The Evolution of Parasite Virulence, Superinfection, and Host Resistance

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abstract: We analyze the evolutionary consequences of host resistance (the ability to decrease the probability of being infected by parasites) for the evolution of parasite virulence (the deleterious effect of a parasite on its host). When only single infections occur, host resistance does not affect the evolution of parasite virulence. However, when superinfections occur, resistance tends to decrease the evolutionarily stable (ES) level of parasite virulence. We first study a simple model in which the host does not coevolve with the parasite (i.e., the frequency of resistant hosts is independent of the parasite). We show that a higher proportion of resistant host decreases the ES level of parasite virulence. Higher levels of the efficiency of host resistance, however, do not always decrease the ES parasite virulence. The implications of these results for virulence management (evolutionary consequences of public health policies) are discussed. Second, we analyze the case where host resistance is allowed to coevolve with parasite virulence using the classical gene-for-gene (GFG) model of host-parasite interaction. It is shown that GFG coevolution leads to lower parasite virulence (in comparison with a fully susceptible host population). The model clarifies and relates the different components of the cost of parasitism: infectivity (ability to infect the host) and virulence (deleterious effect) in an evolutionary perspective.

Keywords: virulence, infectivity, resistance, superinfection, coevolution.

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By definition, parasites are deleterious for their hosts. This deleterious effect can be called the "cost of parasitism" and is often linked to the parasite's virulence. However, this conceptual link is problematic because in the literature, virulence has acquired (at least) two rather different meanings. Virulence often refers to the parasite's capacity to establish an infection, but it may also refer to the consequences of being infected. Both of these aspects affect host reproduction and thus have a bearing on the cost of parasitism, but, as we will claim in this article, their evolutionary consequences can be quite distinct.

The first component determining the cost of parasitism is the process of infection itself, which in turn depends on two factors: the force of infection that measures the rate with which an individual host encounters infectious particles (giving the rate at which fully susceptible hosts become infected) and the probability for a host to become infected upon encounter with these particles. This infection probability is known to depend on traits of both host and parasite. It is often referred to as "susceptibility" or "resistance" when it applies to the host and as "virulence" and "avirulence" when applied to the parasite (e.g., in the classical gene-for-gene (GFG) model; Flor 1956).

The second component of the cost of parasitism is also called "virulence" but refers to the deleterious effect induced by the parasite on the host after successful infection. In other words, this is the cost of infection that is associated with being infected (as opposed to becoming infected). The dual usage of the same word for two different meanings is potentially confusing (Poulin and Combes 1999). To avoid confusion, in this article we will restrict our definition of virulence to the deleterious effect (increased host mortality) induced by the parasite. To denote what is called "virulence" in the gene-for-gene terminology (Flor 1956), we will use the term "infectivity"; more infective parasites can infect a broader host range (including more resistant ones). Infectivity and virulence may not be fully independent. High levels of virulence are sometimes correlated with increased infectivity (e.g., see Ebert 1994, 1999). This could be explained either by a linkage between the genes involved in virulence and infectivity or by sup-

posing that virulence is a by-product (or pleiotropic effect) of increased infectivity. Both components should be taken into account simultaneously to measure the effective selective pressures induced by parasites on their hosts. However, these two aspects of host-parasite interactions mostly have been studied separately.

The evolution of infectivity and resistance has been studied extensively by both epidemiologists and geneticists. Epidemiologists have pointed out that the force of infection is a dynamical variable that depends on the proportion or on the number of infected hosts in the population. Geneticists tend to focus on the evolution of the specificity of the relation between hosts and parasites. They classically formalized host-parasite interactions using GFG models of interactions (e.g., Flor 1956; Day 1974; Burdon 1987; Frank 1991*b*, 1997; Jarosz and Burdon 1991; Thompson and Burdon 1992) or matching allele models of interactions (Frank 1991*a*, 1992, 1993). In these models, the ability of a parasite to infect a host is determined by the genotypes of both the host and the parasite. The outcome of this interaction is often modeled as an all-or-nothing response; that is, the host is either fully resistant or fully susceptible.

In contrast, the approach taken by epidemiologists usually focuses on the evolution of parasite virulence (i.e., the deleterious effect induced by the parasite on its host). In this context, parasite virulence is considered a direct consequence of the parasite's host exploitation strategy. Inevitably, parasites cause damage because they need to reproduce within the host and achieve transmission. However, extreme exploitation strategies will decrease the host's life expectancy and, consequently, the parasite's chances of being transmitted. Several studies have found such a trade-off between virulence and transmission in different host-parasite systems (reviewed by Mackinnon and Read 1999). The existence of such a trade-off leads to the prediction that parasites should evolve toward intermediate levels of virulence (Anderson and May 1979; Ewald 1983; May and Anderson 1983*b*). The study of the evolutionarily stable (ES) level of parasite virulence has stimulated much work in the past decade (Anderson and May 1979; van Baalen and Sabelis 1995; Frank 1996). From these studies, it has become apparent that the ability of the parasite to infect an already infected host is an important factor. Multiple infection can be modeled in different ways depending on the interaction between parasites within the host. Superinfection models assume that the takeover of a resident strain is instantaneous, and consequently, two strains of parasites never share the same host. At the other extreme, co-infection models allow a potentially large number of strains to compete within the host. The evolutionary outcomes of these different models may differ quantitatively, but they show that multiple infections generally select for higher parasite virulence (Eshel 1977; Levin and Pimentel 1981; Bremermann and Pickering 1983; Sasaki and Iwasa 1991; Frank 1994, 1996; May and Nowak 1994, 1995; Nowak and May 1994; Lenski and May 1995; van Baalen and Sabelis 1995; Gandon 1998; Mosquera and Adler 1998; but see Chao et al. 2001 for a contrasting point of view).

