Parasite Virulence

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The Problem

Some parasites exact a terrible price from their hosts, causing severe pathology and reducing the host’s fitness, whereas other parasites are essentially benign. Several kinds of comparisons highlight this observation. Least interesting are comparisons of parasites with very different life histories or types of host tissues invaded (compare human immunodeficiency virus (HIV) and rhinovirus infection in humans). In other cases, the same parasite causes great harm in one species of host, but is tolerable to another, such as the rabies virus, which kills canid hosts but can reside in mustelid populations as non-lethal infections (Kaplan, 1985). Again, this may result from different types of tissues invaded by the parasite. Most intriguing are examples of very different levels of pathology caused to the same host species by closely related parasite species or even different genetic strains within a parasite species.

Examples of this last situation are abundant. Strains of Trypanosoma brucei, the causative agent of African sleeping sickness in humans, differ in the severity of the pathology they cause, so much so that they were long classified as different species, based on symptomatology (Toft and Aeschlimann, 1991). Malaria has the reputation as the malignant ‘million-murdering death’, but only Plasmodium falciparum kills a significant number of victims outright and, within each species, the morbidity and mortality associated with infection vary geographically (Arnot, 1998). The rabies virus, so notorious for its lethality for humans and extremely high mortality for dogs, has evolved an African strain that produces non-fatal oulou fato in dogs (Kaplan, 1985). Entamoeba histolytica and Giardia lamblia, widespread and important intestinal parasites of humans, vary in their pathology by genotype, which led to different species names for polyphyletic clusters of strains (Mehlotra, 1998;
Thompson, 2000). These examples suggest that virulence may be a ‘life-history trait’ of the parasite and is part of the adaptive picture of parasite–host associations. If this is so, what drives the evolution of virulence?

The dominant view among medical parasitologists for generations held that selection favours a reduction in virulence because the parasite’s home site is ephemeral (hosts eventually die) and a parasite should not exacerbate this situation by reducing the host’s lifespan. Recently established associations may show poor adaptation on the part of the parasite, but older associations will reveal accommodation of the parasite to its host and low cost of infection (Burnet and White, 1972). Ball (1943) long ago demonstrated the theoretical and empirical weakness of this reasoning. He found that data comparing the likely ages of different parasite–host systems with their virulence did not support the ‘association age’ hypothesis. Despite the cogent argument of Ball, one of the century’s most eminent parasitologists, and the rejection of the classical prudent-parasite image by evolutionary biologists, it is still current in the medical literature (review in Ewald, 1994).

A growing list of hypotheses have supplanted the venerable prudent-parasite view (reviews in Ewald, 1994, 1995; Groisman and Ochman, 1994; Read, 1994; Bull, 1995; Frank, 1996; Poulin, 1998; Ebert, 1999). Coalescing these many hypotheses into a general theory on the evolution of virulence remains an elusive challenge. A general theory must respect the great systematic and ecological diversity of parasites and incorporate such factors as:

- The phylogenetic history of both parasites and hosts.
- Kind of tissue invaded by the parasite.
- Mode of transmission (consumption of one host by another vs. use of a vector, for example).
- Ease of transmission (including aspects of the biology of both parasite and host).
- Number of host species exploited by a parasite.
- Population structure of the parasite–host system.

Can any single theory gather together the details of the biology of parasites as different as viruses, tapeworms and ticks? Perhaps the diversity of ‘parasites’ in the broadest sense cannot be included within the rubric of a general theory. Ewald (1995) argues persuasively that such a view is pessimistic and unproductive, because a single theory could cast light on a broad range of associations, from viruses to large predators.

**What is Virulence?**

Defining virulence is problematic (Read, 1994; Ebert, 1999; Poulin and Combes, 1999), in part because disciplines in the life sciences differ in their perspective when interpreting the importance of parasitic diseases.
For those with medical or veterinary interests, virulence is any harm done to the host by another organism (usually limiting discussion to small organisms). For medical workers, virulence is an issue of mechanism (such as so-called ‘virulence genes or traits’ (Poulin and Combes, 1999)) and the practical goal of reducing illness. An interesting example is botulism. Carl Bromwich, a physician practising in Kuujjuaq in the Canadian Arctic, reports that he has extensive experience in treating botulism. Local people hunt sea mammals and prefer to eat the meat only after it has aged. *Clostridium botulinum* probably lives on the mammals as a commensal (a parasite with no costs to the host), but rises to huge population densities when it reproduces on the carcass. The bacteria produce a toxin (perhaps as interference competition with other bacteria) and humans become ill, not from an infection with *C. botulinum*, but from ingesting the toxin. Different strains of *C. botulinum* could vary in the quantity and nature of the toxin produced. Medical personnel would reasonably discuss the variation in virulence of these strains, but the selective events leading to such variation in the biochemistry of the bacterial strains had nothing to do with their effects on humans. This example is transparent, but many other cases of supposed parasite ‘virulence’ involve accidental contact of limited significance for the evolution of parasite or host.

The public-health community is concerned with illness associated with infectious disease, as well as the ease of transmission of pathogens. Thus, the term virulence is often applied to some measure of rate of transmission; indeed, for many microbiologists and epidemiologists, that may be the primary definition of the term (Lipsitch and Moxon, 1997). Natural selection will certainly favour more ready transmission by parasites, but this meaning of the term is not relevant for the present discussion.

Wildlife ecologists, in contrast, are normally unconcerned with morbidity or mortality induced in individual hosts, but instead ask if parasitism can regulate host population density (Hudson et al., 1998). For conservation ecologists, the possible reduction in population size of endangered species is a concern (Holmes, 1996). Thus, for an ecologist, consequences of parasitism for host lifespan and fecundity and how they influence host population density are the appropriate measures of virulence, and the evolutionary origin of virulent vs. benign parasites is not of concern.

