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REVIEW

Mechanisms of pathogenesis and the evolution of parasite virulence

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Abstract

When studying how much a parasite harms its host, evolutionary biologists turn to the evolutionary theory of virulence. That theory has been successful in predicting how parasite virulence evolves in response to changes in epidemiological conditions of parasite transmission or to perturbations induced by drug treatments. The evolutionary theory of virulence is, however, nearly silent about the expected differences in virulence between different species of parasite. Why, for example, is anthrax so virulent, whereas closely related bacterial species cause little harm? The evolutionary theory might address such comparisons by analysing differences in tradeoffs between parasite fitness components: transmission as a measure of parasite fecundity, clearance as a measure of parasite lifespan and virulence as another measure that delimits parasite survival within a host. However, even crude quantitative estimates of such tradeoffs remain beyond reach in all but the most controlled of experimental conditions. Here, we argue that the great recent advances in the molecular study of pathogenesis provide a way forward. In light of those mechanistic studies, we analyse the relative sensitivity of tradeoffs between components of parasite fitness. We argue that pathogenic mechanisms that manipulate host immunity or escape from host defences have particularly high sensitivity to parasite fitness and thus dominate as causes of parasite virulence. The high sensitivity of immunomodulation and immune escape arise because those mechanisms affect parasite survival within the host, the most sensitive of fitness components. In our view, relating the sensitivity of pathogenic mechanisms to fitness components will provide a way to build a much richer and more general theory of parasite virulence.

Introduction

Why have some parasites evolved to make their hosts very sick, whereas others cause little harm? For the past 25 years, the evolutionary theory of virulence has been the only framework in which to address this key question (Anderson & May, 1982; Bull, 1994; Ewald, 1994; Frank, 1996). In this paper, we first argue that the evolutionary theory of virulence has been successful only for a very small and particular subset of the general problem of host illness caused by infectious disease. We then propose a new approach that makes specific predictions about the severity of infectious disease in the vast majority of cases for which the standard evolutionary theory is silent.

We base our approach on the biochemical mechanisms by which parasites manipulate host immune responses, invade host tissues, and control the flow of host resources. Those biochemical mechanisms of the parasite comprise *the mechanisms of pathogenesis*. We argue that the mechanisms of pathogenesis determine the relations between virulence and components of parasite fitness, such as transmission to new hosts and survival within the host. By making explicit how the biochemical mechanisms of pathogenesis set the relations between parasite

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fitness and virulence, we expand the conceptual framework of parasite virulence to encompass the many cases that the prior theories of virulence did not address. In this paper, we often use the word *parasite* for both microbial pathogens (microparasites) and multicellular parasites (macroparasites).

Consider first the standard evolutionary theory of virulence, which sets the background and defines the limitations of current thinking. That theory greatly clarifies how the damage a parasite causes to a host evolves in response to a *change* in some key factor of parasite biology. To study the evolution of virulence in response to change means, for example, to focus on how the parasite evolves in response to a change in the density of hosts available for transmission, or how the parasite evolves in response to a change in the dosage of a drug that clears the parasite from the host.

In each of these cases, the problem turns on the evolutionary change of a particular parasite population in response to a change in conditions. Well-defined comparisons have shown, for example, that parasites with increased opportunities for vertical transmission and reduced opportunities for horizontal transmission often become less virulent (Bull *et al.*, 1991; Agnew & Koella, 1997; Messenger *et al.*, 1999). Similarly, a new epidemic that begins by zoonotic transfer of a parasite from one host species to another may at first be highly virulent in the new host, as in the 1918 influenza pandemic that began when an avian influenza virus spread in humans; however, such severe virulence often attenuates upon further transmission in the new host (Fenner & Ratcliffe, 1965; Allison, 1982).

In these two examples, the evolutionary argument is that severe virulence kills the host before the parasite can fully exploit opportunities for transmission to new hosts, so less virulent variants of the parasite tend to increase over time (Anderson & May, 1982; Frank, 1996). Such reasoning provides a very useful framework in which to evaluate how parasites may respond to changing conditions. The clear predictions of that theory have led to a successful literature on parasite response in laboratory experiments that manipulate conditions and in field studies comparing populations that naturally experience different pressures on transmission, competition and clearance.

