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REVIEW

Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future

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Abstract

It has been more than two decades since the formulation of the so-called 'trade-off' hypothesis as an alternative to the then commonly accepted idea that parasites should always evolve towards avirulence (the 'avirulence hypothesis'). The trade-off hypothesis states that virulence is an unavoidable consequence of parasite transmission; however, since the 1990s, this hypothesis has been increasingly challenged. We discuss the history of the study of virulence evolution and the development of theories towards the trade-off hypothesis in order to illustrate the context of the debate. We investigate the arguments raised against the trade-off hypothesis and argue that trade-offs exist, but may not be of the simple form that is usually assumed, involving other mechanisms (and life-history traits) than those originally considered. Many processes such as pathogen adaptation to within-host competition, interactions with the immune system and shifting transmission routes, will all be interrelated making sweeping evolutionary predictions harder to obtain. We argue that this is the heart of the current debate in the field and while species-specific models may be better predictive tools, the trade-off hypothesis and its basic extensions are necessary to assess the qualitative impacts of virulence management strategies.

How and why **parasite virulence** (terms in bold font are in the Glossary) evolves are arguably some of the most important questions addressed by evolutionary biologists. The 1990s saw rich and abounding research in this area, mostly based on the 'trade-off hypothesis' (Anderson & May, 1982), which states that virulence is an unavoidable consequence of parasite **transmission** (see Box 1). In this review, we first briefly outline the seldom-discussed history of virulence evolution. Then, we expose the current debate in the field, which can be summarized as a challenge to the trade-off hypothesis. Finally, to answer this challenge, we discuss the advances made in the past decade and we argue that, in the light of these advances, we need not abandon the trade-off model. Instead, we argue that these new insights ought to be incorporated into the current theory and we identify promising future directions.

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1. A history of virulence

Many, if not most, of the recent theories that attempt to explain how parasites evolve assume that there is a link between virulence and transmission, the so-called 'virulence–transmission trade-off'. However, this idea has become the focus of intense debate. To better understand the issues of current debates in the field, one should be aware that virulence evolution was studied long before the trade-off hypothesis was formulated. Our purpose here is not to present an exhaustive review of the history of the study of infectious diseases but to give a mere glimpse of the richness and originality of this field that has linked many disciplines, from ecology to molecular biology.

The notion that virulence is not fixed but evolves can be traced to Pasteur and Koch in the 19th century.

Box 1: The trade-off hypothesis

The trade-off hypothesis, developed by Anderson & May (1982) and Ewald (1983), is based on the idea that it is not possible for the parasite to increase the duration of an infection without paying a cost. One can make a parallel with Achilles' dilemma, the Greek hero having to choose between a short but glorious life and a long but dull life. Writing the fitness of a parasite helps to illustrate this idea. This fitness is given by the baseline reproduction ratio, or $\boldsymbol{R_0}$ (Anderson & May, 1979), which can be written as

$$R_0 = \frac{\beta S}{\mu + \alpha + \gamma} \quad (1)$$

where S is the density of susceptible hosts in the population, β is the transmission rate of the parasite, μ is the host natural death rate, α is the host death rate due to the infection (i.e. the virulence) and γ is the recovery rate. Equation 1 can be seen as a product between the number of infections caused by a single infected host per unit of time (βS) multiplied by the duration of the infection ($(\mu + \alpha + \gamma)^{-1}$).

According to the trade-off hypothesis, a given value of transmission for a strain requires a minimum value of virulence and recovery (of course there can be maladapted strains with both a low transmission and a high virulence). What is known as the 'trade-off' curve corresponds to a parametric curve showing transmission rates along with the corresponding sum of the minimum virulence and recovery (Fig. 1). The shape of the trade-off can be used to infer the evolutionary outcome of the system. If the curve is linear or increases (i.e. is convex), the system evolves towards infinitely short infections with infinite transmission rates. Only if the trade-off saturates can there be a finite optimal level of virulence, and it is given by the tangent to the curve that passes through the origin (Anderson & May, 1982; van Baalen & Sabelis, 1995; Frank, 1996). Importantly, the shape of the trade-off curve is strongly affected by within-host processes, which means that change in parameter values translate into changes in optimal virulence (Alizon & van Baalen, 2005).

Essentially, the trade-off theory says that the three epidemiological parameters (α, β and γ) are linked so that a change in one will lead to a change in the others. Originally, Anderson & May (1982) proposed a trade-off between recovery and virulence. Recent work by Frank & Schmid-Hempel (2008) supports the role of clearance for virulence evolution in a new parasite-dependent framework, i.e. immune evasion. However, the now-classical trade-off links virulence and transmission (Bremermann & Pickering, 1983; Massad, 1987). Finally, note that trade-offs between transmission and recovery could also affect virulence evolution (Alizon, 2008b), which addresses a concern raised by Weiss (2002) that the trade-off might fail for some viruses that seldom kill their host.

The strength of the trade-off hypothesis lies in its simplicity. Adding a simple constraint to an epidemiological model allows one to make powerful predictions in evolutionary epidemiology. For instance, a classical implication of this theory is that the transmission mode will have a strong effect on virulence evolution (Ewald, 1983, 1994; Day, 2001). More generally, most of the virulence management theory, i.e. the idea that we can control and perhaps redirect virulence of existing infectious diseases, is based on the trade-off hypothesis (Dieckmann *et al.*, 2002).

Although today Pasteur is mostly remembered for his seminal work on vaccines, Mendelsohn (2002) argues that most of his other work has been unjustly overlooked – unjustly, because it was ahead of its time by focusing on parasite virulence and represents some of the first examples of experimental evolution. In the report of Pasteur *et al.* (1881) on the attenuation of the anthrax bacillus, the concept of strain-specific virulence is present, as is the idea that it is possible to change a strain from one type to the other.

Shortly after Pasteur's experiments, evolutionary theories were proposed to explain parasite virulence. For instance, according to Smith (1904):

> [...] there will be a selection in favour of those varieties which vegetate whence they can escape. The surviving varieties would gradually lose their highly virulent invasive qualities and adapt themselves more particularly to the conditions surrounding invasion and escape. That some such process of selection has been going on in the past seems the simplest explanation of the relatively low mortality of infectious diseases.