In the discussion presented here, another outlook on virulence is required: virulence is a trait under selection, either directly or indirectly. Natural selection will work asymmetrically on hosts and parasites, so parasite virulence has two meanings, one for each species in the association. Making a distinction between virulence from the perspective of the host vs. the parasite is not just an exercise in term-mongering but allows us to recognize that the final harm done to the host by infection depends on a composite of two selective forces, one acting on the host and the other on the parasite.
Selection and Virulence – from the Host’s Perspective

For the host, virulence is any consequence of infection that reduces the host’s lifetime reproductive success (fitness). A fitness cost could result from the direct damage done by the parasite (destruction of cells, usurpation of resources), the expenditure of resources in mounting an immune response and collateral damage done to the host by its own immune system. To better grasp how parasites can reduce reproductive success, we can partition fitness into components, such as lifespan, fecundity, number of reproductive episodes, ability to find and court mates and health of offspring. Trade-offs between these components of fitness are a universal challenge faced by organisms (Bell, 1997), so we can imagine that infection may hinder one component of fitness while benefiting another. For example, castration of the host may be beneficial to the parasite if infected hosts partition more resources towards growth and body maintenance, which could provide more resources for the parasite and a longer-lasting host (Boudoin, 1975). Although host lifespan may increase, its overall fitness is reduced to zero. Another example is a parasite that is transmitted via the host’s offspring and manipulates the host’s reproductive biology. Such a parasite could increase the short-term fecundity of the host while reducing the host’s overall lifetime reproduction. Venereal-transmitted parasites could manipulate the host to increase its attractiveness as a mate or expand its period of courting and mating. An expanded mating effort could also reduce the host’s reproductive success if other components of fitness are reduced, such as lifespan. All of these examples demonstrate the importance of keeping the focus on those consequences of infection that would apply a selective pressure on the host.

Selection on the host favours adaptations that prevent or eliminate infection (host immunity in the broadest sense) or reduce the fitness costs of infection. But what would the optimal strategy be for the host – an intense defence that would eliminate the infection or a lesser attack that allows the infection to remain at some low level? Some trade-off must exist that balances the costs of the defence (to the host’s fitness) against the benefit (also to its fitness). Figure 14.1 suggests how selection may favour some intermediate level of host response. Thus, part of the variation seen in parasite virulence could result from differing solutions to the trade-off between the costs and benefits of mounting antiparasite tactics by the host.

Antiparasite tactics include natural resistance, behavioural mechanisms to avoid infection and the immune system. Unfortunately, actual measures of the costs vs. benefits of antiparasite mechanisms are scarce (Gemmill and Read, 1998). One of the better examples of the costs vs. benefits of host resistance is the elegant study of Yan et al. (1997), who examined two genotypes of the mosquito Aedes aegypti in relation to the insect’s resistance to the malaria parasite Plasmodium gallinaceum. Being refractory, or resistant, to the parasite has associated costs to fitness,
including smaller body size, production of fewer eggs per clutch and shorter lifespan. Intuition suggests that this genotype remains in nature because it has the benefit of avoiding pathology induced by the parasite. However, Yan et al. (1997) could detect no fitness costs of infection. This presents a perplexing question. Why does the genetic polymorphism continue in natural habitats if resistance to infection has a fitness cost but no benefit when the mosquito is infected?

More common are circumstantial stories. One such example concerns an odd anatomical feature found on lizards of at least five families – skin invaginations on the neck, axilla and other body regions that seem particularly prone to infestation by mites and ticks. Arnold (1986) suggested

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**Fig. 14.1.** Curves showing one possible relationship for the cost and benefit of differing levels of immune attack against a parasite. The horizontal axis shows the level of the host’s antiparasite effort necessary to hold the parasite’s rate of replication to low levels, or which will allow the parasite to replicate rapidly. The ‘Cost to host’ curve shows the cost to the host’s fitness from mounting an immune attack. A low-level immune attack, one that allows the parasite reproductive rate to remain high, would have low cost, but a potent immune attack that holds the parasite to a low rate of reproduction would have a high cost. The ‘Benefit to host’ curve shows the benefits of limiting the rate of parasite reproduction. A fairly low-level immune attack with a marginal reduction in the parasite growth rate would be highly beneficial, so the benefit curve is steep. However, further reduction in parasite growth rate would have a declining additional benefit for the host. The shape of the cost curve shows that the initial small investment in the immune attack would have low cost, but completely curtailing the parasite would have an extreme cost to the host. The optimal solution is an intermediate level of immune response. Parasites with effective methods to evade the host immune system may select for very limited immune attack. The host may well be able to trump the parasite’s defences, but the cost would outweigh the benefits.
that the pockets function to draw ectoparasites away from areas of the body where they could be more harmful. Immune-system cells are clustered just under the skin of these pockets, perhaps waiting to ambush any pathogens that might be passed by the bites of ectoparasites. Arnold’s (1986) hypothesis was supported by Salvador et al. (1999), who experimentally plugged the neck pockets of the lacertid lizard *Psammodromus algirus* and observed a movement of ticks to areas of the body where they were likely to hinder normal locomotion. Thus, the pockets may serve as a way of reducing the cost of infestation and may be a lower-cost (to fitness) adaptation than any mechanism that would completely eliminate the ectoparasite burden. Actually measuring this trade-off would be a challenge.

**Selection and Virulence — from the Parasite’s Perspective**

Virulence from the parasite’s perspective is any injury to the host that reduces the parasite’s fitness – that is, any consequences to the host of the infection that would cut the number of successful transmissions of the parasite to other hosts. Fitness for a parasite genotype is the number of daughter infections spawned into other hosts (May and Anderson, 1983). The following equation:

\[ N_d = (N_{to} \times P_t) D \]

shows that the number of daughter infections \( N_d \) is a product of the number of transmission opportunities per unit time \( N_{to} \), the probability of each of these opportunities being successful \( P_t \) and the duration of the infection \( D \). Thus, virulence from the parasite’s perspective is any reduction in \( N_d \) that results from its own biology within the host. Some examples include an infection-induced degradation of the host environment (the infection then fails, reducing \( D \), or parasite density falls very low, reducing \( P_t \), death of the host (reduced \( D \)) or changes in the host that reduce \( N_{to} \) (an immobilized host would greatly reduce the transmission opportunities for a directly transmitted parasite!).

Most discussion of parasite virulence assumes that there must be some trade-off between acute, or short-term, transmission success and the duration of the transmission period (typically the host’s survival) – that is, \( N_{to}, P_t \) and \( D \) cannot be maximized simultaneously, because high parasite density increases the probability of transmission but also reduces host survival and thus the duration of the transmission period. There are actually two assumptions being made: (i) parasitaemia is negatively correlated with host survival; and (ii) parasitaemia is positively correlated with the probability of transmission. The final result is that a parasite can maximize either \( P_t \) or \( D \) but not both.