The evolutionary theory is, however, nearly silent about the expected level of virulence in the vast majority of cases. Why does infection by *Bacillus anthracis* (anthrax) often send a human host into mortal shock, whereas the closely related *B. cereus* causes mild disease? Why was smallpox (Orthopoxvirus variola) a human terror with a 25–30% mortality rate, whereas the similar Orthopoxvirus alastrim (*Variola minor*) causes a much less dangerous disease with a mortality rate of only a few percent? Each of these questions compares different species of parasite. In fact, most comparisons one naturally encounters in the study of parasite biology concern differences between species of parasite rather than how a particular parasite population responds to a change in conditions.

In this paper, we show that mechanistic analyses of pathogenesis suggest a way to understand the evolution of virulence for the many cases in which the traditional evolutionary theory of virulence remains silent. We emphasize two predictions. First, pathogenic mechanisms that manipulate host immunity or escape recognition by host immunity will dominate with regard to the evolution of virulence. Second, pathogenic mechanisms that raise the transmission rate late in infection also tend to cause highly virulent disease. Our main goal is to frame such problems clearly, so that future work may link mechanistic aspects of pathogenesis to evolutionary aspects of virulence.

Overview

In this section, we provide a brief overview of how we integrate the mechanisms of pathogenesis into a broader evolutionary theory of virulence.

Tradeoffs

All evolutionary theories of virulence arise from tradeoffs between components of parasite fitness. For example, higher parasite density within a host may increase transmission of the infection to other hosts. Transmission effectively measures the birthrate of the parasite – its fecundity. Higher parasite density may also kill the host more rapidly. Host death cuts short the length of the infection within a host. Length of infection effectively measures the parasite's lifespan. In this case, parasite density within the host induces a tradeoff between parasite fecundity and lifespan. The evolutionary outcome that balances between fecundity and lifespan determines, as a consequence, how much damage the parasite causes to its host, that is, its level of virulence.

Other types of tradeoffs occur between components of fitness. There may, for example, be a tradeoff between two aspects of fecundity measured by transmission: the number of parasites released from the host and the success per released parasite. Greater parasite density could cause more parasites to be released, but also cause the host to become so sick that it would contact other hosts less frequently. In this case, natural selection would favour a balance between the benefits of high density associated with a greater number of parasites released and the costs of high density associated with lower success in transmission per parasite released. Parasite virulence would evolve as a consequence of the balance between the costs and benefits of different components of fecundity. Alternatively, parasite density may affect two aspects of longevity: clearance of the parasite from the host and host survival. The level of

virulence follows from the balance between different aspects of longevity.

In these examples, one would like to know exactly how parasite density affects transmission, host lifespan and other factors that influence components of parasite fitness. However, measuring fitness components directly is difficult except in the most controlled laboratory conditions. Thus, one can rarely measure tradeoffs directly. In addition, parasite density is just one character. Other parasite characters have different effects on various tradeoffs between fitness components. So one cannot easily determine which tradeoff dominates the evolution of parasite traits.

Scope of tradeoff theory

If one can rarely measure tradeoffs, how can an evolutionary theory of virulence based on tradeoffs be useful? The current theory avoids the need to measure tradeoffs by predicting the way in which closely related populations change in response to changed demographic or environmental conditions. The theory implicitly assumes that related parasite populations face the same tradeoffs between components of fitness, without knowing exactly the nature of those tradeoffs. Suppose, for example, that a tradeoff exists between the benefits of greater transmission – fecundity – and the expense of killing the host more rapidly – shorter lifespan. If such a tradeoff exists, then a population that experiences greater opportunities for transmission should evolve higher virulence than a closely related population that experiences lesser opportunities for transmission – a prediction about change in direction that is relatively easy to test. Consistent success in predicting how changes in transmission opportunities lead to changes in virulence suggests that the underlying tradeoff does in fact exist. This comparative formulation of the theory works well with regard to predicting change between closely related populations without the need to understand or measure tradeoffs.

