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E-ARTICLE

Mixed Infections and the Evolution of Virulence: Effects of Resource Competition, Parasite Plasticity, and Impaired Host Immunity

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ABSTRACT: Mixed-genotype parasite infections are common in nature. Theoretical studies analyze the effects of such infections over evolutionary time and predict an increase in virulence due to the competitive advantage of virulent parasites. In contrast, experimental studies compare the overall virulence of mixed and single infections within one generation. Although these within-generation comparisons have limited relevance to existing theory, they demonstrate that within-host parasite interactions are not restricted to competition for resources, as envisaged by theory. Instead, mixed infections may result in phenotypic changes in growth rate or impaired immune clearance. Developing and using a two-parasite epidemiological model with recovery, we confirm that within-host competition for resources selects for higher virulence. However, parasite phenotypic plasticity and impaired host immunity can select for lower virulence. Because these latter two mechanisms would be detected by experimentalists as an increase in pathology, our results warn against the temptation to draw inferences on virulence evolution on the basis of single-generation experiments.

Keywords: evolution of virulence, mixed infections, host immunity, parasite phenotypic plasticity, competition for host resources.

Introduction

Humans, animals, and plants often become coinfected with different genotypes of the same parasite/pathogen species, resulting in within-host parasite interactions (Read and Taylor 2001; Hood 2003). Such interactions—experimentally detected as changes in parasite density because of the presence of a coinfecting genotype—may result from a variety of mechanisms, including limited supply of host resources, host immune responses, or direct interference between parasites (e.g., release of toxins). Because within-host densities may ultimately affect the transmission success of parasites, these interactions have important consequences for the epidemiology and evolution of traits such as resistance to drugs and vaccines (Hastings and D'Alessandro 2000; Kew et al. 2002; Wargo et al. 2007).

Mixed infections have received most attention for their potential effect on the evolution of parasite virulence (i.e., parasite-induced host mortality), and numerous models have been developed to address this topic. Some of these models are based on game theory (Bremermann and Pickering 1983; Sasaki and Iwasa 1991; Frank 1992, 1994, 1996), with a major caveat that they consider what happens at the within-host level only, disregarding the between-host epidemiological dynamics of the parasites. In contrast, epidemiological models that deal with these between-host processes put the details of within-host dynamics in a black box (Levin and Pimentel 1981; Nowak and May 1994; May and Nowak 1995; Mosquera and Adler 1998). Van Baalen and Sabelis (1995) first recognized the need to integrate the two levels in one model because within-host interactions may influence the between-host dynamics and vice versa. The recent development of adaptive-dynamics approaches has significantly contributed to the integration of such evolutionary and epidemiological processes (Dieckmann et al. 2002). Despite their differences (within-host game theoretical vs. between-host epidemiological approaches), mixed-infection models share the common feature that they focus on competition for host resources and that such competition selects for higher levels of virulence. The reason is that mixed infections should select for higher competitive ability, which is assumed to result from higher parasite densities. Because it is also assumed that virulence is proportional to parasite density, higher virulence should be simultaneously selected. The only virulence-reducing mechanisms proposed so far in the case of mixed infections are some forms of cooperation, and these occur whenever benefits are shared among coinfecting parasite genotypes (Chao et al. 2000; Brown et al. 2002; West and Buckling

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2003; Ewald and Cochran 2004). Documented examples include host manipulation, immune suppression, and excretion of chemicals (toxins) by parasites that facilitate their colonization and/or consumption of the host.

Few experimental studies (de Roode et al. 2005*b*; Gower and Webster 2005; Bell et al. 2006; Ben-Ami et al. 2008) have tested the fundamental assumption underlying theoretical models, which is that more virulent parasites are competitively superior and obtain greater transmission from mixed infections than less virulent parasites. Instead, many studies have focused on directly comparing the virulence (mostly measured by a proxy such as weight loss, decrease in life span, or decrease in number of eggs or offspring) of single-genotype and mixed-genotype infections of experimentally infected hosts (Imhoof and Schmid-Hempel 1998; Taylor et al. 1998, 2002; Davies et al. 2002; Hodgson et al. 2002; de Roode et al. 2003; Hughes et al. 2004; Mouton et al. 2004; Schürch and Roy 2004; Vizoso and Ebert 2005; Harrison et al. 2006). While some of these studies have found that the overall parasite density and virulence of the mixed infection are the same as those of the most virulent parasite on its own (Imhoof and Schmid-Hempel 1998; de Roode et al. 2003; Hughes et al. 2004), other studies have found that the overall parasite density of mixed infections is higher than the densities of the respective single infections and that the virulence is higher (Davies et al. 2002; Hodgson et al. 2002; Mouton et al. 2004). Such increases could occur because the immune system is less efficient at clearing mixed than at clearing single infections (e.g., de Roode et al. 2003). Yet other studies have found an increase in virulence without a change in the overall parasite density, such that the virulence experienced by the host is higher than that experienced in a single infection with the most virulent genotype (Taylor et al. 1998; Davies et al. 2002). Such virulence increases are sometimes ascribed to a higher cost of mounting an immune response against a genetically diverse parasite population.

Although these experimental studies clearly show that genetic diversity importantly affects the virulence of parasite infections, they are generally unable to confirm or reject assumptions and predictions of mixed-infection models. An important reason for this is that experimental studies generally are not intended to compare the competitive ability of parasite genotypes of differing virulence. Instead, many are based on the hypothesis that parasites should facultatively increase their densities in response to the presence of a competitor. Whether such phenotypic increases in growth rate should also result in evolutionary increases in virulence is unclear. What is clear is that mathematical models are often necessary to predict the evolutionary consequences of mixed infections and that specific details matter. For example, models have shown that the mode of within-host competition (Brown et al. 2002; Alizon 2008; Lively 2009) and the way in which virulence is defined—for example, lethal versus sublethal effects (Schjørring and Koella 2003)—affect whether mixed infections select for higher or lower virulence.

Here we develop a mathematical model to investigate the consequences of the three most commonly assumed (theory) or tested (experiment) mixed-infection scenarios on the evolution of virulence. For simplicity, we consider two parasite genotypes, one of which produces a lower density and thereby causes lower virulence on its own (called "parasite 1") than the other (called "parasite 2"). Under the first scenario, the only interaction between the two parasites is competition for host resources. Under the second scenario, the parasites respond to the presence of each other by phenotypically increasing their growth rates. Finally, under the third scenario, the host immune system fights against two different parasites less efficiently than against two identical parasites. To complete the picture, we express the relationship between the virulence as defined by theoreticians and the virulence as measured in experiments (Day 2002). We investigate how the three mechanisms of interaction that may occur between parasites infecting the same host individual (competition for resources, parasite phenotypic plasticity, and host immune system impairment) influence the two definitions of virulence (theoretical and experimental) at the scale of one mixed-infection generation (as measured in experiments) and over evolutionary time (as predicted by theoretical models). Our model—which does not account for kin selection—shows mechanisms other than cooperation that favor reduced virulence in case of mixed infections. It also shows that the virulence measured within a single generation rarely predicts the virulence that evolves over evolutionary time.

