CHAPTER 12

The evolution and expression of virulence

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Selection has nothing to do with what is necessary or unnecessary, or what is adequate, for continued survival. It deals only with an immediate better-vs.-worse within a system of alternative, and therefore competing, entities. It will act to maximize the mean reproductive performance regardless of the effect on long-term population survival. It is not a mechanism that can anticipate possible extinction and take steps to avoid it.

—George C. Williams (1966)

Introduction

Studies of the evolution of virulence aim to understand the morbidity and mortality of hosts caused by parasites and pathogens as the result of an evolutionary process. The evolutionary perspective on virulence focuses on the costs and benefits of virulence from the points of view of both parasite and host, with the goal of identifying the selective processes at work. The degree of harm inflicted by the parasite on the host is the trait of interest, ranging from avirulent (asymptomatic) to highly virulent (rapidly killing).

Historically, virulence was considered a deleterious side effect of new host–parasite associations that would evolve to low levels with time. This simple hypothesis is no longer entertained by the field for theoretical (Anderson and May 1982; Bull 1994) and empirical reasons (Herre 1993; Ebert 1994). It has been replaced by a set of new models. Depending on the costs and benefits of virulence, host and parasite physiology, historical constraints, and variance in biological, ecological, and epidemiological aspects of host–parasite interactions, almost any level of virulence can evolve (but not always predictably so), from highly virulent 'Andromeda strains' to mild, avirulent infections (Bull 1994). Of course, it is indeed the case that some of our most highly virulent infections are due to novel parasite associations (e.g., ebola, bird flu, SARS, rabies, fox tapeworm), but it is no longer thought that high virulence cannot be maintained as an evolutionary optimum.

This new perspective offers many possibilities and promises, in that the evolution of virulence is now seen to have a rich set of causes. Furthermore, the new perspective means that the evolution of virulence is now a topic of more than mere academic interest: it offers a conceptual framework to professionals in many fields and may contribute to decision-making in public health fields. One major goal of understanding the evolution of virulence would be to *manage* virulence: to design interventions in which parasites evolve lower levels of virulence and to avoid practices that encourage evolution of higher virulence. This hope was championed by Paul Ewald in the 1990s (Ewald 1994). Toward the goal of management, it would be desirable to discover simple rules that could be used to manage virulence evolution across many pathogens. At a less ambitious level, understanding the evolution of virulence might give insight to the future of new infectious diseases—whether the bird flu, Ebola, or SARS agents will evolve lower virulence if they become established as human epidemics, for example. So there are practical reasons to seek an understanding of virulence evolution.

This chapter offers an overview of current ideas surrounding virulence and its evolution.

The emphasis is on an evolutionary rather than a mechanistic perspective. In discussing different hypotheses we have tried to consider the needs and expectations of different audiences. Public health workers and epidemiologists are concerned with the prevention of disease and the reduction of its average virulence; this interest overlaps broadly with those of evolutionary biologists, for both view populations as a whole. Agricultural biologists concerned with livestock and crop pests fall roughly into the same category. In contrast, most human and veterinary medicine deals with parasites on a case-by-case basis, aiming to reduce their harmful effects in individual patients. At first, evolutionary biology seems to have little to offer them, but as we point out below, this is not always the case.

The concepts in this chapter are based on parasites that are, for the most part, transmitted infectiously between hosts (horizontal transmission). The evolution of vertically transmitted parasites has been discussed in several reviews (Bull 1994; Ebert and Herre 1996).

Outline of this chapter

We begin with some preliminaries: possible definitions of virulence, and the relationship between virulence evolution in a test tube and the creation of live, attenuated vaccines. The latter example lies somewhat outside traditional work on the evolution of virulence, but it sets a precedent for what we hope to obtain from a general theory for the evolution of virulence, and it sheds light on its process. From there, the chapter discusses the evolution of virulence in three successive phases:

- Phase 1 is the first contact of a disease agent with a new host, as with accidental infections.
- Phase 2 occurs as a parasite has just established itself in a new host species and is far from the optimal virulence.
- Phase 3 applies to parasites that are established for a prolonged period of time in a host species and population and evolve in response to changes in the environment, including host demography.

Although these phases are not well-defined biological categories, they help to structure current ideas about the evolution and expression of virulence. For example, the Ebola virus causes occasional local outbreaks in Africa that usually fade out quickly. Because there is no current evidence that it adapts toward its new host during these short episodes, it has never reached phase 2. The human immunodeficiency virus is a relatively young, but persisting, disease that entered the human host only a few decades ago and may still be adapting to it. This disease is still in phase 2 in many parts of the world. In Phase 3 are established diseases, such as malaria, tuberculosis, and leprosy. A parasite in phase 3 that invades a new host population (e.g., human diseases introduced into the Americas from Europe and Africa) may fall back into phase 2.

In our discussion of established diseases (phase 3) we introduce the trade-off model and discuss several of its shortcomings because this model has been the foundation of many models for the evolution of virulence. In the section on mechanisms of virulence we discuss features that future models may incorporate to improve their predictive power.

Many discussions of the evolution of virulence assume that the host population does not show much variation with respect to virulence expression and that it evolves much more slowly than the parasite. Here we discuss the evolution of virulence in cases where host variability is taken into account. We also discuss the possibility that virulence gives the parasite a direct benefit. Finally we address the question of whether we can use our knowledge about virulence to guide parasite evolution to our benefit.

Defining virulence

Central to all theories on the evolution of virulence is an understanding of what virulence is. For many purposes, virulence can be described simply as parasite-mediated morbidity and mortality. This definition encapsulates the entire range of disease symptoms that reduce host fitness, regardless of whether virulence is an evolved characteristic or a coincidental by-product. In specific cases, more precise definitions of virulence should be used and should precede any discussion of its evolution. It is useful here to introduce the distinction between virulence as damage to the host that does

not directly benefit the parasite versus virulence in which the disease symptoms directly benefit the parasite—that the host damage per se (e.g., host death) is needed for parasite transmission.