In this article, we analyze these different views of the evolution of host-parasite interactions in a common framework. In particular, we study the evolution of both aspects of the cost of parasitism (infectivity and virulence) when host and parasite are engaged in a coevolutionary arms race through a GFG type of interaction. We show that in the absence of superinfections, the evolution of parasite virulence is not affected by the GFG interaction. However, when superinfections occur, the presence of resistant hosts in the population decreases the force of infection and, consequently, the evolutionarily stable (ES) virulence strategy.

For the sake of simplicity, we first analyze a model where there is heterogeneity in the level of host resistance but where the host population is static (ecologically and evolutionarily; i.e., there are fixed numbers of resistant and susceptible hosts). This simple model is useful in understanding how host resistance and superinfection together determine the evolution of parasite virulence. In the second part, we relax both simplifying assumptions of the first model. Host population density becomes a dynamical variable, and host resistance becomes a coevolutionary variable. To do this, we use the classical GFG model of coevolution in combination with the evolution of parasite virulence. This model leads to long-term evolutionary predictions for both the host and the parasite when the two components of the cost of parasitism, infectivity and virulence, are coevolving.

A Simple Model for Parasite Evolution

Host and Parasite Life Cycles

We consider a host population with a constant number, N, of individuals among which a fixed proportion f is resistant while the rest are fully susceptible. The level of resistance of resistant hosts is characterized by the parameter ρ, which measures the decrease in the risk of infection relative to susceptible hosts. Hence, the force of infection (i.e., the rate at which individual hosts get a new infection through contacts with infected individuals) acting on susceptible hosts is h_S, and the force of infection acting on resistant hosts is $h_R = (1 - \rho)h_S$. Note that when $\rho = 0$, resistant and susceptible hosts are equally susceptible. At the other extreme, when $\rho = 1$, resistant hosts cannot be infected at all by the parasite. The hosts (both resistant

and susceptible) have an intrinsic (i.e., in absence of infection) death rate δ.

The parasite is assumed to be asexual and haploid. The increased mortality rate due to parasite infection is denoted ν and refers to parasite virulence. Throughout this article, we will assume that there is a positive relationship between a parasite's virulence and its transmission rate β from an infected host to a susceptible host that is given by

$$\beta(\nu) = \frac{\nu}{1 + \nu}. \tag{1}$$

Satiating functions of this form have been used extensively in various models of parasite virulence (Nowak and May 1994; May and Nowak 1995; van Baalen and Sabelis 1995). We further assume that within-host competition between different parasite strains may occur. For the sake of simplicity, we model within-host dynamics as a superinfection process (Nowak and May 1994) where a resident parasite may be outcompeted by a newly arrived parasite with a rate σ, where σ measures the susceptibility to superinfections. One could hypothesize a link between σ and parasite virulence. For example, Nowak and May (1994) assume that more virulent strains always outcompete less virulent strains. Here, we assume that the probability to take over an already infected host is constant and does not depend directly on the parasite's virulence strategy.

The above assumptions result in the following pair of differential equations that governs the number of infected hosts:

$$\begin{aligned} \dot{y}_S &= h_S x_S - (\delta + \nu) y_S, \\ \dot{y}_R &= h_R x_R - (\delta + \nu) y_R, \end{aligned} \tag{2}$$

where y_S and y_R are the numbers of infected hosts of types S (susceptible) and R (resistant), respectively. The number of healthy susceptibles is $x_S = (1 - f)N - y_S$, and $x_R = fN - y_R$ is the number of healthy resistants. In this model, we assume that dead hosts of a given type are replaced immediately by healthy hosts of the same type. The force of infection on susceptible hosts is $h_S = \beta(y_S + y_R)$. We give a summary of the main notations in table 1.

Evolution

To analyze the evolution of parasite virulence, we focus on the fate of a rare mutant strain in a resident parasite population that is at its equilibrium. Let us assume that the resident strain has the virulence strategy ν, while the mutant adopts a deviant strategy ν^*. The dynamics of the mutant strain are described by the following equations:

$$\begin{aligned} \dot{y}^*_S &= h^*_S(\hat{x}_S + \sigma\hat{y}_S) - (\delta + \nu^* + \sigma h_S) y^*_S, \\ \dot{y}^*_R &= h^*_R(\hat{x}_R + \sigma\hat{y}_R) - (\delta + \nu^* + \sigma h_R) y^*_R, \end{aligned} \tag{3}$$

where the hat indicates the equilibrium densities of resident hosts. The force of infection of the mutant on susceptibles is $h^*_S = \beta^*(y^*_S + y^*_R)$, and $h^*_R = (1 - \rho)h^*_S$ is the force of infection of the mutant on resistant hosts.

The strategy ν^* is evolutionarily stable if it can resist invasion by any other mutant strategy (Maynard Smith and Price 1973). In appendix A, we show that the rate of increase of the mutant strategy depends on its basic reproduction ratio R^*_0, which is equal to the number of secondary infections induced by a single mutant in the resident population:

$$R^*_0 = \frac{\beta^*(\hat{x}_S + \sigma\hat{y}_S)}{\delta + \nu^* + \sigma h_S} + \frac{(1 - \rho)\beta^*(\hat{x}_R + \sigma\hat{y}_R)}{\delta + \nu^* + \sigma h_R}. \tag{4}$$

A necessary condition for evolutionarily stability of a parasite's strategy is that this ratio is maximized with respect to ν^*.

If an infected host cannot be reinfected (i.e., $\sigma = 0$), the ES parasite strategy is the one that maximizes the per-host transmission factor: $\beta^*/(\delta + \nu^*)$ (van Baalen and Sabelis 1995). As a consequence, ES parasite virulence de-

Table 1: Summary of main notations

Notation	Definition
Host:	
δ	Natural host death rate
x_S	Density of uninfected susceptible hosts
x_R	Density of uninfected resistant hosts
ρ	Level of host resistance[a]
N	Size of host population[a]
f	Proportion of resistant hosts[a]
r	Maximal rate of host reproduction[b]
c_R	Cost of resistance[b]
Parasite:	
ν	Virulence (disease-induced mortality)
β	Transmission rate
σ	Susceptibility to superinfection
h	Force of infection on susceptible hosts
y_S	Density of infected susceptible hosts[a]
y_R	Density of infected resistant hosts[a]
y_{Si}	Density of susceptible hosts infected by i-type parasites[b]
y_{SI}	Density of susceptible hosts infected by I-type parasites[b]
y_{RI}	Density of resistant hosts infected by I-type parasites[b]
c_I	Cost of infectivity[b]

[a] Specific to the first model (without host coevolution).
[b] Specific to the second model (with host coevolution).

