Challenging the trade-off model for the evolution of virulence: is virulence management feasible?

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Progress in understanding the evolution of infectious diseases has inspired proposals to manage the evolution of pathogen (including parasite) virulence. A common view is that social interventions that lower pathogen transmission will indirectly select lower virulence because of a trade-off between transmission and virulence. Here, we argue that there is little theoretical justification and no empirical evidence for this plan. Although a trade-off model might apply to some pathogens, the mechanism appears too weak for rapid selection of substantial changes in virulence. Direct selection against virulence itself might be a more rewarding approach to managing the evolution of virulence.

The last quarter of the 20th century witnessed an increase in the interest of evolutionary biologists in understanding and controlling infectious diseases. Phylogenetics became the mainstay in tracking infectious diseases, and fears of the evolution of drug resistance in pathogenic microorganisms inspired news headlines and garnered Pulitzer Prizes. In the midst of this was another realization, implying a longer-term use for evolutionary biology: humans themselves might be in a position to manipulate the evolution of infectious diseases toward harmless ends. This proposal offered the possibility that an evolutionary-minded public health programme could render some diseases benign.

Lured by the possibility of such 'virulence management', the virulence of infectious diseases gained respect as a topic for evolutionary study. From the invariant lethal outcome of AIDS to the non-lethal consequences of colds, the optimal virulence hypothesis was advocated as being a kind of 'new-age' tool to fight the consequences of infectious diseases [1–6]. The idea was that with appropriate public health measures and treatment protocols, we could not only reduce the incidence of infectious diseases and cure them when they do occur, but we could also cause the parasites to become avirulent. In some of the more extreme claims, warnings were offered of impending plagues if we ignored the evolutionary message, and suggestions were put forth in broad outline of how to avert these calamities both in human populations and in agriculture. These arguments were extended to suggest that genetically engineered, highly virulent bioweapons might not be a long-lasting threat, because evolution would work quickly against high virulence [7], that the spread of the Ebola virus is strongly limited because it is too virulent, and that imperfect vaccines would lead to higher levels of virulence for unvaccinated people [8]. The possibilities were far reaching.

Although there could be many ways in which the evolution of virulence could be influenced by human intervention, the dominating concept behind most arguments of virulence management is rapid response to indirect selection based on the trade-off model. We question the generality and applicability of this model. We suggest there are a few basic reasons why virulence management based on this simple trade-off model is far less powerful than expected. This is not to say that virulence cannot evolve as a consequence of human intervention, and indeed, virulence does appear to have evolved in response to human intervention (although not explained within the trade-off framework). To manage virulence, other aspects of specific diseases appear more promising than those suggested by the limited scope of the trade-off model.

We question the generality and applicability of [the trade-off model].

The trade-off model

The motivating question is why parasites harm their hosts if a live and healthy host is beneficial to their transmission. The now-conventional answer to this fundamental question is that the reduction in host survival (this equals virulence in the strict sense) is an unavoidable consequence of parasite reproduction within the host [9,10]. This association between transmission and host survival (and thus parasite survival) represents an evolutionary trade-off for the parasite: a low level of reproduction has little impact on host longevity but results in little transmission, whereas a high level of reproduction yields high transmission but only during the brief tenure of the diseased host (Fig. 1). The optimal solution for the parasite is to balance virulence and reproduction such that its

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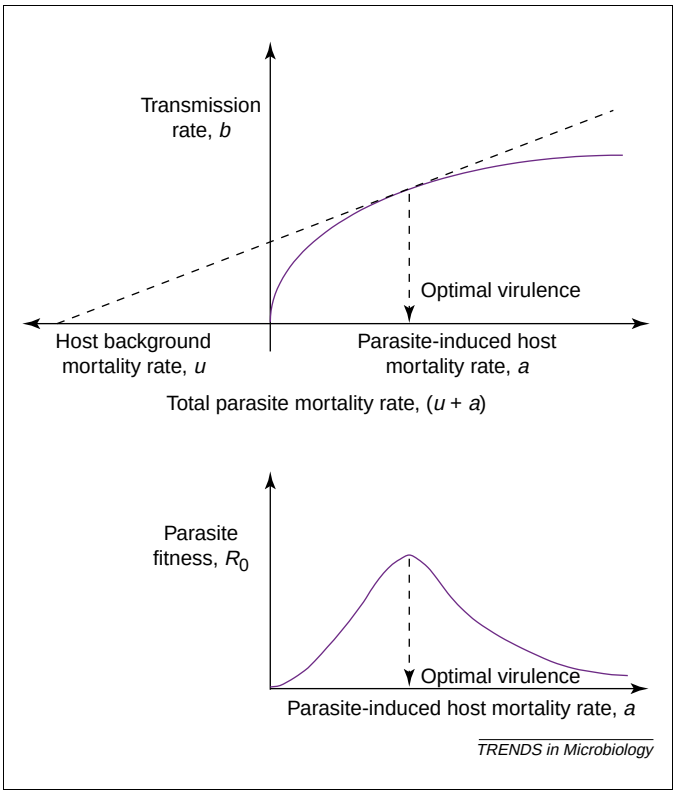

