VOL. 151, NO. 4 THE AMERICAN NATURALIST APRIL 1998

The Acquisition of Hypovirulence in Host-Pathogen Systems with Three Trophic Levels

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Submitted October 14, 1996; Accepted October 1, 1997

ABSTRACT: A major focus of research on the dynamics of host-pathogen interactions has been the evolution of pathogen virulence, which is defined as the loss in host fitness due to infection. It is usually assumed that changes in pathogen virulence are the result of selection to increase pathogen fitness. However, in some cases, pathogens have acquired hypovirulence by themselves becoming infected with hyperparasites. For example, the chestnut blight fungus *Cryphonectria parasitica* has become hypovirulent in some areas by acquiring a double-stranded RNA hyperparasite that debilitates the pathogen, thereby reducing its virulence to the host. In this article, we develop and analyze a mathematical model of the dynamics of host-pathogen interactions with three trophic levels. The system may be dominated by either uninfected (virulent) or hyperparasitized (hypovirulent) pathogens, or by a mixture of the two. Hypovirulence may allow some recovery of the host population, but it can also harm the host population if the hyperparasite moves the transmission rate of the pathogen closer to its evolutionarily stable strategy. In the latter case, the hyperparasite is effectively a mutualist of the pathogen. Selection among hyperparasites will often minimize the deleterious effects, or maximize the beneficial effects, of the hyperparasite on the pathogen. Increasing the frequency of multiple infections of the same host individual promotes the acquisition of hypovirulence by increasing the opportunity for horizontal transmission of the hyperparasite. This effect opposes the usual theoretical expectation that multiple infections promote the evolution of more virulent pathogens via selection for rapid growth within hosts.

Keywords: virulence, hypovirulence, hyperparasite, superinfection, chestnut blight, biological control.

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Am. Nat. 1998. Vol. 151, pp. 343–355.

Pathogens can have profound effects on both the ecological and evolutionary dynamics of their hosts (Price 1980; Burdon 1987). One of the most important influences on the strength of these interactions is pathogen virulence, defined here as a measure of the effect of infection on host fitness. The factors that influence the evolution of virulence have therefore emerged as a major issue in the study of host-pathogen systems. Theoreticians have been especially active in this area, most working under the assumption that virulence is related in some way to pathogen transmission (Levin and Pimentel 1981; Anderson and May 1982; Ewald 1983; May and Anderson 1983; Antia et al. 1994; Frank 1996). Anderson and May (1982) found that the specific relationship between pathogen transmission rate and virulence may often determine the evolutionary dynamics of virulence. If virulence increases linearly with a pathogen's transmission rate, then selection should favor ever-increasing virulence. By contrast, selection will favor an intermediate level of virulence when an increase in transmission results in a disproportionately greater increase in virulence. Recent models have also emphasized how within-host population dynamics of pathogens may influence the evolution of virulence (Antia et al. 1994; Nowak and May 1994).

Pathogen virulence may also be influenced by organisms at a higher trophic level that debilitate the pathogen. Consider a pathogen that is, in turn, infected by a parasite of its own—hereafter termed a *hyperparasite.* The hyperparasite may have a deleterious effect on the pathogen and thereby affect other species down the trophic chain in a manner similar to the top-down effects that predators can have on communities (Hairston et al. 1960; Fretwell 1977; Oksanen et al. 1981; Powers 1992; Holt and Hochberg 1998). Hyperparasitoids, for example, have been shown to have these effects in arthropod food chains, often undermining efforts to employ parasitoids as biological control agents (Beddington and Hammond 1977; May and Hassell 1981). Invasion of a host-pathogen system by a hyperparasite having deleterious effects on the pathogen may mimic the dynamics ex-

pected from an evolutionary reduction in virulence, especially when the hyperparasite is intracellular and hence difficult to detect without detailed study.

It is not clear how important internal hyperparasites might be in reducing pathogen virulence, but there is reason to believe hyperparasitism is common in nature. There are many cases involving hyperparasitism of fungal pathogens of plants (Hollings 1982; Buck 1986). Often, these hyperparasites are double-stranded (ds) RNA elements that do not produce any capsid and hence have no independent existence outside the pathogen. Double-stranded RNAs infect a wide range of fungal species including oomycete (Tooley et al. 1989; Newhouse et al. 1992), ascomycte (Nuss and Koltin 1990; Enebak et al. 1994), and basidiomycete (Lawrence et al. 1988) pathogens. Infections by dsRNA elements generally decrease pathogen virulence (Nuss and Koltin 1990). Many bacterial species are also infected by various plasmids and viruses (Levin and Lenski 1983), many of which encode functions, such as resistance to antibiotics, that benefit their hosts in certain environments. Under other conditions, however, these same elements—and presumably other elements that lack these functions—may reduce the growth rate of their bacterial hosts (e.g., Lenski and Bouma 1987).

In this article, we develop a mathematical model of the dynamics of host-pathogen interactions in which there is a hyperparasite, which constitutes a third trophic level. Our formulation is similar to an earlier model (Lenski and May 1994) of the evolution of virulence except that we introduce a second pathogen, one that carries a hyperparasite, into the population. The resulting model is therefore similar to prior models of competing pathogens (Levin and Pimentel 1981; Levin 1983; Hochberg and Holt 1990) except that we assume an explicit relationship between pathogen transmission and virulence to examine how these properties are affected by hyperparasitism. The biology of hyperparasitism also dictates certain assumptions regarding the transmission properties and competitive abilities of the two pathogen types. For example, we assume that a hyperparasite debilitates the pathogen, which simultaneously reduces the pathogen's rate of transmission and its virulence. Finally, we include density dependence, which allows us to examine the effects of hyperparasitism on host recovery.

