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Taking Stock: Relating Theory to Experiment

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27.1 Introduction

This book is concerned with the way natural selection affects the virulence of disease agents, here loosely defined as damage to the host, and with how we can use this knowledge to design strategies for managing virulence. These questions are rooted in Darwinian thinking about evolution (Poulin 1998; Stearns 1999). If it were possible to resolve these questions at the level of evolutionary storytelling only, this book would not exist. The impetus behind this book came from recent advances in mathematical evolutionary theory, in particular the ongoing merger of the theories of population dynamics and natural selection. This merger enables quantitative, and therefore testable, predictions of the outcome of selection for a given ecological setting. As so often, applied problems form an ideal testbed for the new tools.

Since disease agents have short generation times and usually harbor considerable genetic variation, natural selection can potentially cause rapid changes in the genetic make-up of pathogen or parasite populations. Therefore, the evolution of parasite virulence is an obvious area to test new evolutionary theories. Another matter is whether such tests promise immediate applications. The theory of evolutionary dynamics is not at a stage that can produce lists of management strategies to solve any particular problem with certainty; to be fair, is any theory able to? However, any measure, even the considered absence of action, is guided by some theory in whatever verbal or mathematical form. Given that measures will be taken anyway, we do better to evaluate their effects by comparing them against the predictions of the theory of evolutionary dynamics. Of course, one hopes for more direct applications in a not too distant future, but some scepticism is not altogether unwarranted (e.g., Bull 1994). However, within the scientific approach such scepticism should not bar us from making and testing predictions, and, clearly, one of the most difficult ways of making predictions is to devise management strategies. Moreover, at the applied end, to devise management strategies without incorporating as best as possible the available plethora of evolutionary predictions would be an ostrich-type strategy, since changes in virulence are an undeniable feature of the epidemiology of disease agents.

In this chapter we place the theory discussed in the previous chapters in a wider perspective, take stock of what has been achieved in our field with an emphasis on the interaction between theory and experiments, and touch on a number of

conceptual issues that still require further resolution. This sets the scene for the chapters on virulence management that follow in this part.

27.2 Panoramic View of Virulence Evolution

Before presenting a survey of how the theory relates to experiments at the process level, we first take a grander view and consider the prerequisite for any evolutionary theory to work, the genesis of sufficient genetic variation. This leads us naturally to say a few words about the popular concept of the ecology and evolution of virulence, which is largely shaped by the publicity that surrounds emerging diseases. We argue that from a theoretical viewpoint there is little new to disease emergence, and that work on more mundane aspects of virulence evolution can make a greater contribution to public health improvement. After this, we consider the time scales on which the various relevant processes act and how this is relevant to the questions a prospective virulence manager might ask. This perspective then leads to a few general caveats that a manager should keep in mind.

Sources of genetic variation in virulence-related traits

A prerequisite for natural selection to influence parasite virulence is the existence of genetic variation in traits that affect this virulence. If – to keep things simple – we temporarily equate virulence with the extra host death rate induced by the disease agent (additional to the death rate from other causes), then the differences in virulence among disease agents are striking, be it between species or between genetic variants within species.

Changes in virulence may arise from simple mutations. For example, a single base-pair change in a fungal gene that encodes a product that elicits programmed cell death in a plant (the so called hypersensitive plant response, which creates a "scorched earth" around the infection site) suffices to cause a dramatic change in the virulence of *Cladosporium fulvum* to tomato plants (De Wit 1992; Joosten *et al.* 1994). Such changes often relate to traits involved in evasion from the host's defense system, but they may also concern traits that help the host cell to recover after pathogen invasion, as shown for *Salmonella* bacteria (Fu and Galán 1999). Bacteria, such as *S. enterica* and *Escherichia coli*, have been shown to harbor mutator phenotypes (LeClerc *et al.* 1996; Heithoff *et al.* 1999). Given that their environment continuously changes, mutator alleles may be important for pathogens to evolve new ways to enter the host, find their niche, avoid competition with other microbes, and circumvent host defense barriers (Moxon *et al.* 1994; Taddei *et al.* 1997b). Among the viruses, the human immunodeficiency virus (HIV) is the best known example of a mutator that continually evades immune surveillance (Nowak *et al.* 1991).

Other variations result from genetic recombination between pathogen races and genetic exchange between related species. When together in the same host cell, viruses with a segmented genome (e.g., influenza-, arena- and bunyaviruses) may exchange structural genes in a modular fashion (Koonin and Dolja 1993). Bacteria may swap genes via transformation and exchange of mobile genetic elements

(e.g., plasmids, transposons) and fungal networks may come into genetic contact via anastomosis. A recent example of the latter is the interspecific hybridization between two species of a plant fungus (*Phytophthora* spp.) that led to a new aggressive pathogen of alder trees in Europe (Brasier *et al.* 1999; Brasier 2000). Also, bacteria and fungi themselves can be infected by pathogens that alter the virulence of their host. For example, a bacterial virus (bacteriophage) encodes the gene clusters (so-called pathogenicity islands) that determine the colonization success of cholera bacteria (*Vibrio cholerae*), but another different virus carries the cholera toxin genes (Karaolis *et al.* 1999). Indeed, such converting bacteriophages may act as efficient vehicles for horizontal gene transfer and may explain quantum leaps in virulence through the transfer of blocks of genetic material, rather than through the accumulation of single nucleotide mutations, as shown for *Salmonella* spp. (Mirold *et al.* 1999). All these remarkable ways of generating genetic diversity cause some microbes to become (a)virulent to their host and/or to jump from one host to another.

Emerging or re-emerging diseases?

Some so-called emerging diseases have had devastating effects on their host as well as on their host's populations. An often-heard argument is that these newly emerging diseases prove the existence of major genetic changes in virulence. This is not necessarily true. For one thing, the emergence of a new disease may reflect newly emerging knowledge rather than anything else. For another, the emergence may actually be a re-emergence purely as a result of the mechanisms of population dynamics (i.e., natural selection does not play a role). For example, bubonic plague, caused by the bacterium *Yersinia pestis*, kills its host in a matter of days. It carved a path of death through Europe, once Genoese tradesmen, who contracted the disease in the Crimea, introduced it in 1346. Within 4 years it had moved to Scandinavia and even entered Greenland, killing a third of the European population on its way, according to the medieval chronicler Froissart (McNeill 1976). This 14th century Plague was not a unique event: other Great Plagues have occurred over the past 2 500 years in Asia, the Middle East, North Africa, and Europe and the disease continues to cause 200 reported deaths per year worldwide (Tikhomirov 2001). In some areas bubonic plague has even re-emerged as a significant health concern (Kumar 1995).

