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THE DYNAMICS OF MULTIPLE INFECTION AND THE EVOLUTION OF VIRULENCE

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Abstract.—While for pathogen clones singly occupying a host it may pay to adopt a relatively avirulent host exploitation strategy, clones sharing a host have a conflict of interest that favors more virulent strategies. As the number of infections per host depends on the force of infection and the force of infection, in turn, depends on prevailing virulence, evolutionary analysis needs to be integrated with population dynamics. A full-fledged approach requires exceedingly large capacities for bookkeeping of the infection events and is therefore difficult to establish. In this article the host-pathogen interaction is studied for the simple case in which hosts may become at most doubly infected. It appears that evolution and population dynamics give rise to a feedback mechanism. When double infections are frequent, increased virulence is favored; but when pathogens become more virulent, the force of infection will decrease, favoring lower virulence again. Thus, evolutionarily stable strategy (ESS) virulence depends on the interaction within hosts as well as on the interaction at the population level. As current models of host-microparasite interactions take only first infections into account, they may be inappropriate for evolutionary analyses, which would require modeling of within-host competition between strains and thus of multiple infections.

Because they reproduce at the expense of their hosts, pathogens may not be able to increase infectivity without inflicting more harm. In other words, pathogen reproductive success is constrained by a trade-off between transmission efficiency and disease-induced host mortality. Thus, one might expect that pathogens will evolve toward an optimal level of virulence that maximizes transmission over the entire infective period (Anderson and May 1979; Ewald 1983; May and Anderson 1983). However, such an argument is based on the implicit assumption that hosts are exploited by a single clone of pathogens, which may not be realistic. Even though pathogen-induced physiological or behavioral changes may alter an infected host's vulnerability to subsequent infection (Stewart and Levin 1984; Dobson 1988; Combes 1991), complete prevention is unlikely. Hence if the force of infection—as defined by Anderson and May (1991)—is high, and hosts remain exposed long enough, multiple infections are bound to occur. This will have an impact on the evolution of virulence, because when different clones share a host, a conflict of interest ensues that favors a lower degree of "host preservation"

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(Eshel 1977; Bremermann and Pickering 1983; Sasaki and Iwasa 1991). Since the force of infection—governing the frequency of multiple infection—depends on the density of infected hosts, which in turn is determined by the virulence of the pathogen population, we are confronted with a problem in which evolution and population dynamics are closely intertwined.

Thus, multiple infections change the conceptual scope of the problem. With singly infected hosts, virulence (i.e., disease-induced host mortality) is the outcome of the struggle between single hosts and single pathogen clones. Multiple infection means, in the first place, that the conflict is not only between host and pathogens but among different pathogen clones as well (Eshel 1977; Levin and Pimentel 1981; Bremermann and Pickering 1983; Levin 1983; Sasaki and Iwasa 1991). But in the second place (and this will be the central point of this article), population dynamics can no longer be ignored, because the frequency of multiple infection and the strategies adopted by different pathogen clones will depend on the state of the entire system.

A first insight is provided by Levin and Pimentel's (1981) study of the interaction of pathogen strains differing in virulence. They argue that within doubly infected hosts, virulent pathogens will quickly outgrow avirulent pathogens, so that on the timescale of population dynamics, virulent pathogens instantaneously "take over" hosts infected by avirulent pathogens. A model based on this assumption shows that coexistence of virulent and avirulent strains is possible.

Whether high or low virulence pays off then depends on abundance and virulence of the other pathogens in the population. Reducing virulence prolongs the infective period but also exposes the host longer to subsequent infections; thus it will only pay when there are not too many virulent pathogens in the population at large. Increasing virulence allows pathogens to "steal" hosts from other pathogens, but this approach only pays when these are sufficiently abundant. Thus, in Levin and Pimentel's (1981) model, but also in more complex models (Levin 1983; Stewart and Levin 1984), there is a negative feedback, which may lead to coexistence of virulent and avirulent pathogens. An interpretation of these results is that neither extremely high nor very low virulence is evolutionarily stable (Maynard Smith and Price 1973; Maynard Smith 1982). However, because in such studies usually only two virulence types are considered, inferences on the existence of polymorphisms are premature; there may exist other (e.g., intermediate) strains—not included in the models—that displace both original strains.

Recently, Nowak and May (1994) extended Levin and Pimentel's model by considering more than two strains differing in virulence. Like Levin and Pimentel's model, it is based on the assumption that more virulent pathogens can immediately eliminate less virulent pathogens from a host. This process resembles contest competition, but Nowak and May coined the new term *superinfection* (though *superseding infection* would have been more appropriate). Their model predicts highly polymorphic pathogen populations with complex dynamics. However, if pathogens differ very little in virulence, then within-host competition is unlikely to be of the contest type. In the case of scramble competition, one has to consider the simultaneous presence of more than one strain in a host. To see how this process, termed *coinfection* by Nowak and May (1994), is likely to

drastically change the outcome, it is useful to describe precisely what happens under contest competition. Suppose a polymorphic pathogen population arises as the end result of contest competition between a discrete number of pathogen strains. Then a pathogen strain slightly more virulent than one of the resident strains will always be able to invade, because it has the advantage of a larger number of suitable hosts than its nearest competitor. Thus, the mean level of virulence in the pathogen population will increase. As a consequence, the infected hosts transmit the pathogens over a shorter time span, which then leads to a reduced proportion of infected hosts in the overall population. Eventually the risk of superinfection will decrease to such an extent that opportunities arise for new low-virulence strains. Thus, the prediction of a polymorphic end situation hinges on the assumption of superinfection. If competition were of the scramble type, then the immediate advantage for a slightly more virulent strain vanishes. The end result of this process is not at all obvious, as it does not necessarily result in polymorphism. Hence in this article we intend to investigate the coinfection case.

Our main aim is to investigate the link between the evolution of virulence and the dynamics of multiple infection. As a lack of detailed knowledge on interactions among pathogens within hosts precludes an exact analysis (Anderson and May 1991), we proceed by a two-step argument. First, we will outline how natural selection will shape host exploitation strategies given that hosts are exploited by a single pathogen clone (i.e., multiple infections do not occur). Then we allow hosts to become infected twice (sequentially) and compare the results. For both cases we derive an expression for the basic reproduction ratio (R_0) of a mutant pathogen, which can be used to infer how a mutant can maximize its fitness under given circumstances. When the results are combined with a population dynamical model, it can be calculated how the frequency of multiple infection depends on the host exploitation strategies of the pathogen population, and evolutionarily stable strategy (ESS) conditions can be specified.

THE BASIC REPRODUCTION RATIO

Single Infections

Consider a host-pathogen system in which the rate of infection is given by the mass-action law; that is, an infected host may transmit the pathogen to any of the susceptible hosts in the population with equal probability per unit time. An infected host's transmission rate is then proportional to the density of susceptible hosts (x), where the proportionality constant, commonly denoted β, measures transmission efficiency (a summary of the symbols used in the text is given in app. A). Clearly, pathogens benefit from a high transmission efficiency, so evolution of the pathogens would continually increase β if there were no cost involved. But pathogen transmission is based on the production of propagules, and these are produced at the expense of the host, so that increasing propagule production rate is likely to cause the host to become more ill and have a greater risk to die. Thus, there is likely to be a trade-off between propagule production and host longevity.

Unfortunately, little is known about this trade-off for most systems studied (Anderson and May 1991); the intricacies of the immunological tug-of-war between host and pathogens (see Bryant and Behm 1989) will make it difficult to assess the exact shape by a priori reasoning. In this article we focus not so much on the mechanisms underlying the trade-off per se but rather on their evolutionary consequences; hence we merely assume that a trade-off exists, without specifying it in detail.

Thus let the pathogens have a "host exploitation strategy," denoted by ϵ, that affects both transmission efficiency β and disease-induced mortality α, so that a host infected with pathogens with host exploitation strategy ϵ is characterized by

$$\begin{aligned} \beta &= \beta(\epsilon) \\ \alpha &= \alpha(\epsilon)\,. \end{aligned} \tag{1}$$

It should be noted that ϵ is a dummy variable that may not have direct and general definition; it serves to create the relation between α and β in a way that is convenient for later analysis.

To establish which host exploitation strategy will be favored by natural selection, given a trade-off embodied in equation (1), one should focus on a rare mutant strain and answer the question of how it can maximize its reproductive success, given the presence of a resident strain. Whether a rare mutant can increase depends on its reproduction ratio R_0, the number of new infections per infected host (Kermack and McKendrick 1927; May and Anderson 1983; Heesterbeek 1992). The first step is therefore to determine how the R_0 of a rare mutant depends on its own host exploitation strategy and on the host exploitation strategy of the resident population. The second step is then to resolve how the mutant can maximize its R_0 by optimizing its host exploitation strategy. If this optimal strategy is identical to the strategy adopted by the resident population, it is an ESS, because then any mutant that deviates from the resident strategy has a fitness lower than that of the resident (Maynard Smith and Price 1973; Maynard Smith 1982).

When no multiple infections occur, an infected host will transmit propagules of a single pathogen clone. The R_0 of a mutant can then be calculated by focusing on a host infected by the mutant and determining how many new infections it is likely to produce. Assuming, for simplicity, that the rate of host mortality from other causes is μ and no recovery occurs, one obtains

$$R_0(\epsilon) = \frac{\beta(\epsilon)x}{\mu + \alpha(\epsilon)}, \tag{2}$$

which is simply the product of transmission rate (transmission efficiency $\beta(\epsilon)$ times the density of susceptible hosts x) and expected host longevity $(\mu + \alpha(\epsilon))^{-1}$ (Kermack and McKendrick 1927; May and Anderson 1983). (The assumption of no recovery is not essential to the results from this section. If recovery does occur, we can incorporate its effect in either μ or $\alpha(\epsilon)$, depending on whether it is affected by the pathogen's host exploitation strategy.)

