The evolution of virulence

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Why is there variation in the virulence of infectious diseases? Virulence can have substantial effects on the genetic contribution of both host and pathogen to future generations. Understanding it therefore requires explanation not only in terms of cellular and molecular mechanisms[1,2], but also in evolutionary terms: what is the nature of the selection acting on genes responsible for virulence?

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The term virulence can represent quite different things to different evolutionary ecologists. The following is one definition used by microbiologists (see Ref. 3). "Whereas 'pathogenicity' refers to the capacity of microorganisms to cause disease, the essentially synonymous term virulence is generally used to note variations in degree. Virulence encompasses two features of an organism's disease-producing capacity: infectivity (i.e., the ability to colonize and invade a host) and severity of the disease that is produced." For the most part, evolutionary biologists use some version of that definition, with the addition of some notion of evolutionary fitness (reproductive rate or lifetime reproductive success), but differences exist in whether the emphasis is on host fitness or pathogen fitness. Botanists put more emphasis on pathogen fitness, using virulence to mean the infective capacity of pathogens when applied to suitable host tissues[4]. Frequently, this becomes synonymous with host range, so that more virulent pathogen genotypes are those that can grow on a greater number of host genotypes[5]. In contrast, zoologists usually define virulence in terms of the reductions in host fitness caused by pathogens[6–8]. This is disease severity or pathogenicity. However, fitness is notoriously difficult to quantify. In most mathematical models, virulence is equated with host mortality rate, although nonlethal infections must often have a profound effect on reproductive fitness. In empirical studies, a variety of variables are frequently used as surrogates: morbidity, body mass, tissue damage, pathogen replication rates, and so on. However it is defined, virulence can be viewed as a property of the pathogen, or as a property of the host–parasite interaction (and thus as much a consequence of host resistance as of any parasite traits).

Whether these divergent usages obscure conceptual issues is unclear. In any case, the need for explicit definitions is obvious. In this article, I define virulence to be disease severity as assessed by reductions in host fitness following infection. At issue is how selection acts on genes encoding virulence determinants (those traits of an organism, host or parasite whose loss decreases virulence).

Virulence in Nature

Determining the virulence of infectious disease in natural populations is not easy. For the most part, correlational evidence is simply uninformative. It is often unclear whether high parasite burdens are a cause or a consequence of lower host fitness, and sampling procedures may be biased by the health of animals (sick animals are often rapidly removed by predators; healthy animals may be hard to catch). Consequently, experimental manipulation is usually the only way of convincingly demonstrating parasite-induced reductions in host fitness. But there are problems with this: host fitness is hard to measure in wild populations, and extrapolations from captive or semi-natural situations may be unwarranted. However, there are now numerous carefully controlled studies in wild populations showing that parasitic infection can reduce host fitness[9–11]. Many of these represent very old parasite–host interactions[9–13], and should finally lay to rest the conventional wisdom[14], now infamous among evolutionary ecologists, that parasites always evolve towards avirulence. Indeed, manipulative experiments that have measured a range of fitness components in wild vertebrate populations have found little evidence of avirulent parasites. This may be because such experiments are unlikely to be performed with innocuous parasites. Even so, infection clearly does reduce host fitness in many cases.

The idea that harmful parasites are typically those of recent host–parasite associations is often confirmed by observation[15]; pathogens are frequently less virulent in their native host species than they are in new hosts (e.g. haemorrhagic fever viruses, avian malaria, *Trypanosoma* spp.). While many of these sorts of anecdotal observations may not actually withstand close inspection[16], they cannot in any case demonstrate that the converse is true (i.e. that old associations are typically avirulent). Nor do we know what goes unnoticed: for the most part, virulence is the means by which we initially detect novel host–parasite combinations.

Evolution

There is every reason to think that natural selection has the potential to affect disease severity. First, virulence very probably has major effects on host and pathogen fitness, so that genes determining disease are likely to be under strong selection. Second, there is clearly genetic variation on which selection can act: virulence polymorphisms are common (e.g. Refs 2, 17,18), virulence determinants are frequently encoded by phage, plasmids and transposons[19], and artificial selection can maintain or reduce virulence[20].

How, then, might selection result in the varying levels of virulence that we see? There are a number of possibilities.

Virulence maximizes transmission

Central to most modern theory is the idea that virulence is a consequence of selection maximizing that component of the fitness of a pathogen gained through transmission[7,8]. This is usually defined as R_0, the number of new hosts infected per infected host[7]. Virulence can increase transmission in two ways. First, through illness itself. For example, vector- or predator-borne pathogens may be transmitted more readily from debilitated hosts less able to defend themselves against attack[8]. Second, some level of virulence may be an unavoidable consequence of maximizing transmission. Selection may favour infinitely fecund pathogens that do not reduce any component of host fitness related to their own, but such an ideal will not normally be possible. Instead, maximizing R_0 will require some compromise; depending on the nature of the relevant trade offs, this will frequently be associated with appreciable levels of virulence. Attempts have been made to estimate the form of at least some of these trade offs in only one case: the myxoma–rabbit system[7,18]. In this case, strains of intermediate virulence predominate in wild populations, and this has been explained[7] as a consequence of the trade off between factors determining the period of infectiousness. In extremely virulent strains, the infectious period is shortened because rabbits are rapidly killed by the virus (dead rabbits are not infectious); it is also short for relatively benign strains, because the rabbits rapidly control the infection. More generally, this view of parasite virulence assumes that differences in the form and relative importance of trade offs in different host–parasite systems produce the variation in disease severity observed in Nature.

