

# Multiple Infections, Immune Dynamics, and the Evolution of Virulence

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**ABSTRACT:** Understanding the effect of multiple infections is essential for the prediction (and eventual control) of virulence evolution. Some theoretical studies have considered the possibility that several strains coexist in the same host (coinfection), but few have taken their within-host dynamics explicitly into account. Here, we develop a nested approach based on a simple model for the interaction of parasite strains with their host's immune system. We study virulence evolution by linking the within-host dynamics to an epidemiological framework that incorporates multiple infections. Our model suggests that antigenically similar parasite strains cannot coexist in the long term inside a host. We also find that the optimal level of virulence increases with the efficiency of multiple infections. Finally, we notice that coinfections create heterogeneity in the host population (with susceptible hosts and infected hosts), which can lead to evolutionary branching in the parasite population and the emergence of a hypervirulent parasite strategy. We interpret this result as a parasite specialization to the infectious state of the hosts. Our study has experimental and theoretical implications in a virulence management perspective.

**Keywords:** multiple infections, virulence, evolution, immune system, embedded models, evolutionary branching.

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If parasites need their hosts to survive, why do they harm them? Anderson and May (1982) suggested that parasites harm their hosts because they cannot avoid it. When a parasite cannot reproduce without harming its host, transmission and virulence are linked in a trade-off relationship. Subject to such a trade-off hypothesis, parasites should adopt a prudent host exploitation strategy leading to an intermediate but definite level of virulence (Bremermann and Pickering 1983; Ewald 1983; Nowak and May 1994; van Baalen and Sabelis 1995a). However, such prudent host exploitation results only if infected hosts are exploited by a single clone of parasites. From the moment the host harbors two (or more) parasite strains, prudent exploitation may no longer be the optimal strategy, for instance, because more virulent strains tend to win the within-host contest (Levin and Pimentel 1981; Nowak and May 1994; van Baalen and Sabelis 1995a). This is similar to what in game theory is known as the “Prisoner’s Dilemma”: whatever the strategy of the other strain, defecting (i.e., over-exploiting the host) always leads to the highest payoff (Frank 1996; Turner and Chao 1999). Thus, many studies predict that multiple infections should increase parasite virulence (van Baalen and Sabelis 1995a; Frank 1996; Gandon et al. 2001).

*Virulence and multiple infections.* Experimental support for the prediction that multiple infection increases virulence is equivocal. In some studies, more virulent strains were favored (Ebert and Mangin 1997; Escriu et al. 2000; Davies et al. 2002; Cooper and Heinemann 2005; de Roode et al. 2005b), while in others, less virulent strains had the upper hand (Turner and Chao 1999; Read and Taylor 2001; Gower and Webster 2005; Harrison et al. 2006). In a number of studies, the results again depended on the type of host (de Roode et al. 2004; Hodgson et al. 2004), the order of arrival of the strains (Paul et al. 2004; de Roode et al. 2005a), and the mode of transmission of the parasite (Vizoso and Ebert 2005).

Pointing out these diverging results, several authors argue that multiple infections do not in fact select for raised virulence. This could be the case because multiple infections decrease the relatedness among coinfecting parasites,

which is likely to decrease levels of parasite cooperation (Hamilton 1972; Frank 1996; Turner and Chao 1999; Brown et al. 2002). Similar decreases in virulence could also be due to epidemiological feedbacks (Gandon et al. 2001). This debate is all the more important because concomitant infections by several genotypes or even by several species appear to be the rule rather than the exception (Gilbert et al. 1998; Petney and Andrews 1998; Lord et al. 1999; Cox 2001; Read and Taylor 2001).

Another source of confusion is that the term “virulence” typically stands for both a parasite’s aggressiveness and its effect on the host (harm done). That is, the virulence is a trait of the parasite, but its value is a demographic parameter of its host. This confusion becomes problematic when hosts are infected by more than one strain (or species) of parasites: how can these parasites possibly have different virulences? In reality, disease-induced mortality (or more generally, harm done to the host) is a compound trait, affected by traits of the host and all of the parasites it is harboring. Multiple infections may be more harmful than the most aggressive of the component infections; they can also be as harmful as the most aggressive strain and sometimes actually less harmful than the any of the component strains alone (Cox 2001; Alizon 2008). However, in this article we are interested in knowing which strain is favored when coinfections occur and what will be the consequences for the host. We will characterize parasite strains by their within-host growth rate, which has been shown to be correlated with the effect on host mortality in singly infected hosts (Day et al. 1993; Mackinnon and Read 1999). For multiply infected hosts, we will assume that the first strain to infect a host will be numerically dominant and will determine the host’s mortality—but this does not exclude that subsequent strains with higher growth rates do relatively better. What evolves, in our model, is parasite within-host growth rate; host mortality (virulence) is a consequence.

*Coinfections or superinfections?* Among the first to focus on the evolutionary consequences of multiple infections were Levin and Pimentel (1981). They studied a model with a virulent strain and an avirulent strain, where the virulent strain can take over hosts infected by the avirulent strain (this is called *supeinfection*, as described below). They show that if the cost of being virulent is greater than the superinfection parameter, a mixture of virulent and less virulent strains may circulate in the host population. Such coexistence leads them to the conclusion that evolution favors intermediate levels of virulence. Analyzing a multistrain variant of Levin and Pimentel’s model, Nowak and May (1994) show that the so-called superinfection hypothesis can even lead to the fragile coexistence of many different strains inside the host population.

A very different conclusion is reached by van Baalen

and Sabelis (1995a). In analyzing a model that is very similar to the one analyzed by Levin and Pimentel (1981) and Nowak and May (1994) but that differs in the crucial aspect that parasites do not replace one another inside a host but coexist indefinitely, they show that under coinfection, only one virulence strain will predominate. Under coinfection, all the strains in a host can get transmitted, while under superinfection, only the most virulent strain transmits.

This contrast shows that the precise mechanism of within-host competition has important consequences for the epidemiological and evolutionary outcome of the infection. The coinfection and superinfection approaches to multiple infection both make strong assumptions about the timescales of within-host processes relative to host demography and epidemiology. The superinfection model results arise when within-host dynamics are infinitely fast; the coinfection model assumes that infections establish quickly but the replacement of one strain by another is infinitely slow. Reality, as usual, is likely to be in between these extremes, but the structured population models that are needed to describe it are difficult to analyze (Metz and Diekmann 1986).

While it is easy to argue that the establishment of a first infection is a fast process, it is not so straightforward to extend this argument to the interaction between multiple strains within a host. Fast replacement may occur if the strains differ strongly, but it becomes more unlikely the more that the strains resemble one another. This means in particular that the superinfection hypothesis is problematic in evolutionary approaches. Usually mutations are assumed to have small effect, which means that mutants cannot oust the original strain in a superinfection manner.

Earlier, we have shown that the equilibrium of the within-host interactions may explain the emergence of a trade-off at the level of the epidemiological parameters (Alizon and van Baalen 2005). This analysis, however, was based upon the assumption that multiple infections do not occur. Taking these multiple infections into account fundamentally affects within-host processes (particularly the functioning of the immune system) and thus modifies virulence evolution. Here we extend our previous study by formulating and analyzing an embedded model for the evolution of parasite virulence that incorporates multiple infection.

*A nested approach.* Most models for multiple infections do not take within-host dynamics into account, and in addition, they make two important assumptions: they involve arbitrary trade-offs between parasite transmission and virulence, and they assume multiple infections result in either coinfection or superinfection (but see Mosquera and Adler [1998], who incorporate both coinfection and superinfection and study multiple trade-off relationships).

To avoid making strong assumptions with respect to the timescales associated with multiple infections, we explicitly model within-host dynamics of multiple strains of the parasite and their interaction with the immune system. The outcome of multiple infections (coinfection, superinfection, or something in between) thus depends on the interaction between parasite strains and the lymphocyte clones that mediate the immune response. Modeling within-host dynamics has a second advantage: we do not have to define an arbitrary trade-off between transmission and parasite-induced host mortality because a convex trade-off emerges robustly from the interaction of the parasite with the immune system (Alizon and van Baalen 2005).

