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E-Note

Decreased Overall Virulence in Coinfected Hosts Leads to the Persistence of Virulent Parasites

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abstract: Multiple infections are known to affect virulence evolution. Some studies even show that coinfections may decrease the overall virulence (the disease-induced mortality of a coinfected host). Yet, epidemiological studies tend to overlook the overall virulence, and within-host models tend to ignore epidemiological processes. Here, I develop an epidemiological model where overall virulence is an explicit function of the virulence of the coinfecting strains. I show that in most cases, a unique strain is evolutionarily stable (in accordance with the model I use here). However, when the overall virulence is lower than the virulence of each of the coinfecting strains (i.e., when coinfections decrease virulence), the evolutionary equilibrium may be invaded by highly virulent strains, leading to the coexistence of two strains on an evolutionary timescale. This model has theoretical and experimental implications: it underlines the importance of overall virulence and of epidemiological feedbacks on virulence evolution.

Keywords: multiple infections, virulence, epidemiology, evolution, kin selection, coexistence.

Multiple infections are widespread and have been shown to affect virulence evolution (for a review, see Read and Taylor 2001). Evaluating the evolutionary consequences of multiple infections on virulence raises several experimental questions, one of which is, what should we measure?

Consider two parasite strains i and j: one can measure the virulence of strain i or j alone (i.e., in singly infected hosts), but one can also measure the overall virulence in hosts coinfected by i and j. Virulence is a notoriously ambiguous notion, and most of the time it is defined as the disease-induced mortality of the host (Bull 1994; Read 1994; Day 2002). In a coinfection, this host disease-induced mortality reflects what I will refer to as the overall virulence. This latter notion has little in common with the virulence defined in singly infected hosts. The reason is that on the timescale of an infection, overall virulence is affected by many factors, among which are the number of coinfecting strains, the order of infection, the infection doses, and the relatedness between the strains.

If we are interested in virulence evolution, the overall virulence may seem less important than the transmission success of each of the strains in the coinfected host. Indeed, if we know the transmission success of the strains, we know whether coinfections lead to higher or lower levels of virulence (see, e.g., de Roode et al. 2005). Theoreticians can easily follow the fitness of a given strain and hence do not feel the need to describe the virulence in coinfected hosts in detail. On the contrary, experimental studies often measure only the disease-induced mortality of coinfected hosts for practical reasons. A point that is often overlooked is that within-host processes can induce different overall virulence values, which might affect the epidemiology of the system and lead to different evolutionary outcomes. I explore this problem here using an epidemiological framework that takes coinfections into account.

Experimental Measures of Overall Virulence

The expected, and often verified, outcome of a coinfection is that the overall virulence will be higher than the virulence of any of the coinfecting strains measured in a single infection (Gingery and Nault 1990; Inglis et al. 1997; Taylor et al. 1997, 1998; Davies et al. 2002; Ferguson and Read 2002; López-Ferber et al. 2003; Hodgson et al. 2004; Lawn 2004; Davidar and Morton 2006; Hõrak et al. 2006; Vignuzzi et al. 2006). This is often explained by the difficulty of the immune system to respond to a diverse infection or by the competition between parasite strains for shared limited resources (see below).

Other possibilities are that the overall virulence is determined by the most virulent strain (Inglis et al. 1997;

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Imhoof and Schmid-Hempel 1998; Read and Taylor 2001; Hughes and Boomsma 2004; Hughes et al. 2004) or that it lies in between the virulence of the two strains. This latter case can be due to an averaging of the virulences (Taylor et al. 2002; de Roode et al. 2003) or to the fact that one of the strains is a cheater that exploits the other strain (Thomas et al. 2003; Turner 2005; Harrison et al. 2006). The same phenomenon even seems to occur for some coinfections by different parasites. For instance, in humans, the apparently nonpathogenic flavivirus GB virus C has been shown to prolong survival in patients infected by HIV (Tillmann et al. 2001; Xiang et al. 2001).

In some cases, the overall virulence can be that of the less virulent strain (Berchieri and Barrow 1990; Sernicola et al. 1999). The interpretation of these results is usually that the avirulent strain confers protection to its host against a more virulent strain. This process is observed in plants, where it is known as cross-protection (Fulton 1986). Note that in this case, the order of infection is likely to have a strong impact on the overall virulence.

Finally, in some cases, the overall virulence is lower than the virulence of each of the coinfecting strains. This result is observed in plants (Hood 2003; Schürch and Roy 2004), and multiplicity of infection has also been shown to reduce disease severity of human malaria infections (al Yaman et al. 1997; Smith et al. 1999). But arguably the best example is given by bacteria producing bacteriocins (chemicals that harm other bacterial strains). As shown by Massey et al. (2004), in a diverse infection, interference competition between unrelated bacteria may lead to an overall virulence lower than the virulence of the coinfecting clones.

Modeling Multiple Infections

Theoretical studies on multiple infections are done at either the epidemiological or the within-host level. The problem is that within-host models tend to ignore epidemiological feedbacks and epidemiological models overlook the problems raised by the definition of the overall virulence.

The first epidemiological models incorporating multiple infections assumed that there could be no strain coexistence within a host, that is, that one of the strains instantaneously replaces the other (Levin and Pimentel 1981). This process is known as superinfection, and it can easily lead to the coexistence of many strains in the population on evolutionary timescales when trade-offs are involved, for example, between virulence and transmission (Levin and Pimentel 1981; Nowak and May 1994; Mosquera and Adler 1998; Gandon et al. 2002; Boldin and Diekmann 2008).