This optimistic view is now known as the 'avirulence hypothesis' and became so universally accepted that May & Anderson (1983) could refer to it as the 'conventional wisdom'. The broad acceptance of the avirulence hypothesis is reflected in the etymology and first definitions of what a parasite was. 'Parasite' comes from the Greek word parasitos [παράσιτος: from para 'alongside' and sitos 'food'], which refers to a designated assistant to the priest invited to share the common meals. The first definitions of parasite suggest that, as a respectful dinner guest, the parasite does not wish to overindulge. van Beneden (1875) thus writes:

> The parasite makes a profession out of living at its neighbours' expenses and all its industry consists of exploiting it with economy, without putting its life in danger. It is like a poor person who needs help to survive, but who nevertheless does not kill its chicken in order to have the eggs.

Support for the avirulence theory comes from the numerous observations that new host–parasite associations tend to be virulent (Read, 1994). This support, however, is confounded by the fact that more damaging associations are also more likely to be reported. Furthermore, there is little evidence that the converse is true, i.e. that old host–parasite associations tend to be avirulent (Toft & Karter, 1990; Read, 1994).

The avirulence hypothesis was already being challenged before the second world war. For instance, Kostitzin (1934) argued that mutualistic interactions can evolve out of parasitic interactions, but that the reverse may also occur (so that virulence may well increase through the course of evolution). Later, Ball (1943) issued an explicit critique, noting that many host–parasite interactions are

old and nonetheless virulent. However, he could not provide an alternative explanation and thus finishes with a rather equivocal conclusion:

> Perhaps, as biologists, we may all agree on one aspect of nature, namely, its exceeding variety. Even a parasite may choose the course of manifest destiny and find aggressiveness more attractive and more valuable than an existence of peace and symbiosis.

However, it was Topley (1919) whose early thinking most closely foreshadowed some of the important ideas underlying the modern-day trade-off hypothesis. He suggested that for high-density populations with frequent migration, the strains that replicate most rapidly are most likely to be transferred and that these rapidly replicating strains are also the most virulent. Yet, Topley's ideas were incomplete in that he was unable to bridge the gap between the evolution of virulence at high population densities and what would happen at other population densities. It would take several decades before a more complete argument was presented.

The 1960s and 1970s saw the development of a new field, evolutionary ecology, founded by the famous evolutionary biologists G. C. Williams, J. Maynard Smith and, most notably, W. D. Hamilton (1964), who challenged the then common idea that species evolve to increase their own persistence and that individuals should thus sacrifice themselves for the benefit of the species. After challenging this notion, it is only a small step towards realizing that parasite avirulence is not so inevitable as the conventional wisdom would have it, and indeed during the 1980s a number of alternative hypotheses to the avirulence hypothesis were proposed. Levin & Pimentel (1981) showed that under certain conditions virulent strains can coexist with avirulent strains; Anderson & May (1982) showed, in their now famous paper, that if **recovery** and virulence are linked then intermediate virulence is favoured; and Ewald (1983) argued that virulence should depend on the mechanism of transmission.

In particular, the notion that transmission and virulence are linked became the cornerstone of new theoretical developments. A trade-off was suggested, whereby a parasite strain that evolves a higher transmission rate has to pay a cost in terms of the duration of the infection (see Box 1 and Fig. 1). The trade-off hypothesis strongly stimulated research in the following decade (reviewed in Bull, 1994; Ewald, 1994; Read, 1994; Ebert & Herre, 1996; Frank, 1996). This research was mostly theoretical and experimental work lagged behind. This is one of the reasons why the relevance of trade-offs for virulence evolution was increasingly being questioned (Weiss, 2002; Ebert & Bull, 2003) and why some studies ignore them altogether (for a review on virulence evolution that does not cite the trade-off hypothesis, see Brown *et al.*, 2006). In the following section, we will review the current state of the debate by listing, one by one, the main challenges to the trade-off hypothesis that have been expressed, and the arguments that can be fielded to counter these.

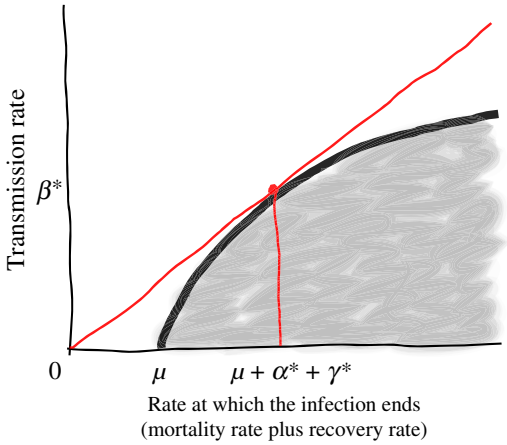

Fig. 1 The trade-off curve. The typical transmission-virulence trade-off is actually the boundary of the set of possible combinations of transmission and inverse of the duration of the infection (the shaded area). If the population starts to evolve at the interior of this set, evolution will favour strains with higher transmission (β) and longer infections (i.e. low virulence, α, and low recovery, γ) until the population 'hits' the boundary (the thick black curve) and increasing rates of transmission can only be 'bought' at the cost of accelerating mortality and/or recovery rates. Adaptive dynamics then can tell us at what point along the trade-off evolution will stop (if at all). If the curve saturates (i.e. is concave), the evolutionary stable strategy (ESS) is given by the tangent of the curve that passes through the origin (diagonal line). This ESS determines the optimal level of virulence (denoted α^*), which coincides with optimal transmission and recovery rates (β^* and γ^*). Note that here the host-background mortality (μ) is assumed to be constant (see Box 1).

2. The current debate

2.1. Lack of evidence

The appeal of the trade-off hypothesis results from its simplicity and generality (see Box 1). One might therefore expect strong evidence in support of the trade-off in natural systems. Virulence has been shown to evolve in experimental (Pasteur *et al.*, 1881; Ebert, 1998) and natural (e.g. Fenner & Ratcliffe, 1965) systems, but that, in itself, is not sufficient to infer the existence of trade-offs. Lipsitch & Moxon (1997) were the first to address this question explicitly and they discovered that neither the evidence for a trade-off nor the amount of relevant experimental studies was overwhelming. There are now more data supporting such a relationship (Table 1), although more evidence is still needed.