Another advantage of such embedded models is that they allow to model the immune response, which is particularly important in case of multiple infections. In ecology, it is well known that a predator that feeds on several prey species may induce what is called “apparent competition” between these prey species (Holt 1977). A similar process is likely to appear inside the host if there is cross immunity, that is, if some lymphocytes recognize more than one parasite strain (Read and Taylor 2001). Apparent competition may have important consequences. For example, just as a top predator may favor coexistence of multiple prey species (Paine 1969), the immune system could permit parasite coexistence within the host (as shown experimentally by Råberg et al. 2006). Note that in the early stages of an infection, the cross immunity is high since most defense mechanisms are not specific.

Finally, van Baalen and Sabelis (1995a) showed that if multiple infections are allowed in the system, an individual-based optimization model is not sufficient to study virulence evolution. The epidemiology must be considered because a strategy that maximizes the parasite’s fitness locally (in a host) can be nonoptimal for the same parasite at a larger scale (in the whole host population). It is very likely that within a host, more rapidly growing parasites are favored, but at a higher level, a parasite cannot maintain itself in the host population if it kills its hosts too rapidly. This is why we use an embedded model (Ganusov et al. 2002; Gilchrist and Sasaki 2002; André et al. 2003; Alizon and van Baalen 2005; Gilchrist and Coombs 2006) that links the within-host level (the parasite strains a host harbors) and the epidemiological scale (a host population). It is worth noticing that all these previous models did not address the question of multiple infections.

### The Within-Host Model

In the model, the immune response is mediated by lymphocytes that attack the parasite within the body. A vertebrate host has a large array of such lymphocyte clones

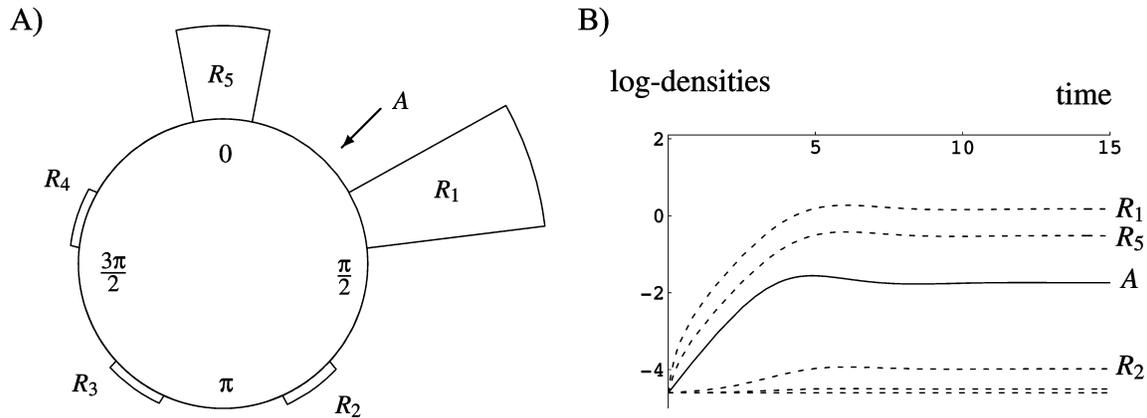
that each can recognize a specific nonself antigen. A microparasite such as a virus typically is recognized and attacked by only a small subset of these lymphocytes. Whenever a lymphocyte encounters an antigen that it recognizes, it will start to reproduce and thus mount an immune response. While the actual clearance may be carried out by other components, the relevant aspect of the mounting of the immune response is that it gives rise to a rapid proliferation of cells that recognize the parasite (for further details, see Frank 2002).

We model the antagonistic relationship between  $n_c$  lymphocyte clones (whose within-host densities are denoted  $y_j$ ) and  $n_p$  microparasite strains (with densities  $x_i$ ). Parasites are distinguished by their growth rates and their antigens, which we assume are independently genetically determined traits. For simplicity, in this study, lymphocyte clones differ only in the receptors that recognize the antigens.

If more than one strain is simultaneously present in the host, defining the immune system and particularly its specificity is an important step in the model formulation. The few models that allow within-host heterogeneity (Nowak et al. 1990; Hellriegel 1992; McLean and Nowak 1992) assume either no cross immunity at all or that a new parasite strain elicits, next to a purely specific response, a general “nonspecific” response. The assumption that specific recognition is perfect has the important consequence that whenever a mutant parasite arises, even if it is very similar to the resident, it will be recognized by a different lymphocyte clone and not at all by the clone that recognizes the resident. However, it seems very unlikely that a mutant would not be recognized at all by lymphocytes targeting the resident. At least some cross reactivity should occur. Indeed, as Pradeu and Carosella (2006) suggest from empirical evidence, continuity in the antigen-receptor interaction seems more appropriate than all-or-nothing recognition to explain immune system functioning.

Our model assumes that the interaction between parasite and immune system is based on an antigen-receptor mechanism with imperfect specificity. That is, we suppose that each lymphocyte clone carries a distinct receptor that recognizes different antigens with different affinities. Thus, different strains of parasites will differentially activate different clones of lymphocytes. The recognition efficiency of a parasite strain by a specific lymphocyte clone depends on the molecular differences between the receptor and the antigen (for further details on modeling an antigen-receptor interaction, see De Boer and Perelson 1995). Our hypothesis is less rigid than the all-or-nothing hypothesis and is consistent with crystallography studies that show that an antibody has different conformations and can recognize different classes of antigens (James et al. 2003).

It is of course an oversimplification to reduce the parasite’s immune characteristics to a unique antigen value



**Figure 1:** Example of an antigen-receptor interaction in a periodic antigen space. *A*, Example of equidistant distribution of  $n_c$  lymphocyte clone receptors  $R_j$  (here  $n_c = 5$ ) when antigen space is taken to be a circle. Boxes reflect the equilibrium intensity of activation of each receptor given the antigen value (arrow). *B*, Within-host dynamics of lymphocyte clones and a unique parasite strain (with an antigen  $A$ ). The solid line indicates the parasite log density; dotted lines indicate the log densities of the lymphocyte clones. *A*, *B*, parameter values are  $A = \pi/4$ ,  $\varphi = 1$ ,  $n_c = 5$ ,  $\delta = 1$ ,  $b = 0.01$ ,  $\sigma_0 = 1$ ,  $\omega = 0.75$ , and  $\eta = 10$ .

since a parasite typically expresses a “cocktail” of antigens. But, experimental data show that only a few of the parasite’s antigens are actively recognized by the immune system, which leads to the activation of very few lymphocyte clones, a phenomenon called “immunodominance” (Frank 2002).

To describe the within-host dynamics, we need an equation for each of the  $n_p$  parasite strains and for each of the lymphocyte clones  $n_c$ :

$$\begin{aligned} \frac{dx_i}{dt} &= \left( \varphi_i - \sum_{j=1}^{n_c} \sigma_{ij} y_j \right) x_i, \\ \frac{dy_j}{dt} &= b + \sum_{i=1}^{n_p} c_{ij} x_i - \delta y_j, \end{aligned} \quad (1)$$

where  $\varphi_i$  is the within-host growth rate of parasites of strain  $i$ ,  $\sigma_{ij}$  is the efficiency of destruction of parasites of strain  $i$  by the lymphocytes of clone  $j$ , and  $c_{ij}$  is the increase of lymphocytes clone  $j$  production due to parasite strain  $i$ . For the sake of simplicity, we suppose that all lymphocyte clones have the same baseline production rate  $b$  and the same death rate  $\delta$  (table A1 defines all symbols used in this article). For a more extensive discussion of the within-host model, we refer to Alizon and van Baalen (2005). In this model, we assumed for simplicity that lymphocyte proliferation does not saturate, but our main results remain qualitatively similar with a saturating function (see app. D).

### Antigenic Determinants

We denote the antigen carried by parasites of strain  $i$  as  $A_i$  and the receptor of lymphocyte clone  $j$  as  $R_j$ . Antigen and receptor are likely to be high-dimensional traits (as they depend on genetic information along stretches of DNA), but for simplicity we will assume they are characterized by a single value. Here, we adopt an approach similar to the one developed by Gog and Grenfell (2002). To avoid boundary effects, we represent antigen space by a circle; that is, both  $A_i$  and  $R_j$  have values between 0 and  $2\pi$ . Even with this simplifying assumption, we are faced with the problem of how clones are distributed along the “antigen space”  $[0, 2\pi]$ . Clones can be distributed randomly or not on the circle. With a homogeneous distribution,  $R_j = j2\pi/n_c$  (fig. 1A).

