Malarial Parasites of Lizards: Diversity and Ecology

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1. INTRODUCTION

In 1902 Ronald Ross received the Nobel Prize for his discovery of the basic life cycle of malarial parasites. Only 22 years before, Laveran had made his epochal identification of the parasite in human blood. Ross was able to unravel the life cycle of *Plasmodium* because he used bird malaria in his studies; bird malaria and its hosts, both vertebrate and insect, were readily available and could be maintained in the laboratory. Early malariologists quickly realized the value of plasmodia of non-humans as models in their research and therefore conducted world-wide surveys of a wide variety of potential hosts to search for other malaria species. Malaria in lizards was first reported from Africa by Wenyon and from South America by Aragão and Neiva, both in 1909. Except for species descriptions and distribution notes, lizard malaria was almost ignored by researchers until Thompson (1946a,b) attempted to use these forms in examining the efficacy of antimalarial drugs. The slow response of lizard infections to antimalarials showed that these hosts were not suitable for continued study on drug therapy. However, Thompson and Winder (1947) went on to demonstrate that lizard malaria provided a unique opportunity to study the effect of temperature on the life stages of *Plasmodium* in its vertebrate host. Lizards are ectotherms and their body temperature can be conveniently manipulated in constant temperature boxes without the major physiological disruption expected when the thermal set point is altered in endothermic birds and mammals. This interesting work unfortunately lay fallow for many years.

Since then, a small number of researchers have demonstrated that lizard malaria is a remarkable and productive system for the study of the ecology and evolution of malarial parasites. Jose Scorza studied the pathology of malaria in lizards and showed that malaria–vertebrate associations that are presumed very ancient can remain highly virulent; this view was in contrast to the then standard parasitological wisdom. Sam R. Telford Jr tirelessly surveyed many lizard populations. Telford's work revealed the great systematic and ecological diversity of lizard *Plasmodium* species that are available for study. Three decades of these systematic studies are summarized in Telford (1988a). Three distinguished general parasitologists conducted intensive, lengthy searches for lizard blood parasites in Pará, Brazil: R. Lainson, J. Shaw and I. Landau. They have shown that the life cycle of malarial parasites is much more diverse than previously suspected. Helen Jordan conducted long-term studies of the prevalence of malaria in populations of lizards in south-eastern USA and collected some of the best such data on any non-human malaria host in the wild. Stephen Ayala first discovered the vectors of a lizard malaria (*Plasmodium mexicanum* in

California, USA). The discovery was a surprise because it is psychodid flies, and not mosquitoes, that are the vectors of this parasite. Ayala also showed that lizard malaria could be used in a great variety of ecological studies; indeed, his work anticipated much recent work on parasite evolutionary ecology. Ayala provided two excellent summaries of the literature on saurian malaria (Ayala, 1977, 1978). The world literature at that time consisted of only 156 publications on 54 species.

The pioneering work by these scholars showed that lizard malaria provides a superior, and perhaps even the best, system to study the evolution and ecology of malarial parasites of non-humans. There are a large number of known species (77 by 1995) and probably many more are left to be described. The known species occur in the temperate, subtropical and tropical regions in North, Central and South America, Africa, South-East Asia, Japan and Australia. The vertebrate hosts include most of the more diverse lizard families. Vectors of one species, *P. mexicanum* in northern California, are fairly well known and can be cultured in the laboratory for manipulative experiments. Lizards are very common in some habitats, easy to observe, capture, and maintain in captivity, and they can be permanently marked and then released back to their original homesite to be repeatedly recaptured. These characteristics allow studies of the course of malarial infection under natural conditions. The blood stages of the parasite are fairly easy to identify; for example, gametocytes are very obvious in many species and cannot be confused with other stages.

Since 1978 my students and I have conducted detailed studies of the ecology of lizard malaria at several sites (at the California site where Ayala once worked, West Africa and the Caribbean islands). I review these studies here. Most of the data and analyses have been published, but considerable new material is included. I also include a review of the findings of other authors. The original goal of our studies was specialized: to cast light on the biology of the plasmodia of lizards. However, early in the work we recognized, as did Jordan and Ayala before us, that lizard malaria is an excellent system for investigating important and timely issues in parasite–host evolutionary ecology, including the evolution of virulence, effect of parasites on sexual selection in the hosts, parasite manipulation of host behavior and parasite-mediated competition.

2. DIVERSITY AND DISTRIBUTION

Fully 77 species of malarial parasite have been described from lizard hosts. Figure 1 shows that most of these have been described since 1960, and almost half by a single worker, S.R. Telford. Telford's careful and precise

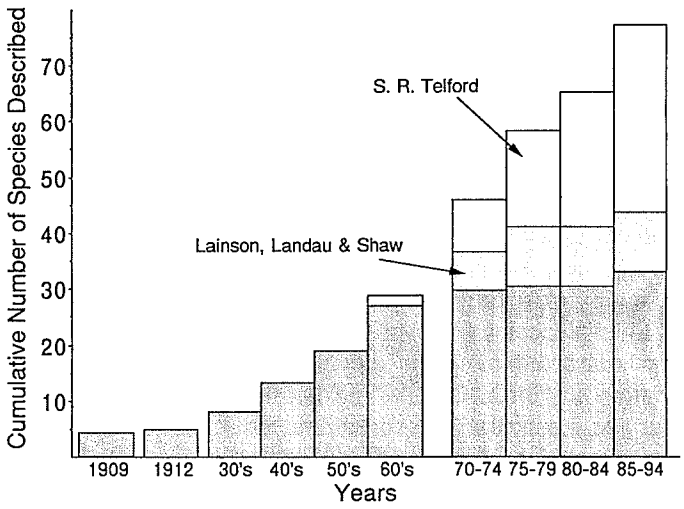


Figure 1 Number of species of *Plasmodium* described from lizard hosts from 1909 to 1994. Modern interest in the diversity and distribution of lizard malaria began with S.R. Telfords's studies in the 1960s. The number of species described by Telford and the group of Lainson, Landau and Shaw working in Brazil is indicated.

descriptions can serve as models for future authors, and parasitologists can be grateful for his conservative methods that detail the variation in parasite morphology that can exist within an individual *Plasmodium* species found among host individuals, populations and species. For example, the Asian species, *P. sasai*, was studied from four populations ranging from Japan to Thailand and in three species of *Takydromus* lizards (Telford, 1982). Various morphological traits of *P. sasai* were found to vary as much within populations as among samples from different sites. This illustrates the importance of studying sufficient material to appreciate the variation in morphology of these parasites before proposing new taxa. Of all the species described by Telford, only one, *P. diminutivum*, was eventually referred to a previously described species (by Telford himself).

The major difficulty with any description of species of *Plasmodium* is the lack of invariant, clear characters. For example, the maximum number of merozoites (daughter cells) produced by a schizont (mother cell) varies enormously among species (Figure 2) and this character has been used both in describing species as well as to define subgenera or species groups (Garnham, 1966). Merozoite number can vary in the same *Plasmodium* species among populations of lizards, and even within infections over time. Figure 3 (drawn from data in Jordan, (1975)) shows the difference in

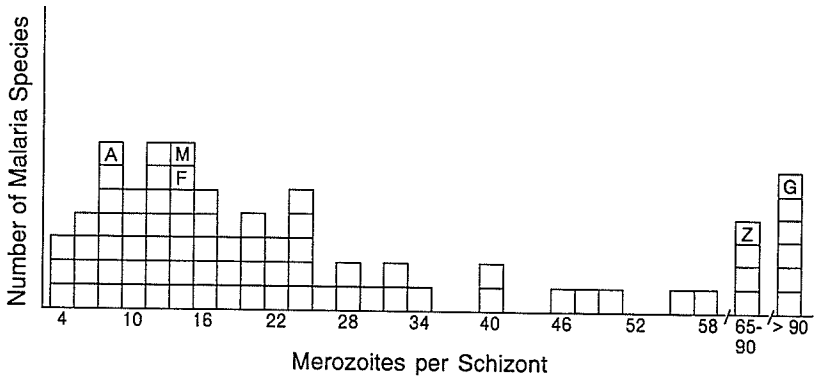


Figure 2 Number of merozoites produced by a schizont for the 77 described species of lizard malaria. Those species studied by the author are indicated: A = *Plasmodium agamae*, M = *P. mexicanum*, F = *P. floridense*, Z = *P. azurophilum*, G = *P. giganteum*. Note the great range in reproductive phenotypes seen among the species.

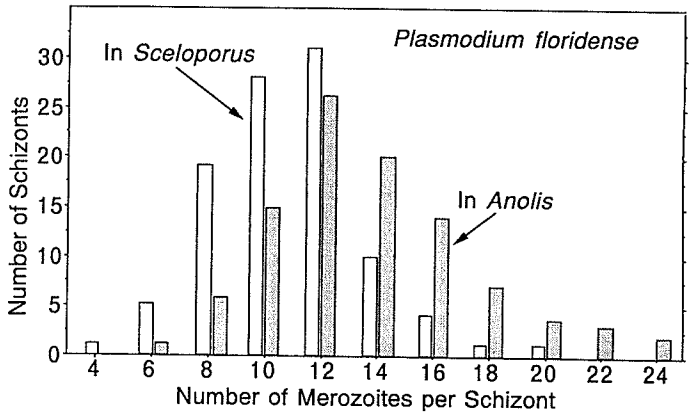


Figure 3 Distribution of the number of merozoites produced by a schizont of *P. floridense* when in two hosts, the eastern fence lizard, *Sceloporus undulatus*, and the anole lizard, *Anolis carolinesis*. Data from Jordan (1975).

number of merozoites produced by schizonts of *P. floridense* in two species of iguanid lizards, *Sceloporus undulatus* and *Anolis carolinensis*. The reproductive phenotype is clearly shifted in the *Anolis* host to favor a larger production of daughter cells. This shift could simply be a non-adaptive change in phenotype driven by differing environments in the blood of the two species of lizards; or, Jordan could have observed an adaptive adjustment by the parasite to the different hosts. Ayala and Spain (1976) showed changing merozoite numbers in *P. colombiense* over the

course of the infection, with smaller number of daughter cells produced by schizonts in chronic infections when compared to early or overwhelming infections.

Even if merozoite number were constant within each species, the phylogenetic significance, if any, of this character would be questionable. Malaria species with large numbers of merozoites, such as seen on the right side of the graph in Figure 2 (*P. giganteum, P. azurophilum, P. balli*, etc.), might well be convergent on this reproductive trait and not part of a monophyletic assemblage of species. Nonetheless, for ecologists, the great variation in life histories seen in lizard malarias is intriguing. What selective pressures would lead to the evolution of large vs small "clutch sizes" of merozoites? What would be the ecological consequences of each reproductive strategy? Perhaps the species with many merozoites could produce clones of parasites very rapidly and race ahead of the host's immune system's ability to respond to the infection. The rate of increase of the parasite population would depend also on the cell division rate. It is possible that the larger species would actually produce slower growing populations than the species with few merozoites if the rate of cell division is much faster in the smaller species (Figure 4). We studied *P. giganteum* (approx. 100 merozoites produced) and *P. agamae* (eight merozoites) which infect the same lizard in west Africa. In this case, examination of Figure 4 shows that if *P. agamae* divides every 3 days, and *P. giganteum* divides every 10 days, then *P. agamae* would have a faster population growth than the species that produces 12 times the number of merozoites.

