Virulence of lizard malaria: the evolutionary ecology of an ancient parasite–host association

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SUMMARY

The negative consequences of parasitic infection (virulence) were examined for two lizard malaria parasite–host associations: *Plasmodium agamae* and *P. giganteum*, parasites of the rainbow lizard, *Agama agama*, in Sierra Leone, West Africa; and *P. mexicanum* in the western fence lizard, *Sceloporus occidentalis*, in northern California. These malaria species vary greatly in their reproductive characteristics: *P. agamae* produces only 8 merozoites per schizont, *P. giganteum* yields over 100, and *P. mexicanum* an intermediate number. All three parasites appear to have had an ancient association with their host. In fence lizards, infection with malaria is associated with increased numbers of immature erythrocytes, decreased haemoglobin levels, decreased maximal oxygen consumption, and decreased running stamina. Not affected were numbers of erythrocytes, resting metabolic rate, and sprint running speed which is supported by anaerobic means in lizards. Infected male fence lizards had smaller testes, stored less fat in preparation for winter dormancy, were more often socially submissive and, unexpectedly, were more extravagantly coloured on the ventral surface (a sexually dimorphic trait) than non-infected males. Females also stored less fat and produced smaller clutches of eggs, a directly observed reduction in fitness. Infected fence lizards do not develop behavioural fevers. *P. mexicanum* appears to have broad thermal buffering abilities and thermal tolerance; the parasite's population growth was unaffected by experimental alterations in the lizard's body temperature. The data are less complete for *A. agama*, but infected lizards suffered similar haematological and physiological effects. Infected animals may be socially submissive because they appear to gather less insect prey, possibly a result of being forced into inferior territories. Infection does not reduce clutch size in rainbow lizards, but may lengthen the time between clutches. These results are compared with predictions emerging from several models of the evolution of parasite virulence. The lack of behavioural fevers in fence lizards may represent a physiological constraint by the lizards in evolving a thermal tolerance large enough to allow elimination of the parasite via fever. Such constraints may be important in determining the outcome of parasite–host coevolution. Some theory predicts low virulence in old parasite–host systems and higher virulence in parasites with greater reproductive output. However, in conflict with this argument, all three malarial species exhibited similar high costs to their hosts.

Key words: lizard malaria, parasite virulence, *Plasmodium*, lizard ecology.

INTRODUCTION

Some species of parasite severely traumatize their hosts, causing mortality or other important reductions in fitness, whereas others are essentially benign. Even closely related parasite species, or intraspecific strains of some parasites, can vary greatly in their effects on hosts (Alger *et al.* 1971; Landau & Boulard, 1978; Garnham, 1980). What proximate and evolutionary factors determine the overall negative effects, or virulence, of parasitic infection? This issue has long perplexed biologists, leading to 4 competing models that attempt to describe the evolution of parasite–host associations.

First, parasitological lore has long held that extremely virulent parasites would often kill their hosts, thus causing their own local extinction (Swellengrebel, 1940; Hoeprich, 1977; Burnet & White, 1972). For example, the distinguished malariologist S. R. Telford (1971) opined: 'Some parasites are indeed harmful to their hosts, yet these parasites cannot in any significant manner be harmful to the population, or else natural selection would not tolerate the perpetuation of such a disadvantageous relationship.' In fact, this view requires, not ordinary natural selection of individuals as envisioned by Darwin, but selection acting at the group level to produce 'prudent' parasites (Williams, 1966).

A less exuberant version of the group selection model proposes that selection acts on the mixture of parasite genotypes within an individual host to reduce virulence and favour transmission success to the next host (Lewontin, 1970; Gill & Mock, 1985). Parasites that kill their host before transmission is effected would obviously have an ultimate fitness of zero. This would be especially critical for parasites that must remain in an individual host for long periods when transmission is unlikely, such as in strongly seasonal environments (Gill & Mock, 1985).

Under the group selection model, the variation in virulence observed in nature would be a product of the age of the parasite–host association. Old parasite–host systems should demonstrate reduced virulence compared to more recently established relationships because group selection requires time to establish an optimal, low cost to the host.

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Second, the theory of individual selection suggests parasites may evolve towards increasing harm to the host if the parasite's virulence and reproductive output are positively correlated (Gill & Mock, 1985). Within an individual host, more rapidly growing parasite genotypes would prosper at the expense of slower growing strains and would eventually produce a greater share of propagules or transmission stages. For example, in malarial parasites (*Plasmodium* spp.), mutation among the billions of parasite cells within an individual vertebrate host would produce a very large number of genotypes, some of which would divide more rapidly or produce more merozoites per schizont cell than others (Alger *et al.* (1971) provide an example). These strains would presumably produce most of the gametocytes for transmission to the insect vector. The limit to these events would occur when some genotypes continue rapid asexual reproduction to the complete exclusion of any gametocyte production. These genotypes would not be transmitted and would die with the host. However, the difference in cell division rate between these 'asexual only' strains and those that produce at least some gametocytes might be slight, allowing the latter to survive and be passed to the insect host. The outcome of selection that favours rapid reproduction should therefore simultaneously favour the evolution of high costs to the host.

The individual selection model concludes that observed variation in parasite virulence is a product of the duration of the parasite–host association, but argues the older associations are more likely to show greater harm to the host.

Third, a contrasting view prevalent among evolutionary biologists holds that coevolution of parasite and host, via individual selection, finally leads to an equilibrium of costs and benefits for both host and parasite (Price, 1980). As an example, even a cursory examination of the medical literature demonstrates the culpability of the human immune system for much pathology associated with parasitic infection. Natural selection may not favour any additional 'improvement' in the immune system if the costs, such as increase in autoimmune disease, are more severe than the effects of the parasite itself. Likewise, the parasite's evolutionary path would be restricted by physiological and morphological constraints that would not be apparent without an intimate knowledge of the biology of the organism. The outcome of this coevolution of host and parasite would be either low or moderate cost of infection to the host.

Again, this model concludes that old parasite–host associations would often illustrate reduced parasite virulence compared to newly emergent systems because such an equilibrium requires many generations of selection acting on both parasite and host.

This tidy scenario is confounded by the asymmetrical rates at which selection can work on parasite and host. Parasites often have very short generation times and large reproductive outputs compared to their hosts and should therefore evolve at a faster pace, putting them at an evolutionary advantage (Price, 1980). For example, within a single vertebrate host of a malarial parasite there can be billions of parasites; typical mutation rates would yield ample variation for natural selection to operate very rapidly among the thousands of genetically distinct clones within the host.

The fourth perspective concludes that parasites in fact dominate their hosts in the coevolutionary race, producing adaptations that manipulate the relationship to maximize transmission success and fitness of the parasite (see Keymer & Read (1989) for some fascinating examples). Thus, virulence would vary depending on the ecology of transmission for each parasite species or life stage and is not related to the age of the host–parasite system. Ewald (1983, 1988), for example, argued that parasites should evolve low costs to their motile vectors, but in some cases be severely pathogenic in their large, less mobile vertebrate hosts.

A potentially very useful model for the study of parasite virulence is the large number of species of malarial parasites (genus *Plasmodium*). Approximately 171 described species of *Plasmodium* exploit a wide range of vertebrate hosts, including mammals, birds, snakes, and especially lizards (Table 1). The four species of malaria infecting humans vary considerably in their virulence (Bruce-Chwatt, 1985). Unfortunately, the consequences of natural malarial infection (i.e. in natural hosts in the wild) for other vertebrate hosts is poorly known, but available evidence suggests plasmodia of non-humans also exhibit interspecific differences in virulence (Coatney *et al.* 1971; Garnham, 1980; Landau & Boulard, 1978). Also, the reproductive outputs among species of *Plasmodium* may vary greatly (below).

I present here the results of a long-term study by my students and me on the effects of malaria on lizards. Some of the data and analysis given here represent a review of previously published results, but much new information is included. We were concerned to measure those consequences of malarial infection that would be of ecological and evolutionary importance for the lizards. That is, pathologies observable in the laboratory were of interest only if they could be related to the activities of the lizards in their natural condition. I attempt to relate haematological, physiological, behavioural, and reproductive data to show that there is a tractable cascade of effects of infection that leads from initial haematological upset to final reduction in the fitness of infected lizards. These results thus provide a rare glimpse into the role malarial parasites play in the ecology, behaviour, and evolution of their non-human vertebrate hosts.

The data will be used to examine three conclusions

that emerge from the theory of the evolution of virulence reviewed above. First, the coevolution model suggests that physiological constraints on both parasite and host may limit their ability to evolve quickly new adaptations to counter every adaptation of the other species. The example given here proposes that lizards do not develop fevers in response to infection with *Plasmodium* because the parasite has a much broader thermal tolerance than possible for iguanid or agamid lizards. Second, the argument that parasites should be avirulent in old host–parasite associations will be tested using two ancient lizard malaria systems. Third, the relationship between reproductive output and virulence will be assessed using malarial parasites with very different reproductive strategies.