Virulence is the result of very short-term selection

The evolutionary maintenance of virulence determinants that confer a growth advantage within hosts is frequently explicable in terms of maximizing transmission rate. However, in the following article, Levin and Bull point out that virulent mutants arising within a host that have a local growth advantage will be selected, at least in the short term, even though they need not increase transmission rate. Indeed, in extreme cases, they may never even be transmitted. For example, Levin and Bull suggest that the pathogens responsible for bacterial meningitis, polio and AIDS may persist because of mutation and within-host selection, even though between-host selection (transmission) may act against them. Where such virulent mutants have substantial effects on the R_0 of the ancestral (colonizing) population, selection should act to reduce the chances of such subversive variants arising, perhaps by eradicating the genetic mechanisms that encode more virulent phenotypes after one or a few simple mutations. However, mutations that give rise to virulence may have their effects late in an infection (and so have a relatively small effect on the fitness of the wild type), or they may be difficult to prevent.

Coincidental selection

Pathogens in hosts they rarely infect, or growing in intrahost habitats they rarely exploit, can be highly virulent. The genes responsible for such effects can thus be thought of as being maintained by coincidental selection[19]: they presumably exist because they have neutral or fitness-enhancing effects in situations where they do not cause disease. For example, Levin and Svanborg Eden[19] argue that genes that code for adhesins (bacterial organelles that bind to host cells) responsible for symptomatic *Escherichia coli* infections in the urinary tract are selected against in that location because the inflammatory response they provoke eventually results in elimination of the bacteria from the host. However, the genes are thought to be maintained because adhesins increase fitness in the gastrointestinal tract, where they do not cause disease. Of course, if the disease phenotype imposes selection against genes coding for these disease determinants, the selection should favour regulatory mechanisms to ensure that expression only occurs in situations where they increase pathogen fitness. Indeed, such regulatory mechanisms are a feature of bacterial virulence[21]. However, if virulence is expressed by pathogens that may die anyway, with little effect on related genotypes in more typical environments, or where virulence is a rare and largely inadvertent consequence of genes coding for normally advantageous phenotypes, the strength of selection against such effects may be quite weak. For example, only a tiny fraction of any *Plasmodium falciparum* population resides in people who die of cerebal malaria. Genes coding for the cytoadherence phenotype, which may cause cerebal malaria[22], could be maintained despite their lethality if they increase parasite fitness when expressed in people who do not develop cerebral malaria.

Maximizing host fitness

The models discussed above share the feature that selection acting on parasite loci is assumed to be paramount in determining the observed level of virulence. However, once infection has occurred, disease severity is frequently due to host response (and over-response). This raises the important and open question of the extent to which variation in disease severity is a consequence of selection maximizing the fitness of infected hosts within the constraints of parasite-imposed trade offs[23,24]. For example, Behnke *et al.*[25] have suggested that hosts may tolerate small worm burdens in the long term, despite the slight reductions in host fitness, because the responses necessary to eject every last worm (inflammation, peristalsis and changes in gut chemistry) result in greater reductions in host fitness. However, if worm burdens are high, the cost of these self-inflicted reductions in fitness is less than that due to the worms, and ejection takes place. Certainly, considerable virulence is believed to be a consequence of host responses (e.g. schistosomiasis, malarial fevers), or their failure (e.g. the so-called 'opportunistic'

infections of AIDS patients). Similarly, how much virulence is a consequence of host responses that are inappropriate in some contexts, but have evolved in response to selection imposed by other pathogens or circumstances?

Putting it all together

Thus, a particular level of virulence might be adaptive for an infected host or for infecting pathogens, or it may be a consequence of parasite genes being expressed in atypical environments, short-term selection of newly mutated pathogen variants, or host responses that are adaptive in other circumstances. The relative importance of these possibilities is likely to vary considerably. In many cases, some complex interplay between them probably determines virulence, and there may be no generalities. Each possibility is supported by largely circumstantial evidence, and few critical tests have been performed; presumably this is a reflection of the youth of the field. Perhaps the most overlooked possibility is the importance of selection acting on host factors. When they are considered at all, the effects of host loci are usually incorporated as relatively fixed constraints within which selection on pathogens acts (e.g. avirulent myxoma viruses are assumed to be less fit because they are rapidly cleared by the host). Typically, selection acting on host genes is assumed to be relatively unimportant in the short term because of differences in generation times between hosts and parasites. Such a view is surely overly simplistic: interactions between microparasites and their multicellular hosts, particularly those with complex immune systems, are occurring between pathogens and somatic cells with similar generation times, allowing considerable potential for rapid evolution[24,26]. At the very least, the trade offs faced by parasites are likely to change as hosts age, become immune and so on.