We suppose recognition efficiency is a function of the distance between the values of antigen and receptor in antigen space,  $|R_j - A_i|$ . That is, the closer the parasite’s antigen is to the value maximally recognized by a lymphocyte’s receptor, the more strongly the lymphocyte is stimulated. We suppose that the recognition efficiency of antigen  $A_i$  by a lymphocyte clone expressing receptor  $R_j$  is proportional to

$$e^{-(R_j - A_i)^2/\omega^2},$$

where  $\omega$  is the accuracy of antigen detection. Note that when  $\omega$  increases, a parasite stimulates more lymphocyte clones (which means the lymphocyte clones are less specific).

We now have to work out the parasite destruction efficiency ( $\sigma_{ij}$ ) from the contributions of all lymphocyte clones. To account for the assumption of a periodic antigen space (see fig. 1A), we approximate killing rate by a weighted sum of three terms:

$$\sigma_{ij} = \frac{\sigma_{\max}}{3} \left( e^{-(R_j - A_i)^2 / \omega^2} + e^{-(R_j - A_i + 2\pi)^2 / \omega^2} + e^{-(R_j - A_i - 2\pi)^2 / \omega^2} \right), \tag{2}$$

where  $\sigma_{\max}$  is a constant.

This model has many parameters, which renders a complete analysis difficult. To obtain interpretable results, we make an additional hypothesis to link some of those parameters. This concerns the stimulation efficiency of the lymphocytes  $\sigma_{ij}$  and their proliferation rate  $c_{ij}$ . These, we assume, are directly linked:

$$c_{ij} = \eta \sigma_{ij}, \tag{3}$$

where  $\eta$  is a constant.

We have to choose default values for all the other parameters. As in Alizon and van Baalen (2005), we try to use empirical values, but we also rely on parameter values used in the literature (detailed in app. C). A problem that remains is how to choose values for parameters  $\omega$  (accuracy of antigen detection) and  $\eta$  (coupling constant between  $c_{ij}$  and  $\sigma_{ij}$ ). These parameters are governed by a level of the functioning of the immune system that is difficult to assess; the parameter values we choose are arbitrary, but we will carry out a sensitivity analysis later on.

### Long-Term Coexistence

Following van Baalen and Sabelis (1995a), we limit to two the maximum number of parasite strains within a host, for mathematical tractability. This allows us to incorporate the main characteristics of multiple infections while simplifying the analysis. To keep notations simple, we always suppose that one of the parasite strains is a resident (dominant in the global parasite population, denoted r) and that the other strain is a (globally) rare mutant (denoted m).

We first analyze the within-host equilibrium to understand when coexistence of mutant and resident parasite strains is possible within a host over the long term. In this case, adaptive immunity will be the dominant response. If we suppose that a host is already infected by the resident, a subsequent infection with the mutant can lead to three different outcomes over the long term: the mutant cannot invade and disappears, the mutant and the resident coexist within the host, and the mutant replaces the resident. The

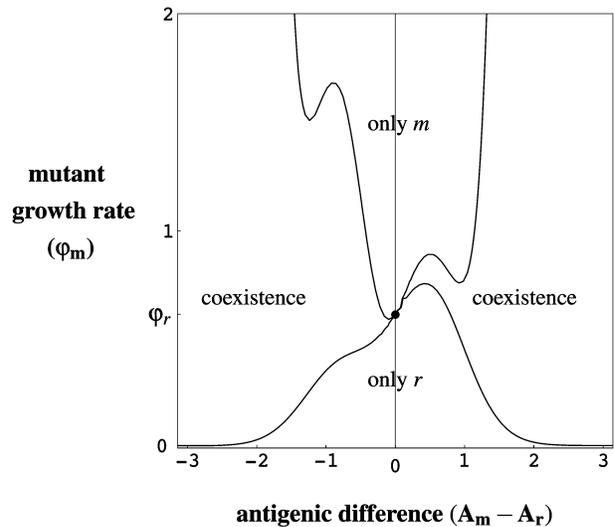
outcome depends on the growth rates and antigen values of the mutant, denoted  $\{\varphi_m, A_m\}$ , and the resident,  $\{\varphi_r, A_r\}$ .

Figure 2 shows that if the mutant and the resident are antigenically close, there is little possibility for long-term coexistence; apparent competition is too strong. The more the strains differ antigenically, the more readily they coexist. When the parasite strains are antigenically identical, the strain with the largest growth rate takes over the host (as observed by Bonhoeffer and Nowak [1994]). Note that the shape of the boundary is due to our simplifying assumption in the definition of  $\sigma$  (eq. 2).

Another result illustrated by figure 2 is produced when the antigen of the resident strain is not optimal. In this figure,  $A_r$  is  $\pi/4$ , but the optimal antigens are  $\pi/5, 3\pi/5$ , and so forth because we assume a homogeneous distribution of lymphocyte clones (fig. 1). Thus, a mutant strain that has the same growth rate as the resident but an antigen closer from an optimal value can take over the host.

Finally, figure 2 shows that mutant parasites with a lower growth rate than that of the resident may coexist in the long term with a host's resident parasite as long as their antigens are different enough. This is because in our model, parasites with very different antigens have essentially independent dynamics.

This analysis indicates whether coexistence within a host



**Figure 2:** Attracting within-host equilibria of the resident and mutant parasite strains as a function of their antigen ( $A_i$ ) trait values and their growth rates ( $\varphi_i$ ). A mutant parasite strain (m) arrives in a host already infected by a resident strain (r) whose characteristics are  $\{\varphi_r, \pi/4\}$ . Note that with the given lymphocyte distribution (fig. 1A), the resident does not have an optimal antigen. The mutant indicated by the dot is indistinguishable from the resident. Except for  $\varphi_r$ , parameter values are identical to those of figure 1B.

is possible, but it gives no information on parasite coexistence in the host population. We also insist that this coexistence graph indicates that what happens over the long term may not be relevant if, for instance, the parasite is able to change its antigenic determinant ( $A_i$ ). Finally, even if it does not appear on this graph, a mutant and a resident with close antigenic values will always coexist on the short term because none has a great advantage. The time it takes for one strain to oust the other may by far exceed the lifetime of the host.

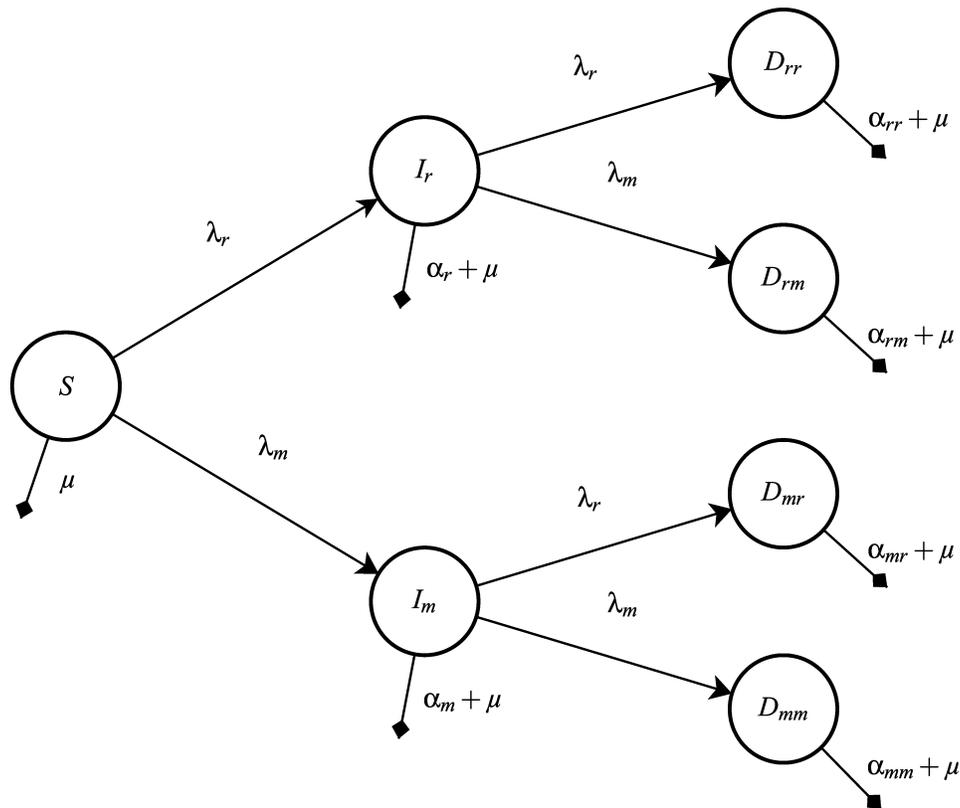
### A Multistrain Epidemiological Model

To understand the effect of multiple infection on the evolution of virulence, we need to model epidemiological dynamics as well. We use a classical susceptible-infected (SI) model, which is summarized in figure 3 (see also app. C); there is no vertical transmission of the parasite and no recovery (infections are persistent). Our model is similar to that of van Baalen and Sabelis (1995a), with the difference that here the order of arrival of the parasite strains

within a host is considered explicitly (this order is likely to affect the outcome of the infection, as several studies suggest; Read and Taylor 2001; Hood 2003; de Roode et al. 2005b).