Showing evidence of a trade-off empirically is highly complicated (Box 2). The first challenge is to find a way to measure transmission and virulence (see below). Then, it is necessary to have variation in these two traits. Several

Table 1 Empirical support for the trade-off hypothesis.

Evidence	Host–parasite system	Studies	Comments
Saturating trade-off relationship between virulence and transmission	Protozoan parasite *Ophryocystis elektroscirrha* in monarch butterfly	de Roode *et al.* (2008)	Negative relationship between emergence and mating probabilities of butterflies and parasite load. Positive relationship between spore load and proportion of monarchs infected. Parasite fitness is maximized for intermediate spore load
	HIV in humans	Fraser *et al.* (2007)	Shows a negative relationship between duration of asymptomatic infection and viral load and a positive relationship between transmissibility and viral load. Transmission potential is optimal for an intermediate viral load
	Myxoma virus in European rabbit	Fenner & Ratcliffe (1965), Dwyer *et al.* (1990)	The relationship saturates only because of a single point
Existence of an optimal virulence maximizing fitness	Bacterium *Pasteuria ramose* in *Daphnia magna*	Jensen *et al.* (2006)	Bacteria with intermediate virulence produce the most spores
Positive correlation between virulence and transmission	*Plasmodium chabaudi* in rodents	Mackinnon & Read (1999a, b, 2003), Ferguson *et al.* (2003)	Transmission increases with virulence estimated with sub-lethal measures (anaemia)
		Mackinnon *et al.* (2002), Ferguson *et al.* (2003)	Mackinnon et al. find that when death occurred the gametocyte density (transmission was high). Fergusson *et al.* find the opposite
	Amblyospora dyxenoides in the mosquito *Culex annulirostris*	Agnew & Koella (1997)	Virulence (measured as fluctuating asymmetry) correlates positively with the number of spores transmitted horizontally
	Nuclear polyhedrosis virus in gypsy moth	Cooper *et al.* (2002)	Timing of transmission: early transmission selects for more virulent strains
	Plasmodium gallinaceum in chickens	Paul *et al.* (2004)	Positive correlation between lethal virulence measure and transmission (but no correlation with anaemia)
	Hyaloperonospora parasitica in *Arabidopsis thaliana*	Salvaudon *et al.* (2005)	Positive correlation between transmission and virulence for different host–parasite combinations
	Citrobacter rodientum in mice	Wickham *et al.* (2007)	Virulence factors increase transmission between hosts
	Plasmodium falciparum in humans	Mackinnon *et al.* (2008)	Positive relationship between expected transmission and virulence in different age classes

For further discussion, see Lipsitch & Moxon (1997) and Sacristán & García-Arenal (2008) for plant pathogens.

problems may complicate these procedures. First, even if the hosts are genetically identical, they may vary in life-history traits such as their immunological status. Both theory (Alizon & van Baalen, 2005) and data (Little *et al.*, 2008) have shown that such small variations among parasites or hosts may generate sufficient variation to blur the trade-off curve. Secondly, even if a link between virulence and transmission can be found experimentally, it is not straightforward to determine whether this relationship is linear or curved. An elementary version of the trade-off hypothesis (Anderson & May, 1982) predicts that the curve needs to saturate for there to be a finite optimal level of virulence (see Box 1). Frequently, saturating functions are fit to transmission–virulence data using least squares regression; however, these fits are often dubious because of outliers and lack of data (B. Bolker, A. Nanda and D. Shah, unpublished data).

One may wonder whether the lack of evidence supporting the trade-off hypothesis comes from a lack of experiments or if it fails to be refuted because of a publication bias (studies that do not show a significant trend are not published). Our opinion is that existing studies (in Table 1) support the idea that a trade-off can be found in most host–parasite systems if it is looked for. For instance, the human immunodeficiency virus (HIV) has often been used as an illustration of the failure of the trade-off hypothesis (Levin & Bull, 1994; Weiss, 2002; Ebert & Bull, 2003) but a recent analysis shows that there indeed is a saturating relationship between virulence (estimated as the set-point viral load) and transmission (Fraser *et al.*, 2007). Our conclusion is that the lack of supportive evidence for the trade-off hypothesis is due to the fact that collecting this data is complicated.

Box 2: Collecting data to support the trade-off hypothesis

Three types of approaches have been used to study the trade-off hypothesis.

1 **The observational approach.** This approach involves collecting different samples of a parasite species in nature and comparing the virulence and transmission trait pairs between samples (see e.g. Anderson & May, 1982; Ewald, 1983, 1994; May & Anderson, 1983; Herre, 1993; Sacristán & García-Arenal, 2008). One needs to be very cautious with the interpretation of such data because of correlation–causation issues. For instance, Sabelis & Metz (2002) show that at least seven different hypotheses can explain observed patterns of virulence evolution in the well-known rabbit-myxoma virus system in Australia. One also needs to be careful when estimating transmission and especially virulence in parasites that have recently crossed a species barrier because the parasite is ill adapted to the host and vice versa.

2 **The comparative approach.** The difference between this approach and the former approach is the scale at which it is defined. Here, the focus is on a larger scale and parasites from different species are pooled in order to understand the effect of some life-history specificities on the trade-off. For instance, Ewald (1983) uses such a cross-species database to show that vector-borne diseases are significantly more virulent than directly transmitted diseases (the explanation being that if the transmission stage is carried by a vector, the parasite can afford to have a less mobile host and thus can be more virulent). As underlined by Ebert (1999), a problem with these studies is that they may not be taxonomically independent.

3 **The experimental approach.** This is of course the approach that offers the best data. Among many advantages, an experimental approach allows one to control both for host effects and parasite variation. Building an experiment to search for evidence of a trade-off experimentally can be divided into three steps.

(a) Find a way to estimate virulence, recovery and transmission in the host–parasite system. Quantifying a transmission rate is often a challenge (though vector-borne disease offer an interesting solution because it is easier to estimate the transmission success to the vector) as is measuring and defining virulence (see the main text).

(b) Observe variability in the parasite population. Here, there are two options and both have weaknesses. One can gather parasite samples from nature or in laboratories, but there may be significant genetic variation between these strains that can interact with host characteristics (especially the immune response). The other option is to generate diversity through mutation. This not only limits genetical variation among strains but also has less biological significance.