A mutant, being rare, does not influence population dynamics, and density of

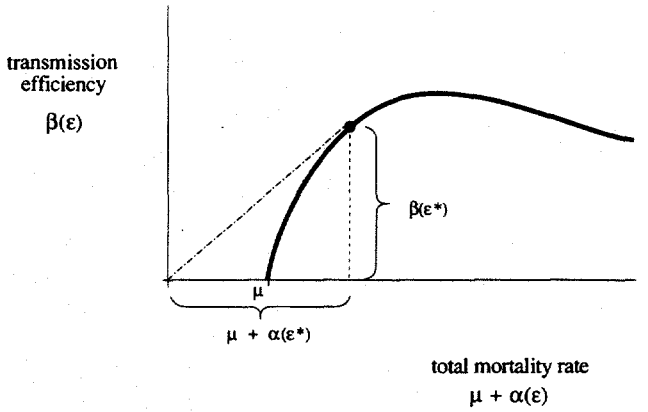

FIG. 1.—Given a hypothetical relation between transmission $\beta(\epsilon)$ and total mortality rate $\mu + \alpha(\epsilon)$, the optimal exploitation strategy ϵ^* (maximizing $\beta(\epsilon)$ over $\mu + \alpha(\epsilon)$) is given by the point where the tangent passes through the origin.

susceptible hosts is set by the resident pathogen. That is, we could express the mutant's R_0 as a function of its own exploitation strategy ϵ and the resident's ϵ^* by substituting $x = F(\epsilon^*)$ into equation (2). The influence of a mutant on its own R_0 is therefore only through transmission efficiency and disease-induced host mortality. Its optimal host exploitation strategy ϵ satisfies

$$\frac{dR_0}{d\epsilon} = 0$$

and

$$\frac{d^2R_0}{d\epsilon^2} < 0, \tag{3}$$

which leads to

$$\frac{\frac{d\beta}{d\epsilon}}{\frac{d\alpha}{d\epsilon}} = \frac{\beta(\epsilon)}{\mu + \alpha(\epsilon)}$$

and

$$\frac{d^2\beta}{d\epsilon^2} < \frac{\beta(\epsilon)}{\mu + \alpha(\epsilon)} \frac{d^2\alpha}{d\epsilon^2}. \tag{4}$$

These conditions have a simple graphic interpretation. When transmission $\beta(\epsilon)$ is plotted against total mortality rate $\mu + \alpha(\epsilon)$, the optimal combination of $\beta(\epsilon)$ and $\alpha(\epsilon)$ (and hence the optimal exploitation strategy ϵ^*) is the point where the tangent of the curve passes through the origin (see fig. 1). A line connecting a point on the curve with the origin has the property that its slope is equal to $\beta(\epsilon)$

divided by $\mu + \alpha(\epsilon)$; this slope is maximal for the tangent line, provided the curve is convex—which is embodied (locally) in inequality (4). This situation is an example of the marginal value theorem, and it is in close analogy to the well-known graphic solutions from optimal foraging theory (Charnov 1976; Stephens and Krebs 1986). The approach ignores any pathogen dynamics within the host and assumes that pathogens achieve the optimal solution instantaneously. How pathogens could actually approach the optimal solution by regulation of their within-host growth schedule is analyzed in more detail by Sasaki and Iwasa (1991). They assume that within-host pathogen density has effects on transmission and disease-induced mortality. In analogy with an optimal host exploitation strategy, an optimal within-host pathogen density exists. A single pathogen clone should attempt to reach this density as quickly as possible and remain at this density as long as no other infection occurs, or the host would be certain to die for other reasons. If maximal within-host reproduction is extremely fast Sasaki and Iwasa's results converge to expressions (4).

Consideration of expression (2) for the mutant's R_0 and conditions (4) for the optimal host exploitation strategy suggests that it is convenient to introduce the "per-host transmission factor," which is defined as transmission efficiency integrated over the entire infective period of the host. If we denote this quantity by B, in the case of single infections we have

$$B(\epsilon) = \frac{\beta(\epsilon)}{\mu + \alpha(\epsilon)}, \tag{5}$$

so that we can write the mutant's R_0 as the product of the density of susceptible hosts and the per-host transmission factor:

$$R_0(\epsilon) = B(\epsilon)x. \tag{6}$$

A rare mutant cannot influence the density of susceptible hosts x (which is set by the resident strain), so it can only maximize its R_0 by maximizing per-host transmission $B(\epsilon)$; exactly this is done in expressions (4). As the assumption of single infections means that the mutant will not be challenged by the resident within a host, its per-host transmission factor depends solely on its own host exploitation strategy. Thus, when only single infections occur, knowledge of neither population dynamics nor the resident host exploitation strategy is required to find the optimal host-exploitation strategy; it is purely a matter of "prudent" host exploitation.

It can be inferred that for humped curves in the α-β plane like the one shown in figure 1, the optimal solution does not maximize transmission efficiency (maximal β, associated with high α), nor is it completely avirulent ($\alpha = 0$). Thus, prudent host exploitation strategies lead to intermediate virulence. Recently this argument has often been emphasized, and it may now have replaced the conventional wisdom that pathogens invariably evolve toward complete avirulence (see, e.g., Ewald 1983; May and Anderson 1983). However, it should be noted that it is based on the assumption that hosts are exploited by only single clones—an

assumption that may or may not be realistic. If multiple infections are frequent, the predictions will change, as we will see below.

R_0 *with Double Infections*

The R_0 given by expression (2) is a fitness measure if the pathogens within a host constitute a single unit of selection. This will be the case if only single infections occur, because then hosts are exploited by a single pathogen clone. However, under multiple-infection pathogen clones can no longer monopolize a host, and a conflict of interest ensues. To an individual clone, the problem is still to balance transmission efficiency and host longevity, but costs and benefits have changed. More specifically, the benefit of producing more propagules is immediate and remains the same, but the benefit from an attempt to prolong the host's longevity is less, because host longevity is also affected by the strategies of other clones.

The simplest model that incorporates the effect of multiple infection is a model in which hosts can become infected twice. Though in principle there is no limit to the number of infections a host can receive, we restrict our analysis to at most two infections per host because it reduces the complexity of the model, while it still allows us to analyze how optimal virulence depends on exclusive versus shared host exploitation. To emphasize the evolutionary consequences, we allow doubly infected hosts to have different epidemiological parameters from singly infected hosts, and pathogens may have different strategies for the exploitation of singly and doubly infected hosts. Then the ESS exploitation strategies for singly and doubly infected hosts can be studied separately. Note that as singly infected hosts can become doubly infected, the ESS exploitation strategy for singly infected hosts will differ from that of the previous section.

Consider a host that has been infected at time $t = 0$. When the host has not yet been infected a second time, the pathogen's transmission parameter is β_1 while the host has a total mortality rate $\mu + \alpha_1$. Upon infection by another pathogen clone, the transmission parameter of the first clone changes to β_2, while the disease-induced mortality changes to α_2. Note that α_2 includes the mortality effects of both pathogen clones. Now the host may start to produce infective particles of the second clone as well, with transmission parameter γ_2. Note that from an epidemiological point of view, overall pathogen transmission efficiency is now $\beta_2 + \gamma_2$.

Determining the basic reproduction ratio of a mutant strain becomes more complex, as pathogens now have two types of susceptible hosts, uninfected and singly infected. The standard definition of R_0 is the number of secondary infections produced by a typical infected host (Heesterbeek 1992), but what is a typical host in this case? The easiest way to determine R_0 here is to shift the viewpoint to a pathogen propagule that has just been released from an infected host and ask how many new propagules it is likely to produce. We have to find expressions for the probability of a mutant to infect each type of host and for the subsequent production of propagules.

A full model for the population dynamics of two pathogen strains is rather

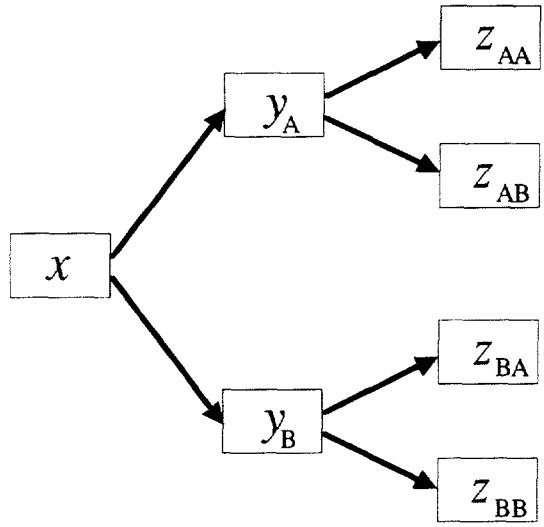

FIG. 2.—In a system with two pathogen strains, A and B, there are uninfected hosts (x), two types of singly infected hosts (y), and four types of doubly infected hosts (z). See text for a further explanation.

complex, as we are forced to keep track of (at least) seven classes of hosts representing all possible combinations of single and double infections (see fig. 2). However, the conditions for invasion of a mutant in a resident population are readily derived. By definition mutants are initially rare, and consequently host population dynamics will be governed only by the resident strain. Assuming population dynamic equilibrium (which must be verified separately), the densities of the two host types a mutant can infect—uninfected hosts, and hosts that are singly infected by the resident—are constant, which implies that the R_0 of the resident equals unity. Then, the mutant has two propagation routes, and all that matters is whether a mutant propagule produces more than one new propagule on the average. This will depend on the interactions between resident and mutant on the within-host level, which will be worked out first, before constructing the expression for the mutant's R_0.