How can a disease kill its host so fast, and yet maintain itself and re-emerge? For bubonic plague the answer is that rodents represent a reservoir of the bacteria, and that flea parasites can serve as a vector not only between rodents, but also from rodents to humans. Thus, whenever rodents such as rats become abundant near urban centers, there is the risk of a pest outbreak. Once the bacterium enters the human host, the disease may progress and its transmission may take place via the handling of infected tissues (septicemic plague) and via pulmonary droplets (pneumonic plague), but historical evidence suggests that these transmission routes are less important than that from rats to humans. Stochastic, spatially structured population models predict that bubonic plague can persist in relatively small rodent

populations from which occasional human epidemics emerge (Keeling and Gilligan 2000). Thus, bubonic plague is driven by its dynamics in the rat population. Consequently, the isolation of infected hosts and/or vaccination are ineffective as methods to eradicate the disease. Rat culling is the solution, provided it takes place at a time of low prevalence of infection among the fleas, since, in the absence of rats, hungry fleas will try their luck among humans.

Plague is just one example showing that a disease outbreak after a long disease-free period does not necessarily demonstrate a newly emerging disease. It may reflect the re-emergence of a disease as a result of the dynamics intrinsic to the parasites and their hosts. However, this is unlikely to be the explanation for all disease outbreaks, because new hypervirulent types of parasites inevitably arise. For example, single point mutations in plasmids of *Yersinia* spp. have been shown to change virulence dramatically (Rosqvist *et al.* 1988; Galyov *et al.* 1993).

Natural reservoirs probably play an important role in the persistence of disease agents (Reid *et al.* 1999; Osterhaus *et al.* 2000), but genetic change is likely to be involved in species jumps followed by outbreaks in populations of the new host. Examples are HIV-1, HIV-2, and the human T-cell lymphotropic virus (HTLV), which have a simian origin (SIV and STLV, respectively; Myers *et al.* 1992; Koralnik 1994; Gao *et al.* 1999). Also, the canine distemper virus was originally not pathogenic for lions, but later entered the lion population in the Serengeti Park (Roelke-Parker *et al.* 1996; Carpenter *et al.* 1998).

Evolutionary time scales

The emergence of new diseases and, to a lesser extent, the generation of variability are the main topics in popular discussions of virulence evolution. Clearly, their importance must not be disregarded. However, the arrival of new genetic material does not drive epidemics on its own. Hypervirulent disease agents are bound to emerge every so often, but they spread only if the appropriate selective conditions arise. The previous chapters in this book take the existence of variation in the virulence of parasites and pathogens as a starting point from which to analyze the processes that ensue: population dynamics of parasite and host genotypes, and the impact of natural selection on genetic variation in virulence and resistance.

The major part of this book deals with interactions between microparasites (viruses, bacteria, fungi) and their hosts. These are characterized by much shorter time scales of dynamics and evolution for the parasite than for the host. So, in evolutionary time emerging microparasites have a head start. In the long term, the host population will respond to parasite-imposed selection pressure by developing resistance. Indeed, for pathogens that are a significant public health burden, such as *Mycobacterium tuberculosis*, *Plasmodium* spp., HIV, hepatitis-B virus, and *V. cholerae*, there are hints of human resistance genes, which hopefully, with the completion of the Human Genome Project, will soon be further substantiated (McNicholl *et al.* 2000). To cope with the parasite's head start, hosts have been naturally selected to produce variable offspring through sexual recombination and enhance offspring resistance by choosing mates with reduced parasite load or indirect

signs of resistance to parasites. These are consequences solely of the time-scale differences between microparasites and their hosts. It is not immediately clear how these consequences change for systems characterized by less extreme time scales between parasite and host evolutionary rates (e.g., macroparasites, such as parasitic nematodes, and arthropods) or by nearly equal time scales (e.g., hymenopterous parasitoids and predatory arthropods).

Much evidence indicates that evolutionary change in parasite–host systems can take place quite rapidly. First and foremost, there is the emergence of multiple resistance of commensal and pathogenic bacteria against well-established and new antibiotics applied in medical and veterinary situations (Kristinsson *et al.* 1992; Austin *et al.* 1999). Key elements behind this rapid increase are pre-existing resistant mutants and the exchange of transposable elements between bacteria and between hosts. Bacterial plasmids that carry resistance and virulence factors can be transferred to sensitive strains of bacteria by cell-to-cell contact, and thereby also enable the transfer of resistance from one host to another. A second example comprises laboratory experiments in which the genetic composition of bacterial populations is recorded. Whereas in chemostats and serial transfer of batch cultures populations are usually taken over by a single genotype, this is not the case in a constant batch culture system without nutrient input or cell removal (Finkel and Kolter 1999). Instead, highly dynamic changes in genetic composition are observed, even within a period of just a few months.

Finally, there is the famous data set on changes in virulence of the myxoma virus and resistance in rabbits in Australia between 1950 and 1985 (Fenner and Fantini 1999). After the release of highly virulent virus strains, resistance increased and virulence dropped to intermediate levels. During the past decennium, however, virulence increased again. These empirical examples show that evolutionary change can take place at a time scale close to that of the ecological dynamics. Thus, evolution cannot be ignored when studying ecological dynamics, and vice versa.

A management viewpoint

Let us now consider the central problems for a prospective virulence manager from the time-scale perspective. On the time scale given by the parasite's generation time, these are:

- How and why virulence levels change as the parasites continue to persist in the host population; and
- How the changed properties of the parasites feed back to the dynamics of the parasite–host system.

Extending the time scale to the usually much longer generation time of the host, the additional problems are:

- How the parasite–host dynamics influence selection for resistance in the host; and
- How this in turn changes the selective environment of the parasite.