Two other traits that have been used in taxonomic discussions are the presence or absence of pigment stored in the parasite's cells and the exploitation of blood cells other than erythrocytes by some species of lizard malaria. Lainson *et al.* (1974) and Lainson and Shaw (1969) have erected three new genera to contain those malaria species that do not produce pigment or infect white blood cells. They authored the genus *Garnia* for those species that use erythrocytes, but do not produce pigment; *Fallisia* which also produces no pigment and is found primarily in thrombocytes and occasionally in lymphocytes; and *Saurocytozoon* which is likewise unpigmented, and does not undergo asexual reproduction in the blood (see also Boulard *et al.* (1987) for ultrastructural evidence for establishing the genera *Garnia* and *Fallisia*). Telford (1978) later found that *Saurocytozoon* can reproduce in lymphocytes and Ayala (1977) reasoned that asexual reproduction in the blood may be transient, leaving behind long-lived gametocytes. These two traits (lack of pigment and use of white blood cells) obviously require significant physiological adaptations by the parasite. Once again, the phylogenetic significance of such adaptations is questionable. Some malaria species of lizards can utilize both red and white cells (*P. azurophilum*, for example), and some species

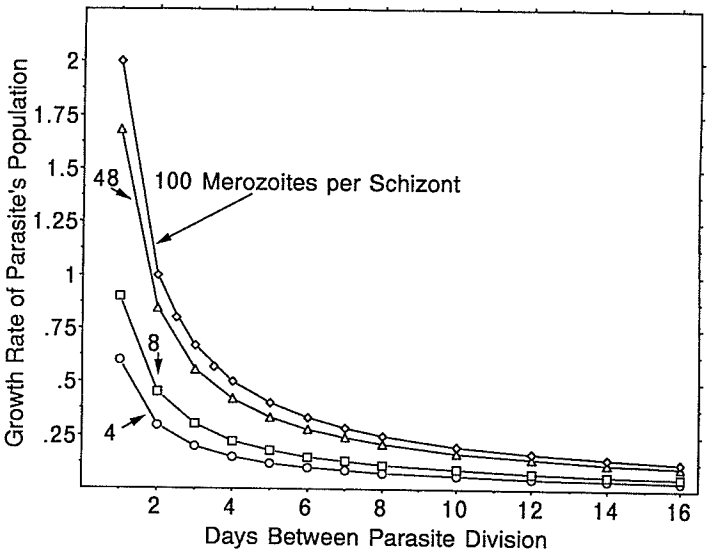


Figure 4 Growth rate of a malarial infection with differing number of merozoites per schizont. Population growth rate is the slope of the relationship: log parasites/10000 RBC over time in days. Redrawn from Schall (1990b), © *Parasitology*, used with permission.

produce pigment in some individual erythrocytes, and no pigment in others (*P. telfordi*, *P. azurophilum*, *P. morulum*). At some sites, *P. telfordi* does not produce pigment, whereas at other locations, pigment production varies among infections (Telford, 1988a). The species placed into each of the genera *Saurocytozoon*, *Fallisia* and *Garnia* might well simply represent convergence. Throughout this paper (in Figures 1 and 2, for example) I follow the argument of Levine (1988) and Ayala (1977) and refer the three genera to *Plasmodium*.

The reports on malaria systematics by early workers, and recent studies by Lainson *et al.* and Telford have shown that *Plasmodium* is a diverse, widespread and successful taxon of parasites. Resolution of systematic questions (what are the true phylogenetic relationships among the currently recognized species?; is *Plasmodium* a monophyletic genus?) is now possible because of the introduction of molecular techniques in the study of protista. The first gene sequence data for lizard malaria have now been described (GenBank data base #L11716 for *P. mexicanum* and #L11717 for *P. floridense*). Table 1 lists the described species of malarial parasites of lizards and is an updated version of one presented by Schall (1990a). This list includes newly described species and eliminates *P. diminutivum* (viewed as a subspecies of *P. minasense* by Telford (1979)),

Table 1 Plasmodium species known from lizards. Species name and author given. Species originally described or sometimes considered as *Fallisia*, *Garnia* or *Saurocytozoon* are also indicated by an [F], [G] or [S] next to the species name.

Species	Author	Lizard host	Location
achiotense	Telford, 1972	*Basiliscus*	Panama
acuminatum	(Pringle, 1960)	*Chamaeleo*	East Africa
agamae	(Wenyon, 1909)	*Agama*	Tropical Africa
arachniformis	Telford, 1988	*Chamaeleo*	Tanzania
attenuatum	Telford, 1973	*Ameiva*	Venezuela
audaciosum [F]	(Lainson, Shaw & Landau, 1975)	*Plica*	Brazil
aurulentum	Telford, 1971	*Thecadactylus*	Venezuela
australis	Garnham, 1966	*Amphibolurus*	Australia
azurophilum [G]	Telford, 1975	*Anolis*	Caribbean Islands
balli [G]	Telford, 1969	*Anolis*	Panama
basilisci	Pelaez & Perez-Reyes, 1959	*Basiliscus*	Panama
beebei	Telford, 1978	*Gonatodes*	Venezuela
beltrani	Pelaez & Perez-Reyes, 1952	*Sceloporus*	Mexico
brumpti	Pelaez & Perez-Reyes, 1952	*Sceloporus*	Mexico
brygooi	Telford & Landau, 1987	*Chamaeleo*	Madagascar
chiricahuae	Telford, 1970	*Sceloporus*	USA
clelandi	Manawadu, 1972	*Varanus*	Ceylon
colombiense	Ayala & Spain, 1976	*Anolis*	Colombia
copemani [F]	Paperna & Landau, 1990	*Carlia*	North-east Australia
cordyli	Telford, 1987	*Cordylus*	East and south Africa
cnemaspi	Telford, 1984	*Cnemaspis*	Tanzania
cnemidophori	Carini, 1941	*Ameiva*	Tropical South America
diploglossi	Aragão and Neiva, 1909	*Diploglossus*	Brazil
effusum [F]	(Lainson, Landau & Shaw, 1974	*Nausticurus*	Brazil
egerniae	Mackerras, 1961	*Egernia*	Australia
fairchildi	Telford, Johnson & Young, 1989	*Anolis*	Central America
fischeri	Ball & Pringle, 1965	*Chamaeleo*	East Africa

floridense	Thompson & Huff, 1944	*Anolis*	South-east USA
giganteum	Theiler, 1930	*Agama*	Tropical Africa
gologoloense	Telford, 1988	*Chamaeleo*	Eat Africa
gonatodi [G]	Telford, 1970	*Gonatodes*	Panama
guyannense	Telford, 1979	*Plica*	Guyana
heischi	Garnham & Telford, 1984	*Mabuya*	EAst Africa
holaspi	Telford, 1986	*Holaspis*	East Africa
iquanae	Telford, 1980	*Iquana*	Venezuela
josephinae	Pelaez, 1967	*Ameiva*	Mexico
lacertiliae	Thompson & Hart, 1946	*Carlia*	Goodenough Island
lainsoni	Telford, 1978	*Phyllodactylus*	Venezuela
lionatum	Telford,1982	*Ptychozoon*	Thailand
loveridgei	Telford, 1984	*Lygodactylus*	Tanzania
lygosomae	Laird, 1951	*Lygosoma*	New Zealand
mabuiae	Wenyon, 1909	*Mabuya*	East Africa
mabuyi [S]	(Lainson, Landau & Shaw, 1974)	*Mabuya*	Brazil
mackerrasae	Telford, 1979	*Egernia*	Australia
maculilabre	Schwetz, 1932	*Mabuya*	Congo
marginatum	Telford, 1979	*Anolis*	Panama
mexicanum	Thompson & Huff, 1944	*Sceloporus*	West USA
michikoa	Telford, 1988	*Chamaeleo*	East Africa
minasense	Carini & Rudolph, 1912	*Ameiva*	Venezuela
morulum [G]	Telford, 1970	*Mabuya*	Panama
multiformis [G]	(Lainson, Landau & Shaw, 1975)	*Plica*	Brazil
modestum [F]	(Lainson, Landau & Shaw, 1974)	*Tropidurus*	Brazil
pelaezi	Malagon & Salmeron, 1988	*Mexico*	Urosaurus

Table 1 Continued.

Species	Author	Lizard host	Location
pifanoi	Scorza & Dagert, 1956	*Ameiva*	Venezuela
pitmani	Hoare, 1932	*Mabuya*	East Africa
rhadinurum	Thompson & Huff, 1944	*Iquana*	Venezuela
robinsoni	(Brygoo, 1962)	*Chamaeleo*	East Africa
sasai	Telford & Ball, 1969	*Takydromus*	Japan
saurocaudatum	Telford, 1983	*Mabuya*	Thailand
scelopori	Telford, 1977	*Sceloporus*	Belize
scorzai	Telford, 1978	*Phyllodactylus*	Venezuela
siamense	Telford, 1986	*Draco*	Thailand
simplex [F]	(Lainson, Shaw & Landau, 1975)	*Plica*	Brazil
tanzaniae	Telford, 1988	*Chamaeleo*	East Africa
telfordi [G]	(Lainson, Landau & Shaw, 1971)	*Ameiva*	Venezuela
torrealbai	Scorza & Dagert, 1957	*Anolis*	Venezuela
tropiduri	Aragão & Neiva, 1909	*Tropidurus*	Venezuela
tupinambi [S]	(Lainson, Shaw & Landau, 1969)	*Tupinambis*	Brazil
uluguruense	Telford, 1984	*Hemidactylus*	Tanzania
uncinatum	Telford, 1973	*Plica*	Guyana
uranoscondoni [G]	(Lainson, Shaw & Landau, 1975)	*Uranoscondon*	Brazil
utingensis [G]	Lainson, Landau & Shaw, 1971	*Anolis*	Brazil
uzungwiense	Telford, 1988	*Chamaeleo*	East Africa
vacuolatum	Lainson, Shaw & Landau, 1975	*Plica*	Brazil
vastator	Laird, 1960	*Draco*	Malaya
vautieri	Pessoa & Biasi, 1973	*Urostrophus*	Brazil
zonuriae	Pienaar, 1962	*Cordylus*	South Africa

and a non-existent species, "*P. archiotensis*", created by a word processing error in the original list. I do not include on this list (nor in Figures 1 and 2) 10 species described from several lizard species in Kenya by Dipeolu and Mutinga (1989). The species descriptions in this paper are inadequate; most likely fewer than 10 species were actually present and these might well be previously known forms.

Figure 5 shows the known distribution of lizard malaria. As would be expected, most species are tropical, but several extend into very seasonal temperate zones (*P. mexicanum* as far north as Wyoming in the USA, and *P. lygosomae* in New Zealand). Lizard malaria has been found in all the warm continents except Europe. I would expect malaria to be found on the Iberian peninsula because of the moderate Mediterranean climate there and also because of the rich lizard fauna in Spain and Portugal. R. Constantinides (personal communication) has found several blood parasites in the lizards of Cyprus but none were *Plasmodium*. Malaria has not yet been found in lizards from China or India. I have surveyed for lizard malaria in northern China without success. This area is a very dry desert and I expect that future surveys will reveal a rich assemblage of species in the wetter regions of southern Asia. We have also searched for malaria in the lizards of some South Pacific islands, also without success, perhaps because of the distance of these islands from mainland source habitats.