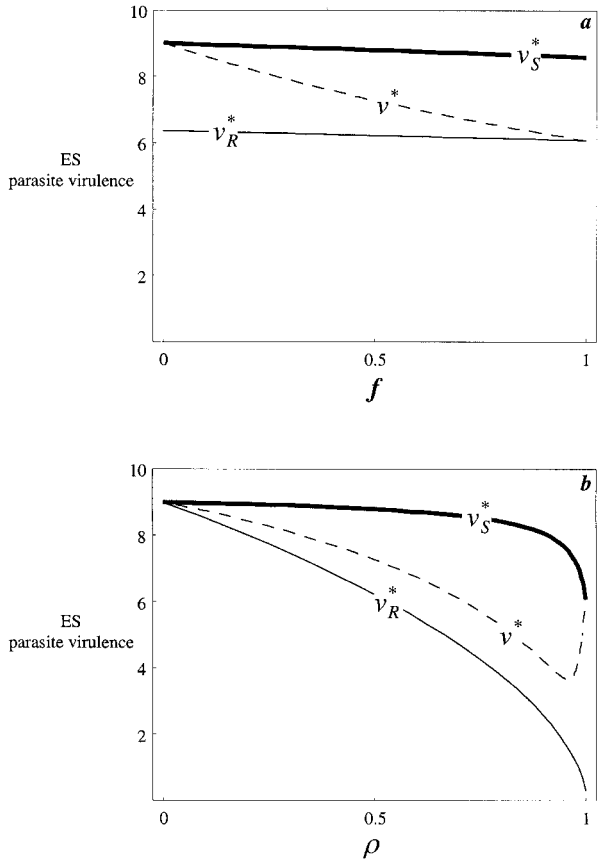

Figure 1: Evolutionarily stable (ES) virulence strategies in the simple model (no host coevolution). The ES virulence strategies are plotted versus (*a*) the fraction of resistant hosts in the population, *f*, for $\rho = 0.5$ and (*b*) the level of resistance, ρ, for $f = 0.5$. Three different evolutionarily stable strategies are plotted: the optimal virulence in a heterogeneous host population, ν^* (*dashed line*); the optimal virulence in a susceptible host population, ν_S^* (*bold line*); and the optimal virulence in a resistant host population, ν_R^* (*light line*), respectively. Parameter values: $\sigma = 1$, $\delta = 0.1$, $N = 100$.

pends neither on the quantity (f) nor on the "quality" (ρ) of resistant hosts. Indeed, for the constraint given by equation (1), ES virulence is given by the simple expression $\nu^* = \delta^{1/2}$. If there is no superinfection, virulence depends on natural host death rate and nothing else (Gandon et al. 2001*a*).

When superinfections occur, we find markedly different results; parasite virulence increases and depends on other parameters including host resistance. Unfortunately, there is no simple expression for the ES parasite strategy. Numerical solutions show that the ES level of virulence decreases with higher f (fig. 1*a*), but the effect of ρ is not monotonous (fig. 1*b*). The ES virulence first decreases with ρ, but when the level of resistance gets very large, it increases with higher ρ. The main effects of the other parameters are summarized in table 2.

Two major processes explain the effects of the fraction of resistant hosts, f, and the intensity of the level of resistance, ρ. First, there is within-host competition. We show that host resistance affects the evolution of parasite virulence only when superinfections occur. Increased host resistance (which can be achieved by increasing either f or ρ) decreases the probability of superinfection and, as a consequence, diminishes the intensity of within-host competition. This favors lower ES parasite virulence (fig. 1).

Second, from the parasite's point of view, the heterogeneous host population can be seen as a parasite metapopulation with two types of habitat (resistant and susceptible hosts). It can be shown that optimal strategies in the different habitats are different. The optimal virulence on susceptible hosts is $\nu_S^* = (\delta + \sigma h_S)^{1/2}$, and the optimal virulence on resistant hosts is $\nu_R^* = (\delta + \sigma h_R)^{1/2}$. Since $h_S \geq h_R$, this yields $\nu_S^* \geq \nu_R^*$. Note that the ES strategy ν^* is not a simple average of the strategies ν_S^* and ν_R^*. Indeed, figure 1*a* shows that ρ has a nonmonotonous effect on the ES virulence ν^*, while ν_S^* and ν_R^* always decrease with ρ. This can be explained by the epidemiological dynamics. When the resistant hosts get very efficient in preventing any infection (i.e., when resistance, ρ, is very high), very few parasites infect resistant hosts. Therefore, parasite evolution is only governed by the selective pressures in susceptible hosts. Indeed the virulence ν^* tends to ν_S^* when $\rho \to 1$.

Gene-for-Gene Coevolution

Host and Parasite Life Cycles

Next, we analyze the case where the host coevolves with the parasite. Different gene-for-gene (GFG) models have been proposed to formalize the reciprocal and specific genetic changes in both partners (reviewed by Otto and

Table 2: Main effects of the parameters of the simple model on equilibrium densities of infected hosts, forces of infection, and evolutionarily stable parasite virulence strategy (ESS)

	Equilibrium densities		Forces of infection		
Parameters	$\hat{y}_R$	$\hat{y}_S$	h	h_R	ESS, ν^*
σ	•	•	•	•	↗
N	↗	↗	↗	↗	↗
ρ	↘	↘	↘	↘	↘
f	↗	↘	↘	↘	↘
δ	↘	↘	↘	↘	↘

Note: The arrows show the main effects of an increase of a given parameter, and a dot indicates there is no effect. In the last column of the table, we only present the effect of the different parameters when $\sigma = 1$.

