Sub-lethal effects of pathogens can lead to the evolution of lower virulence in multiple infections

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According to current evolutionary dogma, multiple infections generally increase a parasite's virulence (i.e. reduce the host's reproductive success). The basic idea is that the competitive interactions among strains of parasites developing within a single host select individual parasites to exploit their host more rapidly than their competitors (thereby causing an increase in virulence) to ensure their transmission. Although experimental evidence is scarce, it often contradicts the theoretical expectation by suggesting that multiple infections lead to decreased virulence. Here, we present a theoretical model to explain this contradiction and show that the evolutionary outcome of multiple infections depends on the characteristics of the interaction between the host and its parasite. If we assume, as current models do, that parasites have only lethal effects on their host, multiple infections indeed increase virulence. By contrast, if parasites have sub-lethal effects on their host (such as reduced growth) and, in particular, if these effects feed back onto the parasites to reduce their rate of development, then multiplicity of infection generally leads to lower virulence.

Keywords: epidemiology; host exploitation; life history; parasite growth; virulence; multiple infection

1. INTRODUCTION

When several parasites share a host, the conflict among the parasites over the level of transmission can lead to less interest in preserving the host and thus to higher levels of virulence (Bremermann & Pickering 1983). Indeed, theoretical studies that combine selection within and among hosts generally conclude that, despite the epidemiological (among-host) selection pressure to use the host efficiently and thus transmit at a high rate, multiple infections will select for higher levels of virulence because of competition within hosts (Bonhoeffer & Nowak 1994; Nowak & May 1994; May & Nowak 1995; Van Baalen & Sabelis 1995; Frank 1996). The idea that virulence is likely to increase when the number of competing parasites increases has become one of the strongest predictions about the evolution of virulence (Frank 1996; Ebert 2000), although it has been noted that a particular relationship between the growth rates of the competing parasites and the mortality of the host (case IV in Bremermann & Pickering (1983)) might lead to the selection of lower virulence in multiple infections.

Doubts about the generality of the prediction, however, have been raised recently, in particular as a response to experimental studies on the co-infection of bacteria by several viral strains. These studies indicate that some mechanisms of host exploitation may select cooperative interactions among the parasites and, therefore, lead to the evolution of lower virulence in multiple infections (Turner & Chao 1999). The mechanisms could be based on a trade-off between competitive ability of the parasite and their growth rate (Chao *et al.* 2000) or on the collective action of parasites—manufacturing products that are shared by the phage during their reproduction—when they

are exploiting their host (Turner & Chao 1999; Brown 2001; Brown *et al.* 2002). The latter idea has been used to indicate a dichotomy in the prediction for the evolution of virulence in multiple infections. Alternatively, if collective action determines a parasite's success, i.e. if a parasite can benefit from the efforts of the other parasites developing in the same host, selection is expected to lead to less exploitation of the host (by individual parasites) as the multiplicity of infection increases (Brown 2001; Brown *et al.* 2002). In other words, parasites evolve to exploit the efforts of other parasites in the co-infection rather than the host. Alternatively, the traditional prediction—increased virulence in multiple infections—is expected to hold for parasites with competitive interactions within their host, where each parasite suffers from the presence of the others (Brown *et al.* 2002). As parasites share a limited resource—their host—individuals exploit the host as rapidly as possible to grow more rapidly and thus transmit more effectively than their competitors.

Although the distinction between cooperative parasites with collective action and competitive interactions is an important step towards understanding the evolution of virulence in multiple infections, we suggest here that the situation is more complex and that, even if parasites are competitive, many situations can lead to the evolution of reduced virulence in multiple infections. Indeed, we suggest that the generality of the current prediction might be based on shared assumptions of current models rather than on shared aspects of the biology of parasites competing within their host.