Most literature deals with the first form of virulence as an unavoidable by-product of parasite replication. Mathematical models of the evolution of virulence for horizontally transmitted parasites typically use parasite-induced host death rate (PIHD) to measure virulence. PIHD is a fitness component of both the parasite and the host. However, whereas host death is considered a measure of virulence that directly impairs host fitness, effects of the parasite on host fitness traits such as the host's attractiveness to mating partners, host fecundity, or more generally morbidity, are not included here because they do not (necessarily) affect the parasite's transmission and, thus, are not expected to affect selection on the parasite. Nevertheless, mathematical models that use PIHD as a measure of virulence are often discussed in the sense that other virulent consequences of infection correlate closely with PIHD. As a consequence, empirical studies often use host fitness reduction (or a component of it) as a measure of virulence, including parasite-induced live-weight lost, anemia, reduced growth, and reduced fecundity (e.g., Ebert 1994; Mackinnon and Read 1999a). For most host-parasite systems this may be a reasonable first approximation of virulence, but to interpret data with regard to parasite evolution, the form and strength of the relationship between disease-related host traits and parasite fitness is important, though it is usually poorly known (Day 2002; Ebert and Bull 2003a). Even measures of host death associated with infections (e.g., case fatality rate) can be misleading for estimating PIHD (Day 2002).

Defining virulence becomes more complex when host evolution is considered. Hosts evolve to reduce the damage that parasites cause to their fitness. Some components of damage may impact both partners (e.g., PIHD), while others may not. For example, parasite-induced reduction in the host's sexual attractiveness, which may strongly reduce host fitness, may occur without any effect on parasite fitness. Therefore, it must be included in models of host evolution and host–parasite coevolution, but not in models of parasite evolution (unless the parasite is sexually transmitted).

A different perspective on virulence comes from studies that try to explain the evolution of specific disease symptoms as directly beneficial to the parasite. For example, host castration has been directly linked to the parasite's resource demands (Obrebski 1975; Ebert *et al*. 2004), impaired host mobility has been suggested to help vectors feed on the diseased hosts (Ewald 1983; Holmstad *et al*. 2006), and altered host behavior may benefit transmission to a second host that preys on the infected first host (reviewed in Moore 2002).

These complications highlight the importance of specifying the bases of virulence when discussing a specific system.

Artificial virulence evolution and live vaccines

One of the oldest and most successful uses of virulence evolution has been technological: to create attenuated live vaccines. Live vaccines are strains of a formerly pathogenic organism that have evolved to become avirulent. They create a mild infection that stimulates the body to develop immunity without causing the disease. Live vaccines include the Sabin (oral) polio vaccine; the measles, mumps, rubella, yellow fever, and chicken pox (varicella) vaccines; one of the influenza vaccines (flu mist); the tuberculosis (BCG) vaccine; and one of the typhoid vaccines (Makela 2000).

Before the advent of genetic engineering, the standard method for developing a live vaccine was to adapt a virulent pathogen to growth in culture. As the pathogen evolved to grow better in culture, it also often evolved to grow poorly in the normal host, where its virulence was therefore reduced (Ebert 1998). The basis of this outcome is a trade-off between ability to grow under one set of conditions and that of another: to grow better in culture, the pathogen must sacrifice its ability to grow in the host. This method did not always succeed in creating a safe vaccine, and it cannot yet be predicted how much the pathogen must adapt to culture to attenuate on the host. But the method was robust enough to succeed in many cases.

The low virulence of attenuated vaccines can be reversed by evolution. If the vaccine strain is again allowed to transmit between hosts, natural

selection may promote the evolution of variants that grow better and re-acquire high virulence. Reversion to virulence, often observed for the Sabin vaccine, has caused several local polio epidemics (Kew *et al*. 2004).

The artificial evolution of virulence during live vaccine creation is not typically considered in treatises on evolution of virulence. Yet there are several reasons to acknowledge it here. First, the creation of attenuated vaccines and the reversion to high virulence illustrates that virulence can evolve rapidly in both directions; reversion to high virulence upon escape of a vaccine strain further indicates that virulence is tied to some form of 'optimum'. Second, practical rules of virulence evolution can be simple, although we must acknowledge that evolution of reduced virulence under radically changing growth conditions may be easier to predict than evolution of virulence within a single host in response to changes in population structure. Third, the results support the basic idea that higher virulence (and in some cases transmission) is associated with higher parasite growth rate (Ebert 1998). So this example is useful for establishing several precedents and offering hope in managing the evolution of virulence.

The three phases of the evolution of infectious diseases

In this section we consider different phases in the adaptation of a parasite to a host. These stages are in essence a progression of disease 'emergence' in a new host, and the progression directly impacts what might be expected in the evolution of virulence.

Phase 1: Accidental infections

Many pathogens can infect and cause disease in a host that is not part of the normal transmission route but is a dead end for the parasite. The accidental infection may fail to cause secondary infections in other individuals (as with rabies, lyme disease, West Nile virus, and anthrax in humans), or it may create a short chain of infections in the accidental host that quickly dies out (e.g., bird flu, SARS, Ebola, and pneumonic plague in humans). Accidental human infections can be particularly virulent. Rabies, Ebola, SARS, bird flu, and pneumonic plague all have case fatality rates of 50% or more. Untreated alveolar echinococcosis caused by the fox tapeworm, *Echinococcus multilocularis*, results in mortality that approaches 100% over a course of 15 years (Ammann and Eckert 1996).

Related to accidental infections are emerging infectious diseases. They are usually defined as diseases of infectious origin whose incidence in humans has increased within the past two decades or threatens to increase in the near future. Chapter 16 in this book deals in more detail with emerging infectious diseases.

The level of virulence expressed when a parasite infects a new host for the first time is not expected to follow evolutionary principles based on natural selection. Because the genes responsible for virulence did not evolve under the conditions in which they are expressed, their effect on the host is not predictable (Levin and Svanborg-Edén 1990). The botulinum toxin produced by *Clostridium botulinum* did not evolve to kill people. The blindness caused by *Toxocara canis* did not result from evolution within human hosts. Thus virulence in novel hosts does not represent an equilibrium for parasite fitness. Furthermore, virulence in novel hosts is unlikely to be associated with high transmissibility (which accounts for the eventual die-out). It follows that the virulence of parasites newly introduced into humans cannot be managed or directed, although preventive measures may be used to reduce their harm or to prevent infections (e.g., vaccines).