Here we link the epidemiological parameters that characterize the hosts (the various  $\alpha_i$  and  $\beta_i$  values) to the state of the dynamics within the host. Since parasite-induced mortality ( $\alpha$ ) and transmission ( $\beta_i$ ) both depend on the within-host growth rates of the parasites ( $\varphi_i$ ), a link between within-host competition and virulence automatically results. This issue was discussed by Read and Taylor (2001), and more recent studies on mixed infections of *Plasmodium chabaudi* have shown a positive correlation between virulence and within-host competitive ability (de Roode et al. 2005b; Bell et al. 2006). Note that if successful exploitation of a host requires cooperation among the parasites (in which case just the growth rate does not suffice to characterize the parasite strains), within-host interactions may be more complex than the question of who grows fastest (Turner and Chao 1999; Brown et al. 2002).

We calculate parasite-induced host mortality from the



**Figure 3:** State transitions in a multistrain model. Healthy hosts ( $S$ ) may first become singly infected ( $I_r$  and  $I_m$ ) and then doubly infected ( $D_{rr}$ ,  $D_{mr}$ ,  $D_{rm}$ , and  $D_{mm}$ ). Arrows indicate infection events; squares indicate death events. Baseline host mortality ( $\mu$ ) is 0.02. All hosts reproduce equally at a rate  $\rho = 0.05$ ;  $\lambda$  is the force of infection,  $\mu$  is the host natural death rate, and  $\alpha$  is the parasite-induced host mortality.

assumption that the negative effects experienced by the host are proportional to the overall replication rates of the parasites ( $\varphi_i x_i$ ) and to lymphocyte densities ( $y_j$ ), to incorporate immunopathological phenomena. To make the model tractable, we assumed that the overall virulence is equal to the virulence of the first strain that infects the host. In mathematical form, for a host infected by strain  $i$  and then by strain  $j$ ,

$$\alpha_{ij} = u\varphi_i x_i + w \sum_j y_j, \quad (4)$$

where  $u$  represents the effect of replicating parasites and  $w$  the detrimental effect of the lymphocytes. These constants are assumed not to vary among the strains and the clones because what we are interested in is the effect of within-host dynamics. Note that the immune system is not only a cost for the host, even though it seems to appear only in the second term, because a strong immune response will lead to low parasite densities ( $x_i$ ), thus decreasing the host death rate.

Transmission is proportional to a strain's within-host density, that is, the transmission of strain  $g$  from a host infected by strains  $i$  and  $j$ :

$$\beta_g[ij] = ax_g, \quad (5)$$

where  $a$  is a constant and  $g$  is either  $i$  or  $j$ .

Under the assumption of within-host equilibrium, the various epidemiological parameters are straightforwardly calculated by substituting the within-host densities with their equilibrium values (Alizon and van Baalen 2005). What to do in the case where that is not possible (one parasite strain slowly replacing another) is discussed below.

Our purpose is to calculate the fitness of a mutant strain ( $m$ ) that appears in a host population infected by a resident strain ( $r$ ) at epidemiological equilibrium. We consider the population just after the appearance of a mutant, so that the mutant's presence has not yet affected population dynamics and epidemiology and the resident host-parasite interaction is still at equilibrium ( $S = S^*$ ,  $I_r = I_r^*$ , and  $D_{rr} = D_{rr}^*$ , where the asterisks indicate equilibrium densities). If the mutant is rare, we can also suppose that the probability for a mutant to infect the same host twice is negligible;  $D_{mm} = 0$ . For the sake of simplicity, we assume that hosts doubly infected by the same strain,  $D_{rr}$ , are identical in all aspects to singly infected hosts,  $I_r$ . This means that  $\alpha_{rr} = \alpha_r$  and  $\beta_r[rr] = \beta_r[r]$ .

Traditionally, microparasite fitness is taken to be the parasite's basic reproduction ratio  $R_0$ , that is, the number of new infections caused by an infected host in a susceptible population. Here, we cannot use this definition because the mutant invades a population already dominated

by another parasite. In addition, there is a complication due to the presence of different transmission routes (one route is via infecting healthy hosts and another is via already infected hosts). The easiest way to solve that problem is to shift to the level of the propagule that has just been released by a host (van Baalen and Sabelis 1995a). The propagule is the parasite form that colonizes new hosts. We then can define a more general expression of the  $R_0$  that is the number of new propagules one propagule will engender. If the  $R_0$  of a parasite strain is greater than unity, it is able to survive in the host population, whereas if  $R_0 < 1$ , it disappears.

#### *The Mutant's Basic Reproduction Ratio $R_0$*

Following van Baalen and Sabelis (1995a), we include the contributions of the two routes of transmission to calculate the basic reproduction ratio  $R_0$  of the mutant strain. Denote the transmission factor of hosts where the mutant comes first by  $B_s$  and the transmission factor of hosts where the mutant arrives second by  $B_l$ . Then, the  $R_0$  of this mutant strain can be written as

$$R_0 = B_s S^* + \varepsilon B_l I_r^*, \quad (6)$$

where  $\varepsilon$  is a factor representing the probability that an invading parasite successfully establishes an infection in an already infected host. We use multiple infection efficiency  $\varepsilon$  as a parameter to vary the impact of multiple infections, contrary to most studies that compare only cases with ( $\varepsilon = 1$ ) or without multiple infections ( $\varepsilon = 0$ ). In reality,  $\varepsilon$  might depend on the relative densities of resident and mutant parasites just after the second infection occurred, but for simplicity, we will assume it is constant. Note that  $\varepsilon$  can be greater than unity if the first infection facilitates further infections. Terms  $S^*$  and  $I_r^*$  are the density at equilibrium of susceptible hosts and hosts infected by the resident strain, respectively.

Transmission factors  $B_s$  and  $B_l$  depend on within-host dynamics. As discussed, classical approaches to multiple infection simplify within-host dynamics to the extreme, assuming either immediate takeover (superinfection) or the instantaneous establishment of a within-host equilibrium (coinfection). Embedded models permit a more realistic approach. For example, take a strain that invades a host already infected by another strain but that does so slowly because it is similar to the first. The first strain then does not "feel" the presence of the second strain for awhile. Thus, mortality of doubly infected hosts is (at least initially) strongly dominated by the virulence of the first strain. Also, its transmission is unaffected by the second strain. Yet the contribution of the host to the second strain may not be negligible, from the point of view of the second

strain. If infected hosts are sufficiently common, they represent a valuable resource even if the value per host ( $B_1$ ) is low.

Since we focus on the case where the resident and mutant are not very different, we can also assume that when the mutant is the first to infect a host, it will not be affected significantly when the host receives a second infection (with the resident). Thus, if the mutant parasite arrives first, its per-host transmission factor ( $B_s$ ) is well approximated by the transmission rate multiplied with the expected duration of the infection:

$$B_s = \frac{\beta_m}{\mu + \alpha_m}, \quad (7)$$

where  $\alpha_m$  is the host mortality due to the parasite,  $\beta_m$  is the transmission of the parasite, and  $\mu$  is the natural host mortality (recovery occurs at a constant rate and is included in the natural mortality term). The reverse, of course, is not true; a second strain invades a host that has its immune system activated by the first ( $\gamma = \tilde{\gamma}$ ), which strongly affects its replication rate.