Previous chapters answer these questions in the form of theoretical statements, giving conditions under which parasites become gradually benign to their host, evolve high virulence, or vary in virulence over time. These theoretical statements help us order our thinking. However, with regard to their practical implications they should be interpreted as no more than hypotheses, as the extent to which the theoretical conditions are fulfilled in a concrete situation is not fully clear in advance.

Once the hypotheses are formulated correctly, the next step, before even considering the management applications, is to formulate consistent hypotheses on how to interfere with or even redirect the evolution of virulence. Among the desired goals such things as low virulence in the parasites of crops, cattle, or humans, and high virulence in the parasites of weeds and pests could be considered. However, the formulation of management measures does not imply that their application be recommended immediately. Clearly, the science of virulence evolution is not yet at a stage where the recipes in this book can be recommended to one's doctor or local politician. In general, physicians should not rush to apply untested evolutionary predictions on how to combat disease outbreaks. Such advice should not be uncritically accepted in the absence of experimentally established fact, unless the measures fit in neatly with existing practice to abate harm. Yet, the phrasing of measures to manage virulence stimulates critical tests in practice, whenever reasonable, possible, and ethical.

Theoretical caveats: what sort of extensions are needed?

The main message of the simplest models of virulence evolution is that, under certain assumptions, diseases will become relatively benign, and even more so when the general living conditions of the host are improved. Do we then have an all-encompassing recipe for eternal bliss? Of course we do not. The reasons why the recipe often fails are hidden in the model's assumptions:

- First, for many disease agents it is not realistic to assume that they do not compete with each other within hosts. Even when hosts are isolated from contact with others soon after disease symptoms first appear, multiple-strain infections can still occur during the period between actual infection and the emergence of the first symptoms. However, to account for the possibility of multiple infections rapidly causes bookkeeping to become a complex procedure, which poses one of the major challenges to the development of the theory. Part C of this book is devoted to models of within-host dynamics, as well as to alternative approaches to the analysis of how multiple infections affect the evolution of virulence.
- A second assumption in need of further theoretical exploration is the structure of the host population. Populations in the real world are rarely homogeneously mixed. Instead, they may exhibit an externally imposed metapopulation structure or develop fancy patterns in space through the host–parasite dynamic itself. Such spatially extended models are discussed in Part B.

- The third critical assumption that requires more research is that natural selection acts only on the disease agents. In this way, the evolutionary responses of the host and of other interacting organisms in the food web are altogether ignored. These coevolutionary responses and their consequences for the evolution of parasite virulence are dealt with in Parts D and E.
- Finally, to complete the list of potentially modifiable assumptions, models may be extended through alternative modes of transmission, such as vertical instead of horizontal transmission or indirect instead of direct transmission (via vectors or intermediate hosts).

Practical caveats: how well can we understand a concrete case?

All the extensions that result from relaxing one or another assumption discussed above can lead to a suite of alternative explanations for empirical observations. For example, the decline to intermediate virulence observed in the rabbit myxoma virus can be explained in at least seven different ways:

1. The observed decline represents a snapshot during the long-term approach toward avirulence, a prediction consistent with the simple models provided the per host disease-induced death rate evolves independent of other parameters.
2. The observed intermediacy is the end result of an evolutionary process constrained by a trade-off between virus-affected parameters (e.g., the per host transmission rate, the per host recovery rate, and the per host disease-induced death rate).
3. The observed decline is a time series of evolutionary end states and the critical variable under gradual change is the disease-free death rate of the rabbits.
4. The introduction of the myxoma virus caused a decline in rabbit density such that the probability of multiple infections drastically declined, as did, concomitantly, the virulence level favored by selection (multiple infections select for higher virulence than single infections).
5. The rabbit population is structured in family groups with high internal contact frequency, yet very low external contact frequency, and so virus strains that wipe out family groups too quickly hamper their own propagation through the rabbit population as a whole.
6. The originally virulent strain of the virus triggered selection for resistance in the rabbit population, which caused the virus prevalence to decline and the probability of multiple infection to decrease. In the longer run this will lead to an arms race between the virus and the rabbit, which potentially could give rise to co-evolutionary cycling of resistance and virulence levels. In this respect it is interesting to note that recent assessments (1992–1994) showed an upsurge of the virulence level (Fenner and Fantini 1999).
7. And, as if this list were not already long enough, there is another hypothesis based on indirect transmission of the virus, first formulated in a recent authoritative ecology textbook (Begon *et al.* 1996). The idea is that blood-feeding lice take up strains of the virus that reside in or near the rabbit's skin and that these strains have lower rates of multiplication than others that exploit more

profitable tissues in the rabbit. Selection for vectorborne transmission therefore favors less virulent strains of the myxoma virus.

This example of rabbit myxomatosis shows that changes in virulence can potentially be explained by many different hypotheses, each of which draws attention to particular, realistic features of the ecological setting of the microparasite–host system. To develop new hypotheses and discriminate between them, mathematical models are an indispensable tool to enable careful reasoning and generate precise, and therefore testable, predictions. Empirical tests are needed to assess which of these hypotheses survive scrutiny. These scientific activities are expected to become a breeding ground for further analyses and for the design of experiments on virulence management.

27.3 Conceptual Issues

Theoreticians can simply make the assumptions they need and define concepts on the basis of their convenience in deriving conclusions. The empiricists thus have to deal with the problems of how to check the assumptions made, or rather of how to identify the systems for which these assumptions may hold at least approximately, and of how to make workable the concepts so conveniently dreamt up. The task of checking assumptions is examined in Section 27.4. First, however, we assess in some detail the two grand concepts central to the analysis of virulence evolution, to wit virulence and fitness. In the world of simple models these are simple concepts. However, the world often is far from simple, in which case the twin questions arise as to how these concepts can best be extended so that conclusions based on them generalize, and as to whether suitable operationalizations, or usable substitutes, can be derived that are relatively accessible experimentally.