(c) Run the experiment and analyse the data. Given the numerous host and parasite effects that can blur the trade-off curve (see the text), it is necessary to have a large sample size. Working with hosts that do not have a complex immune system is likely to give better results, but, as we point out in the text, the immune response is the major issue to address for the trade-off theory. Finally, to plot the data, it is necessary to have an idea of which parameters are involved in the trade-off (virulence and transmission, recovery and virulence, transmission and recovery, or all three of them).

We realize that it is not particularly instructive to say that experiments should be designed to 'find a way to measure transmission, recovery and virulence' and we agree with the criticism that often the trade-off is more complicated than the simple transmission–host–mortality relationship that underlies some of the theory. New elements need to be considered and incorporated. Incorporating new evidence requires new theoretical steps, first to test the resulting trade-off for internal consistency and then to generate testable predictions.

2.2. Defining virulence

Virulence is a notoriously difficult notion to define (Poulin & Combes, 1999). Virulence, in its broadest sense, is the cost to the host due to infection, which translates into 'the reduction in host fitness due to the infection' (Read, 1994). This, however, is not a very practical definition. Currently, it almost seems as if every field has developed its own working definition of virulence. Theoretical biologists use disease-induced host death rate (but see Day, 2002) while experimental biologists often use **sub-lethal** measures because they are easier to quantify or for ethical reasons (for an overview of possible definitions, see, e.g. Casadevall & Pirofski, 1999; Weiss, 2002; Thomas & Elkinton, 2004). Even more confusingly, in the study of plant pathogens and in many population genetics studies, there is a tradition of using virulence to refer not to the damage caused by a parasite, but rather to its transmissibility, i.e. capacity to infect (Sacristán & García-Arenal, 2008). Here, we will only consider virulence as a damage-related concept.

How then can the trade-off relationship be useful if we are not able to define the quantities involved? This also makes it difficult to link theoretical predictions to experiments. The option championed by Ebert & Bull (2003) is to consider that the trade-off cannot be used as a general framework and that each host–parasite interaction should be studied independently. Another possibility is to find links with mortality, morbidity and other forms of pathogenicity (e.g. castration or weight loss). This poses practical problems. To start with: how should we compare studies in which the proxy for virulence is weight loss to studies where it is mortality? There is no fail-safe way to compare these measures with each other. This problem has been extensively debated in the recent literature (O'Keefe & Antonovics, 2002; Ebert & Bull, 2003; Alizon, 2008b). At a more theoretical level, what guarantee do we have that the experimental proxy we choose is subject to the same constraint that underlies the theory?

If a general way of assessing virulence is still lacking, we now know that different ways in which virulence is expressed can differentially affect parasite evolution (Day, 2002). There are also new insights into how sub-lethal effects could affect virulence evolution (Schjørring & Koella, 2003; Bonds, 2006; Lively, 2006). Some studies

have linked virulence to other host traits like resistance or tolerance (Gandon & Michalakis, 2000; Gandon *et al.*, 2002; Horak *et al.*, 2006; Miller *et al.*, 2007; Raberg *et al.*, 2007). This is also supported by experimental studies: Bedhomme *et al.* (2005) show, using sub-lethal virulence measures, that parasites can have an indirect cost on fitness by modifying intra-specific competition. In general, considering direct mortality to be the only harmful effect that the parasite has on the host is too restrictive and other life-history traits affected by the disease should also be taken into account (see, e.g. Price *et al.*, 1986).

Finally, determining what causes virulence is not straightforward. This leads to critiques like those of Weiss (2002), who points to the fact that for some diseases the host's immune response is more to blame for damage to the host than is the parasite. Indeed, it has become increasingly clear that at least part of what we loosely call 'virulence' may actually be caused by the immune system of the host itself (a phenomenon called 'immunopathology', Graham *et al.*, 2005). This finding complicates the analysis, but it does not invalidate the theory. Day *et al.* (2007) show that the possibility that the parasite triggers an immunopathological response can be incorporated into a trade-off framework to predict virulence evolution in such a way that these predictions can be tested experimentally (Long *et al.*, 2008).

The reason for the debate about the mechanisms causing virulence is perhaps due to the misleading idea that virulence is always adaptive. The transmission–virulence trade-off hypothesis implies that virulence can increase transmission and/or decrease recovery. However, this does not mean that *all* the components of virulence are adaptive. Studies on immunopathology show that factors that are non-adaptive for the parasite can also contribute to the virulence and affect its evolution. Overall, the complexity of defining virulence need not derail the trade-off hypothesis, rather, as with any new knowledge, it should serve to refine and improve current theory.

2.3. Multiple infections and levels of selection

In classical models, the fitness of a parasite strain is given by the number of infections caused by an infected host (Box 1). This is not true if different strains have to share hosts, a common phenomenon called **multiple infection** (Read & Taylor, 2001). This has led Levin & Bull (1994) to propose the 'short-sighted evolution' explanation of virulence: they argue that in diverse infections, faster growing strains are favoured because there is competition for shared limited resources. Assuming that growth rates are related to virulence, such competition can lead to increased host mortality and the subsequent extinction of the competing parasite strains (known as the 'tragedy of the commons'). Co-infection experiments in rodent malaria (*Plasmodium chabaudi*) have provided the best support for this process. These experiments show that not only do virulent strains have a competitive advantage over less virulent strains in a multiple infection, but this advantage also translates into higher levels of transmission to the vector (de Roode *et al.*, 2005; Bell *et al.*, 2006).

If short-sighted evolution is important, what eventually stops parasites from becoming more and more virulent? Levin & Bull (1994) suggest, using HIV as an example, that the strains that get transmitted might be different from the strains causing the disease (which has received empirical support from Fraser *et al.*, 2007). If virulence is an adaptation to multiple infection, epidemiology may mediate reduced virulence (Nowak & May, 1994; van Baalen & Sabelis, 1995). Not all hosts are multiply infected and in a singly infected host, being too virulent only means wasting the resources of the host as there is no other strain to compete with. Thus, the prevalence of multiple infection is another factor that will affect the optimal level of virulence. Of course, multiple infection does not in itself affect the virulence–transmission trade-off and it is possible to study models in which there are both multiple infections and a transmission–virulence trade-off (van Baalen & Sabelis, 1995; Alizon & van Baalen, 2008b). This shows that multiple levels of selection need to be taken into account to understand virulence evolution (Coombs *et al.*, 2007).