Among the common features of current models are that the parasite decreases the host's lifespan and that this aspect of virulence limits the parasite's transmission. Many parasites, however, have sub-lethal effects on their hosts by, for example, decreasing their growth rates (Goater & Ward 1992; Alleyne & Beckage 1997). Furthermore, the transmission success is likely to depend on its

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density within the host (for microparasites) or on its size (for macroparasites). As is the case for invertebrates in general (Peters 1983), the fecundity of many helminth parasites, for example, increases with their size (Roberts 1961; Loker 1983; Skorping *et al.* 1991; Morand 1996) and a large size may be achieved most easily in association with a large host (Orr & Hopkins 1969; Poulin 1995). We therefore extended previous models by adding sub-lethal effects to the description of the host–parasite interaction; the parasite reduces the rate at which the host grows and, in turn, the host's size limits the growth and the fitness of the parasite.

2. THE MODEL

We consider a single cohort of a host that is infected by one or several macroparasites at a specific time during its development. Parasites infect hosts randomly at a fixed mean intensity, so that the number of parasites competing within individual hosts is distributed according to the Poisson distribution and the probability that a parasite has a given genotype is determined by the genotype frequency in the population.

Uninfected hosts grow according to Van Bertalanffy's equation (Van Bertalanffy 1938). (Note, however, that we find similar results for growth that follows a decelerating power function (Elliott & Hurley 1995).) Each infection reduces the host's growth parameter proportionally to the parasite's growth effort. Thus, the host's size at time t after infection can be described by

$$H(t) = K \left[1 - \left(1 - \frac{H_0}{K} \right) \exp \left(-r \left(1 - a \sum_i \alpha_i \right) t \right) \right], \quad (2.1)$$

where K is the host's maximum size and H_0 is its size at infection, $i = 1, \dots, n$ is the index of the parasite within the host, α_i is the growth effort of the i th parasite, and a is the proportionality factor relating the parasite's growth effort and its effect on the host's growth.

Within an individual host, each macroparasite also grows according to the Van Bertalanffy equation. Its initial size is defined as W_0 and its asymptotic size is constrained by the restriction that the total size of the parasites in the co-infection cannot exceed the size of the host. The parasite's growth parameter increases with its growth effort α . The growth of the i th parasite in a co-infection can thus be written as

$$W_i(t) = \left(H(t) - \sum_j W_j(t) \right) \left[1 - \left(1 - \frac{W_0}{H(t) - \sum_j W_j(t)} \right) e^{-m_i t} \right], \quad (2.2)$$

where $j = 1, \dots, n$ ($j \neq i$) is the index of co-infecting parasites. Note that, although we formulate the model for the growth of macroparasites, similar results would be reached for the replication of microparasites, if we assumed that their replication rate is density dependent and that their total density is limited by the host's size.

During the parasite's development, the host's mortality rate, β , increases with the growth effort of the parasite according to

$$\beta = \mu \left(1 + b \sum_i \alpha_i^\eta \right), \quad (2.3)$$

where μ is the mortality rate of the host from other causes than the parasite, and b and η are constants that scale virulence to the growth effort of the parasite. Note that when $a = 0$, the parasite does not affect the host's growth. Its sole effect is then to increase the host's mortality, so that our model becomes similar to earlier models on the evolution of virulence (Bremermann & Pickering 1983). In the more general case, when $a > 0$ and $b > 0$, we follow previous suggestions (Bremermann & Pickering 1983) by assuming that both effects increase with the parasite's growth effort and thus letting the adverse effects on the host's mortality and growth be associated positively.

The parasite is transmitted by the release of propagules when the host dies. (We obtain the same qualitative results, if we assume that the parasite is transmitted continuously from infected hosts.) The number of propagules, f , of a parasite is assumed to be proportional to its size: $f = F(W - w_c)$, where w_c is the threshold size that must be reached by the parasite so that it can reproduce. The expected transmission of parasite i is thus

$$T_i = \int F(W_i(t) - w_c) \beta e^{-\beta t} dt, \quad (2.4)$$

where $\beta e^{-\beta t} dt$ is the probability that the host dies between times t and $t + dt$ (Bremermann & Pickering 1983).

