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Interactions between sources of mortality and the evolution of parasite virulence

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A well-known result from the theory of the evolution of virulence is the prediction that the virulence of a pathogen (i.e. the rate of parasite-induced host mortality) always evolves to higher levels when host background mortality rates increase. This prediction, however, is derived from models that assume that host mortality sources combine additively to determine the overall host mortality rate. In this paper, we suggest that such additivity is probably rare for many host–pathogen systems, and explore how the predictions for the evolution of virulence are altered when interactions between host mortality sources are incorporated into the theory. Our results indicate that if mortality-source interactions are sufficiently strong then the evolutionarily stable level of virulence can actually decrease as the background mortality rate increases. Consequently, a detailed mechanistic description of how parasites and other mortality sources combine to cause host mortality is required before reliable predictions about virulence evolution can be made. Moreover, mortality-source interactions make empirical comparisons of the virulence of different parasites a much more subtle issue.

Keywords: pathogen; virulence; mortality; independence; interaction; evolution

1. INTRODUCTION

Many theoretical models that consider multiple sources of mortality assume independence, that is, that the mortality rates due to different sources combine additively to determine the overall mortality rate. One particular area of theory where the assumption of independence is almost universal is in the study of the evolution of parasite virulence. However, numerous empirical studies suggest that this assumption is probably false in many situations. For example, it has been well documented in a number of reviews (Scrimshaw *et al*. 1968; Crompton 1991) that host nutritional status plays an important role in determining the severity of pathogen infections. In particular, protein malnutrition has been demonstrated to inhibit greatly the immune responses to a variety of pathogenic organisms, resulting in higher parasite loads of infected individuals (Scrimshaw *et al*. 1968; Slater & Keymer 1988; Gulland 1992; Holmes 1995).

A number of recently performed co-infection experiments have also indicated a lack of additivity between component mortality sources. Karyeija *et al*. (2000) found that sweet-potato plants contracted sweet-potato virus disease only when infected with both a potyvirus and a crinivirus. Infection with either virus alone produced either no effect or a minimal effect. The authors suggest that the presence of the potyvirus inhibits a signalling mechanism required for repression of the crinivirus. Similar clinical results were obtained when tobacco plants were infected with two potato viruses (Vance 1991). In this case, it was determined that RNA replication of one of the viruses was upgraded in the presence of the other. Along the same lines, a number of studies have found that the damage caused by a bacto-helminthic complex to insect larvae depends on the pathogenicity of both components of the complex as well as on an interaction between the two (Ehlers *et al*. 1997; Gerritsen *et al*. 1998; Bonifassi *et al*. 1999).

Behavioural changes in parasitized hosts may also introduce a non-additive component into mortality effects. Many pathogens induce significant changes in host foraging behaviour (Holmes & Bethel 1972; Dobson 1988; Hurd 1990; Moore & Gotelli 1990). In some cases, such changes may be mediated by increased energetic demands on parasitized hosts, possibly resulting in increased foraging rates and an incidental greater exposure to predation risk (Holmes & Zohar 1990; Milinski 1990). A study of the effects of nematode parasitism in *Drosophila* found that the level of parasite-induced mortality was much greater in field populations than in laboratory controls (Jaenike *et al*. 1995). Similar increases in vulnerability to predation were found in field populations of nematode-infected red grouse (Hudson *et al*. 1992) and snowshoe hares (Murray *et al*. 1997). Thus, it seems very likely that most natural host populations are exposed to a diverse array of mortality sources, resulting in numerous mechanisms through which mortality interactions can be effected.