Finally, some studies on multiple infections suggest that diversity in an infection can even lead to decreased levels of virulence (Frank, 1996; Brown *et al.*, 2002). The idea is that when strain diversity is low within a host, strains tend to be more **related**, which facilitates collective action for the common good (André & van Baalen, 2007). For instance, some bacteria can produce siderophores, which are molecules that can fix iron (a limiting resource for bacteria in a host). These molecules can be used by any bacteria, even those that do not pay the cost to produce the molecules. Theoretical (West & Buckling, 2003) and experimental (Griffin *et al.*, 2004) studies show that this co-operative behaviour can be maintained if the co-infecting strains are related. Increasing strain diversity decreases relatedness thus increasing the risk of collective actions being exploited by cheaters. Note that similar results are observed in cases of interference competition, i.e. when parasites are able to harm those of another strain. This is illustrated by bacteria producing bacteriocins (antibacterial compounds). Massey *et al.* (2004) show that bacteriocins are produced to kill unrelated bacteria. This has been shown to affect levels of virulence, so that hosts actually benefit from harbouring an extra strain (Harrison *et al.*, 2006).

In all of these cases, parasite virulence and transmission do not only depend on parasite replication rate but also on the level of cooperation in the infection. As shown by Alizon (2008a), epidemiological processes can profoundly affect the outcome. For instance, if multiple infections decrease the overall virulence of co-infected hosts, highly virulent strains can be favoured in the host

population. More generally, incorporating these kin selection ideas into the trade-off theory is still an open problem.

2.4. Integrating multiple time scales

Most of the theory of virulence evolution is based on the assumption that short-term evolutionary dynamics do not matter because parasites are at their epidemiological equilibrium. However, for many diseases this will not be the case, as either the host-parasite association is too recent or because the epidemiology is unstable, leading to cycles or even extinction–re-infection metapopulation dynamics (Grenfell & Harwood, 1997). In the case of HIV for instance, the pandemic is still expanding so the classical evolutionary invasion analysis approach will not be appropriate because it assumes that the resident strain is at an epidemiological equilibrium. A way to introduce the epidemiology is to follow changes in viral strain frequencies by using a population genetics approach (Day & Proulx, 2004; Day *et al.*, 2008).

The epidemiological dynamics can be further divided into a within-host phase where the parasite grows and a between-host phase where the parasite is transmitted. One problem is that within-host and between-host time scales may overlap. In the case of co-infections for instance, epidemiological dynamics will take place even though within-host dynamics are not at equilibrium (Alizon & van Baalen, 2008b). This creates complex interactions between selection at the within-host level and at the epidemiological level (Coombs *et al.*, 2007), which makes it difficult to identify potential trade-offs.

The latest theoretical improvement in virulence evolution theory comes from the application of population genetics concepts such as the Price equation to epidemiology (Day & Proulx, 2004; Day & Gandon, 2006, 2007). Classical game-theoretical approaches focus on the long-term outcome by studying 'evolutionary stable strategies' (Maynard Smith, 1982), or in more modern terms, the 'evolutionary attractor' (Geritz *et al.*, 1997). In contrast, the Price equation approach allows one to follow the transitory changes in trait values. This can help experimental validation of the trade-off because predictions about variation in life-history parameters are made in real time and do not require that the host population be at equilibrium. This approach has been used to estimate parameters involved in the trade-off hypothesis in the case of emerging pathogens (B. Bolker, A. Nanda and D. Shah, unpublished data) and has led to the important finding that the time since the beginning of the epidemic (e.g. if the epidemic is increasing or decreasing) can affect the shape of the trade-off and hence the optimal virulence.

Finally, it is worth pointing out that an evolutionary attractor need not be evolutionarily stable and several studies have reported cases where evolutionary branching occurs. For instance, this occurs if multiple infections are allowed, both in super-infection (Gandon *et al.*, 2002b) and co-infection (Alizon & van Baalen, 2008b) frameworks. More generally, multi-host pathogen dynamics easily exhibit evolutionary branching (Gandon, 2004). Finally, even for homogeneous host populations, a density-dependent host mortality rate along with an adequate trade-off function can lead to the evolution of two morphs that coexist over evolutionary time scales (Svennungsen & Kisdi, 2009).

2.5. The simplicity of the trade-off model

One could summarize the criticism of the trade-off hypothesis raised by Ebert & Bull (2003) as 'it is too simplistic'. Of course, they have a point: in reality, host–parasite relationships are characterized by more than just the two parameters, virulence and transmission. For instance, virulence, recovery and transmission are traits that are not determined solely by the parasites, but are actually the result of interactions with the host (see, e.g. Poulin & Combes, 1999). Hosts themselves are heterogeneous, complicating these interactions. The effect of host heterogeneity on virulence evolution is not straightforward. Ganusov *et al.* (2002) find that variations in host capacity to cope with an infection selects for increased levels of virulence. However, they use a uniform distribution of hosts. Gandon (2004) developed a general theoretical framework for the study of life-history evolution of multihost parasites, which includes demography (i.e. variations in the composition of the host population). More recently, Day & Gandon (2007) showed that the Price equation framework discussed above can readily include host heterogeneity. They show that this effect can be captured by expressing the average level of virulence and transmission rates in the parasite population. Experimental tests of these predictions have been equivocal. Grech *et al.* (2006) find a limited impact of host variation on virulence in the rodent malaria system. Working on an oomycete parasite of *Arabidopsis thaliana*, Salvaudon *et al.* (2007) find that host variation is a key factor determining parasite fitness traits. Finally, it is worth stressing that host heterogeneity has important public health implications because access to treatments or vaccines is often limited to one fraction of the host population (because of practical, economical or social reasons), which may strongly affect virulence evolution (Gandon *et al.*, 2003).