Michalakis 1998). Here, we use the classical GFG model of interaction proposed by Flor (1956). Within this framework, there are two types of hosts (resistants, R, and susceptibles, S) and two types of parasites, highly infective I-parasites capable of infecting both R- and S-type hosts and weakly infective i-parasites capable of infecting only S hosts. Note that, in this article, we do not use the classical GFG terminology to avoid the dual use of the term "virulence" (I and i types are often called "virulent" and "avirulent," respectively; e.g., Flor 1956; Thompson and Burdon 1992). Therefore, in contrast to the simple model studied above, the ability of the parasite to infect a host depends on the types of both host and parasite and the outcome of the interaction (infection or not) is an all-or-nothing response (i.e., either $\rho = 0$ or $\rho = 1$).

Note that hosts are characterized by only a single evolving trait (susceptibility), and parasites have two evolving traits (virulence and infectivity). In other words, in the parasite population, two loci are involved. The first locus determines the infection type of parasite (i.e., its infectivity); the second locus determines the virulence of the parasite. Since we assume asexual life cycles, the parasite's two loci are strongly linked. However, if there are mutations at the infectivity locus that change I into i or vice versa, the parasite's genes determining virulence will become effectively unlinked from the infection type. In what follows, we will analyze a situation where mutation can occur at the infectivity locus with a probability μ. The limiting case of no mutation will be studied separately.

In this model, we also relax the assumption of a constant host population size. Instead, we make the classical assumption that the host is regulated by the parasite population. The host has a growth rate, r, but resistant hosts pay a cost of resistance, c_R, which decreases their fecundity. In the same way, we assume that the I-parasites pay a cost, c_I, that reduces their transmission rate. In the GFG model, these costs are necessary to maintain polymorphisms in both host and parasite populations. The dynamics of a resident system are described by the following set of differential equations:

$$\begin{aligned}
\dot{x}_S &= r(x_S + y_{SI} + y_{Si}) - (\delta + h_S)x_S,\\
\dot{x}_R &= r(1 - c_R)(x_R + y_{RI}) - (\delta + h_R)x_R,\\
\dot{y}_{SI} &= h_I[x_S + \sigma(y_{SI} + y_{Si})] - (\delta + \nu + \sigma h_S)y_{SI},\\
\dot{y}_{Si} &= h_i[x_S + \sigma(y_{SI} + y_{Si})] - (\delta + \nu + \sigma h_S)y_{Si},\\
\dot{y}_{RI} &= h_I x_R - (\delta + \nu)y_{RI},
\end{aligned} \tag{5}$$

with $h_S = h_I + h_i$ and $h_R = h_I$, where h_I and h_i are the forces of infection of the two infection types, given by

$$\begin{aligned}
h_I &= (1 - \mu)(1 - c_I)\beta(y_{SI} + y_{RI}) + \mu\beta y_{Si},\\
h_i &= (1 - \mu)\beta y_{Si} + \mu(1 - c_I)\beta(y_{SI} + y_{RI}).
\end{aligned} \tag{6}$$

As before, x_S and x_R are the densities of uninfected susceptible and resistant hosts. The densities of susceptible hosts infected by I- and i-parasites are y_{SI} and y_{Si}, respectively. The density of resistant hosts infected by I-parasites is y_{RI}. Note that it is at the stage of the infection process that the mutations occur that couple the parasite populations.

The equilibrium densities of hosts and parasites can be obtained analytically for $\sigma = 0$ and $\mu = 0$ (app. B). For the more general case where both superinfections and mutation occur, we determined the equilibrium values numerically. The main effects of the model parameters on the equilibrium densities are given in table 3. Extensive numerical simulations showed that the polymorphic equilibrium (with all the different types of host and parasite) is always stable.

Evolution

The dynamics of the mutant parasite strain are described by the following equations:

$$\begin{aligned}
\dot{y}^*_{SI} &= h^*_I[\hat{x}_S + \sigma(\hat{y}_{SI} + \hat{y}_{Si})] - (\delta + \nu^* + \sigma h_S)y^*_{SI},\\
\dot{y}^*_{Si} &= h^*_i[\hat{x}_S + \sigma(\hat{y}_{SI} + \hat{y}_{Si})] - (\delta + \nu^* + \sigma h_S)y^*_{Si},\\
\dot{y}^*_{RI} &= h^*_I(\hat{x}_R + \sigma\hat{y}_{RI}) - (\delta + \nu^* + \sigma h_R)y^*_{RI},\\
h^*_I &= \beta^*[(1 - \mu)(1 - c_I)(y^*_{SI} + y^*_{RI}) + \mu y^*_{Si}],\\
h^*_i &= \beta^*[(1 - \mu)y^*_{Si} + \mu(1 - c_I)(y^*_{SI} + y^*_{RI})].
\end{aligned} \tag{7}$$

Table 3: Main effects of the parameters of the gene-for-gene model on equilibrium densities of infected hosts, forces of infection, and evolutionarily stable parasite virulence strategy (ESS)

	Equilibrium densities					Forces of infection		ESS,
Parameters	$\hat{x}_S$	$\hat{x}_R$	$\hat{y}_{SI}$	$\hat{y}_{Si}$	$\hat{y}_{RI}$	h_i	h_I	ν^*
σ	•	•	•	•	•	•	•	↗
r	↗	↗	↗	↗	↗	↗	↗	↗
c_I	•	↗	•	•	↗	•	•	↘
c_R	↗	↘	↘	↗	↘	↗	↘	↘
δ	↗	↗	↘	↘	↘	↘	↘	↘

Note: The arrows show the effect of an increase of a given parameter, and a dot indicates there is no (or little) effect. In the last column of the table, we only present the effect of the different parameters when $\sigma = 1$, and we assume that there is a large mutation rate ($\mu = 0.01$) on the locus that controls infectivity in the parasite (such high mutation rate prevents evolutionary branching; see the text for an explanation).