The standard models of epidemiology, first proposed by Kermack & McKendrick (1927, 1932, 1933) and later extended in a number of papers by Anderson & May (1979, 1981, 1982), provide the framework within which most theoretical investigations of the evolution of virulence take place. From such models one may construct the so-called basic reproduction ratio (Diekmann *et al*. 1990; we use this term instead of the more common, though less accurate, reproductive rate; see Dietz (1974) and Anderson & May (1981)), the value of which determines whether an infection will spread through a host population or die out. Importantly, this quantity also plays a key role in predicting parasite evolution. Different strains of parasite will have different life-history characteristics and, hence, different reproduction ratios. The results of

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many theoretical studies show that the strain that is evolutionarily stable (i.e. the one that can resist invasion by all mutant strains) is the one with the largest basic reproduction ratio (Anderson & May 1982; Ewald 1983; May & Anderson 1983; Lenski 1988; Bull *et al*. 1991; Ebert 1994). Consequently, if one supposes that there are trade-offs between the various life-history attributes of the parasite then the evolutionarily stable level of virulence can be determined simply by specifying these trade-offs and then determining the level of virulence that maximizes the basic reproduction ratio (Anderson & May 1981, 1982; May & Anderson 1983).

Many studies use this framework as a starting point for investigating how different aspects of host biology might affect this optimal level of virulence. In particular, the question of how host background mortality rate (i.e. the rate at which uninfected hosts die) affects the evolution of virulence has been addressed both theoretically and empirically (Ebert & Mangin 1997). The apparent consensus of theoretical works on the issue (May & Anderson 1983; Kakehashi & Yoshinaga 1992; Lenski & May 1994; Ebert & Weisser 1997) is that increases in host background mortality should lead to increases in host exploitation by the pathogen and, thereby, to increases in parasite-induced mortality (typically equated with virulence; see Read 1994). Interestingly, however, although this prediction appears to be universal in the theoretical literature, there are many empirical studies that call into question its general validity.

One such example is provided by the parasitic ear mite *Dicrocheles phalaenodectes* and its noctuid moth host (Treat 1975; Ebert & Herre 1996). These ear mites infect and damage only one ear of their host, leaving the other ear unaffected, regardless of the population density in the damaged ear. The reasoning behind such a strategy is that a moth with both ears damaged would be less effective at detecting its bat predators (Ebert & Herre 1996). Single-eared moths are somewhat impaired in their ability to avoid predation, but the benefits gained by the mites through reproduction outweigh the potential survival costs. In theory, removal of the predation risk from bats would leave the mites free to invade and damage the other ear of their moth host, since there is no longer a fitness cost imposed on such an expansion (Treat 1975). In other words, decreasing host background mortality (in the form of reduced predation risk) should lead to elevated levels of host exploitation. Similarly, reintroduction of the bat predators should attenuate the level of host exploitation exhibited by the ear mites. Both of these predictions are clearly at odds with the bulk of the theoretical literature.

One reason standard models of the evolution of virulence are unable to account for examples of this sort is their failure to account for interactions between the extent to which the pathogen damages its host and the host's background mortality rate. As illustrated in the above example, increases in the ear mites' level of host exploitation increase the probability that the moth host will succumb to mortality in the form of predation, indicating that these two sources of mortality are not independent. Such interactions are likely to be ubiquitous in host–pathogen systems (Scrimshaw *et al*. 1968) and accounting for them in theoretical models is, therefore, a very important step towards understanding the evolution of parasite virulence in natural populations.

Here, we explore how the predictions for the evolution of virulence are altered when interactions between host background mortality sources and pathogen-induced mortality are included in the standard theoretical framework. Our results reveal that such mortality-source interactions are expected to have major qualitative effects on virulence evolution.

2. THE MODEL

(a) *Formulation*

For many host–parasite systems, both the parasite transmission rate and the pathogen-induced mortality rate (which we define as virulence; Bull 1994; Read 1994) are positively correlated with the degree to which the parasite exploits its host (Bull *et al*. 1991; Herre 1993; Ebert 1994; Ebert & Mangin 1997; Mackinnon & Read 1999; Messenger *et al*. 1999; for a discussion see Lipsitch & Moxon 1997). The extent of host exploitation can be quantified in many ways, including the pathogen's replication rate and its population density within the host's tissue (Ebert 1994; Lipsitch *et al*. 1995; Imhoof & Schmid-Hempel 1998), and we develop a model of the evolution of such 'host-exploitation strategies' (e.g. Van Baalen & Sabelis 1995; Poulin & Combes 1999). Because virulence is often expected to increase with increasing host exploitation, many theoretical studies simply equate the two and, thereby, model the evolution of virulence directly. As will be seen, however, when one allows for interactions between mortality sources, the relationship between host exploitation and virulence will depend on the host's environment. Therefore, it is conceptually more transparent to focus first on the evolution of a parasite's host-exploitation strategy, and, afterwards, to derive from this predictions about the evolution of its virulence level.