A Model for the Acquisition of Hypovirulence by Hyperparasitism

The model includes three host populations: uninfected hosts (U), hosts infected by the virulent pathogen (V), and hosts infected with a pathogen that is hypovirulent because it is infected by a hyperparasite (H). We assume that an individual host can support only a single type of infection, although the type of infection may change (see below). All hosts are born uninfected at a per capita rate that is density dependent, that is, $b - aN$, where N is the number of all hosts (U + V + H). (Table 1 summarizes the definitions of all the model variables and parameters.) All hosts may reproduce, but the birth rate of infected hosts is reduced to a fraction ρ_V for virulent pathogens and ρ_H for hyperparasitized pathogens. Uninfected hosts become infected with the virulent and hypovirulent pathogens at rates $\beta_V UV$ and $\beta_H UH$, respectively, where β_V and β_H are the rates of infectious transmission of the two pathogen types. All hosts die at the rate d due to causes other than infection, while infected hosts are subject to extra deaths. The extra death rate due to infection is a quadratic function of the pathogen's rate of transmission, that is, $e_1\beta_V + e_2\beta_V^2$ for the virulent pathogen.

The hyperparasite is assumed to have no independent existence outside the pathogen. It is vertically transmitted with all propagules produced by the hyperparasite-

Table 1: Parameters of the model and their range of possible values

Parameter	*Definition*	*Range*
U	Density of uninfected hosts	$U \geq 0$
V	Density of hosts infected with a virulent pathogen	$V \geq 0$
H	Density of hosts infected with a hyperparasitized pathogen	$H \geq 0$
b	Maximum host fecundity	$b > d$
a	Intensity of density-dependent effects on host fecundity	$a > 0$
d	Host death rate due to factors other than disease	$d > 0$
β_i	Transmission rate of pathogen i	$\beta_i > 0$
e_1	Linear component of the relationship between disease-induced host death rate and pathogen transmission rate	$e_1 > 0$
e_2	Quadratic component of the relationship between disease-induced host death rate and pathogen transmission rate	$e_2 \geq 0$
s	Efficiency of horizontal transmission of hyperparasite (relative to vertical transmission)	$s \geq 0$
σ_{ij}	Rate at which hyperparasite j displaces hyperparasite i via horizontal transmission	$\sigma_{ij} > 0$
ρ_i	Relative fecundity of hosts infected with pathogen i	$0 \leq \rho_i \leq 1$

infected pathogen. The hyperparasite debilitates the pathogen to some degree, reducing the pathogen's transmission rate to some lower value β_H, which in turn reduces the extra death rate to $e_1\beta_H + e_2\beta_H^2$. The hyperparasite can also be transmitted horizontally. Specifically, it can infect the virulent pathogen when propagules of the hypovirulent pathogen—which carry the hyperparasite—infect hosts that are already infected with the virulent pathogen. The rate of horizontal transfer is $s\beta_H$, where the constant s is a measure of the relative efficiency with which the hyperparasite is transferred from the hypovirulent propagule to the virulent infection. The value of s may be greater or less than unity depending on whether it is easier for a hyperparasitized pathogen to infect a host already infected with the virulent pathogen or to infect an uninfected host, respectively.

Given these conditions, the rates of change for the three host populations are:

$$\dot{U} = (b - aN)(U + \rho_V V + \rho_H H) - dU - \beta_V UV - \beta_H UH; \quad (1)$$

$$\dot{V} = \beta_V UV - s\beta_H HV - dV - (e_1\beta_V + e_2\beta_V^2)V; \quad (2)$$

and

$$\dot{H} = \beta_H UH + s\beta_H VH - dH - (e_1\beta_H + e_2\beta_H^2)H, \quad (3)$$

where the dot notation indicates differentiation with respect to time. This formulation is similar to Hochberg and Holt's (1990) model except that we generalize their model to include density dependence and make the specific assumption that there is some relationship between pathogen transmission and disease-induced mortality.

Invasion Conditions and Equilibria

Henceforth, we assume that $b > d$ so that the host population can persist in the absence of the pathogen and that $\beta_V\hat{U} > d + e_1\beta_V + e_2\beta_V^2$ so the virulent pathogen can invade a population of uninfected hosts at their equilibrium density in the absence of hyperparasites, $\hat{U} = (b - d)/a$.

When the hyperparasite is absent, there is a unique interior equilibrium (Lenski and May 1994) at which

$$\hat{U}_V = \frac{d + (e_1\beta_V + e_2\beta_V^2)}{\beta_V} \quad (4)$$

and

$$\hat{V} = \frac{-B + \sqrt{B^2 + 4AC}}{2C}, \quad (5)$$

where $\hat{U}_V$ is the equilibrium population size of uninfected hosts in the presence of the virulent pathogen and $A = \hat{U}_V(b - a\hat{U}_V - d) > 0$, $B = \hat{U}_V(a + a\rho_V + \beta_V) - \rho_V b$, and $C = a\rho_V > 0$. The eigenvalues of the community matrix (May 1974; Levins 1975) have negative real parts, so this equilibrium is stable.

Now consider a rare hyperparasite invading this equilibrium. Substituting $\hat{U}_V$ and $\hat{V}$ into equation (3) and solving for s, the hypovirulent pathogen will invade when

$$s > \frac{(\beta_V - \beta_H)(d - e_2\beta_V\beta_H)}{\hat{V}\beta_V\beta_H}. \quad (6)$$

Next, we consider the equilibrium population consisting of uninfected hosts and hosts that are infected by the hypovirulent pathogen (i.e., the hyperparasite is fixed within the pathogen population). The equilibria for $\hat{U}_H$ and $\hat{H}$ are identical to equations (4) and (5), respectively, except with the appropriate notational changes. The virulent pathogen can invade this equilibrium only when

$$s < \frac{(\beta_V - \beta_H)(d - e_2\beta_V\beta_H)}{\hat{H}\beta_H^2}. \quad (7)$$

For the virulent and hyperparasitized forms of the pathogen to coexist, inequalities (6) and (7) must both be true.

A hypovirulent pathogen can invade if the hyperparasite it carries does not seriously reduce pathogen transmission (i.e., β_H is close to β_V), and if the hyperparasite has some horizontal transmission ($s > 0$) (fig. 1). Coexistence of virulent and hypovirulent pathogens is most likely when the hyperparasite spreads primarily by horizontal transmission (low β_H and high s) because the hypovirulent pathogen depends primarily on the virulent pathogen for its existence in these situations. In fact, the hyperparasite may depend on horizontal transmission to such an extent that the hypovirulent pathogen cannot become established until after the virulent pathogen has already become established (region *II*, fig. 1). This result echoes Hochberg and Holt's (1990) conclusion that for competing parasites to coexist there must be some tradeoff between a parasite's transmission rate and the ability to compete within multiply-infected hosts. Such a tradeoff is inherent in our model because we assume that the hyperparasite reduces pathogen transmission and that horizontal transmission of the hyperparasite causes hypovirulent pathogens to dominate within multiply-infected hosts.