The idea that virulence is a consequence of selection acting on pathogen loci to maximize transmission has perhaps the best empirical support. Compare, for example, vertically and horizontally transmitted parasites. The reproductive success of the former is intimately linked with the fitness of their hosts, and selection should minimize their effect on host fecundity. This is what has been observed[12,20,27], although maternally inherited pathogens frequently have substantial effects on the sex ratio of offspring produced by hosts; again, this is expected if selection is acting on pathogen loci[28]. Similarly, much virulence is determined by parasite traits that apparently have no function other than for attacking the host so as to enhance transmission[29,30]. However, numerous examples suggest that virulence may not be optimal for maximizing transmission. For example, coincidental selection for some other function presumably maintains the toxins produced by *Clostridium botulinum*, since these bacteria cannot proliferate within a mammalian host[19]. Likewise, *Toxocara canis* rarely infects humans, but when it does, it can make its way to the eye and cause blindness. This does not result in transmission, and so presumably the genes that enable it to survive and reach the eye are maintained by their effects elsewhere. Avian malaria results in the rapid death of Hawaiian endemic birds; thus the virulence determinants of the parasites must be maintained in other birds where symptoms are less severe[31]. Presumably optimizing selection on the virulence phenotype has not been responsible for the obvious virulence of 'opportunistic' pathogens in immunocompromised hosts.

There are still numerous theoretical issues to resolve. Models optimizing fitness typically assume fixed trade offs and that the population dynamics are in equilibrium, but the epidemic and coevolutionary nature of host–parasite interactions may invalidate these assumptions. Under what circumstances might virulence polymorphisms be maintained? Nowak and May[32] suggest that superinfection may be an important factor. In any case, it should be possible, at least in principle, to determine the nature of the selection acting on virulence determinants empirically in particular cases.

Why bother?

Evolutionary ecologists became interested in virulence because of its implications to a number of fields, including epidemiology[7], the evolution of cooperation[20,33] and the maintenance of genetic polymorphism[34,35], as well as explicitly clinical questions[8]. Interest remains in these questions, but it is presumably the first and last that motivate microbiologists (or at least their funding agencies), and in this context there seems much potential for intercourse. One aim of medical and veterinary intervention is to reduce virulence. Yet intervention may impose selection for increasing virulence. Assume, for example, that virulence is largely a consequence of selection optimizing pathogen fitness, and that moderately pathogenic parasite strains are maintained by selection because they are less rapidly cleared by the host (e.g. myxoma infections in rabbits[7]). If vaccination confers partial protection on some hosts by enabling them to clear a pathogen of a given grade of virulence more quickly, selection may actually favour an increase in virulence as the frequency of vaccinated individuals rises. More generally, there is every reason to suspect that changing environmental conditions (e.g. an increase in the number of immunocompromised hosts, greater host densities or different treatment regimes) will alter selection on virulence. If we do not understand the selective regime responsible for current levels of virulence, we cannot predict the consequences of such changes.

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Short-sighted evolution and the virulence of pathogenic microorganisms

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For some microorganisms, virulence may be an inadvertent consequence of mutation and selection in the parasite population, occurring within a host during the course of an infection. This type of virulence is short-sighted, in that it engenders no advantage to the pathogen beyond the afflicted host. Bacterial meningitis, poliomyelitis and AIDS are three candidates for this model of the evolution of virulence.

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From an ecological–evolutionary perspective, the interaction between microparasites (primarily viruses, bacteria and protozoa) and their multicellular hosts can be likened to a genetic arms race. At one level, it is a race between whole species, the microparasite species versus that of the host, with both species (co)evolving over long periods. At another level, it is a race between the microparasite population(s) infecting an individual host and the somatic cells of that host (for vertebrates, primarily those of the immune system), with the outcome of this evolutionary microcosm having little or possibly no consequence for the long-term fate of either protagonist species.

Once a microorganism successfully traverses the gauntlet of physical barriers and constitutive defenses and begins to proliferate in the host, the genetic arms race commences. The parasite and host somatic cell populations will never be the same. Through clonal selection, the genetic composition of the immune system will literally evolve to control the parasite. In turn, the parasite population is under continuous selection to evade detection and destruction by those immune defenses, commonly responding by changing its antigenic characteristics. Some microparasites, such as *Trypanosoma brucei*, *Neisseria meningitidis*, *Streptococcus pyogenes* and *Salmonella typhimurium*, have mechanisms that seem to have evolved to generate variation (evolved to evolve, if you like) specifically for this arms race by augmenting the rate at which antigenic variation is produced[1,2]. For these pathogens,