The monster of fitness

The concept of fitness has all the essential features of a monster. It is a grand idea that is permeating virtually every field of scientific thought. Just as we picture the monster of Loch Ness as a marine dinosaur, we have a pretty good idea of what fitness should be like. Yet, when it comes to obtaining tangible proof, a precise and quantifiable measure of fitness, the specific ecological setting within which the focal organism functions and interacts becomes crucial. Whereas, in principle, theorists can derive fitness measures given any ecological setting, experimental observers face the formidable task of first establishing a complete understanding of their ecological system before they can select a suitable fitness measure and assess how a given organismal trait affects it. Therefore, the real monster exists in the eye of the observer (and not in that of the theorist). Given the large number of sightings in the ecological literature, we may hope the fitness monster will fare better than the monster of Loch Ness (Sheldon and Kerr 1972), and eventually we may even be able to derive a fairly complete picture.

For a better understanding of the problem, take pen and paper and write down a list of candidate representative measures for the fitness of a microparasite. These could include:

- Parasite's population growth rate;
- Net per generation reproduction of the parasite;
- Rate at which new infective hosts are produced per infected host;
- Number of new infective hosts produced over the lifetime of an infected host (i.e., the basic reproduction ratio R_0);
- As well as a number of variants that all differ in the way these measures are averaged over generations.

This exercise shows that it is not at all self-evident which measure is most appropriate.

Now, consider a rare mutant that invades a homogeneously mixed population. Local linearization of the mutant's dynamics at near-zero mutant densities yields a dynamics in which all individuals (disease cases) reproduce effectively independently. The dominant Lyapunov exponent of this linear dynamics provides the correct fitness concept, and, if the coefficients in the linear equations are constant, this reduces to the dominant eigenvalue (Metz *et al.* 1992). In biological terms, this is directly related to the long-term, time-averaged per capita growth rate, but when the population is at equilibrium, R_0, as formalized for the general case by Diekmann *et al.* (1990, 1998), can be shown to provide an equivalent measure. This is the fitness measure used in most of the preceding chapters. For $R_0 > 1$ ($R_0 < 1$), fitness is positive (negative). Thus, evaluation of the value of R_0 suffices to pinpoint the conditions under which the resident population resists invasion by any conceivable mutant. This has led to the use of pairwise invasibility plots (Geritz *et al.* 1998), introduced in Chapter 4.

Recently, the concept of invasion fitness has been extended to metapopulations. Metz and Gyllenberg (2001) and Gyllenberg and Metz (2001) defined a quantity R_m as the average number of new mutant dispersers (infective particles) produced by one newly released mutant disperser. They showed that this quantity qualifies as a substitute fitness measure provided the (resident) metapopulation is at equilibrium. However, much work is still required to identify fitness measures under equilibrium and nonequilibrium conditions in spatially extended systems and in food webs with two or more interacting organisms.

Virulence, what's in a word?

In the medical world virulence is most commonly equated with disease severity. In many models this is abstracted as the per capita disease-induced host death rate. This is a very convenient choice, because this death rate often represents a significant aspect of disease severity as it is commonly perceived, and is also a factor of great importance for a disease agent that – for its own transmission – depends on a living host. Thus, the parasite has to balance within-host growth against host survival, since death of the host halts further transmission of the parasite. However, this definition of virulence is clearly oversimplified, because disease severity may involve many negative effects on the host other than increased mortality, and several of these other effects may not hamper, and may even promote, transmission. As an example of the second point, some parasites castrate their host, which

is detrimental to the host's fitness, but not necessarily negative to the parasite, provided it is transmitted horizontally. If castration prolongs the lifetime of the host, the average number of parasite transmission events to new hosts may even be increased.

Thus, discrimination should be made between the effects of disease severity that reduce the fitness of the host and those that alter the fitness of the parasite. This distinction is at the heart of various attempts to clarify the definition of virulence (Shaner *et al.* 1992; Andrivon 1993; Poulin and Combes 1999). We resist the urge to give our own all-encompassing definition to supersede the rest. Instead, we feel that with the present stage of our knowledge it is better to use the word in a blanket fashion and to concentrate on the particulars of specific cases. Earlier chapters all deal with parasite strategies to exploit the host for multiplication and for transmission, and the authors refer to virulence to indicate the detrimental effect of parasitic exploitation on the host, just as resistance indicates the detrimental effect of the host's defense on the parasite. Clearly, the parasite may incur costs because of its virulence toward its host, just as the host may incur costs because of its resistance against the parasite. Whenever it is necessary to single out those effects on the host that are important for parasite fitness, this has been stated in words rather than by introducing new terms.

The Encyclopedia of Ecology and Environmental Management (Calow 1999) also refers to another definition of virulence: the effect of the parasite on the host population level. In this definition, virulence also includes aspects of the parasite's ability to be transmitted to other hosts in the population. Virulence is then equivalent to R_0, a usage that must be avoided. Virulence and transmission should be distinguished from each other to separate the effects of the parasite's exploitation strategy on the host from those on the ability of the parasite to transmit itself or its offspring to new hosts. It is clear, however, that virulence does not necessarily relate to the damage inflicted on an individual host only. For example, in a metapopulation context virulence may be defined as the impact of the parasite on a local population, and transmission may be defined as the ability of the parasite to reach new *local* populations of hosts. Thus, virulence can be discussed at the level of the local population (or, more briefly, the patch level) as opposed to the individual level (see Chapters 22 and 32). In fact, local populations can then be envisaged as individual hosts, much like individual hosts represent a collection of cells. Clearly, how virulence is defined depends on the issue at stake.

Confusion sometimes arises from the summary statement that parasites have genes *for* (a)virulence. Virulence is a byproduct of the way the parasite exploits its host; it is certainly not its function or goal. Sometimes the severity of a disease is to a large extent the result of the immune reaction against it, in other cases it is a consequence of the growth of the pathogen population inside the host. Pathogens certainly do not express virulence genes to trigger such defense responses. The primary function of these genes is not clear, but they probably confer some advantage to the pathogen instead of specifically eliciting its destruction (Poulin and Combes 1999). Avirulence means that the pathogen is eliminated relatively quietly

when its gene products are recognized by the immune system of the host. Note, however, that the same genes would lead to disease and virulence in hosts not capable of recognizing the pathogen's gene products. Thus, whether the pathogen is avirulent to its host or not depends on the manner in which resistance genes in the host and virulence (determining) genes in the pathogen interact.