Fig. 1. The trade-off model for the evolution of virulence. The model assumes a decelerating functional relationship between transmission rate, *b* and parasite-induced host mortality (virulence), *a* (top panel). Because early host death curtails parasite transmission, the life time transmission success (R_0, the total number of secondary infections produced by a primary infection in a population of susceptible hosts) of a parasite is maximal at intermediate levels of *a* (bottom panel). In a simple model $R_0 = b/(u + a)$, where *u* is the parasite-independent host mortality rate. Note: for other (non-decelerating) functional relationships between *b* and *a* (e.g. linear or accelerating), R_0 has no intermediate optimum. For simplicity, this graphical model does not include the effects of multiple infections. Modified, with permission, from [59].

transmission success is maximized over the lifetime of the infection [9]. Thus, the trade-off model makes the strong assumption (as did the former conventional wisdom) that everything else being equal, host mortality is detrimental for the parasite, that is, virulence is an important component of parasite fitness. Theoretical studies suggest that the virulence optimum is sensitive to the abundance of susceptible hosts, the intrinsic host lifespan, and the rate of clearance of the infection by the host's immune system, as well as other factors [11–13].

This basic trade-off model was developed and applied by Anderson and May [9] and by Ewald [10]. These early applications assumed between-host competition among parasites – that parasites competed through their maximal lifetime transmission success and thus evolved toward an optimum along the transmission–virulence trade-off. Many mathematical models calculating the evolution of virulence under this model and many elaborations of it have since been published (see papers in [5] for a representative overview). The modification of these basic models that received most attention was the effect of multiple strains infecting the host concurrently, leading to within-host competition among pathogens. A common prediction of multiple infection models was increased virulence above the level that evolved under single infection, a result that rests on an additional assumption, namely that the more successful parasite during within-host competition is also more virulent [14–16].

Despite the considerable attention to the conceptual bases of virulence evolution, there are several difficulties with empirical applications of the trade-off model. First, some suggestions were based on faulty intuition, such as the widespread idea that reducing the opportunities for parasite transmission favours lower virulence because high virulence would kill the host too quickly (as noted in [17] and [18]). Second, even for concepts with sound theoretical logic, models merely make qualitative predictions about optima and do not enable quantitative predictions about magnitudes and rates of change. Virulence management is specifically about ways to cause rapid and meaningful changes in virulence, thus rates as well as magnitudes are crucial. For example, the foundation of virulence management is that virulence will evolve in response to indirect selection: changes in opportunities for parasite transmission will select changes in virulence. However, this field has not confronted a basic difficulty with indirect selection. In quantitative genetics terms, indirect selection attempts to generate a response in trait X (virulence) to selection for the correlated trait Y (transmission). If the genetic regression between them is low (i.e. a noisy or shallow trade-off), the response of X to selection on Y will be slow and small in magnitude – in the right direction but not necessarily useful for the short timescale of virulence management [19]. The models do not address the crucial importance of the covariance between virulence and transmission. Third, a trade-off does not necessarily lead to a virulence optimum. The trade-off function has to be saturating, that is, virulence increases more rapidly than transmission rate. In the basic trade-off model, linear or accelerating trade-off functions would not lead to intermediate levels of virulence. This condition for the existence of a virulence optimum has rarely been noted nor been supported with empirical data.

Testing the trade-off model

The optimal virulence perspective offered the promise of explaining variation in virulence across parasites as well as predicting changes in virulence over time. A variety of existing data could be used to test this model and, in addition, it was relatively easy to conduct experiments with model parasites to test the model directly. Although many existing data were consistent with trade-offs in the broad sense, they were in fact equivocal with respect to the more narrow application of virulence management.