The highest species richness of lizard malaria is found in places where the most active researchers have collected (Telford in Panama, Venezuela, Tanzania and Thailand; and Lainson *et al.* in Para, Brazil). Therefore, any global-level biogeographic analysis of the distribution of lizard malarias is not presently feasible. Several regional biogeographical analyses, though, are possible. Ayala (1970) pointed out that *P. mexicanum*-like parasites are found in lizards in relectual Madro-Tertiary floral regions in North America. These areas are presently disjunct, but were contiguous during the Pleistocene. *P. mexicanum* itself is found in fence lizards (*Sceloporus*) in several limited areas in California and in Wyoming (Greiner and Daggett, 1973). This suggests that this parasite–host association is ancient and of value in studies of the evolution of parasite virulence (Schall, 1990b). In Arizona, USA, *P. chiricahuae* infects *S. jarrovi*. The lizard and its malarial parasite are found only on mesic mountain tops surrounded by deserts that are inhospitable for the lizards. The populations of parasite and host have been isolated for 8000–12000 years and provide a novel system to examine evolutionary changes in a *Plasmodium* over a known period of time. Mahrt (1987) found significant differences in the morphology of the lizards on five montane islands in Arizona, but no difference in the size of macrogametocytes of *P. chiricahuae* among sites. Molecular studies on these populations of both host and parasite should prove an intriguing way to

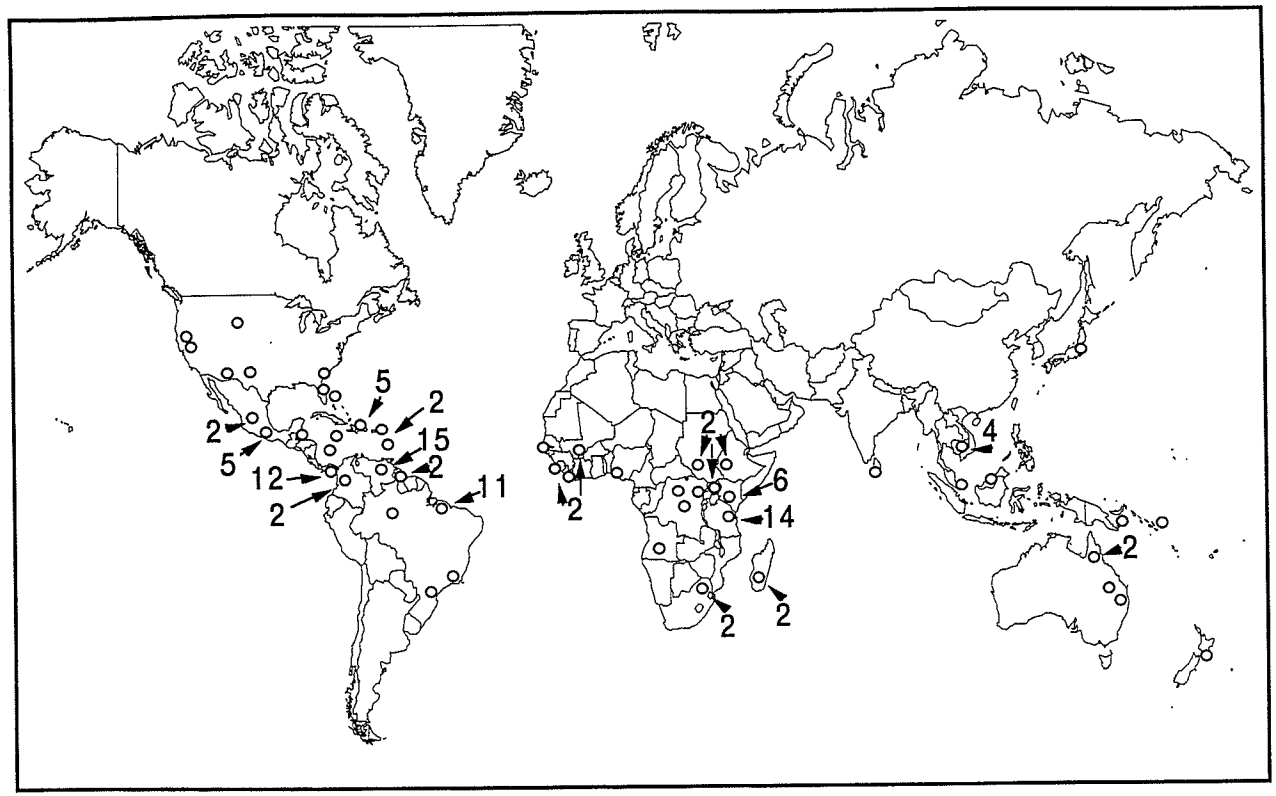


Figure 5 Known distribution of lizard malaria. Unlabelled points indicate a single species is known from the site; numbers show when more than one species is present.

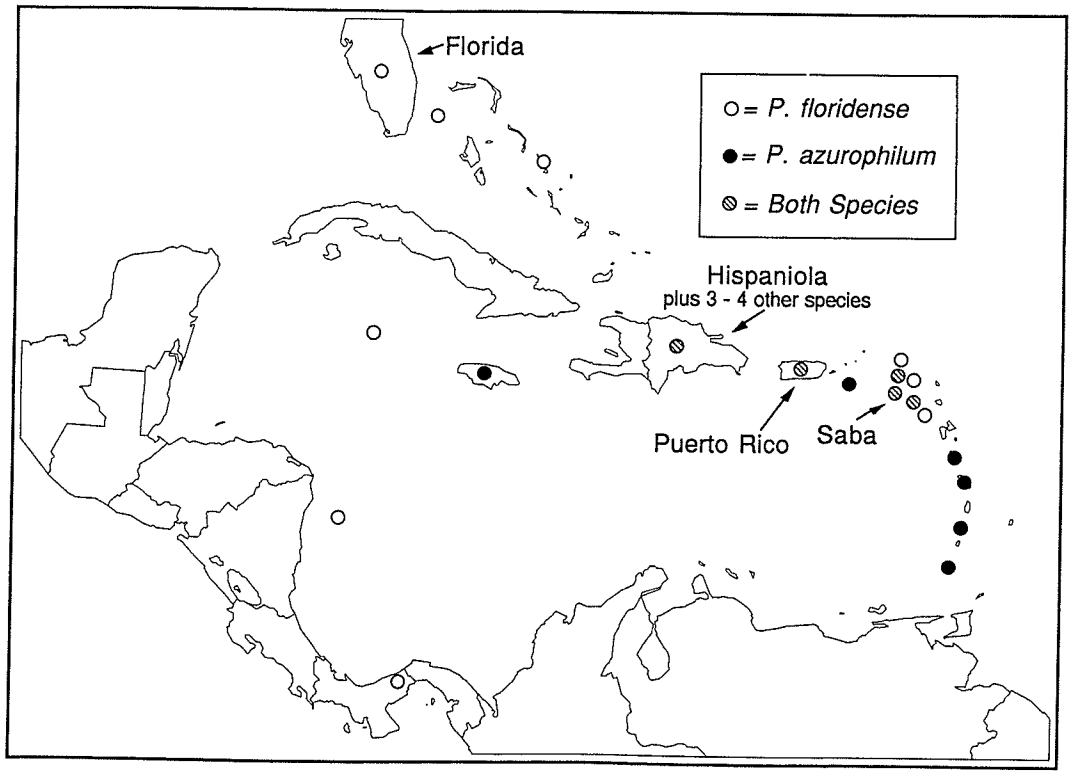


Figure 6 Known distribution of lizard malaria in the Caribbean region.

calibrate any molecular clock used in other studies on the genetics of lizard malarias.

The known distribution of lizard malaria in the Caribbean basin is shown in Figure 6. Two named species, *P. azurophilum* and *P. floridense*, are found infecting *Anolis* lizards in the eastern Caribbean. *P. azurophilum* occurs from Jamaica, east and south to Grenada. *P. floridense* has an even wider distribution, from Mexico, Central America and the south-east USA on the mainland; North Bimini, Cat Cay in the Bahamas, and Grand and Little Cayman in the north-western Caribbean; San Andrés in the central Caribbean; Jamaica, Hispaniola and Puerto Rico in the Greater Antilles; and the Lesser Antilles south only to Montserrat (Telford *et al.*, 1989; Staats and Schall, 1995). Only *P. azurophilum* and *P. floridense* occur on Puerto Rico (Schall and Vogt, 1993). At least five species of lizard malaria have been found on Hispaniola, *P. azurophilum* and *P. floridense*, plus other species similar to forms in Panama and northern South America (*P. tropiduri*, *P. minasense* and *P. fairchildi*). Telford *et al.* (1989) proposed that this higher diversity on Hispaniola compared to other Caribbean islands, including the other Greater Antilles, is a result of Hispaniola having been colonized by four evolutionary lineages of *Anolis*, in comparison to fewer species groups in Jamaica and Puerto Rico (the presence of lizard malaria on Cuba is presently unknown). This suggests that lizard malaria moves onto islands in lizard hosts rather than when the vectors blow from island to island.

Staats and Schall (1995) support this hypothesis by noting that *P. floridense* is not found in the south-east Caribbean in *Anolis* of the Roquet species group. This parasite seems to have originated in Central America and moved to the eastern Caribbean in the ancestors of the Bimaculatus group of anoles. *P. azurophilum* infects lizards in both the Bimaculatus species group and the Roquet group. This suggests that *P. azurophilum* is an ancient parasite (and probably now actually a species cluster) and had an origin in South America. The lack of pigment production and predilection for white blood cells by *P. azurophilum* suggest possible relationships with similar species in South America.

3. STUDY SITES AND GENERAL METHODS

Our primary study site since 1978 has been the Hopland Field Station of the University of California located approximately 160 km north of San Francisco. This 2167 ha plot ranges from 180 to 975 m in elevation and includes woodland, oak savannah and chaparral. Approximately 50 study areas have been established at all habitat types at Hopland for our long-

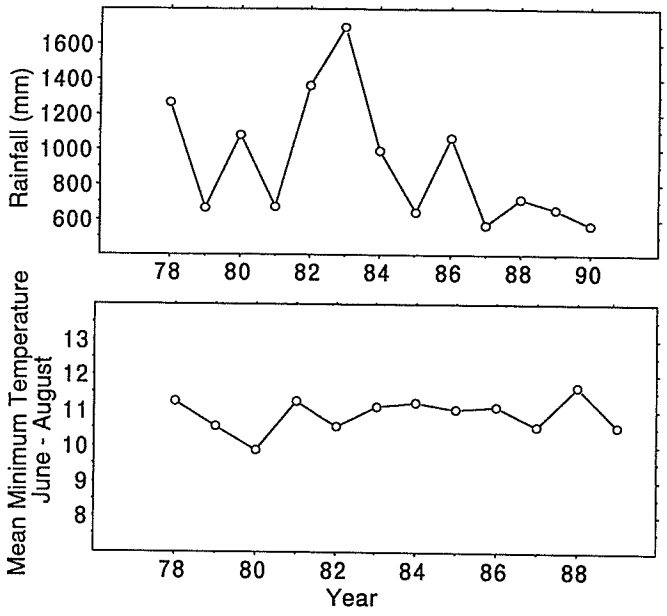


Figure 7 Weather data for the Hopland Field Station, California, USA, from 1978 to 1990. Precipitation during the rainy season (November through May inclusive) prior to each year's research season is very variable compared to mean temperature among years.

term examination of the biology of *P. mexicanum* in its hosts, the western fence lizard *S. occidentalis* and two psychodid sandflies, *Lutzomyia vexator* and *L. stewardi*. The climate at Hopland is moderate Mediterranean with cool, rainy winters when most of the annual precipitation occurs, and dry, warm summers. Annual temperature variation is minimal, but rainfall varies greatly (Figure 7). Lizards become active in late April and remain so until the end of September.