One propagation route for a mutant is through a host harboring a single resident clone already. The per-host transmission factor for this route, denoted by B_S, can be derived analogously to equation (5) (because subsequent infections are assumed not to occur) and is equal to

$$B_S = \frac{\gamma_2}{\mu + \alpha_2}. \tag{7}$$

The only difference from equation (5) is that the first clone (belonging to the resident strain) also may affect transmission of the mutant and mortality of the host.

The other propagation route starts when the mutant is first to infect a host.

Then the mutant may remain unchallenged for some time, until the resident also infects the same host. To determine the per-host transmission factor for this route, we must know how being singly or doubly infected is distributed over the host's infective period.

Let h be the force of infection of the resident (i.e., the probability per unit time for a host to become infected by the resident strain). In fact, the force of infection is a population dynamic variable, but under equilibrium conditions it is constant and may then be represented by a parameter. Let $p_1(t)$ and $p_2(t)$ denote the probabilities to find the host singly or doubly infected t time units after the first infection. At time $t = 0$, single infection is certain, $p_1 = 1$, but p_1 will decrease because the host dies from nondisease causes (with probability per unit time μ), dies from the disease (with probability per unit time α_1), or becomes doubly infected (with probability per unit time h). Initially, p_2 will increase from zero because of transitions from singly to doubly infected hosts, but then decrease again because of death (from either non-disease- or disease-related causes). This gives a pair of linear equations,

$$\begin{aligned} \frac{dp_1}{dt} &= -(\mu + \alpha_1 + h)p_1 \\ \frac{dp_2}{dt} &= hp_1 - (\mu + \alpha_2)p_2 , \end{aligned} \tag{8}$$

that can be solved explicitly (subject to boundary conditions $p_1[0] = 1$ and $p_2[0] = 0$), to yield

$$\begin{aligned} p_1(t) &= e^{-(\mu+\alpha_1+h)t} \\ p_2(t) &= \frac{h}{(\mu + \alpha_2) - (\mu + \alpha_1 + h)}(e^{-(\mu+\alpha_1+h)t} - e^{-(\mu+\alpha_2)t}) . \end{aligned} \tag{9}$$

Figure 3 shows an example of how these probabilities change over time.

The per-host transmission factor of the mutant over the host's entire infective life span, denoted by B_F, is then

$$B_F = \int_0^\infty p_1(t)\beta_1 \, dt + \int_0^\infty p_2(t)\beta_2 \, dt , \tag{10}$$

which (after some algebra; see app. B) yields

$$B_F = \frac{\beta_1 + h\dfrac{\beta_2}{\mu + \alpha_2}}{\mu + \alpha_1 + h} . \tag{11}$$

Note that if the risk of double infection is zero, $h = 0$, equation (11) is identical to the per-host transmission factor (5). But if the rate of infection becomes large, B_F will be dominated by $\beta_2/(\mu + \alpha_2)$, the contribution from doubly infected hosts. As a further check, note that if the first clone is able to monopolize a host (so that the second infection has no effect at all, and $\beta_2 = \beta_1$ and $\alpha_2 = \alpha_1$), B_F simplifies to equation (5) again.

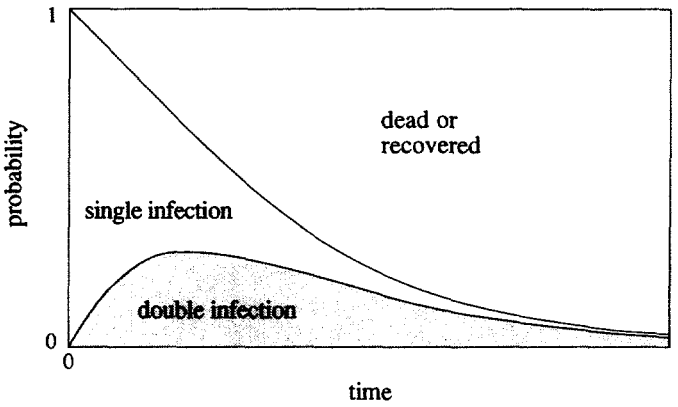

FIG. 3.—An example of how the probabilities of single and double infection are distributed over an infected host's life. (The first infection occurred at $t = 0$.)

Denoting the density of uninfected hosts by x and the density of singly infected hosts by y, we can write analogously to equation (6)

$$R_0 = B_F x + B_S y \tag{12}$$

and in this way combine the two propagation routes. This is the general equation for the R_0 of a mutant pathogen, when hosts may be infected twice (but not more often).

The per-host transmission factors B_F and B_S will be affected by host exploitation strategies of first and second pathogen clones (which may belong to either the resident or the mutant strain). As an illustrative case, assume that the pathogens can detect the presence of other clones in the host. Then, a pathogen clone may use this ability to adopt a strategy conditional upon whether it is alone in the host or it shares the host with another clone. The exploitation strategy for singly infected hosts will still be denoted ϵ; but when a host becomes doubly infected, the first clone may respond and switch to a different strategy, denoted by ϕ. The strategy of a second pathogen clone will be denoted by σ.

For singly infected hosts, transmission efficiency and disease-induced mortality rate are as before,

$$\begin{aligned} \beta_1 &= \beta_1(\epsilon) \\ \alpha_1 &= \alpha_1(\epsilon), \end{aligned} \tag{13}$$

but for a doubly infected host, the exploitation strategies of both clones will have their effect:

$$\begin{aligned} \beta_2 &= \beta_2(\phi, \sigma) \\ \gamma_2 &= \gamma_2(\phi, \sigma) \\ \alpha_2 &= \alpha_2(\phi, \sigma). \end{aligned} \tag{14}$$

Let the mutant have a strategy (ϵ, ϕ, σ), while the resident is characterized by

(ϵ^*, ϕ^*, σ^*). To maximize its R_0 the mutant should optimize ϵ, ϕ, and σ. Before addressing the problem of how to exploit hosts while the second infection has not yet taken place, we examine how the mutant should deal with doubly infected hosts. Consider first the problem of optimizing ϕ, the strategy to switch to when a second clone infects the host. Analysis of expression (7) for the mutant's R_0 reveals that the clone should then maximize B_F with respect to ϕ, as this is the only factor affected by the mutant's ϕ. Hence an optimal ϕ (given σ^*, the resident counterstrategy) will satisfy

$$\frac{\frac{\partial \beta_2}{\partial \phi}}{\frac{\partial \alpha_2}{\partial \phi}} = \frac{\beta_2(\phi, \sigma^*)}{\mu + \alpha_2(\phi, \sigma^*)}. \tag{15}$$

Now suppose the mutant is second. Then it should maximize B_S with respect to σ, which means that σ should satisfy

$$\frac{\frac{\partial \gamma_2}{\partial \sigma}}{\frac{\partial \alpha_2}{\partial \sigma}} = \frac{\gamma_2(\phi^*, \sigma)}{\mu + \alpha_2(\phi^*, \sigma)}. \tag{16}$$

Evolutionarily stable values (Maynard Smith and Price 1973; Maynard Smith 1982) for ϕ and σ should satisfy conditions (15) and (16) simultaneously.

If the two clones share the host on an equal basis, then conditions (15) and (16) will be identical, but this condition will already be violated if the first pathogen can benefit from earlier arrival. Without further specifying the functions $\beta_2(\phi, \sigma)$ and $\gamma_2(\phi, \sigma)$, the consequences for transmission rates and mortality cannot be derived. As little is known about how pathogens can manipulate their hosts in the first place, it is even more difficult to assess how they may interact within a host. Undoubtedly, this is a complex issue, involving host physiology, immune responses, pathogen evasive tactics, and so forth. Sasaki and Iwasa (1991) present an analysis based on the assumption that the disease-induced mortality rate is proportional to the density of pathogens within a host, while transmission efficiency is a satiating function of pathogen density. Then, upon second infection, the optimal within-host density for each strain (i.e., the optimal exploitation strategy) increases (either to some finite value or even to infinity, depending on the effect on transmission efficiency), which increases the disease-induced mortality rate. If increased virulence is favored, disease-induced mortality rate will be larger than in the single-infection case, as well as the combined infectivity $\beta_2 + \gamma_2$. In what follows we will not investigate in detail the ESS (ϕ^*, σ^*) but merely assume that a second clone's presence leads to a reduction in the first clone's transmission efficiency and/or an increase in disease-induced mortality rate, so that $\beta_2/(\mu + \alpha_2)$ will be lower than the maximal per-host transmission factor for the single-infection case.

For given values of ϕ^* and σ^*, the optimal exploitation strategy ϵ^* for singly infected hosts is found by maximization of B_F, which yields

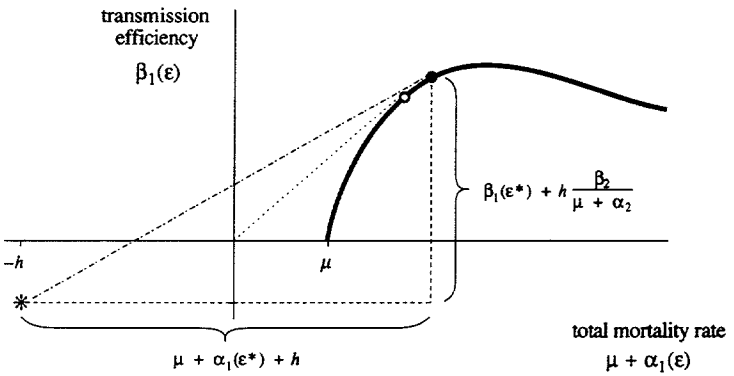

FIG. 4.—Graphic interpretation of the consequences of the risk of double infection. The white dot specifies the ESS without double infections (the same as in fig. 1); the black dot specifies the ESS incorporating the anticipation of double infection. If the pivotal point $(-h, -h\beta_2/[\mu + \alpha_2])$, indicated by an asterisk, lies in the upper-left half of the plane relative to the dotted line (which requires that $\beta_2/[\mu + \alpha_2]$ is small) then the combination of a higher transmission rate and a higher virulence is optimal. If the second clone takes over the host, as in Levin and Pimentel's (1981) model, then β_2 will be zero, and the pivotal point lies on the mortality axis.

$$\frac{\frac{\partial \beta_1}{\partial \epsilon}}{\frac{\partial \alpha_1}{\partial \epsilon}} = \frac{\beta_1 + h\dfrac{\beta_2}{\mu + \alpha_2}}{\mu + \alpha_1 + h} \tag{17}$$

for the optimal ϵ^* (see app. C for a derivation). The graphic interpretation is similar to the single-infection case but differs in that for the optimal ϵ^* the tangent does not pass through the origin but through the "pivotal" point $(-h, -h\beta_2/[\mu + \alpha_2])$, as shown in figure 4. If $\beta_2/(\mu + \alpha_2)$ is low, which is the case if the presence of a second clone would spoil things for the first, the first clone should adopt a strategy ϵ^* that leads to a higher transmission efficiency and a higher mortality of the host. This becomes even more pronounced when the risk of double infection becomes large.