This yields the following basic reproduction ratio (app. B):

$$R_0^* = (1 - c_I)\beta^*\left(\frac{\hat{S}}{\delta + \nu^* + \sigma h_S} + \frac{\hat{R}}{\delta + \nu^* + \sigma h_R}\right) \times \left[1 - \mu - \frac{(1 - 2\mu)\beta^*\hat{S}}{\delta + \nu^* + \sigma h_S}\right] + \frac{(1 - \mu)\beta^*\hat{S}}{\delta + v^* + \sigma h_S}. \quad (8)$$

The maximization of the basic reproduction ratio with respect to the mutant strategy leads to the ES parasite virulence. As in the simple model (with no host coevolution), when only single infections occur ($\sigma = 0$), the ES parasite strategy maximizes the per-host transmission factor: $\beta^*/(\delta + \nu^*)$. Therefore, host resistance and the whole GFG coevolutionary process do not affect the evolution of parasite virulence. The ES virulence depends only on the natural host death rate: $\nu^* = \delta^{1/2}$.

When superinfection occurs, the evolution of virulence depends on the mutation rate at the infectivity locus. If the mutation rate is large, a single virulent strategy evolves (fig. 2). We found that there is a threshold value of the mutation rate below which there is an evolutionary branching of the virulence strategy. Then, a polymorphic state emerges in which two different virulence strategies coexist (fig. 2). In fact, in the absence of mutation at the infectivity locus, there are effectively two different parasite populations (I and i), and virulence evolves independently in these two populations (app. B). The i-type parasites then specialize on susceptible hosts because they never infect resistant hosts. In contrast, the I-type parasites can infect both types of host and evolve toward a generalist strategy that is a compromise between different optimal strategies on different hosts. Here, as in the first simple model, the optimal strategies on the two types of host ($\nu_S^* = [\delta + \sigma h_S]^{1/2}$ and $\nu_R^* = [\delta + \sigma h_R]^{1/2}$) differ only because of variable rates of superinfection. Therefore, superinfection yields different levels of virulence for different types of parasites with $\nu_i^* \geq \nu_I^*$ (see app. B). Mutation at the infectivity locus decouples the fate of virulence mutant from its genetic background (the state of the infectivity locus). If mutation is sufficiently large, this decoupling prevents evolutionary branching and allows these two parasite populations to merge into a single one that evolves toward single virulence strategy ν^*.

The effect of the evolutionary outcome on virulence depends also on GFG coevolution and on several parameters of the model (see table 3 for a summary of these effects). It is interesting to analyze the overall effect of GFG coevolution by comparing the monomorphic virulence strategy ν^* to the ES virulence ($\tilde{\nu}^*$) when both the host and the parasite populations are monomorphic (i.e., only susceptible hosts and i-type parasites). Numerical analyses show that GFG coevolution always decreases the ES parasite virulence (i.e., $\nu^* < \tilde{\nu}^*$). For example (for $r = 5$, $\sigma = 1$, $\delta = 0.1$, $c_R = 0.1$, $c_I = 0.9$, $\mu = 0.01$), $\nu^* = 5.5 < \tilde{\nu}^* = 5.8$. This can be explained by the presence of resistant hosts when simultaneous GFG coevolution occurs. As already shown in the simple model, host resistance decreases the force of infection and selects for lower level of parasite virulence.

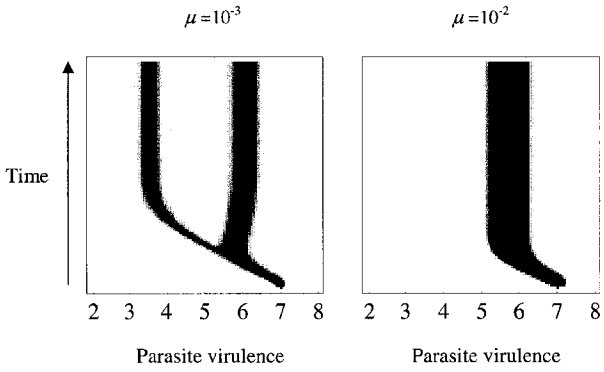

Figure 2: Effect of the mutation rate at the infectivity locus on the evolution of parasite virulence. We present the results (densities of genotypes with different virulence strategies) of deterministic simulations of the model presented in appendix B. Initially, all the parasites have the same level of parasite virulence: $\nu = 7$. Mutation at the virulence locus occurs and allows the evolution of this trait. When mutation rate at the infectivity locus is low ($\mu = 10^{-3}$; *left panel*), an evolutionary branching occurs and yields a polymorphic state (on the virulence locus) where two different virulence strategies evolve. The higher virulence is associated with i-type parasites, and the lower virulence is associated with I-type parasites. Note that we recover the result for optimal strategies for the different types of hosts ($\nu_I^* = 3.65$, $\nu_i^* = 6.15$). High mutation rate at the infectivity locus ($\mu = 10^{-2}$; *right panel*) prevents the evolutionary branching and yields a monomorphic state (on the virulence locus) where a single virulence strategy evolves ($\nu^* = 5.75$). Parameter values: $\sigma = 1$, $\delta = 0.1$, $c_R = 0.5$, $c_I = 0.5$, $r = 5$.

Discussion

Most models of the evolution of parasite virulence (in the epidemiological sense) assume that the host population is homogeneous in its resistance characteristics. Nonetheless, it has been shown that individual hosts often differ in their ability to resist parasitic infection (Flor 1956; Day 1974; Burdon 1987; Jarosz and Burdon 1991; Thompson and Burdon 1992). In this article, we analyze the evolutionary consequences of heterogeneity in host resistance (the ability to decrease the probability of being infected by parasites) on the evolution of parasite virulence (the deleterious effect on its host). First, we analyzed a model where the

level of host resistance does not evolve with parasite virulence. Second, we assumed a GFG type of coevolution between the host and the parasite. Both models give the same predictions, suggesting that the result is robust. When only single infections occur, host resistance does not affect the evolution of parasite virulence. However, when superinfections occur, resistance tends to decrease the evolutionarily stable (ES) level of parasite virulence. More detailed results of both models are discussed below.