Experimental tests

Virulence management and trade-off models are well suited to experimental verification with model parasites, and a variety of such experiments have already been carried out [20–35]. A trade-off between transmission and some measure of virulence has been supported in many but not all systems (e.g. not in [33–35]). Surprisingly, however, (indirect) selection for changes in virulence often failed to obey the expected response even when a trade-off was evident. Many of these studies revealed a complex, often

system-specific pattern of pathogen evolution that did not agree with simple models of virulence management. The results of these studies were sometimes explained post-hoc by meaningful evolutionary arguments, but they show that the predictions derived from the trade-off model were not broadly supported and that specific details of the biology of the systems had to be invoked. The reliance on post-hoc explanations and outright failures in these studies are especially disappointing to hopes for virulence management, because the investigators used well-known systems with which they had previous experience.

Not everything was a failure, however. Some studies observed the predicted direction of virulence evolution in response to selection. Yet most of those studies either employed extreme conditions (e.g. contrasting pure vertical transmission with high levels of horizontal transmission [28,29]) or observed relatively weak responses. For example, one study observed the expected relative change in virulence, but the response was small for the selection applied and even then was apparent only after carefully removing the extensive assay-to-assay variation [27]. Collectively these experiments challenge the premise that the trade-off model is a powerful determinant of virulence evolution under realistic conditions. Applying the trade-off model to infectious diseases under natural conditions appears suspect because of the large number of unmeasured variables.

A similar concern about extreme conditions applies to the famous myxoma virus 'experiments'. The release of a highly lethal myxoma virus into wild rabbits of Australia and Europe was quickly followed by a decline in virulence [36]. Subsequent mathematical analysis suggested that viruses with intermediate levels of virulence did indeed have the greatest transmission success [9]. The interpretation of this example to support the trade-off model for virulence management is difficult, however, as the virus was released to control a species that was not its natural host, and the virulence of the virus at the time of release was chosen to be unnaturally high (the virus was outside the bounds of natural evolution). Thus, we witnessed selection against an unnatural strain with extreme characteristics. Whether selection can act in a similar manner within the natural range of virulence and transmission is unclear.

Rapid evolutionary adaptation of pathogens in the laboratory was taken as an encouraging sign that similar rapid evolution of virulence might be possible under natural conditions. In particular, serial passage experiments with horizontally transmitted parasites and pathogens often led to a strong and rapid increase in pathogen growth rate [37]. In its classical form, pathogens were transferred at regular intervals from one animal to the next, disregarding their virulence and ability to transmit naturally. This favoured increased pathogen growth rates and increased virulence, supporting the idea that virulence is usually held in check by selection for transmission between hosts. The increase in virulence under conditions in which virulence has no cost for the pathogen seems consistent with the trade-off model, which predicts that a reduced cost of virulence should lead to an increase in virulence. However, it also predicts an increase in transmission rate. By contrast, transmission is often even impaired in the evolved lines [37–39]. Thus, a positive correlation between within-host growth rate and virulence during serial passage experiments in animals does not necessarily translate into a positive correlation between virulence and transmission. These experiments merely show the effect of relaxed selection for transmission and are possibly confounded by an altered effective population size for the pathogen (experimental infection could transfer many more parasites than would be transferred with natural infection).

Correlational and comparative studies

Further apparent support for the trade-off model came from comparative studies, indicating that different modes of transmission are associated with variation in virulence. For example, vertically, airborne- and sexually transmitted diseases are less virulent than horizontally, vector-borne and non-sexually transmitted diseases, respectively [10,40,41]. However, the amount of variation in virulence that can be attributed to these factors is typically low, and confounding factors are difficult to exclude. Even if the variation in virulence could be attributed to the mode of transmission, the question remains whether a quantitative change in transmission (as opposed to a change in the mode of transmission) would effectively influence the evolution of virulence and how rapidly virulence could respond to a change in transmission, because the parasite species in these comparative studies are separated by long evolutionary times.