Table 1. Number of described species of *Plasmodium* that exploit various taxa of vertebrates

(Source: numerous reviews and original species descriptions.)

Taxon	*N* species
Humans	4
Other primates	20
Lemurs	3
Rodents	11
Other mammals	9
Birds	42
Snakes	3
Lizards	79
Total	171

LIZARD MALARIA AS A MODEL FOR TESTS OF THE THEORY OF PARASITE VIRULENCE

Although lizard malaria has been known to biologists since 1909 (Aragao & Neiva, 1909; Wenyon, 1909), knowledge of these organisms and their effects on hosts is scant. The entire world literature on saurian malaria was catalogued in 1978, producing only 156 publications, mostly of a taxonomic nature or dealing with host and locality records (Ayala, 1978). This is odd, because approximately half the described species of *Plasmodium* are parasites of lizards (Table 1). Despite this paucity of data, some authors proposed that lizard malarias are relatively benign parasites (Russell *et al.* 1963; Garnham, 1980; Telford, 1971, 1972). Thus unsupported conclusion seems to have derived in part from the belief that old parasite–host systems should evolve towards decreasing virulence of the parasite.

Three aspects of the biology of lizard malarias make these species useful in testing ideas on the evolution of parasite virulence: (1) at least some *Plasmodium*–lizard associations must be ancient; (2) the potential reproductive output of malaria species in lizards varies greatly; and (3) pathogenesis of lizard malaria appears simple compared to that seen in mammals and birds. Each of these will be discussed now in turn.

Manwell (1955) argued that lizards may have been the original vertebrate host of malaria, with the parasite secondarily moving onto bird and mammal hosts. Some extant parasites, almost restricted to reptiles and amphibians, appear to presage the malarial life-history. *Schellackia occidentalis*, a parasite of fence lizards in western North America, is a haemogregarine-like gut parasite that casts its sporozoites into the lizard's blood where they pass to a mite, and are ultimately passed back to the vertebrate host if the lizard ingests an infected mite (Bonorris & Ball, 1955). No development of the parasite takes place in the mite. In haemogregarines of lizards, the parasite undergoes asexual replication in the liver, then passes gametocytes into the blood, where they are taken into the invertebrate host when it takes a blood meal. Finally, some other haemogregarines undergo asexual replication in lizard blood (Manwell, 1977; Telford, 1984). Thus, a complete series of related parasites of lizards makes a connexion from a gut-dwelling protozoan to the complex life-cycle of *Plasmodium*. Garnham (1980) concurred that lizard plasmodia may be the precursors of other malarial parasites.

Manwell's hypothesis is certainly arguable, but at least some species of lizard malaria appear to have been associated with their vertebrate hosts for very long periods of time. Ayala (1970) described the distribution of *P. mexicanum*-like parasites in fence lizards (*Sceloporus*) in western North America. Both parasites and hosts follow a patchy distribution geographically, tracing the now patchy Madro-Tertiary habitats that were once continuous across the region. *P. mexicanum*, for example, has been found in northern California, Wyoming, and south central Mexico. A similar disjunct distribution is seen in the rainbow lizard (*Agama agama*) of mesic tropical Africa and its malarial parasites *P. agamae* and *P. giganteum*. The rainbow lizard is restricted to wet forests and scrub, whereas the parasites are only found in wet zones (Schall & Bromwich, manuscript submitted). Thus, the parasites and their host range over an enormous region stretching the entire width of the African continent. In eastern Africa, though, the distribution of the lizard, and even more so the parasites, exhibit a patchy distribution reflecting the previously continuous nature of wetter habitats (Schall & Bromwich, manuscript submitted). The antiquity of the present range of mesic environments in east Africa is controversial, but seems to date from at least the glacial period in more northern latitudes (Moreau, 1963; Zinderen Bakker, 1978).

The life-history within vertebrate hosts varies substantially among malarial species. Indeed, the distinct symptoms of quartan and tertian malarial

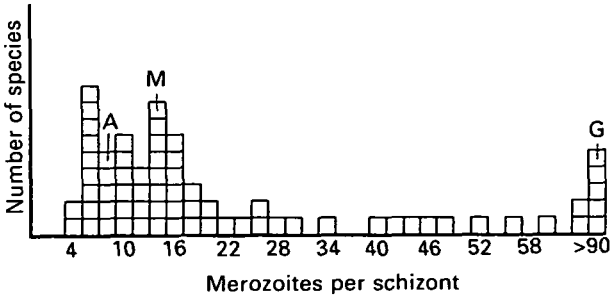

Fig. 1. Merozoites per schizont for various malaria species that infect lizards. The three species discussed in the text are indicated: 'A', *Plasmodium agamae*, 'M', *P. mexicanum* and 'G', *P. giganteum*. Source: numerous species descriptions.

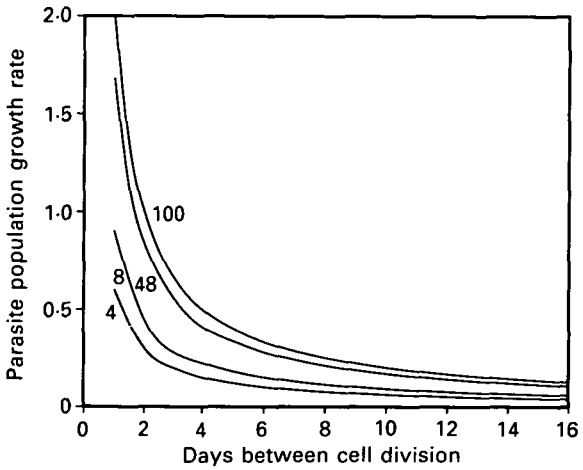

Fig. 2. Parasite population growth for species of malaria with differing reproductive characteristics. Compared are species with 4, 8, 48, and 100 merozoites per schizont with time between cell divisions ranging from 1 to 16 days. Population growth rate is given as the slope for relationship: log parasites/10000 RBC over time in days. The graph illustrates that growth rate for parasites with smaller reproductive output (fewer merozoites) can surpass that of a species with larger output if they have differing cell division periods, but this is true only if the species with fewer merozoites has a short time between cell divisions.

infections of humans are products of divergent reproductive characteristics of different *Plasmodium* species (Bruce-Chwatt, 1985). Fig. 1 illustrates one important kind of diversity in reproductive biology of malarial species, the considerable range in numbers of daughter cells per mother cell in the vertebrate host. Some species of lizard malaria produce only 4 merozoites per mature schizont, whereas others produce over 100. The adaptive significance (if any) and the ecological consequences of this variation in reproductive ecology are unknown. Variation in merozoite numbers has long been used as a systematic character; this trait, though, might not be a 'natural' taxonomic character and convergent evolution could have obscured systematic relationships of *Plasmodium* species.

The best interspecific comparison of the relation between reproductive potential and virulence in *Plasmodium* would be two species, with very different merozoite numbers, that infect the same vertebrate host. *P. agamae* and *P. giganteum* are appropriate; one produces 8 merozoites, the other at least 100, and both infect *A. agama* at many of the same locations throughout mesic Africa. These two species are indicated on Fig. 1. *P. agamae* is also a small parasite, that does not distort the shape of infected erythrocytes. In contrast, *P. giganteum* is truly a giant, the mature schizont filling the cell, pushing the erythrocyte nucleus aside and greatly altering the host cell's shape.

The malarias of humans vary in virulence, but merozoite numbers are similar among species and, except for *P. malariae*, they have the same rate of cell division. The differences in pathology induced by the four human malarial parasites reflect other aspects of their biologies (Bruce-Chwatt, 1985). Comparing virulence between *P. agamae* and *P. giganteum* seems more useful because of the great disparity of merozoite numbers and the similar origin of pathology (below).

Does the difference in potential reproductive output actually translate into greater parasite population growth for *P. giganteum*? If the duration between cell divisions is much greater for *P. giganteum*, it could well be the slower, rather than faster, growing species within a lizard host (Fig. 2). The duration between cell divisions in these two malarial species is unknown (schizogony appears asynchronous in most lizard malarias, so determining the timing of parasite cell maturation is prone to error (Telford, 1972)). However, in most cases, *P. giganteum* would have a higher rate of increase, but the difference would decrease with increasing dissimilarity in time between cell division (Fig. 2).

The nature of pathogenesis of malarial infection of birds and mammals differs among *Plasmodium* species, accounting for variation in severity of infections, and involves the class of blood cells invaded, generalized inflammatory response, alteration of erythrocyte morphology, and extreme water loss (Seed & Manwell, 1977; Maegraith & Fletcher, 1972; Dunn, 1969; Bruce-Chwatt, 1985). In contrast, malarial pathogenesis in lizards appears less complex, and the relationship between parasite population growth and ultimate parasite load and virulence is more clear (below). This is probably a result of the relatively slow and simple reptilian immune system (Sypek & Borysenko, 1986) that seems to lack the severe generalized inflammatory response of the higher vertebrates.