A General Two-Disease Model with Recovery

Consider a host population infected by two different parasite clones, parasite 1 and parasite 2. In the following, to simplify the notation, the subscripts i and j ($i \neq j$) will indifferently refer to parasite 1 and parasite 2. Following previous models on the evolution of virulence, our model is based on the classical epidemiological framework where host individuals belong to four different compartments, according to their clinical status (see, e.g., Levin and Pimentel 1981; van Baalen and Sabelis 1995; Alizon 2008): susceptible to both parasites (in proportion S), susceptible to parasite 2 but infected with parasite 1 (in proportion I_1), susceptible to parasite 1 but infected with parasite 2 (in proportion I_2), and infected with both parasites (in proportion I_{12}). Figure 1 shows the flow of host individuals between the four compartments. For simplicity, we do not

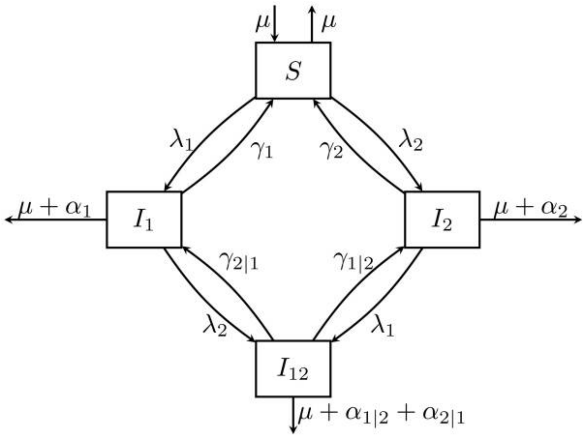

Figure 1: Flowchart of the model. S, I_1, I_2, and I_{12} denote the proportions of host individuals infected with none of the parasites, with parasite 1 only, with parasite 2 only, and with both parasites, respectively. Parameter μ is the host natural birth and death rate, λ_1 and λ_2 are the forces of infection, γ_1, γ_2, $\gamma_{1|2}$, and $\gamma_{2|1}$ are the clearance rates, and α_1, α_2, $\alpha_{1|2}$, and $\alpha_{2|1}$ are the additional host mortalities due to the consumption of host resources by the parasites.

consider host demography, and the host natural birth and death rates are both equal to μ. Parameter λ_i is the force of infection of parasite i.

As commonly considered in models of virulence evolution, parameter α_i is the additional host mortality due to parasite i in the case of a single infection (i.e., when the host individual is infected only with parasite i). This is the definition that theoretical studies give to the notion of virulence of a parasite i, and it is this definition that we will use from this point through "Coevolution of the Two Parasites." We will see, in "Back to the Experimental World," how this definition of virulence relates to the notion of virulence that experimentalists use. In case of mixed infections (i.e., when the host individual is infected with the two parasites), parameter $\alpha_{i|j}$ is the additional host mortality due to parasite i, given that the host individual is also infected with parasite j. In the following, we will prevent ourselves from calling $\alpha_{i|j}$ "virulence," and we will see in "Host Exploitation Strategies and Trade-Offs" how it relates to the virulence α_i we just defined.

Finally, one originality of our model is the inclusion of recovery into such a two-disease framework (see Mosquera and Adler 1998). Parameters γ_i and $\gamma_{i|j}$ are the host recovery rates from parasite i in single and mixed infections, respectively.

The dynamics of this model can be mathematically described by the following set of equations:

$$S = 1 - I_1 - I_2 - I_{12}, \tag{1}$$

$$\frac{dI_i}{dt} = \lambda_i S + \gamma_{j|i} I_{12} - (\lambda_j + \gamma_i + \alpha_i + \mu) I_i, \tag{2}$$

$$\frac{dI_{12}}{dt} = \lambda_1 I_2 + \lambda_2 I_1 - (\gamma_{1|2} + \gamma_{2|1} + \alpha_{1|2} + \alpha_{2|1} + \mu) I_{12}. \tag{3}$$

In accordance with previous models of virulence evolution, the forces of infection follow a classical mass-action process and depend only on the infected individual's clinical state,

$$\lambda_i = \beta_i I_i + \beta_{i|j} I_{12}, \tag{4}$$

where β_i is the transmission rate of parasite i from a host individual infected only with parasite i and $\beta_{i|j}$ is the transmission rate of parasite i from a host individual infected with both parasites i and j. Note that this "susceptible-infected-susceptible" (SIS) framework does not explicitly capture the within-host dynamics of the parasites. The within-host parasite dynamics are implicitly accounted for by the transmission parameters β_i and $\beta_{i|j}$.

The model parameters affect the epidemiological dynamics of the diseases and, in particular, their endemic equilibrium. The analysis developed in the next three sections assumes that the epidemiological dynamics of the diseases are at endemic equilibrium ("Epidemiological Dynamics") and focuses on evolutionarily stable host exploitation strategies by the parasites over the long term ("Evolutionary Dynamics" and "Coevolution of the Two Parasites"). The section "Back to the Experimental World" explores how the three mechanisms of interaction that may occur between parasites infecting the same host individual (competition for resources, parasite phenotypic plasticity, and host immune system impairment) influence the experimental measure of virulence at the scale of one mixed-infection generation and the theoretical measure of virulence over evolutionary time.

Epidemiological Dynamics

One Parasite

When only parasite i infects the host population, the system of equations (1)–(3) reduces to the classical SIS model:

$$S = 1 - I_i,$$

$$\frac{dI_i}{dt} = \beta_i I_i S - (\gamma_i + \alpha_i + \mu) I_i,$$

which yields the following expression for the basic reproduction ratio of parasite i:

$$R_{0_i} = \frac{\beta_i}{\gamma_i + \alpha_i + \mu}. \tag{5}$$

Whenever $R_{0_i} > 1$, the equilibrium densities of the two classes of hosts are

$$\overline{S} = \frac{1}{R_{0_i}} = \frac{\gamma_i + \alpha_i + \mu}{\beta_i} \tag{6}$$

and

$$\overline{I}_i = 1 - \overline{S} = 1 - \frac{\gamma_i + \alpha_i + \mu}{\beta_i} \tag{7}$$

(see Anderson and May 1991; Diekmann and Heesterbeek 2000).

Two Parasites

We now consider that parasite i is present in the host population at the endemic equilibrium and focus on the initial dynamics of parasite j in this population. Parasite j can infect two different kinds of hosts: those susceptible to both parasites (in proportion $\overline{S}$, as defined by eq. [6]) and those already infected by parasite i (in proportion $\overline{I}_i$, as defined by eq. [7]). The initial dynamics of parasite j are described by equations (2) and (3):

$$\frac{dI_j}{dt} = \lambda_j \overline{S} + \gamma_{i|j} I_{12} - (\lambda_i + \gamma_j + \alpha_j + \mu) I_j,$$

$$\frac{dI_{12}}{dt} = \lambda_i I_j + \lambda_j \overline{I}_i - (\gamma_{1|2} + \gamma_{2|1} + \alpha_{1|2} + \alpha_{2|1} + \mu) I_{12}.$$

Note that, soon after the introduction of parasite j in the host population, the proportion of doubly infected host individuals is still negligible compared to the proportion of host individuals infected by parasite i only ($I_{12} \ll \overline{I}_i$), which means that the force of infection of parasite i (eq. [4]) can be approximated as

$$\lambda_i \simeq \beta_i \overline{I}_i.$$

Using the framework developed by Gandon (2004; see also Diekmann et al. 1990; Diekmann and Heesterbeek 2000) for multihost parasites, we can derive the following expression for the basic reproduction ratio of parasite j in a host population already infected by parasite i (see "Calculation of the Basic Reproductive Ratio" for detailed algebra):

$$R_{0_{j|i}} = \frac{\beta_j}{2(\beta_i \overline{I}_i + \gamma_j + \alpha_j + \mu)} \overline{S} + \frac{\beta_{j|i}}{2(\gamma_{1|2} + \gamma_{2|1} + \alpha_{1|2} + \alpha_{2|1} + \mu)} \overline{I}_i + \left\{ \frac{\beta_i \beta_{j|i} \overline{S} + (\beta_1 + \beta_2)\gamma_{i|j}}{(\beta_i \overline{I}_i + \gamma_j + \alpha_j + \mu)(\gamma_{1|2} + \gamma_{2|1} + \alpha_{1|2} + \alpha_{2|1} + \mu)} \overline{I}_i + \left[\frac{\beta_j}{2(\beta_i \overline{I}_i + \gamma_j + \alpha_j + \mu)} \overline{S} + \frac{\beta_{j|i}}{2(\gamma_{1|2} + \gamma_{2|1} + \alpha_{1|2} + \alpha_{2|1} + \mu)} \overline{I}_i \right]^2 \right\}^{1/2}. \tag{8}$$

Note that when there is only one parasite (i.e., $\overline{I}_i = 0$), equation (8) reduces to equation (5).

