The curse of the pharaoh: the evolution of virulence in pathogens with long living propagules

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SUMMARY

We used a mathematical model to evaluate the hypothesis that parasites and pathogens with long living propagules should evolve high levels of virulence, i.e. high rates of pathogen-induced host mortality. Our model shows that the optimal level of virulence is independent of the longevity of the propagules either: (i) if the density or the prevalence of infected hosts is in (or fluctuates around) equilibrium; or (ii) if the death rate of the infected host population is high compared with that of the propagules. The level of virulence that maximizes the parasite's fitness (Malthusian parameter) increases with increasing longevity of its propagules only if the host–parasite system has not reached equilibrium and the death rate of the propagules is high relative to that of the infected hosts. Therefore, for parasites that have recently invaded a susceptible host population, greater propagule longevity may initially favour higher virulence; but once the equilibrium is reached the optimal virulence is independent of propagule longevity.

1. INTRODUCTION

When Howard Carter and Lord Carnavon entered the tomb of the Egyptian pharaoh Tutankhamen, little did they expect that Lord Carnavon would die shortly afterwards of a mysterious infectious disease which became known as 'The Curse of Osiris' (Corelli 1923). Although anecdotal, this report of a highly virulent and very long-lived 'sit and wait' pathogen suggests a possible connection between a pathogen's virulence and the longevity of its propagules outside the host. The ability of pathogen transmission stages to endure phases of low host density, or even temporary host absence, would seem to make them independent of the cost of virulence, i.e. the death of their hosts. Such thinking has led to the intuitively compelling hypothesis that increased longevity of pathogen propagules should favour the evolution of higher virulence (Ewald 1987, 1993, 1994), because a pathogen whose propagules are able to survive for long periods might benefit from exploiting its host more recklessly. Put the other way, this hypothesis also predicts that increased mortality of pathogen propagules, such as by improved sanitation, should lead to the evolution of less virulent pathogens (Ewald 1993, 1994).

This hypothesis seems consistent with the observation that many highly virulent parasites have long-lived propagules. Seeds of the virulent parasitic plants in the genus *Striga* can survive up to 20 years in the soil, and some plant-parasitic nematodes can wait for up to 10 years for their hosts (Stewart & Press 1990; Chappell 1993). Many devastating viruses, including nuclear-polyhedrosis and granulosis viruses, and several spore-forming bacteria can survive many years in the absence of a host (Anderson & May 1981). *Bacillus anthracis* might hold the record for longevity outside a host with a reported survival time for its spores of more than six decades (Duguid *et al.* 1978).

However, parasites with short-lived propagules are not necessarily avirulent, nor are all pathogens with long living propagules virulent. For example, horizontally transmitted microsporidian parasites, which typically produce long living spores (Milner 1972; Fuxa & Brooks 1979; Burges *et al.* 1971; Hurpin 1968; Henry & Oma 1974), range from high to low virulence (Ebert 1995; Mangin *et al.* 1995). Within the *Trichomonadida* (protozoa) are some species which form long-lived transmission stages, while other forms do not. None of the pathogenic species (e.g. *Trichomonas vaginalis*, *T. gallinae*, *Tritrichomonas foetus*) form these long lasting stages, but only the non-pathogenic forms (Mehlhorn & Piekarski 1985). Other pathogens exist which are virulent and yet have short-lived transmission stages, as for example the measles virus (Anderson 1993). The highly lethal honey bee pathogen, *Bacillus larvae*, forms endospores that survive only a few days outside the host (Wilson 1972). The same is reported for the conidia of the fungus *Nomuraea rileyi*, which infects caterpillars (Ignoffo *et al.* 1976).

These several conflicting examples point out the need for: (i) clear predictions based on a mathematical model; and (ii) comparative and experimental tests of

these predictions. To explore more thoroughly the relation between the evolution of virulence and the longevity of pathogen propagules, we formally analyse a mathematical model. In particular, we ask the question: does greater longevity of propagules favour higher levels of virulence?