This argument is intuitively pleasing but is not often explicitly presented (although it underlies most of the hypotheses on virulence that
have been proposed – see below), and even more rarely is it actually tested (Mackinnon and Read, 1999a). The assumption that higher parasite loads lead to reduction in the host survival is supported by data on some pathogens (examples include HIV (Mellors et al., 1996) and rodent malaria (Mackinnon and Read, 1999a)), but the literature is replete with exceptions (Messenger et al., 1999). The actual relationship between parasitaemia and pathology may be non-linear, especially if the host’s immune system is responsible for some part of the injury ‘caused’ by the parasite. In such cases, low parasite loads may be as harmful as moderate or even fairly heavy loads. For example, the destruction of liver and other organs seen in human schistosomiasis is in part a result of the host’s immune attack on the microscopic eggs of the worm (Warren, 1975) and the anaemia presenting with malaria derives in large part from destruction of uninfected red blood cells by an overactive immune system (Wetherall, 1988). The assumption that higher parasitaemia will increase the ease of movement of the parasite from host to host (increase in $P_t$) also seems reasonable, but the overall ecology of transmission can be complex and confound the expected simple relationship between parasitaemia and transmission success (Lipsitch and Moxon, 1997). Natural-history studies on directly transmitted parasites must include data on the distribution and survival of the parasite stages once they leave the host. Vector-borne parasites have ecologies that often fly in the face of biologists’ intuition. For example, malaria parasites replicate asexually in the vertebrate host’s blood and produce gametocytes that are taken up by the biting vector, where they undergo the sexual phase of their life cycle. More rapid asexual replication results in larger numbers of transmissible gametocytes in the blood (Mackinnon and Read, 1999a; Eisen and Schall, 2000). Although higher numbers of gametocytes would seem to favour more efficient transmission, data from experiments on experimental transmission of some malaria parasites most often fail to confirm this relationship (reviewed in Schall, 2000) – that is, infections with a high density of gametocytes in the vertebrate host’s blood are not necessarily those with highest transmission success into the vector.

**Hypotheses on the Evolution of Virulence**

**Transmission-opportunity hypothesis**

This hypothesis proposes that the ecology of transmission is the central factor driving the evolution of virulence (Gill and Mock, 1985; Ewald, 1994). When the parasite has many opportunities to move from host to host, selection will tilt towards rapidly reproducing parasite genotypes. In contrast, when transmission opportunities are rare, a very rapidly reproducing parasite could kill its host before transmission is possible. The most extreme case would be parasites with seasonal transmission or other
periods of ‘impossible transmission’, when the infected host leaves the area where vectors are present (Gill and Mock, 1985). This parasite should reduce its reproduction and virulence, perhaps rebounding when cues indicate that the transmission period is approaching.

For vector-borne parasites, $N_{to}$ will often be high when vectors are abundant in the environment, but vector abundance may not always be a good predictor of selection for high parasitaemia. Variation in vector competence among sites (such as differences in host-seeking behaviour or the physiological environment presented to the parasite) could obscure any pattern between vector abundance and opportunities for transmission. Also, if hosts behaviourally avoid sites where transmission is likely or if they flick off alighting vectors (Hart, 1994), $N_{to}$ could drop and select for low parasitaemia and consequently low virulence. Thus, host behaviour can apply selective pressure on the parasite to reduce virulence.

Overall, in environments or times with low $N_{to}$, infections of prudent, slow-growing genotypes would always be prone to invasion by mutation to rapidly reproducing genotypes of parasites, but such infections would fail to yield many (if any) daughter infections. There is thus a trade-off between $P_t$ and $D$, which is driven by $N_{to}$.

Mobility hypothesis

Parasites that require their host to be mobile for successful transmission cannot cause disabling morbidity and still enjoy successful transmission. Transmission opportunities would be few for directly transmitted parasites that disable their host (Ewald, 1995), but this may not be the case for vector-borne parasites (indeed, vectors may prefer non-ambulatory hosts). Parasites should always be avirulent for vectors that must remain mobile to allow transmission (Ewald and Schubert, 1989). Parasites using non-living vectors, such as flowing water, should be among the most virulent pathogens (Ewald, 1988). Parasites can also increase $N_{to}$ by developing long-lived, highly durable transmission stages, reducing the need for a mobile host. This is termed the ‘curse of the pharaoh’, from the folk-tales that ancient corpses may harbour viable and exotic pathogens (Bonhoeffer et al., 1996).

Host-demography hypothesis

Ebert and Mangin (1997) suggest that selection favours a high reproductive rate (and thus high virulence) for parasites exploiting hosts with naturally short lifespans – that is, if $D$ is naturally short (a short-lived host could support an infection only of short duration), selection would favour high parasite replication to increase the probability of transmission.
Immunity hypothesis

The nature of a host’s antiparasite tactics and the ability of the parasite to respond should influence virulence. As noted above, behavioural defences that reduce the opportunities for transmission should select for decreased virulence. However, a potent immune attack once transmission is successful could produce an opposite selective force (van Baalen, 1998; Gandon and Michalakis, 2000). An effective immune response would limit the duration of the infection, and selection would favour more rapid replication of the parasite (low $D$ favours high $P_t$, just as presented for the host-demography hypothesis) (Anita et al., 1994).

Host-specificity hypothesis

Parasites that specialize on a single (or very few) host species should evolve highly specific ways of dealing with the host immune response and are thus more likely to exploit their hosts efficiently and produce severe pathology (Ewald, 1983). Frank and Jeffrey (2001) turn this view on its head. They propose that newly established parasite–host associations (the equivalent of a parasite with a very large range in host species) will be among the most virulent because of the lack of an efficient, specialized host defence.

Transmission-mode hypothesis

Parasites typically move from host to host in the environment (infectious or horizontal transmission). Some parasites, though, are transmitted via the host’s offspring (congenital or vertical transmission). In such cases, the fitness of the host translates to fitness of the parasite, and the fitness ‘desiderata lists’ (Dawkins, 1990) of both species in the association coincide. Thus, vertically transmitted parasites should be less virulent than those using horizontal transmission (Messenger et al., 1999). If the death of the host is required for transmission (one host must eat the other, for example), the desiderata lists diverge completely, and the parasite may actually manipulate its host to increase its chance of being killed (Poulin, 1998).

Small worlds – diminishing-returns hypothesis

Parasites with low dispersal could have a small world of potential hosts (Herre, 1993; Lipsitch et al., 1995; Boots and Sasaki, 1999). Over time, there would be diminishing returns on rapid transmission, because the opportunity for new hosts would decline as a parasite genotype fully
exploits available hosts. Thus, parasites with a small world of potential hosts must reduce their virulence.

**Clonal-diversity hypothesis**

For parasites that replicate within the host (malaria parasites and viruses are examples), the presence of multiple genotypes, or clones, may lead to competition for resources or simply to be the clone most likely to be transmitted. When infections typically consist of many clones, this would select for high parasite replication and higher virulence (van Baalen and Sabelis, 1995; Frank, 1996). Even if each clone is prudent and replicates slowly, the sum of the clone densities would result in higher virulence. High clonal diversity may also lead to some proto-cooperation by the parasites to elude the host immune system more efficiently, thus resulting in a higher rate of parasite replication and higher virulence.