In one sense, accidental diseases are rare. Given the vast number of parasites with which humans have nearly daily contact, it is clear that only a small minority of them are able to infect hosts other than those they usually live on. Nearly all hosts are resistant to nearly all parasites. The frequent opportunistic infections suffered by immune-compromised people testify to the power of the immune system to protect us from most parasites. Although accidental infections are rare, the same zoonotic disease may emerge repeatedly, posing a severe health risk for those exposed, for example people working in agriculture. Host switches are facilitated by frequent contact between the novel and the reservoir

host, as when they share resources or use the same habitat (Epstein *et al*. 2003). Accidental infections seem more likely when the reservoir host and the novel host are phylogenetically related. Thus, humans become more easily infected with parasites from other primates than from rodents, but more easily with rodent parasites than with fish or even invertebrate parasites. This does not exclude the possibility of a host switch between distantly related hosts, but makes it a rather unlikely event (Krauss *et al*. 2003).

Experimental data suggest that accidental infections are mostly (but not invariably) less virulent than are established ones. The relevant studies (often published under the rubric of local adaptation) compare well-established host–parasite combinations from the same geographic location with novel combinations from different geographic locations. In most cases, infectivity and virulence are, on average, higher in established associations than in novel ones (Lively 1989; Ebert 1994; Morand *et al*. 1996). There can, however, be considerable variation around this average (Ebert *et al*. 1998; Kaltz *et al*. 1999): a novel combination may be avirulent on average, but may occasionally be highly virulent (Ebert 1994).

The low virulence of some accidental infections has been of practical value. Two hundred years ago, Edward Jenner used the cowpox virus to immunize humans against the related smallpox virus. The cowpox virus is largely avirulent in humans, but it is similar enough to the human smallpox virus to induce immunity against it. Cowpox was an accidental infection of women who milked cows, and the immunity of those women to smallpox led to the inference that cowpox was protective against smallpox. Conversely, the high virulence of some (deliberate) accidental infections has also been of practical value. Biological control efforts have at times used extremely virulent parasites of an unwanted pest species (e.g., rabbits released into Australia). The virulent agents are usually found as parasites of related species or strains and happen to be highly virulent in the pest species. This protocol was used in selecting the myxoma virus for release into Australian rabbit populations (Fenner and Ratcliffe 1965). Collectively, these applied examples reveal that there is no universal pattern for the virulence of accidental infections.

What, then, can be said about the virulence of new diseases? There is nothing that can be predicted based on evolutionary principles. There is certainly a bias in reporting novel diseases: more virulent ones are more likely to be noticed and reported. Because harmless infections are not noticed, we get the impression that novel diseases are usually virulent (Ebert 1994).

Phase 2: Evolution of virulence soon after successful invasion

Host switching appears to be common in the history of many infectious diseases when considered over long evolutionary time (see Chapter 16). The successful invasion of a new host species will probably be preceded by many accidental infections that die out (phase 1). To enter phase 2, an accidental infection of one individual spreads to another host, then another, forming a continual transmission chain that successfully invades the new host population. At least initially, this spread will constitute an epidemic, in which the number of infected hosts increases (in contrast to the endemic phase, in which the number of infected hosts is relatively constant, even though new infections are continuing). The invading parasite will generally not be well adapted to the new host, and consequently, there will be rapid evolution of the parasite and possibly the host. Virulence may evolve rapidly in this phase.

The cases where a parasite switches hosts and rapidly causes a novel, spreading disease, are rare. The fox tapeworm and the Ebola virus have so far failed to evolve into spreading human diseases. In contrast, the human immunodeficiency virus (HIV) is one of the rare cases that has established itself in the human population and is still in the initial epidemic phase in parts of the world. Experimental work and field observations have yielded several examples that illustrate the evolution of virulence and make it possible to study the evolution of diseases that emerge from novel parasite–host associations.

Phase 2 also applies to the origin of novel variants of a pathogen in the old host. These are

extreme mutants of existing parasites that are able to start epidemics. For example, a variant with novel antigenic properties may spread through the host population as if it is a completely new parasite, unaffected by existing host immunity and other defenses. Thus, phase 2 is a broad category to represent parasites whose dynamics are initially (strongly) epidemic, regardless of the reason. Virulence and other phenotypes may be far from optimal when such mutants arise, but their advantage in one phenotype (e.g., immune escape) may outweigh other suboptimal traits, such as too high virulence, so that they spread nonetheless. Evolution may subsequently change these suboptimal traits. In contrast to parasites switching hosts and entering phase 2, novel variants of old diseases spreading epidemically and causing a threat 'from within' may be rather common.

The classic example of virulence evolution in phase 2 is the European rabbit-myxoma virus system. The highly virulent myxoma virus was isolated from a South-American hare species and released for biocontrol of a rabbit pest in Australia. Over the following years the virus first declined rapidly in virulence. Later (in what was probably phase 3) virulence then increased slowly again, as tests on non-coevolving control rabbits showed. At the same time the wild rabbit evolved to suffer less from the original virus (Fenner and Kerr 1994). In this case, the virulence of the original virus, which was deliberately chosen to be a virulent pest control agent, was apparently well above the optimal level. This impressively documented example shows that virulence can evolve rapidly with large changes—the initial decline in virulence took only a few years—and that hosts evolve to reduce the costs of parasitism. Thus, virulence is influenced by both host and parasite evolution. We will return to this example when discussing the trade-off model.

There are few natural examples of phase 2 evolution that have been documented as well as the rabbit–myxoma system. However, there are a number of serial passage experiments that serve to illustrate what happens if a parasite is evolving under new conditions. Serial passage studies reveal the changes in virulence associated with parasite adaptation to novel hosts or novel culture conditions.

Serial passage experiments

In the earlier discussion of serial passage experiments, it was noted that virulence in a former host often declines when a parasite is adapted in a new environment. Here we note that the flip-side of this evolution is also true: when a parasite is adapted by serial passage to a new host, virulence in that new host typically increases. While this offers a tool for studying the evolution of virulence under experimental conditions, it has obvious limitations with regard to its application to natural populations.