A finding cited by Ewald [1,6] to support evolutionary changes in virulence is strain replacement – a new strain, differing in virulence, replaces the former strain in circulation. This type of evidence also suffers strongly from confounding factors. Unless the genomes of the two strains are found to be virtually identical except for virulence, such data are equivocal, because the strain replacement could occur for reasons other than differences in virulence. One of the most devastating strain replacements in history was the 1918 influenza ('Spanish flu'). Ewald speculated that its high virulence evolved in the poor sanitary conditions in the trenches of the Western Front of World War I, where the high density of soldiers became an incubator for the quickly lethal virus [1,6]. However, these were also the first influenza strains of the H1N1 serotype, hence were invading a population lacking in protective antibodies. It is also unclear why this event has not occurred again in regions of the world where poor sanitation and high population density are part of everyday life. Thus, it is not clear whether the high virulence of the Spanish flu was adaptive to the virus.

Virulence of pathogens of human

Consideration of pathogens of humans could yield the most relevant insights to the feasibility of virulence management. One of the most obvious difficulties facing the hopes for virulence management is that an evolved change in virulence has rarely been documented, despite massive human interventions and social changes over the past century.

The trade-off model might not apply to one of our most notorious diseases. Infection by HIV-1 is considered nearly 100% fatal if untreated, but mortality peaks ten years after infection. It could well be that virulence is, in some complex way, associated with transmission (higher viral loads might be associated with more transmission and more rapid disease progression [42,43]), but this does not mean that transmission of HIV is limited by virulence, as assumed by the trade-off model. There is in fact considerable (but indirect) evidence that although the disease is epidemic, the epidemiologically relevant transmission of HIV-1 occurs soon (months) after infection and hence years before mortality [44]. If selection for increased transmission leads to a decrease in time to death of infected patients, as predicted by the trade-off model [11,45,46], then the current epidemic spread of HIV in many parts of the world (also now endemic in some populations) has grim prospects. However, to our knowledge there has been no acknowledged change in the disease progression of HIV infections in the two decades since its discovery. Admittedly, it is also not obvious whether a change in the rate of disease progression would have been detected in the countries where the disease is most rampant and devastating.

Polio virus infects the human gut, where it does no obvious harm, and >99% of infections are asymptomatic. Disease occurs when the infection passes into the blood and then into the central nervous system. As is typical of many diseases, virulence is greater (i.e. disease is more likely) with the age of the host. Contrary to expectations based on the trade-off model, improved social hygiene is thought to have increased the virulence of polio infections by increasing the average age of first exposure. However, the increased virulence appears not to be an evolved outcome, but merely to be a consequence of the altered epidemiology [47]. Despite almost complete worldwide eradication of polio virus through use of a vaccine, there has been no recognized decline in virulence as would be expected from the trade-off model.

The bacterial disease diphtheria constitutes one of the few documented cases of an evolved decline in virulence, which is now being reversed in the former Soviet Union. This decline occurred in response to human intervention, but the standard version of the trade-off model does not provide us with the correct understanding. The bacterium *Corynebacterium diphtheriae* inhabits the throats of humans in either of two forms, one benign and the other pathogenic. The pathogen secretes a toxin that inflames tissues and thereby presumably improves its local growth conditions and ultimately improves its transmission. A vaccine engenders immunity against the toxin per se, so the pathogenic bacterium no longer inflames the tissues. Widespread vaccination has been followed by decreased incidence of the pathogenic form, and relaxation of vaccine coverage has led to subsequent increases [48,49]. This decline in virulence does not fit the standard trade-off model – which predicts that the optimal virulence should increase when the cost of virulence is removed [8]. Lower virulence seems to have evolved because of direct selection against virulence instead of through indirect selection via host mortality. An extended version of the trade-off model, with an altered fitness function for the pathogen, did indeed show that this can be predicted if the biology of the system is accurately taken into account [50]. A similar mechanism has been proposed to explain the post-vaccination (inducing antitoxin immunity) decline in the virulence of pertussis [50,51]. Both examples illustrate that virulence management can be effective, but specifically when virulence per se is the direct, rather than indirect, target of intervention.

Why the difficulty?

The trade-off model is based on two assumptions: (1) parasite-induced host mortality is costly for the parasite; and (2) transmission and virulence are inextricably coupled. Although these assumptions do apply in some cases, they are not general. In addition to the limitations of this model evident in many of the examples we have discussed, there are further grounds for questioning one or both assumptions, suggesting that virulence has little or no selective consequence for many pathogens. First, virulence is not always a simple function of parasite reproduction, but has many different causes, including the host immune response, specific tissues invaded by the pathogens (which often challenges the naive view that levels of parasite reproduction are directly correlated with virulence) or specific interaction among hosts and parasite genotypes [52,53]. In some diseases, virulence results from infection of tissues that are dead-ends for the parasite, from which no transmission occurs [54]. Without understanding what causes virulence, what the alternative virulence phenotypes are, and how virulence impacts parasite fitness, it could be impossible to predict how virulence will evolve. Second, even if virulence is embedded in a trade-off with transmission, the trade-off might involve more than just two dimensions, and any attempt to reduce it to two dimensions could give misleading results [55].