But what about comparing different species of parasite that have diverged in the biochemical and physiological mechanisms that induce tradeoffs? Here, the current theory says that each species evolves to balance its own unique tradeoffs. However, because we cannot measure those tradeoffs directly, that theory provides little guidance.

How can we predict differences in virulence between species of parasite that do not share a common tradeoff? If we understood the mechanistic basis of tradeoffs, then we might be able to predict how such tradeoffs differ between species. Unfortunately, we are not likely to know exactly how particular mechanisms of pathogenesis determine quantitative aspects of fitness components and their associated consequences for virulence. So, we must look for some general aspects of pathogenesis that lead to comparative predictions: how does virulence evolve differently between two parasite species when each species expresses different mechanisms of pathogenesis?

Sensitivity of fitness components

To develop a theory that compares virulence between different species, one has to understand the relative sensitivity of different components of parasite fitness. Suppose, for example, that a parasite can suppress the host's immune system. More extreme suppression may have two opposing effects on parasite fitness. On the one hand, more strongly inhibiting host immunity reduces the rate at which the parasite is cleared from the host. On the other hand, stronger immune inhibition may increase the rate at which the host eventually dies from the consequences of infection – an increase in virulence. The parasite always gains strongly by slowing the rate at which it is cleared. By contrast, the parasite loses relatively little by host death if such death sometimes occurs near to or after the time at which the parasite would be cleared from the host. In this regard, the parasite will often be strongly sensitive to clearance, and relatively weakly sensitive to host death.

The evolutionary balance will often tip in favour of the strongly sensitive fitness component and against the weakly sensitive component. In our example, a tradeoff between clearance and host death will often tip in favour of fighting against clearance at the expense of increased host death. By contrast, during initial invasion by the parasite, high virulence leading to host death early in the infection would kill the infection before it is transmitted to new hosts. Thus, the lifespan of the infection, mediated by virulence, would be a strongly sensitive fitness component during invasion. Such sensitivity may often tip the balance in favour of mildly virulent mechanisms of pathogenesis during invasion.

The exact nature of a tradeoff will, of course, depend on the specific mechanism of pathogenesis and its consequences for fitness components. Indeed, our argument focuses particularly on how one can classify certain kinds of pathogenic mechanisms with regard to the relative sensitivities they induce in opposing components of fitness.

One important aspect of sensitivity concerns the age of the infection at which a pathogenic mechanism acts – that is, the length of time since the initiation of infection. Mechanisms with early age-specific effects tend to be strongly sensitive, whereas mechanisms with late age-specific effects tend to be weakly sensitive (Frank, 1996; Levin *et al.*, 1996; Day, 2003). Thus, tradeoffs between early and late components of fitness typically favour the early components – a point emphasized in the antagonistic pleiotropy theory of aging (Williams, 1957; Rose, 1994). In the following section, we discuss how age-specific effects provide insight into the relations between pathogenesis and virulence.

Predicted association between pathogenesis and virulence

The literature on pathogenic mechanisms and immune evasion is vast and covers all major parasite groups, e.g. viruses (Tortorella *et al.*, 2000), bacteria (Hornef *et al.*, 2002), protozoa (Sacks & Sher, 2002), fungi (Rappleye & Goldman, 2006) and helminths (Loukas & Maizels, 2000). We do not review this literature or provide an exhaustive analysis of different mechanisms. Instead, we present examples of how we may relate pathogenic mechanism to consequences for virulence.

Mechanisms to escape host immunity: theory

We develop our two key predictions in this section. First, pathogenic mechanisms designed to manipulate or escape host immunity typically dominate in causing virulent effects on the host. Second, in comparing between species, those parasites that rely more strongly on immunomodulation and immune escape will tend to be relatively more virulent. In this section, we develop our argument in support of these predictions.