In plants the situation is a little different because the defense responses are less graded. What largely matters is whether the pathogen can gain entrance to the host or not, but once it is has gained entrance, there is little difference, at least on the time scale of agricultural practice, in the amount of damage inflicted. In addition, phytopathology started from a concern about crop losses (i.e., damage to the local population), which led to the use of the term virulence gene for those genes that allow the pathogen to gain entrance, with resistance genes as the matching term on the host side. In this book the authors who deal with plant systems (Chapters 17 and 31) chose to use the term "matching" virulence to distinguish virulence through nonrecognition by the host from virulence as a byproduct of the parasite's exploitation strategy, which they call "aggressive virulence".

27.4 The Dialogue between Theorists and Empiricists

Here we take stock of our current understanding of the process of virulence evolution as treated in this book and the recent literature. As in any field of science, evolutionary epidemiology gains its momentum from the dialogue between theorists and empiricists. Here, we attempt to identify the key elements of this dialogue and also try to identify the points on which theorists and empiricists seem to misunderstand each other. We hope to foster communication by rephrasing theoretical issues in more general terms, by scrutinizing assumptions that underlie the theory, and by comparing explicit theoretical predictions against what can be gleaned from the literature on empirical tests.

Trade-off relationships prevent evolution toward avirulence

Parasitologists long held the view that all parasites evolve to become mild for their host. This view was rejected on theoretical grounds because it assumes that the disease-induced host death rate (i.e., virulence) is independent of the parasite transmission per infected host. It seems more likely that there is a trade-off between transmission and virulence. The underlying rationale is that higher replication rates of the disease agent lead to higher virulence as well as higher transmission, and that both these relationships have a genetic basis. Empirical support for these relationships and assumptions is provided by a study of the malaria parasites of mice (Mackinnon and Read 1999a). Increased virulence as a result of increased pathogen replication is observed for the case of African trypanosomes (Diffley *et al.* 1987). That the increased pathogen load correlates with increased transmission is substantiated by studies on:

- Myxoma virus of rabbits (Fenner and Fantini 1999);
- Phages of *E. coli* bacteria (Bull and Molineux 1992);
- Malaria parasites of humans (Dearsly *et al.* 1990; Day *et al.* 1993);

- Microsporidian parasites of water fleas (Ebert 1994); and
- HIV (Quinn *et al.* 2000).

Thus, all in all, there is good reason to think that parasites generally do not evolve to avirulence, because of the trade-offs with, for example, transmission.

Virulence management is not simply about transmission intervention

Empiricists often take the view that measures to reduce pathogen virulence are a matter of reducing their transmission. This is true insofar as reduced transmission causes both per host infection and within-host competition among pathogen strains to decrease. However, surprising as it may seem, theory shows that in the long run it may be more effective to base measures of virulence management on how they influence the degree to which a pathogen "is in control of exploiting its host". If the pathogen is not in control because of competition with other pathogens or erratic mortality of the host, then it pays for pathogens to multiply rapidly at the expense of the host. But if the pathogen controls the resources contained in the host, even when delaying its use of them, then virulence may be expected to decrease.

For example, antibiotic therapy in itself reduces the pathogen population and thereby its transmission, but this may actually favor increased virulence. The pathogens lose their host anyway, so their only opportunity for transmission is during the time between infection and the administration of antibiotics. Thus, even though the parasite population decreases and fewer hosts become infected, it is possible that pathogen virulence increases. As another example, eradication of mild strains that could monopolize exploitation of their host (e.g., by triggering an immune response that harms later invaders more than the initial ones) might create opportunities for more virulent strains that lack this ability. In this situation it would be advisable to tolerate rather than combat mild strains. Thus, it is somewhat misleading to focus on transmission intervention in the design of virulence management strategies. Instead, it is more appropriate to develop strategies that "give a pathogen control over the exploitation of a host's resources"; as long as pathogens do not have to compete with other pathogens and/or can exploit their host without interference from environmental causes, the well-being of the host is in the evolutionary interest of the parasite.

Virulence increases when ordinary transmission is bypassed

Theory predicts that selection favors fast-replicating strains of the disease agent when transmission is guaranteed (i.e., when it does not depend on host survival). These conditions are met in so-called serial passage experiments (SPEs), in which pathogens or parasites are transferred from one host to another, either artificially by injection or through natural transmission in dense host cultures, and thereby the constraints on real-world infectious processes are relaxed. Hosts usually have low genetic diversity (clonal or inbred lines), but the pathogens and parasites are genetically variable and undergo mutation. The striking general outcomes of these

SPEs are a rapid increase in the parasite-induced reduction of host fitness and that the passaged parasites outcompete the ancestral strains used to initiate the SPEs, which indicates parasite adaptation to the hosts used in SPEs (Ebert 1998a).

The rate at which virulence increases is most rapid for ribonucleic acid (RNA) viruses, slower for deoxyribonucleic acid (DNA) viruses and bacteria, and slowest for eukaryotes, which suggests that generation time and mutation rate determine the rate of change. Albeit obtained in the laboratory, SPEs taught an important lesson: disease agents are less virulent than they can be because they depend on host survival for their transmission. Outside the laboratory, the very same lesson applies when transmission is facilitated by the long-term survival of propagules, mediated by water or vectors. In all these cases disease agents tend to be more virulent, a view championed by Ewald (1983, 1991a, 1994a, and Chapters 2 and 28).

Vertical transmission lowers virulence

Another theoretical prediction supported strongly by empirical evidence is that low virulence is favored by selection when transmission is vertical [i.e., from mother to offspring (Ebert and Herre 1996)]. All else being equal, a parasite transmitted exclusively vertically should not harm its host, because the number of new infections depends on the fecundity of the host. For example, Bull *et al.* (1991) propagated populations of phage-infected *E. coli* in two ways. In one treatment, phage replication was wholly dependent on host reproduction. In the other, phages could be transmitted both vertically and horizontally. The two selection regimes had the expected effects on host fitness: infected bacteria in the vertically transmitted lines increased in density much more quickly than did those in the lines in which horizontal transmission could occur. Genetic changes in both host and parasite were responsible for the evolved differences in virulence. Further support for low virulence with vertical transmission comes from observations on an initially virulent parasitic bacterium in an amoeba. After 5 years of mainly vertical transmission, this bacterium evolved to be mild to its host (Jeon 1972, 1983).