STUDY SYSTEMS

The California study site is the University of

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California Hopland Field Station, a tract of oak woodland lying in the Mendocino valley approximately 160 km north of San Francisco. My students and I have studied lizard malaria there since 1978, but the site was earlier investigated by Ayala (1970). Western fence lizards, *Sceloporus occidentalis*, are common there, usually seen perching on fallen logs, tree stumps, rocks, and fence posts. They are small (adult males are 60–75 mm, and adult females 60–85 mm snout to vent length), insectivorous lizards that are active from late April to late September. Fence lizards are relatively easy to capture using a slip noose on the end of a fishing pole. They can be readily maintained in captivity in aquaria and large pens provided full-spectrum lights are used and a variety of insect prey is supplied as food.

Fence lizards at Hopland are frequently infected with *P. mexicanum*, a parasite first described from Mexican *Sceloporus* by Thompson & Huff (1944). A description of the vertebrate stages is given by Ayala (1970), and of the invertebrate forms by Klein *et al.* (1988). Prevalence of malarial infection varies around the 3600 ha property. Malaria is absent from higher elevations where chaparral vegetation replaces the woodland, but also is absent from some more wooded regions of the station. At sites where malaria is present, 25–50 % of adults will be infected; this level of infection has remained quite constant over the past 12 years and is similar to that seen by Ayala (1970) over 20 years ago. Fence lizards at Hopland are also often infected with *Schellackia occidentalis* and rarely by an undescribed trypanosome.

The invertebrate hosts of *P. mexicanum* appear to be two species of phlebotomine flies (psychodidae), *Lutzomyia vexator* and *L. stewarti*. Both of these flies live in the burrows of the California ground squirrel, *Citellus beecheyi*, emerging in early evening to take a blood meal from lizards and other ectothermal vertebrates (Chaniotis & Anderson (1968), and personal observations). Flies fed on infected lizards show oocysts on their midgut from six to eight days later when kept at 26 °C (Klein *et al.* 1988, and personal observation). A description of the ecology of these flies in nature and transmission success in the laboratory will be published elsewhere.

We studied the malarial parasites of the African rainbow lizard at 23 sites in Sierra Leone, West Africa. The natural habitat of Sierra Leone is tropical rainforest and swamp, but much of this has been removed to favour farming. *A. agama* is common on houses, tree trunks along the edges of fields and roads, and large boulders at some sites. These are fairly large (adults range from 77 to 138 mm snout to vent length), aggressive, and wary lizards that are difficult to catch. We collected rainbow lizards usually by knocking them from walls with a long pole after a frantic chase. For some studies, lizards were shot with a 0·22 airgun.

A. agama is exploited by a diverse community of blood parasites. We observed approximately 5 species of haemogregarines, 2 microfilarial worms, a virus that forms enormous assembly pools visible under the light microscope (Stehbens & Johnston, 1966), as well as *P. agamae* and *P. giganteum*. *P. agamae* was the first saurian malaria described (Wenyon, 1909), initially from the southern Sudan. *P. giganteum* was found somewhat later in West Africa (Theiler, 1930). Both species have a wide distribution (Schall & Bromwich, manuscript submitted); *P. giganteum* seems less common, but this could simply reflect inadequate collection efforts. The vertebrate stages are shown in Theiler (1930); the invertebrate stages are unknown, although Petit *et al.* (1983) studied oocyst formation in *Culicoides nubeculosus*.

Both *P. agamae* and *P. giganteum* were found at all surveyed sites, but the percentage of lizards infected with either species varied from 8 to 90 %, and the relative proportions of the two parasites also varied among sites. No clear association of habitat type, elevation, or abundance of the lizard with parasite prevalence was apparent (Schall & Bromwich, manuscript submitted).

The methods used in these studies are presented in detail in the papers cited for each section of the Results. For some kinds of data gathered (for example, the haematological and physiological studies) standard methods were used and will not be repeated here. For less familiar techniques, a brief summary is presented in the Results sections below. Readers seeking details can consult the cited publications. In comparisons between infected and non-infected lizards, animals infected with any parasite other than malaria were excluded.

CONSEQUENCES OF MALARIAL INFECTIONS FOR LIZARDS

Haematology

Available data on percentage immature red blood cells (%iRBC), number of red blood cells/mm^3, haematocrits, and haemoglobin concentration are presented in Table 2. Immature erythrocytes are readily apparent in lizards because of their more rounded shape, relatively larger nucleus, and more blue cytoplasm when stained with Giemsa at pH 7–7·2. Note that these cells are not referred to here as reticulocytes because most apparently mature lizard erythrocytes retain considerable reticulum in the cytoplasm (Maizels, 1980). Immature erythrocytes are rare in the blood of non-infected lizards, only about 1–2 %, but appear to increase when the animal is infected with *Plasmodium* (Table 2). Curiously, %iRBC and *Plasmodium* parasitaemia are not correlated in *Sceloporus* (Schall, 1983*a*) and only weakly correlated in *Agama* ($r_s = 0{\cdot}51$ for *P. giganteum*

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Table 2. Haematological measurements for two species of lizards that are parasitized by three *Plasmodium*

(Results for infected and non-infected animals are compared. Given are means, S.D., sample size, and results of Mann–Whitney *U*-tests comparing infected and non-infected samples for each lizard species and parasite.)

	iRBC (%)	RBC/mm³ blood (×10³)	Hematocrit (%)	Haemoglobin (g/100 ml blood)
Sceloporus occidentalis and *Plasmodium mexicanum*				
Non-infected	2·6	843·4	32·3	7·3
♂♂	(4·1)	(251·4)	(5·1)	(1·4)
	25	15	21	22
Infected	9·5	972·1	33·4	5·5
♂♂	(7·3)	(245·1)	(6·5)	(1·3)
	68	12	17	27
	$P < 0·001$	$P > 0·05$	$P > 0·25$	$P > 0·001$
Agama agama				
Non-infected	0·534	—	♂♂ 35·4	11·66
	(0·719)		(0·726)	(3·77)
	73		246	153
			♀♀ 33·6	11·81
			(0·721)	(4·69)
			330	60
Agama agama and *P. agamae*				
Infected	4·01	—	♂♂ 34·1	9·13
	(4·81)		(8·17)	(2·81)
	35		97	26
			♀♀ 31·5	7·23
			(0·709)	(1·09)
			42	7
	$P < 0·001$		$P > 0·05$ (both)	$P < 0·01$ (both)
Agama agama and *P. giganteum*	4·85	—	♂♂ 35·7	11·47
	(4·63)		(0·951)	(2·07)
	18		26	3
			♀♀ 31·8	6·75
			(0·670)	—
			9	1
	$P < 0·001$		$P > 0·05$ (both)	—
Agama agama and mixed infection	5·91	—	♂♂ 34·4	8·96
	(8·99)		(0·656)	(2·65)
	51		50	31
			♀♀ 33·7	6·47
			(0·707)	(2·34)
			13	10
	$P < 0·001$		$P > 0·05$ (both)	$P < 0·01$ (both)

infections and 0·45 for *P. agamae* infections; $N = 163$). In mammals and birds a significant fraction of the anaemia associated with malarial infection seems to result from the host's immune system destroying both infected and non-infected RBC; anaemia occurs even in very early, weak infections (Weiss, 1983; Nussenzweig, Cochrane & Lustig, 1978; Zuckerman, Spira & Ron, 1973). In lizard malaria, weak and moderate-level infections are characterized by approximately the same level of iRBC. This is important for further considerations because the level of infection can usually be ignored when comparing infected and non-infected lizards.

The number of blood cells is similar for infected and non-infected lizards (haematocrits and direct counts of cells, Table 2). Blood haemoglobin concentration, though, is significantly reduced for all species examined. This must be a result of immature erythrocytes containing less haemoglobin than mature cells (Schall, 1983*a*). Correlations between % iRBC and haemoglobin concentration are significant for all 3 malaria species studied (Spearman correlations, $P < 0·05$). It is interesting to note from Table 3 that the degree of reduction in haemoglobin concentration is similar for all malarial species as well as for mixed infections in the two African species. This reduction is about 20–25 %.

The increase in immature erythrocytes appears to be typical for lizard malaria. Scorza (1971) reported such anaemia in *Tropidurus torquatus* infected with

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Table 3. Oxygen consumption while at rest and during maximal activity for *Sceloporus occidentalis* (California fence lizards) and *Agama agama* (African rainbow lizard)

(Adult male animals infected with malaria and those not infected are compared. Given in the table are means, S.D., sample sizes, and results of Mann–Whitney *U*-tests for resting oxygen consumption and two measures of maximal oxygen consumption, labelled here Max1 and Max2 (described in text). Also given for *S. occidentalis* is aerobic scope, the increment between resting and maximal oxygen consumption. All measures are in ml/g·h corrected to STP conditions. Rainbow lizards infected only with *P. giganteum* were fairly rare, so small sample sizes for this group precluded statistical comparisons for some measures.)