Thus, the evolutionary consequences of double infections are twofold. First, a pathogen clone may have to share its host with another clone, which leads to a conflict of interests that is likely to favor higher virulence. Second, a first clone, when still "alone," should anticipate later infections, which also favors higher virulence because a shared host is less profitable.

Because increased virulence is favored both when there is a risk of hosts becoming shared and when they are actually shared, it is unlikely that the assumption of pathogen knowledge of other infections is critical to the predictions. If the pathogens cannot respond to each other's presence in the same host, they have to incorporate the risk of having to compete in a fixed exploitation strategy. Unfortunately, optimality conditions for such a strategy turn out to be rather complex, but as such a strategy is necessarily a compromise between ϵ^*, ϕ^*, and

σ^*, multiple infection would favor increased virulence in this case as well. (A more formal analysis of pathogen ESSs for such cases is outlined in app. D.)

That sharing of hosts favors higher virulence is not a new idea (see, e.g., Bremermann and Pickering 1983; Sasaki and Iwasa 1991). But to our knowledge there are no explicit studies of the feedback between the level of virulence and the frequency of multiple infection. This feedback will be the subject of the next section.

POPULATION DYNAMIC FEEDBACK

The Force of Infection

Optimal host exploitation strategies depend on the frequency of multiple infection, because sharing hosts with nonrelated clones favors higher virulence. This effect depends on h, the force of infection. Because h is not an arbitrary constant but rather depends on the strategies adopted by the pathogens infecting other hosts, one also needs to investigate the population dynamic consequences of evolving host exploitation strategies.

The model formulated by Levin and Pimentel (1981) offers a good starting point. Hence we assume that all hosts reproduce at the same rate, infection is a mass-action process, and no recovery occurs. However, unlike in Levin and Pimentel's model, in which the only type of second infection that matters is of virulent pathogens infecting a host harboring avirulent pathogens, we keep track of all double infections. To simplify the analysis we first formulate the population dynamic model for a single pathogen strain (i.e., the resident), and then use the results from the previous sections to investigate the opportunities for invasion of mutants.

Denote the density of uninfected, singly infected, and doubly infected hosts by x, y, and z, respectively. If singly infected and doubly infected hosts transmit the pathogen with efficiencies β_1 and $\beta_2 + \gamma_2$, respectively, then the "force of infection" (Anderson and May 1991) will be proportional to

$$h = \beta_1 y + (\beta_2 + \gamma_2)z. \tag{18}$$

Assuming, for simplicity, that infected hosts are just as susceptible to (double) infection as uninfected hosts and that all hosts reproduce (with a per capita rate r), we can write

$$\begin{aligned} \frac{dx}{dt} &= r(x + y + z) - \mu x - hx, \\ \frac{dy}{dt} &= hx - hy - (\mu + \alpha_1)y, \end{aligned} \tag{19}$$

and

$$\frac{dz}{dt} = hy - (\mu + \alpha_2)z.$$

The efficiency of second infections is, of course, an evolutionary variable, much like other aspects of infection. One could introduce an additional constant

S that measures the relative efficiency of second infections (cf. Levin and Pimentel 1981), but to keep things simple we will set $S = 1$ and thus assume that second infections occur exactly as efficiently as do first infections.

No Difference between Singly and Doubly Infected Hosts

Suppose that doubly infected hosts are indistinguishable from singly infected hosts (i.e., $\beta_2 + \gamma_2 = \beta_1 = \beta$ and $\alpha_2 = \alpha_1 = \alpha$). Then all infected hosts can be lumped into a single class $Y = y + z$, and the model becomes

$$\begin{aligned} \frac{dx}{dt} &= r(x + Y) - \mu x - hx \\ \frac{dY}{dt} &= hx - (\mu + \alpha)Y \end{aligned} \tag{20}$$

with

$$h = \beta Y. \tag{21}$$

This system has a stable equilibrium

$$\begin{aligned} \bar{x} &= \frac{\mu + \alpha}{\beta} \\ \bar{Y} &= \frac{\mu + \alpha}{\beta} \frac{r - \mu}{\mu + \alpha - r} \end{aligned} \tag{22}$$

whenever it is positive (Anderson and May 1979; Diekmann et al. 1988), which requires

$$\mu < r < \mu + \alpha. \tag{23}$$

Thus for hosts and pathogens to coexist, host per capita reproduction should exceed nondisease mortality, and pathogens should be virulent enough to be able to cause the host population to decline. In the following, we assume that these conditions are met.

Under equilibrium conditions the force of infection equals

$$\bar{h} = \beta \bar{Y} = \frac{(\mu + \alpha)(r - \mu)}{\mu + \alpha - r}, \tag{24}$$

which increases very sharply if the disease-induced mortality α decreases (fig. 5*A*). Then, the fraction of hosts that is only singly infected (which follows from equilibrium condition $\bar{z} = \bar{h}\bar{y}/(\mu + \alpha)$) is equal to

$$\frac{\bar{y}}{\bar{y} + \bar{z}} = \frac{\mu + \alpha}{\mu + \alpha + \bar{h}} = 1 - \frac{r - \mu}{\alpha}. \tag{25}$$

As shown in figure 5*B*, a considerable fraction of the infected hosts is infected more than once, especially if α is low. Hence, evolution toward reduced virulence increases the fraction of multiply infected hosts. As we have seen, this is likely to favor the evolution of increased virulence and, if the pathogens are able to

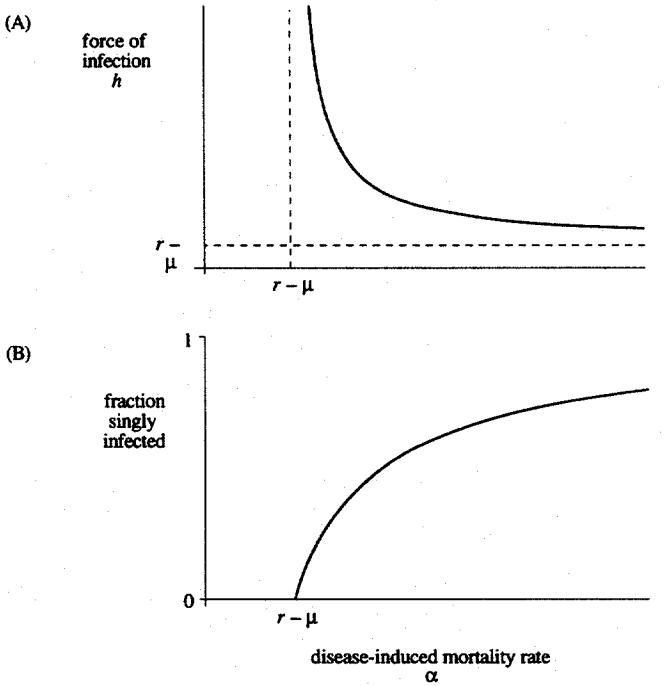

FIG. 5.—The force of infection (*A*) and the fraction of the hosts that is only singly infected (*B*) as a function of the disease-induced mortality rate α. When α decreases, the equilibrium densities of both uninfected and infected hosts increase sharply and, as a consequence, the force of infection as well. When the force of infection increases, singly infected hosts become quickly infected a second time.

adopt conditional strategies, may lead to epidemiological differences between singly and doubly infected hosts.

Differences between Singly and Doubly Infected Hosts

When singly and doubly infected hosts are no longer indistinguishable, the analysis becomes more complex. Because more than two infections do not occur—a simplifying assumption of the present analysis—optimal exploitation strategies exist for pathogen clones sharing doubly infected hosts that can be solved (in principle) without knowledge of population dynamics. Hence, the parameters characterizing doubly infected hosts (β_2, γ_2, and α_2) are fully determined by the within-host competition and will be considered as constants in what follows. However, the parameters characterizing singly infected hosts (β_1 and α_1) do depend on population dynamics, because the ESS ϵ^* depends on the force of infection h. In the following section all parameters are considered as constants, except for the evolutionary variables β_1 and α_1 or, more precisely, the exploitation strategy for singly infected hosts, ϵ. The approach is as follows. First we will analyze the population dynamic model that results when all pathogens have adopted the strategy ϵ^*, which leads to $\beta_1^* = \beta_1(\epsilon^*)$ and $\alpha_1^* = \alpha_1(\epsilon^*)$. This leads, under condi-

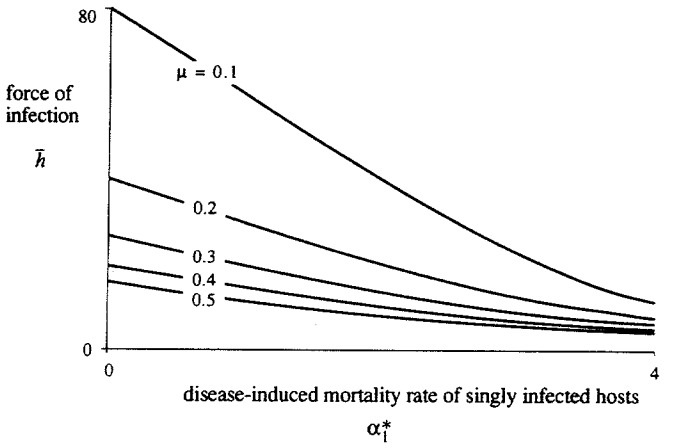

FIG. 6.—The force of infection under ecological equilibrium conditions, as a function of the disease-induced mortality rate. The effect is especially strong when the natural mortality rate μ is low. (Parameters: $r = 2$, $\alpha_2 = 2$.)

tions of ecological equilibrium, to a certain rate of infection $\bar{h}$. Under these circumstances mutants may be favored with a different exploitation strategy ϵ_{opt}. The evolutionarily stable strategy therefore requires $\epsilon^* = \epsilon_{opt}$.