Another approach is to assume that strains can coexist indefinitely in the same host (Bremermann and Pickering 1983). This process is known as coinfection, and it has been shown to lead to an evolutionarily stable strategy (van Baalen and Sabelis 1995). In the coinfection model developed by van Baalen and Sabelis, there can be no strain coexistence in the population on evolutionary timescales. This may seem surprising since several studies based on a coinfection framework do find strain coexistence (May and Nowak 1995; Mosquera and Adler 1998; Zhang et al. 2001; Allen et al. 2003; Martcheva and Pilyugin 2006). The only striking difference is that, contrary to the model developed by van Baalen and Sabelis, these models do not allow for hosts to be coinfected twice by the same strain. As a consequence, a rare strain always has an advantage because it can infect hosts already infected by the common strain. This presumably explains the absence of coexistence in the framework developed by van Baalen and Sabelis (which I use in this article). An extensive comparison of these two classes of models will be the basis for a subsequent study (for further details on this issue, see app. C).

The other way to study multiple infections is to develop a within-host model. Parasite strains competing for a shared limited resource are in a "tragedy of the commons" situation. The well-known outcome is that the strain with the highest growth rate will always outcompete the others at the within-host level. Multiple infections hence favor faster-growing, usually more virulent strains (Bremermann and Pickering 1983). Note that in this scenario, kin selection theory predicts that relatedness among the coinfecting strains will decrease overall virulence by decreasing the competition intensity (Chao et al. 2000).

On the contrary, many within-host models involving kin selection predict that overall virulence should be low in diverse infections (Frank 1992; Brown 2001; Brown et al. 2002; Gardner et al. 2004). The first explanation is that some strains behave as cheaters: they are hardly capable of infecting a host on their own and can be seen as avirulent. However, these defective strains may exploit the work of efficient virulent strains (Turner 2005). Clearly, cheaters benefit from the diversity of an infection, and this results in a decrease in collective actions that decreases virulence (Brown 2001; Brown et al. 2002). A second explanation is direct competition among unrelated parasite strains, which also decreases the ability of the parasites to exploit the host. This is the case for bacteria clones engaged in interference competition through bacteriocins (Massey et al. 2004). Intuitively, one might think that if multiply infected hosts have lower overall virulence, selection favors less virulent strains (a conclusion reached by most of the kin selection models cited above). However, predicting the outcome of virulence evolution is not that straightforward.

To study the effect of overall virulence on parasite evolution, we need a coinfection framework (otherwise there is no overall virulence). In addition to the strong epide-

miological assumption described above, coinfection models usually assume a single overall virulence function. The originality of the model presented here is that it combines biological processes occurring within the host with an epidemiological framework. This allows me to study the consequences of different overall virulence functions on virulence evolution.

The Model

This study is based on the coinfection framework developed by van Baalen and Sabelis (1995) for microparasites (e.g., viruses or bacteria) causing persistent infections. I study the conditions of invasion of a rare mutant (m) in a host population already infected by a resident strain (r). I further assume that the resident strain is at equilibrium, that is, that the densities of susceptible (S), infected (I_r), and coinfected hosts (D_{rr}) have reached their equilibrium values denoted S^*, I_r^*, and D_{rr}^* (the exact values are in app. A, along with the equations of the system). As in the original model, an important epidemiological feature here is that hosts can be coinfected twice by the same strain. Without this assumption, a rare strain always has an advantage: it can infect hosts already infected by the common strain while the common strain has few hosts to coinfect.

Parasite fitness can be evaluated using the basic reproduction ratio, R_0. However, the expression derived by Anderson and May (1979) is valid only for single infections, so we need to use the more general expression derived by van Baalen and Sabelis. In a coinfection model, the R_0 of a mutant strain is the sum of two components: the fitness achieved through the infection of susceptible hosts (S) and the fitness achieved through the infection of hosts already infected by the resident (I_r);

$$R_0 = \frac{\beta_m + \lambda_r[\beta_{mr}/(\mu + \alpha_{mr})]}{\mu + \alpha_m + \lambda_r} S^* + \frac{\beta_{rm}}{\mu + \alpha_{rm}} I_r^*, \quad (1)$$

where β refers to the transmission rate of the mutant strain, α refers to the virulence rate (often measured as host disease-induced mortality), λ_r is the force of infection of the resident strain (see app. A), and μ is the host natural death rate. All the subscripts define the order of infection (for instance, "mr" indicates a host infected first by the mutant and then by the resident).

Here, we are interested in the overall virulence, which is captured by the terms α_{rm} and α_{mr}. Most existing studies make one assumption and consider that the first strain to infect the host determines the overall virulence (van Baalen and Sabelis 1995) or that the overall virulence is constant (Zhang et al. 2001, 2007; Martcheva and Pilyugin 2006) or is equal to the virulence of the most virulent strain (May and Nowak 1995; Allen et al. 2003). Only two studies compare approaches (Bremermann and Pickering 1983; Mosquera and Adler 1998): both consider that overall virulence is either the sum of the virulence of the two strains or an increasing function of this sum (100 times the sum [Mosquera and Adler 1998] or a power of the sum [Bremermann and Pickering 1983]).

Here, an easy approach could be to set these overall virulence terms to an arbitrary low or high value. However, because we work with microparasites, we also want hosts doubly infected by the same strain to be identical to hosts singly infected by this strain. Setting the overall virulence to an arbitrary value only in hosts coinfected by different strains would create a strong discontinuity. An alternative approach is to define overall virulence as a function of the virulence of the two coinfecting strains,

$$\alpha_{ij} = f(\alpha_i, \alpha_j). \quad (2)$$

Following the experimental data listed in the first section of this article, I study three different cases: a case where the overall virulence (α_{ij}) is the average of the virulence of the coinfecting strains (α_i and α_j), a case where α_{ij} is always lower than both α_i and α_j, and a case where α_{ij} is always higher than both α_i and α_j.