To calculate the evolutionarily stable host-exploitation strategy (ESS), we consider the simplest case, in which members of the host population are infected by a single parasite genotype (clone), eliminating the potential for within-host competition. From standard epidemiological models (Anderson & May 1981, 1982) parasite fitness may be quantified by determining the basic reproduction ratio, R (usually denoted R_0, but we use R to simplify our notation), of the infection (Anderson & May 1981, 1991; May & Anderson 1983; Frank 1996). For many epidemiological models, R is a function of host- and parasite-specific parameters of the form

$$R = \frac{\beta \mathcal{N}}{f(\varepsilon, \delta, \gamma)}, \tag{2.1}$$

where β is the transmission rate of the parasite, $\mathcal{N}$ is the size of the susceptible host population, ε is the host-exploitation strategy, δ is the infection-free (background) host mortality rate, γ is the clearance or host-recovery rate from parasitism and $f(\varepsilon, \delta, \gamma)$ is a function that describes the 'mortality rate' of the infection. Equation (2.1) can be viewed as the product of the number of new infections per unit time resulting from a single infected host, $\beta\mathcal{N}$, and the mean life expectancy of an infection in a given host, $1/f(\varepsilon, \delta, \gamma)$. Thus, R is the expected number of

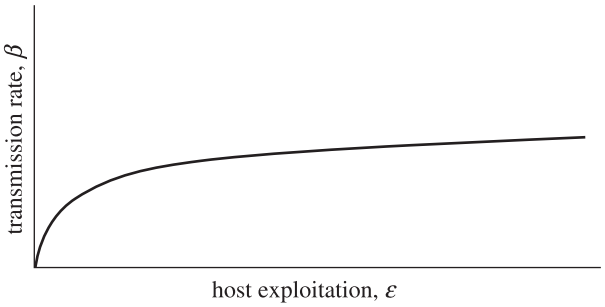

Figure 1. Relationship between transmission rate and host-exploitation strategy. The transmission rate, $\beta(\varepsilon)$, initially increases with increasing level of exploitation, ε, but saturates at high levels of exploitation. Strictly speaking, this curve represents a constraint rather than a functional relationship, since there might exist strains with transmission–exploitation-strategy relationships that lie below the curve. However, for a given level of exploitation, strains whose rates of transmission lie below the curve will have lower fitness than those that lie on it, and hence we can treat this curve as a functional relationship.

secondary infections caused by a single infected host in an entirely susceptible population (May & Anderson 1983, 1990; Murray 1993; Frank 1996).

The evolutionarily stable level of exploitation, ε^*, is that which maximizes R, and must therefore satisfy the local condition

$$R_\varepsilon = 0, \tag{2.2}$$

where the subscript denotes differentiation (the second-order maximum condition is also satisfied for all the examples used in this article). Using the definition of R in equation (2.1), this gives

$$\frac{\beta_\varepsilon}{\beta} = \frac{f_\varepsilon}{f}. \tag{2.3}$$

Equation (2.3) states that, given a small increase in the level of host exploitation, the proportional change in parasite transmission is equal to the proportional change in total mortality at the optimum. That is, at the optimum, the benefit gained by increasing transmission through an increase in host exploitation must balance the cost of increased mortality through the same increase in host exploitation. To proceed further we must now specify the functional forms of f and β in more detail.

For the purposes of this paper, we assume that the relationship between β and ε has the form shown in figure 1. The results remain qualitatively similar with any saturating non-decreasing function. In the absence of mortality-source interactions, virulence, ν (which we define as the additional host mortality rate caused by the parasite), is assumed to be an increasing function of ε only (i.e. $\nu(\varepsilon)$). When there are interactions between background sources of host mortality and host mortality caused by the parasite, however, ν will be a function of both ε and δ (i.e. $\nu(\varepsilon, \delta)$). Figure 2*a*,*b* illustrates a general method, analogous to a two-way analysis of variance, of representing the total effect on host mortality of background and pathogen-induced mortality sources.