**A Successful General Theory of Virulence?**

The review of hypotheses presented here must be incomplete (the literature on parasite virulence is large and growing rapidly), but at least presents a flavour of the discussion. Note that all of the reviewed hypotheses centre on how selection works on the parasite. Virulence from the host perspective has received too little attention (but see an exception below). Can these hypotheses be merged to produce a general theory of parasite virulence? A successful general theory in science must explain a broad array of observations and suggest numerous predictions for future testing. The review suggests that no simple selective process drives the evolution of virulence. We could easily produce numerous thought experiments to design parasites with high (or low) virulence that came to that state via very different evolutionary trajectories. Frank (1996) concludes that ‘the models [on virulence] cannot be applied without careful consideration of the biology of particular host–parasite interactions’. Any general theory can take a very broad sweep, ignore the annoying complexity of natural history and still yield useful insights. The notion of ‘desiderata lists’ in Dawkins (1990), for example, shows that, when the fitness of the parasite depends on the fitness of the host, virulence should evaporate and the parasite may evolve towards a mutualistic relationship. Such a perspective has heuristic value (that was Dawkins’s stated objective), but may not be of much use for medical or experimental parasitologists. Ewald, in testing the mobility hypothesis (Ewald, 1983, 1988, 1994; Ewald and Schubert, 1989), used large among-species comparisons – another broad sweep through parasites with quite different biologies.

Untidy results that emerge from broad tests of the theory do not mean that the theory has failed, but point out which species are likely to be
particularly interesting for future study. For example, Jaenike (1996) found that nematode parasites of *Drosophila* are less likely to fit a model of parasite virulence when they exploit multiple host species – and thus no ‘optimal’ virulence is possible for any particular species of host. Ebert and Mangin (1997) conducted a selection experiment with a microsporidian parasite of *Daphnia* to test the host-demography hypothesis. They found that the selective regime that should have favoured low virulence (longer lifespan of *Daphnia*) actually resulted in higher virulence, because multiple infections became more common in the longer-lived infections, resulting in within-cell competition among the parasites. Thus, the specific natural history of the parasite ‘can lead to wrong predictions’ (Ebert and Mangin, 1997). A last example is a selection experiment for high and low virulence of a malaria parasite of mice (Mackinnon and Read, 1999b). Over time, both the selected lines increased in virulence; again, the details of the natural history of this malaria parasite most probably confounded the expectations of simple models of virulence.

The theory becomes most interesting when we recognize that parasite and host coevolve, so we must consider how selection acts from the parasite and host perspective, but simultaneously. The model of van Baalen (1998) uses this tactic. The model takes into account the trade-off between the cost of mounting an immune response by the host and the benefit of eliminating/reducing the parasite, as well as the prevalence of the parasite and the parasite’s ability to coevolve with the host. The results are intriguing. Coevolution of parasites and hosts can lead to two stable situations, one with avirulent, common parasites and low host investment in the immune response, and one with rare, but virulent parasites and high host defence costs. These two genotypes of parasite could well exist in a mixed strategy, which would account for the presence of high- and low-virulence genotypes in metapopulations of *P. falciparum* (Gupta *et al*., 1994), as well as other parasites.

### A Case-study in Parasite Virulence: Lizard Malaria

#### Systematic and ecological diversity

The malaria parasites, genus *Plasmodium*, are taxonomically and ecologically diverse; ±170 described species exploit reptiles, birds and mammals as their vertebrate hosts (Schall, 1996). Of these, approximately 70 species of *Plasmodium* infect lizards on all the warm continents (except Europe) and are found in a wide range of habitats (wet tropical forest to dry, deciduous, temperate savannah). The great ecological and systematic diversity of lizard malaria parasites makes them a good model system for among-species tests of the theory of virulence. This has been one of my goals over the past 23 years – to compare the costs of infection for lizard–*Plasmodium* associations from distinct ecological situations.
Here I report a summary of the results for six *Plasmodium* species, and then examine the data to evaluate the hypotheses presented earlier.

### Six malaria parasites of lizards

*Plasmodium mexicanum* infects the western fence lizard, *Sceloporus occidentalis*, in the western USA and Mexico (Ayala, 1970). Its life cycle is the only one known in detail for lizard malaria parasites, but is typical for *Plasmodium*. Repeated cycles of asexual replication occur in red blood cells. With each reproduction, the mother cell (schizont) releases about 14 daughter parasites (merozoites); the cell is destroyed and the merozoites enter new erythrocytes to begin a new reproductive cycle. After an initial period of asexual growth, some parasite cells develop into sex cells, or gametocytes, which cease replication in the blood. The vectors, two species of sandfly (*Lutzomyia vexator* and *Lutzomyia stewarti*), take up the parasite cells when they consume a blood meal from an infected lizard (Fialho and Schall, 1995). Only the gametocytes survive, and some undergo sexual reproduction followed by asexual replication to produce cells that travel to the insect’s salivary glands. The parasites are passed back to a lizard during the next blood feeding by the vector. They then travel to the liver and other organs to undergo a cycle of asexual replication before entering the blood cells. Transmission success into the vector is only weakly related to the density of gametocytes in the lizard’s blood (Fig. 14.2). Among lizard hosts of *P. mexicanum*, there is substantial variation in life-history traits, such as rate of asexual replication and final parasite levels (Eisen, 2000), with genetic variation explaining part of this variation in life histories among infections (Eisen and Schall, 2000).

My students and I have studied *P. mexicanum* at the Hopland Field Station in Mendocino County, California, since 1978. Although prevalence of *P. mexicanum* varies among years and sites (Schall and Marghoob, 1995), typically about 25% of lizards are infected. The environment at Hopland is strongly seasonal, with wet, cool winters and hot, dry summers. Transmission is thus seasonal. The lizards suffer substantial winter mortality during their inactive brumation period. The parasite density in the blood drops during the winter months to very low levels, but rebounds again the next spring (Bromwich and Schall, 1986; Eisen, 2000).

Two parasite species were studied in the rainbow lizard, *Agama agama*, in Sierra Leone, West Africa: *Plasmodium agamae* and *Plasmodium giganteum* (Schall, 1990; Schall and Bromwich, 1994). Both parasites were common in the lizards surveyed at 22 sites in several habitat types, including savannah, riparian forest and urban zones; typically, 25–75% of lizards were infected. The two species have strikingly different life histories. *P. agamae* is a small parasite, producing only eight merozoites during asexual replication in the blood, whereas *P. giganteum* is a true giant, filling the host cell and producing > 100
merozoites. There are more mixed infections (both parasites within a single lizard) than expected by chance. *P. giganteum* uses primarily immature red blood cells and *P. agamae* the mature cells. Immature cells are rare in uninfected lizards, but increase rapidly once the lizard becomes infected with either species of *Plasmodium*. Thus, *P. giganteum* may have difficulty in becoming established in a lizard unless *P. agamae* is already present (Schall and Bromwich, 1994). The use of a rare habitat (immature red blood cells) by *P. giganteum* may also keep its growth rate and parasitaemia to lower levels than expected, based on the large reproductive output of each giant schizont.