Another issue that the classical trade-off hypothesis ignores is that different parasites are transmitted by different routes, each of which may affect the shape of trade-off differently (Ewald, 1983). Too add further complexity, many parasites may have the capacity to be transmitted by multiple routes. Finally, the behaviour and ecology of hosts influence the rate of transmission for any given transmission route and may determine the relative importance of each route to the epidemiological dynamics. For example, hand-washing decreases the risk of person-to-person transmission but may alter rates of

water-borne transmission. To believe that levels of virulence and associated transmission rates, generated by vastly different process, will all lie along the same smooth curve is perhaps unrealistic. However, this area of research is distinct from questions about what favours different strains within a species (where it is much easier to imagine that individuals are indeed on the same curve). In practice, comparing trade-offs across transmission routes could only occur after first having obtained such species-specific curves from empirical data, and then comparing their shapes (see Box 2).

Trade-offs can be shown to emerge naturally from within-host processes (but see the discussion on multiple infections above or on spatial structure below). The underlying idea is that there is a constraint on the way the parasite exploits its host: a parasite cannot keep increasing its transmission rate, it cannot keep decreasing its induced mortality rate (without compromising its transmission), it cannot withstand the host's immune system indefinitely, and so on. When these constraints interact, for instance, if transmission cannot be increased without the parasite exposing itself to the immune system, a trade-off results. *A priori*, however, little can be said about the general shape of this trade-off, nor, as a matter of fact, which parasite life-history parameters it involves.

Recently, several within-host models have addressed this question, trying to assess the underlying constraints that lead to trade-offs (Mideo *et al.*, 2008). Of course, a trade-off still requires an arbitrary number of assumptions, but, in contrast to epidemiological models, these assumptions are necessarily more mechanistic and hence open to experimental verification. A main result of this approach is that the trade-off can occur at two levels. In most **nested models** (Mideo *et al.*, 2008), it comes from the way within-host dynamics are linked to epidemiological variables. This is achieved by assuming that one (or more) parasite trait (usually replication rate) affects virulence or transmission. However, in some models the trade-off occurs during the within-host dynamics. Examples of this approach are provided by Gilchrist *et al.* (2004) who study a case where viral production rate decreases the lifespan of infected cells and by Alizon (2008b) who studies a case where the immune activation rate depends on the parasite overall growth rate. In these cases, a convex trade-off curve emerges without making further assumptions on how the epidemiological processes are related to within-host processes.

Finally, some concerns about realism can be addressed without nested models. For instance, it is often stressed that virulence is not always adaptive. This is illustrated by the immunopathological phenomena discussed above. Weiss (2002) also points out the case of oncogenic viruses that lead to tumours decades after the infection. This can be addressed by following the timing of disease life-history events (Day, 2003). In the case of the oncogenic virus, if the tumour occurs late, when the parasite transmission is low, then it has no effect on the parasite fitness.

2.6. Spatial or social structure

No host population is without structure of some kind, spatial or social. Theoretical epidemiology shows that such structure may have far-reaching consequences (Diekmann & Heesterbeek, 2000). Such structure will also affect virulence evolution. Rand *et al.* (1995) have shown that ever higher transmission rates will not evolve even if there is no constraint or trade-off. The reason is that when their transmission rate is too high parasites will 'burn through' host clusters too quickly and kill them before they have contacted or merged with other host clusters. The evolutionary end result, they show, is characterized by a form of criticality with violent local stochastic fluctuations, just short of the boundary of host–parasite extinction. More recently, van Ballegooijen & Boerlijst (2004) developed a model without virulence where parasite traits (recovery and transmission) are allowed to evolve and where no assumption is made *a priori* to link these traits. They find that spatial dynamics cause evolutionary trajectories to follow a single relationship between transmission and recovery. Moreover, in their system, natural selection favours diseases with high transmission rates that cause short infections.

It would require an independent study to review all the implications of spatial structure. Here, we only highlight some results that may have arisen due to the trade-off hypothesis. van Baalen (2002) shows that components like the number of contacts and other topological parameters characterizing the structure of the host network may affect virulence evolution (see also Haraguchi & Sasaki, 2000). In summary, decreasing the relatedness of competing parasites (both within and between hosts) can have various effects on virulence (Buckling & Brockhurst, 2008). Recently, Kamo *et al.* (2007) derived a general framework to predict virulence evolution in spatial models depending on the shape of the transmission–virulence trade-off. Finally, using a nested model with explicit spatial structure, Read & Keeling (2006) show that even if there is no trade-off at the level of a host (a linear relationship between transmission and recovery), spatial structure is sufficient to lead to an evolutionarily stable intermediate level of virulence.

Most of these developments have remained mainly in theory but the first experimental results are arriving too. Boots & Mealor (2007) have designed an experiment with moth larvae infected with a virus (PiGV) to study the evolutionary consequences of population viscosity. In their set-up, infectivity of the virus evolves to the lowest value in the most structured environment. This illustrates how trade-off-based models that some might call 'simplistic' can lead to a better understanding of virulence evolution.

3. Perspectives

While the validity and applicability of the trade-off hypothesis have been questioned, subsequent studies have addressed these challenges and rectified some of its limitations. The fact that the trade-off hypothesis is now perceived as a new conventional wisdom is due largely to its simplicity. In a way, the current debate is similar to the one that challenged the avirulence hypothesis: the vast majority of theoretical studies assume a trade-off but few experimental systems have been designed to test this assumption and empirical data are lacking. One might therefore wonder if the trade-off hypothesis should be discarded, as was done with the avirulence hypothesis. A crucial fact that distinguishes these two theories is that there exists evidence that clearly contradicts the avirulence hypothesis, e.g. increasing virulence in nematode parasites of fig wasps over thousands of years (Herre, 1993), or the fact that tuberculosis was already present in ancient Egypt (Manchester, 1984; Donoghue *et al.*, 2004) and is still virulent. Similar refuting evidence has not been found for the trade-off hypothesis (though, given its general nature, it is admittedly not completely clear what kind of evidence would lead to an outright rejection of the trade-off hypothesis).

One of the strongest criticisms of the trade-off hypothesis is that it is too general to develop virulence management policies (Ebert & Bull, 2003, 2008; Salvaudon *et al.*, 2007). Such arguments point to the fact that although some parasites show the expected simple relationships between traits, others are subject to different ones. Theory often only accurately predicts changes when the biology of a particular system is taken into account in detail (for a specific example on vaccination, see Gandon *et al.*, 2002a). That predictions about specific diseases are only accurate when such biological specificities are taken into account is not really surprising; however, moving towards species-specific models is not always feasible, nor is it always appropriate.