In specifying the parasite mortality function, f, previous theory has ignored mortality-source interactions

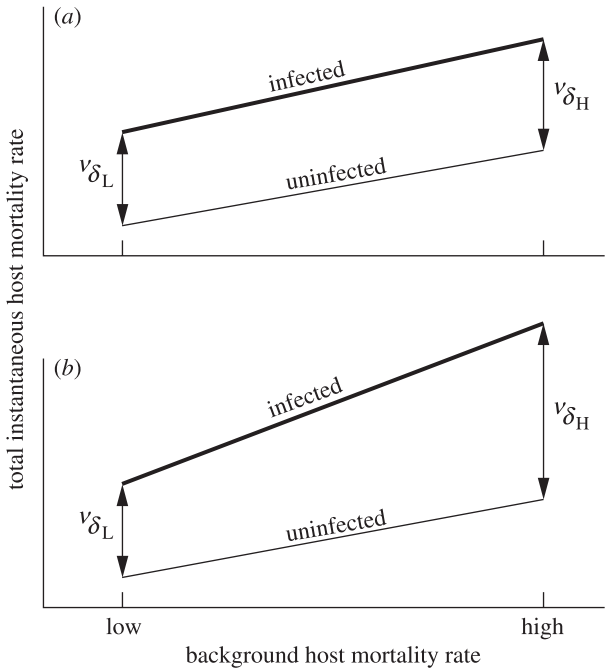

Figure 2. A graphical representation of the potential relationships between pathogen-induced mortality (i.e. virulence) and background mortality. The total host mortality rate is plotted for uninfected and infected individuals under conditions of low and high background mortality. A thick line connects the two infected classes and a thin line connects the two uninfected classes. (*a*) When there is no mortality-source interaction the connecting lines are parallel and the additional host mortality due to infection, ν_{δ_L} and ν_{δ_H} in the low and high host background mortality treatments, respectively, is independent of background mortality rate. In this case $\nu_{\delta_L} = \nu_{\delta_H}$, and virulence can be measured as the distance between the thick and thin lines at any level of background mortality. (*b*) When there is an interaction between pathogen-induced and background mortality the lines are no longer parallel, and $\nu_{\delta_L} \neq \nu_{\delta_H}$. Virulence is still given by the distance between the thick and thin lines, but it now depends on the background mortality rate.

by assuming that the three ways in which an infection might end (death of host due to background mortality, occurring at rate δ; death of host due to virulence of parasite, occurring at rate $\nu(\varepsilon)$; and clearance of the parasite, occurring at rate γ) combine additively to give (Anderson & May 1982; Frank 1996)

$$f(\varepsilon, \delta, \gamma) = \nu(\varepsilon) + \delta + \gamma. \tag{2.4}$$

To incorporate interactions between mortality sources, we first partition the background mortality into that which interacts with virulence (occurring at rate δ_I) and that which does not (occurring at rate δ_{NI}). 'Interactive' background mortality (denoted by the subscript I) represents mortality sources that an infected host might be more (or less) susceptible to than an uninfected host. For example, a host labouring under an infection might be less able to perform anti-predator behaviours effectively, or might be more susceptible to other parasites and pathogens. Although we are primarily interested in positive interactions, interactive mortality sources also include mortality sources that might diminish the total level of parasite-induced mortality. An example of this might be

extreme nutritional deficiency, in which the host provides an inhospitable environment for the parasite (Scrimshaw *et al*. 1968; Latham 1975; Crompton 1991). 'Non-interactive' background mortality (denoted by the subscript NI) is simply a random removal of hosts. In this case f has the more general form

$$f(\varepsilon, \delta_{\mathrm{NI}}, \delta_{\mathrm{I}}, \gamma) = \nu(\varepsilon, \delta_{\mathrm{I}}) + \delta_{\mathrm{I}} + \delta_{\mathrm{NI}} + \gamma. \tag{2.5}$$