No Host Coevolution

In the first model, host resistance is characterized by the quantity, f, and the quality, ρ, of the resistant hosts. We show that, if superinfections occur, a higher fraction of resistant hosts, f, always decreases the ES parasite virulence (fig. 1*a*). A higher fraction of resistant hosts decreases the rate of superinfection, which favors less virulent strategies. A similar result has been obtained by May and Nowak (1994). This observation has implications for the design of virulence-management strategies that aim at reducing the cost of parasitism. In particular, it suggests that vaccination campaigns may entail a twofold benefit. First, there is a short-term benefit through a decrease in the average risk of becoming infected. Second, there is a long-term benefit through the reduction of parasite virulence (May and Nowak 1994; Gandon and Michalakis 2000, 2002). The effect of the level of resistance, ρ, is more complex since the ES virulence level does not always decrease with ρ (fig. 1*b*). In terms of the vaccination analogy, this suggests that an imperfect vaccine (McLean and Blower 1993) could have a long-term beneficial effect through a selection for lower parasite virulence (Gandon et al. 2001*b*). However, the use of imperfect vaccines imposes a short-term cost because it necessarily implies an increase in the proportion of infected hosts. This example illustrates one of the dilemmas that may occur in virulence management (Gandon and Michalakis 2002; van Baalen 2002).

Host Coevolution

In a GFG type of host-parasite interaction, the parasite is characterized by two genetically determined traits: a gene that governs the GFG interaction (this is what we call "infectivity" but what is sometimes called "virulence") and a gene that governs its virulence. Although the resulting model is rather complex, it yields interesting insights. In this article, we mainly focus on the evolutionary consequences of GFG interaction on the parasite (the coevolution of host resistance is not studied in detail).

Evolution of Linkage between Virulence and Infectivity. Qualitatively different evolutionary outcomes may emerge depending on the linkage between the two parasite loci. If linkage is low, the evolutionary dynamics on these two loci are decoupled, and virulence evolves toward a single compromise virulence. If linkage is high, virulence and infectivity will no longer evolve independently, and correlation between these two traits can emerge.

Mutation or recombination can reduce the linkage between these two loci. Here, we only explore the effect of mutation at the infectivity locus and show that, below a certain mutation-rate threshold, the parasite population evolves toward a polymorphic situation where two different virulences coexist. In such a polymorphic population, we find that I-type parasites tend to be less virulent than i-type parasites. This is due to variable likelihood of superinfections. Because I-type parasites can infect resistant hosts, which have lower risk of being superinfected, they are less exposed to within-host competition. As a result, lower virulent strategies are favored in the I-type parasites.

Since resistant hosts can only be infected by I-type parasites, one may expect a negative correlation between the level of resistance of the host and the virulence of the parasite. Such a correlation has been found in several studies (Ebert 1994, 1999). These findings have often been explained by pleiotropic effects in the host since genes involved in the ability to counter establishment of the parasites could also help limiting the damage later. Our results suggest an alternative explanation since this pattern could also result from a linkage disequilibrium between the parasite's loci governing infectivity and virulence. This hypothesis can easily be tested experimentally because correlation does not depend on the host's genotype. In particular, we expect that, when infecting susceptible hosts, I-type parasites are less virulent than i-type parasites.

Actually, a positive association between the ability to infect and the level of virulence has often been observed (e.g., see the review on serial transfer experiments in Ebert 1999). Such a pattern, however, could also be produced by pleiotropy in the parasite if the ability to infect a resistant host (I type) was functionally associated with reduced virulence. In principle, it should be possible to distinguish between the "pleiotropy hypothesis" and our "linkage disequilibrium" alternative. For our model, we assumed that infectivity and virulence are controlled by different genes. If experimental selection for virulence does not affect the level of infectivity, or vice versa, different sets of genes are likely to be involved in the control of these two traits. This would lead to rejection of the pleiotropy hypothesis.

We found some empirical evidence that supports the decoupling between virulence and infection. First, in the filamentous fungal pathogen *Colletotrichum magna*, which

causes anthracnose in cucurbit plants, a nonpathogenic mutant (i.e., which causes no damage to the plant host) has been isolated (Freeman and Rodriguez 1993). This mutant has the same host range (i.e., infectivity) of the wild type, indicating that virulence and infectivity are distinct traits. Further genetic analysis shows that the difference in virulence between the mutant and the wild type involves a single locus. Second, additional support for this hypothesis might be provided by viruses. The ability of a virus to enter a cell is normally regulated by a set of genes that are not involved in the replication process. Therefore, at the cellular level, infectivity and virulence are mostly uncoupled. As a consequence, we would not expect virulence and infectivity to be pleiotropic in bacteriophages, which would make them potential experimental organisms for testing the hypotheses generated by our model. At the organismal level, some indication that viral infectivity and virulence are uncoupled is given by diseases for which attenuated vaccines have been produced. Attenuated vaccines are made up of viruses that replicate poorly within their hosts but are still competent in achieving an infection, suggesting that these two traits are not functionally linked.

However, experimental evidence supporting the hypothesis that infectivity and virulence are evolving independently is rare. We believe this is mainly due to the paucity of research in this direction. As pointed out in the introduction to this article, the study of the evolution of parasite virulence and parasite infectivity has often been carried out independently (both theoretically and empirically). Our model shows that interesting and testable predictions emerge from the interaction between the evolution of parasite virulence and parasite infectivity. We hope that this observation will encourage further experimental investigations in this direction.