2. THE MODEL

We consider three variables as functions of time: t $X(t)$, the density of uninfected (susceptible) hosts; $Y(t)$, the density of infected hosts; and $F(t)$, the density of free transmission stages. The simplest model of the host–parasite dynamics is:

$$dX/dt = \Phi(X, Y, F), \tag{1}$$

$$dY/dt = bXF - dY - vY, \tag{2}$$

$$dF/dt = kY - uF. \tag{3}$$

The rate of change of the uninfected host population is, in its most general form, a function $\Phi(X, Y, F)$ of the densities of the uninfected hosts, X, the infected hosts, Y, and the free infectious propagules, F. Newly infected hosts are generated at a rate proportional to the densities of susceptible hosts, X, and propagules, F, with a proportionality constant, b, which represents the infectivity (per contact and time) of propagules. Infected and uninfected hosts have a background death rate, d. Infected hosts have an additional pathogen-induced death rate, v, which is a measure of the pathogen's virulence. Propagules are produced at a rate k per infected host and die at a rate u. The average survival time of a propagule therefore is $s = 1/u$.

Note that we do not include the term $-bXF$ in the rate of change of the propagules in equation 3. This term would account for the loss of propagules because of their intake by susceptible hosts. We assume that the death rate, u, is the main cause of the elimination of propagules and that $-bXF$ can be neglected. The results of our model remain qualitatively unchanged if the term $-bXF$ is included in equation 3 (S. Bonhoeffer, unpublished results).

The basic reproductive rate of a parasite is defined as the average number of secondary infections caused by a single infected host in an entirely susceptible host population (Anderson & May 1979, 1981, 1991). For our model, the basic reproductive rate of the parasite is given by

$$R_0 = \hat{X}bk/((d+v)\,u),$$

where $\hat{X}$ is the equilibrium density of susceptible hosts in the absence of the parasite ($Y = F = 0$). An intuitive understanding of R_0 can be obtained by recognizing that $1/(d+v)$ is the average life time of an infected host; k is the rate at which an infected host produces infectious propagules; $1/u$ is the average life time of a propagule; and $\hat{X}b$ is the average rate at which propagules encounter and infect new hosts. All of these processes must occur in sequence for a pathogen to spread. The product of all these terms gives the expected number of secondary infections from a single infected host in an otherwise entirely susceptible host population.

If $R_0 > 1$ then the parasite can spread in an initially uninfected host population. Hence, for given $\hat{X}$, b, k, d and v, the survival time, s, of a parasite's propagule must fulfill

$$s = 1/u > (d+v)/(\hat{X}bk).$$

Therefore, a highly virulent parasite (large v) may need propagules with sufficiently long survival time (large s) to invade and persist in the host population. If $R_0 > 1$ and equations 1–3 converge to an equilibrium, then the equilibrium density of uninfected hosts is:

$$X^* = (d+v)\,u/(bk). \tag{4}$$

3. EVOLUTIONARY DYNAMICS

Consider two parasite strains competing for the same host. We assume that an individual host can be infected by only one strain of the parasite, so that intrahost competition between strains does not occur. The epidemiological dynamics are:

$$dX/dt = \Phi(X, Y_1, Y_2, F_1, F_2), \tag{5}$$

$$dY_1/dt = b_1 XF_1 - (d+v_1)\,Y_1, \tag{6}$$

$$dY_2/dt = b_2 XF_2 - (d+v_2)\,Y_2, \tag{7}$$

$$dF_1/dt = k_1 Y_1 - u_1 F_1, \tag{8}$$

$$dF_2/dt = k_2 Y_2 - u_2 F_2. \tag{9}$$

The two strains may differ in all parasite-specific parameters: infectivity (b_1, b_2), propagule production rate (k_1, k_2), death rate of propagules (u_1, u_2), and virulence $(v_{1,2})$.

Given that the infected hosts converge to equilibrium, then for a generic choice of parameters, there can be no coexistence of both parasite strains. Strain 2 can invade and replace strain 1 in the host population if

$$b_2 k_2/((d+v_2)\,u_2) > b_1 k_1/((d+v_1)\,u_1). \tag{10}$$

If this inequality is fulfilled, then strain 2 has positive growth rate when the uninfected hosts are at the equilibrium, X^* (equation 4), set by strain 1. More generally, the strain with the highest R_0 (equation 10) will hold the density of uninfected hosts to the lowest equilibrium value, X^*, and that strain can invade and exclude all other competitors.