Consider a biochemical mechanism of a parasite that interferes with or avoids host immunity. Suppose an enhancement of this mechanism has two effects. First, it causes an incremental decrease in the clearance of the parasite from the host at age x. Here, 'age x' means the time since initiation of infection in the host. Second, the enhanced mechanism has pathogenic effects that cause an equivalent incremental increase in virulence at age $x + \delta$, measured as an increase in host death at that time. These assumptions lead to a tradeoff between clearance from the host and host death by virulence. Clearance and virulence are two different ways to reduce parasite survival, because both processes kill the infection. The question here is: how does natural selection influence the balance in the tradeoff between clearance and virulence? Put another way, what is the sensitivity of parasite fitness to a reduction in clearance at age x relative to an increase in virulence at age $x + \delta$?

Both clearance and virulence describe components of parasite survival, but have effects at different ages. The fitness sensitivity of changes in survival is always weaker at later ages (Hamilton, 1966; Rose, 1994). The decline in fitness sensitivity with age occurs, because the probability of being alive is always greater at earlier ages than at later ages. So, a process that enhances the death rate at a later age always has less effect than a process that enhances the death rate by the same amount at an earlier age. For example, a high probability of death at a very old age has little significance, because few, if any, individuals will be alive at that advanced age.

These facts lead to the following prediction. A parasite always gains by reducing clearance at an earlier age in exchange for an equivalent increase in death via virulent destruction of the host at a later age. In terms of pathogenesis, any mechanism that can reduce the ability of the host's immune system to clear the infection will be favoured, even if that mechanism causes an equivalent reduction in host survival at any later time. The greater the time lag, δ, between effects, the more potent the virulent consequences that can be tolerated by the parasite. Because the parasite typically gains the immediate benefit of immune suppression or escape before the subsequent virulent consequences of immune manipulation (Young *et al.*, 2002; Scholz *et al.*, 2003; Merrell & Falkow, 2004; Perlman & Dandekar, 2005), most immunomodulatory or escape mechanisms can be favoured in spite of their virulent consequences. By contrast, pathogenic mechanisms that enhance parasite transmission do not have the same inevitability with regard to being favoured in spite of virulent consequences.

The different consequences of clearance and transmission arise because of the different ways in which a mechanism acting at age x propagates benefits to later ages. A survival benefit that acts only at age x carries forward as a benefit throughout life: if one survives better at a single instant, then one has a greater chance of being alive at all later ages. This survival benefit enhances potential transmission across all ages after age x. By contrast, a transmission benefit at age x has a benefit only at that particular age, and does not carry forward to later ages.

The asymmetry between clearance and transmission means that mechanisms reducing clearance will usually spread more easily than mechanisms that enhance transmission when matched for similar consequences with respect to virulence. Thus, we predict that virulent consequences of pathogenesis will most often be associated with immune manipulation or escape from host immunity rather than with mechanisms that increase transmission.

Mechanisms to escape host immunity: examples

The prediction that immune manipulation or escape from host immunity dominates virulence cannot be tested directly, given the current empirical understanding of pathogenic mechanisms. To make such a test, one would have to compile a comprehensive listing of pathogenic mechanisms and their consequences for virulence. Such information does not exist at present. Further, in the current approach to studying mechanistic aspects of pathogenesis, there is no reason to pay particular attention to: whether a mechanism functions to reduce clearance or enhance transmission; the age since time of infection at which the mechanisms exert their effects; and the consequences for virulence. One point of this paper is to call attention to the need for such information.

Given these limitations, we use a simple approach to gauge the status of the current empirical literature. To choose a sample of highly virulent pathogens, we use the

pathogens listed as potential bioweapons by Casadevall & Pirofski (2004). In Table 1, we list, for each pathogen, the mechanistic aspects of pathogenesis that appear to dominate as causes of virulence. In most cases, virulent aspects of pathogenesis seem to be associated with immunomodulatory mechanisms, although one cannot draw firm conclusions from such anecdotes. In addition, the severe virulence of *Clostridium botulinum* is caused by a neurotoxin. Overall, the table illustrates the commonality of immunomodulatory mechanisms, but also shows that other mechanisms will play important roles in many cases. Further examples are discussed elsewhere (Schmid-Hempel, unpublished data).