3. RESULTS AND INTERPRETATION

We illustrate the evolutionary outcome of the processes governed by these equations with invasion plots (figure 1). These consider a uniform population of resident parasites with a common growth effort and show the range of growth effort that would allow a rare mutant to invade the population. This approach enables us to estimate the evolutionarily stable growth effort that cannot be invaded by any mutant. If the parasite affects only the host's mortality ($a = 0$, $b = 1$), the conventional prediction is confirmed (figure 1*a–d*). As the number of parasites in the co-infection increases, the locally stable growth effort and thus the parasite's virulence increase. Furthermore, an unstable equilibrium appears at a high growth effort, above which virulence evolves toward the maximally possible value. As the intensity of the co-infection increases, the region of stability decreases and the threshold level of the growth effort that enables this runaway process decreases. At very high intensities of co-infection, the two equilibria disappear, so that the only possible equilibrium is at the maximal value.

If, however, the parasite affects the host's growth ($a = 1$, $b = 0$), we often find the opposite pattern (figure 1*e–h*): increased multiplicity of infection decreases the parasite's growth effort and thus reduces its detrimental effect on the host. Naturally, this does not imply that the effect of the total parasite load is less than the effect of a single infection, but only that the evolutionary pressure leads individual parasites to grow more slowly than they would had they consistently experienced single infections. Indeed, with the parameters used in figure 1, high multiplicities of infection reduce the growth rate of the host to values close to zero (for six infections, for example, the total growth effort is 0.96).

Figure 1. Invasion plots calculated from the simulation of equations (2.1)–(2.4). The parasite was initialized with 99.9% ‘residents’ and 0.1% ‘mutants’. The frequency of hosts infected with a certain number of parasites followed a Poisson distribution with a fixed mean number of parasites per co-infection. The development of each infection was calculated according to equations (2.1)–(2.3), and equation (2.4) was used to calculate the frequencies of the two parasite types following transmission. The parameter values used in the equations are: $r = 0.03$, $K = 2000$, $H_0 = 600$, $W_0 = 50$, $\mu = 0.01$, $\eta = 6$, $F = 1$, $w_c = 10$. Each panel varies the growth effort of the residents and gives the range of growth effort that enables the mutant to invade as a grey area. The circles indicate locally stable growth efforts, where no mutant can invade a population consisting of residents; the squares indicate saddle points, where any higher growth effort will lead to runaway selection for increased effort. The intensity of grey gives a qualitative indication of the reproductive success of a resident parasite, where black indicates the maximal reproductive success in the given epidemiological setting. In *a* the parasite reduces the survival of the host ($a = 0$, $b = 1$), in *b* it reduces the growth rate of the host ($a = 1$, $b = 0$). In *a*(i) and *b*(i) an average infection harbours one parasite, in *a*(ii) and *b*(ii) two parasites, in *a*(iii) and *b*(iii) four parasites and in *a*(iv) and *b*(iv) six parasites.

An additional aspect of our simulations that is worth noting is the difference in the parasite’s transmission success. Increased competition when the parasite affects the host’s mortality leads the parasite to a situation where its success is much lower than the maximal value that would be possible in the same epidemiological setting, i.e. for a given intensity of infection (figure 1). Alternatively, when the parasite reduces the host’s growth rate, its transmission success is reduced only slightly from what the maximal transmission would be. This difference can be interpreted as the distinction between an exploitative strategy for parasites that affects mortality, where each parasite grows as rapidly as necessary to outcompete its competitors, and a more cooperative strategy for parasites with sub-lethal effects, where the parasites utilize their limiting resource more prudently.

4. DISCUSSION

In our model, multiple infection, in contrast to traditional predictions for parasites competing within a host, can lead to decreased virulence. Whether it does depends

on the nature of the interactions between the host and the parasite. If the parasite increases the host’s mortality, competition within the host leads to increased virulence; if it decreases the rate of the host’s growth and if the parasite’s growth depends on the host’s size, competition tends to decrease virulence. We thus give an additional mechanism to the two earlier ones: a trade-off among competitive ability and growth of a parasite (Chao *et al.* 2000) and collective action of parasites (Brown *et al.* 2002). Taken together, these studies indicate that multiple infections would select decreased virulence in several different types of host–parasite interaction and thus that the conditions conforming to the traditional prediction, that is, that multiple infections lead to increased virulence, are not as general as previously thought.