If two mutant and resident strains resemble each other, a second infection will have a net within-host growth rate close to zero, implying that within-host competition is very low. Let  $\Delta\varphi$  be the difference between the growth rates of the mutant and resident, and let  $\Delta\sigma$  denote the antigenic difference between the parasite strains. Using a result obtained by Stollenwerk and Jansen (2003) we show (app. B) that the within-host dynamics of the mutant is approximated by

$$x_m(t) \approx x_0 e^{(\Delta\varphi - \sum_{j=1}^{n_c} \Delta\sigma \tilde{\gamma}_j)t}, \quad (8)$$

where  $t$  is the time the second strain is present in the host and  $x_0 = x_m(0)$  is the initial within-host density of the mutant.

To calculate the per-host transmission factor for this case, it is useful to introduce a survival function ( $F_s$ ) that depends on the host's natural death rate ( $\mu$ ) and on the resident parasite virulence ( $\alpha_r$ ), given by

$$F_s(t) = e^{-(\mu + \alpha_r)t}. \quad (9)$$

Using this definition, the per-host transmission factor  $B_1$ , proportional to all the propagules the parasite produced during the infection, can be written as

$$B_1 = \int_0^\infty F_s(t) a_m x_m(t) dt, \quad (10)$$

and we obtain

$$B_1 = \frac{a_m x_0}{\mu + \alpha_r - \Delta\varphi + \sum_{j=0}^{n_c} \Delta\sigma \tilde{\gamma}_j}. \quad (11)$$

Using the expressions for the per-host transmission factors, the mutant parasite's fitness can thus be expressed as

$$R_0 = \frac{\beta_m}{\mu + \alpha_m} S^* + \varepsilon \frac{a_m x_0}{\mu + \alpha_r - \Delta\varphi + \sum_{j=0}^{n_c} \Delta\sigma \tilde{\gamma}_j} I_r^*. \quad (12)$$

Note that the mutant's fitness depends explicitly on the state of the immune system in already infected hosts. We will use this expression to study the invasion fitness of mutants and find evolutionarily singular virulence strategies.

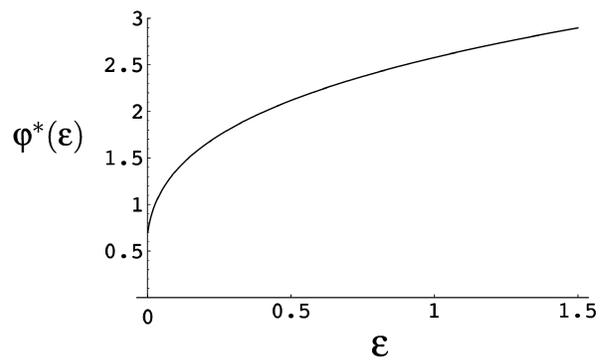
### The Evolution of Virulence

To find the evolutionarily stable within-host growth rate for the parasites, we need to solve

$$\left. \frac{dR_0(\varphi_r, \varphi_m)}{d\varphi_m} \right|_{\varphi_r = \varphi_m = \varphi^*} = 0. \quad (13)$$

Numerically, it is easy to calculate the parasite's optimal growth rate value  $\varphi^*$  depending on  $\varepsilon$ , the efficiency of multiple infections (fig. 4). This value is an evolutionarily singular strategy (Geritz et al. 1997) and thus a candidate evolutionarily stable strategy (ESS; Maynard Smith 1982).

Thus, our study corroborates earlier studies (van Baalen and Sabelis 1995a; Frank 1996; Gandon et al. 2001; Gantsov et al. 2002) in that the optimal parasite growth rate



**Figure 4:** Effect of the efficiency of multiple infections ( $\varepsilon$ ) on the parasite evolutionarily stable strategy, that is, the optimum within-host growth rate ( $\varphi^*$ ). For simplicity, antigen values here are assumed to be optimal for both the resident and the mutant ( $A_r = A_m = \pi$ ). Other parameter values are  $\rho = 0.05$ ,  $\mu = 0.02$ ,  $n_c = 5$ ,  $\delta = 1$ ,  $b = 0.01$ ,  $\sigma_0 = 1$ ,  $\omega = 0.75$ ,  $u = 0.5$ ,  $w = 0.25$ , and  $c = 10$ .

(which is linked to virulence) increases when the efficiency of multiple infections increases. Since it also results from classical coinfection or superinfection approaches (Nowak and May 1994; van Baalen and Sabelis 1995a), the conclusion that multiple infections select for increased levels of virulence seems a general one. However, this result corroborates only some of the experimental observations listed in the introduction to this article, a matter that we address in "Discussion."

Locating an evolutionary equilibrium is not enough to actually predict the evolutionary outcome of the system. We also need to know whether the equilibrium is stable and whether populations converge to it (Geritz et al. 1997). Evolutionary stability can be assessed by considering the invasion fitness of mutants that are different from the resident population. From the resulting "fitness landscape," one can infer whether an evolutionary equilibrium is a true optimum (and thus an evolutionarily stable strategy) or whether it is a fitness minimum (and evolutionary branching is likely to occur). (When all equilibria considered in this study are evolutionarily stable, they are also convergence stable.)

When multiple infections have no effect (i.e., when the second parasite arriving in a host cannot produce any propagule), the fitness landscape has a unique optimum (fig. 5A), confirming that the evolutionary equilibrium is stable in this case. Note that if the resident strain does not have an optimal antigen value, it may be invaded by a mutant with an optimal antigen value (*dashed line*). However, the value of the optimal growth rate is the same.

When there is a low efficiency of multiple infections (fig. 5B), the evolutionary equilibrium resident strategy may become unstable; mutants with a sufficiently fast growth rate can invade. Again, a mutant with an optimal antigen can invade a resident with a nonoptimal antigen.

Finally, with fully efficient multiple infections ( $\varepsilon = 1$ ; fig. 5C), the equilibrium point is clearly evolutionarily unstable; once the resident strain has reached an optimal growth rate value ( $\varphi^*(\varepsilon)$ ), any mutant growing slower or faster is able to invade. As before, when a mutant with an optimal growth rate invades the population, the optimal growth rate values are the same, as before. In other words, the evolutionary equilibrium is a local minimum for the mutant's  $R_0$ , and it could lead to an evolutionary branching case (fig. 5D).

The value of  $\varepsilon$  for which the equilibrium point becomes unstable and becomes a branching point is obtained by solving

$$\left. \frac{d^2 R_0}{d\varphi_m^2} \right|_{\varphi_r = \varphi_m} = 0. \quad (14)$$

For our set of parameter values we find that, mathematically, the ESS becomes unstable for  $\varepsilon \approx 0.006$ . This would imply that even multiple infections with very low efficiency could lead to an evolutionary branching in the population. This branching pattern is robust, as we show in appendix D (which describes the exact range of parameter values which allow the branching to occur), and it is always observed for low efficiencies of multiple infections ( $\varepsilon \geq 0.01$ ). Also, different definitions of within-host models (e.g., having a saturating lymphocyte proliferation function) lead to similar results.