Earlier we discussed the ways in which the sensitivities of fitness components change as time passes during an infection. The theory that fitness sensitivities change with time predicts that parasites may modulate stage-specific gene expression of pathogenic mechanisms. The

Table 1 Pathogenesis and immune evasion in parasites considered efficient bioweapons (after Casadevall & Pirofski, 2004).

Pathogen (Disease)	Pathogenesis, virulence	Immune evasion mechanisms	Reference
Bacillus anthracis (Anthrax)	Inhalation of bacterial spores that reach lung alveoles, where ingested by macrophages. These are transported to lymph nodes where spores germinate and bacteria replicate to large numbers	Components of anthrax toxin (LT) suppress pro-inflammatory cytokines, dendritic cell responses and B- and T-cell deployment, or suppress phagocyte functions and cytokine pathways. At high concentrations, LT destroys macrophages, promotes vascular leakage	Abrami *et al.* (2005), Moayeri & Leppla (2004)
Clostridium botulinum (Botulism)	Three forms (food-borne, infant, wound) caused by botulin toxin (a neurotoxin) causing severe muscle paralysis. Colonizes intestine	Produces exotxoin (C3) that (via cascade involving Kruppel-like transcription factors) suppresses pro-inflammatory cytokines and phagocytosis. Also affects general regulation of cell cytoskeleton, suppresses cell proliferation, inhibits monocytes to stimulate T-cells. Botulin toxin targets synaptobrevins and prevents release of neurotransmitters	Wilson *et al.* (2002), O'Grady *et al.* (2007)
Coxiella burnetii (Q fever)	Obligate intracellular bacterium; survives in phagocytes. Acute febrile symptoms, pneumonia	Induces its internalization by cytoskeleton reorganization. Subverts macrophages, prevents maturation of phagosomes. Down-regulation of IL-2, IFN-γ to inhibit T-cell response. Induces up-regulation of IL-10, TGF-β. Inside vacuole scavenges reactive oxygen and evades antimicrobial peptides by tolerance of low pH. Shows antigenic phase shifts	Finlay & Falkow (1997), Maurin & Raoult (1999), Raoult *et al.* (2005)
Francisella tularensis (Tularemia)	Enters respiratory tract; there interacts and replicates within dendritic cells (DC), alveolar macrophages. Lymph node swellings, pneumonia	Causes aberrant activation of DCs, which also clears the cells from lung tissue. Suppresses ability of DCs to release pro-inflammatory cytokines (IL-6, TNF-α), but induces immunosuppressive cytokine TGF-β. In macrophages, induces PGE2, so inhibits IL-2 secretion thus disrupting T-cell proliferation. Induces Th2-like response with induction of IL-5. Escapes from vacuoles of macrophages; releases factor inhibiting macrophage responsiveness to produce pro-inflammatory cytokine. Shows phase variation in LPS inflammatory potential	Bosio & Dow (2005), Woolard *et al.* (2007), Rhen *et al.* (2000)
Yersinia pestis (Plague)	Is taken up by macrophages in early infection, where it survives and is transported to lymph nodes. There, bubos form in which parasite escapes from macrophages and multiplies extra-cellularly. Severe septicaemia follows	Yersinia outer proteins (Yops) target a variety of host functions, such as disrupting cytoskeleton, down-regulating inflammatory responses (by repressing TNF-α, IF-γ with secreted Lcrv protein). Disables and induces apoptosis in macrophages upon contact. Additionally interferes with recognition by macrophages through a surface protein (F1). Proteolytic enzyme facilitates spread and favours bacteraemia and disease	Nakajima & Brubaker (1995), Armstrong (2001), Du *et al.* (2002), Sing *et al.* (2002), Titball *et al.* (2003)
Variola minor (Smallpox)	Disseminates via lymph and infected leucocytes; replicates in cell cytosol. Spread leads to dangerous secondary viraemia	A variety of immunomodulatory proteins target complement and cell functions, block cytokines and interferon binding, phagocytosis, T-cell functions. Severe pathogenesis results from massive parasite-induced disruption of signalling by pro-inflammatory cytokines (cytokine storm), with sepsis and cytopathic effects in many tissues	Moss & Shisler (2001), Alcami (2003), Stanford *et al.* (2007)