	Resting	Max1	Max2	Scope
Sceloporus occidentalis and *P. mexicanum*				
Not infected	0·54	1·53	1·02	1·00
	(0·131)	(0·351)	(0·291)	(0·336)
	15	14	8	14
Infected	0·59	1·30	0·62	0·71
	(0·105)	(0·252)	(0·249)	(0·247)
	14	14	5	14
	$P > 0·10$	$P < 0·05$	$P < 0·05$	$P < 0·01$
Agama agama				
Not infected	0·20	—	0·93	0·62
	(0·077)		(0·312)	(0·281)
	12		52	12
Infected with *P. agamae*	0·24	—	0·83	0·51
	(0·111)		(0·292)	(0·236)
	9		28	9
	$P > 0·10$		$P < 0·05$	$P < 0·05$
Infected with *P. giganteum*	0·245	—	0·68	0·73
	(0·021)		(0·239)	(0·302)
	3		11	3
Mixed infection	0·23	—	0·74	0·51
	(0·086)		(0·228)	(0·163)
	7		21	7
	$P > 0·10$		$P < 0·05$	$P < 0·05$

P. tropiduri, and I have seen deteriorated blood pictures in *P. azurophilum* in *Anolis gingivinus* on St Maarten island in the eastern Caribbean, and unidentified *Plasmodium* species in *Ameiva ameiva* in Brazil and *Kentropyx* sp. in Suriname.

Oxygen consumption

As might be expected, the decrement in haemoglobin concentration associated with malarial infection appears to cause a reduction in the ability of the lizards to deliver oxygen to tissues. Maximal oxygen consumption was measured in two ways. The small lizards (*S. occidentalis*) were forced to run continuously, or until exhausted, while in a small glass chamber; small electrical shocks provided the stimulus to run (= 'Max1' in Table 3). However, the larger lizard (*A. agama*) would not run within a chamber, so these animals were forced to maximal running for 30 s in an oval track in an incubator set at their normal body temperature, then the lizards were placed within a closed glass chamber for 2 min. Oxygen consumption during this 2 min period was used to gain an insight into maximal oxygen consumption levels (= 'Max2' in Table 3). For uniform comparison, a similar procedure was performed on a sample of fence lizards.

Maximal oxygen consumption was reduced for infected animals (Table 3; Schall, Bennett & Putnam, 1982). Note that the decrement in maximum oxygen consumption associated with malarial infection was similar for all *Plasmodium* studied and was about the same level as observed for reduction in haemoglobin concentration (approximately 20–25%). Fig. 3 plots Max1 for *S. occidentalis* against blood haemoglobin content and suggests that the reduction in oxygen consumption is primarily a result of reduction in haemoglobin levels because data for both infected and non-infected animals fall on the same regression line. Thus, complicating factors, such as alteration in other aspects of blood chemistry, may not be important.

Resting oxygen consumption was not measurably different for infected and non-infected lizards.

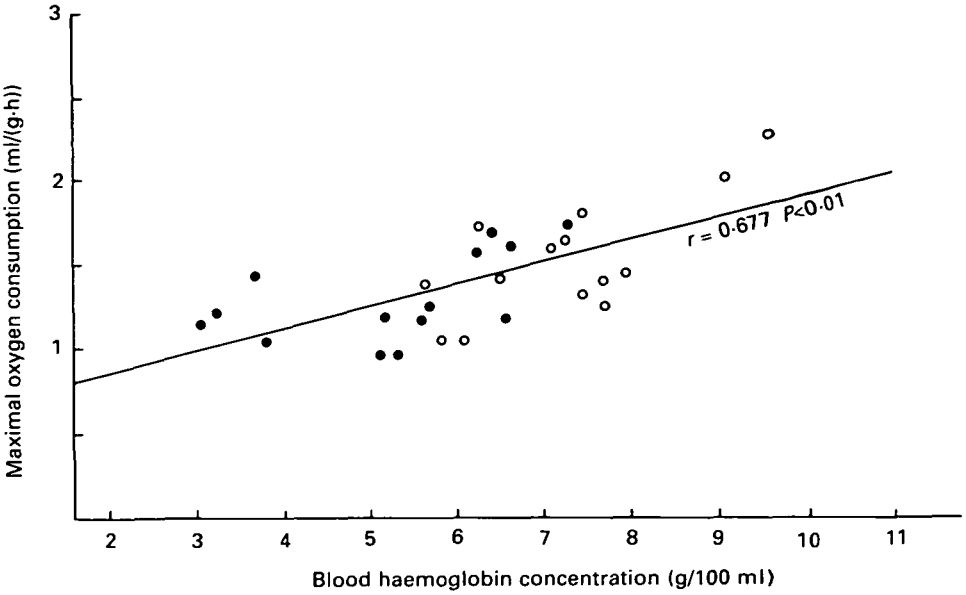

Fig. 3. Relationship between blood haemoglobin concentration and maximal oxygen consumption for *Sceloporus occidentalis*, comparing animals infected (●) and not infected (○) with *Plasmodium mexicanum*. Illustration shows that both sets of data fall on the same regression line with infected animals tending toward lower maximal oxygen consumption and blood haemoglobin levels. (From Schall *et al.* (1982).)

Resting metabolic levels for lizards are very low, perhaps only 10% of a mammal of similar mass at the same temperature (Bennett, 1983), so the lack of effect of malaria on resting oxygen consumption in lizards is not surprising.

Stamina and running speed

The initial seconds of running by a lizard are anaerobically maintained (Bennett, 1983). Longer movement can be maintained aerobically only at very low velocities. Long sprints, though, are driven by a combination of aerobic and anaerobic means and most lizards reach exhaustion after only 1–2 min of activity (Bennett, 1983). Table 4 shows the effect of infection on locomotive performance of *Sceloporus* and *Agama*. Sprint speed, measured in a 2 m track fitted with electronically controlled timers (Schall *et al.* 1982), was not affected in fence lizards. Infection, though, appears to reduce the ability of both fence lizards and rainbow lizards to maintain longer runs. In a 3 m oval track, placed in a walk-in incubator set at the normal body temperature of the lizards, infected animals ran shorter distances when continually chased than non-infected lizards. Once again, the effect appears similar for all malarial species studied and for mixed infections of the two African species.

The ecological consequences of these results depend on how often the lizards need to make long sprints. In a study of the behavioural time budget of *Sceloporus* at Hopland, very little difference was seen between infected and non-infected lizards (Schall &

Table 4. Sprint running speed and running stamina compared for lizards infected and not infected with malarial parasites

(Sprint speed was measured in a 2 m track and reported as velocity in m/s. Running stamina is given as distance run in 30 s in an oval track. The results of Mann–Whitney *U*-tests are also given.)

	Sprint speed	Stamina
Sceloporus occidentalis and *P. mexicanum*		
Not infected	1·44	21·3
	(0·38)	(5·82)
	15	15
Infected	1·28	17·0
	(0·41)	(3·68)
	15	14
	$P = 0·10$	$P < 0·01$
Agama agama		
Not infected	—	27·8
		(7·24)
		58
Infected with *P. agamae*	—	23·6
		(5·98)
		25
		$P < 0·05$
Infected with *P. giganteum*	—	26·3
		(6·29)
		9
		$P > 0·05$
Mixed infection	—	21·9
		(5·4)
		20
		$P < 0·05$

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Table 5. Percentage of lizards with broken or regenerated tails, an index of predation pressure, comparing animals infected and not infected with malarial parasites

(Male lizards typically suffer more tail breaks than females, so data are examined by sex. Sample sizes are given in parentheses. There was no significant difference in proportion of lizards with injured tails for malarious and non-infected animals for either sex (χ^2 tests; all $P > 0{\cdot}05$).)

	Males (%)	Females (%)
Sceloporus occidentalis		
Infected	49	36
Non-infected	42	33
N	731	628
Agama agama		
Infected	15	15
Non-infected	17	10
N	1239	967

Sarni, 1987). In 600 runs seen and measured, none could have lasted over 4 s, and most lasted only 0·5 s or less, well within the range of anaerobically maintained short runs. The mean number of short runs was only 6/h of observation. Recovery from such short bursts of running in the laboratory required additional oxygen consumption, but the duration of recovery only lasted about 1 min/s of running. Thus, if infection increased recovery time by about 25% (an estimate based on results on haemoglobin deficit in infected animals), infection should have no ecologically important effects on ability to flee from predators or sprint to catch food. Indeed, for both fence lizards in California and for rainbow lizards in Africa, there is no difference between infected and non-infected lizards in frequency of broken or injured tails, an index of predator attacks (Table 5).

Social behaviour and ability of males to obtain mates

One important difference in behaviour between malarious and non-infected lizards was observed during the study of time budgets. Infected fence lizards, both male and female, were socially active less often than non-infected lizards (Schall & Sarni, 1987). This was especially apparent in the adult males (Fig. 4). Social interactions can last for as long as 8 min in fence lizards; for males these events demand intense activity, including bobbing, body shaking, chases, and circular struts around females or competing males. Intense social activity must require active mobilization of oxygen to muscular tissues and would be expected to suffer because of malarial infection.