With this form, we can incorporate any non-additive relationship between virulence and background mortality by choosing an appropriate form for ν. For the purposes of illustration, however, we take one of the simplest possible forms, namely

$$\nu(\varepsilon, \delta_{\mathrm{I}}) = a\varepsilon + b\varepsilon\delta_{\mathrm{I}} = (a + b\delta_{\mathrm{I}})\varepsilon, \tag{2.6}$$

where a and b are parameters specifying the strengths of the two terms. The $b\varepsilon\delta_{\mathrm{I}}$ term of equation (2.6) represents the mortality-source interaction. If b is positive, then any given exploitation strategy will result in a higher virulence (i.e. a higher parasite-induced mortality rate) when the host is found in an environment with a higher interactive background mortality.

(b) *The effect of background mortality*

Under the assumptions in § 2a, ε^* can be calculated by using equations (2.4) or (2.5) and (2.6) to solve equation (2.3). From this we can then determine how the optimal exploitation strategy changes in response to an increase in background mortality by implicitly differentiating equation (2.3) with respect to $\delta_{\mathrm{i}} = \mathrm{I}, \mathrm{NI}$. This gives

$$\frac{\mathrm{d}\varepsilon^*}{\mathrm{d}\delta_i} = \frac{\beta[ff_{\varepsilon\delta_i} - f_\varepsilon f_{\delta_i}]}{f[f\beta_{\varepsilon\varepsilon} - f_{\varepsilon\varepsilon}\beta]}, \tag{2.7}$$

where the subscripts denote partial differentiation. The term in square brackets in the denominator of equation (2.7) will be negative provided that ε^* yields a maximum and β/f is positive. Therefore, the sign of $\mathrm{d}\varepsilon^*/\mathrm{d}\delta_{\mathrm{i}}$ is determined by the sign of $f_\varepsilon f_{\delta_i} - ff_{\varepsilon\delta_i}$. This has the same sign as $-[ff_{\varepsilon\delta_i} - f_\varepsilon f_{\delta_i}]/f^2$ and therefore ε^* will be an increasing function of background mortality whenever

$$[f_\varepsilon/f]_{\delta_i} < 0 \text{ for } i = \mathrm{I}, \mathrm{NI}. \tag{2.8}$$

Intuitively, inequality (2.8) says that whenever the proportional costs of an increase in exploitation strategy are diminished by an increase in background mortality, it pays for the parasite to increase its exploitation.

For the parasite mortality function given by equation (2.4) (i.e. no interaction) we have

$$f_\varepsilon/f = \nu_\varepsilon(\varepsilon^*)/(\nu(\varepsilon^*) + \delta + \gamma). \tag{2.9}$$

Because ν is an increasing function of ε, it is clear that the proportional costs of an increase in exploitation strategy decrease with an increase in δ. Hence, with this form of f, the optimal level of exploitation always increases as background mortality increases. As a result, the optimal level of virulence, $\nu(\varepsilon^*)$, increases as well. This is the mathematical result underlying the widely held belief that optimal virulence is always an increasing function of background mortality (May & Anderson 1983; Kakehashi & Yoshinaga 1992; Lenski & May 1994; Ebert & Weisser 1997).

By way of comparison, the proportional cost of an increase in exploitation strategy for the f given in equations (2.5) and (2.6) is

$$f_\varepsilon/f = (a + b\delta_{\mathrm{I}})/(a\varepsilon^* + b\varepsilon^*\delta_{\mathrm{I}} + \delta_{\mathrm{I}} + \delta_{\mathrm{NI}} + \gamma). \tag{2.10}$$

Note that equation (2.10) does not behave in the same way with respect to increases in the two different types of background mortality, δ_{NI} and δ_{I}. An increase in δ_{NI} inflates the denominator of equation (2.10), while leaving the numerator unaffected. This leads, as in the previous example, to an increase in the optimal exploitation strategy. This is not surprising since δ_{NI} is non-interactive mortality, just as δ is in equation (2.9). However, an increase in δ_{I} affects both the numerator and the denominator of equation (2.10), making it less obvious how the optimal exploitation level is affected. From equation (2.7), the critical value in determining how the optimal exploitation strategy will respond to an alteration in δ_{I} is

$$f_\varepsilon f_{\delta_{\mathrm{I}}} - ff_{\varepsilon\delta_{\mathrm{I}}} = a - b(\delta_{\mathrm{NI}} + \gamma). \tag{2.11}$$

When this value is negative, an increase in the interactive background mortality rate will decrease the evolutionarily stable level of host exploitation. This result may be restated as a condition on the coefficient of the interaction term: given an increase in interactive background mortality, the optimal level of host exploitation will decrease whenever $b > a/(\delta_{\mathrm{NI}}+\gamma)$.