Ideally, f should have the following two properties: (1) $f(\alpha_i, \alpha_j) = f(\alpha_j, \alpha_i)$, which means the order of arrival in the host does not affect the overall virulence, and (2) $f(\alpha_i, \alpha_i) = \alpha_i$, so that if the two strains are identical, doubly infected hosts have the same characteristics as singly infected hosts. This second property implies that, contrary to the findings of Mosquera and Adler (1998), overall virulence cannot be defined as the sum of the virulence ($\alpha_i + \alpha_i$). Such an assumption could be appropriate to study macroparasitic infections because then coinfections should increase the total parasite density. For microparasitic infections, however, the overall virulence should be equal to the virulence of the strains, because if two identical strains coinfect a host, the total density is likely not to be affected given the large population sizes involved.

Properties 1 and 2 are verified in the first case, that is, when α_{ij} is the average of α_i and α_j (fig. 1):

$$f_1(\alpha_i, \alpha_j) = \frac{\alpha_i + \alpha_j}{2}. \quad (3)$$

For the other two cases, I would also like f to be a Gaussian function of the distance between the two strains ($|\alpha_r - \alpha_m|$), which is maximized (or minimized) when $\alpha_r = \alpha_m$. Unfortunately, it can be shown that such a function does not exist. In want of a better approximation, I define the two functions (f_2 and f_3) as

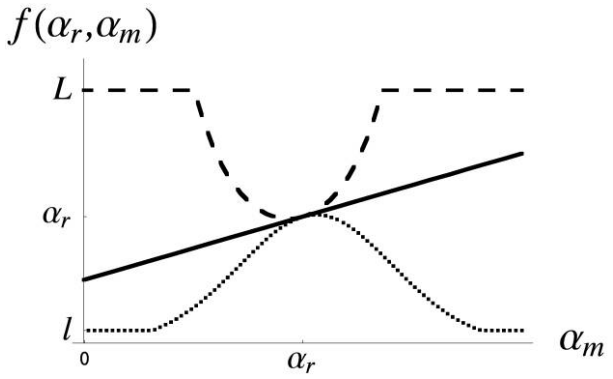

Figure 1: Overall virulence in a coinfected host. Overall virulence can be an average function (f_1; *solid line*), an increasing function (f_2; *dashed line*), or a decreasing function (f_3; *dotted line*). The first strain that arrives in the host has a virulence α_r, and the second strain has a virulence α_m. The minimum and maximum overall virulence values are denoted l and L, respectively. In this figure, $s = 1$.

$$f_2(\alpha_i, \alpha_j) = \frac{\alpha_i + \alpha_j}{2}\exp\left[\frac{(\alpha_i - \alpha_j)^2}{s}\right], \quad (4)$$

$$f_3(\alpha_i, \alpha_j) = \frac{\alpha_i + \alpha_j}{2}\exp\left[-\frac{(\alpha_i - \alpha_j)^2}{s}\right], \quad (5)$$

where s is a parameter giving the width of the function. Note that f_3 has a Gaussian shape, but it is centered in $[\alpha^2 + (s/2)]^{1/2}$ instead of $\alpha_i = \alpha_j$. It is possible to find functions that are centered in α_i over most of their definition domain, but they are discontinuous and their shapes make less biological sense. These functions lead to similar results that are more difficult to visualize because of the discontinuities involved. In the main text, I used the above f_3 function, thus sacrificing some of the mathematical accuracy to clarity and biological sensibility (but see app. B for the cases with the alternative functions).

As shown in figure 1, in the case of f_2 (*dashed line*), the more the virulence of the mutant parasite (α_m) differs from that of the resident (α_r), the higher the overall virulence will be (the maximum virulence value is denoted L). Such an overall virulence function describes cases where the immune system has difficulty fighting two infections at the same time. There is a further assumption here that the difference between the coinfecting strains determines the overall virulence. A possible biological interpretation is that the virulence of a strain is somehow correlated with the components of the immune system that are fighting it.

In the case of f_3 (*dotted line*), we have the opposite pattern. Here, a possible underlying assumption is that strains differing in virulence have a low relatedness, implying that they are less likely to cooperate according to kin selection theory. As highlighted in the first section of this article, the absence of cooperation can benefit the host for two reasons: either because avirulent strains exploit the work of virulent strains (Thomas et al. 2003; Turner 2005; Harrison et al. 2006) or because there is interference competition between nonrelated strains (Massey et al. 2004). Note that in their study on bacteriocin-producing bacteria, Massey et al. (2004) measure the lowest overall virulence in the case where the difference in virulence among the strains is the highest.

We now need to define the transmission rate of the mutant in a coinfected host. This function should have two properties: (1) $\beta_{rm} = \beta_{mr}$, so that transmission is not affected by the order of arrival in the host; and (2) if $\alpha_r = \alpha_m$, then $\beta_{mr} = \beta_m/2$, because hosts coinfected by the same strain are identical to singly infected hosts.

For the definition of the transmission rates, I study two cases. In both of these cases, the expression for R_0 is given by equation (1). In the first case, transmission is independent of virulence and is set to be constant in any type of infected hosts (β). This implies that virulence is only a cost, and theory predicts the evolution toward complete avirulence.

In the second case, I assume a trade-off between virulence and transmission, that is, that increasing virulence also increases the transmission rate. If the trade-off curve is concave, parasites evolve toward intermediate levels of virulence (Massad 1987; Ewald 1994; van Baalen and Sabelis 1995; Alizon and van Baalen 2005). Here, I choose

$$\beta(\alpha) = a\alpha^{3/4}, \quad (6)$$

where a is a constant. The choice of the exponent of α is arbitrary, but similar results can be obtained for other values in]0, 1[.