Evolutionary Dynamics

Host Exploitation Strategies and Trade-Offs

Following van Baalen and Sabelis (1995), each parasite has a "host exploitation strategy" ε_i, a pleiotropic trait that affects both the transmission efficiencies β_i and $\beta_{i|j}$ and the parasite-induced additional host mortalities α_i and $\alpha_{i|j}$. Note that ε_i is a dummy variable that does not have a direct biological interpretation but helps the analyses in setting the relation between parasite transmission and parasite-induced host mortality. See table 1 for exact definitions of the notation for the different host exploitation strategies used throughout this article.

Table 1: Definitions of the different host exploitation strategy notation

Notation	Definition
ε_i	Host exploitation strategy of resident parasite i
ε_i^m	Host exploitation strategy of mutant parasite i
$\hat{\varepsilon}_i$	ES host exploitation strategy of parasite i alone
ε_i^*	ES host exploitation strategy of parasite i in the presence of parasite j
$\tilde{\varepsilon}_i$	CoES host exploitation strategy of parasite i in the presence of parasite j

Note. ES = Evolutionarily stable; see "Evolutionary Dynamics." CoES = Coevolutionarily stable; see "Coevolution of the Two Parasites."

We assume that the host exploitation $\varepsilon_{i|j}$ in mixed infections is related to the host exploitation ε_i in single infections:

$$\varepsilon_{i|j}(\varepsilon_i) = A_i \cdot \varepsilon_i, \tag{9}$$

where A_i ($A_i \geq 1$) is a dimensionless parameter that accounts for the phenotypic plasticity through which each parasite can facultatively increase the exploitation of its host when it detects the presence of another parasite genotype. Thus, equation (9) explicitly decomposes the host exploitation trait into a genetic component ε_i and an environmental component A_i.

One originality of our model is the definition of two different trade-offs between parasite-induced additional host mortality and parasite transmission as defined in equations (10)–(13). For simplicity, we consider that parasite-induced additional host mortalities α_i and $\alpha_{i|j}$ are proportional to the host exploitations ε_i:

$$\alpha_i(\varepsilon_i) = \varepsilon_i, \tag{10}$$

$$\alpha_{i|j}(\varepsilon_i) = \frac{2 - C}{2}\varepsilon_{i|j}(\varepsilon_i), \tag{11}$$

where $\varepsilon_{i|j}(\varepsilon_i)$ is defined by equation (9) and parameter C ($0 \leq C \leq 1$) accounts for the strength of competition between the two parasite clones. In the absence of competition for host resources between the two parasite clones (i.e., $C = 0$), the overall parasite-induced host mortality in the mixed infection is equal to the sum of the effects of the two parasites, $A_1\varepsilon_1 + A_2\varepsilon_2$. On the contrary, when the competition is maximal (i.e., $C = 1$), the overall parasite-induced host mortality in the mixed infection is equal to the mean effect of the two parasites, $(1/2)(A_1\varepsilon_1 + A_2\varepsilon_2)$. Recall that, from "A General Two-Disease Model with Recovery" through "Coevolution of the Two Parasites," the "virulence" of parasite i refers to α_i, which is, from equation (10), equal to the pleiotropic trait ε_i.

As is usually assumed by models of virulence evolution, the parasite transmission efficiencies are also increasing functions of the host exploitation strategies, but these functions saturate in a Michaelis-Menten form in order to account for the finite nature of host resources:

$$\beta_i(\varepsilon_i) = \frac{\varepsilon_i}{1 + \varepsilon_i}, \tag{12}$$

$$\beta_{i|j}(\varepsilon_i, \varepsilon_j) = \frac{\varepsilon_{i|j}(\varepsilon_i)}{1 + \varepsilon_{i|j}(\varepsilon_i) + C \cdot \varepsilon_{j|i}(\varepsilon_j)}. \tag{13}$$

Note that the host total resources are fixed to the same arbitrary quantity in single and mixed infections:

$$\lim_{\varepsilon_i \to +\infty} \beta_i(\varepsilon_i) = \lim_{\substack{\varepsilon_i \to +\infty \\ \varepsilon_j \to +\infty}} \beta_{i|j}(\varepsilon_i, \varepsilon_j) = 1.$$

The conclusions of our analysis do not depend on the exact value of this asymptote. Also note that, when $\varepsilon_j = 0$, equation (13) reduces to equation (12). Finally, everything else being equal, the host immune system may not fight against two different parasites as efficiently as against two identical parasites:

$$\gamma_{i|j}(\gamma_i) = B_i \cdot \gamma_i,$$

where parameter B_i ($0 < B_i \leq 1$) accounts for the efficiency with which the host immune system deals with mixed compared to single infections.

One Parasite

The fitness W_i^{m} of a mutant individual parasite i (with strategy $\varepsilon_i^{\mathrm{m}}$) in a resident population of parasite i (with strategy ε_i) is defined as the invasibility of the rare mutant in the resident population and can thus be derived in the same way as we derived the basic reproduction ratio (see "Epidemiological Dynamics" and "Calculation of the Basic Reproductive Ratio"). Here this gives

$$W_i^{\mathrm{m}}(\varepsilon_i^{\mathrm{m}}, \varepsilon_i) = \frac{\beta_i^{\mathrm{m}}(\varepsilon_i^{\mathrm{m}})}{\gamma_i + \alpha_i^{\mathrm{m}}(\varepsilon_i^{\mathrm{m}}) + \mu}\left[1 - \bar{I}_i(\varepsilon_i)\right] \tag{14}$$

$$= \frac{\beta_i^{\mathrm{m}}(\varepsilon_i^{\mathrm{m}})}{\beta_i(\varepsilon_i)} \cdot \frac{\gamma_i + \alpha_i(\varepsilon_i) + \mu}{\gamma_i + \alpha_i^{\mathrm{m}}(\varepsilon_i^{\mathrm{m}}) + \mu}, \tag{15}$$

where $\beta_i^{\mathrm{m}}(\varepsilon_i^{\mathrm{m}}) = \beta_i(\varepsilon_i^{\mathrm{m}})$ and $\alpha_i^{\mathrm{m}}(\varepsilon_i^{\mathrm{m}}) = \alpha_i(\varepsilon_i^{\mathrm{m}})$. We can check that when the mutant strategy equals the resident strategy ($\varepsilon_i^{\mathrm{m}} = \varepsilon_i$), the fitness of the mutant is equal to 1: $W_i^{\mathrm{m}} = 1$. An equilibrium $\hat{\varepsilon}_i$ for the strategy ε_i can be found by solving

$$\left.\frac{\partial W_i^{\mathrm{m}}(\varepsilon_i^{\mathrm{m}}, \varepsilon_i)}{\partial \varepsilon_i^{\mathrm{m}}}\right|_{\substack{\varepsilon_i^{\mathrm{m}} = \hat{\varepsilon}_i \\ \varepsilon_i = \hat{\varepsilon}_i}} = \frac{\mu + \gamma_i - \hat{\varepsilon}_i^2}{\hat{\varepsilon}_i(1 + \hat{\varepsilon}_i)(\mu + \hat{\varepsilon}_i + \gamma_i)} = 0,$$

which here yields the standard result

$$\hat{\varepsilon}_i = \sqrt{\gamma_i + \mu}. \tag{16}$$

The equilibrium strategy $\hat{\varepsilon}_i$ is convergence stable (CS; i.e., a population of a nearby strategy can be invaded by mutants even closer to $\hat{\varepsilon}_i$), because the sign of

$$\left.\frac{\partial W_i^{\mathrm{m}}(\varepsilon_i^{\mathrm{m}}, \varepsilon_i)}{\partial \varepsilon_i^{\mathrm{m}}}\right|_{\varepsilon_i^{\mathrm{m}}=\varepsilon_i} = \frac{\mu + \gamma_i - \varepsilon_i^2}{\varepsilon_i(1+\varepsilon_i)(\mu+\varepsilon_i+\gamma_i)}$$

is positive for $\varepsilon_i < \hat{\varepsilon}_i$ and negative for $\varepsilon_i > \hat{\varepsilon}_i$ (Geritz et al. 1998). Finally, this equilibrium is also evolutionarily stable (ES; i.e., protected from invasion by mutants of nearby strategies), because

$$\left.\frac{\partial^2 W_i^{\mathrm{m}}(\varepsilon_i^{\mathrm{m}}, \varepsilon_i)}{\partial^2 \varepsilon_i^{\mathrm{m}}}\right|_{\substack{\varepsilon_i^{\mathrm{m}}=\hat{\varepsilon}_i \\ \varepsilon_i=\hat{\varepsilon}_i}} = -2\frac{\hat{\varepsilon}_i}{(\hat{\varepsilon}_i+\mu+\gamma_i)^2} < 0.$$

Equation (16) shows that the CS and ES virulence (as defined by eq. [10]) increases as the duration of infection (proportional to $1/(\gamma_i + \mu)$) shortens. Note that this derivation implies that there cannot be coinfection between a parasite genotype and its mutant. This is reasonable if we assume that the phenotypes of a parasite clone and its mutant are very close, or at least much closer than are the phenotypes of two different parasite clones.