GFG Coevolution, Virulence, and the Evolution of Sex. When the mutation rate is sufficiently large, the parasite will evolve toward an ES level of virulence that is always less than the ES virulence in the absence of GFG coevolution. In other words, GFG coevolution may have evolutionary consequences for parasite traits other than those strictly involved in the GFG interaction. In a similar fashion, this could affect the evolution of other traits in the host. For example, several authors (May and Anderson 1983*a*; Howard and Lively 1994; Lively and Howard 1994; Parker 1994; Peters and Lively 1999) have shown that high virulence levels are needed to explain the maintenance of sexual reproduction by parasites (the Red Queen hypothesis; Van Valen 1975; Hamilton 1980; Hamilton et al. 1990; Ebert and Hamilton 1996). Seen in the light of our results, such high virulence may not be expected at all. Indeed, we show that GFG coevolution tends to reduce parasite virulence. This would diminish the potential benefits of sexual reproduction for the host. Therefore, the evolution of sex (as a defense against parasites) is likely to be governed by a negative feedback mechanism. As yet, it is not at all clear whether sexual reproduction will be favored if parasite virulence is coevolving. A formal analysis of this prediction remains to be carried out.

Concluding Remarks

Multiple Infections and Indirect Effects. We show that the occurrence of superinfections is a critical factor in the effect of host resistance on the evolution of parasite virulence. For clarity, we modeled superinfections in a simple way. We have checked the robustness of our results by considering an infection process in which co-infection can occur or when the probability of taking over a resident strain in a given individual host depends on the difference in the levels of virulence between the resident and the mutant. The basic result is that, no matter how one models multiple infection, it adds another level of selection: within-host selection. Such within-host selection is known to select for higher virulence strategies (Eshel 1977; Bremermann and Pickering 1983; Frank 1994, 1996; May and Nowak 1994, 1995; Nowak and May 1994; van Baalen and Sabelis 1995). Note that Chao et al. (2001) have suggested that the reverse pattern might result under some circumstances. That is, when parasites need to trade off their capacity for within-host growth in order to be competitive, their combined effects on the host will be less severe. However, this only works if there is a negative functional link between virulence and competitiveness and not necessarily if the two traits evolve separately (M. van Baalen, V. A. A. Jansen, and S. Gandon, unpublished manuscript).

Whether positively or negatively, any factor that affects the probability of multiple infection will indirectly affect the evolution of virulence. For example, such indirect effects might explain why Ebert and Mangin (1997) found decreased virulence when they increased host mortality in an experimental host-parasite system and not increased virulence as they expected (Gandon et al. 2001*a*). Other factors include life-history traits of the host (e.g., natural growth rate; see fig. 2) and of the parasite (e.g., propagule survival [Gandon 1998] or the total number of propagules produced [Jansen and Mulder 1999]). This article shows that host resistance is another factor that acts on the evolution of virulence through its effect on the force of infection.

Different Forms of Resistance. We analyzed an epidemiological version of the classical GFG model of host-parasite coevolution. As noted above, other models of interaction have been proposed. In particular, the matching allele model (Frank 1991*b*, 1992, 1993) does not involve any

cost of resistance and cost of infectivity. The analysis of the evolution of parasite virulence under these different assumptions remains to be carried out. However, we believe our prediction that coevolution reduces the ES parasite virulence when multiple infection occurs will hold since this result is a direct consequence of the polymorphism for resistance in the host population that is, itself, a necessary condition of any GFG model of host-parasite coevolution.

A host may adopt different resistance mechanisms to reduce the cost of parasitism. As recently noted by Gandon and Michalakis (2000), different modes of resistance can have qualitatively different evolutionary consequences for the evolution of parasite virulence. In this article, we analyze the effect of all-or-nothing resistance. With this type of resistance, the effect of multiple infection is reduced in the resistant hosts, selecting lower parasite virulence. Other forms of resistance (e.g., resistance that reduces but does not prevent within-host growth of the parasite) may, on the contrary, favor higher virulence (van Baalen 1998; Gandon and Michalakis 2000; Gandon et al. 2001*b*). As a consequence, the long-term evolutionary outcomes between hosts and their parasites are likely to be highly dependent on host resistance mechanisms.

Space. In this article, we focus on the evolution in a single host population. However, several empirical studies show that coevolutionary interactions are often characterized by important spatial and temporal variations (Thompson 1994, 1999; Thrall and Burdon 1997; Burdon and Thrall 1999). Such variations have been documented for genes involved in the GFG interaction but little is known concerning spatial variation on genes that control parasite virulence (however, see Ebert 1994). Since we show that variations in infectivity may affect the evolution of parasite virulence, one may expect that spatial variations in infectivity could drive spatial variation in virulence. A complex feedback mechanism will determine the spatiotemporal variability in such systems (Keeling and Rand 1995). This variability is important because it will affect the invasion success of mutant strategies in both host and parasite populations. Further investigations are therefore required to analyze the effect of space in the interaction between GFG coevolution and parasite virulence.

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APPENDIX A

Simple Models

Epidemiological Analysis

There is no simple analytic solution for the equilibrium densities of the different types of parasites. However, the necessary condition on virulence for the maintenance of the parasite population is $\nu < \beta N(1 - f\rho) - \delta$. In other words, the overall death rate of the host ($\nu + \delta$) has to be smaller than the rate at which new infections occur ($\beta N[1 - f\rho]$). This yields the following condition on ν:

$$\frac{A - \sqrt{A^2 - 4\delta}}{2} < \nu < \frac{A + \sqrt{A^2 - 4\delta}}{2},$$

with $A = N(1 - f\rho) - \delta - 1$. Within this range, there is always a single equilibrium where parasites can be maintained in the population. Extensive numerical simulations suggest that this equilibrium is always locally stable. The main effects of the parameters of the model on equilibrium densities are summarized in table 2.