If all parameters were independent of each other, then natural selection would decrease virulence, v, and increase transmission rate, b, propagule production rate, k, and the propagule's survival outside the host, $s(= 1/u)$. In general, however, a parasite's detrimental effects and its rate of propagule production are not necessarily independent parameters (May & Anderson 1979; Ewald 1983; Bull & Molineux 1992; Ebert 1994; Lenski & May 1994). For example it is often assumed that propagule production rate and parasite-induced mortality are positively correlated, and that

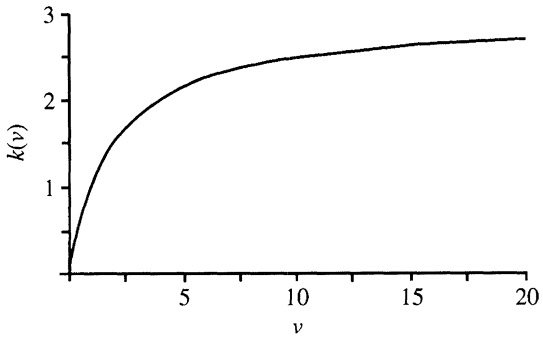

Figure 1. The production rate of infectious propagules, k, increases with increasing virulence, v, but saturates for high levels of virulence. $\alpha = 3$, $\beta = 2$ in equation 11.

there is a tradeoff between parasite transmission and virulence. However, the generality of this tradeoff has been questioned (Levin & Bull 1994; Bonhoeffer & Nowak 1994*b*). For our purposes, we simply allow the general possibility that the rate of propagule production, $k(v)$, may be some function of the parasite's virulence, v, but we need not make any specific assumptions about the form or even the direction of that relation. In host–parasite systems that are in equilibrium, the optimal level of virulence maximizes R_0 (and minimizes X^*) and is obtained by differentiating R_0 with respect to v:

$$\frac{\partial}{\partial v}\left(\frac{bk(v)}{(d+v)\,u}\right) = 0 \quad \text{and} \quad \left(\frac{\partial^2}{\partial v^2}\frac{bk(v)}{(d+v)\,u}\right) < 0.$$

If the death rate of an infectious propagule, u, is not a function of the virulence, v, then $1/u$ is a constant factor and the optimal virulence does not depend on u. Hence, the optimal virulence is not affected by the survival of a parasite's propagules outside the host.

We now illustrate our reasoning with a specific example. We assume that the production rate of propagules, k, increases as the virulence, v, increases, but saturates for high levels of virulence. A simple functional relation fulfilling these requirements is

$$k(v) = \alpha v/(\beta+v) \tag{11}$$

(see figure 1). Let us further assume that the propagule death rate, u, and infectivity, b, do not depend on the virulence, v. Then R_0 is given by

$$R_0 = \hat{X}b\alpha v/(u(d+v)\,(\beta+v)) \tag{12}$$

(see figure 2). The optimal virulence, which maximizes R_0, is

$$v_{\text{ESS}} = \sqrt{\beta d},$$

where the subscript ESS denotes the evolutionarily stable strategy. For the assumed tradeoff between propagule production and virulence (see equation 11), the optimal virulence depends only on the background host mortality, d, and the coefficient describing the shape of the tradeoff, β. In particular, the optimum is independent of the survival time, s, of the parasite's propagule stage.

In summary, we have shown that a parasite with given virulence needs to have propagules with a sufficiently long survival time outside the host to be able to invade a susceptible host population. Mathematically, this requires $R_0 > 1$. The optimal virulence, however, is independent of the propagules survival time. Mathematically, this is because the value of v that maximizes R_0 is independent of u.

So far we have assumed that the host–parasite system is in equilibrium. Next we analyse host–parasite systems that are not in equilibrium. Lenski & May (1994) showed that the optimal virulence when the host–parasite system is at equilibrium is not optimal when the host–parasite system is away from equilibrium. We consider three cases: (A) the dynamics of the infected hosts, Y, are fast compared with the dynamics of the propagules, F (i.e. $u \ll d+v$); (B) the