The habitat in Sierra Leone, although tropical, is also strongly seasonal because of pronounced wet and dry seasons. Transmission of the parasites may also be seasonal (Schall, 1990). Rainbow lizards are large, long-lived animals.

Three species of malaria parasite were studied in the Caribbean islands: *Plasmodium floridense*, *Plasmodium azurophilum* and an unidentified species, referred to here as *Plasmodium ‘red’*. These three species infect *Anolis* lizards throughout the eastern Caribbean islands (Staats and Schall, 1996a,b; Perkins, 2001). *P. floridense* is found on the northern islands in the eastern Caribbean; it is a small parasite, producing
very few merozoites. The name ‘P. azurophilum’ was originally erected for a parasite infecting both erythrocytes and two classes of white blood cells of the anoles (Telford, 1975). Recently, this parasite has been revealed to be two species, each with a wide distribution in the eastern Caribbean islands (Perkins, 2000, 2001). I retain here the name P. azurophilum for the species that infects only white blood cells and use P. ‘red’ for the species that infects erythrocytes. These two species are morphologically indistinguishable (Perkins, 2000) and are giant parasites, each schizont producing about 65 merozoites. Phylogenetic evidence indicates that P. azurophilum originated from P. ‘red’ on St Kitts or a nearby island (Perkins, 2001).

The number of white cells in the blood limits the density of P. azurophilum. Although infection is associated with an increase in white blood cells (Ayala and Hertz, 1981; Schall, 1992), the number of the cell type needed by the parasite is never near the abundance of erythrocytes in the lizard’s circulation. The derived life history of P. azurophilum limits its population density in the host and perhaps its ability to be transmitted by the biting vectors. The use of white blood cells by P. azurophilum must also lead to quite different pathology for the lizard host from that of the more typical Plasmodium infecting erythrocytes.

The vectors for the Caribbean lizard malaria species are unknown. Prevalence is about 30% for P. ‘red’ and 10% for the other two species on Puerto Rico (Schall et al., 2000), and on Saba, Netherlands Antilles, about half the lizards are infected by at least one species of Plasmodium (Staats and Schall, 1996a). No difference in prevalence was noted among seasons for either the Puerto Rico or Saba sites (where long-term studies have been under way for the past decade), so transmission may be year-round. However, periodic hurricanes and droughts strike the islands and could result in unpredictable periods of reduced transmission.

In summary, six species of lizard malaria parasite will be discussed here: P. mexicanum in temperate, seasonal California; P. agamae and P. giganteum in a seasonal, tropical region of West Africa and P. floridense, P. azurophilum and P. ‘red’ on the tropical, aseasonal Caribbean islands, which are regularly disturbed by severe weather. A seventh species, Plasmodium chiricahuæ of temperate and seasonal high elevations in Arizona, was studied by Foufopoulos (1999), so some comparative data are also presented for that species. P. chiricahuæ is particularly interesting because it is the closest sister taxon to P. mexicanum (Perkins and Schall, 2002).

Measuring the virulence of malaria infections

Comparisons of malaria-infected vs. non-infected lizards have revealed many health consequences of infection, including effects on haematology, physiology, behaviour and reproduction. Figure 14.3 summarizes the data for the P. mexicanum–fence-lizard association in California. Simple
comparisons of infected and non-infected lizards may be misleading for two reasons. First, such comparisons may obscure actual cause-and-effect relationships, i.e. perhaps sickly lizards may simply be more prone to infection with parasites. Foufopoulos (1999) has manipulated infections of *P. chiricahuae* in mountain spiny lizards and observed changes in health status similar to some of those shown in Fig. 14.3, which argues that malaria infection is the cause of the pathologies to be described here. Secondly, some kinds of pathology may be dependent on parasitaemia, and density-dependent virulence would be missed by simple comparisons of infected vs. non-infected hosts. Our studies have found that some effects of parasitism are more severe with increasing parasitaemia (change in social behaviour appears related to parasitaemia (Schall and Dearing, 1987)), but other effects appear to be more or less independent of parasitaemia (effects on blood haemoglobin levels (Schall, 1982)). Some other measures are difficult to relate to parasitaemia – clutch size, for example, is determined by long-term events and parasitaemia is determined from a single blood smear (Schall, 1983).
The goal of the research was to take as many measures of pathology as possible, with the expectation that the total picture will reveal some measure of virulence from both the parasite and host perspectives.

**Measuring host mortality**

The most difficult data to obtain on virulence may be the most interesting: reduction in host lifespan. Several methods were used to detect any increase in mortality induced by *Plasmodium* infection in lizards. Naturally infected fence lizards suffer two to six times higher mortality in laboratory cages and, for all parasite species, infected animals are more prone to attack by predators (Schall, 1996). Prevalence (percentage infected) rises with the lizard’s age for every system studied, but prevalence typically levels off or even drops for the oldest lizards (Fig. 14.4). Long-term mark–recapture studies of infected fence lizards (Bromwich and Schall, 1986; Eisen, 2000) and laboratory-held anoles and rainbow lizards show that infections are seldom eliminated. Thus, the dip in prevalence suggests that mortality increases for older lizards when they are infected. Among sites at Hopland and Saba, there is a negative relationship between maximum body size and the prevalence of malaria infection (Fig. 14.5). Thus, lizards tend to be smaller in areas where prevalence of the parasites is highest. Lizards typically grow throughout

![Graph showing prevalence of Plasmodium giganteum in rainbow lizards, Agama agama, in Sierra Leone, West Africa. The pattern shown here is typical for lizard malaria parasites. Prevalence increases with size (= age), as expected if lizards seldom lose infection. Males are more often infected than females. Prevalence drops for the oldest animals, suggesting that the parasite increases mortality for these animals.](image)
their life and reduce growth rate as they age. Two studies (\textit{P. mexicanum} in fence lizards and \textit{P. agamae} and \textit{P. giganteum} in rainbow lizards) show no reduction in growth rate for infected lizards. Therefore, Fig. 14.5 suggests that infected lizards simply have an abbreviated lifespan and thus never grow to their largest possible size.