These results suggested that male fence lizards,

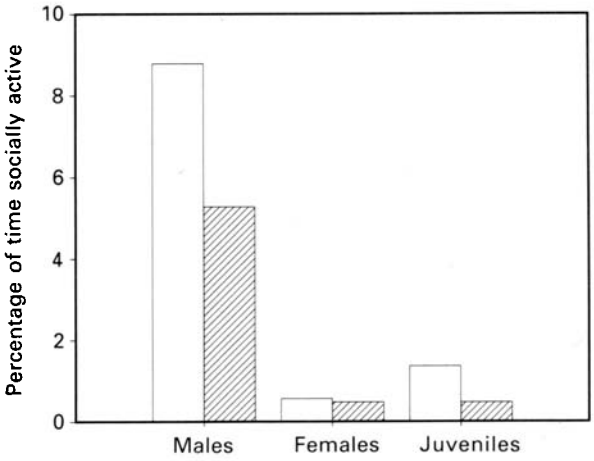

Fig. 4. Percentage of time spent in social activities for western fence lizards, *Sceloporus occidentalis*, comparing those infected (▨) and not infected (□) with a malarial parasite.

when infected with *Plasmodium*, would be less able to compete with other males for access to females because they would more easily reach exhaustion during intense social interactions. We tested this proposal in two ways. Numerous lizards were captured, a blood smear made from each, and a number painted on their backs with a non-toxic paint. After the lizards were returned to the site of capture, they were observed over a 6-week period to determine site fidelity, home range, and dominance status (Schall & Houle, manuscript submitted). The observers were not aware of the infection status of the lizards until the end of the observation period. Infected animals were sighted less often and had smaller home ranges than non-infected lizards. For 35 males that were sighted often enough to judge social status (males were always obviously socially dominant or submissive based on the outcome of aggressive encounters with other males), all 17 rated dominant were non-infected, whereas 11 rated submissive were infected and 7 were non-infected (*G*-test, $P < 0{\cdot}01$).

In a second kind of observation, we placed pairs of males, matched by size and body colour, but one infected and the other not infected with *P. mexicanum*, into large (4·9 × 4·9 m) enclosures (Schall & Dearing, 1987). One or two females were also introduced into the enclosures which usually provoked at least one of the male lizards to active interaction with both the other male and the female. Again, the observer did not know the infection status of the lizards, but was able in most of the 17 trials to determine the socially dominant male. There was a strong association of several measures of dominance with infection status. For example, the time spent displaying to the female and to the other male was significantly higher for non-infected males. In 9 of 10 cases in which one male was judged strongly dominant, that male was non-infected. In this

experiment, parasitaemia and outcome in the trials were correlated: the higher the parasitaemia, the more submissive was the lizard.

The pen experiments also suggested that it is not exhaustion during social interactions that cause infected lizards to be socially submissive. Infected lizards in the enclosures did not display to the other male until exhaustion, but instead quickly abandoned any social interaction and submitted to frequent chases and even attacks. In two cases when only the infected male was present with the female, he readily courted her, but quickly resumed his submissive role when a non-infected male was introduced into the pen. This argues that infected animals are able to assess their ability to succeed in intense social interactions, and simply abandon efforts to display toward a non-infected animal.

Unfortunately, I have no data on social interaction for *Agama agama* to compare infected and non-infected males. However, weak circumstantial evidence may indicate that infected rainbow lizards also suffer reduced social status. The mass of food in the stomachs of those lizards shot during the study was measured and corrected for body mass (food mass/body mass). Infected lizards had less food in their stomachs than non-infected animals (infected = 2·66% of body mass; non-infected = 3·24%; Mann–Whitney U-test, $P < 0{\cdot}001$). This 18% reduction in food in the stomachs of infected animals probably does not result from a reduction in their ability to capture prey. Dashes for insects last only a few seconds at most, which is supported by anaerobic means (above). More likely, infected animals are pushed to poorer quality territories where food is less abundant.

Sexual selection

Hamilton & Zuk (1982) provoked considerable discussion with their hypothesis that parasites may alter the appearance of sexually dimorphic traits in their hosts, thus allowing females of the host species to monitor the parasite loads of their prospective mates. That is, parasitized animals will appear scrofulous or less showy and thus indicate their infection status. This hypothesis has broad importance because it addresses general issues in sexual selection theory, but is relevant here because it suggests that infection may reduce fitness by making the host less attractive to potential mates.

The H–Z hypothesis has been tested by comparing typical parasite loads of species with showy males versus related species with bland males (Hamilton & Zuk, 1982; Read, 1987; Ward, 1988; Zuk, 1990). In each case, showy species were exploited by a greater variety of parasites than dull species. A second way to test the hypothesis is with intraspecific comparisons: are parasitized males less showy than non-infected males and do females use this information in selecting mates?

The first intraspecific comparison was by Schall (1986) who studied the effect of a haemogregarine parasite of the Aruba island whiptail lizard, *Cnemidophorus arubensis*. Male dorsal colour varies considerably in this species from bright blue to dull brown. Infected lizards were actually more brightly coloured on the average than non-infected animals, thus running counter to the prediction of Hamilton & Zuk (1982).

We examined this issue with the fence lizard–malaria system in California (Ressel & Schall, 1989). Male fence lizards are brown on the dorsal surface (very cryptic on logs and tree trunks), but bright blue, black and yellow on the ventral surface. During courtship, or male–male conflicts, males will raise their body in a stiff-legged 'push up' and expose the ventral colours by puffing the throat and compressing the body laterally. Juvenile males lack much pigment on the ventral surface, but the colour increases with age. However, there is enormous variation in degree of colour on the venter of adult males. Some are copiously endowed with black ringing bright blue patches, whereas others are almost juvenile in their appearance.

We reasoned that the California system was suitable for an intraspecific test of the Hamilton–Zuk hypothesis because there is a sexually dimorphic trait that varies among adult males (ventral colour), this trait is important in courtship, the lizards are frequently infected with a debilitating parasite (malaria), and the parasite alters lipid metabolism (below) and lipids are an important component of melanophores. Also, infection alters testis growth, perhaps altering hormone levels that might control ontogenetic colour change (Ressel & Schall, 1989).

In our study, the venters of 827 males were photographed under standard conditions and the area of each colour measured by projecting the photographic images onto a digitizing image analysis system. Also, the pattern, or arrangement, of colours on the ventral surface varied for animals with the same proportions of colours. We therefore made colour photographs of 35 pattern classes, then captured an additional 500 adult males the next season and classified the animals by pattern.

Our results showed that infected males exhibited different ventral colours than non-infected animals, correcting for body size, an index of age. However, the infected lizards were actually more showy than the non-infected animals. That is, they were darker and exhibited the most pronounced sexually dimorphic trait. Perhaps this is simply a product of infected males growing slower, resulting in the infected animals in any body size class being older and therefore more showy. We tested this idea by determining the effect of infection on growth rate of

the lizards, via a mark-recapture programme. The results of this study agreed with my earlier finding (Schall, 1983*a*) that malarial infection does not affect growth in these lizards, thus eliminating the possibility that differences in colour between malarious and non-infected males were a product of differences in the average age of the two groups of lizards.

We concluded that infection with *P. mexicanum* does alter the sexually dimorphic trait of the fence lizards, but by making them more, not less, showy. Coupled with the result from my earlier study on the Aruban whiptail, this outcome suggests an alternative hypothesis to that of Hamilton & Zuk (1982). Perhaps the brighter patterns in infected lizards are a signal to females that these males are superior potential mates because they have been able to tolerate infection with one or more parasites (the handicap principle of Zahavi (1975)).

Agama agama males are also brightly coloured (hence their common name of rainbow lizard). We found these lizards very difficult to observe under natural conditions, and so have no comparable information on how malaria might alter their behaviour or colour patterns.

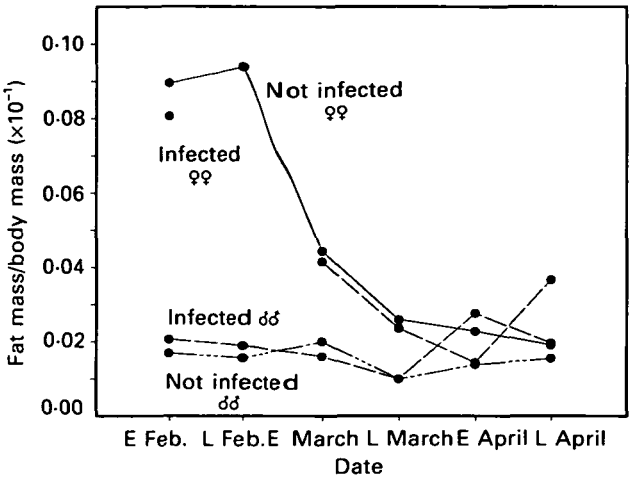

Fig. 5. Mean fat mass discounted for body mass in *Agama agama*, comparing males (lower sets of data) and females (upper sets), infected or not infected with malarial parasites (*P. agamae* and/or *P. giganteum*). Very little fat is stored by these lizards, but there is a seasonal cycle in females.