Results

Case without a Trade-Off

Figure 2*A*–2*C* depicts pairwise invasibility plots (PIPs; Geritz et al. 1997) showing R_0 as a function of the virulence of the resident (on the *X*-axis) and of the mutant strain (on the *Y*-axis). In all three graphs, $R_0 > 1$ below the diagonal, implying that a mutant with a lower virulence can always invade the resident strain. This result was expected: without a trade-off, parasites evolve toward avirulence. In figure 2*C*, however, the fact that coinfections decrease virulence leads to an interesting pattern: mutants slightly more virulent than the resident vanish (*black area*), but highly virulent mutants can establish in the host population. Contrary to mutants that are less virulent than the

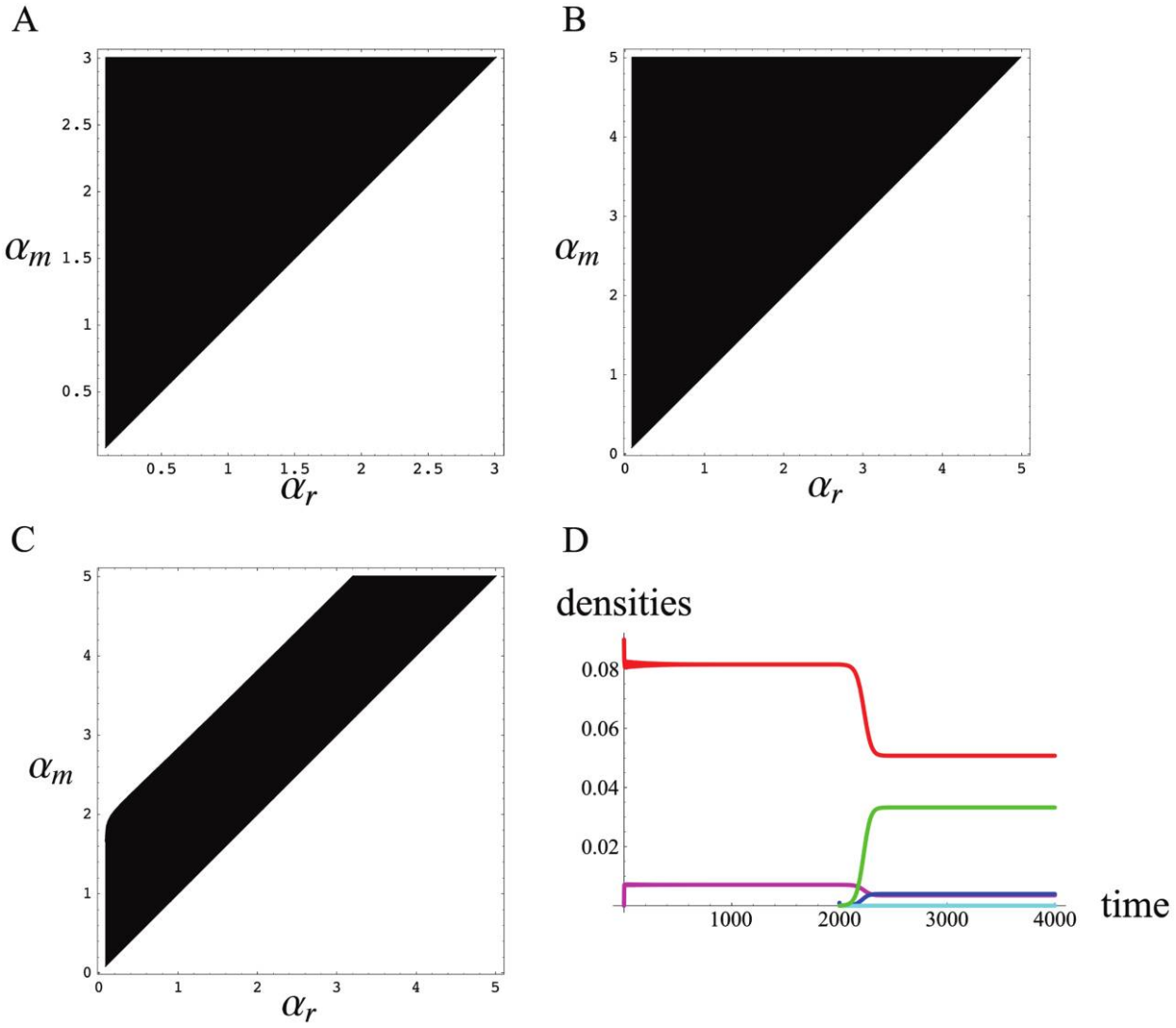

Figure 2: Pairwise invasibility plots with average (f_1; *A*), high (f_2; *B*), or low (f_3; *C*) overall virulence, and coexistence of a virulent and a highly virulent strain in a host population with low overall virulence (f_3; *D*). *A–C* show the R_0 of a mutant parasite (α_m), depending on the virulence of a resident parasite (α_r). In the black areas, the mutant strain disappears ($R_0 < 1$), while in the white areas, it persists in the host population. Parameter values are $\beta = 1$, $s = 1$, $t = 0.01$, $r = 0.1$, and $\mu = 0.02$. In *D*, I start with a virulent strain ($\alpha_1 = 1$). Hosts infected by the virulent strain (I_r) are in red, and hosts coinfected by the virulent strain (D_{rr}) are in pink. At $t = 2{,}000$, I introduce a rare, highly virulent strain ($\alpha_2 = 4$). Hosts infected by only the mutant strain (I_m) are in blue, while hosts coinfected by the mutant strain (D_{mm}) are in cyan. Hosts coinfected by two different strains are in green.

resident that take over the population, these highly virulent mutants may coexist with the resident on ecological timescales (see fig. 2*D*).