In summary, infected fence lizards suffer higher mortality in captivity, and infected lizards are more prone to attack by predators and other aggressive animals. There is a drop in prevalence for older lizards, and lizards are generally smaller (younger) at sites with a higher prevalence of malaria infection. These observations suggest that lizard malaria can cause an increase in host mortality. However, the most direct measure of mortality comes from mark–recapture studies on fence lizards in California, and these reveal no indication of an increase in mortality associated with infection (Bromwich and Schall, 1986; Eisen, 2001). That is, the duration for which a lizard was known to be alive did not differ for lizards infected or not infected with \textit{P. mexicanum}. The second study (Eisen, 2001) was most striking because it followed marked lizards over several warm seasons and is the most detailed such study ever done for a malaria parasite of non-humans. No similar studies have been done for the Caribbean or African systems, but infected anoles and rainbow lizards

![Graph showing body size vs. percentage infected](image)

**Fig. 14.5.** Body size (SVL, snout-to-vent length) vs. percentage of lizards infected with malaria parasites among sites for two study locations, California (fence lizards, \textit{Sceloporus occidentalis}, infected with \textit{Plasmodium mexicanum}) and Saba island (the endemic anole, \textit{Anolis sabanus}, infected with \textit{P. floridense}, \textit{P. azurophilum} and \textit{P. 'red'}). For each location, lizards grow larger at sites where malaria is less common. Malaria does not reduce body growth, so the two relationships suggest that lizards on the average live longer where they are less likely to be infected with the parasites.
brought into the laboratory suffer no increase in mortality compared with non-infected animals.

**Measuring reduction in host reproduction**

Malaria infection can disrupt the reproduction of both male and female lizards. The data are most complete for fence lizards infected with *P. mexicanum*; these data are highlighted here, but some comparative data for other species are also presented. Figure 14.6 shows results for fence lizards and rainbow lizards and reveals a significant reduction in fecundity for infected females amounting to one to two eggs for fence lizards infected with *P. mexicanum* and about four to five for rainbow lizards infected with both *P. agamae* and *P. giganteum*. This averages out to approximately 20% and 60% reduction in fecundity, respectively. No measure of an effect on female reproductive output for *Anolis* was possible. Anoles produce one egg per clutch, so only longitudinal studies of individual lizards will reveal any reduction in the number of reproductive periods per lifetime.

The origin of this substantial reduction in fitness may derive from the ability of the lizard to assimilate and store resources. Fence lizards store

![Graph showing clutch size of eggs produced by female fence lizards and rainbow lizards, comparing females infected with a malaria parasite with those not infected. Regression lines are fitted to the data. Clutch size increases with body size for both lizard species. Clutch size is significantly reduced for fence lizards infected with *Plasmodium mexicanum*. For rainbow lizards, clutch size is reduced for females infected with both *P. agamae* and *P. giganteum.*](image-url)
fat during the end of the warm season and then recycle this source of energy into eggs the next year. Infected fence lizards store less fat and the energetic loss is equal to one to two eggs, the deficit in observed fitness (Schall, 1982, 1983). Similar data are available only for the tropical rainbow lizard, which stores very little fat, so no effect can be detected for malaria infection (Schall, 1990). The loss in fat stored by fence lizards is not a result of infected lizards having reduced foraging success. The mass of faeces produced within 24 h after capture, an indication of foraging success, is not lower for infected fence lizards (Eisen and Schall, 1997).

A similar result emerges for the anole of Saba island infected with all three Caribbean *Plasmodium* species (Schall and Staats, 2002). Thus, the cost to fitness of malaria infection for fence lizards appears to result from the parasite consuming resources normally used to produce offspring.

Infected male fence lizards also suffer a fitness cost. They are less active socially (Schall and Sarni, 1987), are less able to hold a territory against non-infected conspecific males (Schall and Houle, 1992) and fare poorly in male–male interactions (Schall and Dearing, 1987). Infected males also produce less testosterone and higher levels of corticosterone, a ‘stress’ hormone (Dunlap and Schall, 1995), and have smaller testes (Schall, 1983). The sexually dimorphic ventral colour of infected male lizards is altered, which may allow females to determine infection status (Ressel and Schall, 1989). Male Saban *Anolis* appear to be less harmed by malaria infection, because there is no effect on male–male interactions or on body colour (Schall and Staats, 2002).

**Other consequences of infection for lizard hosts**

Infection with *Plasmodium* initiates a cascade of effects on lizard hosts, beginning with haematological changes, which drive physiological and behavioural alterations. The data are most complete for fence lizards and *P. mexicanum*, so these results are presented first (reviewed in detail in Schall, 1996). When red blood cells are consumed by the parasite, the number of immature erythrocytes increases in the blood. These cells house less haemoglobin than mature cells, so blood haemoglobin levels drop, sometimes by as much as 20%. As a consequence, the ability of the blood to deliver oxygen to tissues declines (maximal oxygen consumption is reduced). This translates into effects on locomotive performance. Sprint running in lizards is funded by anaerobic respiration and is not affected by infection, but aerobically sustained stamina running (measured as the distance the lizard can run) is reduced.

Immature red blood cells increase in the circulation and blood haemoglobin declines for infections of *P. floridense* and *P. ‘red’* in Caribbean anoles (Schall, 1992; Schall and Staats, 2002) and for infections of *P. agamae* and *P. giganteum* in African rainbow lizards, and running stamina is reduced for malarious rainbow lizards (Schall, 1990). Thus, similar physiological and behavioural changes are apparent for these
parasite–host systems. Blood haemoglobin levels do not drop for infections of *P. azurophilum*, as might be expected, because this parasite infects only white blood cells. However, white blood cells infected with *P. azurophilum* produce less acid phosphatase, an important enzyme in the functioning of these immune-system cells (Schall, 1992). This suggests that anoles infected with *P. azurophilum* may have reduced resistance to infection with other parasites if their immune system is compromised. Unfortunately, no data on this issue are available (indeed, no data on the impact of malaria infection on resistance to other parasites exist for any lizard host).

**Comparisons among *Plasmodium*-lizard systems**

Clearly, malaria parasites can harm their lizard hosts (Fig. 14.3). The consequences of infection are broad, including changes in hormone levels, haematology, physiology, running stamina, social and courtship behaviour, colour, fecundity and perhaps survival. But the consequences of infection differ among parasite and host species. *P. mexicanum* is particularly virulent for fence lizards, but the Caribbean lizard malaria parasites seem rather benign overall. For example, in a survey of the consequences of infection for the Saba island lizard, *Anolis sabanus*, few indications of harm were noted (Schall and Staats, 2002), and infected *Anolis gundlachi* on Puerto Rico do not suffer a reduction in body condition (mass vs. length) (Schall and Pearson, 2000). Thus, the species discussed here, with some additional data on *P. chiricahuae*, can be used to compare levels of virulence for malaria parasites and to test the hypotheses presented above on the evolution of virulence.