In addressing the limitations of the trade-off hypothesis in relation to its applicability to management, Ebert & Bull (2003) seem to present a choice between using only the generalized trade-off model or an approach that views each system as novel and distinct, for which new theory has to be built from the ground up (i.e. a species-specific approach). From our point of view, the heart of this particular debate is about making *qualitative* vs. *quantitative* predictions, and also about identifying plausible outcomes vs. particular ones (see also Gandon & Day, 2003). Instead of being thought of as two different approaches for tackling a question about parasite evolution, the trade-off hypothesis and species-specific models ought to be thought of instead as two extremes along a spectrum that give rise to answers to completely different questions (see Box 3).

Thus, the nature of the question being addressed will dictate which approach is most appropriate. Even for specific application-driven questions the trade-off hypothesis and its extensions discussed above provide the general reasoning necessary to understand qualitative features of empirical data. This has been the case for anti-disease treatments where a model based on the trade-off hypothesis made both general predictions and predictions specific to the case of *Plasmodium falciparum* in Tanzania (Gandon *et al.*, 2001). The general predictions were later supported by experimental data (Mackinnon & Read, 2004).

Finally, incorporating more details of the biology of host–parasite interactions is always possible with the trade-off hypothesis. In a recent review, Frank & Schmid-Hempel (2008) study the influence of the type of pathogenic effects along with the timing of the effects on the shape of the trade-off. Frank and Schmid-Hempel find that when pathogenicity comes from escaping the immune response, higher levels of virulence are reached, which supports the original formulation of the trade-off hypothesis that involved recovery and virulence (Anderson & May, 1982). Schmid-Hempel (2008) suggests that a better understanding of immune evasion can help us understand why closely related parasites differ drastically in their virulence. Arguably, the greatest experimental and theoretical challenge for the trade-off hypothesis is now to better incorporate the immune system.

4. Conclusion

That some evidence to support the trade-off hypothesis is lacking is more of a call to improve the theory and experiments than to discard it. Undoubtedly, identifying the exact shape of trade-off curves will continue to prove to be a difficult task. Among several reasons, one is that usually it is assumed that transmission trades off with virulence but recovery is likely to play a major role as well (Anderson & May, 1982; Alizon, 2008b; Frank & Schmid-Hempel, 2008; Schmid-Hempel, 2008). We think that it is unfair to criticize the trade-off hypothesis for not making species-specific predictions because that is not what it is should be used for. A central dilemma in formulating good models is choosing the appropriate level of complexity with which to address the question. The trade-off hypothesis has a major role to play in addressing new problems because it provides a common framework in with which to compare experimental (qualitatively) or theoretical results. It is likely that new developments in the virulence evolution field will continue to use the trade-off hypothesis incorporating along the way further aspects such as inclusive fitness, transient dynamics, immunopathology, spatial structure. This will lead to more realistic models, not only to make specific predictions concerning virulence evolution that are more amenable to empirical testing, but also, and more fundamentally, to better understand parasite biology.

Box 3: Model complexity: malaria as an example

We use the case of *Plasmodium falciparum* to illustrate how layers of detail can be added to a model in order to answer questions that are increasingly quantitative in nature. We show that models required to answer qualitative and quantitative questions are not mutually exclusive, but rather that there is a gradation from one extreme to the other. For further details on virulence evolution in malaria, see Mackinnon & Read (2004).

The trade-off hypothesis in an S-I-R model

This epidemiological model describes the dynamics of susceptible, $S(t)$, infected, $I(t)$, and recovered, $R(t)$, individuals. There is a trade-off because the fraction of new infections per contact (β) is linked to virulence (α) via a mutual dependence on parasite density.

$$\frac{\mathrm{d}S(t)}{\mathrm{d}t} = \theta - \mu S(t) - \beta(\alpha)S(t)I(t) \tag{2a}$$

$$\frac{\mathrm{d}I(t)}{\mathrm{d}t} = \beta(\alpha)S(t)I(t) - (\mu + \alpha + \gamma)I(t) \tag{2b}$$

$$\frac{\mathrm{d}R(t)}{\mathrm{d}t} = \gamma I(t) - \mu R(t) \tag{2c}$$

where θ is the input of hosts in the population (assumed to be constant) and the other notations are given in Box 1. The transmission rate is denoted $\beta(\alpha)$ because it is a function of the virulence.

Parasite fitness for this model is given by eqn 1 and it allows one to predict the evolution towards intermediate levels of virulence (see Box 1). Moreover, because malaria is highly transmissible (transmission is achieved by the vector), Ewald (1983) suggests that selection should favour evolution towards high levels of virulence in the human host.

The trade-off hypothesis in a model with infection age

The traits of a disease vary during the course of an infection. The McKendrick equation allows one to follow the infection age (a) in an SIR model (McKendrick, 1926; Day, 2001):

$$\frac{\mathrm{d}S(t)}{\mathrm{d}t} = \theta - S(t)\int_0^\infty \beta(\alpha(a))\, I(a,t)\mathrm{d}a \tag{3a}$$

$$\frac{\partial I(a,t)}{\partial t} = -\frac{\partial I(a,t)}{\partial a} - (\alpha(a) + \gamma(a) + \mu)\, I(a,t) \tag{3b}$$

$$\frac{\mathrm{d}R(t)}{\mathrm{d}t} = \int_0^\infty \gamma(a)I(a,t)\mathrm{d}a - \mu R(t) \tag{3c}$$

$$I(0,t) = S(t)\int_0^\infty \beta(\alpha(a))I(a,t)\mathrm{d}a \tag{3d}$$

Equation 3a shows that transmission occurs between a susceptible host and a host of a given infection age ($I(a,t)$) at a rate that depends on the age of infection, $\beta(\alpha(a))$. Equation 3b indicates the fate of infected hosts: either they survive and go into the next age category (a increases) or the infection ends. The recovered pool is fed by infected hosts recovering (eqn 3c). Finally, eqn 3d is a boundary condition.

Introducing the age of the infection is an opportunity to add more biology into the model because it is unlikely that virulence, transmission and recovery will all occur at the same time nor will they remain constant throughout infections. Taking into account the timing of disease life-history events has a strong influence on virulence evolution (Day, 2003).