Two Parasites

With the same rationale as in "Two Parasites" (above) and "Calculation of the Basic Reproductive Ratio," we can derive an expression of the fitness W_j^{m} of a rare mutant parasite j in a resident population of parasites i and j (see "Expression of the Fitness of a Rare Mutant" for detailed algebra):

$$\begin{aligned} W_j^{\mathrm{m}}(\varepsilon_j^{\mathrm{m}}, \varepsilon_i, \varepsilon_j) &= \frac{\beta_j^{\mathrm{m}}}{2(\beta_i\bar{I}_i + \beta_{i|j}\bar{I}_{12} + \gamma_j + \alpha_j^{\mathrm{m}} + \mu)}\bar{S} \\ &+ \frac{\beta_{j|i}^{\mathrm{m}}}{2(\gamma_{1|2} + \gamma_{2|1} + \alpha_{i|j} + \alpha_{j|i}^{\mathrm{m}} + \mu)}\bar{I}_i \\ &+ \left\{\frac{\left[(\beta_{j|i}^{\mathrm{m}}\bar{S} + \gamma_{i|j})\beta_i + \gamma_{i|j}\beta_j^{\mathrm{m}}\right]\bar{I}_i + (\beta_{j|i}^{\mathrm{m}}\bar{S} + \gamma_{i|j})\beta_{i|j}\bar{I}_{12}}{(\beta_i\bar{I}_i + \beta_{i|j}\bar{I}_{12} + \gamma_j + \alpha_j^{\mathrm{m}} + \mu)(\gamma_{1|2} + \gamma_{2|1} + \alpha_{i|j} + \alpha_{j|i}^{\mathrm{m}} + \mu)}\right. \\ &+ \left[\frac{\beta_j^{\mathrm{m}}}{2(\beta_i\bar{I}_i + \beta_{i|j}\bar{I}_{12} + \gamma_j + \alpha_j^{m} + \mu)}\bar{S}\right. \\ &\left.\left. + \frac{\beta_{j|i}^{\mathrm{m}}}{2(\gamma_{1|2} + \gamma_{2|1} + \alpha_{i|j} + \alpha_{j|i}^{\mathrm{m}} + \mu)}\bar{I}_i\right]^2\right\}^{1/2}, \end{aligned} \tag{17}$$

where $\bar{S} = \bar{S}(\varepsilon_1, \varepsilon_2)$, $\bar{I}_i = \bar{I}_i(\varepsilon_1, \varepsilon_2)$, and $\bar{I}_{12} = \bar{I}_{12}(\varepsilon_1, \varepsilon_2)$ are the equilibrium proportions of susceptible hosts, hosts infected by parasite i, and hosts doubly infected by parasites 1 and 2, respectively. Furthermore, $\beta_i = \beta_i(\varepsilon_i)$, $\beta_{i|j} = \beta_{i|j}(\varepsilon_i)$, $\gamma_{i|j} = \gamma_{i|j}(\varepsilon_i)$, $\alpha_{i|j} = \alpha_{i|j}(\varepsilon_i)$, $\beta_j^{\mathrm{m}} = \beta_j(\varepsilon_j^{\mathrm{m}})$, $\beta_{j|i}^{\mathrm{m}} = \beta_{j|i}(\varepsilon_j^{\mathrm{m}})$, $\alpha_j^{\mathrm{m}} = \alpha_j(\varepsilon_j^{\mathrm{m}})$, and $\alpha_{j|i}^{\mathrm{m}} = \alpha_{j|i}(\varepsilon_j^{\mathrm{m}})$. Note the resemblance to equation (8), although here we use not only the endemic equilibrium proportions $\bar{S}$ and $\bar{I}_i$ but also $\bar{I}_{12}$. Note also that when there is only one parasite (i.e., $\bar{I}_i = \bar{I}_{12} = 0$), then equation (17) reduces to equation (15). However, contrary to the single-infection case (eq. [15]), $\varepsilon_j^{\mathrm{m}} = \varepsilon_j$ does not here imply $W_j^{\mathrm{m}} = 1$. This is because the fitness of the mutant parasite j depends not only on the resident strategy of parasite j (ε_j) but also on the resident strategy of parasite i (ε_i). The equilibria ε_j^* of strategies ε_j of parasite j, given strategy ε_i of parasite i, were calculated according to the same rationale as in "One Parasite." However, given the complexity of the fitness function (eq. [17]), all the calculations were carried out numerically. Thus, the equilibria ε_j^* were found by numerically solving

$$\left.\frac{\partial W_j^{\mathrm{m}}(\varepsilon_j^{\mathrm{m}}, \varepsilon_j, \varepsilon_i)}{\partial \varepsilon_j^{\mathrm{m}}}\right|_{\substack{\varepsilon_j^{\mathrm{m}}=\varepsilon_j^* \\ \varepsilon_j=\varepsilon_j^*}} = 0.$$

Their convergence stability was checked by verifying that

$$\left.\frac{\partial W_j^{\mathrm{m}}(\varepsilon_j^{\mathrm{m}}, \varepsilon_j, \varepsilon_i)}{\partial \varepsilon_j^{\mathrm{m}}}\right|_{\varepsilon_j^{\mathrm{m}}=\varepsilon_j}$$

is positive for $\varepsilon_j < \varepsilon_j^*$ and negative for $\varepsilon_j > \varepsilon_j^*$, and their evolutionary stability was checked by verifying that

$$\left.\frac{\partial^2 W_j^{\mathrm{m}}(\varepsilon_j^{\mathrm{m}}, \varepsilon_j, \varepsilon_i)}{\partial^2 \varepsilon_j^{\mathrm{m}}}\right|_{\substack{\varepsilon_j^{\mathrm{m}}=\varepsilon_j^* \\ \varepsilon_j=\varepsilon_j^*}} < 0.$$

In all the numerical analyses that follow, calculated equilibria were both CS and ES, and for simplicity we will refer to them simply as ES. The fact that our equilibria were both CS and ES does not rule out the possibility, for other parameter values, of more complex outcomes, such as evolutionary branching. Note also that in "One Parasite" we used a hat to denote ES strategies when only one parasite infects the host population. Here we use an asterisk to denote the ES strategy of a parasite, given that the other parasite also infects the host population (see table 1). As in "One Parasite," this derivation implies that there cannot be coinfection between a parasite genotype and its mutant. Again, this is reasonable if we assume that the phenotypes of a parasite clone and its mutant are very close, or at least much closer than are the phenotypes of two different parasite clones.

Coevolution of the Two Parasites

Because parasite 1's ES strategy is a function of parasite 2's ES strategy and vice versa, the appropriate method to study the evolution of the two parasites at the same time is to calculate the coevolutionarily stable (CoES) strategies $(\tilde{\varepsilon}_1, \tilde{\varepsilon}_2)$ by finding ES strategies of parasites 1 and 2 simultaneously (e.g., van Baalen 1998; Gandon et al. 2002). Note that here CoES strategies are denoted by a tilde; see table 1. The CoES point is found by locating the intersection of the ES curve of parasite 1, given parasite 2's strategy, and the ES curve of parasite 2, given parasite 1's strategy (see fig. 2).