Table 14.1 compares the various measures of virulence for the six species of lizard malaria parasite. *P. mexicanum* is clearly the most virulent species, affecting every aspect of the physiology, behaviour and reproduction of infected fence lizards. Data on the consequences for mortality, however, are equivocal. *P. agamae* and *P. giganteum* also appear virulent for rainbow lizards, although the most severe harm depends on mixed infection of the two parasite species. The three Caribbean species also have effects on their hosts’ haematology, reducing haemoglobin (*P. floridense* and *P. ‘red’*) or altering the physiology of white blood cells (*P. azurophilum*), but studies on the costs of infection for the *Anolis* hosts detected no other consequences of infection. Thus, these three species may almost be benign.

Cross-species comparisons can be confounded if the phylogenetic relationships among those species are not understood (Harvey and Pagel, 1991). Two species of *Plasmodium* could have similar effects on their hosts simply because they are close sister taxa: that is, virulence may be a conservative trait, not subject to rapid alteration by differences in ecological conditions. To eliminate this potential source of error, a portion of the overall phylogeny for malaria parasites of Perkins and Schall (2002) is
Parasite Virulence

Table 14.1. Inventory of known costs to lizard hosts of infection by six species of malaria parasite (Plasmodium species). The various consequences of infection and the parasite–host systems are described in the text. ‘Age × prevalence’ is the relationship between body size (an indication of lizard age) and percentage of lizards infected at a site.

<table>
<thead>
<tr>
<th>Lizard host</th>
<th>Sceloporus</th>
<th>Agama</th>
<th>Agama</th>
<th>Anolis</th>
<th>Anolis</th>
<th>Anolis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitaemia per 10,000 RBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modal</td>
<td>&lt; 50–500</td>
<td>85</td>
<td>840</td>
<td>87</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>2500</td>
<td>119</td>
<td>2317</td>
<td>1130</td>
<td>2180</td>
<td></td>
</tr>
<tr>
<td>Mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In laboratory</td>
<td>+ 300–600%</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Injuries</td>
<td>+10–18%</td>
<td>Nil</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Age × prevalence</td>
<td>Negative</td>
<td>No effect</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Survival in field</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Behaviour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foraging success</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Male–male status</td>
<td>Reduced</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Percentage time social</td>
<td>Reduced</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Body colour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Showy trait</td>
<td>Altered</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Symmetry</td>
<td>Reduced</td>
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<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Haematology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Immature RBC</td>
<td>+ 365%</td>
<td>+ 750%</td>
<td>+ 908%</td>
<td>+ 142%</td>
<td>No effect</td>
<td>+ 148–268%</td>
</tr>
<tr>
<td>RBC density</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Haemoglobin in blood</td>
<td>−25%</td>
<td>−22%</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Acid phosphatase in WBC</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>Increased</td>
<td>No effect</td>
</tr>
<tr>
<td>Number WBC</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Physiology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose</td>
<td>−10%</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Blood testosterone</td>
<td>−36%</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Blood corticosterone</td>
<td>+ 225%</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Resting oxygen use</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Maximal oxygen use</td>
<td>−38%</td>
<td>−11%</td>
<td>−27%</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Spring speed</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Stamina</td>
<td>−20%</td>
<td>−15%</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
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<tr>
<td>Body temperature</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Body condition</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Reproduction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clutch size</td>
<td>−20%</td>
<td>No effect</td>
<td>No effect</td>
<td>Mixed infection</td>
<td>−60 to −75%</td>
<td>No effect</td>
</tr>
<tr>
<td>Testis mass</td>
<td>−37%</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Fat stored</td>
<td>−20 to 45%</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
</tbody>
</table>

RBC, red blood cells; WBC, white blood cells.
presented in Fig. 14.7, with the species of interest indicated. Examination of this tree reveals no pattern for the virulent vs. avirulent lizard malaria species. The most virulent, *P. mexicanum*, is a sister species of *P. chiriacaueae*. Recent studies by Foufopoulos (1999) on mountain spiny lizards (*Sceloporus jarrovi*) infected with *P. chiriacaueae* in Arizona reveals no effects of infection on male spiny lizards, but a reduction in body condition and clutch size of females. *P. floridense*, an avirulent species, is most closely related to the *P. chiriacaueae + P. mexicanum* pair. *P. agamae* and *P. giganteum* are sister taxa, as are *P. ‘red’* and *P. azurophilum*. These two pairs of species are more closely related than each is to *P. floridense* or *P. mexicanum*, and yet *P. ‘red’* and *P. azurophilum* are low-virulent species and *P. agamae* and *P. giganteum* are more virulent. Overall, the virulent parasites do not cluster, and neither do the avirulent species.

**Fig. 14.7.** Phylogeny for lizard and bird malaria parasites (extracted from a larger phylogeny recovered from cytochrome b sequences for 52 parasite taxa) (Perkins and Schall, 2002)). The outgroup for this tree are species of *Haemoproteus*, another group of malaria parasites, from lizards and birds. Species of *Plasmodium* isolated from bird hosts are indicated as ‘Bird Plasmodium’ on the tree. Other taxa on the tree are *Plasmodium* isolated from lizards. Species discussed in this review are indicated in bold.
The lack of any phylogenetic influence on virulence suggests that ecological reasons underlie the variation in virulence of the lizard malaria parasites. Therefore, I now revisit the hypotheses presented previously to determine if the present ecology of the lizard malaria parasites differs in ways that would predict variation in virulence.

**Evaluation of hypotheses**

**Host perspective**

No data are available on the cost to lizards of mounting an immune response to malaria infection. This is not unusual, as data on this issue are rare (Gemmill and Read, 1998). I have followed both captive and free-ranging animals infected with the *Plasmodium* species discussed here, as well as lizards infected with various haemogregarines, microfilarial worms and a trypanosome, and have rarely noted elimination of the parasite. The only exception was a virus that forms huge assembly pools within erythrocytes of *A. agama*. These infections were acute and were eliminated within a few weeks. This anecdotal evidence suggests that lizards do not mount a particularly effective immune response to protozoan blood parasites. This is surprising, because there is a substantial fitness cost (reduction in clutch size) associated with infection by *P. mexicanum* and mixed infections of the two African species.