The parameter e_2 governs whether pathogen virulence rises linearly ($e_2 = 0$) or disproportionately ($e_2 > 0$) with increasing transmission rate, and it is especially important for understanding the spread of the hyperparasite. When $e_2 = 0$, selection on the pathogen favors maximal transmission (Anderson and May 1982). The hyperparasite, therefore, invariably lowers the fitness of the pathogen by reducing pathogen transmission, and the existence

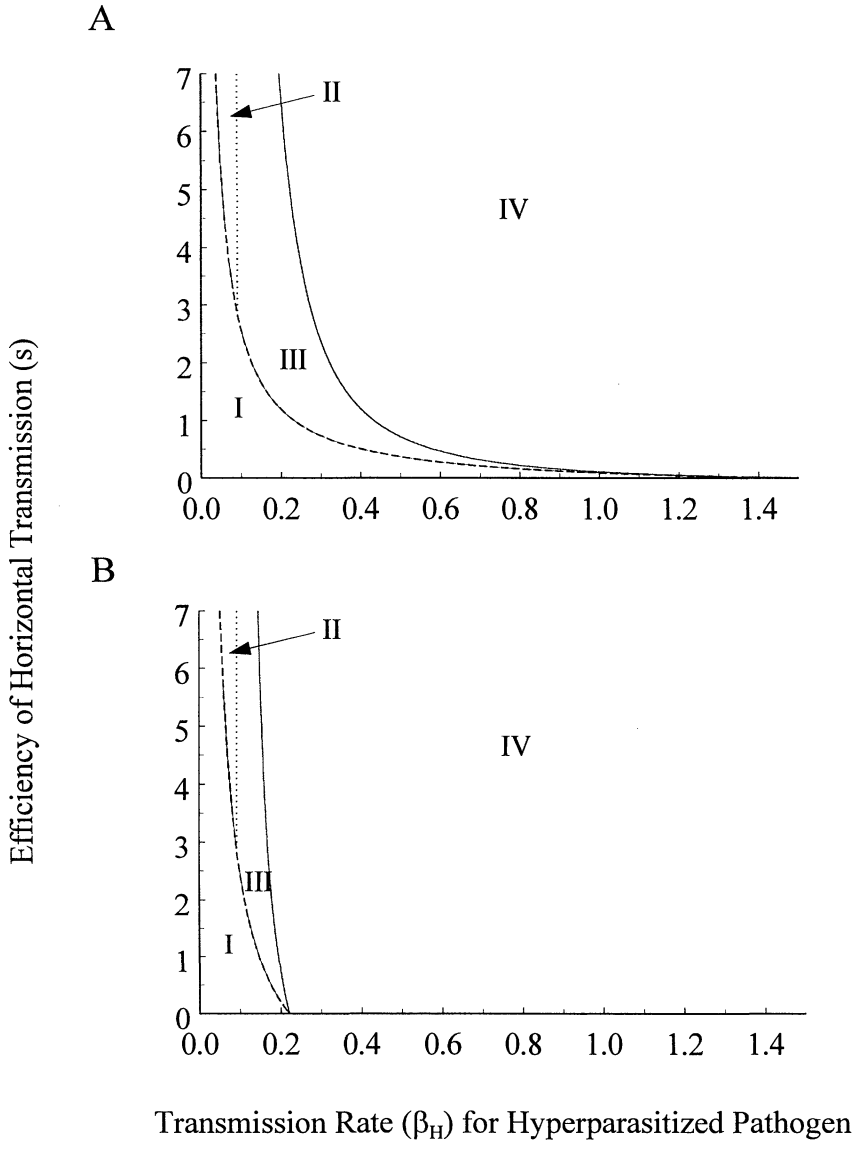

Figure 1: Effect of the relationship between pathogen transmission and virulence on the invasion conditions for the virulent and hyperparasitized pathogens. *A*, $e_2 = 0$. *B*, $e_2 = 2.0$. Dashed lines indicate the boundary where the hyperparasite can invade a population of virulent pathogens; invasion occurs above and to the right of the line. Solid lines indicate boundary conditions for invasion of the virulent pathogen into an equilibrium population of hyperparasitized pathogens; invasion occurs below and to the left of the line. Regions of the graph are as follows. *I* = the hyperparasite cannot invade a virulent pathogen population. *II* = hyperparasite can only invade if the virulent pathogen is already resident within the host population; the resulting equilibrium population contains a mixture of virulent and hyperparasitized pathogens. *III* = hyperparasite will always invade when rare; the resulting equilibrium population contains a mixture of virulent and hyperparasitized pathogens. *IV* = hyperparasite always invades when rare; at equilibrium, all members of the pathogen population are infected by the hyperparasite. Other parameter values are as follows: $b = 5$, $a = 0.8$, $d = 0.5$, $\rho_H = \rho_V = 0$, $e_1 = 0.1$, $\beta_V = 1.5$.

of the hyperparasite requires sufficient horizontal transmission to offset this effect (fig. 1*A*). When $e_2 > 0$, however, selection on the pathogen favors intermediate transmission (Anderson and May 1982). By reducing the pathogen's transmission, the hyperparasite may actually push the pathogen closer to its evolutionarily stable strategy (ESS), in which case the existence of the hyperparasite does not require horizontal transmission (fig. 1*B*). To see this, notice first that when $e_2 > 0$, the hyperparasite spreads to fixation if

$$\beta_H > \frac{d}{\beta_V e_2}. \tag{8}$$

This condition renders the inequality in equation (6) true and the inequality in equation (7) false for all other relevant parameter values (including $s = 0$). Then consider the fact that the pathogen's ESS transmission rate is that which minimizes the density of uninfected hosts (Lenski and May 1994) or

$$\beta^* = \sqrt{\frac{d}{e_2}}. \tag{9}$$

Combining equations (8) and (9) implies that

$$\frac{\beta_V}{\beta^*} > \frac{\beta^*}{\beta_H}. \tag{10}$$

Recalling that $\beta_V > \beta_H$, and recognizing that β_V must be greater than β^* in order to fulfill this inequality, equation (10) implies that the hypovirulent pathogen can invade if it is closer to the ESS than the virulent pathogen. In effect, the hyperparasite is a mutualist of the pathogen.

