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LETTER

Virulence evolution via host exploitation and toxin production in spore-producing pathogens

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Abstract

Many pathogens produce resilient free-living propagules that allow their dissemination in the absence of direct contact between susceptible and infected hosts. One might expect pathogens capable of producing such long-lived propagules to evolve high levels of virulence because their reproductive success is de-coupled from the survival of their host. Despite some comparative data supporting this prediction, theory has questioned its general validity. I present theoretical results that incorporate two transmission routes neglected by previous theory: death-mediated propagule production and direct host-host transmission. This theory predicts that spore-producing pathogens should evolve high levels of virulence under quite broad conditions. Moreover, a novel prediction of this theory is that the production of propagules can generate selection for the evolution of pathogen characteristics such as toxins whose sole function is to kill the host. This latter result reveals an unanticipated mechanism through which virulence is expected to evolve in spore-producing pathogens.

Keywords

Curse of the Pharaoh, disease, parasite, mortality.

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INTRODUCTION

Many pathogens produce free-living propagules, and it has been suggested that such propagule production results in the evolution of higher levels of virulence (defined as parasite-induced mortality rate) because a pathogen's reproductive success is thereby de-coupled from the survival of its host (Ewald 1987, 1994; Prescott *et al.* 1999). Nevertheless, theoretical results have called these verbal hypotheses into question (Bonhoeffer *et al.* 1996). This previous theoretical research has, however, focused solely on pathogens that produce propagules while an infected host is alive, and have thereby ignored two features that are common to many spore-producing pathogens: death-mediated propagule production and direct host-host transmission. I present theory demonstrating that each of these transmission routes can have important evolutionary consequences, and that they often lead to the evolution of higher virulence in pathogens that produce long-lived propagules. This theory also makes the unanticipated prediction that, under some circumstances, such pathogens should evolve host-killing traits such as toxin production. The dominant paradigm in studies of virulence evolution has been that a pathogen must exhibit a high level of host exploitation to obtain a high rate of transmission, but that such exploitation results in high pathogen-induced host mortality as well. Therefore, virulence is expected to evolve only when a positive relationship between transmission and host mortality is unavoidable. The results presented here illustrate that virulence in spore-producing pathogens can evolve through traits such as toxin production as well, even in the absence of an association between the degree of host exploitation and host mortality.

I present a general mathematical model describing the epidemiology of a pathogen that is able to persist freely in the environment, and that can be transmitted between hosts via three distinct routes: (i) directly from one living host to another, (ii) indirectly from one host to another through the release of free-living infectious propagules into the environment during an infection, and (iii) indirectly through the release of free-living infectious propagules into the environment upon death of an infected individual. I use the terms propagule and spore interchangeably, and make no distinction between propagules that are specialized environmental 'spores' and those that are simply normal pathogen cells that can persist outside of a host. I note that Ebert & Weisser (1997) analysed a model of parasite evolution where transmission occurs only through propagules that are released upon host death. Because such parasites are

'obligate killers', however, they focused on the evolution of the timing of host death rather than on the evolution of virulence (as defined here, i.e. parasite-induced mortality).

The importance of each of the above three routes will vary depending upon the pathogen, but many pathogens use all three routes to some degree. For example, the pathogens causing smallpox and diphtheria can remain viable in the environment for relatively long periods of time (Prescott *et al.* 1999) whereas others such as influenza, while persisting for some period of time outside of a host, cannot do so for long. Similarly, the extent to which pathogen propagules are released upon death of an infected host varies. Diseases such as Kuru in humans (Caughey & Chesebro 1997) and bovine spongiform encephalopathy in cattle (Donnelly *et al.* 1999) are, in effect, transmitted through the release of pathogens upon host death, since transmission occurs via consumption of a dead infected host. For other pathogens such as *Bascillus anthracis* and many nuclear polyhedrosis and granulosis viruses, death-mediated propagule production is a major transmission route (Prescott *et al.* 1999; Mock & Fouet 2001). Using S, I, and F to denote the densities of susceptible hosts, infected hosts, and free-living propagules, the epidemiological model I use is

$$\begin{aligned}\frac{dS}{dt} &= \Phi(S, I, F)\\ \frac{dI}{dt} &= \beta SI + \sigma SF - \mu I - \nu I - \gamma I\\ \frac{dF}{dt} &= \kappa I + (\mu + \nu)I\omega - \delta F\end{aligned} \tag{1}$$