The evolutionary dynamics of this system are difficult to study because the less virulent strain evolves toward avirulence. The problem is that in this epidemiological system, parasite virulence is necessary to limit the density of susceptible hosts. Once a strain with a zero virulence emerges, it takes over the population, and the density of hosts explodes. However, there can be coexistence on an evolutionary timescale between the low-virulent and the highly virulent strain before the system explodes (figure not shown).

Case with a Transmission-Virulence Trade-Off

Figure 3*A–3C* shows PIPs for the case with a convex trade-off between virulence and transmission (eq. [6]). These

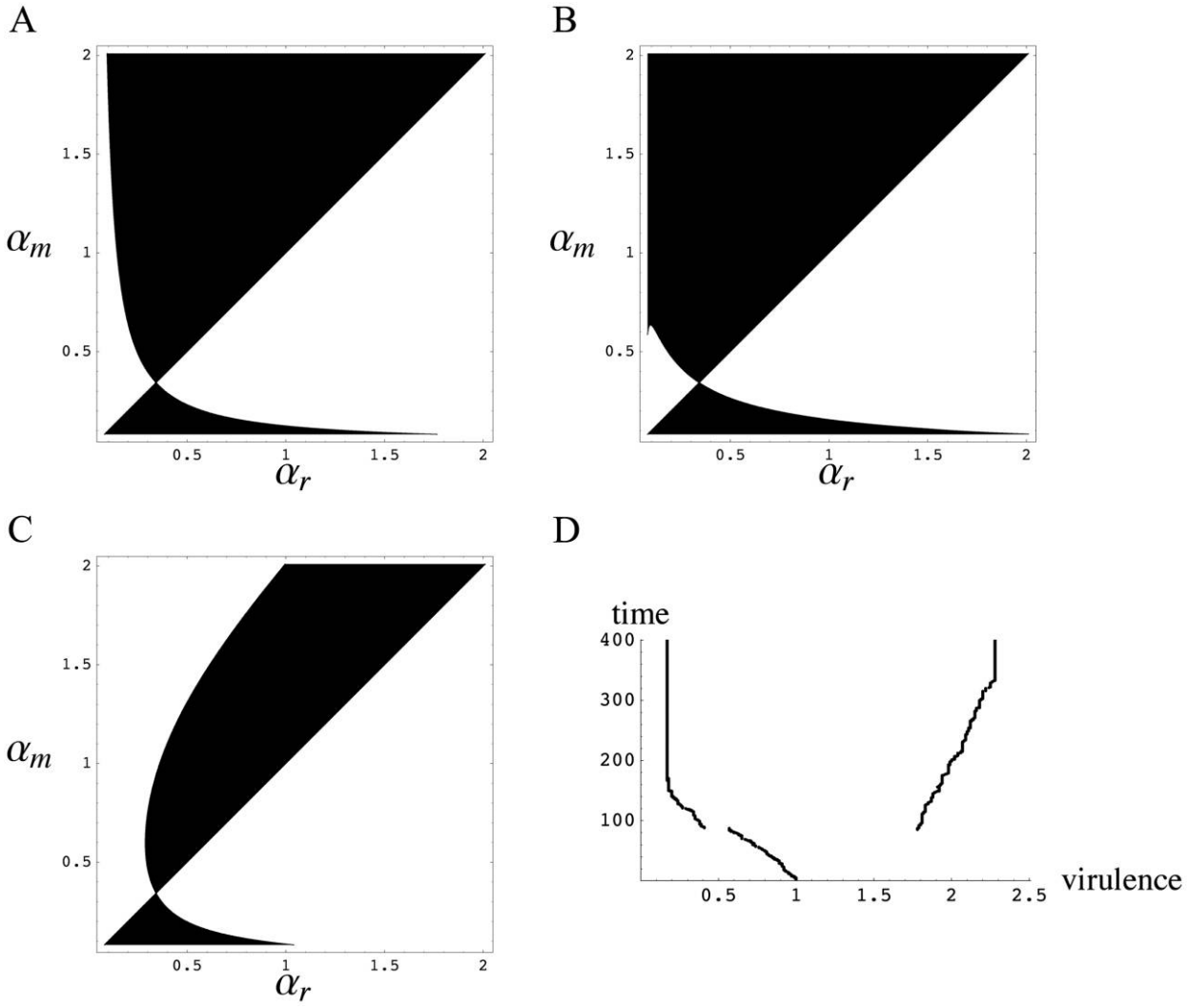

Figure 3: Pairwise invasibility plots with a transmission-virulence trade-off for average (*A*), high (*B*), and low (*C*) overall virulence, and evolutionary dynamics of the virulence trait for low overall virulence (*D*). Parameter values are $a = 1$, $s = 1$, $t = 0.01$, $r = 0.1$, and $\mu = 0.02$. In *D*, mutation rate is 10^{-5}, and the mutation step is 0.01 for small mutations. For further details, see figure 2.

figures are similar to the case without a trade-off except that now there is an evolutionarily singular strategy, or ESS (for $\alpha \approx 0.35$). This ESS is locally stable because less virulent and slightly more virulent strains cannot invade. However, as in the case without a trade-off, the ESS can turn into a branching point when multiple infections decrease virulence (function f_3) if large mutations are allowed (fig. 3*C*). In figure 3*D*, the mutation size is set to 0.01, but 10% of the mutation may have a large effect, and their size is between 0.1 and 4. We see that a strain converges toward the ESS, but it coexists with highly virulent strains that arise through rare mutation events. Note that in this system, there can be only one highly virulent strain. More precisely, the two strains that may coexist are the one that is closest to the ESS and the one that is farthest from the ESS.