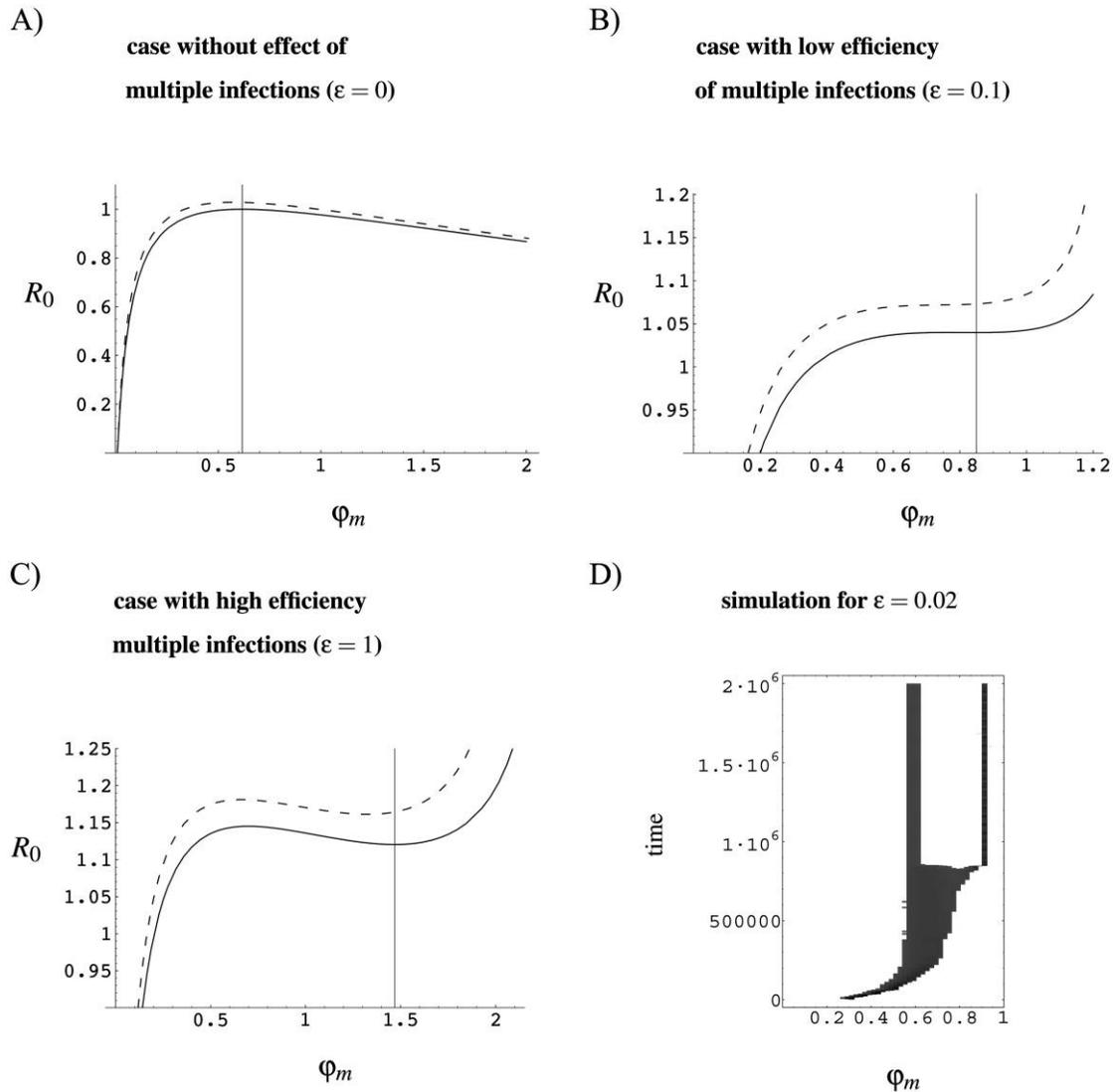
## Discussion

Embedded models linking within-host and epidemiological dynamics bring new insights to the current debate on the effect of multiple infections on virulence evolution. The fact that these models require an explicit within-host approach raises two important questions. The first question deals with timescales, because within-host and epidemiological dynamics are overlapping. The second question deals with the modeling of the immune system and its importance to the study of the dynamics of coinfecting strains.

### *Between Ecology and Evolution*

Current within-host models do not take parasite antigenic aspects explicitly into account in case of multiple infections. They suppose that strains of parasites within a host are either antigenically completely different (such as the model by van Baalen and Sabelis 1995a) or identical (e.g., Nowak and May 1994). In other words, they suppose either that parasites always coexist within a host (coinfection) or that two strains cannot coexist at all (superinfection). Reality, most likely, lies in between those two extremes, and our study suggests that interesting phenomena may arise. For instance, when a mutant that resembles the resident infects an already infected host, it will encounter an activated immune system that renders its net within-host growth rate very low. As the mutant strain remains rare, it can increase its growth rate without paying the cost of killing its host (this would occur only if it reaches high densities within the host).

The problem of overlapping timescales has already been noticed in several studies, but it was never formulated in terms of within-host dynamics. Jansen and Mulder (1999) model replacement in plant communities and suppose that the mutant does not immediately replace the resident. They analyze the resulting overlap between ecological dynamics (plant populations) and evolutionary dynamics (plant species) by calculating approximations of the resident and the mutant dynamics. However, we cannot di-



**Figure 5:**  $R_0$  values for a mutant parasite arriving in a host population infected by a resident strain at equilibrium. A–C, Vertical line indicates the evolutionarily stable strategy ( $\varphi^*(\varepsilon)$ ). *Solid line*, the mutant has the same antigen as the resident ( $A_r = A_m = \pi/4$ ); *dashed line*, the mutant has a different antigen ( $A_m = \pi/5$ ) that is optimal for the lymphocyte receptor distribution (fig. 1A). Other parameter values are the same as those in figure 4. D, Typical results of a numerical simulation of a case leading to branching. At each time step, population densities of hosts are updated following the equations described in appendix C, and a mutant with a new growth rate may appear. Different populations coexist, and when a population density reaches zero, the corresponding trait is removed, which leads to the observed pattern of growth rate evolution.

rectly apply their approach because their model for plant communities is in discrete time. Stollenwerk and Jansen (2003) analyze a model using an approach adopted from statistical physics that allows them to derive equations for macroscopic quantities, such as mean density and outbreak size of different strains of bacterial pathogens, from a microscopic description of a stochastic birth-death process. Mathematically, their model has many similarities to our within-host model, so we can use their approach to

fill the gap that exists between typical superinfection models and typical coinfection models, where one strain always has a local competitive advantage but transitory coexistence always occurs at the timescale of host survival times. Finally, Day and Proulx (2004) developed an original epidemiological approach but it does not allow coinfection.

We derived an approximate fitness measure that does not require an a priori assumption regarding the timescale of within-host dynamics relative to the epidemiological

and evolutionary ones. However, much remains to be done to generalize this result. The problem of overlapping time-scales has been noted before (Gomulkiewicz and Holt 1995; Yoshida et al. 2003), but we feel it deserves more attention as it challenges the key assumption in adaptive dynamics that ecological and evolutionary timescales can be separated.

#### *Immune System Modeling and Multiple Infections*

The coexistence of many strains within a host depends on the specificity of the immune response. To date, cross-immunity processes have been studied mainly in epidemiological models, most of which focus not on multiple infections but rather on the immunological status of a host previously infected by other strains (Gupta et al. 1994; Andreasen et al. 1997; Gog and Grenfell 2002). Using embedded models allows us to base predictions on more realistic assumptions about the functioning of the immune system. Our study stresses the importance of studying non-specific immunity in case of multiple infections because it creates a form of “apparent competition” among coinfecting strains. The intensity of this competition is likely to influence the outcome of the infection, in particular for similar strains. In general, strong apparent competition leads to competitive exclusion of one strain by the other, whereas low levels of apparent competition facilitates the coexistence of parasites within a host.

With our approach, it also becomes clear that parasites could use the immune system as a weapon against competitors (Brown and Grenfell 2001). To do so, they should elicit an immune response (activate cytotoxic T lymphocytes) and survive it. Our model predicts that such a strategy is linked to a very high parasite virulence. This is what Råberg et al. (2006) recently showed experimentally; in mixed infections, avirulent strains of *Plasmodium chabaudi* suffer more from apparent competition than virulent strains. However, this simple view could become more complicated in case of infections with different species since, for instance, humans acquire resistance to specific *Plasmodium* genotypes but not to all of them (Read et al. 2002).

#### *Divergence*

We find that the easier it is for the second infection to establish itself, the higher the optimal level of virulence is, in agreement with previous studies (van Baalen and Sabelis 1995a; Gandon et al. 2001; Ganusov et al. 2002). We also find that if the efficiency of multiple infections is high enough, the parasite population can diverge into a low-virulence strain and a hypervirulent strain. This is an example of specialization. The low-virulence strain prudently

exploits singly infected hosts, whereas the hypervirulent strain is adapted to exploit already infected hosts. Thus, embedded models and taking parasite short-term coexistence into account straightforwardly leads to an evolutionary branching.

It is also interesting to note that antigenic differences do not seem to affect the branching pattern because, in the early stages of an infection, innate immunity plays a central role in the immune response. It is only when the coinfecting strains both settle in the host that acquired immunity becomes essential (see fig. 2).