A striking example of the role of vertical versus horizontal transmission is provided by the cytoplasmatic endosymbionts that are transmitted from mother to daughter and sons, but enter a dead-end in the latter (O'Neill *et al.* 1997). These symbionts have little impact on the survival of their female host, but promote their transmission by causing the female host to produce more daughters. If the symbionts end up in male hosts, they render mating with uninfected females incompatible – an effect referred to as spiteful because it reduces the fitness of uninfected hosts relative to infected hosts. Alternatively, they cause the males to die. The latter occurs only when male death allows the symbiont to be transmitted horizontally (e.g., via consumption of the lethargic male). Thus, virulence is sex specific: symbiont-induced mortality is much higher in the horizontally transmitting sex than in the vertically transmitting sex (Hurst 1991; Ebert and Herre 1996).

Multiple infection increases virulence

There is good support for the prediction that virulence increases when horizontal transmission leads to the multiple infection of hosts and consequently to within-host competition. A striking interspecific correlation between nematode virulence, multiple infection, and opportunities for horizontal transmission was observed in the nematode parasites of fig wasps (Herre 1993, 1995). Within fig wasp species, the reproductive success of individual females can be related to the presence and/or absence of nematode infections, which thereby provides an estimate of nematode virulence. For some wasp species, typically a single wasp pollinates a fig inflorescence so that she only carries the nematodes that enter the inflorescence, and the only nematodes that leave are carried by her offspring (vertical transmission). In other wasp species (which pollinate different species of figs) many wasps pollinate a single inflorescence, which allows nematodes from different host lineages to mix before dispersal (horizontal transmission). The virulence caused by nematodes increases with the degree of within-fig competition and concomitantly with the potential for horizontal transmission of nematodes between wasps within a fig.

Conflicting trends often result from poor experimental design

Several reports on evolutionary change in virulence seem at first sight to contradict theory, but actually do not because of pitfalls in the experimental design. For example, contrary to expectation, experiments with the gut parasites of water fleas showed a higher virulence under the vertical transmission regime (Ebert and Mangin 1997). This, however, was because the experimental manipulations, inadvertently, created higher within-host competition under a vertical transmission regime. In other cases, virulence did not change [e.g., conjugative plasmids (Turner *et al.* 1998)] or exhibited only small increases when switched from horizontal to vertical transmission [e.g., bacteriophages (Messenger *et al.* 1999)]. Possibly, this resulted from low within-host competition despite horizontal transmission and a shallow trade-off relation between transmission and virulence. As a final example, artificial selection for high (or low) weight loss in malaria-infected mice – a measure correlated with the death rate in mice – did not give rise to the expected low (or high) transmission. Instead, it gave rise to increased replication rates of the malaria parasites. Possibly, this effect arose because fast replication was the best strategy for the parasite to reach the syringes used for transmission under either selection regime (Mackinnon and Read 1999b).

Evolutionary dynamics takes place at multiple levels

It is generally agreed that virulence evolves by selection that acts both within hosts and between hosts. Variants of the disease agents compete for resources within hosts and, in addition, they compete to infect new hosts. Within-host competition favors the fast-growing strains, which may reduce the infectious period for the host, either through host mortality or by triggering a stronger immune

response. Between-host competition of pathogens favors those strains that balance their within-host growth rate and per host transmission rate against the consequences for the infectious period. Relative to the single-infection case, virulence is predicted to increase when hosts become infected by multiple strains.

However straightforward this prediction may seem, reality may be more complex because natural selection and population dynamics may interact. The critical point is whether density-dependent feedback brings the pathogen–host system to states in which multiple-strain infections prevail or become rare. Hence the evolution of virulence cannot be predicted without taking the population dynamics of host and pathogen into account (and vice versa). To ignore the feedback of population dynamics when formulating general predictions can be very dangerous. Theorists are usually well aware of this problem, but empirical researchers sometimes are not; this may lead to the rejection of hypotheses for the wrong reasons. Much the same pitfalls may arise if pattern formation and coevolution in parasite–host systems are ignored. Spatial patterns may create additional selection levels and reciprocal selection on parasites and hosts may enrich the dynamic repertoire of virulence (and resistance) evolution.

27.5 Gaps in Current Knowledge

Not only should a dialogue between theorists and empiricists result in a mutual agreement as to which predictions stand up to scrutiny, but also it should identify the gaps in our present knowledge. Below we argue that current progress in evolutionary epidemiology hinges crucially on a better empirical insight into the underlying processes. We emphasize three areas of empirical research in need of more in-depth work: within-host competition, between-host transmission, and responses of the host to infection. We conclude this section by highlighting some major gaps in our current theoretical understanding.

Within-host processes

No doubt it is overly simplistic to model within-host competition between multiple strains of parasites as a race of fast replication rates. For within-host interactions between parasites, all the competition mechanisms known to occur in ecological communities can be expected, such as:

- Resource limitations may trigger exploitation competition;
- Parasite clones may interfere with each other by producing toxins that kill off their competitors or block entry to later infections (interference competition);
- An increase in density of one parasite clone may trigger an immune response that affects all the clones in the host (apparent competition).

Indeed, replication of a disease agent like a virus is much like a predator–prey–resource system. By infecting a cell, the virus preys on its resource, while it itself is being subjected to predation by T-cells that are part of the host's immune system (Levin *et al.* 1999).

Although there are examples of independently transmitted strains [e.g., variant surface antigen serotypes of malaria parasites (Gupta *et al.* 1996; see Chapter 25)], reality probably offers a plethora of competition outcomes, such as:

- Priority phenomena ("first come, first serve" irrespective of replication rate);
- Improved or reduced transmission under mixed versus single infection;
- Evasive specialization to different resources within the host or to different host genotypes (Taylor *et al.* 1997a, 1998b; Taylor and Read 1998).