The life history of the host also has important effects on the invasion conditions for the hyperparasite (fig. 2). Reducing the strength of density dependence, *a*, restricts the spread of the virulent pathogen (fig. 2*A* vs. *B*). This effect occurs because reduced density dependence increases the density of hypovirulent pathogens, which makes it difficult for virulent pathogens to invade (eq. [7]). Reduced density dependence also increases the density of virulent pathogens, which allows hypovirulent pathogens to invade more readily (eq. [6]). The latter effect is less pronounced, however, because the density of hosts infected by virulent pathogens is less sensitive to density dependence than is the density of hosts infected by hypovirulent pathogens.

Increasing the relative fecundity of infected hosts (ρ_V and ρ_H) increases the equilibrium number of infected hosts ($\hat{V}$ or $\hat{H}$, eq. [5]), making the condition for the invasion of the hyperparasite more general (eq. [6]) and the condition for the invasion of the virulent pathogen more stringent (eq. [7]). A realistic scenario might be that virulent pathogens reduce the fecundity of hosts more than do hyperparasitized pathogens, that is, $\rho_V < \rho_H$. If ρ_V is much lower than ρ_H, the curves denoting the critical values of *s* may intersect, creating a new region in the parameter space (region *V*, fig. 3*A*). This region contains an unstable equilibrium in which either pathogen class can invade a pathogen-free population, but neither can invade when the other pathogen is at equilibrium (fig. 3*B*). Hence, the pathogen that persists is the one that becomes established in the population first. These so-called priority effects have been found in previous models of hyperparasitism (Hochberg and Holt 1990).

Selection among Hyperparasites

We now examine the conditions under which a mutant hyperparasite can invade a system in which a hyperparasite has already spread to fixation in the pathogen population. A mutant hyperparasite will infect pathogens already infected with the wild-type hyperparasite at a rate of $\sigma_{ij}\beta_j H_i H_j$, and the opposite occurs at a rate of $\sigma_{ji}\beta_i H_i H_j$, where H_i and H_j are the densities of the two hyperparasitized hosts and σ is the rate that one hyperparasite displaces another via horizontal transmission. The per capita rate of change of a rare hyperparasite invading the equilibrium population is

$$\frac{\dot{H}_j}{H_j} = \beta_j \hat{U}_i - d - (e_1\beta_j + e_2\beta_j^2) - \sigma_{ji}\beta_i \hat{H}_i + \sigma_{ij}\beta_j \hat{H}_i. \tag{11}$$

Assuming for simplicity that $\sigma_{ij} = \sigma_{ji} = \sigma$ and substituting in the equilibrium value for $\hat{U}_i$ (eq. [4]), the per capita rate of change for the mutant hyperparasite becomes

$$\frac{\dot{H}_j}{H_j} = \frac{(\beta_i - \beta_j)(\beta_i\beta_j e_2 - \sigma\beta_i\hat{H}_i - d)}{\beta_i}. \tag{12}$$

If $e_2 = 0$, then a mutant hyperparasite will invade only when it allows a higher rate of transmission by its pathogen host ($\beta_j > \beta_i$). The evolutionary tendency, therefore, is for hyperparasites to minimize their deleterious effects on the pathogen. If $e_2 > 0$, then a hyperparasite that reduces the transmission of its pathogen host can invade when

$$\beta_j > \frac{\beta_i\hat{H}_i\sigma + d}{\beta_i e_2}. \tag{13}$$

At any instant, the optimum pathogen transmission from the perspective of the hyperparasite is

$$\sqrt{\frac{\beta_i\hat{H}_i\sigma + d}{e_2}}. \tag{14}$$

Note that this optimum depends on the transmission rate of the resident pathogen, so that a new optimum transmission rate is established each time a new hyperparasite proceeds to fixation. An ESS is achieved when the optimum transmission rate for an invading hyperparasite is the same as the transmission rate for the resident hyperparasite, which can be obtained by setting equation (14) equal to β_i and solving for β_i. The resulting ESS is a lengthy expression because $\hat{H}_i$ is a complex function of β_i. However, assuming for simplicity that infected hosts cannot reproduce and there is no density dependence,