Discussion

Multiple infections are known to affect virulence evolution (Read and Taylor 2001). However, epidemiological studies tend to overlook the problems raised by the definition of overall virulence in coinfected hosts. Also, most within-host models of coinfection ignore epidemiological feedbacks. Several experimental studies have shown that hosts carrying diverse infections may experience less detrimental

effects (al Yaman et al. 1997; Smith et al. 1999; Schuttler et al. 2002; Hood 2003; Massey et al. 2004; Schürch and Roy 2004). This could be due to an absence of cooperation (Brown et al. 2002) or even to direct competition between parasite strains (Massey et al. 2004). Here, I study virulence evolution with an epidemiological model where I introduce the biology of coinfected hosts by comparing various functions of overall virulence (in accordance with experimental observations).

In addition to the expected evolutionary outcome (avirulence if there is no trade-off between virulence and transmission or intermediate virulence if there is a trade-off), I find that when multiple infections decrease overall virulence in the coinfected host, strains with a high virulence may emerge and persist in the population. In this case, the overall virulence experienced by a coinfected host is inversely proportional to the difference in virulence between the coinfecting strains (eq. [5]). In other words, if the two coinfecting strains have an intermediate virulence, coinfected hosts experience an intermediate overall virulence. On the other hand, if one of the strains has an intermediate virulence and the second strain is very virulent, the overall virulence will be low. A highly virulent strain thus kills its host very quickly but may persist through coinfected hosts (fig. 2*D*, *green line*).

If multiple infections do not decrease the overall virulence, there is no coexistence. An interesting feature of this model is that a highly virulent strain cannot survive alone in the system: it needs a less virulent strain to create a reservoir of coinfected hosts. In a way, the mild strain specializes in exploiting susceptible hosts, while the highly virulent strain specializes in exploiting already infected hosts. One could say that the highly virulent strain acts as a parasite of the mild strain. A similar result is described by Alizon and van Baalen (2008) but in an approach where the overall virulence experienced by coinfected hosts is ignored (the first strain to infect the host determines the overall virulence).

This coexistence result is particularly interesting because with the coinfection framework used here (that of van Baalen and Sabelis 1995), evolutionary coexistence of strains is supposed to be difficult (see app. C). It is only the decrease in overall virulence that allows highly virulent strains to persist. This coexistence is similar to what is observed in models of superinfection (when strains cannot share a host). Thus, taking into account the effect of multiple infections on overall virulence could help to link frameworks of co- and superinfections.

A limitation of this model is that the transmission function is not affected by multiple infections. If the virulence of a highly virulent parasite decreases during a coinfection, one might expect its transmission rate to decrease as well. Here, if the decrease in transmission is proportional to the decrease in virulence, the advantage of the highly virulent strain vanishes. However, if the decrease in transmission is less pronounced (e.g., if it is only proportional to the square root of the difference in virulence between the strains), I find qualitatively similar results. Such a concave relationship between virulence and transmission does not seem unreasonable because it actually corresponds to the assumption classically made in most models of virulence evolution: virulence has to increase faster than transmission for there to be an ESS (Bull 1994; van Baalen and Sabelis 1995; Alizon and van Baalen 2005). A way to overcome this limitation could be to use a biological system, such as the bacteriocin-producing bacteria, to quantify both the decrease in virulence due to the coinfection and the fitness of the coinfecting strains.

Another possible improvement to this model would be to decouple the virulence and the relatedness of the coinfecting strains. By assuming that these two traits are coded by different loci, one could study the effect of the linkage disequilibrium between these two traits on virulence evolution. This would also allow one to build an even more realistic overall virulence function that would depend on the virulence of the coinfecting strains and on their relatedness. A possible way to address this question could be to use an embedded model where the overall virulence stems from the interactions between parasite strains and the immune system inside the host (for other embedded models with multiple infections, see Alizon and van Baalen 2008; Boldin and Diekmann 2008).

The main implication of this study is that the epidemiology needs to be taken into account carefully to understand the evolution of virulence when there are multiple infections. Van Baalen and Sabelis (1995) already identified an epidemiological feedback that forbids too virulent strains from taking over a host population (these strains win the within-host competition but kill their host too quickly when they are alone). Here, I show that the ecological characteristics of the coinfected host may have profound implications on virulence evolution. This study brings new insights to the current debate on the effect of multiple infections on virulence evolution. Even if multiple infections favor the less virulent clone in a coinfection (Brown et al. 2002), it does not necessarily imply that the less virulent strain will be favored in the host population. Experiments focusing on virulence evolution usually study a coinfected host in isolation. Finding a way to combine epidemiological feedbacks with experimental data is still an open problem.

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APPENDIX A

The Epidemiological System

The equations of the system without the mutant are

$$\frac{dS}{dt} = r(S + I_r + D_{rr}) - \lambda_r S - \mu S, \tag{A1}$$

$$\frac{dI_r}{dt} = \lambda_r S - \lambda_r I_r - (\mu + \alpha_r) I_r, \tag{A2}$$

$$\frac{dD_{rr}}{dt} = \lambda_r I_r - (\mu + \alpha_{rr}) D_{rr}, \tag{A3}$$

where α is the virulence, β is the transmission rate, r is the host reproduction rate, μ is the host natural death rate, and λ_r is the force of infection of the resident given by

$$\lambda_r = \beta_r I_r + \beta_{rr} D_{rr}. \tag{A4}$$

Note that in addition to susceptible (S) and infected (I_r) hosts, we also consider hosts doubly infected by the resident (D_{rr}). As underlined by van Baalen and Sabelis (1995), this is done to avoid giving an advantage to rare mutants. In other words, here, both the resident and the mutant can infect already infected hosts.