Our predictions are consistent with previous models. Gandon (2004) showed that host heterogeneity can lead to evolutionary branching in the parasite population. Branching is therefore to be expected as parasites capable of multiple infection inherently face a heterogeneous host population (uninfected and infected hosts). Similar results have also been obtained when considering a heterogeneous population of susceptible and resistant hosts: Gandon et al. (2002) found that under certain conditions, a parasite population could diverge into a highly virulent strain that specializes on susceptible hosts and a less virulent strain that exploits the resistant hosts (host resistance protects the parasite from multiple infection and allows it to exploit it more prudently). Our results are also in agreement with conclusions drawn from Regoes et al.’s (2000) model; they studied virulence evolution in a system with host heterogeneity and assumed a trade-off between virulence in host 1 and virulence in host 2, and they found that depending on the trade-off function’s shape, parasites may evolve toward specialism or generalism. They also suggested that heterogeneity might lead to evolutionary branching in the parasite population. Finally, Boldin and Diekmann (2008) recently showed that evolutionarily stable coexistence between strains is possible in a superinfection model, depending on the superinfection function. Note that they also used an embedded model, but they separated the within-host and epidemiological dynamics (because of their superinfection assumption). This last result suggests that the absence of evolutionary branching in classical coinfection or superinfection models comes from the strong, discontinuous assumptions these models make (see the introduction to the article for further details). If we modify a coinfection model to take into account short-term coexistence or a superinfection model to make the superinfection function more continuous, the evolutionary branching occurs.

Our conclusions are in accordance with experimental results. For instance, Turner and Chao (1999) managed to evolve a very virulent lineage of bacteriophage by imposing high levels of competition between unrelated genotypes. The lineage they evolved through single infections also saw its virulence increase but not as much as the

lineage obtained through multiple infections. This result was interpreted in terms of kin selection; when the two evolved genotypes are competing, the very virulent one behaves as a “cheater” and seems to use shared intracellular products without producing them. López-Ferber et al. (2003) observed a slightly different case. They show a mutualistic interaction between defective and efficient viruses: the defective virus needs the other virus to produce proteins it lacks, but the efficient virus also benefits from the defective virus.

#### *Kin Selection or Antigenic Proximity?*

Kin selection theory suggests that related parasite strains are more likely to cooperate (Frank 1996; Chao et al. 2000; Brown 2001; Brown et al. 2002). This has been shown experimentally for viruses (Turner and Chao 1999; López-Ferber et al. 2003; Vignuzzi et al. 2006) and bacteria (Griffin et al. 2004; Harrison et al. 2006).

In their study, Turner and Chao (2003) noticed that some of their results that were explained by cooperation-defection strategies can also be explained in terms competitive exclusion due to exploitation or interference. Here, we show how a cheater (“hypervirulent”) strategy emerges from coinfection without any explicit cooperation between parasite strains. However, we observe such specialization only if resident and mutant parasite strains are antigenically close enough. What is sometimes analyzed in terms of kin selection can be explained here by apparent competition due to the immune system and can be seen as a side effect of parasites’ antigenic proximity. Our approach illustrates the potential of incorporating antigenic determinants to study virulence evolution.

A possible confounding factor could be parasite cooperation itself. Cooperation will lead to lower levels of virulence if parasites are in a “tragedy of the commons” scenario (Chao et al. 2000). On the contrary, as supported by a growing amount of studies, parasite cooperation can lead to increased levels of virulence through collective actions that may lead to common good production (Brown 2001), interparasite signaling (Brown and Johnstone 2001), or immune system impairment (Bonhoeffer and Nowak 1994). Such processes could account for the selection of lower virulence through multiple infections. However, it is worth noting that this result could be frequency dependent in that cheaters that do not participate

in the collective action are favored only while rare. Once these cheater strains have invaded the population, the effect of multiple infection might be less clear, and experiments lead to diverging results (e.g., Turner and Chao 1999 and Vignuzzi et al. 2006 for divergent results for phages). There is thus a need for detailed studies on the effect of parasite cooperation on virulence to better understand the variations in the evolutionary outcome discussed in the introduction to this article. Incorporating apparent competition caused by the host’s immune system is thus necessary to better understand social interactions among parasites. As shown by Alizon (2008), it is also necessary to include an epidemiological model with these within-host models because, for instance, decrease in overall virulence can lead to the persistence of virulent strains in the host population.

#### *Perspectives*

Many parasites have high mutation rates, which can be seen as a means to deal with their hosts’ immune system, either by overwhelming it (Nowak et al. 1990) or by escaping from it (Korthals Altes and Jansen 2000). Embedded models allow the introduction of antigen variation, a feature we did not develop in this study (the antigens of both parasite strains were generally fixed). This process might be very important because it could generate “escape mutants” able to invade the host population (André and Godelle 2006). It could also allow us to study parasite recombination between different genotypes.

The possibility of hypervirulent strains emerging is clearly a worrisome outcome from a virulence management perspective. However, as we discussed in the introduction to this article, most experimental studies assess only the short-term consequences of mixed infections on host mortality and do not allow conclusions pertaining to the evolutionary consequences of multiple infections. There is thus a clear need for detailed data on what strains are favored in multiply infected hosts.

#### *Acknowledgments*

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## APPENDIX A

## List of the Notations Used

Table A1: List of the notations used

Symbol	Value	Description
Within-host model:		
$\varphi_i$	$\nu$	Parasite within-host growth rate
$x_i$	$\nu$	Density of strain $i$ parasites
$y_j$	$\nu$	Density of clone $j$ lymphocytes
$n_c$	5	Number of lymphocyte clones
$n_p$	2	Number of parasite strains
$A_i$	$\nu$	Parasite antigen value
$R_j$	$\nu$	Lymphocyte receptor value
$b$	.01	Lymphocyte baseline production rate
$\delta$	1	Lymphocyte mortality rate
$\omega$	.75	Accuracy of antigen detection
$\sigma_{ij}$	.01	Killing rate of parasite $i$ by lymphocytes of clone $j$
$\sigma_{\max}$	3	Maximal value of $\sigma_{ij}$
$c_{ij}$	$\nu$	Proliferation rate of lymphocytes $j$ activated by a parasite $i$
$\eta$	10	Constant coupling $c_{ij}$ and $\sigma_{ij}$
Epidemiological model:		
$S$	$\nu$	Density of susceptible hosts
$I_i$	$\nu$	Density of hosts infected by parasite strain $i$
$D_{ij}$	$\nu$	Density of hosts infected first by $i$ and then by $j$
$\mu$	.02	Host natural death rate
$\rho$	.05	Host reproduction rate
$\lambda_i$	$\nu$	Force of infection of parasite strain $i$
$\alpha_i$	$\nu$	Infected host mortality due to parasite strain $i$
$\beta_i$	$\nu$	Transmission rate of parasite strain $i$ alone in a host
$\beta_i[ij]$	$\nu$	Transmission rate of parasite $i$ in a host infected first by $i$ and then by $j$
$u$	.5	Deleterious effect of a replicating parasite
$w$	.25	Lymphocyte detrimental effect
$a$	1	Transmission constant
$R_0$ calculation:		
$R_0$	$\nu$	Parasite basic reproduction ratio
$B_S$	$\nu$	Parasite transmission factor in hosts where it arrived first
$B_1$	$\nu$	Parasite transmission factor in hosts where it arrived second
$x_0$	1	Parasite initial density in a host
$\varepsilon$	$\nu$	Multiple infection efficiency

Note: Variables are indicated with a  $\nu$ , and constants are indicated by their default values.

## APPENDIX B

Calculation of Reproductive Success of a Parasite Arriving Second ( $B_1$ )*Equilibrium of the Resident Population*

We suppose that the resident strain is at equilibrium ( $x_r = \tilde{x}_r$ ) and the same thing for the immune system strains ( $\forall j \in [1, n_c] y_j = \tilde{y}_j$ ). So,  $\forall j \in [1, n_c]$ ,

$$0 = \left( \varphi_r - \sum_{j=1}^{n_c} \sigma_{rj} \tilde{y}_j \right) \tilde{x}_r, \quad (\text{B1})$$

$$0 = b + \eta \sigma_{rj} \tilde{x}_r - \delta \tilde{y}_j. \quad (\text{B2})$$

If  $\tilde{x}_r \neq 0$ , then  $\forall j \in [1, n_c]$ , and

$$\varphi_r = \sum_{j=1}^{n_c} \sigma_{rj} \tilde{y}_j, \quad (\text{B3})$$

$$\tilde{y}_j = \frac{b + \eta \sigma_{rj} \tilde{x}_r}{\delta}. \quad (\text{B4})$$

By introducing the second equation into the first, we obtain

$$\varphi_r = \sum_{j=1}^{n_c} \sigma_{rj} \frac{b + \eta \sigma_{rj} \tilde{x}_r}{\delta}, \quad (\text{B5})$$

which means

$$\tilde{x}_r = \frac{\delta \varphi_r - b \sum_{j=1}^{n_c} \sigma_{rj}}{\eta \sum_{j=1}^{n_c} \sigma_{rj}^2}. \quad (\text{B6})$$