Comparative studies have been conducted in Sierra Leone, west Africa, on *P. agamae* and *P. giganteum* which infect the rainbow lizard, *Agama agama*. We established 22 study sites in Sierra Leone in a variety of habitats: higher elevation savannah–woodland, coastal swamp, riverside woodlands, closed forest at lower elevations, and agricultural areas and towns. *P. agamae* and *P. giganteum* were chosen for study because they occur at opposite ends of the range in merozoite numbers shown in Figure 2 and because they infect the same lizard species. In more recent years we have moved onto a study of the distribution and ecology of malaria in the *Anolis* lizards of the eastern Caribbean. Our two major bases have been the Luquillo Forest in Eastern Puerto Rico and the tiny 13 km^2 island of Saba

(Netherlands Antilles) in the north-eastern Caribbean. *P. floridense* and *P. azurophilum* occur at both sites. A long-term study on parasite prevalence is under way on Saba to provide a tropical comparison with the more mature study in California.

Figure 8 presents drawings of the malaria species used in our studies.

Lizards at all sites are captured by hand or with a slip noose on the end of a fishing pole. The animals are kept in cloth or net sacks until evening when they are measured (snout to vent length (SVL) in mm), gender determined, tail condition noted (intact, broken, or regenerated — this shows the frequency of attacks by conspecifics or predators), and a drop of blood taken from a toe clip. A thin smear is made (thick smears are not useful because lizard erythrocytes are nucleated), dipped in absolute methanol and stored. The smears are stained with Giemsa, at 1:10 for 50 min at pH 7.0. The parasites are very obvious with this staining procedure. For some studies we clip toes in an individually recognizable order so that the lizards can later be recaptured and identified. Almost all lizards are eventually returned to their site of capture.

All smears are scanned at ×900–1200 for 6 min, during which approximately 10000 erythrocytes are examined. For some studies this procedure is repeated three times for apparently non-infected smears. Smears from infected lizards at sites with more than one malaria species (Africa and the Caribbean) are examined again for up to an hour to determine species present (if both species are seen, the scan stops; otherwise it may continue for an hour).

Our protocol will rarely, if ever, yield false positives for an experienced observer. However, false negatives are likely for infections with either very weak or no parasitemia. Serological techniques could eliminate this error, but can result instead in false positives if different parasites cross-react, or if a parasite was present only transiently. Some false negatives are certainly included in our studies, but the percentage of lizards involved is likely to be small and thus have little effect on the qualitative results reported. Hundreds of *P. mexicanum* infections have been studied in the laboratory and in free-ranging lizards; 61 infections of *P. agamae* and *P. giganteum* were followed in the laboratory; and dozens of *P. floridense* and *P. azurophilum* infections were followed in lizards in the laboratory or housed in large outdoor cages. No *P. mexicanum*, *P. agamae*, *P. giganteum* or *P. azurophilum* infections were "lost" in these studies. Some very low *P. floridense* infections, though, dropped to undetectable levels. This suggests that the vast majority of infections are detected by our protocol. Also, the various patterns described in our studies would be difficult to explain if the scanning protocol was seriously flawed. Nonetheless, data on percentage of lizards infected are used only in comparisons (male vs female lizards,

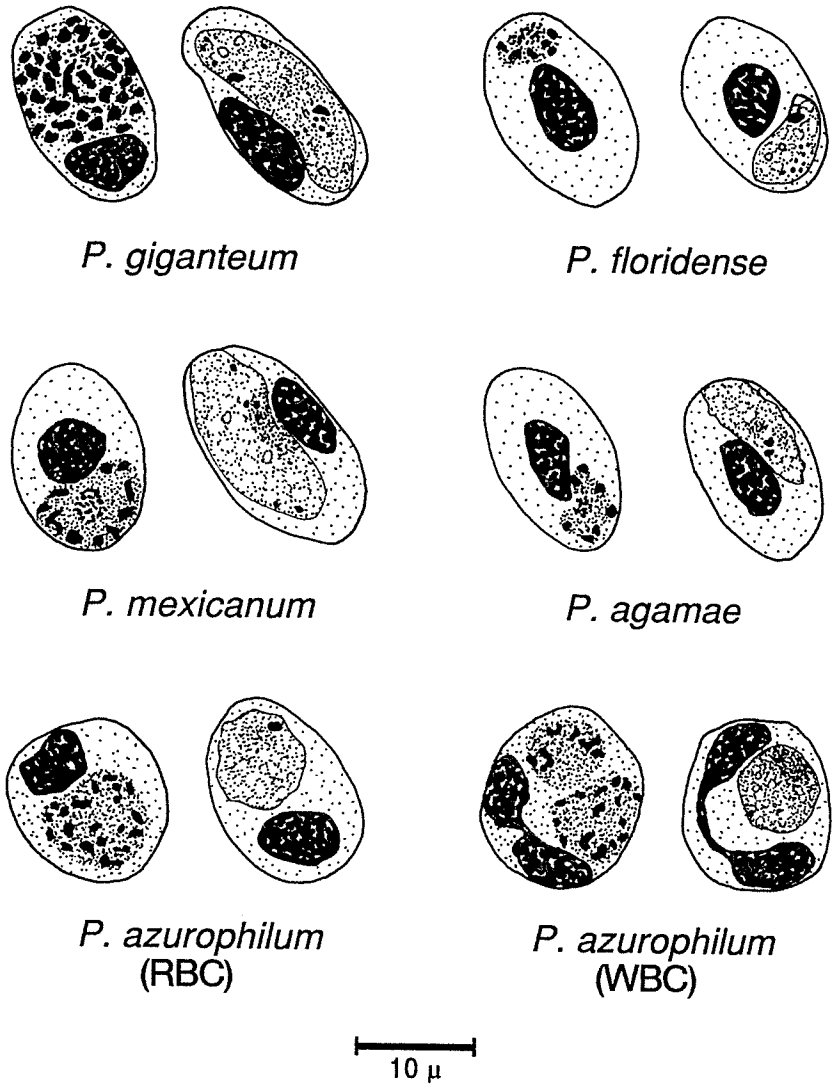


Figure 8 Camera lucida drawings of lizard malaria species studied by the author. Schizont is shown on the left and macrogametocyte on the right. Schizonts are mature except for *P. giganteum* in which an immature cell is shown; mature schizonts are packed with nuclei. *P. azurophilum* is shown in its two cell hosts, an erythrocyte on the left and white blood cell on the right (a double infection in a white blood cell is shown).

older vs younger lizards, among years, etc.) and should not be used as exact measures of prevalence in analyses of transmission rates.

The hematology of reptiles remains poorly known. Many blood cell types of reptiles resemble those of mammals morphologically and some authors have been tempted to use the terminology of mammalian hematology for the cells seen in reptiles. Other authors have coined a variety of cell names specific to reptiles. The homology of reptile and mammal blood cells, even when their morphology is very similar, is mostly unknown. I follow the conservative terminology of Sypek and Borysenko (1988); any similarity in terms with those used for mammals does not necessarily imply functional similarity. One specific conflict in terminology concerns the nature of immature red blood cells in lizards. Lizard erythrocytes are nucleated and synthesis of hemoglobin may continue for the life of the cell. A high proportion of mature erythrocytes can contain reticulum (75% or more of cells) judging from reticulum visualized by use of new methylene blue (Maizels, 1980). Thus, most cells, including most mature red cells, could be termed reticulocytes, so I will instead use the term "immature red blood cell". These cells have a larger nucleus, rounder shape, and more blue-staining cytoplasm. Cells with this morphology contain less hemoglobin than mature erythrocytes (Schall, 1990b).

All statements in this review are supported by the appropriate statistical analyses that are presented in the original publications; these results are not repeated here. For data and analyses that are given here for the first time, the results of statistical testing are given.

4. PATTERNS IN PREVALENCE

A central issue in ecology concerns the forces that shape the distribution and abundance of organisms, both over time and space. Indeed, a popular general university ecology textbook defines ecology in its title as the "experimental analysis of distribution and abundance" (Krebs, 1985). The abundance of a malarial parasite can be defined as its prevalence (percentage of hosts infected) or parasitemia (parasite load in an individual infected host). This section will deal with patterns, both temporal and spatial, in prevalence; the next will discuss parasitemia in the context of the course of infection.

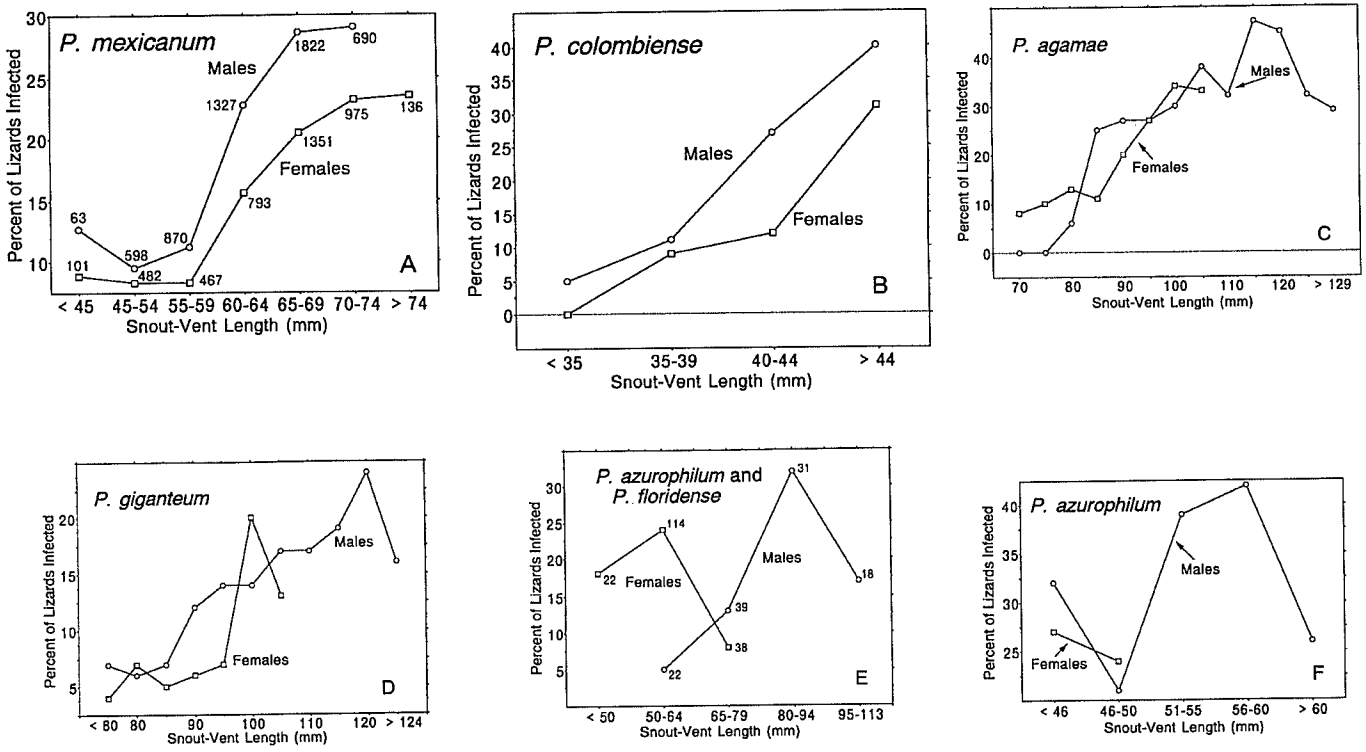
4.1. Host Age and Gender

Figure 9 presents prevalence data for body size (SVL), and in most cases for gender, for a variety of lizard malaria systems. In all cases, prevalence

increases with body size and therefore with age. Older lizards are likely to have had a greater cumulative chance of becoming infected. In some species males are more likely to be infected than females at most body sizes (*P. mexicanum* in California *Sceloporus occidentalis*, *P. floridense* and *P. azurophilum* in Puerto Rico *Anolis gundlachi*). Small females are more often infected on St Kitts island (*P. azurophilum* in *A. bimaculatus*) (all G-tests, $P < 0.05$).