Reproductive output

Fecundity is an important component of fitness, and one of the easiest to measure for females. Number of offspring produced each reproductive period for males, however, is very difficult to determine. To understand the analysis of effects of malarial infection on reproductive output of lizards, a brief comparison of the reproductive cycle of temperate and tropical lizards is necessary.

S. occidentalis, is fairly typical for temperate zone lizards in having a brief reproductive period during the spring and early summer. Most female fence lizards at Hopland produce only a single clutch of eggs each season; a small proportion may produce two. Clutch size increases as females grow. After the breeding season, both males and females continue to feed and store considerable lipid in the form of fat tissue that lies in the posterior portion of the body cavity. This fat presumably supports the animals during winter dormancy and in reproduction the next spring. In studies on other lizards, females recycled stored fat into production of eggs in the spring; females with fat experimentally removed in autumn were unable to produce a clutch of eggs the next year (Hahn & Tinkle, 1965). Male fence lizards display a seasonal pattern in size of the testes; they are largest in early spring, decrease throughout the reproductive season, then increase again in late summer and early autumn (Schall, 1983*a*).

In tropical lizards, including *A. agama*, the animals are reproductively active for longer periods. Gravid rainbow lizards were collected in every month of our study, in both wet and dry seasons. Testis size is relatively constant in tropical compared to temperate lizards. In *S. occidentalis* testis size varied 25-fold during the season, but in *A. agama*, there was much less than even a doubling from smallest to largest size.

In California, testis size of infected male *S. occidentalis* was significantly smaller compared to non-infected animals in late summer for 2 years sampled, with a reduction of about 37% of mass (Schall, 1983*a*). The reproductive consequences of this reduction are not known but it is reasonable to guess that larger testes may produce more sperm or hormones during the reproductive season. In contrast, testis mass of *A. agama* was not affected by malarial infection. As testis mass did not vary much over the year, data are pooled to compare infected ($\bar{x}$ = 0·60% of body mass, N = 145) and non-infected ($\bar{x}$ = 0·60%, N = 134) (U-test; $P > 0{\cdot}05$) lizards.

By late summer, female *S. occidentalis* store more fat than do males, so data must be analysed by sex. For both males and females, infected lizards store less fat than non-infected animals (Schall, 1983*a*, *b*). To place this result into biological perspective, the deficit in fat stored by infected females is equal to the calorific value of 1–2 eggs (Schall, 1983*b*). Female *A. agama* also store more fat than males and there is a weak seasonal trend in fat stored (Fig. 5). However, the amount of fat stored is very small, at most only 1% of body mass. There was no significant difference in amount of fat stored for infected and non-infected rainbow lizards (Fig. 8; U-tests for each date, $P > 0{\cdot}05$).

The reproductive result of the differing influence of malaria on lipid cycling in the two lizard species is

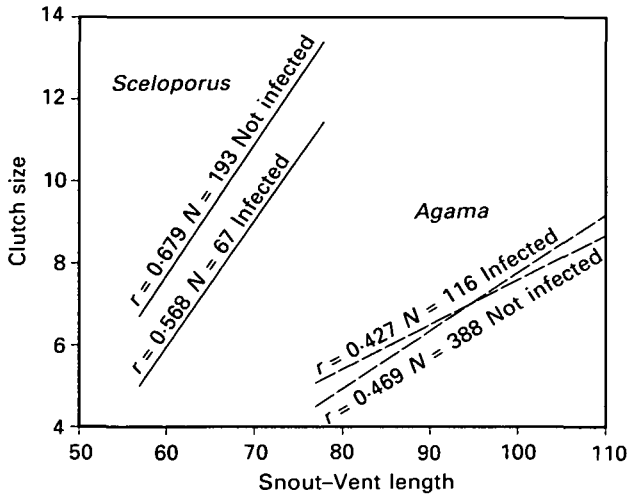

Fig. 6. Clutch-size – body size regressions for two species of lizards, *Sceloporus occidentalis* and *Agama agama*. Compared also are animals infected with malaria and not infected. The regression lines differ significantly for *Sceloporus* (analysis of covariance, $P < 0{\cdot}05$), but not for *Agama*.

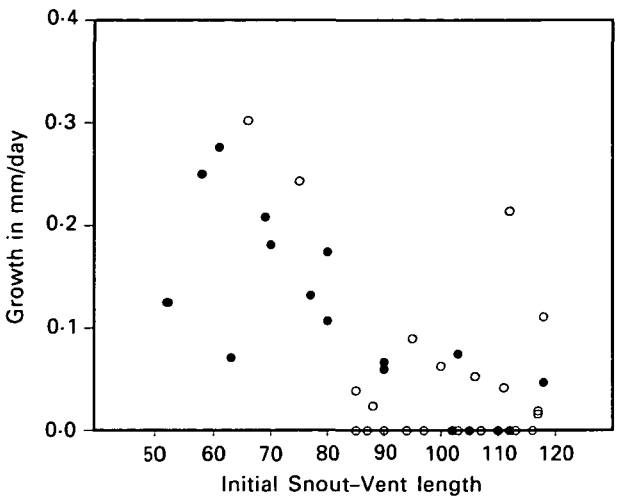

Fig. 7. Growth rate of *Agama agama* infected (○) and not infected (●) with *Plasmodium agamae* and/or *P. giganteum*. Growth rate declines with body size for these lizards, but there is no significant difference between infected and non-infected animals (analysis of covariance; $P > 0{\cdot}05$).

apparent in Fig. 6. Infection confers a reduction in clutch size in *S. occidentalis* of about 1–2 eggs, the reduction predicted by the fat body data presented above. This represents a reduction in fecundity of about 20% for a lizard of 70 mm snout to vent length. Clutch size of female *A. agama*, in contrast, does not seem to be affected by malarial infection (Fig. 6). Note also on Fig. 6 that fence lizards produce a much larger number of eggs than the African species. This emphasizes that temperate lizards generally store fat and produce 1 or 2 large clutches each year, whereas tropical species do not store fat and often produce many smaller clutches throughout the year (for example, Schall (1983*c*)). Thus, malarial infection may not alter the number of eggs produced in each clutch for *A. agama*, but probably increases the time needed between clutches.

Several measures of egg quality were also compared between infected and non-infected lizards. These included egg mass, percentage of eggs hatching, size of young at hatching, and time taken to hatch (the latter three measures determined in laboratory-reared eggs). None of these measures differed for infected and non-infected lizards (Schall (1983*a*) for *S. occidentalis* and data on egg mass only for *A. agama*).

Survival and growth

In the laboratory, infected *S. occidentalis* suffer increased mortality (a 6-fold increase for males, and about a 3-fold increase for females) (Schall, 1983*a*). Assessing the effect of infection on the survival of wild fence lizards, however, proved difficult. In one study (Schall & Houle, manuscript submitted), infected male lizards tended to remain at a site for shorter periods of time, although the difference was only marginally significant (*U*-test, $P = 0{\cdot}075$). We interpreted this to mean that infected males were driven from their home sites more often by non-infected competitors. However, increased mortality could also account for the missing lizards. In a second, more detailed mark and recapture study (Bromwich & Schall, 1986), the duration between first and last capture was almost identical for infected (57·8 days) and non-infected (58·9 days) lizards. This result suggests mortality during the activity season is not higher in malarious lizards.

Growth rate should be important for survival in lizards, so growth was measured in infected and non-infected *S. occidentalis* during two mark and recapture studies (Schall, 1983*a*; Ressel & Schall, 1989). Similar data are presented for *A. agama* in Fig. 7. In all cases, malaria did not seem to hinder the growth of infected lizards.

Body temperature

One of the characteristic features of malarial infection of humans is the onset of periodic fevers. Most accounts view malarial fevers as a pathological result leading from the release of some toxin when infected erythrocytes rupture (i.e. Garnham, 1980). This mirrors the consensus view that fever represents a disruption of normal physiological processes by pathogens or other sources of disease. However, a venerable alternative explanation, recently argued again by Kluger (1979) and Boorstein & Ewald (1987), holds that fever is actually an adaptation that acts to disable at least some kinds of pathogens.

Kluger (1987) used a lizard model to examine the

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biology of fever, and found that the desert iguana (*Dipsosaurus dorsalis*) developed a behavioural fever in response to infection with *Aeromonas hydrophila*. Infected lizards allowed to regulate their own body temperature in a thermal gradient sought out the warmer areas of the gradient and raised their body temperature by about 4 °C. Lizards proved excellent models for studies on fever because their body temperature can be readily controlled by placing the animals in constant temperature rooms. Kluger (1987) showed that when animals were forced to maintain their normal daytime body temperature, infection proved fatal. The fever body temperature, however, quickly eliminated the infection. Also, infected animals treated with sodium salicylate did not develop behavioural fevers, but died from the infection. Similar results have been reported from other ectotherms, including insects, that have been challenged with various pathogens (see Boorstein & Ewald (1987)).