**Transmission-opportunity hypothesis**

Comparisons among the lizard malaria systems do not give support for this hypothesis. *P. mexicanum* in fence lizards appears to be the most virulent of the seven species surveyed here and yet transmission is strongly seasonal. Overall prevalence of *P. mexicanum* at the California site is somewhat lower than for the tropical systems, suggesting that transmission is less intense. For the two tropical systems, vector biting activity may be more uniform in the Caribbean, where there is no clear dry season with reduced transmission. However, the three Caribbean lizard malaria species appear to be least harmful to their host.

**Mobility hypothesis and transmission-mode hypothesis**

These hypotheses should be irrelevant for comparisons among lizard malaria, as all are transmitted by blood-feeding Diptera.

**Host-demography hypothesis**

Mark–recapture programmes at all sites reveal that anoles, fence lizards and rainbow lizards can all live for several years. However, rainbow lizards are much larger animals and would be expected to have longer lifespans, and fence lizards suffer heavy winter mortality in California. This suggests that the virulence of malaria in fence lizards should be the
most severe and in rainbow lizards the least harmful. This is not the trend observed.

**Immunity hypothesis**
As noted above, lizards may mount a weak immune response to blood parasites. This should select for avirulent strains of parasite. *P. mexicanum* is the most virulent of the parasites studied, and there is no evidence that the immune response of fence lizards to *P. mexicanum* is stronger than that produced by the other lizards.

**Host-specificity hypothesis**
Surveys of common lizard species at each of the study sites revealed that each of the parasite species is highly host-specific. For example, at the Puerto Rico study area, five species of *Anolis* are common and yet only one, *A. gundlachi*, is a host for the three Caribbean lizard malaria parasites (Schall and Vogt, 1993). Thus, there is no variation in degree of host specificity for any of the parasites, so this hypothesis cannot explain the observed variation in virulence.

**Small worlds – diminishing-returns hypothesis**
Although data on vector behaviour for any lizard malaria parasite are scant (indeed, the vector is known with any certainty only for *P. mexicanum*), some intriguing data suggest that dispersal of the parasite is limited. First, high-prevalence vs. low-prevalence sites can be very local at the California and Saba study sites (Schall and Marghoob, 1995; Staats and Schall, 1996b). Sites only a few hundred metres apart can differ substantially in the proportion of lizards infected there, and this pattern can remain for many years. Secondly, ‘hot spots’ for malaria prevalence at the California site can be very small and local – just 100 m² (Eisen and Wright, 2001). If this indicates that dispersal of the parasite is low, this would suggest that selection should favour reduction in virulence. Again, the data do not support this prediction, because *P. mexicanum* in California is the most virulent parasite and the three species on Saba are the least harmful to their host.

**Clonal-diversity hypothesis**
No direct measures of clonal diversity of lizard malaria infections are available. Highly variable surface proteins known for *P. falciparum* and *Plasmodium vivax*, or variable microsatellite loci for *P. falciparum* allow such measures for human malaria, but similar variable loci have not been identified for any lizard malaria species. However, an indirect measure of clonal diversity is possible by determining the sex ratio of gametocytes in infections (Read *et al.*, 1992; Schall, 2000). Sex-ratio theory predicts that low clonal diversity, and thus low inbreeding of gametes within the vector, will select for a strongly female-biased gametocyte sex ratio. In contrast, high clonal diversity will lead to a 50:50 sex ratio. Data
on gametocyte sex ratio are available for only three of the lizard malaria parasites: *P. mexicanum*, *P. agamae* and *P. giganteum*. Of these, *P. mexicanum* has the lowest proportion of female gametocytes, and the proportion is much lower than those for human or bird malaria parasites that have been described (Read *et al.*, 1992). For *P. mexicanum*, the sex ratio is correlated with two potential measures of virulence: infection growth rate and final parasitaemia (Schall, 2000). Perhaps the transmission biology of *P. mexicanum* leads to high genetic diversity within infections and competition among those clones for resources and transmission, and hence the high virulence observed for this parasite. Other studies suggest that clonal diversity drives the virulence of infections within a species of malaria parasite (Taylor *et al.*, 1998; Pickering *et al.*, 2000), so variation in the way infections are established (leading to high vs. low clonal diversity) could drive differences in virulence among species.

**Prospects**

The good news is that the venerable ‘association-time’ hypothesis, which dominated parasitology for generations, has now been supplanted by a growing literature of sophisticated verbal and mathematical models on the evolution of virulence. The less satisfying news is that tests of the theories are relatively rare and, more often than not, cast doubt on the most discussed of the models. None the less, manipulative and comparative studies reveal that tests of the theory on parasite virulence provide some of the most intriguing findings in all of ecology and evolutionary biology (Ewald, 1983; Bull and Molineaux, 1991; Herre, 1993; Jaenike, 1996; Ebert and Mangin, 1997; Taylor *et al.*, 1998; Mackinnon and Read, 1999b; Messenger *et al.*, 1999). Elegant laboratory systems that allow careful manipulation of relevant factors and yet retain a close resemblance to natural parasite–host associations are particularly productive and desirable (Ebert and Mangin, 1997; Mackinnon and Read, 1999b; Messenger *et al.*, 1999). Also needed are careful tests of the assumptions that underlie each of the hypotheses, such as the relationship between transmission efficiency and cost to the host (Mackinnon and Read, 1999a).

This review ends with a plea. We need more data. Data on the actual costs of parasitism – costs for natural parasite–host systems – are notoriously scant (Dobson and Hudson, 1995). Obtaining such data is laborious and time-consuming and not particularly helpful to those wishing to increase their academic fitness. The relevant measures of virulence may not be obvious and they certainly differ among species (insects vs. vertebrates). Most of our data on parasite virulence come from human medicine (the best-known comparative studies centre on human pathogens (for example, Ewald, 1983, 1988, 1994)), simply because those are
the available data. Our perspective on parasite virulence may well be biased by this scarcity of comparative data. Thus, we need not just more data on costs of infection, but data on a greater variety of taxa, both of parasites and hosts. Some taxa are simply more charismatic and likely to draw the attention of researchers (*Plasmodium* among parasites and especially lizards among hosts!), but a broader view will certainly lead to exciting and unexpected findings on the evolution of parasite virulence.

### Acknowledgements

I thank those who shared their views and offered lively debate on parasite virulence. Their insights shaped this review: Lori Stevens, Chris Staats, Becky Eisen, Paul Ewald, Andy Read, Susan Perkins, Rob Fialho, Sarah Osgood, Mike Sukhdeo and Doug Gill. The research on lizard malaria was funded by numerous grants from the National Science Foundation (NSF), the National Institutes of Health (NIH), the National Geographic Society and the University of Vermont.

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