Derivation of R_0^*

To derive the R_0 of a parasite, we first consider that per unit of time, $\beta^*(y_S + y_R)(x_S + \sigma y_S)$ susceptible hosts are infected, and $(1 - \rho)\beta^*(y_S + y_R)(x_R + \sigma y_R)$ resistant hosts are infected. The probability for a parasite to start an infection in a susceptible host is therefore $(x_S + \sigma y_S)/[(x_S + \sigma y_S) + (1 - \rho)(x_R + \sigma y_R)]$. During the lifetime of such an infection, on average, $\beta^*[x_S + \sigma y_S + (1 - \rho)(x_R + \sigma y_R)]/(\delta + \nu^* + \sigma h_S)$ secondary infections are achieved. The probability to start an infection in a resistant host is $(1 - \rho)(x_R + \sigma y_R)/[(x_S + \sigma y_S) + (1 - \rho)(x_R + \sigma y_R)]$, and $\beta^*[x_S + \sigma y_S + (1 - \rho)(x_R + \sigma y_R)]/(\delta + \nu^* + \sigma h_R)$ secondary infections are produced. Multiplying the number of infections with the probability of achieving this particular infection and adding up the two cases yields R_0^* (see eq. [4]).

APPENDIX B

GFG Model

Equilibrium Densities

When $\sigma = 0$, the equilibrium densities of the GFG model (eqq. 5, 6) are

$$\hat{x}_S = \frac{\delta + \nu}{\beta},$$

$$\hat{x}_R = \frac{c_I(\delta + \nu)}{\beta(1 - c_I)},$$

$$\hat{y}_{SI} = \frac{(\delta + \nu)[r(1 - c_R) - \delta]}{\beta[\delta + \nu - r(1 - c_R)]},$$

$$\hat{y}_{Si} = \frac{r\nu c_R(\delta + \nu)}{\beta(\delta + \nu - r)[\delta + \nu - r(1 - c_R)]},$$

$$\hat{y}_{RI} = \frac{c_I(\delta + \nu)[r(1 - c_R) - \delta]}{\beta(1 - c_I)[\delta + \nu - r(1 - c_R)]}.$$

Derivation of R_0 *When Some Mutations Occur*

Here, we derive the conditions of invasion when some mutations occur on the locus that controls the infectivity of the parasite. In this case, the two loci that control the virulence and infectivity are not fully linked to each other. A mutant (on the virulence locus) that emerges in an i-type parasite will also appear in an I-type parasite as soon as a mutation event effects the locus for infectivity. Consequently, the invasion of a new virulent strategy cannot be decoupled for the different types (i or I) of parasites. The dynamics of the mutant (on the virulence locus) are described by

$$\begin{pmatrix} \dot{y}^*_{SI} \\ \dot{y}^*_{Si} \\ \dot{y}^*_{RI} \end{pmatrix} = \mathbf{A} \begin{pmatrix} \dot{y}^*_{SI} \\ \dot{y}^*_{Si} \\ \dot{y}^*_{RI} \end{pmatrix},$$

with

$$\mathbf{A} = \begin{bmatrix} (1 - \mu)(1 - c_I)\beta^*\hat{S} - (\delta + \nu^* + \sigma h_S) & \mu\beta^*\hat{S} & (1 - \mu)(1 - c_I)\beta^*\hat{S} \\ \mu(1 - c_I)\beta^*\hat{S} & (1 - \mu)\beta^*\hat{S} - (\delta + \nu^* + \sigma h_S) & \mu(1 - c_I)\beta^*\hat{S} \\ (1 - \mu)(1 - c_I)\beta^*\hat{R} & \mu\beta^*\hat{R} & (1 - \mu)(1 - c_I)\beta^*\hat{R} - (\delta + \nu^* + \sigma h_I) \end{bmatrix},$$

with $h = h_I + h_i$, $\hat{S} = \hat{x}_S + \sigma(\hat{y}_{SI} + \hat{y}_{Si})$, $\hat{R} = \hat{x}_R + \sigma\hat{y}_{RI}$, and

$$h_I = (1 - \mu)(1 - c_I)(\beta y_{SI} + \beta y_{RI}) + \mu\beta y_{Si},$$

$$h_i = (1 - \mu)\beta y_{Si} + \mu(1 - c_I)(\beta y_{SI} + \beta y_{RI}).$$

The condition for invasion of the mutant strain is determined by the dominant eigenvalue, λ, of $\mathbf{A}$ (if $\lambda > 0$, the mutant invades the resident population). After some algebra, it is possible to derive λ as a function of the basic reproduction ratio of the mutant, $R^*_0 : \lambda \propto 1 - R^*_0$. This yields the basic reproduction ratio given in equation (7).

Derivation of R_0^* *in the Absence of Mutation*

In the absence of mutation at the infectivity locus, the condition of invasion of the virulence mutant depends on the state of the infectivity locus (i or I). First, if the mutation emerges in a weakly infective (i-type) parasite, the dynamics of invasion are simply given by $\dot{y}_{Si}^* = h_i^* \hat{S} - (\delta + \nu_{Si}^* + \sigma h)\, y_{Si}^*$, with $\hat{S} = \hat{x}_S + \sigma(\hat{y}_{SI} + \hat{y}_{Si})$. The mutant will invade if $R_{0i}^* > 1$, with $R_{0i}^* = \beta_{Si}^*/(\delta + \nu_{Si}^* + \sigma h)\hat{S}$. This yields the following ES virulence strategy of weakly infective parasites: $\nu_i^* = (\delta + \sigma h_S)^{1/2}$.

Second, if the mutation emerges in a highly infective (I-type) parasite, a derivation analogous to the one in appendix A yields

$$R_{0I}^* = (1 - c_1)\beta^* \left(\frac{\hat{S}}{\delta + \nu^* + \sigma h_S} + \frac{\hat{R}}{\delta + \nu^* + \sigma h_R} \right).$$

Unfortunately, there is no simple analytic expression for the ES virulence strategy of broadly infective parasites ν_I^*. However, given that $h_S \geq h_R$, it is possible to show that $\nu_i^* \geq \nu_I^*$.

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