In some of the cases shown in Figure 9 there is an apparent drop in percentage of lizards infected for the largest body size class. This can only be accounted for if larger infected lizards eliminate the infection (or at least reduce it to undetectable levels) or if larger infected animals suffer higher mortality than non-infected animals. Infections were not spontaneously cured in either marked free-ranging animals or laboratory-kept lizards (see Section 3), so patent infection with malaria may be life-long for most lizards. This would cast doubt on the first explanation. However, infections have not been followed over the same time scale as the age curves shown in Figure 9 (the lifespan of the lizards), so a reduction in prevalence in the oldest lizards because of gradually increasing immunity in older lizards cannot be definitively rejected. The largest lizards, and therefore the oldest, may be under more stress than the smaller animals because of their reproductive activities and the social interactions necessary for the oldest lizards to maintain territories. Infected older animals may suffer increased mortality because of such stress. Evidence for this idea comes from observations of lizards in captivity. Lizards in laboratory cages are assumed to be under increased stress compared to those in the wild. Mortality of captive infected male fence lizards was six times higher than for non-infected animals and mortality of infected females was more than twice as high (Schall, 1983a); this supports the idea that prevalence of malaria in older lizards drops because of increased mortality in these animals.

Figure 9 also shows prevalence data for age for humans in areas with epidemic and endemic malaria. For humans in areas with endemic malaria (comparable with the lizard malaria systems shown in the figure), prevalence decreases with age after an initial rise in the youngest age groups. The pronounced difference in the prevalence figures for human and lizard malaria almost certainly reflects the very different functioning of the mammalian and reptilian immune systems. Over time, the human immune system eventually mounts an effective attack against the parasite and either eliminates the infection or drives it to very low levels. In contrast, malaria appears to be a life-long infection in lizards which mount an inefficient and slow immune system attack.


P. mexicanum
Males
Females
1822
690
1327
975
136
1351
870
793
63
598
101
482
467
A
Percent of Lizards Infected
< 45 45-54 55-59 60-64 65-69 70-74 > 74
Snout-Vent Length (mm)
P. colombiense
Males
Females
B
Percent of Lizards Infected
< 35 35-39 40-44 > 44
Snout-Vent Length (mm)
P. agamae
Males
Females
C
Percent of Lizards Infected
70 80 90 100 110 120 > 129
Snout-Vent Length (mm)
P. giganteum
Males
Females
D
Percent of Lizards Infected
< 80 80 90 100 110 120 > 124
Snout-Vent Length (mm)
P. azurophilum and
P. floridense
Males
Females
114
22
31
18
39
38
22
E
Percent of Lizards Infected
< 50 50-64 65-79 80-94 95-113
Snout-Vent Length (mm)
P. azurophilum
Males
Females
F
Percent of Lizards Infected
< 46 46-50 51-55 56-60 > 60
Snout-Vent Length (mm)

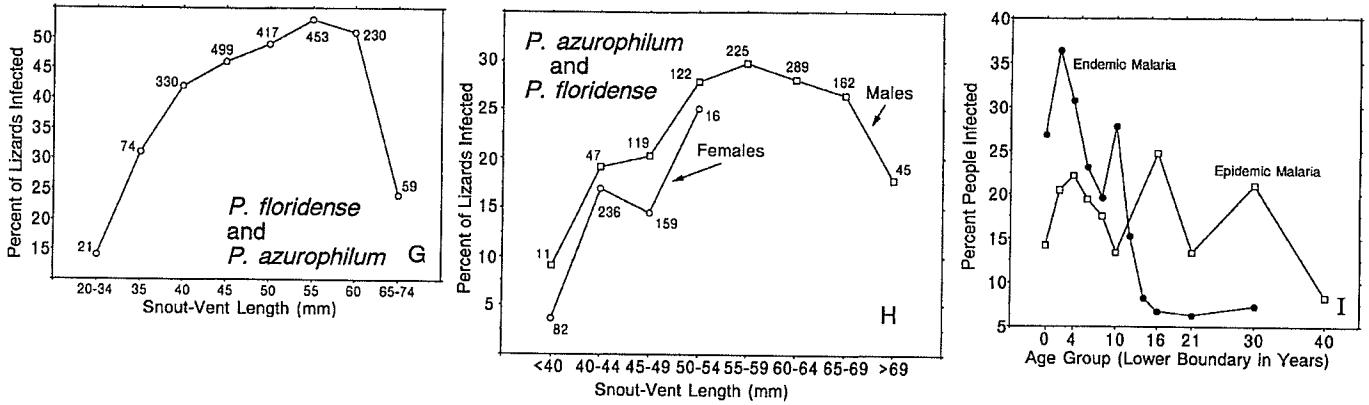


Figure 9 Prevalence data for a variety of lizard malaria systems and for human malaria from one study. (A) *Plasmodium mexicanum* in *Sceloporus occidentalis* at the Hopland Field Station in California from 1978 through 1995; (B) *P. colombiense* in *Anolis auratus* (data from Ayala and Spain (1976)); (C) *P. agamae* in *Agama agama* in Sierra Leone, West Africa; (D) *P. giganteum* in *Ag. agama* in Sierra Leone; (E) *P. azurophilum* and *P. floridense* in *A. bimaculatus* on St Kitts Island in the Lesser Antilles; (F) *P. azurophilum* in *A. gingivinus* on St Maarten island, Netherlands Antilles; (G) *P. floridense* and *P. azurophilum* in *A. sabanus* on Saba Island, Netherlands Antilles; (H) *P. floridense* and *P. azurophilum* in *A. gundlachi* on Puerto Rico (figure from Schall and Vogt (1993), © *Biotropica*, used with permission); (I) human malaria in an endemic and epidemic zone (data from Macdonald, 1951). Body size (SVL) in lizards is correlated with age. Samples sizes are next to points on some graphs.

4.2. Prevalence Over Time and Space

Mathematical modelling in ecology has its origin with Ronald Ross's (1911) studies of the prevalence of malaria in humans which were later modified by Lotka (1923), the "founder" of theoretical ecology, and by George Macdonald (1957) in a series of classical studies. It is satisfying that theoretical ecologists have returned to the Macdonald models to present them in a more modern format (Aron and May, 1982; Anderson and May, 1992). Macdonald noted that within the geographic range that is apparently suitable for human malaria, the parasite can either be absent at a particular site, present endemically with high prevalence, or present at low levels with periodic epidemic outbreaks. Macdonald's model shows that these three conditions are determined by the average number of blood meals taken by the vectors: few bites result in an absence of malaria, more bites produce low prevalence with occasional epidemics, and even more bites yield endemic malaria with high prevalence (Anderson and May, 1992).

The Macdonald model does not show cycles or other complex dynamics. However, available data sometimes suggest long-duration cycles in malaria prevalence in humans (Pampana, 1969). These data are often confounded by ongoing control measures or population movements. Figure 10 shows such data for Mississippi, USA (Faust, 1949). Prevalence fell during eradication efforts from 1915 to 1945, but some vestige of a cycle remains. Two long-term studies of prevalence are available for lizard

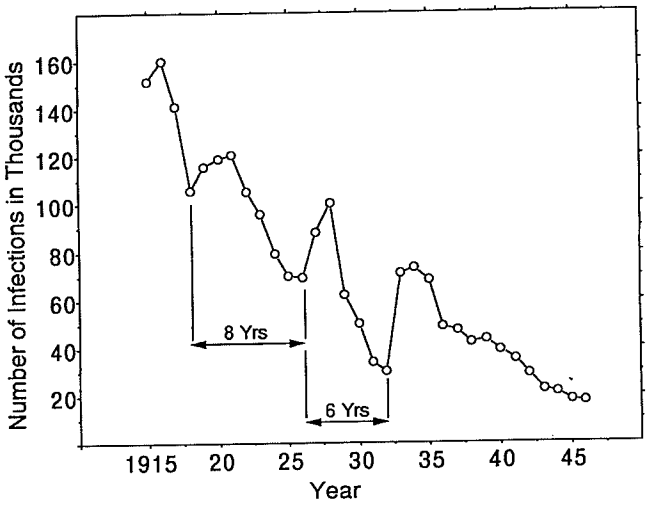


Figure 10 Change in prevalence of human malaria in Mississippi, USA, over many years. Data from Faust (1949).

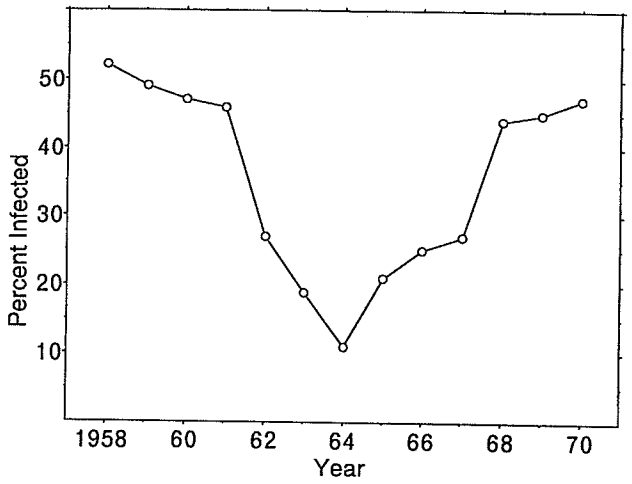


Figure 11 Change in prevalence of *P. floridense* in the eastern fence lizard, *Sceloporus undulatus*, in Georgia, USA over a 13 year period from 1958 to 1970. Redrawn from figure in Jordan and Friend (1971).

malaria (and, to my knowledge, the only such studies for malarial parasites of non-human vertebrate hosts). Data for *P. floridense* in *S. undulatus* in Georgia, USA (Jordan and Friend, 1971) are graphed in Figure 11 and show significant variation in prevalence over a 13 year period that does not fit any of the cases explained by Macdonald's model. *S. undulatus* is a short-lived animal which suggests the changes seen from year to year in Figure 11 reflect changes in transmission of the parasite. A cycle of about a 10 year duration may have been present.

A similar possible cycle appeared in our 13 year study of *P. mexicanum* at the California site (Schall and Marghoob, 1995; Figure 12). We examined weather data to determine if rainfall or temperature could account for the variation seen in the prevalence of *P. mexicanum*. Precipitation during the rainy season prior to each field season (November to May; lizards were sampled from May through September), rainfall the previous year, temperature during the collecting period, and temperature during the previous warm season were not correlated with parasite prevalence. As shown in Figure 7 rainfall varied considerably at the Hopland site, with some years of the study falling in drought, and others in times of very heavy rainfall. A bioassay of habitat quality was also examined: vegetative growth was measured by the Hopland Station staff using a standard technique at many study plots. This assay also was not correlated with parasite prevalence. If environmental conditions cannot account for the variation in *P. mexicanum* prevalence, perhaps some factors inherent in the transmission

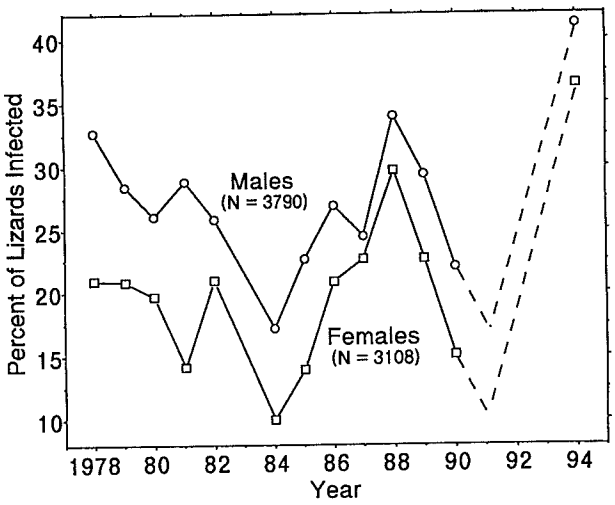


Figure 12 Change in prevalence of *P. mexicanum* in the western fence lizard, *Sceloporus occidentalis*, in California, USA, over a 17 year period from 1978 to 1994. Dotted line indicates 3 year period when no data were taken, but shows pattern expected if a 10 year cycle exists at the site.

biology of the parasite give rise to variation in its abundance and even perhaps the possible 10 year cycle seen in the first 13 years of the study. Such complex dynamics are predicted by non-linear models of parasite biology (May, 1985). Both the Georgia and California studies lasted only 13 years which would allow only one 10 year cycle to be observed. To test the conclusion that a long cycle takes place at Hopland, we returned to the site to sample again in 1994. The result is also shown in Figure 12; the prevalence observed in the summer of 1994 matched the level expected if a 10 year cycle exists.