Here β is the direct transmission rate between hosts, σ is the transmission rate of propagules to susceptible hosts, κ is the rate of propagule release by a living infected host, and ω is the number of propagules released upon death of an infected host. The parameters μ, ν, and γ are the disease-independent death rate, the pathogen-induced death rate (i.e. virulence; Frank 1996; Bull 1994; Read 1994; Ebert & Herre 1996; Levin 1996) and the rate of clearance of infection through host defence mechanisms, respectively. The parameter δ is the loss rate of propagules from the environment through death/degradation, and $\Phi(S,I,F)$ is a function describing the rate of change of susceptible hosts as a result of infection, births, deaths, immigration and emigration. This formulation assumes that all hosts that die release propagules regardless of how they died. Except where noted below, the results presented are qualitatively unchanged if only those hosts that are killed by the parasite actually release propagules.

Assuming that the system (eqn 1) reaches a stable endemic equilibrium, the density of susceptible hosts is

$$\hat{S} = \frac{\delta(\mu + \nu + \gamma)}{\beta\delta + \sigma\kappa + \sigma\omega(\mu + \nu)}. \tag{2}$$

If different parasite strains compete with one another, then in the absence of co or super-infection (Nowak & May 1994; May & Nowak 1995), the evolutionarily stable strain is the one that reduces $\hat{S}$ to the lowest possible level because both pathogen types are competing for a common resource (susceptible hosts) (Armstrong & McGehee 1980; Bremermann & Thieme 1989). Thus the evolutionarily stable strain is the one with the largest value of $R \equiv 1/\hat{S}$, which is given by

$$R = \beta L + \kappa L D\sigma + \chi\omega D\sigma. \tag{3}$$

I have simplified notation by defining, $L = 1/(\mu + \nu + \gamma)$, the expected duration of an infection; $D = 1/\delta$, the expected duration a propagule remains viable; and $\chi = (\mu + \nu)/(\mu + \nu + \gamma)$, the probability that an infected host dies before the infection ends as opposed to recovering. Note that in the absence of propagules (i.e. $\kappa = 0$, $\omega = 0$) eqn 3 reduces to $R = \beta L$, which is the standard measure of fitness used in many previous epidemiological models (Frank 1996).

Epidemiologically, eqn 3 is the expected number of new infections produced by a single infected host, per susceptible host, and reveals the three routes through which the pathogen can gain reproductive success. The first term is the expected number of new infections from direct transmission (the rate of transmission between hosts is β, and L is the expected life span of an infection, making βL the expected number of new infections). The last two terms are the expected number of new infections from indirect transmission via propagules. The latter terms contain $D\sigma$, which is the expected number of new infections per unit propagule abundance. The first of these is multiplied by κL, the abundance of propagules released during an infection (i.e. this occurs at rate κ over L units of time), whereas the second is multiplied by χ and ω, which are the probability that the host dies before the infection ends, and the number of propagules released upon death (note that propagule loss through new infections is assumed negligible).

Virulence evolution via host exploitation

There is some evidence suggesting that higher levels of within-host replication (denoted by ε) often results in higher transmission rates between hosts, β, but higher virulence, ν, as well (Anderson & May 1982; Ebert 1994; Ebert & Mangin 1997; Lipsitch & Moxon 1997; Mackinnon & Read 1999; Messenger *et al.* 1999). If within-host replication is too large, however, then transmission between hosts will decrease again because such infections result in lower activity levels of the host and thus less contact with other susceptible individuals (Day 2001). Consequently, I assume that $\beta(\varepsilon)$ increases with ε, attaining a maximum for some intermediate value of ε. Similarly, higher within-host

replication, ε, results in greater propagule release during an infection, $\kappa(\varepsilon)$, as well as greater propagule release upon death of an infected host, $\omega(\varepsilon)$; $\kappa(\varepsilon)$ might also reach a maximum for an intermediate value of ε, but $\omega(\varepsilon)$ will likely increase monotonically because higher replication results in higher propagule release upon host death.