Figure 2*A* shows the simple case where parasites 1 and 2 infect the same host population but, when infecting the same host individual, do not exploit the same host resources; that is, there is no competition ($C = 0$). In addition, there is no phenotypic plasticity for host exploitation ($A_i = 1$), and the host immune system is as efficient in mixed infections as in single infections ($B_i = 1$). Thus, the only interaction between parasites 1 and 2 is that they can be in the same host individual at the same time. Figure 2*A* shows that this increases the ES host exploitation strategies of both parasites (see the arrows on fig. 2*A*). For each parasite, this increased exploitation compensates for its decrease in fitness due to the other parasite's virulence in mixed infections and translates into an increase in its virulence α_i (eq. [10]). This is a case of a "tragedy of the commons," where the costs (here additional host mortality) of a strategy (here host exploitation) are shared between all the protagonists (here parasites 1 and 2), but the benefits (here increased parasite transmission of one parasite) are not. This result is in agreement with previous game-theoretical models (Bremermann and Pickering 1983; Sasaki and Iwasa 1991), kin selection models (Frank 1994, 1996), and the coinfection model of May and Nowak (1995), which showed that the simple fact of sharing a host individual selects for higher levels of parasite virulence.

Scenario 1: Competition for Host Resources

By contrast with figure 2*A*, figure 2*B* shows the CoES host exploitation strategies $\tilde{\varepsilon}_1$ and $\tilde{\varepsilon}_2$ of parasites 1 and 2, respectively, when the two parasites exploit exactly the same host resources ($C = 1$ instead of $C = 0$, as in fig. 2*A*). In this case, each parasite not only suffers from the other parasite's virulence but also has to compete with that parasite for the limited supply of host resources. Figure 2*B* shows that the evolutionary response to this competition for host resources is an augmentation of the ES host exploitation strategies of both parasites, which increases the competitiveness of each parasite when faced with the other

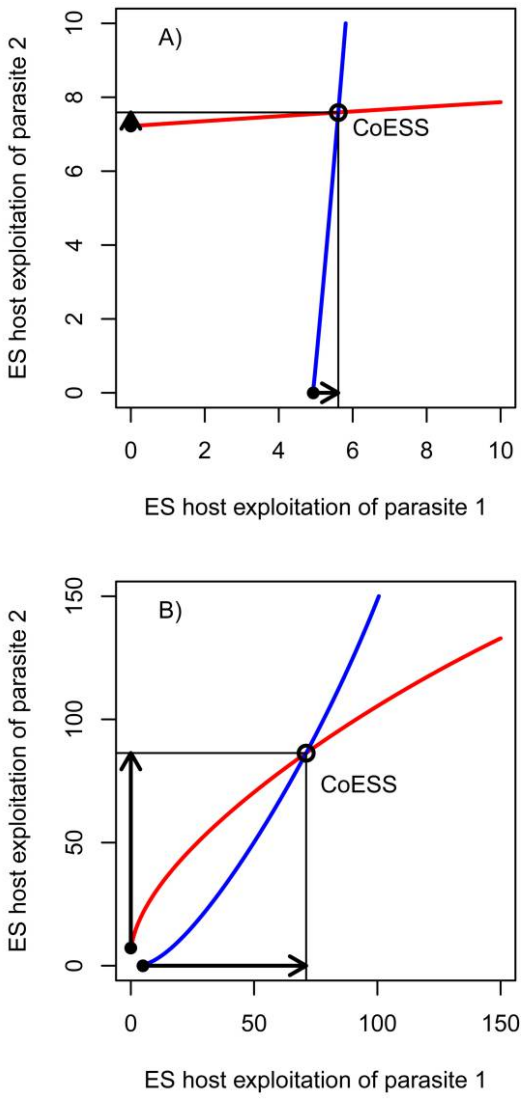

Figure 2: Evolutionarily stable (ES) host exploitation strategies ε_i^* (*blue and red curves*) and coevolutionarily stable (CoES) host exploitation strategies $\tilde{\varepsilon}_i$ in the absence of competition for host resources (*A*; $C = 0$) and when competition is maximal (*B*; $C = 1$). There is no parasite phenotypic plasticity ($A_i = 1$) and no host immune system impairment ($B_i = 1$). The host birth and death rates are kept equal at $\mu = 1/70$ year^{-1}. Parasite 1 (*blue curve*) has an infectious period ($\gamma_1 = 365/15$ year^{-1}) half as long as parasite 2's (*red curve*; $\gamma_2 = 365/7$ year^{-1}), which implies, from equation (16), that parasite 2 has a higher ES host exploitation strategy $\hat{\varepsilon}_i$ on its own than parasite 1; these ES host exploitation strategies $\hat{\varepsilon}_i$ are represented by the two filled circles. The blue and red curves represent the ES host exploitation strategies ε_i^* when mixed infections are allowed. The blue curve represents ε_1^* as a function of ε_2, and the red curve represents ε_2^* as a function of ε_1. The open circle represents the CoES host exploitation strategies $\tilde{\varepsilon}_i$ of the two parasites, and the arrows show the increase (from $\hat{\varepsilon}_i$ to $\tilde{\varepsilon}_i$) in host exploitation strategies after coevolution of the two parasites. See table 1 for definitions of $\hat{\varepsilon}_i$, ε_i^*, and $\tilde{\varepsilon}_i$.

in mixed infections. Again, from equation (10), such increases in ES host exploitation strategies translate into increases in parasite virulences α_i, as predicted by kin selection models (Frank 1994, 1996).

Scenario 2: Parasite Phenotypic Plasticity

Consider now the case where the parasites can phenotypically increase their host exploitation strategy when detecting the presence of another parasite in the same host individual (i.e., $A_i > 1$ instead of $A_i = 1$, as in fig. 2). Figure 3*A* shows the effect of this phenotypic plasticity A_i on the CoES host exploitation strategies ε_i in the case of no competition (*dashed line*; $C = 0$, as in fig. 2*A*) or full competition (*solid line*; $C = 1$, as in fig. 2*B*). In both cases, the host immune system is as efficient in mixed infections as in single infections ($B_i = 1$, as in fig. 2). This figure shows that phenotypic plasticity always decreases the CoES host exploitation strategies of both parasites, which can be understood as follows. From figure 2, we saw that mixed infections represent a cost for each parasite and that each parasite compensates for this cost by evolving a higher host exploitation strategy ε_i. However, because host exploitation strategies ε_i are positively correlated with parasite-induced host mortalities α_i and $\alpha_{i|j}$ (see eqq. [10], [11]), they represent a cost for the parasite in single infections. Phenotypic plasticity allows for a parasite to compensate for the increased parasite-induced host mortalities in mixed infections by facultatively increasing its exploitation without paying any cost of increased exploitation in a single infection. Thus, for a given level $\varepsilon_{i|j}$ of host exploitation in mixed infections, phenotypic plasticity ($A_i > 1$) allows a lower CoES host exploitation strategy (eq. [9]). When the phenotypic plasticity A_i increases, the CoES host exploitation strategies tend toward limits whose values decrease as the proportion I_{12} of mixed infections at epidemiological equilibrium increases. If this proportion is high enough, the CoES host exploitation strategies may become lower than the ES host exploitation strategies when the parasites evolve on their own (fig. 2*A* when there is no competition; *dashed lines*). Above a threshold value of the phenotypic plasticity, the more virulent parasite outcompetes the other one, leaving only one parasite in the host, as in superinfection situations (Nowak and May 1994). In figure 3, values of A_i are all below this threshold. This effect of phenotypic plasticity decreasing the host exploitation strategy accords with the results obtained by Taylor et al. (2006) in the context of single infections.