We have found substantial variation in prevalence of lizard malaria among sites at Hopland (Schall and Marghoob, 1995), Sierra Leone (Schall and Bromwich, 1994), Puerto Rico (Schall and Vogt, 1993) and Saba Island (C. Staats, personal communication). Part of the variation in prevalence at Hopland can be accounted for by elevation (Figure 13) because *P. mexicanum* is rare or absent in lizards at sites above 500 m where night-time air temperature is significantly cooler than at lower elevations. As described by Jordan (1964), the distribution of lizard malaria in the USA is strongly influenced by temperature. We surveyed for the presence of the vector at sites at higher and lower elevations and found that *Lutzomyia* sandflies are present, and sometimes abundant, at the higher elevations but appear to have fewer nights when air temperatures allow them to be active (Schall and Marghoob, 1995). Thus, the mean

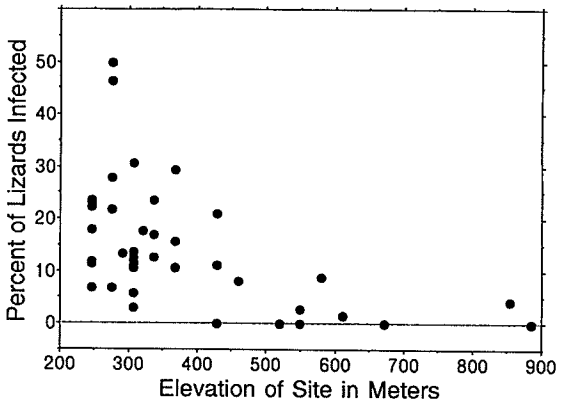


Figure 13 Prevalence of *P. mexicanum* at the Hopland Field Station at many sites that vary in elevation. Figure from Schall and Marghoob (1995), © *Journal of Animal Ecology*, used with permission.

number of blood meals taken by the sandflies at higher elevations may be low enough to prevent the parasite from being maintained as predicted by Macdonald's model. Alternatively, the lower temperature may prevent the parasite from completing development in the vector (below).

Below 500 m at Hopland the parasite's prevalence also varies from very common (~50%) to rare (<10%). In some cases, sites with abundant malaria are only a few hundred meters from those with rare malaria. There was no relationship between abundance of ground squirrel burrows (the site of egg laying and daytime resting for the sandflies) and abundance of malaria (Schall and Marghoob, 1995). Although malaria prevalence varied among the years, sites with high malaria remained consistently at relatively high prevalence and sites with low prevalence remained low for the entire length of the study from 1978 to 1994.

Lizard malaria also differs in prevalence at the tropical sites we have studied. In Sierra Leone, prevalence of *P. agamae* and *P. giganteum* ranged from 18% to 90%. Although habitat type varied (lowland rainforest to upper elevation savannah, for example), there was no clear relationship between habitat type and parasite abundance in the rainbow lizard, *Agama agama* (Schall and Bromwich, 1994). In eastern Puerto Rico, *P. floridense* and *P. azurophilum* were significantly more abundant in lizards in wet rainforest compared to a drier bamboo–woodland habitat (30% vs 7%) (Schall and Vogt, 1993). Recent studies on Saba, Netherlands Antilles, show that even on this tiny island malaria prevalence ranges considerably (Figure 14). All of the "lower malaria" sites are in dryer habitats on the

windward side of the island. However, some high malaria sites are in such habitats as well (one site shown on Figure 14 with 38% of lizards infected).

The factors controlling malaria prevalence in humans are a venerable mystery in parasitology. Likewise, only a small portion of the variation in abundance of malaria in lizards among our study sites in California, Sierra Leone, and on Saba can be accounted for by habitat quality.

4.3. Host Range

In the Luquillo rainforest of eastern Puerto Rico, five species of *Anolis* lizards are common in the lower zones of the forest. Schall and Vogt (1993) examined a large sample of these species (2456 lizards) and found that malaria was very rare in four of the anoles (<1% in three, and 2% in the fourth). The fifth species, *A. gundlachi*, was frequently infected (overall 26% of males and 14% of females). All five lizard species were found in the same general area and three of them, including *A. gundlachi*, are often found within a few meters on the lower tree trunks and ground litter. The five anoles are closely related, all falling into two species series of the Cristatellus species group of *Anolis*. Therefore, the distribution of malaria in the five lizards is perplexing: all five species are capable of hosting the

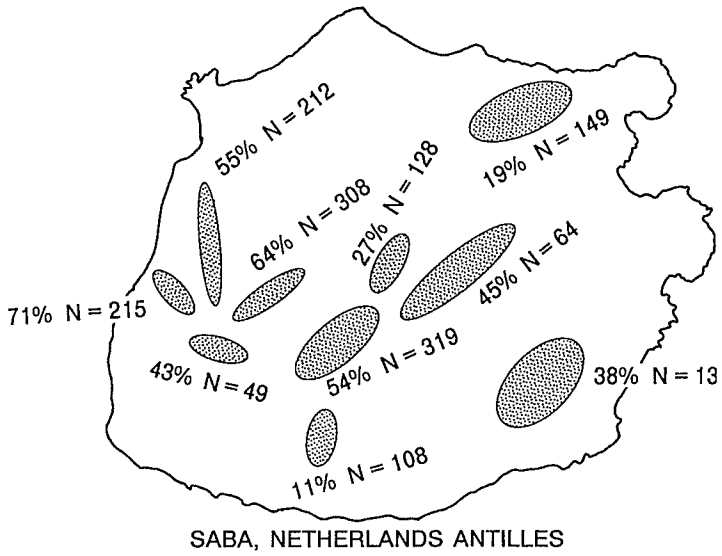


Figure 14 Map of Saba Island (Netherlands Antilles) showing some study areas with percentage of *Anolis sabanus* infected with *Plasmodium floridense* and *P. azurophilum* and sample sizes.

parasite, the hosts are assumed to be similar physiologically because of their close phylogenetic relatedness, they live in the same habitat and must all be available to the vector(s). A somewhat similar situation was observed on St Maarten, Netherlands Antilles. There both *A. gingivinus* and *A. wattsi* are hosts of *P. azurophilum*, but the parasite much more commonly infects *A. gingivinus* even though both species of lizard are found at the same sites. The implications of such differences in the use of co-existing lizards for the hosts' community ecology is discussed below (Section 9.8).

5. COURSE OF INFECTION

5.1. Pre-erythrocytic Stages

The classic picture of the course of malarial infection in a vertebrate host begins with the pre-erythrocytic stages, often very cryptic, followed by eruption of the parasite into the peripheral blood, exponential increase in parasitemia, resolving either in death of the host or a crash of the parasite population. The parasite may be eliminated from the blood or remain there in very low densities, perhaps to flash into exponential growth sometime in the future. This story is based on infections studied in humans and in experimental laboratory infections of mammals and some birds. The function of the reptilian immune system differs greatly from that of the mammalian system — most importantly in the speed of its response to parasitic infections (Sypek and Borysenko, 1988). Does the course of infection with *Plasmodium* differ in reptile vs mammalian systems? No clear answer has emerged.

Telford (1989) has described the pre-erythrocytic forms of *P. sasai* in the Japanese lizard *Takydromus tachydromoides*. Several authors had previously described large schizonts in cells other than erythrocytes in lizards (reviewed by Telford (1989)), but none of these were unambiguously the initial stages of the infection rather than secondary pathological invasions of unusual cell classes. In contrast, the entire series of pre-erythrocytic stages were found for *P. sasai*. (The difficulty in identifying these stages should be appreciated by future researchers; fully 20 species of parasites were found in *T. tachydromoides*, five of which were haemoparasites and four were intestinal coccidia that could potentially cast some life states into the blood.) Telford proposed a plausible model of the course of infection in lizard malaria based on his study of *P. sasai*. Uninucleated parasites found in the liver parenchymal cells (probably equivalent to the hypnozoites found in primate *Plasmodium* infections) represent the first post-

sporozoite stage. These cells may undergo karyokinesis, producing schizonts in the liver, or may remain quiescent to emerge during a spring recrudescence in temperate species. The liver schizonts were of two size classes (16 vs 40 nuclei) which suggests at least one cycle of cell division and reinfection in the liver. The parasite subsequently invades macrophages and moves throughout the host's body where parasites encyst in endothelial cells of every organ. These cells become the permanent infection of the lizard, releasing additional reproductive cells into the blood as the infection wanes after attack by the host's immune system. If *Plasmodium* evolved first as a parasite of reptiles (Manwell, 1955), it still retains pre-erthythrocytic stages in the reticulo-endothelial system (characteristic of bird malarias) and hepatic parenchyma (typical of mammal malarias).

5.2. Blood Stages

The course of malarial infection within the blood of lizards seems to vary, both among species of parasite and among individual hosts. Jordan (1970) studied natural infections of *P. mexicanum* within three fence lizards that were brought into the laboratory. In each case, infections remained at low levels during the early spring, then revealed exponential increase during late spring and early summer. One lizard died when parasitemia reached 3900/10000 red blood cells (RBC); the final outcome of the other infections was not reported. Jordan (1975) also studied three natural infections of *P. floridense* in *S. undulatus*. A period of acute rise lasted 65 days, reaching parasitemia of about 13000/10000 RBC, and was followed by a decline period. Number of merozoites decreased in schizonts during the period of decline.

Telford (1972) documented the history of artificially induced (via blood transfer containing carefully controlled numbers of asexual stages) *P. sasai* infections in two species of *Trakydromus*. Similar to the infections of *P. floridense* and *P. mexicanum* described above, *P. sasai* remained at low levels (undetectable) for several days, then rose quickly to a peak, then declined to remain at low levels.

These results are interesting, especially as they resemble the course of infection of human malaria, but many of the studied infections are artificial, with the entire pre-erythrocytic cycle missing, and all involved animals in laboratory cages. Bromwich and Schall (1986) followed natural infections of *P. mexicanum* in free-ranging fence lizards at the Hopland site. The goal of this project was to determine if the course of infection was conditional based on time of year or quality of host. That is, would infections beginning later in the season increase more rapidly to maximize transmission success (mortality of lizards over the winter dormancy period

is high), and would infections follow a predictable schedule in producing gametocytes (rapid production of gametocytes in infections starting later in the season)? We marked 550 lizards, and recaptured 334 of them at least once, 104 of which were infected. We had 505 smears from infected free-ranging lizards to examine and at least three smears were available for over at least half of the warm season for 65 animals.