Typically, serial passage experiments involve the artificial infection of a novel, but related, host and subsequent transmission from one individual to the next in that new host species (e.g., by syringe transfer of blood). The host strains used are usually well defined and of low genetic diversity (inbred lines, full sib families, clonally propagated cell lines). Host-to-host transmission is controlled by the experimenter, and selection for the natural mode of transmission is relaxed. Despite huge variation in the purpose and methodology of serial passage experiments, several results are consistent (Ebert 1998):

- Virulence usually increases during serial passage in the new host. It can evolve rapidly and on time scales of weeks or months. This effect can be so strong that even selection for reduced virulence during serial passage experiments results in an increase in virulence (Mackinnon and Read 1999b).
- The increase in virulence depends on the host's genotype. As noted earlier, parasites passed through one host-type become 'attenuated' or show reduced virulence in the original host and may thus serve as a vaccine in that host.
- The increase in virulence likely results from within-host evolution, which drives an increase in within-host growth rate and thus, virulence (Bull and Molineux 1992; Ni and Kemp 1992; Novella *et al*. 1995). Competition trials between parasite strains with different within-host growth rates have shown that the most rapidly growing strains out-compete slower growing strains (Ni and Kemp 1992; Novella *et al*. 1995). Thus, it seems that within-host competition between parasite genotypes drives

within-host growth rate, and that the growth rate is positively correlated with virulence.

Given these findings, the question arises, 'Why does virulence not increase under normal, 'non-passage' conditions?' The answer may relate to the evolution of between-host transmission. During serial passage experiments, the experimenter ensures host-to-host transmission among genetically homogeneous host lines (e.g., inbred lines, cell cultures) regardless of the level of virulence, so selection for host-to-host transmission is relaxed and adaptation to specific host lines favored. Thus, serial passage experiments mimic endless within-host growth. Under such conditions within-host competition drives selection for increased growth rates, a process that might have costs in terms of reduced host-to-host transmission (Ebert 1998).

The examples of parasites spreading in novel hosts suggest several important insights into the evolution of virulence. Virulence can evolve quickly up or down when the parasite faces a new host from which it is able to transmit. While within-host competition is well established as the mechanism for the evolutionary increase in virulence, the mechanisms that reduce virulence are not yet understood. The trade-off model discussed in the following section is a possible solution.

Phase 3: The evolution of optimal virulence

After persisting for some time in a new host population, the parasite should approach equilibrium virulence, and evolutionary changes in virulence should become less striking. The parasite may then reach a selection boundary, in which trade-offs among the parasite's various fitness components constrain further evolution of virulence and transmission (Ewald 1980; Anderson and May 1982). The main difference between phase 2 and phase 3 is whether the parasite has evolved to the trade-off boundary. Anderson and May (1982) found evidence consistent with a trade-off between parasite-induced host mortality (= virulence) and host-induced parasite mortality (= host recovery) in the data from the rabbit–myxoma virus system. Highly virulent myxoma strains cause many lesions (presumably necessary for transmission), are cleared slowly by the host immune system, and quickly kill the rabbit; whereas mildly virulent strains are quickly cleared. The fittest strain has intermediate virulence and is cleared at intermediate rates. Epidemiological data showed that strains with intermediate virulence evolve to dominate the system, a finding supported by mathematical models (Fenner and Ratcliffe 1965; Anderson and May 1982).

In its simplest form, the optimum in the trade-off model is found by maximizing the number of secondary infections produced by a primary infection. Such selection is based entirely on maximizing between-host transmission. In most cases the trade-off is between 'how long' and 'how fast' the parasite can transmit (Mackinnon and Read 2004). High virulence, which is assumed to have a high *rate* of transmission, impedes *net or total* transmission because it kills the current host too quickly, impairs host interactions with other hosts, or induces the host immune system to react strongly. These positive correlations between virulence, within-host growth, and transmission rate are well-supported with data from many organisms (Bull and Molineux 1992; Ebert and Mangin 1997; Lipsitch and Moxon 1997; Thrall and Burdon 2003). In some cases, they can be interpreted as suggesting the diversion of resources from the host into the reproducing parasite.

The trade-off model is versatile and makes it possible to predict changes in optimal virulence for different conditions. For example, Read and Mackinnon discuss possible evolutionary consequences of vaccination programs for virulence evolution in Chapter 11. Most commonly, it has been recognized that changes in external conditions can favor a different level of virulence, and a parasite with a short generation time can evolve a new level quickly. There are some recognized external effects on the virulence optimum: Short host life span favors higher parasite virulence, although we have no obvious reason to think that host life span will change enough to select meaningful changes in parasite virulence. An empirical test of this prediction failed, however (Ebert and Mangin 1997), possibly because of an epidemiological feedback that arose as a consequence of altered host longevity. The idea that host longevity shapes virulence evolution is based on the notion that host death

curtails parasite transmission. Likewise, every other factor that influences the parasite's life span may influence virulence evolution. A further prediction of the trade-off model is that higher virulence is favored when the number of infected hosts is increasing rapidly (an epidemic); relatively low virulence is favored when the number of infected hosts is static (endemic) (Lenski and May 1994; Bonhoeffer *et al*. 1996; Bull 2006). Empirical data to support this prediction are lacking.

A common misunderstanding of the trade-off model is that the opportunity for transmission affects virulence evolution, such that lower host densities per se were suggested to lead to a decrease in the optimal virulence (Ewald 1994). However, as pointed out by Lipsitch *et al*. (1995), when the parasite has reached dynamical equilibrium, each infected host gives rise to one new infected host on average, so the effect of host density drops out. Host density does affect the optimal virulence during the epidemic phase, just not when the parasite and host have reached dynamic equilibrium (Bull 1994; Lenski and May 1994).

Experimental tests

Heineman and Bull (submitted) experimentally evolved virulence (time to lysis) specific to different host densities in the bacteriophage T7. In this study, the virus population was maintained in epidemic phase and not allowed to reach dynamic equilibrium, so the virulence optimum differed with high and low host density: high host density allowed the phage to spread quickly, favoring high virulence; low host density greatly slowed phage growth and favored low virulence. They found mixed support for a trade-off model explaining virulence evolution. At high host density, expected to select high phage virulence (rapid lysis), the experimentally evolved outcome was quantitatively close to the predicted optimum. At low host density, expected to select low phage virulence (late lysis), the phage evolved but still retained much faster lysis than was predicted. In general, the phage's evolution between the conditions of high and low host density was less extreme than predicted.