Scenario 3: Host Immune System Impairment

Similarly, figure 3*B* shows that the CoES host exploitation strategies $\tilde{\varepsilon}_1$ and $\tilde{\varepsilon}_2$ decrease with the efficiency B_i of the host immune system. The explanation of this observation follows the same arguments as above. The cost associated with mixed infections is compensated for by a decrease in the clearance rate $\gamma_{i|j}$, which allows for transmission over a longer-lasting infection. Thus, because the impaired host

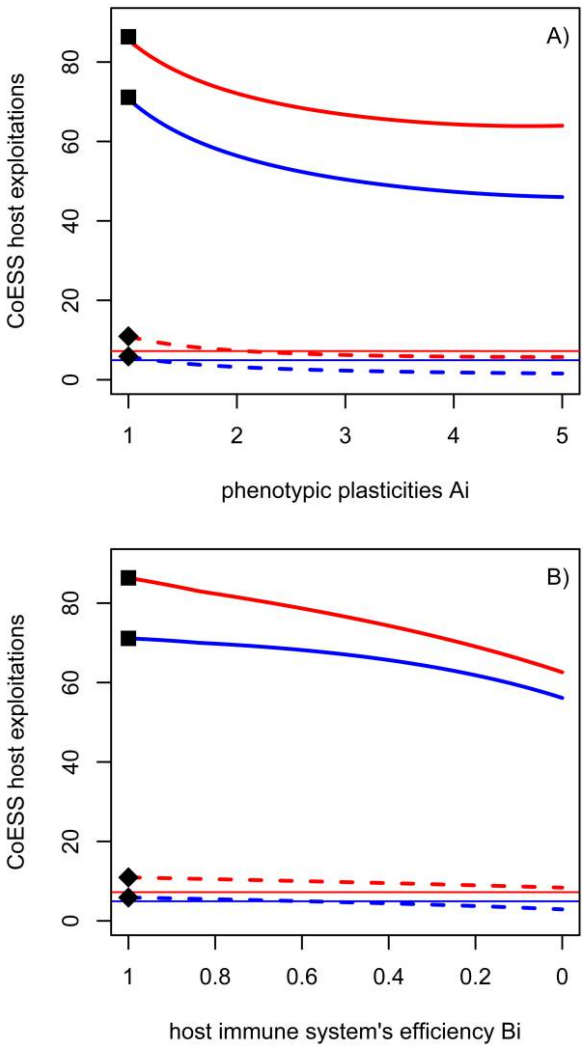

Figure 3: Coevolutionarily stable (CoES) host exploitation strategies $\tilde{\varepsilon}_i$ as functions of the parasites' phenotypic plasticities A_i (*A*) and the host immune system efficiencies B_i (*B*), in the absence of competition (*dashed line*; $C = 0$, as in fig. 2*A*) and when the competition between the two parasites is maximal (*solid line*; $C = 1$, as in fig. 2*B*). The thin blue and red horizontal lines correspond to the evolutionarily stable host exploitation strategies $\hat{\varepsilon}_1$ and $\hat{\varepsilon}_2$ of parasites 1 and 2 on their own, respectively. The diamonds correspond to the CoES host exploitation strategies $\tilde{\varepsilon}_i$ of figure 2*A* ($C = 0$ and $A_i = 1$), and the squares correspond to the CoES host exploitation strategies $\tilde{\varepsilon}_i$ of figure 2*B* ($C = 1$ and $A_i = 1$). In *A* the host immune system is as efficient in mixed infections as in single infections ($B_i = 1$), and in *B* there is no parasite phenotypic plasticity ($A_i = 1$). See figure 1 for other parameter values.

immune system already compensates for the cost due to increased virulence, there is no need for the parasite to do so by increasing its host exploitation strategy ε_i. Equation (16) expresses the fact that the CoES host exploitation strategy $\hat{\varepsilon}_i$ decreases with the clearance rate γ_i. When the efficiencies B_i of the host immune system in mixed infections tend toward 0 ($B_i \to 0$), the CoES host exploitation strategies tend toward a limit whose value decreases as the proportion I_{12} of mixed infections at epidemiological equilibrium increases. If this proportion is high enough, the CoES host exploitation strategies can become lower than the ES host exploitation strategies when the parasites evolve on their own (fig. 3*B*).

Back to the Experimental World

The analysis presented in "Coevolution of the Two Parasites" shows the effect of three types of within-host interaction on the long-term coevolution of host exploitation strategies $\tilde{\varepsilon}_i$. The next step is to relate these host exploitation strategies ε_i to measures of virulence. As reviewed by Day (2002), there are several different ways in which parasite virulence can be related to the host exploitation strategy. Most theoretical works simply use the additional host mortalities α_i as a measure of the virulence of parasite i (see eq. [10]):

$$\alpha_i(\varepsilon_i) = \varepsilon_i.$$

Recall that it is this definition of virulence that we used from "A General Two-Disease Model with Recovery" through "Coevolution of the Two Parasites." In contrast, the measure of virulence that most "verbal" discussions implicitly assume is based on a definition of case mortality (eq. [2.2] of Day 2002):

$$\chi_i(\varepsilon_i) = \frac{\alpha_i(\varepsilon_i)}{\alpha_i(\varepsilon_i) + \gamma_i + \mu} = \frac{\varepsilon_i}{\varepsilon_i + \gamma_i + \mu},$$

which quantifies the likelihood of host death due to parasite i when the host is infected by parasite i only. An adaptation of this case-mortality expression to our model of mixed infections can easily be derived:

$$\chi_{i|j}(\varepsilon_i, \varepsilon_j) = \frac{\alpha_{i|j}(\varepsilon_i)}{\alpha_{1|2}(\varepsilon_1) + \alpha_{2|1}(\varepsilon_2) + \gamma_{i|j}(\gamma_i) + \mu} \tag{18}$$

$$= \frac{A_i\varepsilon_i}{A_1\varepsilon_1 + A_2\varepsilon_2 + 2(B_i\gamma_i + \mu)/(2 - C)}. \tag{19}$$

This quantifies the likelihood of host death due to parasite i when the host is infected by both parasites i and j.

When studying the effect of mixed infections on the evolution of parasite virulence, experimentalists often compare the case fatality in mixed infections, $\chi_{1|2}(\hat{\varepsilon}_1, \hat{\varepsilon}_2) + \chi_{2|1}(\hat{\varepsilon}_1, \hat{\varepsilon}_2)$, with the highest level of case fatality in single infections, $\max(\chi_1(\hat{\varepsilon}_1), \chi_2(\hat{\varepsilon}_2))$, which means that they look at the ratio

$$\Sigma = \frac{\chi_{1|2}(\hat{\varepsilon}_1, \hat{\varepsilon}_2) + \chi_{2|1}(\hat{\varepsilon}_1, \hat{\varepsilon}_2)}{\max(\chi_1(\hat{\varepsilon}_1), \chi_2(\hat{\varepsilon}_2))}. \tag{20}$$

Note that the host exploitation strategies then are the ES strategies $\hat{\varepsilon}_i$ when each parasite evolves on its own. For this reason, we call Σ a "proximate" measure of virulence. In contrast, when they investigate the evolution of virulence of parasite i due to mixed infections, theoreticians look at the ratio

$$\Gamma_i = \frac{\alpha_i(\tilde{\varepsilon}_i)}{\alpha_i(\hat{\varepsilon}_i)}, \tag{21}$$

which includes both the ES strategies $\hat{\varepsilon}_i$ when each parasite i evolves on its own and the CoES strategies $\tilde{\varepsilon}_i$ after the parasite i has coevolved with the parasite j in the same host population. This is thus an "ultimate" measure of virulence. Figure 4 compares these two measures of virulence changes due to mixed infections and how they are influenced by the three different mechanisms that may occur. The figure illustrates that competition decreases the proximate measure of virulence but increases the ultimate measure of virulence: the red and blue dashed lines are below the red and blue solid lines, whereas the black dashed line is above the black solid line. Moreover, both parasite phenotypic plasticity and host immune system impairment increase the proximate measure of virulence while they decrease the ultimate measure of virulence. Thus, there is no positive correlation between the proximate virulence that experimentalists may measure and the ultimate measures of virulence that theoreticians aim to predict. Table 2 summarizes the effects observed in figure 4.