As an example, we return to the ear-mite–moth system described in § 1. It has been determined under laboratory conditions free from predators that infected moths do not suffer greater mortality than their uninfected counterparts (Treat 1975). Only when bat predators are introduced does the effect of parasitism manifest itself as higher mortality (figure 3). In this case, $a = 0$, indicating a lack of effect of host-exploitation strategy on host mortality in the absence of interactive background mortality. As a result, equation (2.11) is always negative, predicting that the optimal level of host exploitation will decrease with an increase in predation, in accordance with the verbal reasoning presented in § 1.

(c) *The ESS level of virulence*

The analysis in § 2b focuses on the evolutionarily stable exploitation strategy, but what is usually of more interest is the ESS level of virulence (i.e. the ESS level of parasite-induced mortality). The presence of mortality-source interactions complicates this question, and there are now at least two comparisons to be considered (figure 4): first, the virulence levels of parasites that have evolved under high versus low levels of background mortality in a common environment, and second, the virulence levels of parasites that have evolved under high versus low levels of background mortality, each evaluated in the environment under which it evolved. Of course, the most complete comparison would be one made under several different common environments.

The first comparison is probably the most typical, particularly if the possibility of mortality-source interactions is not considered, since the goal is usually to measure differences in host mortality due to differences in the parasite alone (e.g. by controlling conditions in the laboratory). Of course, when interactions are present a

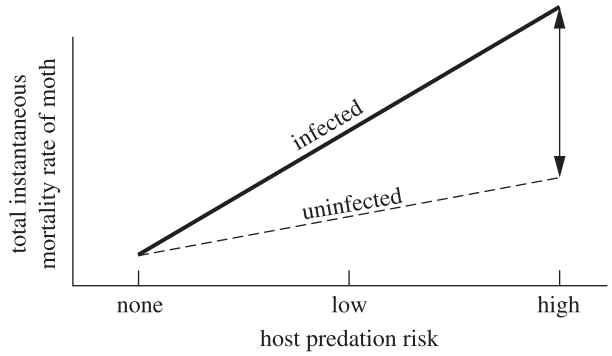

Figure 3. The graphical representation, introduced in figure 2, of mortality interactions in the ear-mite–moth system. Under predator-free conditions, ear-mite infection has a negligible effect on host mortality.

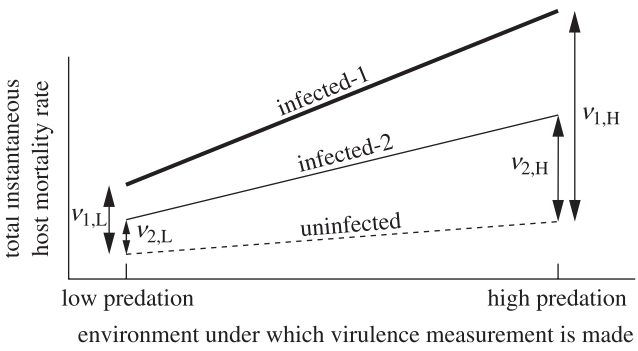

Figure 4. Comparison of virulence levels in the presence of interactions. The dashed line connects the mortality rates of uninfected hosts in two predation-risk environments. Solid lines connect the mortality rates of hosts infected with parasite 1 (thick line) or parasite 2 (thin line) as measured in the two environments. The virulence of strain i when measured in predation environment j is $\nu_{i,j}$. The observed virulence depends on the environment in which the measurement is made ($\nu_{i,\mathrm{L}} \neq \nu_{i,\mathrm{H}}$) indicating an interaction; virulence is greater in the high-predation environment. Suppose parasite 1 evolved in the low-predation environment and parasite 2 evolved in the high-predation environment. Parasite 1 is more virulent than parasite 2 when compared in a common environment, but parasite 2 is more virulent than parasite 1 if each is measured in its natural habitat ($\nu_{2,\mathrm{H}} > \nu_{1,\mathrm{L}}$).