These results suggested to me that lizards might develop behavioural fevers in response to malarial infection and this fever might hinder the parasite's population growth. To test this idea, I compared the body temperature of infected and non-infected lizards in two ways. In the field, cloacal temperature of collected lizards was measured using a rapid-reading thermometer within seconds after capture (*Sceloporus*) or after the lizard was shot (*Agama*). If the delay in taking the temperature was longer than a few seconds, or if the lizard was badly damaged by the shot, the measurement was discarded. For an introduction into these methods in the study of lizard body temperature, see Schall (1977). Body temperatures were also recorded in laboratory thermal gradients. In these cases, a thermistor probe was inserted into the cloaca, taped into place on the tail, and the lizard released into a $1{\cdot}83 \times 5{\cdot}82$ m thermal gradient, heated at one end by heat lamps (substrate temperature > 50 °C) and cooled at the other by subsurface copper tubes containing running water at 8 °C. Two very similar pens were used with a single infected lizard placed into one pen, and a single non-infected animal into the other. Body temperature was then read for both animals at regular intervals from a blind.

Body temperature distributions for infected and non-infected *S. occidentalis* and *A. agama* are shown in Fig. 8. Means did not differ for either species (*U*-tests, $P > 0{\cdot}05$), nor did the distributions (χ^2 goodness-of-fit tests, $P > 0{\cdot}05$). Likewise, when the data for *Agama* were separated by species of parasite, or mixed infection, versus non-infected, no difference in body temperature was observed (Kruskal–Wallis test, $P > 0{\cdot}05$). To determine if behavioural fevers are found only in the most severe infections, I searched the data for lizards with massive infections, then compared those with the non-infected sample. Again, body temperatures were not distinguishable

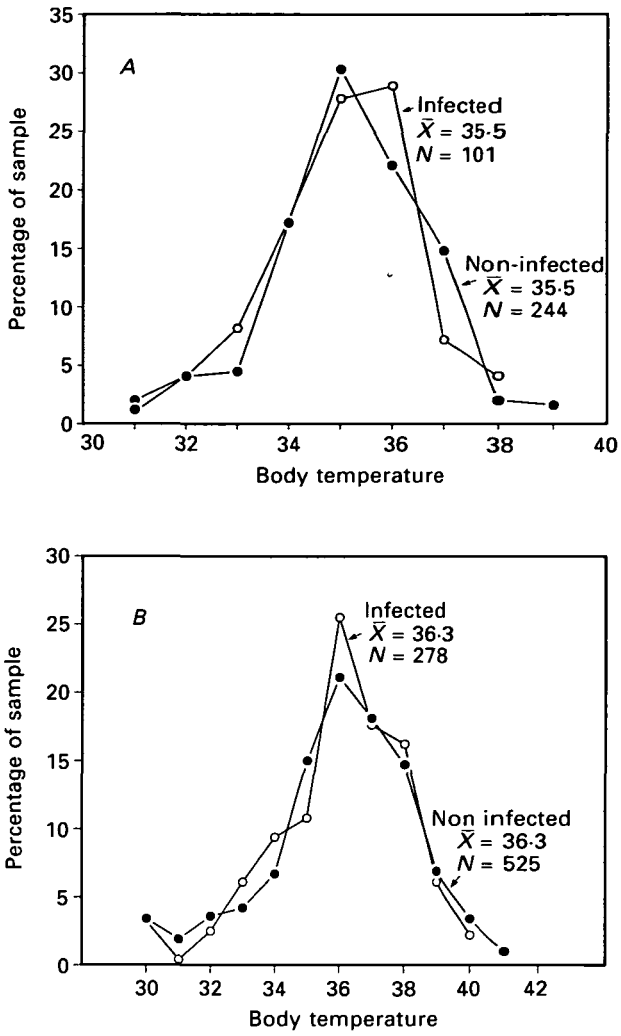

Fig. 8. Body temperature distributions for free-ranging lizards, comparing those infected (○) and not infected (●) with malarial parasites. (A) *Sceloporus occidentalis*; (B) *Agama agama*.

(*U*-test, $P > 0{\cdot}05$). Infected and non-infected fence lizards did not choose perching locations with different thermal characteristics. For example, in one 2-day period I measured the air temperature at the perching location of 41 infected and 53 non-infected lizards; the means were similar (25·2 for infected, 24·9 non-infected, *U*-test, $P > 0{\cdot}05$).

In the laboratory, 6 experiments were run with fence lizards, in each case an infected animal with high parasitaemia was used. Number of temperature readings varied for each experiment from 13 to 62, taken over a 2-day period. Fence lizards in the pens readily moved about, basking in the heat spot, then moving to cooler regions of the pen. They also readily ate crickets when provided. In all 6 experiments, body temperature for infected and non-infected animals did not differ (*U*-tests, all $P > 0{\cdot}05$). *Agama* did not prove suitable for thermal gradient studies because in all but one experiment one or both lizards removed the thermistor probe or broke the trailing wire. In the single successful run,

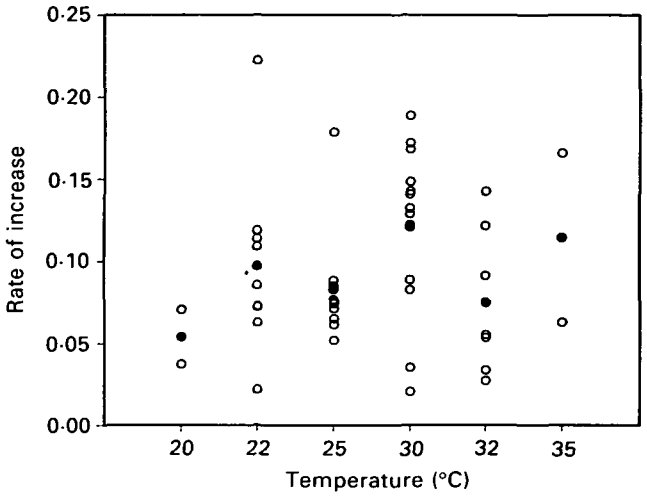

Fig. 9. Growth rate of parasite population of *Plasmodium mexicanum* in western fence lizards kept at various constant temperatures. Each point represents a single experiment; (●) means for each temperature. Rate of increase is given as the slope of the relationship: log parasites/10000 RBC over time in days.

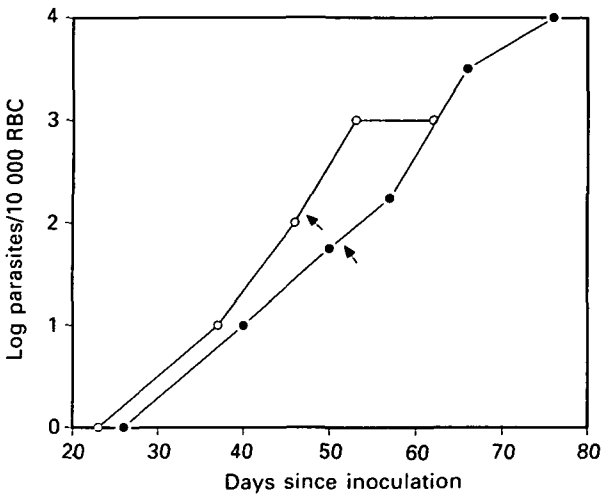

Fig. 10. Two experiments in which a western fence lizard was experimentally infected with *Plasmodium mexicanum* and kept at 22 °C initially, then changed to 32 °C (indicated by arrow). Parasite density expressed as log parasites/10000 RBC is plotted against time since the animal was infected with malaria via blood inoculation.

a rainbow lizard with a massive (about 30% of cells infected) mixed infection showed the same body temperature as the non-infected animal (sample sizes were 149 temperatures for the infected animal, and 179 for the non-infected animal, *U*-test, $P > 0{\cdot}05$).

I conclude that these two species of lizards do not alter their body temperature in response to malarial infection. I next sought to determine the effect of differing temperatures on lizard malarial parasites. Data on the influence of temperature on the vertebrate stages of malarial parasites are meagre. Thompson & Winder (1947) demonstrated that the population growth rate of *P. floridense* in *Anolis carolinensis* was altered by placing the lizards in incubators set at various temperatures: higher temperatures resulted in faster parasite growth. They did not attempt to determine the upper thermal tolerance of the parasite. Caldwell (1944) examined the thermal biology of *P. cathemerium* by removing infected blood from canaries and incubating the blood at various temperatures before inoculating the blood into non-infected birds. These experiments revealed that *P. cathemerium* has a remarkable thermal tolerance, surviving when the blood was raised to temperatures as high as 47 °C for 30 min.