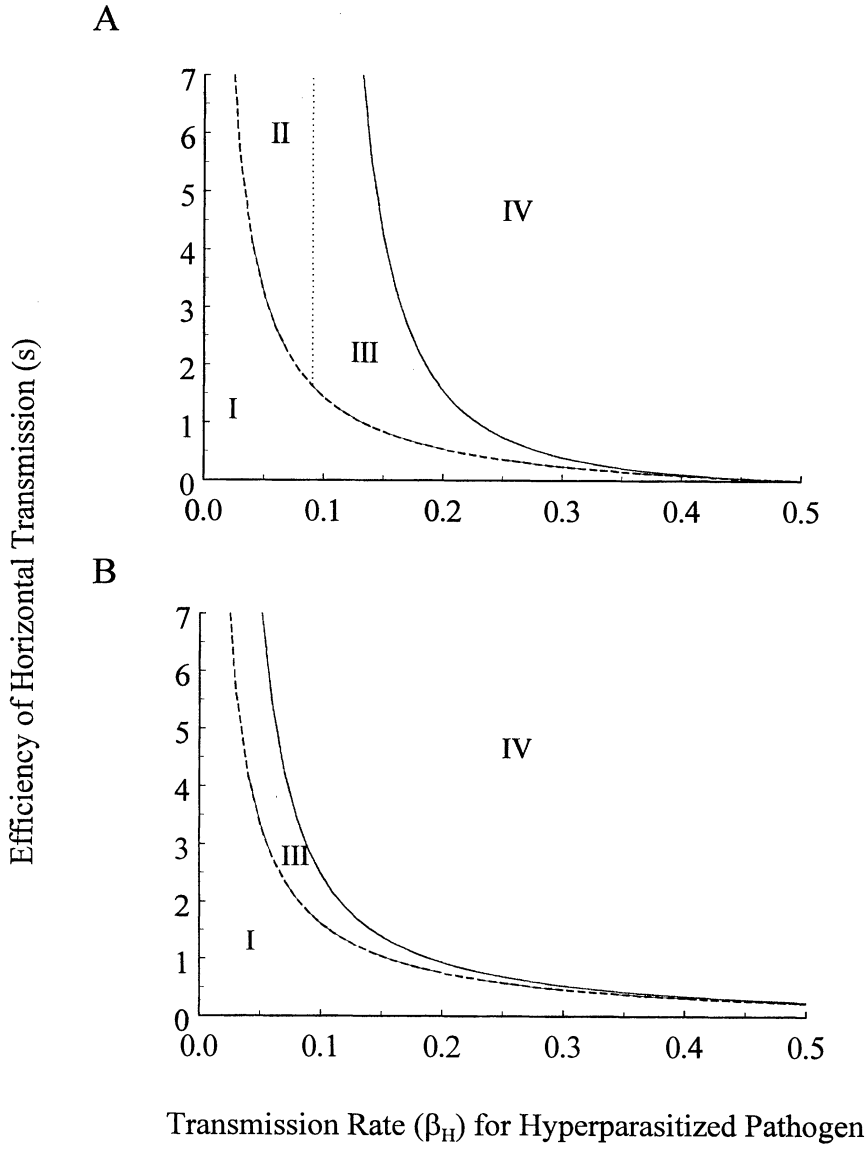

Figure 2: Effect of density dependence on the invasion conditions for the virulent and hyperparasitized pathogens. *A*, $a = 0.80$. *B*, $a = 0.05$. Regions I–IV are the same as figure 1. All other parameters are as in figure 1*A* except $\beta_V = 0.5$.

the ESS pathogen transmission from the hyperparasite's perspective is

$$\beta^* = \sqrt{\frac{\sigma(b - d) + d}{e_2}}. \quad (15)$$

When competing hyperparasites cannot invade each other via horizontal transmission ($\sigma = 0$), the ESS from the hyperparasite's perspective is the same as the ESS for the pathogen alone (eq. [9]). If $\sigma > 0$, however, the hyperparasite raises the ESS pathogen transmission and virulence. Thus, while the proximate effect of a hyperparasite is inevitably to reduce the pathogen's transmission rate and virulence, it may ultimately raise the ESS transmission rate and virulence under certain conditions.

Equilibrium Population Size

The effect of hyperparasitism on the density of the host population is analyzed in the appendix but can be summarized as follows. When $e_2 = 0$, selection on the pathogen always favors increased transmission and hence virulence. In that case, the presence of a hyperparasite reduces pathogen transmission and virulence, thereby increasing host density (fig. 4*A*). This beneficial effect of the hyperparasite on the host is diminished, however, as the transmission rate of the hypovirulent pathogen approaches that of the virulent pathogen. When $e_2 > 0$, the ESS of the pathogen dictates intermediate transmission and virulence. In this situation, the density of hosts is

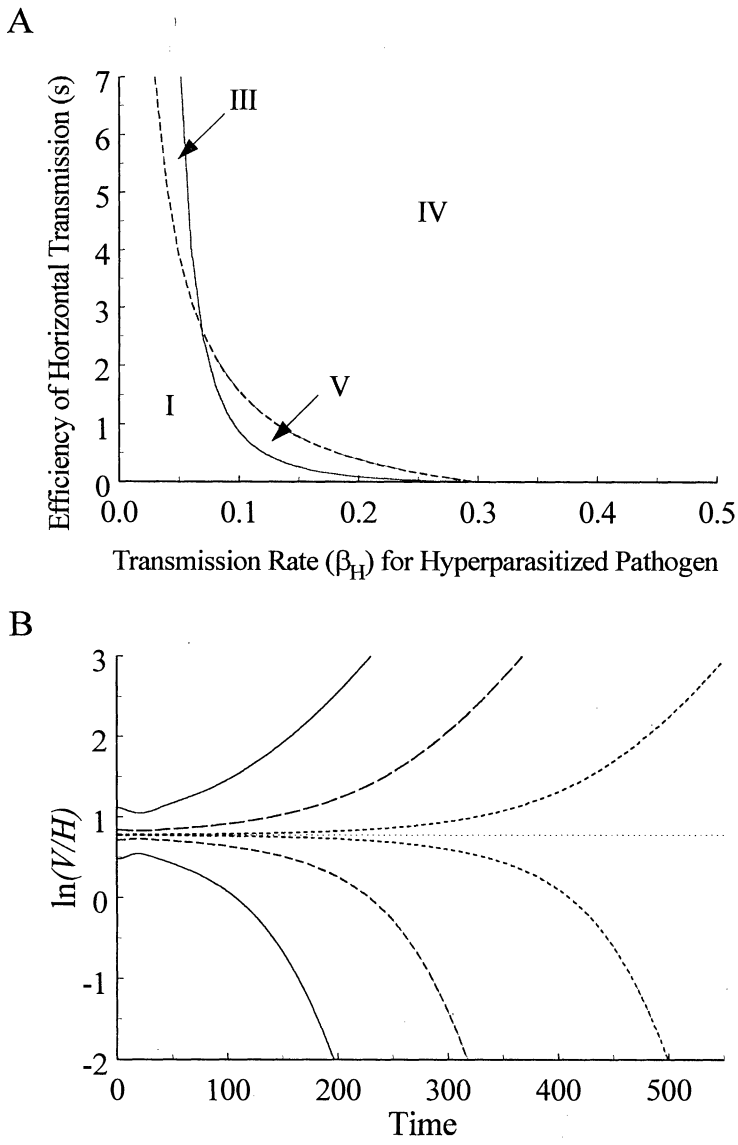

Figure 3: Effect of unequal fecundity of hosts infected with the two pathogen types. *A,* Invasion conditions for the virulent and hypovirulent pathogens. In region V, there is an unstable equilibrium, in which neither pathogen can invade the equilibrium established by the other, although either type can invade an equilibrium population of uninfected hosts. Other regions are defined in figure 1. *B,* Various perturbations of this unstable equilibrium (*horizontal dashed line*) all lead to fixation of one pathogen type and extinction of the other type. The parameter values were as follows: $\rho_V = 0$, $\rho_H = 1.0$, $\beta_V = 0.3$, $b = 2$, $a = 0.05$, $d = 0.75$, $e_1 = 0.1$, $e_2 = 0$. In *B*, $s = 0.4$ and $\beta_H = 0.15$.