#### *About the Mutant Population*

The mutant strain population dynamic is given by the following equation (where we suppose that the host's parameters are determined by the parasite resident strain):

$$\frac{dx_m}{dt} = \left( \varphi_m - \sum_{j=1}^{n_c} \sigma_{mj} \tilde{y}_j \right) x_m. \quad (\text{B7})$$

We introduce  $\Delta\varphi$  and  $\Delta\sigma_j$ :

$$\Delta\varphi = \varphi_m - \varphi_r,$$

$$\Delta\sigma_j = \sigma_{mj} - \sigma_{rj}.$$

Then, we can write  $dx_m/dt$  such that

$$\frac{dx_m}{dt} = \left[ \varphi_r + \Delta\varphi - \sum_{j=1}^{n_c} (\sigma_{rj} + \Delta\sigma_j) \tilde{y}_j \right] x_m,$$

which means

$$\frac{dx_m}{dt} = \left( \Delta\varphi - \sum_{j=1}^{n_c} \Delta\sigma_j \tilde{y}_j \right) x_m + \left( \varphi_r - \sum_{j=1}^{n_c} \sigma_{rj} \tilde{y}_j \right) x_m.$$

If we replace  $\varphi_r$  with its value from equation (B5), we finally obtain

$$\frac{dx_m}{dt} = \left( \Delta\varphi - \sum_{j=1}^{n_c} \Delta\sigma_j \tilde{y}_j \right) x_m. \quad (\text{B8})$$

If we integrate (and choose  $x_m(0) = x_0$ ),

$$x_m(t) = x_0 e^{(\Delta\varphi - \sum_{j=1}^{n_c} \Delta\sigma_j \tilde{y}_j)t}. \quad (\text{B9})$$

### Integral Calculation

Begin with

$$B_I = \int_0^{\infty} F_s(t) a_m x_m(t) dt. \quad (\text{B10})$$

If we replace  $F_s(t)$  and  $x_m(t)$  with their expressions, we obtain

$$B_I = a_m \int_0^{\infty} e^{-(\mu + \alpha_r)t} x_0 e^{(\Delta\varphi - \sum_{j=1}^{n_c} \Delta\sigma_j \tilde{y}_j)t} dt, \quad (\text{B11})$$

which means

$$B_I = a_m x_0 \int_0^{\infty} e^{-(\mu + \alpha_r - \Delta\varphi + \sum_{j=1}^{n_c} \Delta\sigma_j \tilde{y}_j)t} dt. \quad (\text{B12})$$

Thus,

$$B_I = -a_m x_0 \left[ \frac{e^{-(\mu + \alpha_r - \Delta\varphi + \sum_{j=1}^{n_c} \Delta\sigma_j \tilde{y}_j)t}}{\mu + \alpha_r - \Delta\varphi + \sum_{j=1}^{n_c} \Delta\sigma_j \tilde{y}_j} \right]_0^{\infty}. \quad (\text{B13})$$

As

$$\lim_{t \rightarrow +\infty} e^{-t} = 0,$$

we finally obtain

$$B_I = \frac{a_m x_0}{\mu + \alpha_r - \Delta\varphi + \sum_{j=0}^{n_c} \Delta\sigma_j \tilde{y}_j}. \quad (\text{B14})$$

## APPENDIX C

### The Multistrain Epidemiological Model

#### *System Used for the Population Dynamics*

We suppose that hosts are born healthy and are denoted  $S$  (i.e., no vertical transmission), that all individuals reproduce equally, and that there is no recovery from an infection. Healthy hosts can be infected by both strains (and are denoted

$I_i$ ). Once a host has been infected twice ( $D_{ij}$ ), it cannot be infected anymore. We summarize this in figure 3. The dynamics of hosts and the two parasite strains are thus governed by

$$\begin{aligned}
 \dot{S} &= (S + I_r + I_m + D_{rr} + D_{rm} + D_{mr})\rho - (\lambda_r + \lambda_m + \mu)S, \\
 \dot{I}_r &= \lambda_r S - (\alpha_r + \lambda_r + \lambda_m + \mu)I_r, \\
 \dot{I}_m &= \lambda_m S - (\alpha_m + \lambda_r + \lambda_m + \mu)I_m, \\
 \dot{D}_{rr} &= \lambda_r I_r - (\alpha_{rr} + \mu)D_{rr}, \\
 \dot{D}_{rm} &= \lambda_m I_r - (\alpha_{rm} + \mu)D_{rm}, \\
 \dot{D}_{mr} &= \lambda_r I_m - (\alpha_{mr} + \mu)D_{mr},
 \end{aligned} \tag{C1}$$

where the dot stands for differentiation with respect to time and with

$$\begin{aligned}
 \lambda_r &= \beta_r I_r + \beta_r [rr] D_{rr} + \beta_r [rm] D_{rm} + \varepsilon \beta_r [mr] D_{mr}, \\
 \lambda_m &= \beta_m I_m + \varepsilon \beta_m [rm] D_{rm} + \beta_m [mr] D_{mr}.
 \end{aligned}$$

The  $\rho$  is the host reproduction rate (supposed to be the same for all);  $\mu$  is the natural host mortality;  $\lambda_i$  is the force of infection of parasite strain  $i$  ( $\lambda_r \gg \lambda_m$ ), that is, the risk to become infected by this strain;  $\alpha_i$  is the host mortality induced by a parasite  $i$  alone in the host;  $\alpha_{ij}$  is the host mortality induced by two parasites  $i$  and  $j$  sharing the host;  $\beta_i$  is the transmission rate of the parasite  $i$  alone in the host; and  $\beta_i [ij]$  is the transmission rate of the parasite  $i$  in a shared host where the strain  $i$  arrived before the strain  $j$ .

Note that the forces of infection depend on  $\varepsilon$ , the probability for a parasite that the infection process of an already infected host is successful. However, as we work with an invasion fitness and as we assume that  $D_{ij}$  hosts are identical to  $I_i$  hosts, this does not affect our calculations.

Even if it is not intuitive, it is important to take into account the hosts doubly infected by the same parasite strain. Otherwise, it confers an advantage to the mutant strain, which has more potential hosts (van Baalen and Sabelis 1995a, 1995b); the mutant would have all the singly infected hosts to infect that would not be available to the resident (or a mutant close to the resident). We also assume that the mutant is initially rare and that there can be no hosts infected twice by a mutant, that is, that  $D_{mm} = 0$ .

### Equilibrium of the Resident Population

If we suppose that there is one parasite strain in the system (i.e., that the mutant has not appeared yet), then the only positive subpopulations are  $S$ ,  $I_r$ , and  $D_{rr}$ . The equation system is then

$$\begin{aligned}
 \dot{S} &= (S + I_r + D_{rr})\rho - (\lambda_r^o + \mu)S, \\
 \dot{I}_r &= \lambda_r^o S - (\alpha_r + \lambda_r^o + \mu)I_r, \\
 \dot{D}_{rr} &= \lambda_r^o I_r - (\alpha_{rr} + \mu)D_{rr},
 \end{aligned} \tag{C2}$$

where  $\lambda_r^o = \beta_r I_r + \beta_r [rr] D_{rr}$  and  $\lambda_r^o$  is the force of infection of the resident strain when it is alone. We assume that this system settles at an equilibrium where all the population sizes are positive (denoted  $S^*$ ,  $I_r^*$ , and  $D_{rr}^*$ ).

## APPENDIX D

## Robustness of the Results

We tested the robustness of our main result, that is, the evolutionary branching, by varying each of the parameters independently. We find that this pattern is obtained for most of the parameter values.

There is evolutionary branching if  $c \geq 0.01$  (10),  $\sigma \geq 0.05$  (1),  $b \leq 10$  (0.01),  $\delta > 0$  (1),  $w \leq 2$  (0.1),  $u > 0$  (0.5),  $a > 0$  (1), or  $r > \mu$ . The default values are in parentheses.

We also tested the branching with the following lymphocyte production function:

$$\frac{dy}{dt} = b + \sum_{i=1}^{n_p} c_i \frac{x_i}{x_i + \Phi} y - \delta y,$$

where  $\Phi$  is the parasite density at which the rate of growth of immunity is half maximal. With this saturating function, the evolutionary branching is conserved if  $\Phi > 0$ .

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