How does propagule longevity, D, affect virulence evolution? Previous theory has implicitly assumed no direct or death-mediated transmission ($\beta = 0$, $\omega = 0$) reducing eqn 3 to $R = \kappa LD\sigma$ (Bonhoeffer *et al.* 1996). This has lead to the conclusion that propagule longevity has no effect on the optimal level of ε (and thus virulence). However, accounting for direct and death-mediated transmission results in the prediction that greater propagule longevity, D, increases the relative importance of propagules as a component of pathogen fitness (see eqn 3). Therefore, if pathogen transmission through propagules selects for the evolution of higher virulence than does transmission through direct contact, then we expect greater propagule longevity to cause the evolution of higher virulence.

Pathogen transmission through propagules is likely to select for the evolution of higher virulence than direct transmission for at least two reasons. First, although β (the direct transmission rate) and κ (the propagule production rate while alive) might both have maxima at intermediate values of ε, the maximum of β will usually occur at a lower value of ε than that of κ meaning that transmission through propagules selects for higher virulence. The reason is that direct transmission requires an infected host to be active and to contact a susceptible host, whereas effective propagule production merely requires propagules to be transmitted into an environmental pool regularly contacted by susceptible hosts. Even if a host is extremely ill, its propagules can be transferred to the environmental pool by abiotic (e.g. wind or water) as well as biotic (e.g. attendants or other animals) means, thereby partially de-coupling activity level from the ability to infect, and selecting for higher pathogen replication (and thus virulence). Second, whenever there is some death-mediated transmission, this always selects for extreme replication (and thus virulence) because replication increases the abundance of propagules released upon death, as well as the probability that death occurs as opposed to recovery. Therefore, higher propagule longevity will select for higher virulence in pathogens transmitted by both propagules and by direct contact (Fig. 1).

The above considerations reveal when we should expect increased propagule longevity to result in the evolution of increased virulence, and they also demonstrate that we expect pathogens transmitted solely through propagule production to evolve a higher virulence than those that are transmitted solely by direct means, irrespective of propagule longevity. Indeed, propagule longevity has no effect on virulence evolution for pathogens transmitted solely through free-living propagules (as previous theory has shown; Bonhoeffer *et al.* 1996), but such pathogens should nevertheless evolve a higher level of virulence than those transmitted solely by direct contact for reasons outlined in the preceding paragraph.

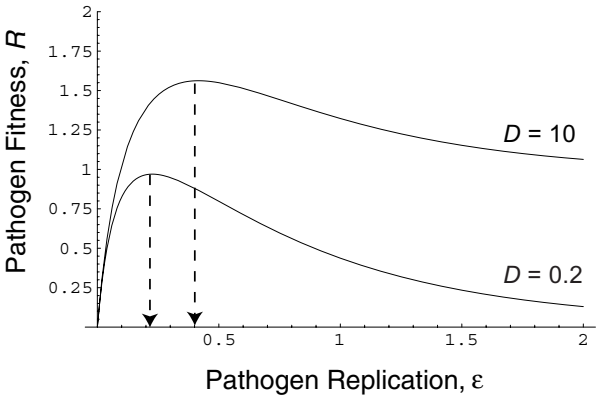

Figure 1 Pathogen fitness, plotted against replication rate, ε, for two different values of propagule longevity ($D = 10$ and $D = 0.2$). The maximum fitness occurs at a higher replication level and thus a higher virulence under higher propagule longevity. Functional forms and parameter values are $v(\varepsilon) = \varepsilon$, $\beta(\varepsilon) = 2(1 - e^{-\zeta_\beta \varepsilon})e^{-\varepsilon}$, $\omega(\varepsilon) = (1 - e^{-\zeta_\omega \varepsilon})$, $\kappa \equiv 0$ and $\sigma = 0.1$, $\zeta_\beta = 1$, $\zeta_\omega = 5$, $\mu = 0.01$, $\gamma = 0.1$. See section *Virulence evolution via host exploitation* for justification of the difference in parameter values between β and ω (which result in β having its maximum at a smaller value of ε than does ω).