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Virulence is not a fixed property of an infectious agent. By far the largest variance in virulence is observed among infected host individuals. For example, in polio, tuberculosis and malaria, the effects of infection range from asymptomatic to severe disease. The factors that can contribute to this strong variation are diverse and include genetic variation and interactions among hosts and parasites, environmental effects such as dose dependence, host nutrition and age at first exposure, and interactions with other infectious diseases [53,56,57]. The virulence–transmission trade-off has so far been considered across pathogen genotypes, but hardly across host conditions, host genotypes or environmental conditions. It could well be that within the complex settings in which infectious diseases evolve, only a small fraction of patients account for the majority of transmission and that this is independent of virulence.

A change in perspective

This paper offers a devil's advocate view against the contemporary incarnation of optimal virulence models, which are based on a transmission–virulence trade-off. Indeed, we go so far as to suggest that the predictions for virulence management generated by such models are doomed to fail, although we acknowledge that our view is conjectural. To put virulence management in a more positive light, we suggest that the current paradigm is an unnecessarily narrow view of virulence, a view that might hinder progress in this exciting field by channelling research along one narrow track. We believe that there are more promising avenues to explore. First, we think that any programme of virulence management must understand how virulence relates to parasite fitness, and cannot assume that virulence follows a naive trade-off model. Second, as exemplified by the diphtheria case, virulence management can be more successful when targeting virulence directly rather than when targeting a correlated trait.

Many of the most successful studies of the evolution of parasites and pathogens did not focus on parasite-induced host mortality (the common definition of virulence), but instead addressed other traits more directly linked to parasite fitness. This approach might lack the apparent generality of the trade-off model because it is embedded in the specific biological details of the parasite, but by acknowledging specific biological details, it provides a broader foundation for controlling transmission and virulence. The evolution of many traits can be more easily predicted when there is a direct connection to parasite fitness – successes and promising examples include drug resistance, infectivity, and evasion from the immune system. Further, there are long lists of diverse behavioural alterations of infected hosts that have been directly shown to be linked to parasite fitness (in same cases to host fitness) (see [58] for a recent review). Likewise, studies of the evolution of benevolence of vertically transmitted parasites (where parasite fitness is strongly linked to host reproduction) offer an unmatched series of successes [28,29,40]. For exclusively horizontally transmitted parasites, no study of the evolution of virulence is equally convincing as those involving vertical transmission. The reason for these latter successes could be that during vertical transmission virulence is more closely linked to parasite fitness and thus more strongly subject to selection than in the case of horizontal transmission.

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This leads to our second suggestion for an altered perspective of virulence evolution: how to manage virulence. We suggest that virulence management should be based on identifying ways to select against virulent forms of parasites and pathogens directly, rather than selecting on correlated traits (P. Ewald suggested a similar idea to J.J.B., pers. commun.). Direct selection against virulent forms makes virulence a key component of parasite fitness and a response to selection is much more likely. The best-known examples for the evolution of virulence can be explained with direct selection against virulent pathogens – as with the antitoxin vaccines used against pertussis and diphtheria. Even the evolution of benevolence in exclusively vertically transmitted parasites fits this category, because selection operates directly against virulence in this design. Direct selection is very powerful to change trait means rapidly, in particular when the trait under selection has a high heritability. As this seems to be the case for virulence in many pathogens and parasites, we expect that this form of virulence management could have a promising future.