Note, however, that our goal was not to describe the complete behaviour of the model nor all of the evolutionary scenarios for parasites that reduce their host’s growth rate. We therefore do not claim that reduced virulence is always the outcome of multiple infection by such parasites, and indeed we have found some parameter values for which a low level of multiple infection increases virulence

before virulence again diminishes at higher intensities of infection. Rather, we emphasize that critical details of the interaction between host and parasite can determine the outcome of the evolutionary process. So that we can understand the evolution of virulence, it is essential that we know whether the parasites exploit their host collectively (Brown *et al.* 2002), how they affect their host and how the host's condition affects their growth. Subtle changes in these characteristics of the host–parasite interaction can alter the evolutionary pressure on the parasite's virulence.

We have described the two extreme possibilities of the parasites' interaction with their host, where they affect their host's mortality or its growth but not both. Many parasites, however, have both sub-lethal and lethal effects on their host, in which case the relative strengths of the two effects will determine whether multiple infection selects for increased or decreased virulence (or no change). As an example, we can consider malaria, which is not only the main cause of death of children in Africa (WHO 1999) but also slows their growth. One of the mechanisms for both of these effects is the severe anaemia that can be caused by parasite infection. This, in turn, feeds back to slow the replication rate of the parasite, as malaria requires iron to replicate (Oppenheimer 1989). And indeed, conforming with the diversity of effects on the host, multiple infection of malaria can be associated with increased disease severity, with decreased severity or with no change in severity, depending on differences among populations in the intensity of transmission (Read & Taylor 2001).

Many of the assumptions in our model can be weakened without changing the general conclusions. Thus, for example, the crucial assumption that sub-lethal effects on the host reduce the parasite's fitness must not necessarily be mediated by decreased growth of the host but can be stated more generally as a degradation of the quality of the host as an environment for the parasite. As already mentioned in the discussion, malaria parasites can replicate only slowly in anaemic, iron-deficient hosts (Oppenheimer 1989). Similarly, hosts harbouring more virulent parasites may, for example, forage less and thereby decrease the availability of resources for parasite growth (Hudson & Dobson 1995), or they may mount a stronger immune response causing greater damage to the parasite tissue (Almogly *et al.* 2002). These cases again lead to the conclusion that multiple infections can decrease virulence.

Furthermore, the model does not consider any epidemiological feedback. In other words, the force of infection (i.e. the probability per unit of time that a host becomes infected) and the mean multiplicity of infection are held constant. Including a feedback would change the selection pressure for virulence by affecting the mean infection intensity in the host population (Van Baalen & Sabelis 1995). Thus, whether the parasite affects the host's growth or mortality, increased (total) virulence of the infection would lead to lower intensity of infection and to lower multiplicity of infection, limiting the potential for runaway selection. This feedback, however, would not alter the qualitative difference of the predictions for parasites affecting growth or mortality.

In conclusion, we have presented results indicating that the traditional view—that competitive interactions among

parasites in multiple infection lead to evolutionary pressure for increased virulence—may be due to an implicit bias of earlier models. Indeed, most models on the evolution of virulence in multiple infections consider systems where the parasite's fitness is affected only by adverse effects on the host's survival (Bonhoeffer & Nowak 1994; Nowak & May 1994; May & Nowak 1995; Van Baalen & Sabelis 1995; Frank 1996; Chao *et al.* 2000). Although in such host–parasite associations multiple infections may select for higher virulence, we have shown here that in other kinds of host–parasite associations multiple infections need not do so. Indeed, they may favour lower virulence in systems where sub-lethal effects on the host prevail and feed back to reduce the parasite's fitness. Thus, if the parasite slows the host's growth and if the decreased host growth in turn impairs the parasite's growth and fecundity, multiple infections can decrease the level of virulence.

It remains to be seen whether it is the sub-lethal or the lethal effects that dominate the evolutionary pressure on virulence in multiple infections. It should come as no surprise, however, that in contrast to conventional wisdom, experimental evidence in several host–parasite systems (malaria in mice, trypanosomes in cattle, hepatitis B in ducks, various bacteria in mice, pigs, humans and chickens) has demonstrated that the severity of disease can be reduced by competitive interactions within hosts, whereby avirulent strains can outgrow or exclude the more virulent ones (Read & Taylor 2001).

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