Virulence evolution via toxin production

Most previous theory, including that above, assumes that virulence (pathogen-induced host mortality) evolves indirectly as an unavoidable, correlated response to selection for increased within-host replication through its benefits on pathogen transmission (Bull 1994; Read 1994; Ebert & Herre 1996; Frank 1996; Levin 1996). Nevertheless, some pathogens have traits that result in substantial host mortality without having any obvious connection to within-host replication. For example, several bacteria species such as *Bacillus anthracis* and *Corynebacterium diphtheria* produce toxins that are substantial determinants of host mortality (Prescott *et al.* 1999). While it is possible that toxins enhance within-host replication (e.g. by compromising the immune system), the existence of successful pathogens that do not produce obvious toxins demonstrates that such constraints are not universal. I now use the theory developed above to demonstrate that such toxin production can actually be selectively advantageous because it kills the host. This has the important implication that virulence through toxin production (or any other trait increasing host mortality) is

predicted to evolve in many spore-producing pathogens even in the absence of a positive association between replication within the host and host mortality.

Suppose that pathogens are characterized by two traits, their replication strategy, ε, and their level of toxin production, τ. As above, I assume that the beneficial effects of within-host replication occur through the dependence of β, κ, and ω, on ε. Now, I assume that pathogen-induced host mortality, ν, is an increasing function of toxin production. I also allow ν to increase with replication, ε, but as will be seen such constraints are not necessary for pathogens to evolve virulence (i.e. to cause host mortality).

Regardless of whether there is a mortality cost to within-host replication, the direction of selection on toxin production can be found by differentiating equation 3 with respect to τ;

$$\frac{\partial R}{\partial \tau} \propto \gamma\omega D\sigma - (\beta + \kappa D\sigma). \quad (4)$$

If, $\partial R/\partial\tau > 0$, then greater toxin production is selectively favoured and vice versa. Thus, toxin production is always selected against whenever $\omega = 0$; it is never advantageous for a pathogen to 'directly' kill its host in the absence of death-mediated propagule production because there is no direct selective advantage to host killing. If death-mediated propagule production is the only route for pathogen transmission, however, then toxin production is always selectively favoured because it increases the probability that the host will die and thus spread the pathogen before the infection ends. Of course, for toxin production to ever be favoured, there must also be some chance that the infection will end prior to the host dying (i.e. $\gamma \neq 0$). Alternatively, if we assume that only those hosts that are killed by the parasite actually release propagules, then γ in eqn 4 is replaced by $\gamma + \mu$. Then, even in situations where $\gamma = 0$, toxin production can be selectively favoured.

Importantly, toxin production can also be selectively advantageous even when all three routes of pathogen transmission are used, provided that $\gamma\omega D\sigma > \beta + \kappa D\sigma$. An examination of this inequality reveals two clear predictions. First, the presence of free-living propagules (and death-mediated propagule production) is necessary for the evolution of toxin production, providing another reason why such pathogens might be more virulent. Second, for pathogens that have some direct transmission, greater propagule longevity makes it more likely that this inequality is satisfied. Thus greater propagule longevity makes it more likely that toxin production will be advantageous, providing another reason to expect a positive association between propagule longevity and virulence (compare Figs 2a and b).

CONCLUSIONS

The theory presented here demonstrates that endemic, propagule-producing pathogens are expected to evolve higher levels of virulence owing to the de-coupling of their reproductive success from the survival of their hosts. Additionally, this theory makes the prediction that traits such as toxin production can evolve in spore-producing pathogens solely to kill the host. The dominant paradigm in studies of virulence evolution has been that pathogen-induced mortality is an unavoidable consequence of within-host replication, and there is a well-developed epidemiological theory for exploring such effects (Frank 1996). The