The central prediction of the trade-off model—that parasite lifetime transmission success peaks at intermediate virulence—has recently been shown in a case study using a parasite that builds up progeny in the host and then must kill the host to release its progeny (as does bacteriophage T7). Such parasites are very well suited to be model species for the study of virulence, for one can estimate their lifetime transmission success and relate it to other variables. Jensen *et al*. (2006) showed that for a bacterial pathogen in a planktonic crustacean host lifetime spore production of the bacterium peaks at intermediate time to host death (see Box 12.1). This study is in line with the idea that virulence evolves to an optimum, but it does not allow us to conclude that the mechanism proposed by the trade-off model is the driving force in shaping this optimum.

Within-host evolution

The trade-off model has been extended along various lines, most importantly to include within-host selection, i.e., competition among parasites within hosts. As discussed in the previous section, it is thought that within-host competition usually selects for higher parasite growth rate, and thus for higher virulence (Bremermann and Pickering 1983; Nowak and May 1994; van Baalen 1994). This has led to predictions that factors that increase the rate of multiple infections (i.e., higher host density, higher transmission rates, and low host background mortality, which allows for longer life span and thus more multiple infections) also select for higher virulence (van Baalen and Sabelis 1995; Frank 1996; Ebert and Mangin 1997). Likewise, under conditions of increased competition among unrelated parasite genotypes, virulence may increase, e.g., less spatial structure and less vertical transmission (Frank 1996). The picture may change if co-infecting parasites cooperate to exploit their host (Turner and Chao 1999; Brown 2001) or evolve to exploit each other, as is the case with defective interfering particles of viruses (DIPs), which are, in essence, parasites of viruses (Bull 1994). Both phenomena may lead to a reduced virulence, but it is currently not clear whether they are representative of natural systems.

Trade-off models also predict that if parasites can adjust their virulence facultatively, they should express higher virulence when encountering a competitor within the same host. This prediction has been met in a malaria–mouse system (Taylor

Box 12.1 Experimental evidence from waterfleas

The trade-off virulence model predicts that transmission-stage production and host exploitation are balanced, such that the parasite's lifetime transmission success (LTS) is maximized. For parasites that suppress host reproduction, this simple model has been modified to account for the fact that they convert host reproductive resources into transmission stages (Ebert *et al*. 2004). Parasites that kill the host too early will not benefit from these resources, while postponing the killing of the host results in diminished returns, because the parasite grows more rapidly than its host. Therefore, killing the host after an intermediate time period results in maximal LTS. Earlier experimental studies have had difficulty finding direct evidence for maximal LTS at intermediate virulence. Jensen *et al*. (2006) used a host–parasite system (*Daphnia*, a planktonic crustacean, and the bacterial parasite *Pasteuria ramosa*), in which the competition between host and parasite for resources is particularly strong. The parasite benefits by converting a large proportion of host biomass into parasite transmission stages (endospores). To gain more resources, *P. ramosa* must suppress reproduction of its host. The transmission stages produced by the parasite accumulate in the host and their number increases with the age of infection. The spores produced by the parasite are not released until host death, which permits one to estimate the parasite's LTS accurately. To test for an optimal LTS at intermediate times to death, the authors infected individual *Daphnia magna* of one host clone with the bacterium and followed these individuals until their parasite-induced death. They found that the parasites showed strong variation in the time to kill their host, and that transmission-stage production peaked at an intermediate level of virulence (Fig. 12.1). Variation in time to death and LTS was shown to be at least partially based on genetic variation among parasite genotypes (Jensen *et al*. 2006).

Another interesting finding of this study was the observation that some hosts died before the parasite had produced any transmission stages. Apparently some parasite lines were so virulent that they induced host death before they finished spore development. This may put an upper limit on virulence evolution.

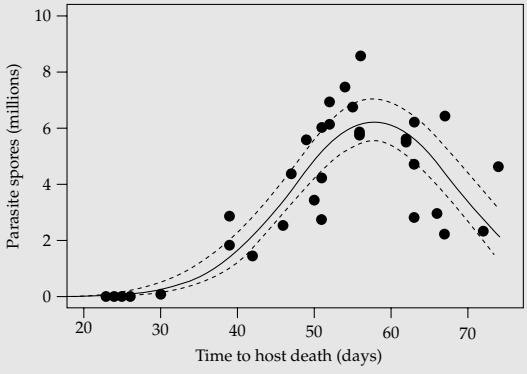

Figure 12.1 Relationship between lifetime spore production of *Pasteuria ramosa* and longevity of its host *Daphnia magna*.

et al. 1998) but not in other systems (Lauria Pires and Teixeira 1997; Imhoof and Schmid-Hempel 1998; Vizoso and Ebert 2005).

Combining the trade-off model with within-host evolution

In the broad picture, intense host exploitation, and thus, virulence, can be viewed as a selfish strategy favored by within-host competition but selected against by between-host competition (Antia and Koella 1994; Bonhoeffer and Nowak 1994; Bull 1994; Nowak and May 1994; van Baalen 1994; Frank 1996). In this respect, the evolution of virulence is like other evolutionary problems in which selection operates differently at different levels of population structure, as in the classic group versus individual selection in the evolution of cooperation and altruism (Williams 1966). The level of virulence that evolves in hierarchical population structures depends on the conditions that influence the type and frequency of multiple infections and the costs imposed by early parasite death. Epidemiological conditions under which between-host competition is very important lead to low virulence, as

in vertically transmitted parasites (Jeon 1972; Bull *et al*. 1991; Herre 1993), while experimental exclusion of between-host transmission dynamics leads to high virulence, as in serial passage experiments (Ebert 1998; Mackinnon and Read 1999b). These cases are extremes along a continuum, on which most horizontally transmitted parasites evolve under less extreme conditions. Understanding the evolution of virulence under non-extreme conditions is a major challenge to the field in the coming years.

The beauty of the trade-off model is that it makes predictions. Direction and magnitude of change may depend on the environment, which may be influenced by treatment. The chapters by Koella and Turner (Chapter 17) and by Read and Mackinnon (Chapter 11) in this book give various examples for the application of the trade-off model. In the following aspects of virulence evolution not considered by the trade-off model are discussed.

Mechanisms of virulence remain to be considered

Understanding the mechanisms that generate virulence can shed light on how virulence evolves. Here we suggest that such analysis should partition the different components of morbidity and mortality rather than lumping them together into a single measure of virulence, as is done frequently in applying the trade-off model. Because such understanding has not been incorporated into most evolutionary literature on virulence—most mathematical models ignore the mechanistic basis of virulence—it is an area that begs to be developed. Here we focus on places where incorporating mechanisms is likely to improve our understanding of the evolution of virulence.