To calculate the force of infection under equilibrium conditions ($\bar{h}$), set the time derivatives of y and z equal to zero, and express $\bar{y}$ and $\bar{z}$ in terms of $\bar{h}$ and $\bar{x}$:

$$\bar{y} = \frac{\bar{h}\bar{x}}{\bar{h} + \mu + \alpha_1^*}$$
$$\bar{z} = \frac{\bar{h}^2\bar{x}}{(\bar{h} + \mu + \alpha_1^*)(\mu + \alpha_2)}, \tag{26}$$

to obtain an expression for total host density

$$\bar{x} + \bar{y} + \bar{z} = \left(1 + \frac{\bar{h}}{\bar{h} + \mu + \alpha_1^*} + \frac{\bar{h}^2}{(\bar{h} + \mu + \alpha_1^*)(\mu + \alpha_2)}\right)\bar{x}. \tag{27}$$

Setting the time derivative of x to zero and substituting expression (27) yields

$$r\left(1 + \frac{\bar{h}}{\bar{h} + \mu + \alpha_1^*} + \frac{\bar{h}^2}{(\bar{h} + \mu + \alpha_1^*)(\mu + \alpha_2)}\right) - \mu - \bar{h} = 0. \tag{28}$$

This leads to a quadratic equation in $\bar{h}$, with one positive solution, unfortunately given by a rather messy expression. Nonetheless, it is a crucial result, as it links the host exploitation strategy of the resident pathogen to the force of infection $\bar{h}$. Equation (28) shows that the force of infection is only dependent on α_1^* but not on β_1^*, which is just as in the simple host pathogen model (eq. [24]). (Note, however, that equilibrium densities are still determined by β_1^*.) The solutions in figure 6 show that $\bar{h}$ decreases with increasing α_1^*; in other words, if the

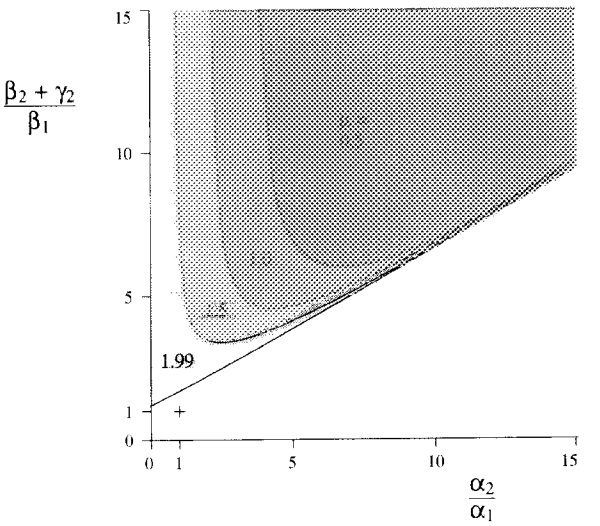

FIG. 7.—Numerical fiburcation analysis indicating how different a doubly infected host must be relative to a singly infected host to destabilize the population dynamic equilibrium. The shaded areas show the regions of unstable ecological equilibrium for different values of nondisease mortality μ. (Parameters: $r = 2$, $\beta_1 = 1$, $\alpha_1 = 1$.)

population of pathogens becomes more virulent, the rate of infection decreases, and with it, the frequency of multiple infection. This effect becomes more pronounced the lower nondisease mortality rate μ is.

Ecological Stability

An evolutionary analysis based on R_0 measures assumes that hosts and pathogens are in population dynamic equilibrium. This is not necessarily the case, and stability of the equilibrium has to be verified separately.

If singly and doubly infected hosts are indistinguishable, the population dynamical equilibrium is always stable (Diekmann et al. 1988). However, distinction permits the possibility of destabilizing time delays, for example, when only doubly infected hosts transmit the disease. The question is therefore how large the differences between singly and doubly infected hosts must be to become destabilizing. A numerical stability analysis of the model (eqq. [18] and [19]; see fig. 7) shows that the equilibrium becomes unstable when relative transmission efficiency of doubly infected hosts is sufficiently large. Unless the nondisease mortality rate μ is very close to prey reproduction rate r, transmission efficiency of doubly infected hosts has to be several times the transmission rate of singly infected hosts, and even larger when the relative disease-induced mortality also increases. Though one cannot a priori exclude the possibility that evolution leads to parameter combinations in the unstable region, it does not seem very likely. Hence, for the present population dynamic model the assumption of a stable ecological equilibrium will often be justified. But other host-pathogen models lead

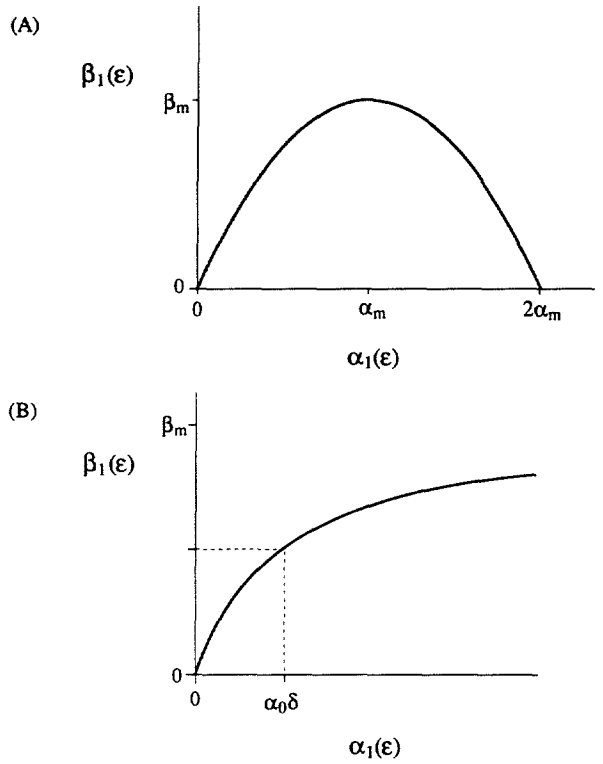

FIG. 8.—Two simple fitness sets: *A*, a parabolic one in which maximal transmission rate β_m corresponds with mortality rate α_m ($\beta_1(\epsilon) = \beta_m\epsilon(2 - \epsilon)$ and $\alpha_1(\epsilon) = \alpha_m\epsilon$), and *B*, a hyperbolic one in which the transmission rate can always be increased, but with a steeply increasing mortality rate ($\beta_1(\epsilon) = \beta_m\epsilon/(\epsilon + \delta)$ and $\alpha_1(\epsilon) = \alpha_0\epsilon$).

to limit cycles or even chaos (Godfray and Grenfell 1993), and then the question of evolution becomes much more complex, as the concept of R_0, which assumes equilibrium conditions, can no longer be used. This problem, however, falls outside the scope of the present article.

How ESS and Population Dynamics Combine

When the pathogen population changes in virulence, the force of infection will change with it. This will bring about a change in the frequency of multiple infection, and selection on host exploitation strategies shifts. To make the feedback explicit, consider the two fitness sets in figure 8, a parabola and a hyperbola in the α-β plane. For such simple fitness sets optimal exploitation strategies ϵ^* (specified by eq. [17]) can be solved explicitly (see app. E) and lead to solutions of the kind as shown in figure 9. For both fitness sets ϵ^* is an increasing function of $\bar{h}$, provided $\beta_2/(\mu + \alpha_2)$ is small enough. Hence, the larger the risk of double infections, the stronger the virulence that is favored. This effect is most conspicuous for the fitness set that does not have a definite optimum (fig. 8*B*), in which