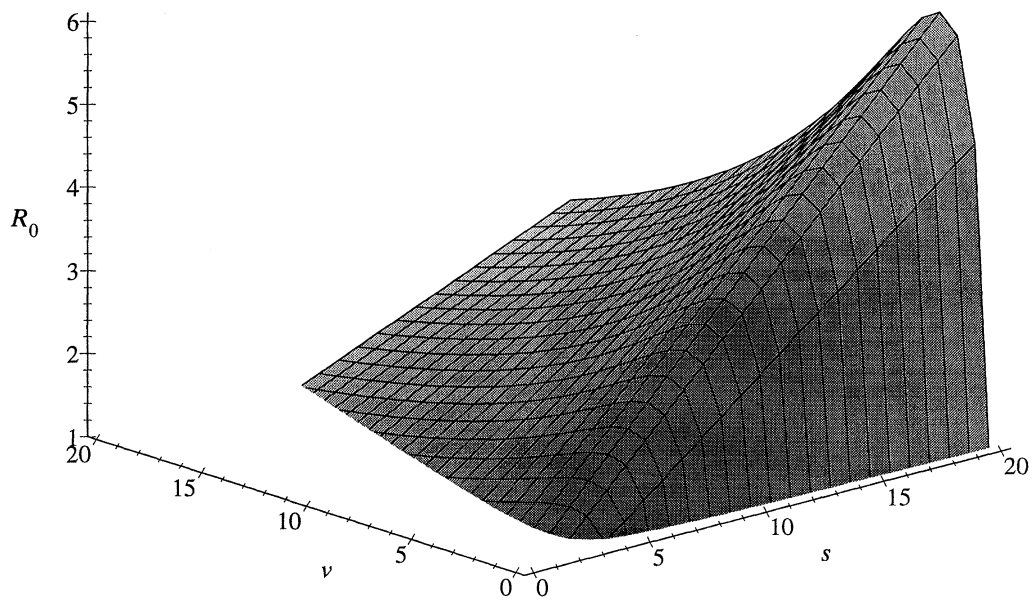

Figure 2. The basic reproductive rate of the parasite, R_0, in relation to propagule survival, $s = 1/u$, and virulence, v. R_0 is only shown for those combinations of s and v where $R_0 > 1$, the criterion for parasite persistence. $\hat{X}b\alpha = 9$, $\beta = 2$, $d = 3$; see equation 12.

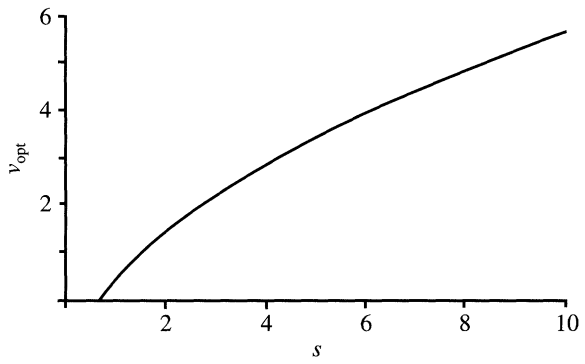

Figure 3. The optimal virulence, v_{opt}, which maximizes the parasite's rate of increase when the uninfected host population is not in equilibrium, as a function of propagule longevity, s. $\beta = 2$, $b\alpha X = 3$ in equation 17.

dynamics of F are fast compared with the dynamics of Y (i.e. $u \gg d+v$; see Jansen & Sabelis 1992); and (C) the number of infected hosts fluctuates around a time constant average.

In case A, we can assume that the infected hosts, Y, are in quasi-steady state, that is $dY/dt = 0$ at any time t. Then $Y = bXF/(d+v)$ and equations (1–3) reduce to

$$dX/dt = \Phi(X, F), \tag{13}$$

$$dF/dt = \left(\frac{bk}{d+v}X - u\right)F. \tag{14}$$

The parasite with maximal rate of increase for a given uninfected host density $X(t)$ is determined by the maximum of the Malthusian parameter,

$$r(t) = \frac{dF/dt}{F} = \frac{bk}{d+v}X(t) - u.$$

The r-maximizing virulence is obtained by differentiation and solving for v:

$$\frac{\partial}{\partial v}r(v) = \frac{\partial}{\partial v}\left(\frac{bk(v)}{d+v}X(t)\right) - 1 = 0.$$

Because the above expression is independent of u, the r-maximizing virulence does not depend on the propagule death rate, u. Hence, in host–parasite systems that are not in equilibrium, but where the dynamics of the infected host population, Y, are fast relative to those of the propagules, the r-maximizing virulence is independent of the survival time of the parasite's propagules.