lowest at some intermediate transmission rate. Depending on the transmission rates of the virulent and hypovirulent pathogens in relation to this ESS, therefore, the reduction in pathogen transmission caused by the hyperparasite may actually harm the host population (fig. 4*B*). In both cases, the transmission rate of the hypovirulent pathogen that maximizes host density appears to be that rate which is just high enough to eliminate the virulent pathogen (i.e., the boundary between regions *III* and *IV,* fig. 4).

Discussion

Because not all pathogens are deadly, and because pathogen virulence in some systems appears to have declined over time (Fenner and Ratcliffe 1965; Allison 1982; Ewald 1983), there is intense interest about what processes or constraints may favor intermediate or reduced virulence (Ewald 1983; Levin and Lenski 1983; May and Anderson 1983; Dwyer et al. 1990; Bull et al. 1991; Herre 1993; Antia et al. 1994; Bull 1994; Lenski and May 1994;

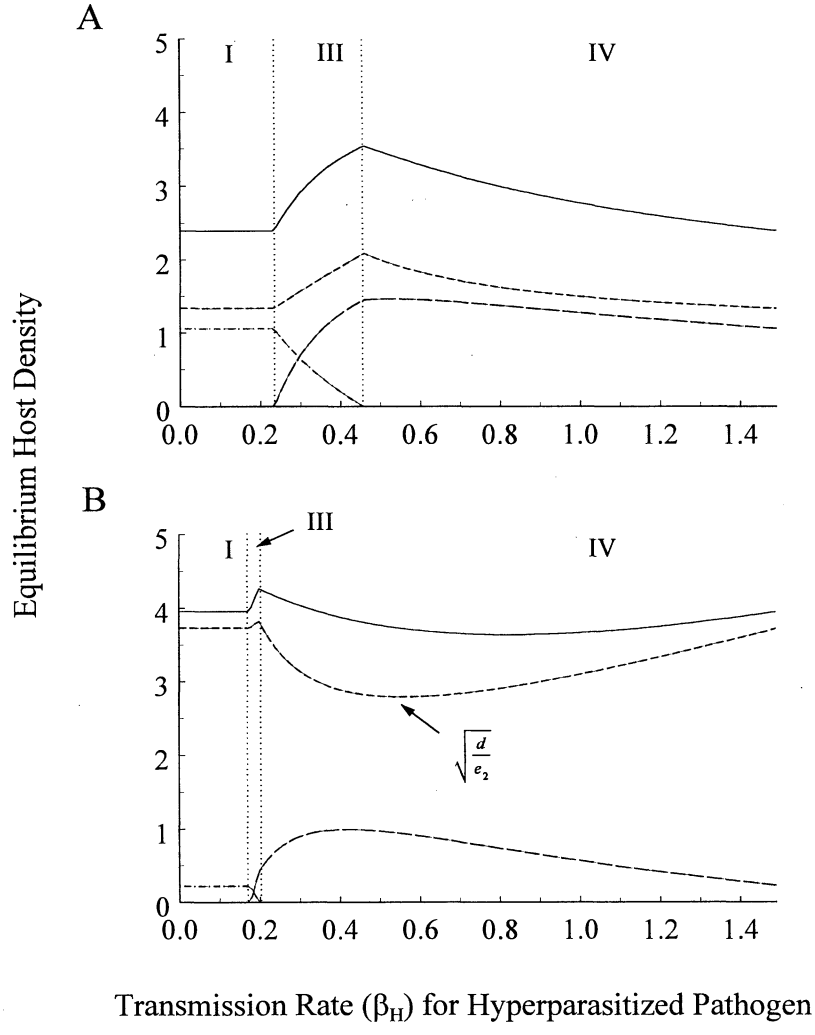

Figure 4: Equilibrium number of hosts as a function of hyperparasite transmission (β_H). Regions I–IV as in figure 1. Solid lines, short dashes, long dashes, and dash-dots represent total host density, $\hat{U}$, $\hat{H}$, and $\hat{V}$, respectively. *A*, $e_2 = 0$. *B*, $e_2 = 2.0$. Other parameter values are as follows $\beta_V = 1.5$, $b = 4$, $a = 0.8$, $d = 0.5$, $e_1 = 1$, $s = 1.7$, $\rho_V = \rho_H = 0$.

Frank 1996). In this article, we consider the possibility that what appears to be an evolutionary reduction in pathogen virulence could in some cases be an ecological phenomenon. That is, the pathogen may be infected by a debilitating hyperparasite that reduces the pathogen's virulence.

A critical factor in determining the impact of a hyperparasite on the dynamics of the host-pathogen interaction is the form of the functional relationship between the pathogen's rate of transmission and its virulence. Depending on this relationship, selection acting on the pathogen may favor either ever-increasing or intermediate virulence (Anderson and May 1982). This effect, in turn, determines the invasion conditions for the hyperparasite and, in particular, the extent to which the hyperparasite depends on its own horizontal transmission. If selection currently favors an increase in the transmission rate of the pathogen, then hyperparasites inevitably reduce the fitness of the pathogen. In that case, whether the hyperparasite can spread is determined by how seriously it debilitates the transmission of its pathogen host and its own rate of horizontal transmission. In circumstances where reduced pathogen virulence is favored, however, the pathogen may actually benefit from the acquisition of the hyperparasite, provided that the resulting hypovirulence is nearer to the pathogen's evolutionarily stable strategy. In other words, the hyperparasite might provide the mechanism whereby the pathogen achieves the submaximal growth rate that provides the optimum balance between transmission rate and host mortality. In

this case, hyperparasites can invade like any other favorable "allele" and do not require horizontal transmission. Michalakis et al. (1992) suggested that this sort of mutualistic interaction between parasites and their hosts may be common in nature.