empirical relations between fitness sensitivities and gene expression remain unclear at present. However, many empirical examples do show variable expression of pathogenic mechanisms at different stages of an infection. The examples range from stage-specific gene expression in parasitic protozoa such as *Trypanosoma* or *Leishmania* (Sacks & Sher, 2002) to host cytokine manipulation by viral infections (Boomker *et al.*, 2005) or the regulation of bacterial virulence factors (Beier & Gross, 2006; Fouet & Mock, 2006).

In bacteria, quorum sensing can lead to expression of specific genes under high bacterial densities, a different array of immune-modulating molecules, and therefore differences in the associated virulence effects (West *et al.*, 2006). In this regard, quorum sensing may provide cues that allow bacteria to modulate their pathogenic mechanisms and virulent consequences as fitness sensitivities change in response to changing demographic conditions in the host over the course of an infection.

Mechanistic coupling between transmission and virulence

One can make simple predictions about the virulent consequences of mechanisms that primarily affect clearance, but general predictions concerning transmission are more difficult. We discussed above the fact that clearance, as an aspect of parasite survival, leads to ubiquitous consequences with regard to age-specific effects. By contrast, transmission, as an aspect of pathogen fecundity, does not lead to general age-specific predictions in relation to a tradeoff with virulence. Day (2002a, 2003) has made some theoretical progress on the relation between age-specific transmission and virulence; here we emphasize predictions about transmission that arise from consideration of pathogenic mechanisms.

One special case arises by mechanistic coupling between transmission and virulence (Day, 2002b). For example, transmission may occur by release of spores after host death. In *Autographa californica*, a nucleopolyhedrovirus that attacks caterpillars, the virus is ingested during feeding and invades the midgut cells (Szewczyk *et al.*, 2006). From there, the virus replicates and spreads until more than 10^{10} polyhedra fill the host, comprising in some cases more than 30% of host dry weight. In the last stages of infection, the virus produces chitinase and cathepsin that together breakdown the host cuticle. The host liquefies at death, releasing the viruses to be taken up by other hosts. Many terminally killing pathogens have similar life histories (Ebert & Weisser, 1997).

In mammals, anthrax (*B. anthracis*) provides a spectacular case of mechanistic coupling between transmission and virulence (Mock & Fouet, 2001; Fukao, 2004; Moayeri & Leppla, 2004; Abrami *et al.*, 2005). In the later stages of infection, bacterial density in the host grows to extremely high levels. As bacterial density rises to high levels, the increasing concentration of anthrax lethal toxin knocks out the immune system by destroying the macrophages, probably facilitated by the sensitization of macrophages via bacterial capsule and cell wall components. Other toxin consequences, not fully understood mechanistically, include vascular leakage, systemic hypoxia and a shock-like collapse stimulated by an excessive bacterial-induced cytokine (IL-1) secretion by the macrophages. Furthermore, anthrax oedema toxin induces the production of an excess amount of cAMP in host cells, which eventually disrupts the flow of ions and cellular functions. These extreme pathogenic effects typically lead to host death. Transmission appears to occur primarily by spores released after the host dies. Interestingly, *B. anthracis* has a close relative, *B. cereus* (Helgason *et al.*, 2000), that does not cause severe virulence in the manner of *anthracis*. Instead, *B. cereus* transmits by the oral-faecal route, typically colonizes the intestine, causes diarrhoea, and only rarely induces severe pathology (Kotiranta *et al.*, 2000).

From an evolutionary perspective, mechanistic coupling between transmission and extreme virulence strongly shapes the life history of parasites (Day, 2002b). If the first major transmission episode severely damages the host, then the chance of having subsequent transmission is low. Consequently, the parasite will often gain by expressing strongly its full complement of pathogenic mechanisms in the period leading up to the first transmission episode, growing to maximum density and maximizing opportunities for successful transmission. From that point, the evolutionary step to coupling transmission and host death follows easily. Thus, an initial mechanistic coupling between transmission and strongly deleterious effects on the host may lead rather quickly to the evolution of coupling between transmission and host death via the recruitment of multiple pathogenic mechanisms designed to maximize transmission during the single transmission episode. Perhaps this powerful evolutionary tendency explains why the closely related *B. anthracis* and *B. cereus* differ so greatly in their life history, and why, in general, horizontally transmitted virulence islands (Boyd & Brüssow, 2002) often cause such drastic changes in bacterial life history.