Biological research into the mechanisms that underlie these competitive interactions is still in its infancy. No doubt, the consequences for the evolution of virulence are decisive. For example, if parasite clones invest more energy in interference than in replication, multiple infections may lead to reduced instead of increased virulence (Chao *et al.* 2000).

Apart from competition, parasites may also interact within hosts in other ways. They may gain by cooperation, despite the potential to cheat that such an interaction always entails (Brown 1999). For example, infectious bacteria need to reach a critical density to overcome host defenses and establish the infection. Many different bacterial pathogens are now known to regulate virulence-determining processes in a manner dependent on cell density through cell–cell communication via a diffusible signal molecule [e.g., *N*-acylhomoserine lactone (Williams *et al.* 2000)]. This phenomenon, referred to as "quorum sensing", may be an important determinant of the population variance observed in virulence assessments. It clearly requires an evolutionary explanation of its own (Brookfield 1998; Brown 1999), and its potential role in the evolution of virulence is virtually unexplored.

Between-host replication

Whether within-host interactions are competitive or cooperative, within-host selection in itself is unlikely to maximize parasite replication rates. This is because fast multiplication rates *per se* do not promote between-host transmission. Many parasites have specialized transmission stages and, thus, invest in traits that increase the per capita success of transmission, even when this is at the expense of their replication rates. It may well be that the increased replication rates favored by selection in SPEs actually result from the loss of investment in transmission-related traits (Ebert 1998a). Clearly, there is a need for selection experiments to elucidate the key traits for transmission, rather than just the traits that determine the numerical outcome of within-host competition.

More insight is also required into the costs and benefits involved in the various modes of transmission, as this may help us understand why certain transmission routes evolve in the first place. Parasites are necessarily involved in a struggle for transmission and they may employ one transmission route or a combination of these from a range of possibilities:

- Transmission via long-lived propagules versus exclusive reliance on transmission from a living host;

- Direct versus indirect transmission;
- One form of indirect transmission versus another (e.g., waterborne versus vectorborne);
- Horizontal versus vertical transmission;
- Control over the host as a transmission vehicle or control over the vector (parasite-induced host behavior).

How transmission routes evolve together with virulence has been explored for some special cases [e.g., horizontal and vertical transmission to hosts that cannot be coinfected by other parasites (Yamamura 1993; Lipsitch *et al.* 1995b, 1996)]. The results of these analyses do not indicate a virulence–avirulence continuum between horizontal and vertical transmissions, but a more comprehensive theory is needed to allow for multiple infections of hosts and a wider variety of transmission routes.

Although pathogens may maximize the per host production of transmission stages, they can also promote transmission by expanding their host range. Many medically and veterinarily important pathogens can infect more than one species of host. However, such a generalist strategy probably carries a cost to the pathogen. Each host species will impose a different selection regime on the pathogens, so that adaptation to one host occurs at the expense of adaptation to another. Multi-host pathogens may therefore tend to be less virulent to their hosts. For example, SPEs rapidly lead to pathogen lines that are much milder to their original host than the ancestor pathogens (Ebert 1998a) and nematode parasites seem to be less virulent to fruit flies when they attack various host species (Jaenike 1993). However, multi-host pathogens may also be less dependent on the survival of their host by virtue of a larger overall host population. Exactly how this influences the evolution of virulence in multi-host pathogens is not yet clear.

In some cases, multi-host pathogens express unusually high virulence. This may occur in hosts that do not contribute to the transmission of the pathogen. For example, rodent-associated hantaviruses are extremely virulent to humans, which represent a dead end for transmission of the virus. Unusually high virulence may also result from short-sighted evolution within hosts (Lipsitch and Moxon 1997). Pathogens mutate and experience severe selection within hosts, which leads to invasion of vital host tissues other than those needed for the pathogen's transmission (e.g., bacterial meningitis). However, the persistence of such mutator pathogens in evolutionary time still requires an evolutionary explanation (Moxon *et al.* 1994; Taddei *et al.* 1997b), and their role in the evolution of virulence is only now beginning to be explored (Bergstrom *et al.* 1999).

Reactions of the host

Changes in disease severity do not result solely from genetic changes in the pathogen. Damage to the host results from pathogen aggressiveness and host resistance. Given a sufficiently tight coupling between the interacting populations, reciprocal selection may drive parasite–host coevolution.

There is now good evidence that genotype–genotype interactions are of overriding importance in the expression of virulence, especially in the pathogens of plants (Thompson and Burdon 1992; Clay and Kover 1996), but also in:

- Trypanosomes that infect bumblebees (Shykoff and Schmid-Hempel 1991; Schmid-Hempel and Schmid-Hempel 1993);
- Microsporidia that infect waterfleas (Little and Ebert 2000);
- Webworms that feed on wild parsnip (Berenbaum and Zangerl 1998).

Moreover, it has been shown for various invertebrates and vertebrates that host resistance has a genetic basis and carries a cost (Toft and Karter 1990; Henter and Via 1995; Kraaijeveld and Godfray 1997; Sorci *et al.* 1997; Webster and Woolhouse 1999).

How reciprocal selection affects the coevolutionary dynamics of parasite–host systems is little explored empirically (but see Henter and Via 1995; Burdon and Thrall 1999; Fenner and Fantini 1999), which leaves an expanding body of theory (e.g., on maintenance of polymorphism and coevolutionary cycling) largely untested (Frank 1992b, 1993b, 1994a, 1996a, 1996c; Dieckmann *et al.* 1995; Haraguchi and Sasaki 1996; Sasaki and Godfray 1999; Sasaki 2000; see Chapters 4 and 14 to 17).

Parasitic relationships may evolve to become mutualistic (Michalakis *et al.* 1992), and mutualistic relationships may vary between parasitism and mutualism depending on the time scale and the spatial scale under consideration (Bronstein 1994a, 1994b). To understand why reciprocal exploitation provides net benefits to each partner or otherwise requires a sharp insight into the common interests shared by the partners, their private interests, and the conflicts of interests that may arise (Herre *et al.* 1999).