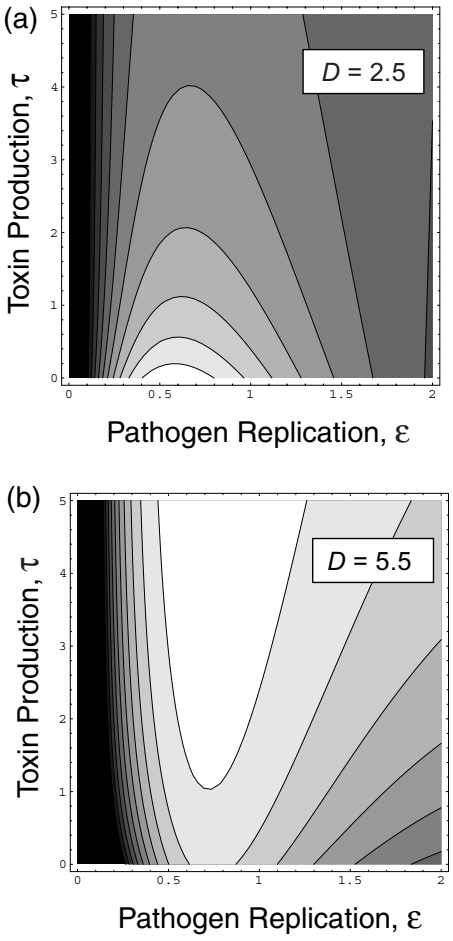

Figure 2 Contour plots of pathogen fitness, eqn 3, against replication level, ε, and toxin production, τ, for two different values of propagule longevity ($D = 2.5$ and $D = 5.5$). Lighter shading represents higher fitness. Toxin production is selected against when propagule longevity is low ($D = 2.5$) but it is selected for when longevity is high ($D = 5.5$). Functional forms and parameter values are $\nu(\varepsilon,\tau) = \varepsilon + \tau$, $\beta(\varepsilon) = 2(1 - e^{-\zeta_\beta\varepsilon})e^{-\varepsilon}$, $\omega(\varepsilon) = (1 - e^{-\zeta_\omega\varepsilon})$, $\kappa \equiv 0$ and $\sigma = 0.1$, $\zeta_\beta = 1$, $\zeta_\omega = 5$, $\mu = 0.01$, $\gamma =$ a) $D = 2.5$. (b) $D = 5.5$.

epidemiological theory presented here highlights a different route to virulence evolution, and demonstrates that such constraints are not necessary for virulence evolution in spore-producing pathogens. Indeed it predicts that virulence determinants unrelated to such host exploitation should evolve in these pathogens.

Another way to illustrate this result is to step back from the particular trade-offs between transmission and virulence assumed above, and simply ask how selection acts on the various parasite fitness components. Equation 3 reveals the obvious result that natural selection always favours an increase in all transmission rate components (β, κ, ω and σ) because this will increase fitness. Contrary to many previous models, however, natural selection can favour the evolution of an increase or a decrease in parasite-induced mortality (i.e. virulence, v) depending upon this magnitude of each transmission route. In particular, differentiating eqn 3 with respect to v yields an expression that is proportional to eqn 4. Therefore, eqn 4 can be viewed as a general expression that, when positive, indicates that natural selection favours the evolution of increased parasite-induced mortality. This increased mortality might come about through many different mechanisms, including toxin production (as postulated above) as well as simply though increased host exploitation if exploitation is positively associated with virulence. In the latter case, however, if transmission is maximal at some intermediate level of exploitation (e.g. because extremely high exploitation reduces the likelihood that an infected host will contact a susceptible host; Day 2001) then selection will favour the evolution of host killing strategies that are unrelated to host exploitation as well.

Another interesting (and currently unresolved) issue concerns the relative timing of toxin production vs. pathogen replication. Toxin production should be strategically timed to balance conflicting demands, allowing large numbers of pathogens to build up in the host for transmission and release both while alive and upon death, but still ensuring that the host dies rather than recovers so as to reap the benefits of death-mediated propagule production. This issue could be explored by combining the present theory with that of Ebert & Weisser (1997). Many pathogens have mechanisms through which reliable cues for the timing of toxin production are possible (e.g. quorum sensing in bacteria; Miller & Bassler 2001) and therefore it would be worthwhile extending theory in this direction.

An alternative hypothesis for why spore-producing pathogens have high virulence involves the possibility that such pathogens are more frequently subject to multiple infections, and that the ensuing within-host competition selects for the evolution of higher virulence (Gandon 1998). It is not yet known whether heightened levels of multiple infection actually do occur in spore-producing pathogens, but if so, then a feedback between multiple infection and toxin production is also possible. Most theory considering within-host competition assumes that the evolution of higher parasite replication is countered by its associated mortality costs. Once toxin production has evolved, however, these mortality costs can be greatly reduced (T. Day, unpublished results), and within-host competition will then be less opposed by such mortality costs, potentially leading to the evolution of even greater virulence.

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