Discussion

In its current form, the widely discussed theory of parasite virulence predicts how the virulence of populations evolves in response to changes in conditions. Here, we have made a first step in developing a theory that predicts differences in virulence between parasite species. Our theory follows from general properties of sensitivity in fitness components. In particular, pathogenic mechanisms that incrementally reduce clearance of the parasite tend to have greater fitness sensitivity than pathogenic mechanisms that incrementally alter transmission. Thus, we predict that pathogenic mechanisms that manipulate

host immunity or escape from the host's immune defences will dominate as causes of virulence.

We now set our argument in the context of recent research. Antia has developed the most extensive series of papers on how functional aspects of parasite characters influence virulence (Antia *et al.*, 1994; Antia & Lipsitch, 1997). In particular, Ganusov & Antia (2003) studied several alternative mathematical assumptions with regard to how resource acquisition and interactions with host immunity alter parasite growth and survival within the host and transmission between hosts. They concluded: 'Our major result is that predicting the optimal level of virulence of a parasite will require a detailed quantitative understanding of the interaction of the parasite and its host'. We agree. However, we have argued that the way forward does not follow the path of detailed quantitative understanding. Rather, the current theory derives its strength from qualitative comparison between closely related populations based on the assumption of common tradeoffs that cannot be measured directly. From this current theory, we developed the new argument that qualitative comparison between different species can be made based on simple and general arguments about the relative sensitivity of fitness components.

Our argument about relative sensitivity of fitness components derives from classical life history theory. In that theory, survival and fecundity have different sensitivities at different ages. We apply that theory to infections by equating parasite survival to clearance from the host or death of the host, and by equating fecundity to transmission. Frank (1996) reviewed and developed the early work on that line of thought, which was also developed by Levin *et al.* (1996) and, recently and more fully, in a series of papers by Day (Day, 2002a, 2003; Andre & Day, 2005). Although these life history concepts have now been well studied for parasite infections, our specific argument here appears to be novel. In particular, the fact that pathogenic mechanisms that fight against clearance should be strongly sensitive and therefore strongly associated with virulence has not been previously emphasized.

Andre & Godelle (2006) did come rather close to our argument about the sensitivity of clearance. They studied the fate of novel mutations that arise during the course of an infection within a particular host. They found that natural selection acted more strongly on mutations influencing clearance than on mutations influencing transmission. This conclusion certainly relates to the relative sensitivity of fitness components. However, that aspect of sensitivity by itself is a standard part of life history analysis. In their application, they focused on how mutations in the parasite population accumulate within hosts, particularly during infections of long duration.

In contrast with Andre & Godelle (2006), we argue that the fitness sensitivities provide insight into which particular types of pathogenic mechanisms will, in general, be associated with virulence. Further, we use the sensitivity argument to develop a way to think about comparisons of virulence between species based on pathogenic mechanisms. This step to comparison between species is particularly important, because the existing theory has been of little practical use in understanding differences between species.

In the future, we envision a more general theory that links the mechanisms of pathogenesis to the evolution of virulence. That theory would emphasize a functional classification of pathogenic mechanisms. Each mechanism would be analysed with regard to two attributes. First, each mechanism has a functional role in the life of the parasite: manipulating host immunity, escaping detection by host defences or extracting host resources. Second, each mechanism acts at particular ages, where 'age' means time since the start of infection. From the combination of function and age of action, one may deduce predictions about sensitivity of fitness components and consequences for virulence. This expanded theory also provides a conceptual framework in which to organize and give meaning to the rapidly accumulating molecular and immunological data on pathogenesis and immune evasion mechanisms.

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