad \text{(B5)}
\end{aligned}$$

We also know that at an equilibrium, the evolutionary R_0's value is 1 (a mutant identical to the resident neither invades nor disappears), which means that

$$\frac{\beta'(\varphi)}{\alpha'(\varphi)} = 1. \qquad \text{(B6)}$$

This leads to the equivalence

$$\frac{d^2\beta(\varphi)}{d\alpha(\varphi)^2} < 0 \iff \beta''(\varphi) < \alpha''(\varphi). \qquad \text{(B7)}$$

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