Different Ecological Factors Promote the Acquisition versus the Evolution of Hypovirulence

Our model for the acquisition of hypovirulence via hyperparasitism differs substantively from earlier models that describe the evolution of hypovirulence in more conventional terms. In particular, these two classes of models make diametrically opposed predictions with respect to the effects of two ecological variables on the conditions favoring hypovirulence.

Earlier models for the evolution of virulence have generally emphasized that increases in virulence are favored when host population density is high and when multiple infections on a single host (so-called superinfections) are prevalent. The first effect operates because a higher host density increases the opportunity for horizontal transmission, which should tend to shift the selective balance between infection of new hosts, on the one hand, and continued survival of infected hosts, on the other hand (Lenski 1988; Lenski and May 1994). The second effect operates because superinfections promote within-host competition between pathogen genotypes, which should tend to favor those pathogens that grow faster and are more virulent (Bremermann and Pickering 1983; Nowak and May 1994; see also Frank 1992, 1996; Herre 1993; van Baalen and Sabelis 1995).

In contrast, our model indicates that both high host density and frequent superinfections promote the acquisition of a hyperparasite—and concomitantly, hypovirulence—by a pathogen. Both of these effects operate because they allow more efficient horizontal transmission of the hyperparasite. From the standpoint of a hyperparasite, virulent pathogens are a resource to be exploited via horizontal transmission, and any factor that increases their density will increase hyperparasite transmission. Frequent superinfection places the virulent and hypovirulent pathogens in close contact within a single host and provides a greater opportunity for hyperparasites to spread via horizontal transmission.

Selection among Hyperparasites

Natural selection will usually favor those variants of the hyperparasite that minimize their deleterious effects, or maximize their beneficial effects, on the pathogen. When selection favors increasing transmission in the pathogen, selection favors hyperparasites that reduce pathogen transmission least. When selection favors reduced pathogen transmission toward an intermediate optimum, selection favors hyperparasites that move the pathogen transmission closer to this optimum. The exception to this rule is when hyperparasites compete with each other via high rates of horizontal transmission ($\sigma > 0$). In that case, hyperparasitism reduces pathogen virulence in the short term, but horizontal transmission among hyperparasites actually increases the evolutionarily stable rate of pathogen transmission and hence virulence.

The generality of these evolutionary trends, however, may depend on the life-history characteristics of hyperparasites. We have assumed that horizontal transmission of the hyperparasite is achieved when a hypovirulent strain coinfects a host that harbors a virulent strain. Because this horizontal transmission requires the dispersal and establishment of pathogen propagules containing hyperparasites, we assume that it occurs at a rate ($s\beta_H$) that is proportional to the transmission rate of the pathogen (β_H). Thus, vertical and horizontal transmission of the hyperparasite both depend on the successful transmission of its pathogen host. Another consequence of this life history is that it is impossible for a hyperparasite to rely exclusively on horizontal transmission (since $s\beta_H > 0$ implies $\beta_H > 0$). A different result might be found if the hyperparasite possessed a mode of horizontal transmission that was independent of the transmission of its pathogen host—an encapsulated virus, for example. In this case, if hyperparasite transmission is enhanced more by horizontal than by vertical transmission, natural selection may maximize the rate of horizontal transmission of the hyperparasite even though this may have increasingly deleterious effects on the pathogen. Here, the hyperparasite may possess a life history seen in many pathogens—one with no vertical transmission at all.

Hyperparasitism and the Recovery of the Host Population

Hyperparasites have been implicated as potentially useful agents of biological control (Nuss 1992). Our model suggests that where selection favors ever-increasing pathogen transmission, pathogens with lower rates of transmission have less deleterious effects on the host population, and hyperparasites may be useful for biological control. We show that the most effective hyperparasite for biological control would have a very high component of horizontal transmission and a severe enough effect on the pathogen so as to allow recovery of the host population but not so severe as to allow virulent strains to persist.

Where selection favors an intermediate optimum pathogen transmission, however, pathogens that have

this optimum transmission cause the greatest reduction in the density of uninfected hosts (Lenski and May 1994). Although it is possible for a hyperparasite to effect host recovery by reducing pathogen transmission below the optimum, a hyperparasite that lowers pathogen transmission to a value closer to this optimum may actually harm the host population. Moreover, the evolutionary tendency is for hyperparasites to move pathogens toward an optimum rate of pathogen transmission, which means that the hyperparasites most useful for biological control will not be evolutionarily stable. Evolution in hyperparasites, therefore, is expected to have deleterious effects on the host population.

The Chestnut Blight Host-Pathogen System

Throughout this article, we have avoided tailoring the model to any specific biological system. One natural system for which the model may be particularly instructive, however, is the chestnut blight host-pathogen system. Recovery of chestnuts in many areas of Europe is thought to be due to the spread of dsRNA hyperparasites (Van Alfen et al. 1975; Heininger and Rigling 1994). Infection by these hyperparasites debilitates the fungal pathogen (*Cryphonectria parasitica*), allowing trees to "wall off" the fungal infections and recover. However, repeated attempts to introduce dsRNAs into North American populations of chestnut have failed, although some limited natural recovery has occurred in chestnut populations in Michigan (Fulbright et al. 1983; Brewer 1995).

Even in its general form, our model indicates that several factors influence the likelihood of dsRNA invasion, and any of these may potentially account for the lack of recovery in North American chestnut populations. In particular, our model shows that the rate of horizontal transmission of the dsRNA, the relationship between the rate of transmission and virulence of the fungus, the strength of density dependence in the chestnuts, the relative transmission rates of virulent and hypovirulent fungi, and the relative fecundities of chestnuts infected with virulent and hypovirulent fungi may all have important impacts on whether the dsRNA hyperparasite can invade this system. Moreover, by predicting that selection among hyperparasites will tend to minimize the debilitating effects (or maximize the beneficial effects) of hyperparasitism on the fungal pathogen, our model suggests that dsRNAs can sometimes invade chestnut populations with little or no recovery of the host population (see Enebak et al. 1994). Unfortunately, this type of hyperparasite may also be resistant to invasion by hyperparasites that are most useful for biological control, that is, those that allow recovery of the host population.