Discussion

Infections of hosts by multiple parasite genotypes are common in nature (Read and Taylor 2001; Hood 2003). Theoretical models developed since the beginning of the 1980s have investigated the effect of such infections on the long-term evolution of parasite virulence. Whatever their detail, most of these models come to the same conclusion, that mixed infections select for increased levels of virulence (Levin and Pimentel 1981; Bremermann and Pickering

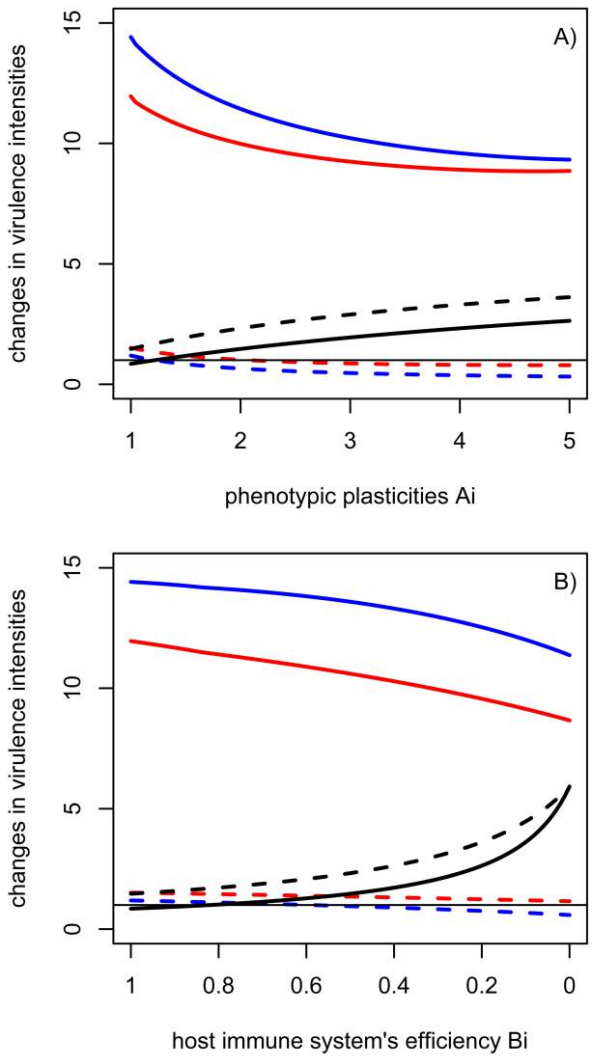

Figure 4: Comparisons of theoretical predictions and experimental measures of virulence changes. These virulence changes are measured as ratios (see eqq. [20], [21]). The thin black horizontal line represents no change (ratio equal to 1). Above and below this line are, respectively, increases and decreases in virulence. The blue and red lines show the increase in virulence of parasites 1 and 2, respectively, as theoretically predicted from equation (21) in the absence (*dashed lines*; $C = 0$) and presence (*solid lines*; $C = 1$) of competition as a function of parasite phenotypic plasticities A_i (A) and host immune system efficiencies B_i (B). The black lines show the increase in virulence as experimentally measured (eq. [20]). See figure 1 for other parameter values.

1983; Sasaki and Iwasa 1991; Frank 1992, 1994, 1996; Nowak and May 1994; May and Nowak 1995; van Baalen and Sabelis 1995; Mosquera and Adler 1998). Cooperation is the only mechanism so far proposed to explain reduced virulence in cases of mixed infections (Chao et al. 2000; Brown et al. 2002; West and Buckling 2003; Ewald and Cochran 2004). It is only recently that the effect of mixed infections on the evolution of parasite virulence has attracted the attention of experimentalists (see, e.g., Imhoof and Schmid-Hempel 1998; Taylor et al. 1998, 2002; Davies et al. 2002; Hodgson et al. 2002; de Roode et al. 2003, 2005*b*; Hughes et al. 2004; Mouton et al. 2004; Schürch and Roy 2004; Gower and Webster 2005; Vizoso and Ebert 2005; Harrison et al. 2006; Ben-Ami et al. 2008). However, although these studies are motivated by theoretical models, they rarely test the main theoretical prediction, that parasites with greater virulence outcompete less virulent parasites in mixed infections. Instead, most experimental studies investigate the virulence of mixed infections within the time frame of a single infection generation and test the prediction that the overall virulence of such infections should be higher than that of single infections.

The model developed here proposes a simple and general framework to study epidemiological situations in which two parasites infect the same host population and the host mounts an immune response against the parasites. Previous two-parasite models (see, e.g., van Baalen and Sabelis 1995) did not include immune clearance because of the complexity it adds to the mathematical analysis. Here we use the methodology developed to analyze models of multihost parasites (see, e.g., Gandon 2004) to bypass such complexity. Note that our general framework can easily be adapted to obligate killers by simply canceling the recovery rates in our model. This would, incidentally, simplify its analysis. Also, the experimental measure of virulence (eqq. [18], [19]) would then be built from Day's (2002) equation (2.6), virulence measured as expected life span, instead of his equation (2.2), virulence measured as case mortality. This would set the numerators of equations (18) and (19) equal to 1.

The analysis of our model allows us to identify three main sources of discrepancy between theoretical and experimental results. The first pertains to the exact nature of the within-host interaction of parasite clones within a host individual. Indeed, whereas theoretical models invariably associate mixed infections with some form of competition, biological evidences suggest that interactions other than competition may occur (see, e.g., Taylor et al.

Table 2: Effects of competition for resources, parasite phenotypic plasticity, and host immune system impairment on the theoretically predicted and experimentally measured changes in virulence

	Theoretical prediction	Experimental measure
Competition	Increase	Decrease
Phenotypic plasticity	Decrease	Increase
Immune system impairment	Decrease	Increase

Note. This table summarizes the results of figure 4.

1998; Davies et al. 2002; Hodgson et al. 2002; Mouton et al. 2004). The second reason is related to differences in definitions of virulence between theoreticians and experimentalists. Whereas theoreticians usually consider virulence as a parasite trait, the virulence that experimentalists measure is an intensity of pathology that depends on both host and parasite traits (Day 2002). Finally and most importantly, theoreticians and experimentalists do not, in fact, look at the same thing. Whereas theoreticians are interested in the magnitude of virulence in the long term and at the population level, experimentalists actually measure—for obvious practical reasons—the magnitude of pathology at the scale of one infection generation.

Our analysis confirms previous theoretical results that, in the absence of cooperation, competition selects for increased host exploitation by parasites (and hence increased parasite virulence, as considered by theoreticians). Moreover, it proposes two new mechanisms that select for decreased host exploitation by parasites (and hence decreased parasite virulence, as considered by theoreticians): (*i*) parasite phenotypic plasticity, whereby each parasite clone responds to the presence of each other by increasing its growth rate, and (*ii*) host immune system impairment, where the host immune system fights against two different parasites less efficiently than against two identical parasites. Finally, when considering the virulence measured by experimentalists and still in the absence of cooperation, we find opposite results: pure competition (i.e., without phenotypic plasticity on parasite growth) decreases the overall level of virulence, whereas both parasite phenotypic plasticity and host immune system impairment increase the level of virulence that experimentalists measure. This result thus warns against drawing inferences on the evolution of virulence based on mixed-infection experiments.

In our model, both the phenotypic plasticity A_i and the host immune system efficiency B_i are fixed parameters. By using the same model and letting these parameters be free to evolve, we would be able to study the evolution of parasite phenotypic plasticity and resistance in a context of mixed infections. Moreover, we have focused here on two clones of the same parasite species, which justifies the choice made on the symmetry of parameters A_i and B_i: $A_1 = A_2$ and $B_1 = B_2$. However, such an assumption can be relaxed to analyze more general situations of different parasite species. In such cases, the two parasites may also have different modes of transmission (as modeled by the force of infection λ_i), and the model can be easily extended to account for other forms of within-host parasite interactions, such as the release of toxins. In some situations it may happen that the order of infection by the two parasites is important (van Baalen and Sabelis 1995; de Roode et al. 2005*a*; Ben-Ami et al. 2008). Again, the model can be extended to such situations by simply increasing the number of infected host classes. The analysis will be more complex, but the principle remains simple. Similarly, the model can be extended to more than two parasites infecting the same host population.