Gametocytes, merozoites and immune effector dynamics

One can add further detail by modelling within-host dynamics. Malaria has several life stages in its human host. Schematically, one can distinguish between an asexual stage (merozoite) that is responsible for growth and a sexual stage (gametocyte) that is responsible for transmission to the mosquito vector. Equations describing gametocyte (G) and merozoite (M) dynamics as a function of the infection age are used to augment the standard SIR model. Further realism is added by explicitly modelling immune effector (E) dynamics.

$$\frac{\mathrm{d}M}{\mathrm{d}a} = (1-g)\varphi\omega M(a) - \delta M(a) - kM(a)E(a) \tag{4a}$$

$$\frac{\mathrm{d}G}{\mathrm{d}a} = g\varphi M(a) \tag{4b}$$

$$\frac{\mathrm{d}E}{\mathrm{d}a} = \rho E(a) \tag{4c}$$

where g is the proportion of asexual parasites that are converted to sexual life stages, φ is the replication rate of merozoites, ω is the burst size of an infected red blood cell, δ is the death rate of a merozoite, k is the killing rate of the immune effector and ρ is the production rate of the immune effector.

With such a nested model (Mideo *et al.*, 2008), transmission and virulence are explicitly linked through their mutual dependence on within-host parasite densities. Mathematically, we have

$$\alpha(a) = c\varphi M(a) \tag{5a}$$

$$\gamma(a) = qM(a) \tag{5b}$$

$$\beta(a) = \frac{\zeta G(a)}{G(a) + z} \tag{5c}$$

where c, q, ζ and z are scaling parameters.
Defining

$$I_T = \int_0^\infty I(a,t)\mathrm{d}a$$

as the total number of individuals with infections of all ages, the mean virulence for all infected individuals is

$$\bar{\alpha} = \int_0^\infty \alpha(a)I(a,t)\mathrm{d}a\ I_T$$

Mean transmission $\bar{\beta}$, and recovery rates $\bar{\gamma}$ are defined similarly. Integrating the eqn 3b with respect to a, parasite fitness for this model is $R_0 = \bar{\beta}S/(\bar{\gamma} + \bar{\alpha} + \mu)$. For fixed parameter values, the solutions $M(a)$, $G(a)$ and $E(a)$ to the within-host equations 0 can be found using numerical integration. These can then be used to calculate $\bar{\alpha}, \bar{\gamma}$ and $\bar{\beta}$.

This model provides a more mechanistic approach for understanding the shape of the trade-off curve (which is usually assumed

Box 3: *continued*

with little justifications). Here, the dependence of virulence on the overall growth rate, $\varphi M(a)$, creates the saturation of the trade-off curve (as in most nested models; see, e.g. Gilchrist & Sasaki, 2002; André *et al*., 2003; Alizon & van Baalen, 2005).
This model is also easier to parameterize as $\bar{\beta}$ and $\bar{\alpha}$ are clearly defined as averages over all infection ages, and therefore, more amenable to making quantitative predictions. Finally, because of the added realism through the within-host scale, this model makes more accurate predictions. It can be used to answer questions of a more qualitative nature, for example, comparing the efficacy of a vaccine that targets the asexual vs. the sexual parasite life stages (Alizon & van Baalen, 2008a).

A completely realistic model

The model in the above section is likely still unsatisfying to many malariologists, who would insist instead on capturing more biological realism by explicitly tracking target cell (i.e. red blood cell) abundance and incorporating the distinctly discrete life cycle of malaria parasites (Molineaux & Dietz, 1999). A completely realistic model of malaria infections would include these additions as well as separately tracking male and female gametocytes, specific and non-specific immune responses, considering multiple infections, spatial structure, and specificities in the dynamics of the mosquito life cycle. The ultimate goal of such a model might be to predict the number of lives that will be saved given that a vaccine that blocks the sexual phase of the *Plasmodium* parasite (e.g. via antibodies targeting antigens expressed in the mosquito, Miura *et al*., 2007) will be administered to 50% of the population in high transmission regions of Senegal. The completely realistic model could likely answer such important questions, but contains many parameters that would need to be estimated and the final model prediction is likely to contain a high degree of uncertainty, due to the errors in these parameter estimates. At the other end of the spectrum, the trade-off model is simple and transparent, yet the implications – natural selection will favour intermediate levels of virulence – are less outstanding. More complicated models are better at making specific predictions, however, applied questions can be either of a qualitative or quantitative nature and simple models such as the trade-off hypothesis will often serve as launching pads to understand the results of more complex systems (for an example on vaccination, see Gandon *et al*., 2001).

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Glossary

Parasite: We here use the broad definition of parasites, which encompasses both micro-parasites, or pathogens, that replicate intensively within their host (e.g. viruses, bacteria and some protozoa) and macro-parasites (e.g. worms).

Virulence: The general definition is the harm a parasite does to its host. Here, we will consider that it is the host mortality due to the infection. Other detrimental effects are referred to as 'sub-lethal effects' here.

Transmission rate: The rate at which a susceptible host becomes infected when it encounters an infected host. Formally, it is the product of the contact rate and the transmission probability.

Recovery rate: The rate at which a parasite is cleared from its host.

Sub-lethal effects: Detrimental effects of a parasite on its host other than host mortality (e.g. sterilization or weight loss).

Multiple infections: Many hosts are often infected by more than one parasite strain (or species) at the same time, which affects the dynamics of the disease. Epidemiological models usually address this problem by either assuming that one strain immediately replaces the other in a host (super-infection) or that both strains always coexist within a host (co-infection).

Relatedness: This notion is essential to the kin selection theory, which states that, since it is mostly the genes that are passed from one generation to another, individuals that have genes in common have a converging interest

and should help one another. Relatedness is usually defined at the level of a gene and it can be seen as the probability that two individuals have the same allele for a given gene.

Nested model: A model that incorporates and links within-host dynamics and epidemiological dynamics.

R_0: It is the basic reproduction ratio. It indicates how many new hosts an infected host will infect over the course of an infection in a population where all the hosts are susceptible. This is an epidemiological measure of parasite fitness. If this rate is strictly greater than 1, the disease spreads (an infection produces more than one infection).

SPE: Serial passage experiments involve repeatedly transmitting a disease manually to a new susceptible host. By doing so, the cost of transmission for the parasite is alleviated.

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