Whether a symbiont evolves to become mutualistic, commensalistic, or parasitic also depends on the degree to which it can control exploitation of the host and on the opportunities for transmission to new hosts (e.g., Yamamura 1993; Lipsitch *et al.* 1995b, 1996). Similarly, the host opens or closes transmission routes available to the symbionts and thereby influences the evolution of virulence, benevolence, or cooperation. Although it is clear that vertical transmission can promote the evolution of mutualism and that vertical transmission may evolve depending on the symbiont-induced benefits that accrue to the host (Yamamura 1993; Law and Dieckmann 1998), the full set of conditions has not yet been established. This is because host control over symbiont transmission and the full range of competitive interactions among symbionts within hosts have not yet been taken into account.

Recent molecular phylogenetic studies showed that bacterial symbionts belong to deeply branching clades that are strictly parasitic or strictly mutualistic (Moran and Wernegreen 2000). This pleads against the theoretical notion that transitions from parasitism to mutualism occur frequently. Thus, symbiotic interactions between bacteria and animal hosts may be more constrained than assumed in evolutionary models so far. Such constraints may result from major genomic changes associated with a certain bacterial lifestyle, which implies a massive loss of genes

that code for a variety of functional capabilities and thereby limits the evolutionary options for bacterial lineages.

Gaps in the theory

The most critical gap in our knowledge is the lack of good biological insight into the role of within-host competition and within-host evolution. Once this gap begins to close, we may begin to explore the theoretical outcomes of virulence evolution in more realistic settings. The models needed for such explorations have to be based on a framework that allows complex book-keeping procedures, especially when it comes to modeling multiple infections and the ensuing competitive interactions within hosts. Physiologically structured models phrased in terms of partial differential equations offer such a framework (Metz and Diekmann 1986; see also Diekmann and Heesterbeek 1999; Metz and Gyllenberg 2001; Gyllenberg and Metz 2001), and their application to the analysis of adaptive dynamics offers much promise.

In addition, we are only beginning to understand the role of spatial processes in population dynamics and evolution (Dieckmann *et al.* 2000). Studies of excitable media have shown the potential for self-organization, and parasite–host systems have all the essential properties to exhibit this behavior. A major future task is to understand how contact networks develop in space, determine the spread of disease agents, and influence the evolution of their virulence.

27.6 Discussion: Toward Virulence Management

"It is time to close the book on infectious diseases." This statement was made in 1969 by the USA surgeon general after inspecting a successful disease control campaign. It reflects the widespread optimism that good sanitation, vaccines, and antimicrobial agents would conquer infectious diseases (Binder *et al.* 1999). However, the public health successes of the 1960s and 1970s were followed in the 1980s and 1990s by ominous developments, such as the resurgence of diseases (e.g., tuberculosis) and the emergence of the HIV pandemic.

Just as with the current HIV, both the medieval Plague and the 1918 epidemic of Spanish Flu had major impacts on human populations and significantly altered the course of human history (McNeill 1976). Increased contacts with faunal elements (e.g., rodents), tradesmen, colonizers, and soldiers were at the root of new diseases that had ravaging effects on endemic populations (Diamond 1997). Such epidemics have even caused landscapes to metamorphose. When the Asian rinderpest virus entered Eritrea via cattle brought by Italian invaders, it took only 5–10 years for the virus to spread through Africa with devastating effects on cattle and pastoral tribes. This changed vast areas in Africa from grassland into bushy Savannah and woodland, thereby giving way to wildlife and tsetse. The tsetse flies, in turn, prevented humans and their cattle from regaining the areas formerly occupied. So, the rinderpest outbreak at the end of the 19th century caused irreversible changes in the African landscape (Anonymous 2000a; see Chapter 3). Diseases can cause dramatic mortality and ecosystem changes, and they can also leave traces in the

genetic make-up of human populations. These include the polymorphism in genes involved in discriminating self from nonself [the major histocompatibility complex (MHC)] and the polymorphism for sickle-cell anemia maintained by heterozygote advantage in areas with high malaria incidence (Hamilton and Howard 1997). The importance of diseases is also reflected in that resistance against diseases is shown to be a target for mate preferences and hence subject to sexual selection (Penn and Potts 1999).

The fact that several of these dramatic historical events took place over a time scale of 10–100 years shows that disease agents can, in principle, impose new selection pressures over vast areas and evolve on a time scale shorter than or near to the generation time of their host. To ignore natural selection in designing campaigns to combat (re-)emerging diseases is therefore (as already stated) a serious mistake. The same conclusion can be drawn from the development of microbial resistance against antibiotics (Kristinsson *et al.* 1992), the resistance of malaria mosquitoes against dichlorodiphenyltrichloroethane (DDT; Coetzee *et al.* 1999; Roberts *et al.* 2000), the resistance of malaria parasites against chloroquine (Peters 1985; Conway and Roper 2000), and from many other examples. The challenge is to develop sustainable ways to control disease agents, and thereby take selection into account (Kolberg 1994). For example, the World Health Organization (WHO) advocates the use of pyrethroid-impregnated bednets to reduce the incidence of malaria (the so-called Roll Back Malaria campaign). However, this has already led to selection on the mosquitoes for resistance against pyrethroids (e.g., in French-speaking West Africa). In addition, selection for mosquito behavior is expected to circumvent the use of bednets, for example by modifying the diurnal pattern of biting activity (Anonymous 2000b). Deeper biological insight into mosquito behavior may well provide clues as to how to "attract and kill", and there is much potential for the control of mosquitoes by using their natural enemies (Takken and Knols 1999). However, in developing sustainable control strategies it is important to ask which new selective pressures will be created and how the development of resistance to control methods can be slowed or even prevented. From this viewpoint, the central question is whether evolutionarily stable control strategies can be designed.

The idea of an evolutionarily stable control strategy is based on the assumption that the world is constant at least on the time scale under consideration. Yet the world does not remain constant on a larger time scale. Population growth, increased mobility, and possibly also climatic change will create new opportunities for diseases (Epstein 2000), whereas genetic change will help the disease agents to evade the barriers put in their way. Therefore, instead of chasing the facts it may pay off to think ahead and ask how to steer epidemiological changes brought about by population dynamics and natural selection. This requires that our control strategies not only be evolutionarily stable, but also be robust. In this chapter we have summarized what we consider the main rules of thumb that have surfaced so far, as well as the main provisos. The following chapters examine specific fields of application in further detail.

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