Ayala (1970) reported for this same population of infected lizards that there is a spring recrudescence. His sample size was small (88 for the entire season). We combined the 505 smears from infected animals in an analysis to mimic Ayala's. Dividing the data by week from mid-May to early September revealed no variation in parasitemia among time periods, and thus no clear recrudescence. Mean proportion of gametocytes increased with the season, until mid-summer when it dropped, then began to increase again in late July. The majority of new infections were observed in late June which accounts for the drop in mean proportion of gametocytes. Thus, gametocytes eventually emerge in most infections, then increase in abundance over time. Date of first appearance of the parasites in blood of 26 lizards was observed (trophozoites appeared in the blood of previously non-infected lizards); these "new" infections were seen throughout the season. Jordan (1964) also found that new infections of *P. floridense* in *A. carolinensis* were common throughout the warm season in Georgia, USA, indicating continuous transmission.

This kind of vertical analysis is informative, but the great variation in the course of infection at Hopland requires that individual infections be followed over time. Sufficient information was available for a reliable estimate of growth rate of parasite populations in 18 rising infections. Infections that first became obvious in the blood early in the season had the slowest mean rate of increase ($r = 0.132$ day^{-1}, $N = 4$), those starting in mid-season had a faster rate (0.189, $N = 10$), and the late starters showed the most rapid growth (0.213, $N = 2$). These differences, however, were not statistically significant and, of course, sample sizes are small. However, this is the pattern expected if the infections follow a conditional behavior adaptive in a seasonal environment. Another 17 lizards scored as non-infected by late summer were recaptured the following spring and were infected by then. There was a significant positive relationship between the intensity of the spring infection and how late the previous year the animal was known to be non-infected. This suggests that those animals that become infected late in the season support higher parasite loads the next spring, a situation that would result if the late-starting infections experienced more rapid asexual reproduction.

The 65 infections followed in detail fell into three classes. Most (38 or 59%) maintained a stable level of parasitemia. In 26, parasitemia was observed rising, but in some of these, the infection levelled off to a stable

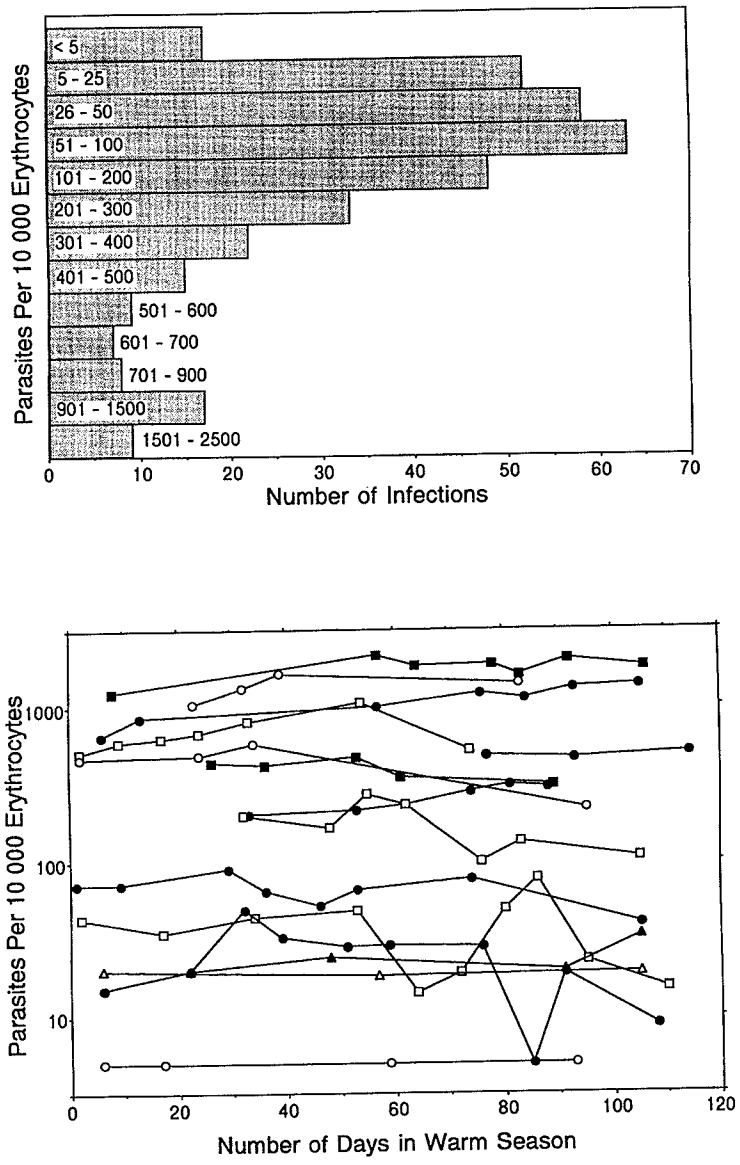


Figure 15 Plasmodium mexicanum parasitemia in natural infections form free-ranging fence lizards at the Hopland Field Station. Upper panel: distribution of parasitemia in 323 lizards when first sampled in the warm season. Lower panel: parasitemia for sample of constant infections followed over time. Each line represents a single lizard.

condition. Figure 15 shows examples of these infections. Only one infection dropped in density during the warm season. However, all 10 of the infections followed from one year into the next warm season dropped to low parasitemia. We were not able to follow these infections to see if they experienced a spring recrudescence as described by Ayala (above). Maximum parasitemia was related to body size; smaller lizards suffered higher maximum parasitemia (juveniles' mean parasitemia = 3.8% of erythrocytes infected; small adults = 1.3%, and the largest adults = 0.36%). However, there was substantial variation within each size group such that some adults, for example, had high-level infections. The infections shown in Figure 15 are all for adult lizards.

We were impressed with the variation in stable parasitemia seen among the infections; some lizards maintained very weak infections, whereas others had infections in which up to 18% of the erythrocytes were infected. This result clearly differs from all previous studies on lizard malaria, including Jordan's (1970) study on the same species (and it is also very different from what is seen in human malaria). Our study was unique in that it followed in detail the course of infection of free-ranging, naturally infected lizards. It is possible that the true reproductive strategy of the parasite becomes obvious only when its host experiences natural environmental conditions, including changing duration of daylight and average temperature.

Vertical analysis showed that gametocytes increase in abundance over time in the infections (above). Longitudinal observations revealed the variation seen among infections in the timing of this production of gametocytes. In 90% of infections (both stable in parasitemia or rising), the percentage of parasites that were gametocytes increased steadily in the infection to become eventually dominant in the parasite population. Such a pattern would be expected in a parasite in a seasonal environment in which its host has a strong likelihood of dying during the winter. A surprise came when we examined the available data that allowed an accurate estimate of the dynamics of initial gametocyte production for 11 new infections. In six of these, gametocytes appeared shortly after the infection was detected, and in some the proportion of gametocytes rose dramatically over only 7–19 days. In the other infections, gametocytes appeared only slowly, in one case not entering the blood for 78 days. The time from first detection of the infection until first appearance of gametocytes did not differ with date of the infection's origin. There was therefore no fixed schedule in which gametocytes would appear in infections.

Schall and Bromwich (1994) followed 61 natural infections of *P. agamae* and *P. giganteum* for 7–211 days in African rainbow lizards brought into a large laboratory pen (2.2 × 5.2 × 2.4 m). We attempted to build an indoor habitat that mimicked the natural world in light quality, tempera-

ture, humidity and hiding locations. Nonetheless, mortality of the lizards in the cage was high (only 23% of the infections could be followed for >50 days) indicating the environment was far from natural or optimal for the animals. All but three of the observed infections remained at constant parasitemia, including the animal kept for 211 days. Among the constant infections, parasitemia varied from barely detectable to infections >500 parasites/10000 RBC. Of these infections, 38 were solitary *P. agamae*, 10 were solitary *P. giganteum*, and 13 were mixed infections. Thus these data, although not ideal, show a pattern similar to that seen in *P. mexicanum*, that is, mostly constant infections over a wide range of parasitemia.

P. azurophilum is one of the more intriguing malarial parasites of lizards because it infects both erythrocytes and several classes of white blood cells (Telford, 1975). Ayala and Hertz (1981) studied this parasite on Martinique in the Lesser Antilles. They noted that there was complete separation of the erythrocyte vs white blood cell forms in different individual hosts. That is, infections with both red and white blood cells infected were not observed. We have examined this species in *Anolis* lizards on St Maarten (Schall, 1992) and on Saba (C. Staats, personal communication) in the eastern Caribbean and also noted the preponderance of "RBC only" and "WBC only" infections. On St Maarten I counted parasites in 31 infections and found that in 24 (77%) the parasite was seen only in either erythrocytes or two types of white cells, monocytes and neutrophils (terminology from Sypek and Borysenko (1988)). Ayala and Hertz (1981) wondered if perhaps two species of *Plasmodium* might be involved. This is unlikely because *P. azurophilum* has similar morphology in all cell types. Perhaps *P. azurophilum* could first infect one cell class (perhaps erythrocytes), then move into white cells. Ayala and Hertz noted an unusually large number of the cell types used by *P. azurophilum* in infected lizards. On St Maarten, the ratio of RBC/monocytes and neutrophils appears to favor the WBC classes as the infection grows (parasitemia vs. ratio, Spearman correlation, $r = -0.54$). The effect is most obvious when comparing non-infected *A. gingivinus* (144 RBC/WBC), animals with only RBC infected (213 RBC/WBC), and lizards with only WBC infected (96 RBC/WBC). These results suggest that *P. azurophilum* may first enter the RBC, then manipulates its host to produce more of its host WBC, or at least it may wait until these WBC are produced by the host.

5.3. Merozoite Number and Parasitemia

P. giganteum and *P. agamae* have very different reproductive phenotypes (Figure 2), with *P. giganteum* being a true giant among species of plasmodia. Despite the much larger number of merozoites produced by *P.*

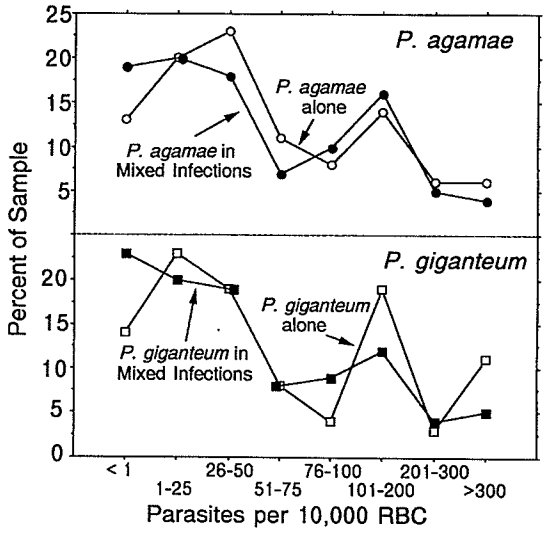


Figure 16 Distribution of parasitemia of two species of *Plasmodium* infecting the same host, the rainbow lizard, *Agama agama*, in Sierra Leone, West Africa. For each species, data are separated for infections in which that species occurs alone, and for those in which both parasites were present.

giganteum, this species does not produce consistently higher parasitemia (Figure 16).

5.4. Effects of Temperature

Every beginning biology student learns that temperature affects physiological processes; typically, over a range of tolerance, these processes increase with rising temperature in a predictable way (the Q_{10}). Biologists' intuition argues that temperature should influence the rate of development and cell division of malarial parasites. The effect of temperature on the parasite's stages in vectors has been studied exhaustively (an example is in Section 8 below). It is remarkable, though, that very few studies have examined the influence of temperature on the stages in the vertebrate host, especially as one of the striking features of malarial infection of humans is the onset of periodic elevations in body temperature. Perhaps fever in humans is an adaptation that acts to disable at least some kinds of pathogens (Kluger, 1979), including malaria.