For many infectious diseases a great detail is known about the molecular mechanisms generating virulence. A detailed marriage between mechanisms (proximate) and evolution (ultimate) should be possible. For example, microbiologists have identified 'virulence factors' in many pathogenic bacteria, genes essential to cause disease but that are not essential for the growth of the microbe. Virulence factors of bacteria are often clustered on plasmids or in 'islands' of the genome and are easily identified by comparison of pathogenic and non-pathogenic strains. For many virulence factors their function is known in great detail. Often they are toxins or toxin-delivery systems that target host cells and interfere with the host's normal cellular function. Although there are few attempts to incorporate such information into models of virulence, knowing the functions of the genes responsible for virulence should help in understanding the connection between benefit to the parasite (e.g., transmission or growth in the host) and virulence phenotype. This knowledge, in turn, may allow a more accurate modeling of whether and when the cost of virulence outweighs the benefit to the parasite.

A general assumption that underlies most models of virulence evolution is that higher parasite densities within hosts lead to higher virulence and to higher rates of transmission. From this, a positive correlation between virulence and transmission rate is postulated, and has been found in several studies (see previous section). At a gross level, this seems plausible. Yet, there are biological reasons why this view may be misleading when dealing with more subtle variations in parasite density. The immediate cause of virulence may be the systemic levels of parasites, or it may be due to parasite levels in a specific tissue. This is particularly problematic if virulence results from a complex interaction between parasite levels and host tissue. For example, polio and bacterial meningitis are caused by the infection of tissues in the central nervous system (CNS) that are not part of the normal infection–transmission cycle. For these cases, higher parasite densities in the normal tissue of infection are usually not problematic, but infection of the CNS is. If parasite density in the normal tissue is correlated with infection of the CNS, then the generic model of 'high parasite density = high virulence' may work. If parasite density in the normal tissue is uncorrelated with CNS infection, then the model fails (Levin and Bull 1994).

Virulence is an interaction between host and parasite. One might naively assume that virulence is due merely to the parasite's killing of host cells. Yet it is increasingly realized that the causes of virulence are often an 'over-reaction' by the host immune system (Margolis and Levin 2007).

For example, the Spanish flu virus (influenza A), which killed up to 50 million people in the 1918 pandemic, is believed to trigger an aberrant innate immune reaction in its host, resulting in persistent elevation of inflammatory-response genes and a highly virulent infiltration of the immune cells in the respiratory tract (Kobasa *et al*. 2007). Just as an allergic reaction and asthma can be fatal in the absence of a true threat, the immune response to a parasite can contribute to host morbidity and mortality in ways inappropriate for controlling the infection. Immune over-reaction may in fact put the parasite in a 'cruel bind'; it must find a way to avoid triggering a strong immune response yet still generate enough of an infection to be transmitted (Margolis and Levin 2007). How an immune over-response should be incorporated into models for the evolution of virulence has yet to be properly developed.

Variation of hosts impacts the expression and evolution of virulence

The trade-off model assumes that the level of expressed virulence reflects an adaptation of the parasite, ignoring the fact that the expression of virulence is usually the result of an interaction between host and parasite genotypes. Variation among hosts within populations is common, and virulence across different combinations of host and parasite genotypes has consistently been shown to vary markedly (Hill *et al*. 1991; Singh *et al*. 1997; Carius *et al*. 2001; Rauch *et al*. 2006). Furthermore, for many diseases only a small fraction of the infected hosts become sick, while the majority are symptom-free carriers. For example, only about 5% of humans infected with tuberculosis develop the disease. About 10% of Europeans carry *Neisseria meningitidis* at any given time, but only about 1 in 10,000 will develop meningitis. Only one in 200 polio virus infections leads to irreversible paralysis (WHO Factsheets 2003).

In the absence of parasite evolution, virulence would decrease as a result of host selection for reduced costs of parasitism. In the absence of host evolution, parasites would evolve to exploit their hosts optimally. In a coevolutionary scenario, however, virulence is subject to antagonistic selection, and the resulting outcome is not readily predicted (Ebert and Hamilton 1996). The expressed level of virulence in coevolving host–parasite populations may lie somewhere between the hosts' and the parasites' optima and may vary within the population, but epidemiological feedbacks can also produce very different outcomes (Restif *et al*. 2001; Gandon *et al*. 2002), making predictions difficult.

The host-genotype specificity of virulence is enhanced when microparasites spend many generations on an individual host and adapt to deal specifically with this host genotype. Adaptation to one host genotype has been found to increase virulence on that host genotype and to reduce the parasites' ability to exploit other host genotypes (Ebert 1998), but the generality of this is not clear. A corollary of this was suggested by a study of Garamszegi (2006): generalist species of malaria are less virulent than specialist species. Apparently, in genetically diverse host populations, parasites may have to adapt anew whenever they are transmitted, which goes hand in hand with a reduced level of virulence (Ebert 1998). This finding may—at least in part—explain the notorious sensitivity of agricultural monocultures to devastating epidemics (Barrett 1981). The cloning of livestock also needs consideration, for harmless diseases could evolve into deadly epidemics when infecting clonal herds.

Virulence has a direct benefit for the parasite

The explanations for the evolution and expression of virulence that have been discussed so far see virulence as an unavoidable by-product of parasitic infections, one linked to the parasite's other fitness components. In this sense, virulence has no direct benefit for the parasite or for the host. It is, however, possible that certain symptoms expressed by the diseased host do directly benefit parasite reproduction, survival, or transmission. Such examples of virulence may lend themselves to a practical theory, because the evolution of many traits, such as drug resistance, infectivity, and evasion from the immune system, are more easily predicted when there is a direct connection instead of an indirect connection to parasite fitness. Infected hosts

display many, diverse changes in behavior linked directly to parasite fitness (Moore 2002). Likewise, selection for avirulence in vertically transmitted parasites, where parasite fitness is directly linked to host reproduction, has been highly successful (Bull *et al*. 1991; Bull and Molineux 1992; Herre 1993). The idea that virulence gives an advantage to the parasite can potentially explain a wide range of disease-related symptoms, even diseases without host mortality such as the common cold, so there is a great potential for developing this line of theory. Ewald (1994) has presented some broad direct-benefit hypotheses based on mode of disease transmission.