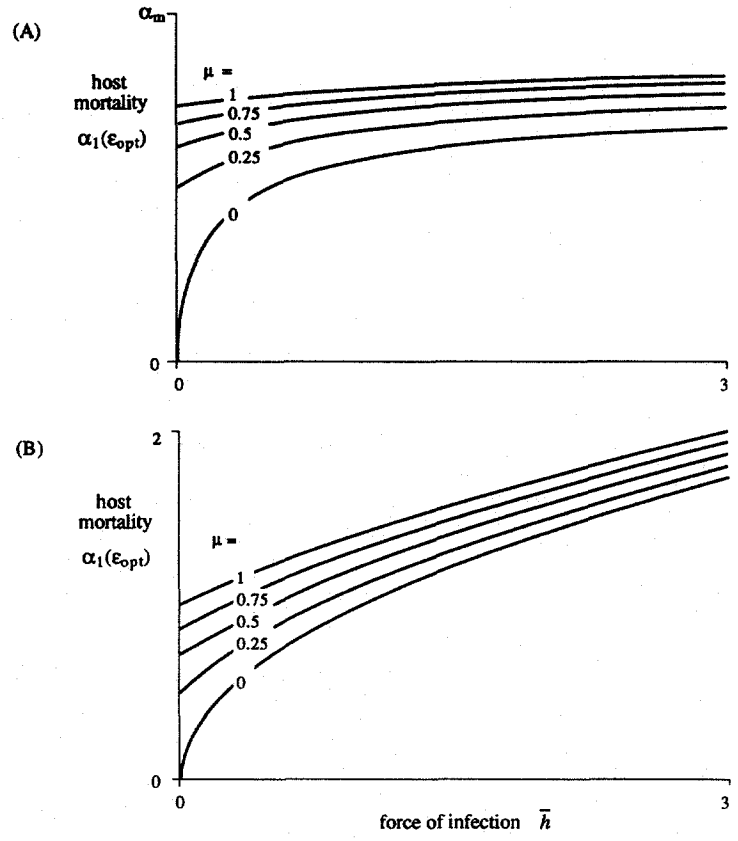

FIG. 9.—Mortality of singly infected hosts, as a consequence of optimal host exploitation strategies given the force of infection, for different values of the nondisease mortality μ. *A*, For the parabolic fitness set, with parameters: $\beta_m = 1$, $\alpha_m = 1$, $\beta_2 = 0.5\beta_m$, $\alpha_2 = \alpha_m$ (i.e., doubly infected hosts are shared equally, but the competition leads to maximal transmission rate and high virulence). *B*, For the hyperbolic fitness set, with parameters $\beta_m = 1$, $\delta = 1$, $\alpha_0 = 1$, $\beta_2 = 0.5\beta_m$, and $\alpha_2 = \infty$ (i.e., doubly infected hosts are shared equally, but the struggle leads to extremely large mortality rates.)

almost any host mortality rate may result from optimal host exploitation strategies (fig. 9*B*), depending on $\bar{h}$ and μ.

Now the argument can be completed. On the one hand, evolution toward lower virulence leads to an increased frequency of double infection (fig. 6). On the other hand, when the risk of double infection increases, higher virulence is favored (fig. 9). Figure 10 shows how, for an arbitrary example, the ESS α_1^* can be found.

For the simple fitness sets and the simple population dynamic model, it is possible (in principle) to calculate α_1^* exactly. But for more complex situations this will be a difficult task, even though more general models (e.g., allowing arbitrary numbers of infections, or incorporating host recovery) are likely to produce the same qualitative trends. In general, the evolution of virulence will

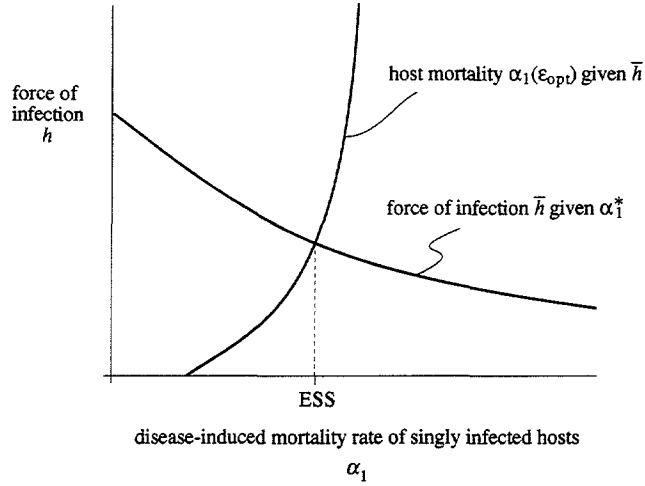

FIG. 10.—The ESS value $\alpha_1(\epsilon^*)$ is found by intersecting the curve specifying the optimal virulence $\alpha_1(\epsilon_{opt})$ given $\bar{h}$ with the curve specifying h as a function of the resident level of virulence $\alpha_1(\epsilon^*)$.

depend on both small-scale interactions between pathogens (competition within hosts) as well as large-scale interactions (competition for hosts on the population level). Changes in the population dynamics lead to a different relation between population virulence and the force of infection; changes in the outcome of the within-host interaction lead to a different relation between force of infection and optimal virulence. Both types of change will affect the ESS level of virulence.

Even though quantitative predictions are hard to obtain, two trends emerge from the present analysis. First, comparison of figure 9*A* and *B* suggests that optimal exploitation strategies are relatively little affected by the force of infection if the trade-off between transmission efficiency and disease-induced mortality has a top (fig. 8*A*). As it does not pay to increase virulence beyond this point, whether there is competition or not, increasing levels of multiple infection lead to progressively smaller increases in virulence. But if transmission efficiency can be increased indefinitely, increasing competition between pathogens promotes evolution toward higher virulence, in spite of its accelerating effect on disease-induced mortality. As a consequence, optimal host exploitation becomes strongly dependent on the force of infection.

Second, figures 6, 9, and 10 suggest that interaction between evolution and population dynamics is likely to be most pronounced if nondisease mortality (μ) is low. This makes sense, because the lower the nondisease mortality, the tighter the host population is regulated by the disease and, as a consequence, the more prominent the effect of evolutionary changes in the pathogen population will be.

DISCUSSION

The Evolution of Virulence

Whereas recent studies have countered conventional wisdom by pointing out that evolution toward complete avirulence is unlikely (Levin and Pimentel 1981; Ewald 1983; May and Anderson 1983), the present analysis of the coinfection case, as well as Nowak and May's (1994) analysis of the superinfection case, predict that pathogens may actually become more virulent when they get established in the host population. In the early stage of an epidemic, multiple infections are rare, and pathogens can afford to adopt prudent host exploitation strategies that lead to reduced virulence. However, when the pathogen becomes endemic, the frequency of multiple infection rises, and pathogens will have to share hosts more frequently. The resulting conflict of interests is not only likely to favor increased virulence in shared hosts but may also favor higher virulence in singly infected hosts, because subsequent infections have to be anticipated.

The coinfection case and the superinfection case both lead to increased virulence, but the manifestation is entirely different. Whereas superinfection (contest competition among pathogen clones) will lead to polymorphisms in virulence (Nowak & May 1994), coinfection (scramble competition) drives the pathogen population to a monomorphic virulence level. The polymorphism under superinfection arises because of the inherent advantage of slightly more virulent strains, but under coinfection such an advantage does not exist. In fact, the polymorphisms under superinfection are evolutionarily unstable, as they can always be invaded by new strains. Apparently, it is the nature of the within-host competition process that really matters. However, to our knowledge there is very little information on the biological details of this process. Models for the pathogen–immune system interaction (Nowak et al. 1990; Antia and Koella 1994; Antia et al. 1994; Sasaki 1994) might be tailored to shed more light on the outcomes of within-host competition.

A general result is that under multiple infection (independent of whether it results in scramble or contest competition) there will be an interaction between population dynamics and evolution; a change in population dynamics will be followed by an evolutionary response. Such a response will not occur if only single infections are allowed; then the optimal host exploitation strategy is independent of the state of the system.

Empirical Evidence

Contrary to the model predictions, observations often indicate evolution toward reduced virulence (Bull et al. 1991; Herre 1993); mortality caused by newly introduced pathogens may initially be very high and decrease in the course of a few generations. The classical example is the myxoma virus. Introduced in Australia to control rabbit populations, it caused initially more than 90% mortality, whereas a few years later only about 40% of the rabbits died from the infection (Levin and Pimentel 1981; Dwyer et al. 1990). However, a recent study on the interaction between cytoplasmic pathogens and *Daphnia magna* (Ebert 1994) in-

dicates that evolution may also lead to increased virulence. Ebert's work also suggests that such evolutionary transients are associated with a reduction in the variation of the effects of infection. When *Daphnia* hosts were infected with pathogens collected from distant sites, many combinations of virulence and spore load were observed, whereas less variation was observed in hosts infected with pathogens from nearby sites.

If we disregard evolutionary transients and consider cases in which hosts and pathogens are likely to have had sufficient time to approach evolutionary equilibrium, the question becomes whether pathogens have adopted truly prudent host exploitation strategies or whether they are actually more virulent, as the present theory would predict. This will be a difficult issue to decide. The detailed analysis of Dwyer et al. (1990) shows that in the rabbit-Myxoma system, prudent host exploitation would lead to a level of virulence slightly lower than actually observed. Yet the discrepancy is very small, and it may have many other causes.

A clear example is provided by fig wasps and their nematode parasites (Herre 1993). In some fig wasp species, females usually reproduce singly within a fig, whereas in other species figs are often occupied by more than one female. In the single-foundress case, parasites are completely dependent on the offspring of their carrier for their transmission, and these parasites are avirulent. In the multiple-foundress cases, the parasites can infect the offspring of the other foundresses, and such parasites are much more virulent (Herre 1993). These observations seem to be in agreement with our model predictions.

As Anderson and May (1991) note, the discussion is likely to become "sterile" without further knowledge about how pathogens interact with their hosts and with each other. Unfortunately, experimental data on the relation between transmission and host mortality are often hard to obtain (Anderson and May 1991), and this situation is not likely to change soon. However, present results suggest that to understand more of the evolution of virulence, it may be worthwhile to investigate in more detail what happens in the case of multiple infections.

On the one hand, pathogens could try to protect their hosts against further infections. Some pathogens may, for example "hide" in particular cells or organs, while activating the immune system of their host, barring the entry of subsequent pathogens. If this is the case, they can afford less virulent exploitation strategies. Another example may be provided by bacteria-bacteriophage systems, in which bacteria that carry "temperate" bacteriophage DNA are partially immune to infection by free phage (Stewart and Levin 1984; Bull et al. 1991). On the other hand, induced illness or other behavioral changes may also make the host more susceptible to pathogen vectors, which thus increases the efficiency of subsequent infections. For example, diseased hosts may groom less and thus become more prone to an attack of pathogen vectors. In humans it is known that some pathogens increase the susceptibility to other infective diseases (Anderson and May 1991), so why not also for the same disease? Often, multiple infection with the same disease will be difficult to detect.