In case B, we assume that the dynamics of the propagules, F, are fast compared with the dynamics of the uninfected hosts, Y, so that $dF/dt = 0$ and $F = kY/u$. Equations (1–3) then become

$$dX/dt = \Phi(X, Y), \tag{15}$$

$$dY/dt = \left(\frac{bk}{u}X - d - v\right)Y. \tag{16}$$

In this case, the optimal level of virulence that maximizes $r = (dY/dt)/Y$ does indeed depend on the propagule death rate, u. Assuming, for example, the tradeoff between infectious particle production rate, k, and virulence, v, given by equation (11), we obtain for r-maximizing virulence:

$$v_{opt}(t) = -\beta + \sqrt{[b\alpha\beta s X(t)]}, \tag{17}$$

where $s = 1/u$. Hence, $v_{opt}(t)$ increases with increasing propagule survival, s (see figure 3). However, as long as the system is not in equilibrium, there is no competitive exclusion and all strains with $r(t) > 0$ increase in abundance. In equilibrium, the r-maximizing and the R_0-maximizing virulences coincide.

Finally, we consider case C in which the number of infected hosts fluctuates around a time constant average. Then the time average of $r(t)$ is zero and we obtain

$$\langle r \rangle = \langle X \rangle bks - d - v = 0,$$

where $\langle . \rangle$ denotes the time average. The time average of the uninfected hosts is $\langle X \rangle = X^* = (d+v)/(bks)$. To invade a strain has to fulfill

$$\langle X \rangle \frac{bks}{d+v} > 1.$$

Hence, the strain with the largest R_0 outcompetes all other strains. We have shown above that the level of virulence which maximizes R_0 does not depend on u. Therefore, the optimal virulence is independent of the survival time of the pathogen propagules, if the number of infected hosts fluctuates around a time constant average.

4. DISCUSSION

The virulence of a parasite is typically defined in terms of its effect on host mortality. By this definition, some parasites are extremely virulent, whereas others are much less so, even though they may be quite closely related to forms that are highly virulent (Fenner & Ratcliffe 1965; Levin & Lenski 1985; Rosqvist *et al.* 1988). Whereas molecular biology may identify the genetic bases for intra- and interspecific differences in virulence (see, for example, Rosqvist *et al.* 1988), evolutionary biology seeks to identify ecological factors and physiological constraints that have produced these differences (Anderson & May 1982; Ewald 1983, 1993, 1994; May & Anderson 1983; Levin & Lenski 1985; Lenski 1988; Herre 1993, 1994; Nowak & May 1993, 1994; Bonhoeffer & Nowak 1994*a*, *b*; Bull 1994; Ebert 1994; Lenski & May 1994; Levin & Bull 1994; Lipsitch & Nowak 1994; Read 1994; Frank 1996).

Evolutionary analyses have made it clear that virulence cannot be understood in isolation, but instead must be considered in relation to other aspects of a parasite's life history, such as its rate of transmission. For example, if one strain of parasite produces more infectious propagules than another strain, then the former may have both greater virulence and increased transmissibility relative to the latter. In that case, selection may favour high or intermediate levels of

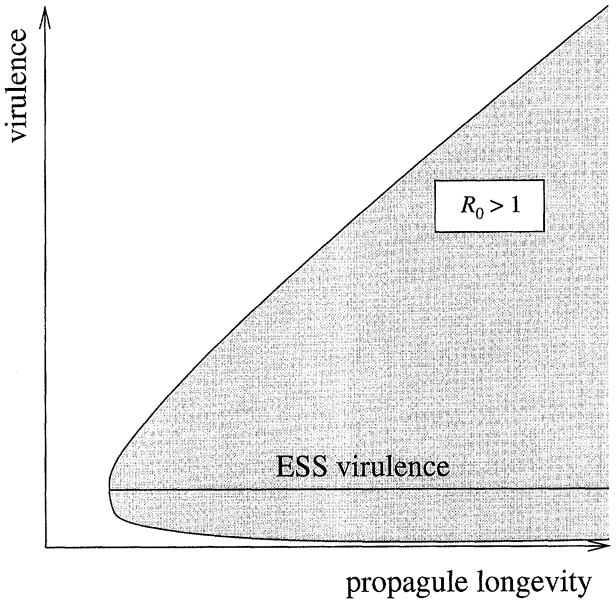

Figure 4. The shaded area shows combinations of virulence and propagule longevity for which parasite persistence is possible ($R_0 > 1$). The optimal virulence, indicated by a solid line, is independent of the propagule longevity.

virulence, whereas virulence would be minimized by selection if it were uncoupled to the rate of propagule production (May & Anderson 1983).