clean separation of parasite and environment is, by definition, not possible since virulence is a function of both host exploitation and background mortality (i.e. $\nu(\varepsilon, \delta_{\mathrm{I}})$). Nevertheless, even if interactions are known to be important, this comparison still has the benefit of disentangling the dependence of virulence on the host-exploitation strategy from its dependence on the background mortality rate. Furthermore, it addresses how the difference in the level of virulence due solely to the evolution of host exploitation is affected by the level of background mortality under which the parasites evolved. In any event, given that virulence increases with the level of host exploitation, it is clear in this comparison that the ESS level of virulence is predicted to change in exactly the same way as the ESS level of host exploitation with respect to changes in background mortality.

The second comparison essentially compares the levels of virulence of different parasites as they are exhibited in their natural settings, and thereby confounds the evolved differences in host exploitation and the background mortality of the environment. Because of mortality interactions, however, if differences in total parasite-induced mortality are of interest then this comparison might be appropriate. Determining how the ESS level of virulence is expected to change with changes in background mortality in this case is a subtler exercise. For a given evolutionarily stable host-exploitation strategy, ε^*, the associated ESS level of virulence (the total parasite-induced mortality) is

$$\nu^* = (a + b\delta_{\mathrm{I}})\varepsilon^*. \tag{2.12}$$

Therefore, the ESS virulence changes with an increase in background mortality as

$$\frac{\mathrm{d}\nu^*}{\mathrm{d}\delta_{\mathrm{I}}} = (a + b\delta_{\mathrm{I}})\frac{\mathrm{d}\varepsilon^*}{\mathrm{d}\delta_{\mathrm{I}}} + b\varepsilon^*. \tag{2.13}$$

The ESS virulence will increase with increasing background mortality whenever (2.13) is positive. The form of equation (2.13) illustrates that there can be situations in which the ESS level of host exploitation decreases with an increase in background mortality (i.e. $\mathrm{d}\varepsilon^*/\mathrm{d}\delta_{\mathrm{I}} < 0$) but the ESS virulence still increases (provided that $b\varepsilon^*$ is large enough). It is also possible, however (but less likely; P. D. Williams and T. Day, unpublished data), for both the ESS level of host exploitation and the ESS virulence to decrease with an increase in background mortality, provided that equation (2.13) is negative.

From an empirical standpoint, the above considerations demonstrate that, when comparing the virulences of parasites that have evolved under different mortality regimes, we can expect the result to depend on how the comparison is made. Which comparison is of most interest will depend on the question being addressed.

3. CONCLUSIONS

Investigations of the interactions between pathogen infections and other sources of mortality are well represented in the empirical literature. The bulk of the evidence suggests that non-independence of mortality due to parasites and mortality due to other sources is a general principle governing many host–pathogen interactions. These observations, together with our theoretical results, strongly suggest the need to reassess the widely held view that increases in host background mortality inevitably lead to the evolution of increased pathogen virulence.

These results also suggest a number of precautions for the empirical study of virulence evolution. First and foremost is the need to determine the extent to which mortality-source interactions are important. This can only be done through a thorough understanding of how the parasite in question exploits its host, and how this exploitation interacts with other mortality risks to which the host is exposed.

Similarly, although not always done in theoretical treatments, our results demonstrate that a clear distinction must be made between host exploitation and virulence (i.e. parasite-induced mortality) in order to make

empirical tests of the theory in this context meaningful and easier to interpret.

Finally, our results also demonstrate that, in the presence of mortality-source interactions, the question of how we expect virulence to evolve in response to background mortality becomes a much more subtle issue. The environment in which the virulence of parasite strains is assessed will play an important role in determining the virulence that is observed, and therefore different comparisons are predicted to yield different results (figure 4). As a consequence, one must be very specific about the question of interest in any given study before determining how best to make the comparison.

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