In host-pathogen interactions complexities abound, both on the small scale of single hosts as well as on the large scale of host and pathogen populations. Quite drastic simplifying assumptions were needed to permit an analytical solution of

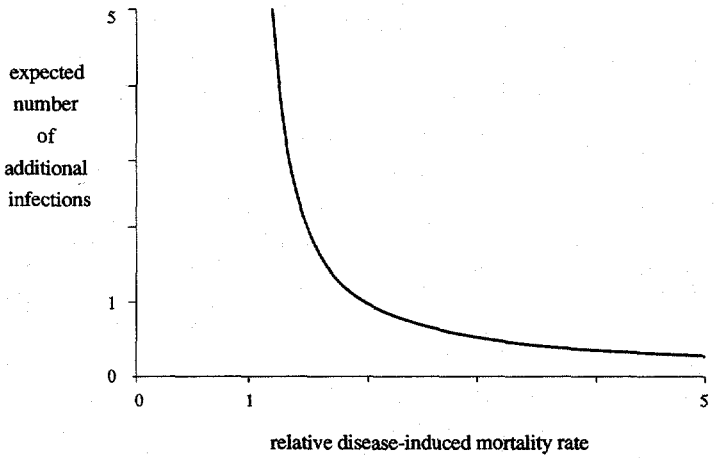

FIG. 11.—Expected number of subsequent infections as a function of the relative mortality rate (α^* divided by $r - \mu$, the net growth rate of the host population in absence of the disease). The more clones share a host, the more intense within-host competition will be, which favors increased virulence.

the relationship between multiple infection and the evolution of virulence. Nevertheless, the relation is likely to be common. To form an idea of the degree of host sharing in an arbitrary host-pathogen system, assume, as a null hypothesis, that infected hosts are equally susceptible to infection as uninfected hosts (a situation that becomes more likely the smaller the induced effects of pathogen infection are). Then the expected infective period lasts approximately $(\mu + \alpha^*)^{-1}$ time units, which means that an infected host is likely to be infected

$$N = \frac{\bar{h}}{\mu + \alpha^*}$$

times after the first infection. Because the force of infection $\bar{h}$ depends on the disease-induced mortality rate caused by the prevailing host exploitation strategies, we can write the number of additional clones in terms of α^*. In the case of the simple model analyzed here (eqq. [20–21]), a host is likely to receive

$$\frac{1}{\frac{\alpha^*}{r - \mu} - 1}$$

additional pathogen clones during its infective period. For highly virulent pathogens ($\alpha^* \gg r - \mu$) this number vanishes, but for moderately virulent pathogens ($\alpha^* \approx r - \mu$) it will become substantial (see fig. 11). An individual clone optimizing its exploitation strategy should definitely take this into account. Whereas influencing host longevity may work for pathogens singly occupying a host, any attempt will be overwhelmed by other host exploitation strategies, when hosts are shared with a multitude of others. Mutants that do not try to prolong the host's life but maximize their own transmission instead then have an advantage.

Thus, apart from that it never pays to reduce infectivity too much, complete avirulence is not likely to evolve for another reason: hosts would become shared by increasingly large numbers of pathogen clones.

Host-Microparasite Models

Many models for host-pathogen interactions assume that within-host pathogen reproduction is so fast that subsequent infections will have no discernible effect on within-host population dynamics. This leads to the standard model formulation for so-called host-microparasite interactions (Anderson and May 1979; May and Anderson 1979), in which all infections after the first are ignored altogether. From the perspective of the present analysis it will be clear that, even though they may give an adequate description of host-pathogen population dynamics, such models may be inappropriate for evolutionary analyses. The R_0 measure derived from a population dynamic model is the one of the resident pathogen strain, whereas an evolutionary analysis should be based on the R_0 of a mutant strain. The latter cannot be specified without knowledge (or, in absence of such knowledge, additional assumptions) about within-host competition.

Host Coevolution

To emphasize the interrelationship of multiple infection and pathogen evolution we assumed that host characters remain constant. Yet hosts will evolve as well, which may have counterintuitive consequences.

From the perspective of the present analysis, evolution of the hosts is likely to change the relation between transmission efficiency and disease-induced mortality (or, rather, the effect of pathogen exploitation strategies on both). That hosts benefit from a reduced disease-induced mortality is clear, while what they can gain by influencing pathogen transmission efficiency is less obvious. If we assume that disease-induced mortality decreases while pathogen transmission remains relatively unaffected, the $\beta(\epsilon)$-$\alpha(\epsilon)$ trade-off curve will become compressed to the left. A first-order effect is that optimal host exploitation leads to reduced disease-induced mortality (cf. fig. 1). However, we have also found that reducing disease-induced host mortality leads to an increased force of infection and that this favors increased virulence (disease-induced mortality) instead. One cannot even a priori exclude the possibility that virulence increases when the host population coevolves, so further research is needed to resolve this issue.

We assumed that second infections are just as efficient as first infections, but this is a rather arbitrary assumption. Differential efficiencies, which may be caused by both pathogens or hosts, have interesting consequences. To a host, being "prudently exploited" by a single pathogen clone may be preferable to being ravaged by the struggle among many different pathogen strains. To a pathogen clone, it is advantageous to hamper other pathogen clones. Thus, there is scope for "cooperation" between host and pathogen, in which host and pathogen clone help each other in fighting subsequent infections.

ACKNOWLEDGMENTS

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APPENDIX A

TABLE A1

SYMBOLS AND THEIR INTERPRETATION

Symbol	Interpretation
r	Per capita growth rate of the host
μ	Nondisease mortality rate of the host
x	Density of uninfected hosts
y	Density of singly infected hosts
z	Density of doubly infected hosts
h	Force of infection
α	Disease-induced mortality rate
β	Transmission efficiency of first pathogen clone
γ	Transmission efficiency of second pathogen clone
B_F	Per-host transmission of first pathogen clone
B_S	Per-host transmission of second pathogen clone
R_0	Basic reproduction ratio
ϵ	Host exploitation strategy for singly infected hosts
ϕ	Host exploitation strategy of the first clone in doubly infected hosts
σ	Host exploitation strategy of the second clone in doubly infected hosts

NOTE.—A bar over a symbol (e.g., $\bar{x}$) denotes the value under population dynamic equilibrium. The subscripts 1 and 2 denote values for singly and doubly infected hosts. An asterisk denotes the ESS.

APPENDIX B

THE PER-HOST TRANSMISSION FACTOR WITH DOUBLE INFECTION

The per-host transmission factor for the case in which the mutant is the first to infect a host follows straightforwardly from the integration of equation (10), using the probability functions (9):

$$\begin{aligned} B_F &= \int_0^\infty \beta_1 e^{-(\mu+\alpha_1+h)t}\,dt + \\ &\quad \int_0^\infty \frac{h\beta_2}{\alpha_2-\alpha_1-h}\left(e^{-(\mu+\alpha_1+h)t} - e^{-(\mu+\alpha_2)t}\right)dt \\ &= \frac{\beta_1}{\mu+\alpha_1+h} + \frac{h\beta_2}{\alpha_2-\alpha_1-h}\left(\frac{1}{\mu+\alpha_1+h} - \frac{1}{\mu+\alpha_2}\right) \\ &= \frac{\beta_1}{\mu+\alpha_1+h} + \frac{h\beta_2}{\alpha_2-\alpha_1-h}\,\frac{(\mu+\alpha_2)-(\mu+\alpha_1+h)}{(\mu+\alpha_1+h)(\mu+\alpha_2)} \\ &= \frac{1}{\mu+\alpha_1+h}\left(\beta_1 + \frac{h\beta_2}{\mu+\alpha_2}\right), \end{aligned} \tag{B1}$$

which is identical to equation (11).

APPENDIX C

Maximization of R_0 with Risk of Subsequent Infection

Setting the derivative with respect to ϵ of the per-host transmission factor B_F,

$$\frac{dB_F}{d\epsilon} = \frac{d\beta_1}{d\epsilon}\frac{\partial B_F}{\partial \beta_1} + \frac{d\alpha_1}{d\epsilon}\frac{\partial B_F}{\partial \alpha_1}, \tag{C1}$$

equal to zero yields

$$\frac{\dfrac{d\beta_1}{d\epsilon}}{\dfrac{d\alpha_1}{d\epsilon}} = -\frac{\dfrac{\partial B_F}{\partial \alpha_1}}{\dfrac{\partial B_F}{\partial \beta_1}}. \tag{C2}$$

If we substitute the partial derivatives

$$\frac{\partial B_F}{\partial \alpha_1} = -\frac{\beta_1 + h\dfrac{\beta_2}{\mu + \alpha_2}}{(\mu + \alpha_1 + h)^2} \tag{C3}$$

and

$$\frac{\partial B_F}{\partial \beta_1} = \frac{1}{\mu + \alpha_1 + h} \tag{C4}$$

into equation (C2), we obtain equation (17). The condition with the secondary derivatives follows in a similar fashion.

APPENDIX D

Formal ESS Analysis

That pathogens are omniscient and can detect the presence of other clones within a host is a rather arbitrary assumption. In fact, there are many ways in which the pathogens may be limited in their abilities to react to other clones. For example, a second clone might be able to detect that the host is already infected (e.g., through the presence of antibodies), while the pathogens within the host are not able to detect the arrival of a newcomer (because this is, initially at least, a tiny minority). Different assumptions lead to different relations between pathogen host exploitation strategies and the epidemiological parameters. Depending on the number of situations a pathogen can distinguish, it can adopt a number of different strategies, and a pathogen's strategy can thus be captured in a vector of parameters.