Our model appears to succeed at explaining the different changes in virulence observed in different experimental setups. However, since our model does not explicitly account for within-host parasite dynamics (which are implicitly modeled by eqq. [10]–[13]), it cannot help in understanding the different changes in parasite densities. An extension of our model to a nested one coupling within- and between-host parasite dynamics (see, e.g., Alizon and van Baalen 2005, 2008) would help in that effort. By estimating parameter values of such a model, one would be able to identify the relative intensities of the different mechanisms of within-host parasite interactions. This will be a crucial step, because it is likely that several within-host mechanisms interact with each other. For example, phenotypic increases in host exploitation may occur only in response to competition, and these two mechanisms have opposing effects on the subsequent selection on parasite virulence. Thus, whether there will be selection for higher or lower virulence will depend on the relative importance of these mechanisms. It is clear that only a tight coupling between experiments and mathematical modeling will allow us to draw inferences on the long-term evolution of virulence at the population level from mixed-infection experiments (see, e.g., Brown et al. 2006).

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APPENDIX

Calculation of the Basic Reproductive Ratio

If parasite *i* is present in the host population at the endemic equilibrium, the dynamics of parasite *j* are described by equations (2) and (3):

$$\frac{dI_j}{dt} = \lambda_j \bar{S} + \gamma_{i|j} I_{12} - (\lambda_i + \gamma_j + \alpha_j + \mu) I_j,$$

$$\frac{dI_{12}}{dt} = \lambda_i I_j + \lambda_j \bar{I}_i - (\gamma_{1|2} + \gamma_{2|1} + \alpha_{1|2} + \alpha_{2|1} + \mu) I_{12},$$

where $\bar{S}$ and $\bar{I}_i$ are the equilibrium proportions of susceptibles and hosts infected by parasite i, respectively. With the approximation $\lambda_i \simeq \beta_i \bar{I}_i$ (see "Two Parasites" within "Epidemiological Dynamics"), the above system becomes

$$\frac{dI_j}{dt} = [\beta_j \bar{S} - (\beta_i \bar{I}_i + \gamma_j + \alpha_j + \mu)] I_j + (\beta_{j|i} \bar{S} + \gamma_{i|j}) I_{12},$$

$$\frac{dI_{12}}{dt} = (\beta_1 + \beta_2) \bar{I}_i I_j + [\beta_{j|i} \bar{I}_i - (\gamma_{1|2} + \gamma_{2|1} + \alpha_{1|2} + \alpha_{2|1} + \mu)] I_{12},$$

which, in matrix notation, gives $\dot{\mathbf{I}}_j = (\mathbf{B}_{j|i} - \mathbf{D}_{j|i}) \cdot \mathbf{I}_j$, where $\mathbf{I}_j = (I_j, I_{12})^T$,

$$\mathbf{B}_{j|i} = \begin{bmatrix} \beta_j \bar{S} & \beta_{j|i} \bar{S} + \gamma_{i|j} \\ (\beta_1 + \beta_2) \bar{I}_i & \beta_{j|i} \bar{I}_i \end{bmatrix},$$

and

$$\mathbf{D}_{j|i} = \begin{bmatrix} \beta_i \bar{I}_i + \gamma_j + \alpha_j + \mu & 0 \\ 0 & \gamma_{1|2} + \gamma_{2|1} + \alpha_{1|2} + \alpha_{2|1} + \mu \end{bmatrix}.$$

The dominant eigenvalue of $\mathbf{B}_{j|i} \cdot \mathbf{D}_{j|i}^{-1}$ yields equation (8).

Expression of the Fitness of a Rare Mutant

With the same rationale as in "Calculation of the Basic Reproductive Ratio," if resident parasites 1 and 2 are present in the host population at the endemic equilibrium, the dynamics of a mutant of parasite j are described by equations (2) and (3):

$$\frac{dI_j^{\mathrm{m}}}{dt} = \lambda_j^{\mathrm{m}} \bar{S} + \gamma_{i|j} I_{ij}^{\mathrm{m}} - (\lambda_i + \gamma_j + \alpha_j^{\mathrm{m}} + \mu) I_j^{\mathrm{m}}, \tag{A1}$$

$$\frac{dI_{ij}^{\mathrm{m}}}{dt} = \lambda_i I_j^{\mathrm{m}} + \lambda_j^{\mathrm{m}} \bar{I}_i - (\gamma_{1|2} + \gamma_{2|1} + \alpha_{i|j} + \alpha_{j|i}^{\mathrm{m}} + \mu) I_{ij}^{\mathrm{m}}, \tag{A2}$$

where I_j^{m} is the proportion of hosts infected with parasite j and I_{ij}^{m} is the proportion of hosts doubly infected with parasite i and mutant parasite j. Soon after the introduction of mutant parasite j in the host population, the proportion of host individuals infected doubly with parasite i and mutant parasite j is still negligible, relative to the proportion of host individuals infected by parasite i only ($I_{ij}^{\mathrm{m}} \ll \bar{I}_i$) and the proportion of host individuals infected doubly by resident parasites i and j ($I_{ij}^{\mathrm{m}} \ll \bar{I}_{12}$). This means that the force of infection of parasite i can be approximated as

$$\lambda_i \simeq \beta_i \bar{I}_i + \beta_{i|j} \bar{I}_{12}.$$

The force of infection of mutant parasite j comes from equation (4):

$$\lambda_j^{\mathrm{m}} = \beta_j^{\mathrm{m}} I_j^{\mathrm{m}} + \beta_{j|i}^{\mathrm{m}} I_{ij}^{\mathrm{m}}.$$

Inserting these two expressions of the forces of infection into equations (A1) and (A2) yields

$$\frac{dI_j^{\mathrm{m}}}{dt} = [\beta_j^{\mathrm{m}}\bar{S} - (\beta_i\bar{I}_i + \beta_{i|j}\bar{I}_{12} + \gamma_j + \alpha_j^{\mathrm{m}} + \mu)]I_j^{\mathrm{m}} + (\beta_{j|i}^{\mathrm{m}}\bar{S} + \gamma_{i|j})I_{ij}^{\mathrm{m}},$$

$$\frac{dI_{ij}^{\mathrm{m}}}{dt} = [(\beta_i + \beta_j^{\mathrm{m}})\bar{I}_i + \beta_{i|j}\bar{I}_{12}]I_j^{\mathrm{m}} + [\beta_{j|i}^{\mathrm{m}}\bar{I}_i - (\gamma_{1|2} + \gamma_{2|1} + \alpha_{i|j} + \alpha_{j|i}^{\mathrm{m}} + \mu)]I_{ij}^{\mathrm{m}},$$

where $\bar{S}$, $\bar{I}_i$, and $\bar{I}_{12}$ are the equilibrium proportions of susceptible hosts, hosts infected with parasite *i*, and hosts doubly infected with the resident parasites, respectively. In matrix notation, the above system gives $\dot{\mathbf{I}}_j^{\mathrm{m}} = (\mathbf{B}_{j|i}^{\mathrm{m}} - \mathbf{D}_{j|i}^{\mathrm{m}}) \cdot \mathbf{I}_j^{\mathrm{m}}$, where $\mathbf{I}_j^{\mathrm{m}} = (I_j^{\mathrm{m}}, I_{ij}^{\mathrm{m}})^T$,

$$\mathbf{B}_{j|i}^{\mathrm{m}} = \begin{bmatrix} \beta_j^{\mathrm{m}}\bar{S} & \beta_{j|i}^{\mathrm{m}}\bar{S} + \gamma_{i|j} \\ (\beta_i + \beta_j^{\mathrm{m}})\bar{I}_i + \beta_{i|j}\bar{I}_{12} & \beta_{j|i}^{\mathrm{m}}\bar{I}_i \end{bmatrix},$$

and

$$\mathbf{D}_{j|i}^{\mathrm{m}} = \begin{bmatrix} \beta_i\bar{I}_i + \beta_{i|j}\bar{I}_{12} + \gamma_j + \alpha_j^{\mathrm{m}} + \mu & 0 \\ 0 & \gamma_{1|2} + \gamma_{2|1} + \alpha_{i|j} + \alpha_{j|i}^{\mathrm{m}} + \mu \end{bmatrix}.$$

The dominant eigenvalue of $\mathbf{B}_{j|i}^{\mathrm{m}} \cdot \mathbf{D}_{j|i}^{\mathrm{m}^{-1}}$ yields equation (17).

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