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References

1 Ewald, P.W. (1994) *The Evolution of Infectious Disease*, Oxford University Press
2 Williams, G.C. and Nesse, R.M. (1991) The dawn of darwinian medicine. *Quart. Rev. Biol.* 66, 1–22
3 Nesse, R.M. and Williams, G.C. (1994) *Why We Get Sick*, Times Books
4 Trevathan, W.R. et al., eds, (1999) *Evolutionary Medicine*, Oxford University Press
5 Dieckman, U. et al., eds, (2002) *The Adaptive Dynamics of Infectious Disease: In Pursuit of Virulence Management*, Cambridge University Press
6 Ewald, P.W. (2000) *Plague Time: How Stealth Infections Are Causing Cancers, Heart Disease, and Other Deadly Ailments*, The Free Press/ Simon and Schuster, Inc
7 Dennis, C. (2001) The bugs of war. *Nature* 411, 232–235
8 Gandon, S. *et al.* (2001) Imperfect vaccines and the evolution of pathogen virulence. *Nature* 414, 751–755
9 Anderson, R.M. and May, R.M. (1982) Coevolution of hosts and parasites. *Parasitology* 85, 411–426
10 Ewald, P.W. (1983) Host–parasite relations, vectors, and the evolution of disease severity. *Annu. Rev. Ecol. Systemat.* 14, 465–485
11 Lenski, R.E. and May, R.M. (1994) The evolution of virulence in parasites and pathogens: reconciliation between two competing hypotheses. *J. Theor. Biol.* 169, 253–265
12 Antia, R. *et al.* (1994) Within-host population dynamics and the evolution and maintenance of microparasite virulence. *Am. Nat.* 144, 457–472
13 Bull, J.J. (1994) Perspective – Virulence. *Evolution* 48, 1423–1437
14 Frank, S.A. (1996) Models of parasite virulence. *Quart. Rev. Biol.* 71, 37–78
15 Nowak, M.A. and May, R.M. (1994) Superinfection and the evolution of parasite virulence. *Proc. R. Soc. Lond. B* 255, 81–89
16 van Baalen, M. and Sabelis, M.W. (1995) The dynamics of multiple infection and the evolution of virulence. *Am. Nat.* 146, 881–910
17 van Baalen, M. and Sabelis, M.W. (1995) The scope for virulence management: a comment on Ewald's view on the evolution of virulence. *Trends Microbiol.* 3, 414–416
18 Lipsitch, M. *et al.* (1996) The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution* 50, 1729–1741
19 Falconer, D.S. (1981) *Introduction to Quantitative Genetics*, 2nd edn, Longman
20 Turner, P.E. *et al.* (1998) Tradeoff between horizontal and vertical modes of transmission in bacterial plasmids. *Evolution* 52, 315–329