In this paper, we constructed a mathematical model to analyse formally the hypothesis that long-lived propagules should evolve high levels of virulence. The idea behind this hypothesis is that 'sit and wait' parasites are largely independent of the survival of their hosts, and hence they can afford to be more virulent. This hypothesis assumes the aforementioned tradeoff between virulence and propagule production, but it further postulates that optimal virulence (v_{opt}) should scale positively with the survival time of free propagules outside the host (s).

Our analyses show that the relation between propagule longevity and parasite virulence is much more complex than supposed by this hypothesis. In particular, whether v_{opt} depends on s depends, in turn, on whether the host–parasite interaction is at equilibrium as well as the nature of any deviations from the equilibrium.

1. If the system is at ecological and evolutionary equilibrium, then the hypothesized relation does not hold. In that case, the evolutionarily stable strategy for virulence (v_{ESS}) is independent of s (see figure 4).

2. The hypothesized relation between v_{opt} and s also does not hold if the infected host population fluctuates around some average density.

3. If the host–parasite system is in disequilibrium, but turnover of the infected host population is fast relative to turnover of the propagules, then v_{opt} is again independent of s.

On the other hand, our analysis also identified two circumstances in which a relation between virulence and propagule survival is expected.

4. There exists a minimum value for propagule survival that must be met for a parasite to invade a susceptible host population, and that threshold increases with increasing virulence. As a consequence, combinations of low propagule survival and high virulence simply cannot exist in self-sustaining parasite populations (see figure 4). The absence of these trait combinations might generate a statistical correlation between parasite virulence and propagule longevity in certain comparative studies.

5. If the host–parasite system is in disequilibrium, and if the dynamics of propagules are fast compared with the dynamics of infected hosts, then v_{opt} is indeed an increasing function of s, as hypothesized.

This last situation may apply to some human pathogens. First, for many human pathogens the population density of infected humans is not in equilibrium (nor fluctuates around equilibrium). Secondly, the propagules of most human parasites and pathogens probably turn over much faster than their long-lived hosts. (However, this assertion may be complicated by turnover in the infected host population because of recovery, which was not included in our simple model.) Ewald (1993, 1994) has postulated that public health measures that reduce propagule survival – such as improved water treatment for waterborne diseases – should not only greatly reduce the incidence of disease but also favour the evolution of less virulent strains. His analyses of temporal and spatial trends in the relative virulence of *Vibrio cholerae* strains isolated from communities with varying water quality seem to conform to this prediction (Ewald 1994). If one accepts that the interaction between *V. cholerae* and humans fulfills (5) above, then our analysis supports the theoretical plausibility of this relation, which clearly has important implications for public health. However, it should also be pointed out that there are alternative explanations for reduced virulence of *V. cholerae* in regions with improved water quality. For example, poorer sanitation increases the likelihood of superinfection with individual hosts, whereby more rapidly growing – and presumably more virulent – strains may displace ones that grow more slowly (Nowak & May 1994).

Ideally, one would like to test the specific predictions of our model using either comparative (Harvey & Pagel 1991) or experimental (Levin & Lenski 1985; Bull *et al.* 1991; Ebert 1994) approaches. However, using comparative approaches to test these predictions may prove to be difficult, as it has to be established whether particular host–parasite interactions are in ecological and/or evolutionary equilibrium. In addition, one would have to control for all other factors that might influence the evolution of virulence. Therefore selection experiments, in which propagule survival can be manipulated and equilibria perturbed by varying host and parasite densities, may offer a better opportunity to verify or falsify the predictions of our model. For example, by growing populations of *Daphnia* and its microsporidian parasites (Ebert 1994, 1995) in the laboratory, one might manipulate the survival of the parasites outside their hosts by varying the frequency at which dirty culture medium is replaced with fresh medium.

Finally, in our analyses, we allowed the possiblity of a tradeoff (or other functional relation) between

parasite virulence and propagule production. However, we did not assume any functional constraint between propagule longevity and other traits. It is not obvious to us that such constraints are an essential feature of the hypothesis. But one could imagine, for example, that increased propagule longevity requires an energetic expenditure, which would reduce the parasite's rate of propagule production. Perhaps future studies will examine how tradeoffs between propagule longevity and other parasite traits might alter our conclusions. For now, we have shown that the relation between propagule longevity and parasite virulence is quite complex even without these additional constraints.

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