Let the mutant's strategy be denoted by the vector $\mathbf{E}$, while the resident has strategy $\mathbf{E}^*$. Then we can expand equation (12) for the mutant's R_0 into

$$R_0(\mathbf{E}, \mathbf{E}^*) = \frac{\beta_1(\mathbf{E}) + h(\mathbf{E}^*)\dfrac{\beta_2(\mathbf{E}, \mathbf{E}^*)}{\mu + \alpha_2(\mathbf{E}, \mathbf{E}^*)}}{\mu + \alpha_1(\mathbf{E}) + h(\mathbf{E}^*)} x(\mathbf{E}^*) + \frac{\gamma_2(\mathbf{E}^*, \mathbf{E})}{\mu + \alpha_2(\mathbf{E}^*, \mathbf{E})} y(\mathbf{E}^*), \tag{D1}$$

where $x(\mathbf{E}^*)$ and $y(\mathbf{E}^*)$ denote the equilibrium densities of uninfected and singly infected hosts, respectively, and $h(\mathbf{E}^*)$ stands for the equilibrium force of infection of the resident.

The expressions for the equilibrium densities may be quite complex, but equilibrium requires that the R_0 of the resident be equal to one:

$$R_0(\mathbf{E}^*, \mathbf{E}^*) = \frac{\beta_1(\mathbf{E}^*) + h(\mathbf{E}^*)\dfrac{\beta_2(\mathbf{E}^*, \mathbf{E}^*)}{\mu + \alpha_2(\mathbf{E}^*, \mathbf{E}^*)}}{\mu + \alpha_1(\mathbf{E}^*) + h(\mathbf{E}^*)} x(\mathbf{E}^*) + \frac{\gamma_2(\mathbf{E}^*, \mathbf{E}^*)}{\mu + \alpha_2(\mathbf{E}^*, \mathbf{E}^*)} y(\mathbf{E}^*) = 1. \tag{D2}$$

Existence of a population dynamic equilibrium guarantees that a solution of equation (D2) exists. Therefore, the condition for invasion of a mutant, which requires $R(\mathbf{E}, \mathbf{E}^*) > 1$, leads to

$$R_0(\mathbf{E}, \mathbf{E}^*) > R_0(\mathbf{E}^*, \mathbf{E}^*)\,. \tag{D3}$$

If no mutant can invade, that is, if

$$\max_{\mathbf{E}} R_0(\mathbf{E}, \mathbf{E}^*) \leq R_0(\mathbf{E}^*, \mathbf{E}^*)\,, \tag{D4}$$

with equality only when $\mathbf{E} = \mathbf{E}^*$, then strategy $\mathbf{E}^*$ is evolutionarily stable. Thus, if a mutant that maximizes its R_0 according to condition (D4) will adopt strategy $\mathbf{E}^*$, given that the resident population has adopted this strategy, strategy $\mathbf{E}^*$ is an ESS.

If we collapse pathogen strategies into a single parameter, which implies that pathogens have no ability to detect each other whatsoever (i.e., in terms of the parameters used in the text, it would mean $\epsilon = \phi = \sigma = \mathbf{E}$), ESS conditions become

$$\frac{dR_0(\mathbf{E}, \mathbf{E}^*)}{d\mathbf{E}} = 0 \quad \text{and} \quad \frac{d^2R_0(\mathbf{E}, \mathbf{E}^*)}{d\mathbf{E}^2} < 0 \tag{D5}$$

for $\mathbf{E} = \mathbf{E}^*$. This simplification leads to very complicated expressions, which do not allow an easy interpretation.

If the pathogens have the ability to detect the type of host they infect, but not the arrival of other pathogens, a strategy vector has two elements, $\mathbf{E}_1 = \epsilon = \phi$ and $\mathbf{E}_2 = \sigma$, that can vary independently. Maximization a mutant's R_0 then leads to the following pair of conditions:

$$\begin{aligned}
&\frac{dR_0}{d\mathbf{E}_1} = 0 \\
&\Leftrightarrow \frac{d}{d\mathbf{E}_1}\left[\frac{\beta_1(\mathbf{E}_1) + h(\mathbf{E}^*)\dfrac{\beta_2(\mathbf{E}_1, \mathbf{E}_2^*)}{\mu + \alpha_2(\mathbf{E}_1, \mathbf{E}_2^*)}}{\mu + \alpha_1(\mathbf{E}_1) + h(\mathbf{E}^*)}\right] = 0 \\
&\Leftrightarrow \left[\frac{d\beta_1(\mathbf{E}_1)}{d\mathbf{E}_1} + h(\mathbf{E}^*)\frac{\dfrac{d\beta_2(\mathbf{E}_1, \mathbf{E}_2^*)}{d\mathbf{E}_1} - \dfrac{\beta_2(\mathbf{E}_1, \mathbf{E}_2^*)}{\mu + \alpha_2(\mathbf{E}_1, \mathbf{E}_2^*)}\dfrac{d\alpha_2(\mathbf{E}_1, \mathbf{E}_2^*)}{d\mathbf{E}_1}}{\mu + \alpha_2(\mathbf{E}_1, \mathbf{E}_2^*)}\right]\left[\frac{d\alpha_1(\mathbf{E}_1)}{d\mathbf{E}_1}\right]^{-1} \\
&\qquad = \frac{\beta_1(\mathbf{E}_1) + h(\mathbf{E}^*)\dfrac{\beta_2(\mathbf{E}_1, \mathbf{E}_2^*)}{\mu + \alpha_2(\mathbf{E}_1, \mathbf{E}_2^*)}}{\mu + \alpha_1(\mathbf{E}_1) + h(\mathbf{E}^*)}
\end{aligned} \tag{D6}$$

and

$$\begin{aligned}
&\frac{dR_0}{d\mathbf{E}_2} = 0 \\
&\Leftrightarrow \frac{d}{d\mathbf{E}_2}\left[\frac{\gamma_2(\mathbf{E}_1^*, \mathbf{E}_2)}{\mu + \alpha_2(\mathbf{E}_1^*, \mathbf{E}_2)}\right] = 0 \\
&\Leftrightarrow \frac{\dfrac{d\gamma_2(\mathbf{E}_1^*, \mathbf{E}_2)}{d\mathbf{E}_2}}{\dfrac{d\alpha_2(\mathbf{E}_1^*, \mathbf{E}_2)}{d\mathbf{E}_2}} = \frac{\gamma_2(\mathbf{E}_1^*, \mathbf{E}_2)}{\mu + \alpha_2(\mathbf{E}_1^*, \mathbf{E}_2)}\,.
\end{aligned} \tag{D7}$$

(Second-order derivatives are not given, but they can be derived by differentiation of

conditions [D6] and [D7].) If anything is to be concluded from this sort of condition, then it is that the optimal solution for $\mathbf{E}_1$ depends on $h(\mathbf{E}^*)$ and therefore on the strategies of other pathogens in the population. In that case we cannot even evaluate condition (D7) to calculate the optimal $\mathbf{E}_2$ independently, because this requires $\mathbf{E}_1^*$ to be known.

APPENDIX E

Optimal Host Exploitation for Two Simple Fitness Sets

Consider first the fitness set depicted in figure 8*A*:

$$\begin{aligned} \beta_1(\epsilon) &= \beta_m \epsilon(2 - \epsilon) \\ \alpha_1(\epsilon) &= \alpha_m \epsilon . \end{aligned} \tag{E1}$$

Applying condition (17) for optimal ϵ yields

$$\frac{2\beta_m(1 - \epsilon)}{\alpha_m} = \frac{\beta_m \epsilon(2 - \epsilon) + h\dfrac{\beta_2}{\mu + \alpha_2}}{\mu + \alpha_m \epsilon + h}, \tag{E2}$$

which after some rearranging leads to a quadratic expression in ϵ,

$$\frac{1}{2}\epsilon^2 + \frac{\mu + h}{\alpha_m}\epsilon - \left(\frac{\mu + h}{\alpha_m} - \frac{h}{2\beta_m}\frac{\beta_2}{\mu + \alpha_2}\right) = 0, \tag{E3}$$

whose positive root is given by

$$\epsilon = -\frac{\mu + h}{\alpha_m} + \sqrt{\left(\frac{\mu + h}{\alpha_m}\right)^2 + 2\left(\frac{\mu + h}{\alpha_m} - \frac{h}{2\beta_m}\frac{\beta_2}{\mu + \alpha_2}\right)}. \tag{E4}$$

This expression has been used to calculate the results in figure 9*A*.

The fitness set depicted in figure 8*B* is given by

$$\begin{aligned} \beta_1(\epsilon) &= \frac{\beta_m \epsilon}{\epsilon + \delta} \\ \alpha_1(\epsilon) &= \alpha_0 \epsilon . \end{aligned} \tag{E5}$$

Again applying condition (17) and rearranging lead to the quadratic expression

$$(1 + Q)\epsilon^2 + 2Q\delta\epsilon + \left(Q\delta - \frac{\mu + h}{\alpha_0}\right)\delta = 0, \tag{E6}$$

with

$$Q = \frac{h}{\beta_m}\frac{\beta_2}{\mu + \alpha_2}, \tag{E7}$$

whose positive solution is

$$\epsilon = \frac{-Q\delta + \sqrt{(Q\delta)^2 - (1 + Q)\left(Q\delta - \dfrac{\mu + h}{\alpha_0}\right)\delta}}{1 + Q}. \tag{E8}$$

If the conflict between pathogen clones in doubly infected hosts escalates, we would have

$$\frac{\beta_2}{\mu + \alpha_2} \to 0 \tag{E9}$$

and hence $Q \to 0$, so that then

$$\epsilon \to \sqrt{\frac{\mu + h}{\alpha_0}\delta}. \tag{E10}$$

This relation has been used to calculate the examples in figure 9*B*.

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