The direct benefit model and the trade-off model are not incompatible. A symptom with a direct benefit to the parasite may have side effects detrimental to the parasite. Furthermore, the overexpression of a beneficial symptom may kill the host, thus making an intermediate level of expression more favorable. Ewald (1983), for example, proposed that vector-borne diseases gain an advantage by reducing their hosts' ability to defend themselves against biting vectors. This may be achieved by increased fatigue and fever; however, the overexpression of fever may lead to unwanted host death, which is clearly not in the interest of the parasite.

We have not presented many examples of disease symptoms that clearly benefit the parasite. Furthermore, the general model of direct benefit is prone to the abuse of simplistic speculation, as is illustrated by alternative explanations of disease symptoms. Coughing may promote the transmission of the parasite, but may also be a host response to clear the respiratory track. Fever may be beneficial to the parasite, but is also recognized as beneficial to the host by impairing parasite growth (Nesse and Williams 1994). Parasitic castration and gigantism in certain invertebrate hosts may help the parasite gain resources, or may help the host to allocate more resources to defense, if the castration is temporary (Minchella 1985; Ebert *et al*. 2004). To settle these arguments, quantitative data on host and parasite fitness effects are required. Unfortunately, such data are difficult to obtain, particularly in vertebrate hosts, and environmental variation may complicate the picture further.

Can we manage the evolution of virulence?

The onslaught of drug-resistant microbes in the past few decades has outstripped industry's ability to develop new drugs and has thus led to worldwide awareness of simple rules to manage drug resistance evolution. The use of imperfect vaccines and vaccines that do not cover the full spectrum of circulating strains is motivating models to predict and thus manage the impact of those vaccines (Lipsitch 1999; Chapter 11). Can we likewise realistically manage virulence evolution of human and agricultural parasites?

Virulence management of human diseases has been proposed as a viable strategy (Dieckman *et al*. 2002). The ideas of managing virulence in human, agricultural, or natural populations have mostly been based on the trade-off model. In virulence management proposals, that model is interpreted as making it possible to predict expected changes in virulence when host density or demography, opportunities for transmission, or frequency of multiple infections changes. Recently we argued (Ebert and Bull 2003a,b) that this idea may be too simple and is not likely to result in significant reductions of virulence of medically and economically important diseases for the following reasons.

1. There are no good examples of predicted changes in virulence associated with trade-offs.
2. The few published successful experiments either used extreme conditions (e.g., comparison between vertically and horizontally transmitted diseases) or found a weak response.
3. Comparative evidence of virulence among different host–parasite combinations under different environmental conditions explains only a small portion of the variance in virulence, suggesting that simple environmental correlates play only a minor role in virulence evolution.
4. Host mortality—the single measure of virulence in most models—is not high enough for most human parasites of medical interest to have much impact on parasite evolution. For example, human influenza mortality rates are typically less than 1%, which is not be expected to be a limiting factor in the transmission to new hosts.

5. The idea of a two-dimensional trade-off may be too simplistic; the underlying genetic and physiological structure may be multidimensional. For example, trade-off models commonly ignore the immune response and behavioral and life-history changes of the hosts.
6. The trade-off model assumes constant parameter rates throughout the course of the infection. When rates early in the infection differ from those late in the infection, or when the level of virulence affects the immune response, predictions may change drastically.
7. The trade-off model ignores genetic variation among hosts and thus neglects one of the most prominent factors in disease expression.

The field of the evolution of virulence is a young field, and we do not want to halt progress by being overly pessimistic. Instead we would like to see the field take an open-minded attitude and not fixate on trade-offs. Virulence in the trade-off model is a correlated trait, and correlated traits are notorious for their slow evolutionary response. Few people have studied virulence traits under direct selection, where an evolutionary response should be much more rapid. For example, in the case of diphtheria (see Box 12.2), the vaccine produced direct selection against strains that produced the disease-causing toxin. As a result, virulence of the overall population of the bacterium dropped significantly, due to a reduced frequency of strains carrying the toxin. Without specific knowledge of the biological details of this system, a general model would not have predicted this outcome, and indeed, the evolution of virulence in strains that continue to carry the toxin is a separate matter. Our view is that the best hope for virulence management will be the application of models to specific cases, and that, in contrast to the management of drug resistance evolution, there will be few practical generalities. One of the best arenas for such studies may be agriculture, where extreme crowding leads to strong selection for change.

Box 12.2 Case histories

Perhaps the strongest justification for a theory on the evolution of virulence would be evidence that parasite virulence commonly evolves on a short time scale. Such an observation is not easy to make, but they will hopefully be forthcoming as more attention is devoted to this subject. As noted elsewhere in this chapter, various environmental factors can affect virulence, such as nutrition, health care, and immunity. One well-documented example is measles, which has a high mortality rate, approaching 30%, in humans that are poorly nourished, but a vastly lower mortality rate in well-nourished people. Thus, broad social trends in environmental factors can alter virulence over time, giving the appearance that virulence has evolved when it has not. We discuss a few cases here. There are not many examples, as changes in virulence over time have rarely been documented. And the examples we do have do not clearly support any general model for the evolution of virulence; rather, they suggest that models must be specific to the details of the nature of the disease and virulence.

Diphtheria
One well-documented evolutionary change in virulence has occurred in the bacterium that causes diphtheria, *Corynebacterium diphtheriae*. The pathogenic form of this bacterium carries a set of genes that produce a toxin causing swelling in the throat. Widespread vaccination targeting the toxin specifically has reduced the frequency of the pathogenic form of the bacterium relative to the non-pathogenic form, in essence an evolved reduction in virulence. While this reduction in virulence can be explained post hoc on evolutionary principles, it is not clear whether the theory applied *a priori* would have predicted a decrease or an increase in virulence in response to the vaccine.

Transmissible gastroenteritis coronavirus (TGEV)
This virus infects the guts of pigs, causing diarrhea, and is a common source of mortality in piglets (Kim *et al.* 2000). A mutant form of this virus, porcine respiratory coronavirus (PRCV), differs only by a deletion and a few point

Continues

Box 12.2 ***Continued***

mutations, yet it infects the pig respiratory system and is often much less virulent that TGEV. Antibody cross reactivity between the two viruses means that the population of one form of the virus interferes with the other, and it is suspected that less virulent PRCV was responsible for the disappearance of TGEV in some pig farm areas. None of the existing evolution of virulence theories could have predicted either the evolution of a new tissue tropism in this virus, or the differential success of viruses according to their virulence. Indeed, the mutant strain may not have been at its virulence optimum when it first arose.