21 Jaenike, J. (1996) Suboptimal virulence of an insect-parasitic nematode. *Evolution* 50, 2241–2247
22 Ebert, D. and Mangin, K.L. (1997) The influence of host demography on the evolution of virulence of a microsporidian gut parasite. *Evolution* 51, 1828–1837
23 Taylor, L.H. and Read, A.F. (1998) Determinants of transmission success of individual clones from mixed-clone infections of the rodent malaria parasite, *Plasmodium chabaudi*. *Int. J. Parasitol.* 28, 719–725
24 Taylor, L.H. *et al.* (1998) Virulence of mixed-clone and single-clone infections of the rodent malaria *Plasmodium chabaudi*. *Evolution* 52, 583–591
25 Mackinnon, M.J. and Read, A.F. (1999) Selection for high and low virulence in the malaria parasite *Plasmodium chabaudi*. *Proc. R. Soc. Lond. Ser. B* 266, 741–748
26 Elena, S.F. (2001) Evolutionary history conditions the timing of transmission in vesicular stomatitis virus. *Infect. Genet. Evol.* 1, 151–160
27 Messenger, S.L. *et al.* (1999) Virulence evolution in a virus obeys a trade-off. *Proc. R. Soc. Lond. Ser. B* 266, 397–404
28 Bull, J.J. *et al.* (1991) Selection of benevolence in a host–parasite system. *Evolution* 45, 875–882
29 Bull, J.J. and Molineux, I.J. (1992) Molecular-genetics of adaptation in an experimental-model of cooperation. *Evolution* 46, 882–895
30 Ebert, D. (1994) Virulence and local adaptation of a horizontally transmitted parasite. *Science* 265, 1084–1086
31 Mackinnon, M.J. and Read, A.F. (1999) Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. *Evolution* 53, 689–703
32 Lipsitch, M. and Moxon, E.R. (1997) Virulence and transmissibility of pathogens: what is the relationship? *Trends Microbiol.* 5, 31–37
33 Medica, D.L. and Sukhdeo, M.V.K. (2001) Estimating transmission potential in gastrointestinal nematodes (Order: Strongylida). *J. Parasitol.* 87, 442–445
34 Weiss, R.A. (2002) Virulence and pathogenesis. *Trends Microbiol.* 10, 314–317
35 Jakel, T. *et al.* (2001) Reduction of transmission stages concomitant with increased host immune responses to hypervirulent *Sarcocystis singaporensis*, and natural selection for intermediate virulence. *Int. J. Parasitol.* 31, 1639–1647
36 Fenner, F. and Ratcliffe, F.N. (1965) *Myxomatosis*, Cambridge University Press
37 Ebert, D. (1998) Evolution – experimental evolution of parasites. *Science* 282, 1432–1435
38 Contreras, V.T. *et al.* (1994) *Trypanosoma cruzi*: metacyclogenesis *in vitro*. I. Changes in the properties of metacyclic trypomastigotes maintained in the laboratory by different methods. *Mem. Inst. Oswaldo Cruz* 89, 253–259
39 Day, K.P. *et al.* (1993) Genes necessary for expression of a virulence determinant and for transmission of *Plasmodium falciparum* are located on a 0.3-megabase region of chromosome 9. *Proc. Natl Acad. Sci. USA* 90, 8292–8296
40 Herre, E.A. (1993) Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* 259, 1442–1445
41 Lockhart, A.B. *et al.* (1996) Sexually transmitted diseases in animals: ecological and evolutionary implications. *Biol. Rev.* 71, 415–471
42 Quinn, T.C. *et al.* (2000) Viral load and heterosexual transmission of human immunodeficiency virus type 1. *New Engl. J. Med.* 342, 921–929
43 Saah, A.J. *et al.* (1998) Association of HLA profiles with early plasma viral load, $CD4^+$ cell count and rate of progression to AIDS following acute HIV-1 infection. *AIDS* 12, 2107–2113
44 Levin, B.R. (1996) The evolution and maintenance of virulence in microparasites. *Emerg. Infect. Dis.* 2, 93–192
45 Levin, B.R. *et al.* (1996) The intrinsic rate of increase of HIV/AIDS: epidemiological and evolutionary implications. *Mathematical Biosci.* 132, 69–96
46 Levin, B.R. *et al.* (2001) Epidemiology, evolution, and future of the HIV/AIDS pandemic. *Emerg. Infect. Dis.* 7 (Suppl.), 505–511
47 Nathanson, N. *et al.* (1995) The evolution of infectious diseases: their emergence, epidemicity, and control. In *Molecular Basis of Virus Evolution* (Gibbs, A.J. *et al.*, eds), pp. 31–46, Cambridge University Press
48 Bergamini, M. *et al.* (2000) Evidence of increased carriage of *Corynebacterium* spp. in healthy individuals with low antibody titres against diphtheria toxoid. *Epidemiol. Infect.* 125, 105–112
49 Galazka, A. (2000) The changing epidemiology of diphtheria in the vaccine era. *J. Infect. Dis.* 181, S2–S9
50 Gandon, S. *et al.* (2002) Antitoxin vaccines and pathogen virulence. Reply. *Nature* 417, 610
51 Soubeyrand, B. and Plotkin, S.A. (2002) Antitoxin vaccines and pathogen virulence. *Nature* 417, 609–610
52 Levin, B.R. and Svanborg-Edén, C. (1990) Selection and evolution of virulence in bacteria: an ecumenical excursion and modest suggestion. *Parasitology* 100, S103–S115
53 Ebert, D. (1999) The evolution and expression of parasite virulence. In *Evolution in Health and Disease* (Stearns, S.C., ed.), pp. 161–172, Oxford University Press
54 Levin, B.R. and Bull, J.J. (1994) Short-sighted evolution and the virulence of pathogenic microorganisms. *Trends Microbiol.* 2, 76–81
55 Pease, C.M. and Bull, J.J. (1988) A critique of methods for measuring life history trade-offs. *J. Evol. Biol.* 1, 293–303
56 Read, A.F. *et al.* (1999) What can evolutionary biology contribute to understanding virulence? In *Evolution in Health and Disease* (Stearns, S.C. *et al.*, eds), pp. 205–215, Oxford University Press
57 Mackinnon, M.J. *et al.* (2000) Quantifying genetic and nongenetic contributions to malarial infection in a Sri Lankan population. *Proc. Natl Acad. Sci. USA* 97, 12661–12666
58 Moore, J. (2002) *Parasites and the Behavior of Animals*, Oxford University Press
59 Ebert, D. and Herre, E.A. (1996) The evolution of parasitic disease. *Parasitol. Today* 12, 96–101