Here, I study microparasites (i.e., viruses, bacteria) and thus assume that doubly infected hosts behave like singly infected hosts ($\alpha_{rr} = \alpha_r$ and $\beta_{rr} = \beta_r$). Furthermore, I also assume that there is no relationship between virulence and transmission (adding that it does not affect the results) and that all strains have the same transmission β. Concerning the transmission rate in coinfected host, it is important to set $\beta/2$; otherwise, hosts doubly infected by the same strain would see their transmission rate doubled.

APPENDIX B

Alternative Overall Virulence Function (f)

Since the overall virulence function fI introduced in equation (5) is not centered in $\alpha_r = \alpha_m$, I also checked the results using the following function:

$$f_4(\alpha_r, \alpha_m) = \frac{\alpha_r + \alpha_m}{2} \exp\left(-\frac{|\alpha_r - \alpha_m|}{s}\right). \tag{B1}$$

This function is discontinuous because of the absolute value. Also, from a biological point of view, the sharpness of the peak of the function (fig. B1) is not satisfying because we expect that when parasites with similar virulence coinfect a host, the overall virulence will be close to that of the parasites. Finally, by studying separately the cases where $\alpha_r < \alpha_m$ and $\alpha_m \leq \alpha_r$, one can show that this function has a maximum in $\alpha_m = \alpha_r$ as long as $s \leq \alpha_r + \alpha_m$ when $\alpha_m > \alpha_r$. However, problems occur when $s \geq \alpha_r + \alpha_m$, that is, when virulence values are low. This is why this case is not discussed in the main body of this article.

Figure B2 shows that with this new function, the result for the case without a trade-off is very similar to figure 2 except that the black strip is larger here. For the case with a trade-off, however, the PIP looks slightly different (see fig. B2*B*). There now exists a small range of virulence values that always allow a mutant to invade. This occurs because f_4 is not continuous when $\alpha_r = \alpha_m$. More precisely, as stated above, if $s \leq \alpha_r + \alpha_m$, then when $\alpha_m > \alpha_r$, f_4 does not decrease anymore but instead increases. This affects the sign of the invasion fitness.

The evolutionary dynamics corresponding to figure B2*B* are in figure B3. Note that with overall fitness function f_4, the exact dynamics of the trait depend on the starting point (figure not shown).

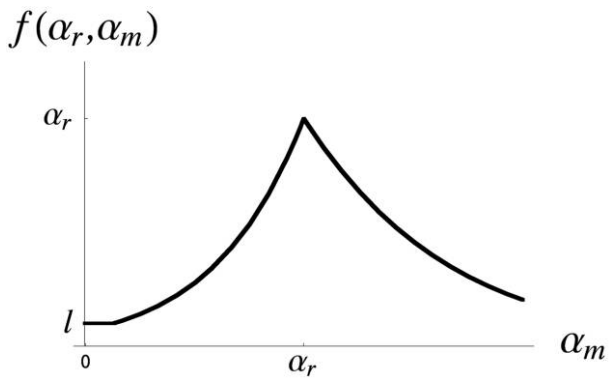

Figure B1: Overall virulence in a coinfected host. The first strain has a virulence α_r, and the second strain has a virulence α_m. The function is centered in $\alpha_m = \alpha_r$, but it is not symmetric. Parameter values are $s = 1$.

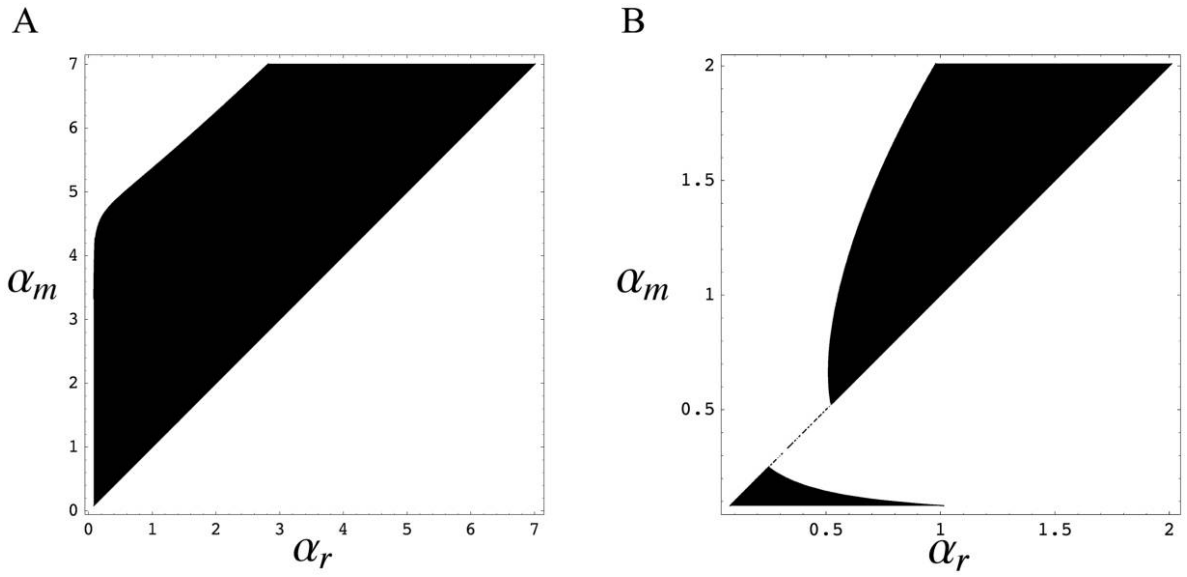

Figure B2: R_0 of a mutant parasite (α_m) in a population infected by a resident parasite (α_r) in a case without (*A*) or with (*B*) a transmission-virulence trade-off. In *A*, parameter values are identical to those in figure 2, and in *B*, they are identical to those in figure 3.