Influenza A

Perhaps the most dramatic changes in the virulence of human pathogens have been observed in the flu virus, although interpreting these changes as virulence evolution is equivocal. The flu virus causes annual epidemics and evolves rapidly in its major antigenic determinants, so the human population remains largely susceptible over time, and the mortality rates in typical years are moderately constant. Yet the mortality and extent of population-wide infection occasionally increases dramatically when 'pandemics' occur. Most year-to year evolution in the influenza virus is within a type of virus. Antigenic type is designated H1N1, or H3N2, with 'H' referring to the antigenic type of viral hemagglutinin and the 'N' referring to the type of viral neuraminidase. Three pandemics occurred in the 1900s, the most lethal being the 1918 flu that was the first introduction of the H1 serotype. Mortality rates from this infection were not only unusually high, but the virus also disproportionately killed 20 and 30 year olds compared to other epidemics. The mortality rate from this virus eventually dropped to lower levels. It is not known if the virus evolved lower virulence per se, or if the human population acquired sufficient immunity to H1, but when the H1N1 type was reintroduced in the1970s after disappearing for several decades, virulence was not appreciably high, even though much of the population had had no prior exposure.

Experiments with a genetically reconstructed 1918 virus genotype in macaques suggest that this virus was so virulent because infected hosts mounted an aberrant innate immune response that was insufficient for protection (Kobasa *et al*. 2007). In comparison, the contemporary H1N1 virus elicits a transient and appropriate activation of the immune defense (Kobasa *et al*. 2007). The 1918 and the contemporary H1N1 virus differ in a number of other proteins. Thus, it is plausible that the virulence of the 1918 type in humans was indeed high for reasons other than the novelty of its antigens.

Influenza occurs in many organisms besides humans. Current concerns about 'bird flu' center on an H5N1 variant that spreads rapidly and is highly lethal in birds. When it infects humans, mortality rates are 50% or more, but so far all introductions into humans have died out. Evolution of virulence theories can potentially account for virulence in birds, however, which is due chiefly to one or a few amino-acid mutations in a proteolytic cleavage site in the hemagglutinin protein. Similar outbreaks of highly virulent flu strains have been reported previously, forcing the destruction of entire chicken farms. Since many of the birds transmitting these viruses are domestic and maintained at high densities, this case may be well suited to applications of evolution of virulence theories.

Summary

1. Virulence is a complex trait. Its expression depends on the host and parasite genotypes, the evolutionary history of the association and the current conditions.

2. The evolution of virulence by natural selection on the parasite can be partitioned into three stages: phase 1, the first contact of host and parasite, as in accidental infections; phase 2, the evolution toward an optimal virulence soon after successful invasion of a new host species; phase 3, evolution of virulence after the disease is well established. Most efforts to understand, predict, and manage the evolution of virulence have been applied to phase 3.

3. The most common model of virulence evolution assumes a simple trade-off between virulence and transmission and that selection optimizes the net

transmission between hosts. This model may be applied to phases 2 and 3; most efforts have been to phase 3. Empirical data support the assumptions of the trade-off model, but do not well support the predictions.

4. Few current models include the mechanism of virulence. We suggest that models based on biological details of specific diseases may result in better predictions and therefore that future efforts should be directed to consider virulence evolution in specific cases.

5. Although host variability plays an important role in the expression of virulence, the impact of host variability for the evolution and expression of virulence has not been satisfactorily incorporated into the models.

6. We caution against the use of untested general models to guide attempts for the management of infectious diseases.

7. With respect to public health and domestic livestock implications, considerations of virulence should focus on more than just its evolution. Dense host populations are susceptible to invasion by highly virulent parasites, even though the high virulence may not be optimal for the parasite.

Acknowledgments

We thank Steve Stearns and Jacob Koella for many detailed comments on three earlier versions of this chapter. Bruce Levin and Elisa Margolis pointed our attention to some inconsistencies.

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Evolution in Health and Disease

Second Edition

EDITED BY

Stephen C. Stearns and Jacob C. Koella

OXFORD
UNIVERSITY PRESS

OXFORD
UNIVERSITY PRESS
Great Clarendon Street, Oxford OX2 6DP
Oxford University Press is a department of the University of Oxford.
It furthers the University's objective of excellence in research, scholarship,
and education by publishing worldwide in
Oxford New York
Auckland Cape Town Dar es Salaam Hong Kong Karachi
Kuala Lumpur Madrid Melbourne Mexico City Nairobi
New Delhi Shanghai Taipei Toronto
With offices in
Argentina Austria Brazil Chile Czech Republic France Greece
Guatemala Hungary Italy Japan Poland Portugal Singapore
South Korea Switzerland Thailand Turkey Ukraine Vietnam
Oxford is a registered trade mark of Oxford University Press
in the UK and in certain other countries
Published in the United States
by Oxford University Press Inc., New York

This edition 2008
1st edition 1999

British Library Cataloguing in Publication Data
Data available
Library of Congress Cataloging in Publication Data
Evolution in health and disease / edited by Stephen C. Stearns and
Jacob C. Koella.—2nd ed.
p.; cm.
Includes bibliographical references and index.
ISBN 978–0–19–920745–9 (hardback: alk. paper)
ISBN 978–0–19–920746–6 (pbk.: alk. paper)
1. Medical genetics. 2. Human evolution. 3. Disease—Causes and theories
of causation. I. Stearns, S. C. (Stephen C.), 1946 II. Koella, Jacob C.
[DNLM: 1. Evolution, Molecular. 2. Disease. 3. Health. QU 475 E95 2008]
RB155.E96 2008
616′.042—dc22 2007033610
Typeset by Newgen Imaging Systems (P) Ltd., Chennai, India
Printed in Great Britain
on acid-free paper by
Antony Rowe, Chippenham, Wiltshire
ISBN 978–0–19–920745–9 (Hbk.) 978–0–19–920746–6 (Pbk.)
10 9 8 7 6 5 4 3 2 1

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