Our model also suggests that high levels of vegetative compatibility diversity in the fungus, often cited as a potential explanation of why dsRNAs have failed to invade *C. parasitica* populations in the eastern United States (Anagnostakis et al. 1986), may not prevent the spread of hyperparasites in the long term. The dsRNAs are horizontally transferred between fungal colonies when cytoplasmic bridges are formed between a colony containing the hyperparasite and a recipient colony. Many fungi (including *C. parasitica*) have vegetative compatibility systems whereby fungal genotypes do not form cytoplasmic bridges if they belong to different compatibility groups. A diversity of vegetative compatibility groups within a fungal population would generally reduce the exchange of cytoplasmic elements and lower the rate of horizontal transmission of hyperparasites (i.e., lower s in our model). Nauta and Hoekstra (1994) suggested that vegetative compatibility systems may have evolved specifically to prevent the spread of cytoplasmic parasites, but they concluded that very high levels of diversity would be required. Similarly, we have shown that a hyperparasite can invade as long as its rate of horizontal transmission is somewhat greater than zero, unless the hyperparasite is quite debilitating to the pathogen.

We recognize that factors other than transmission and virulence are likely to influence the ecology and evolution of hyperparasitism in most natural systems. For example, our analysis has not considered the effects of either host or pathogen recovery (i.e., clearance of the pathogen or hyperparasite, respectively). As with trade-offs between transmission and virulence, a trade-off between transmission and clearance (Antia et al. 1994; Frank 1996) may alter the ESS for the pathogen, the hyperparasite, or both. We also do not consider host resistance, which is certain to alter the dynamics of the system. Finally, we ignore the within-host dynamics of multiple infections. These dynamics may be particularly relevant to the chestnut blight system where large trees may harbor numerous infections, but only a few virulent cankers may kill the entire host. Despite the assumptions and limitations of our model, we suggest that incorporating hyperparasitism into host-pathogen models will shed new light on selection pressures shaping the evolution of virulence and may eventually have broad implications for efforts in biological control.

Acknowledgments

We thank R. Holt and three anonymous reviewers for their contributions. This work was supported by the National Science Foundation (NSF) Center for Microbial Ecology (BIR-9120006), NSF grants DEB-9421237, DEB-

9528966, and DEB-9509304, and the Samuel Miller Foundation.

APPENDIX

Equilibrium Population Size

To examine the effect of the invasion of a hyperparasite on the host population, we assume for simplicity that $\rho_V = \rho_H = 0$; numerical simulations indicate this assumption does not qualitatively alter the results. First, we consider the equilibrium host densities when there are uninfected hosts and a single pathogen type, i (regions *I* and *IV*, fig. 1). The equilibrium density of uninfected hosts is given by equation (4), with appropriate notational changes, and the equilibrium density of infected hosts is

$$\frac{b\beta_i - ad - a(e_1\beta_i + e_2\beta_i^2) - d\beta_i}{\beta_i(a + \beta_i)}. \tag{A1}$$

The total density of hosts is the sum of equations (4) and (A1), which simplifies to

$$\frac{(e_1\beta_i + e_2\beta_i^2) + b}{a + \beta_i}. \tag{A2}$$

The partial derivative of equation (A2) with respect to β_i is $(e_1a + 2e_2\beta_i a + e_2\beta_i^2 - b)/(a + \beta_i)^2$. When $e_2 = 0$, we use the invasion condition for the pathogen ($\beta_i\hat{U} > d + e_1\beta_i + e_2\beta_i^2$, where $\hat{U} = [b - d]/a$) to show that this partial derivative is negative, that is, increased pathogen transmission reduces total host density (fig. 4*A*). When $e_2 > 0$, the situation becomes more complicated, as this partial derivative may be either negative or positive. As shown in figure 4*B*, total host density is minimized at an intermediate transmission rate within region IV. The density of uninfected hosts is also minimized at an intermediate transmission rate, which is given by equation (9). However, the transmission rates that minimize total and uninfected host densities are not the same (Lenski and May 1994).

Next we consider the equilibrium host densities when the virulent and hypovirulent pathogens coexist (regions *II* and *III*, fig. 1). Equations (1), (2), and (3) are at equilibrium when

$$\hat{U} = \frac{s\beta_H(b - d) + ae_1(\beta_V - \beta_H) + ae_2(\beta_V^2 - \beta_H^2) + e_2\beta_H\beta_V(\beta_V - \beta_H) - d(\beta_V - \beta_H)}{a(s\beta_H + \beta_V - \beta_H)}; \tag{A3}$$

$$\hat{H} = \frac{\beta_V\hat{U} - d - (e_1\beta_V + e_2\beta_V^2)}{s\beta_H}; \tag{A4}$$

and

$$\hat{V} = \frac{-\beta_H\hat{U} + d + (e_1\beta_H + e_2\beta_H^2)}{s\beta_H}. \tag{A5}$$

The total density of all hosts is the sum of these three equilibria, or

$$\frac{s\beta_H(b - d) - d(\beta_V - \beta_H) + e_2\beta_H\beta_V(\beta_V - \beta_H)}{s\beta_H a} \equiv \hat{U} - \frac{(d - e_2\beta_H\beta_V)(\beta_V - \beta_H)}{s\beta_H a}, \tag{A6}$$

where $\hat{U}$ is the equilibrium density of hosts in a pathogen-free population, and the second term is the combined severity of infection by the virulent and hypovirulent pathogens on the host population. The partial derivative of the severity of the infection with respect to β_H is

$$\frac{\beta_V(e_2\beta_H^2 - d)}{s\beta_H^2 a}. \tag{A7}$$

Within the region of coexistence of virulent and hypovirulent pathogens, therefore, the severity of the infection is diminished with increasing transmission of the hypovirulent pathogen unless $e_2 > 0$ and $\beta_H > \sqrt{d/e_2}$.

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Associate Editor: Robert D. Holt