S. occidentalis can be readily infected with *P. mexicanum* by blood transfer. I removed blood from naturally infected lizards by inserting a heparinized capillary tube into the post-orbital sinus. This blood was mixed with an equal volume of heparinized physiological saline and 10 μl injected i.p. into non-infected adult male lizards. These animals were then placed in plastic aquaria fitted out with sand and rocks and installed into constant temperature boxes lit for 12 h each day. Lizards in incubators were fed daily with crickets. Every 5 to 30 days blood samples were taken from a tiny nick to a toe of each animal. Growth rate of the parasite population was determined by taking the slope of the best fit regression line of log parasites/10000 RBC by days since inoculation from the day when parasites were first seen in the blood.

Parasite population growth rate is compared for various temperatures in Fig. 9. Growth rate varied substantially among experiments for each temperature, perhaps reflecting the overall health of the lizard, its immune state, or the genetic nature of the parasite population. No effect of temperature was apparent from these experiments (Kruskal–Wallis test, $P > 0{\cdot}05$). Between 20 and 35 °C the parasite did not differ in its population growth rate. Two other experiments are summarized in Fig. 10. Two animals were kept first at 22 °C, then moved to 32 °C. As seen on the figure, there appears to have been no alteration in the population growth rate.

In another series of experiments, high temperature shocks were administered to the parasite by warming lizards in incubators set at 39–41 °C. Body temperature of the lizards was monitored with a thermistor probe inserted into the cloaca. These thermal shocks lasted from 1 to 3 hours. The results of these experiments are shown in Fig. 11. In each case the parasite not only survived these high temperatures, but seemed to continue growing at a fairly constant rate. I estimate the lizard's thermal maximum to be about 42 °C, because lizards at that temperature appeared to be in extreme discomfort. In one experiment the incubator malfunctioned, quickly driving the lizard's body temperature to 45 °C, and killing the animal. I raised the incubator's temperature to 50 °C, kept the lizard at that

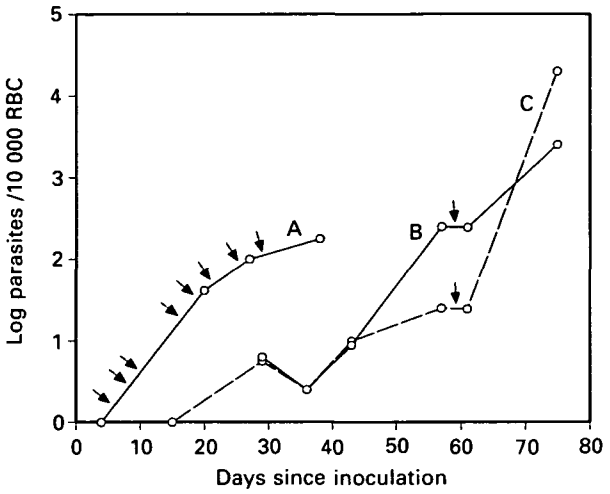

Fig. 11. Three experiments in which western fence lizards were experimentally infected with *Plasmodium mexicanum*, then kept at a constant temperature except when given heat shocks for 3 h (indicated with arrows). A, kept at 35 °C, heat shocks of 39 °C; B, kept at 25 °C, heat shocks twice at 41 °C; C, kept at 25 °C, heat shocks twice at 40 °C. Data are presented in the same form as in Fig. 10.

temperature for 3 h, then removed blood from the dead animal and injected it into several non-infected lizards. All of these became infected with normal-looking parasites.

These observations and experiments demonstrate that fence lizards do not develop behavioural fevers in response to malarial infection, and *P. mexicanum* has broad thermal buffering abilities as well as a broad tolerance. I suggest these are not independent phenomena. The evolution of such a broad thermal tolerance by *P. mexicanum* may represent an unbeatable evolutionary move when its host may be phylogenetically bound to a low body temperature (Pianka, 1988). Unfortunately, *A. agama* proved refractory to infection by blood inoculation, so complementary experiments with that species were not possible.

DISCUSSION

The results presented here demonstrate that malaria has severe consequences for its lizard hosts. Virulence appears similar for species with very different reproductive strategies (*P. giganteum* vs. *P. agamae*) and has remained high for ancient parasite–host associations (all three malaria species examined). Is this result typical for lizard malarial parasites, or did I happen to choose those with unusually great negative effects on the vertebrate hosts? Unfortunately, comparative data are scant, but are reviewed here.

Scorza (1971) presented evidence that *P. tropiduri* causes haematological upset in *Tropidurus torquatus* similar to that observed in our studies. Seven of 11 lizards naturally infected showed reduced haemoglobin but little reduction in RBC numbers. In 4 experimentally infected lizards, haemoglobin concentration decreased and immature RBC increased over time. Telford (1972) found that *P. sasai* produced anaemia (= increased numbers of immature RBC) in *Takydromus tachydromoides*, but concluded, 'I doubt that *Plasmodium sasai* can be considered pathogenic to its host. No other effect upon hosts was observed.' However, other possible effects do not seem to have been sought in this study. Rand, Guerrero & Andrews (1983) studied *P. tropiduri* in *Anolis limifrons* in Panama and concluded that this parasite is benign. Although this is possible, I find their data questionable. Reproductive output was measured in lizards maintained in captivity and fed to satiation each day. Under such conditions lizards typically display unusually large reproductive effort (Schall, 1978 and other personal observations), so the effects of infection may have been obscured. Also, sample sizes for some data are small and the methods used may have been incorrect (blood haemoglobin level, for example).

Are the data presented here relevant to a discussion of the evolution of parasite virulence? Arguments that parasites should nurture their hosts to assure successful transmission to new hosts are concerned only with a low rate of mortality in infected individuals. Reduction in other aspects of host fitness (reduction in fecundity or ability to attract mates) are irrelevant to this argument. An extreme example is host castration that may increase the host's survival, but obviously reduces its fitness. Some group selection arguments (such as quoted in the Introduction section) centre on the perpetuation of the host population, so all components of the host's fitness must be examined. Coevolution models are also concerned with overall host fitness because it is only reduction in fitness that would have evolutionary consequences.

I have attempted to gather data on a broad span of effects of malaria on lizards that would be relevant to all of the virulence models. The data are clearly more complete for *S. occidentalis* and its parasite than for *A. agama*. However, the haematological and physiological consequences of infection for the two lizards are so similar that this suggests that *A. agama* may suffer the same battery of effects as the fence lizard. These effects will now be summarized and related to the models on parasite virulence.

There is little evidence that malaria increases mortality in lizards. The increased death rate in laboratory maintained *S. occidentalis* suggests that mortality may increase in stressed wild infected animals, especially during winter dormancy when mortality in fence lizards is high (Ruth, 1977). However, the populations of *S. occidentalis* and *A. agama* are both very dense despite the high prevalence of malaria. The population consequences for

lizards of malarial infection thus appear absent or slight. If the parasites were suddenly eliminated from my study sites, the lizard populations may increase, but would probably be reduced again by competition for food, perching sites, or other resources. Thus, both group selection models would view lizard malaria as a non-virulent parasite that is well adapted to its host.

The individual selection model is not upheld by the data on lizard malaria. The two African species, with potentially very different reproductive levels, are not measurably different in their effects on *A. agama*. Also, lizard malaria, despite its ancient origin, is not deadly for its hosts.

The fitness of individual lizards appears substantially reduced by malarial infection. In *S. occidentalis*, infected males are less able to defend territories or compete for mates, and have smaller testes. Their ventral colour pattern is altered and they store less fat during the late summer. Female fence lizards also store less fat and have smaller clutches of eggs, the best direct evidence that infection reduces individual fitness. Although clutch size of *A. agama* is not reduced by malarial infection, I believe that the time between clutches is expanded, thus reducing yearly egg production. The simple coevolution model predicts reduction in a parasite's cost to its host's fitness over evolutionary time, but this ignores the poorly understood role of biological constraints that limit evolutionary changes (Gould, 1989). The remaining cost to the fitness of *S. occidentalis* of malarial infection may indicate the evolutionary compromise between a parasite and its host. The much broader thermal tolerance of *P. mexicanum* than its host could represent an example of the constraints experienced by hosts as they coevolve with their parasites.

A long series of students, technicians, and other helpers were vital in both the field and laboratory portions of this research. Carl Bromwich spent a total of 6 months in Sierra Leone gathering the lion's share of the data on *Agama*, on several occasions risking his life to obtain samples, and displaying a rare degree of enthusiasm and resourcefulness in both laboratory and field studies. He is now a physician, but I will always regard him as a 'lizard man'. Others who assisted greatly were R. W. Schall, S. Ressel, D. Dearing, L. Soucy, L. Harvie, T. Simbo, D. Sama, M. Kailie, A. Johnson and A. Bennett. We were hosted at the Hopland Field Station by A. H. Murphy, and in Sierra Leone at the Njala University College by P. White. The research was funded by grants from the National Geographic Society, and the United States NSF (BSR-8306925, 8516627, and 8806235).

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