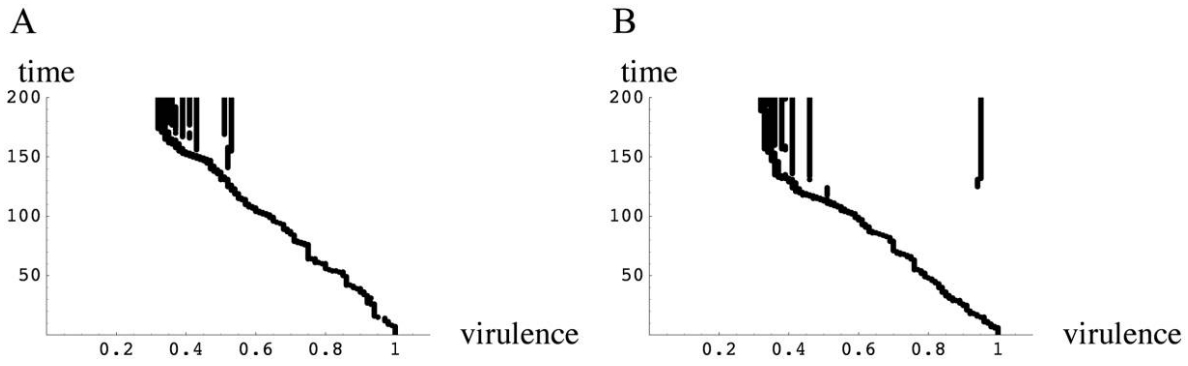

Figure B3: Evolutionary dynamics of the trait with small mutations (*A*) and with occasional large mutations (*B*). Parameter values are $a = 1$, $s = 1$, $t = 0.01$, $r = 0.1$, and $\mu = 0.02$.

APPENDIX C

Comparison between Coinfection Models

There are two broad classes of coinfection models. In the first class, a host can be infected twice by the same strain (van Baalen and Sabelis 1995). In the second class, a host can be coinfected only by different strains (e.g., May and Nowak 1995; Mosquera and Adler 1998). This latter class favors parasite coexistence because, as discussed in the main text, a rare mutant always has the advantage of being able to coinfect hosts.

To illustrate the difference between these two models, I compute the R_0 for a rare mutant in both these cases and plot the corresponding PIP (fig. C1*A*, C1*B*). Note that I assume a trade-off between virulence and transmission (eq. [6]). Figure C1*C* shows the evolutionary dynamics when the initial population is polymorphic (virulence values of the strains are 0.3 and 0.6). In the case where hosts can be coinfected twice by the same strain, one of the strains disappears, and the other reaches the ESS (which corresponds to the PIP in fig. C1*A*). In the case where coinfections are always diverse, many strains coexist in the system. This is consistent with the pattern observed on the PIP (fig. C1*B*). In a future study, I will try to understand in further details the basis of the difference between these two coinfection models.

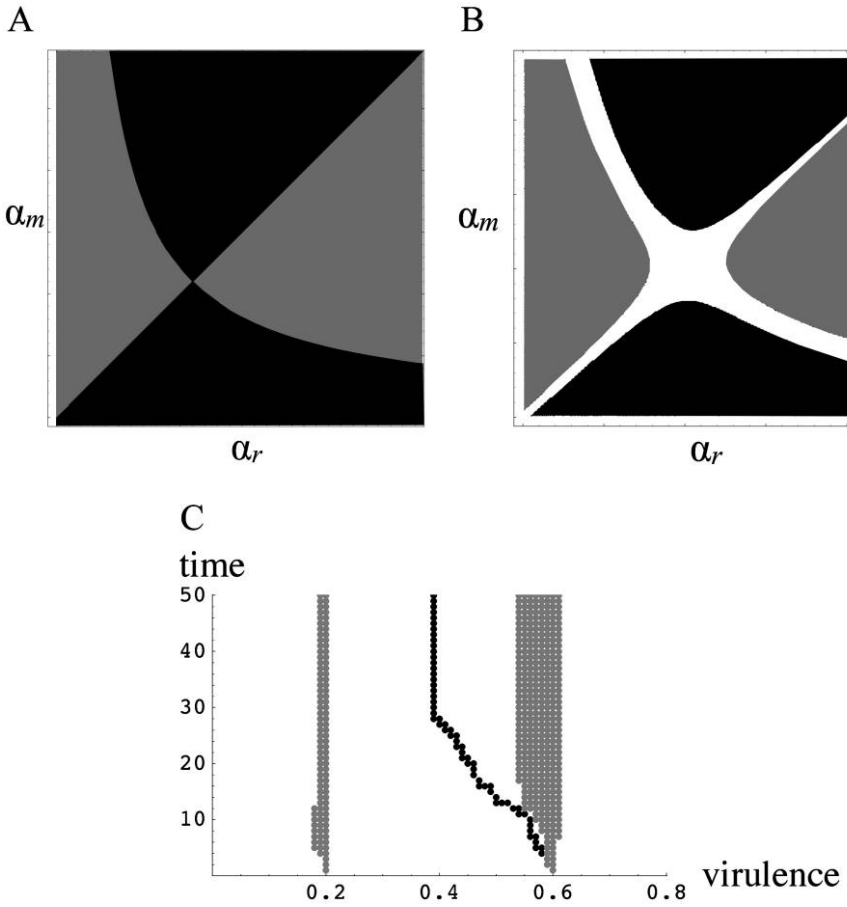

Figure C1: Pairwise invasibility plots with (*A*) and without (*B*) hosts coinfected by the same strain. In the black regions, the mutant can invade and replace the resident; in gray areas, it is the opposite; and in white areas, both the mutant and the resident can invade each other (which implies coexistence). The evolutionary dynamics are shown in *C*; case *A* is in black, and case *B* is in gray. Parameter values are $r = 0.25$ and $\mu = 0.2$.

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