An obvious difficulty exists for any manipulative studies of body temperature of mammal or bird hosts of *Plasmodium*: changing the thermal set-

point of an endotherm could seriously disrupt multiple physiological functions. Caldwell (1944) examined the thermal biology of *P. cathemerium* by another means. He removed infected blood from canaries and incubated the blood at various temperatures before inoculating the blood back into non-infected birds. *P. cathemerium* was shown to have a remarkable thermal tolerance, surviving when the blood was raised to temperatures as high as 47°C for 30 min. We have duplicated this study with *P. berghei* in laboratory mice (unpublished). The parasite survived in whole blood incubated for 30 min at 38°C and 40°C, and for 5 min at 44°C. Thompson and Winder (1947) demonstrated that lizard malaria provides a better model to study temperature effects on *Plasmodium* because the body temperature of ectothermal lizards is easily manipulated in the laboratory without any use of drugs. Their experiments showed that *P. floridense* infections grew faster at higher temperatures as expected. Thompson and Winder did not determine if infected lizards choose different body temperatures (via behavioral means) than non-infected animals (perhaps lower preferred temperatures might be expected).

I compared the body temperature of infected and non-infected lizards collected in the wild, by measuring body temperature immediately after capture with a rapid-reading thermometer. Body temperature distributions for *S. occidentalis* in California and *A. agama* in Africa did not differ for those infected with malaria and non-infected animals. For example, the mean for both infected ($N = 101$) and non-infected ($N = 244$) fence lizards was 35.5°C, and for both infected ($N = 278$) and non-infected (N = 525) *A. agama* the mean was 36.3°C. The thermal characteristics of perching locations of infected and non-infected fence lizards at Hopland also were similar. Naturally infected lizards put into laboratory thermal gradients had their body temperatures monitored and, again, infection status did not alter body temperature chosen. I conclude that lizard malaria does not result in changes in body temperature in infected animals (Schall, 1990b), and that lizards do not develop the kind of behavioral fevers in response to malarial infection that is seen in some lizards when exposed to pathogenic bacteria (Kluger, 1979).

I replicated the experiments of Thompson and Winder with *P. mexicanum* in fence lizards with unexpected results (Schall, 1990b). Lizards were artificially infected by blood transfer and then placed in cages within constant temperature chambers set at 20°C, 22°C, 25°C, 30°C, 32°C and 35°C. The warmest temperature approximately matched the mean body temperature of lizards in the wild. Lizards kept at a constant temperature above 35°C did not survive for the duration of preliminary experiments. The rate of increase of the parasite population did not differ among these treatments. Other lizards were maintained at 22°C for the first half of the experiment (45–50 days), then switched to 32°C chambers; again no

change in the population growth rate was observed. Some lizards (and their parasite population) were placed in constant temperature boxes at 25°C or 35°C, and periodically given heat shocks of 39–41°C for 3 h. Some lizards were treated once, and others up to eight times. No obvious effect on the parasite population growth rate was observed.

The complete lack of response in population growth rate of *P. mexicanum* to temperatures over a 15°C range reveals a remarkable thermal buffering ability by the parasite. Such buffering could well be useful to a parasite of lizards; their hosts' body temperature fluctuates widely during the course of each 24 h period from daytime basking periods to nocturnal resting at cooler temperatures. Also, lizards cannot inhibit the growth of the parasite by reducing their own body temperature. In addition to thermal buffering, the parasite also has a very high thermal tolerance (higher than that of the lizards because 41°C is very close to the lethal point for the lizards). Therefore, short periods of behavioral fever would be useless as an antiparasite tactic, and perhaps even dangerous for the lizard.

6. SEX RATIO OF GAMETOCYTES

Plasmodium produces gametocytes, or sex cells, in the vertebrate host's blood. As the entire malarial cell is either a microgametocyte (male) or macrogametocyte (female), we can consider these cells as individual male or female organisms. When taken up with the blood by a vector during its blood meal, a microgametocyte produces several mobile gametes. A gamete may enter a macrogametocyte to effect fertilization and production of a transient diploid cell. The sex-determining mechanism for gametocytes is unknown, but experiments demonstrate that gametocyte sex is not heritable (Walliker, 1976; Alano and Carter, 1990). This does not mean that there cannot be genetically based tendencies to develop into a micro vs macrogametocyte; that is, although any pre-gametocyte may develop into either a male or female cell, the probability of becoming one or the other could be genetically based. These details of the malarial life cycle are well known to malariologists. One important aspect, though, has been almost ignored by researchers: the factors that determine the ratio of male and female gametocytes in the vertebrate's blood.

It is surprising that published data on sex ratios of gametocytes are very scanty. I have surveyed many general review volumes on the biology of *Plasmodium* as well as a large number of more specialized papers on the course of infection. General discussion on sex ratio is almost absent in these works. Many authors mention that female outnumber male gametocytes in most infections, but quantitative data are not presented. The rare

counts of gametocytes presented in the literature almost always have been from few infections followed for short periods of time (review in Schall, 1989). The paucity of reliable data on the sex ratio of gametocytes derives from the difficulty in distinguishing gametocytes from asexual stages of some species under the light microscope and the similar appearance of immature male gametocytes to macrogametocytes (Schall, 1989).

The lack of interest in sex ratio in *Plasmodium* is perplexing because sex ratio theory is one of the most active and successful branches of evolutionary biology (detailed reviews are found in Charnov (1982) and Karlin and Lessard (1986)). This theory can be readily adapted to apply to malarial parasites as suggested first by Ghiselin (1974). Intuition might suggest that the sex ratio should favor females to maximize the number of unions of mobile gametes and the macrogametocytes (Scudo, 1967), and this should be a ratio of 1 male:K females, where K is the number of mobile gametes produced by a male cell. This outcome depends on natural selection working at the group level — that is, favoring the entire parasite population in its host. Although the notion of group selection is highly controversial, the consensus view among evolutionary biologists holds that group selection leading to adaptive changes requires special ecological circumstances (such as type of population structure). A more widely held view can be readily applied to sex ratio in malarial parasites; this view derives from Fisher's (1930) reasoning that in any population with unequal proportions of males and females, individuals of the rarer sex will produce more offspring on the average than individuals of the more common sex. Thus, frequency-dependent selection acting on individuals would lead to an equilibrium proportion of males and females of 1:1 (actually, an equal investment in males and females, but this detail of the theory is not relevant to *Plasmodium* gametocytes because each pre-gametocyte can produce only one adult sex cell).

A thought experiment reveals how Fisher-type selection would act on the gametocytes of *Plasmodium* in the vertebrate host. Suppose the proportion of viable gametocytes in an infection is one microgametocyte to eight macrogametocytes (1:8 ratio of males to females). If every macrogametocyte unites with a gamete (K = 8), then each male would have eight offspring cells, and each female could have only a single offspring (ookinetes = offspring cells). Thus, males would have eight times the fitness of females. Any pre-gametocyte in the vertebrate that has the genetic tendency to develop into a male cell would have higher fitness and would increase in the population. A symmetrical situation would hold if female gametocytes are more abundant in the population. Over time the proportion of males and females would fluctuate, reaching a stable equilibrium when the ratio of males to females is 1:1.

Hamilton (1967) suggested another mechanism driving the evolution of sex ratio. He noted that Fisher's argument depends on random mating among genotypes in the population. In situations with mating between relatives, the sex ratio should favor females. For example, in parasitic wasps in which a reproductive female places eggs into an insect host, the resulting offspring mate within the host. Thus, for maximal fitness, the adult wasp should manipulate the sex ratio to produce just enough sons to mate with all the daughters. This "kin selection" hypothesis can be applied to malarial parasites as well (Read *et al.*, 1992, 1995; Dye and Godfray, 1993). When the genetic diversity of gametocytes is high, and mating between related cells is uncommon, the Fisher ratio should be observed. When genetic diversity is low we should observe a ratio of 1 microgametocyte:*K* macrogametocytes. Note that the expected sex ratio under the kin selection model resembles the expected ratio under the group selection model when inbreeding is high. It differs, though, because it is the relatedness that is important in giving the expected result under kin selection. Read *et al.* (1992) derive the expected sex ratio of gametocytes under different levels of inbreeding by the gametocytes. Under the kin selection model the sex ratio could be locally adapted to the typical degree of inbreeding in that area (Read *et al.*, 1995) (which would be regulated by the number of vectors biting individual hosts) or the sex ratio could be molded within each infection based on the genetic diversity found in that infection (pre-gametocytes could monitor the infection before making the developmental decision to become a male or female gametocyte). Day *et al.* (1992) note that genetic data indicate single genotype infections in malaria are common and this may explain the frequent bias toward female gametocytes often noted for *Plasmodium.*

Some insight into these issues can be gained by inspecting the distribution of sex ratios among natural malarial infections. Figure 17 shows such distributions for three species of lizard malaria (in these species the adult male and female gametocytes can be readily distinguished). Mean sex ratio differs significantly among species (Schall, 1989; Figure 17). Also, there is considerable variation in sex ratio among infections in each of the three malaria species. Such broad variation does not support either the group selection or Fisher individual selection models which both predict uniform sex ratios (*K* females:1 male for group selection, and 1:1 for Fisher individual selection).

The distributions for *P. agamae* and *P. giganteum* support the kin selection model because over half of the infections are female biased in both species. However, female-biased infections are much more common in *P. giganteum* (67% of infections) than in *P. agamae* (53% of infections) suggesting that population structure of infections differs in these two malarial parasites such that self-crossing is more common in *P. agamae*

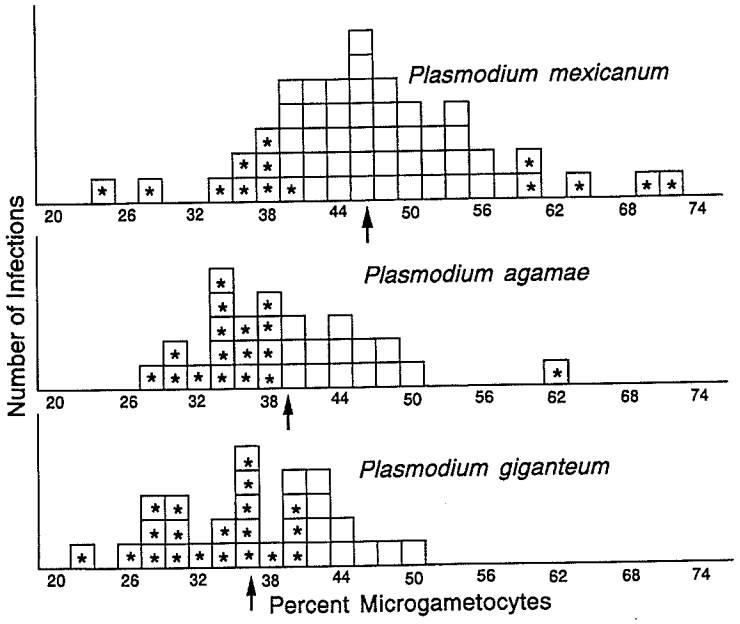


Figure 17 Sex ratio of gametocytes for infections of *P. mexicanum*, *P. agamae* and *P. giganteum*. Means are indicated by arrows and stars indicate a sex ratio significantly different from 1:1 (χ^2 tests).

(Read *et al.*, 1995). If so, this would be intriguing because both malaria species infect the same lizard host at our sites in Sierra Leone. The distribution for *P. mexicanum* is more difficult to interpret under any of the models; 74% of infections were not significantly different from a Fisher ratio of 1:1, but 17% were female biased, and fully 9% were male biased.

The weakness in using single spot checks on sex ratio shown in Figure 17 is that the history of the infection is unknown. Sex ratio could well be changing during the course of infection (especially if the parasites monitor the local genetic diversity of the population). To examine the sex ratio over time in individual infections, I monitored infections in lizards kept in the laboratory as well as infections of free-ranging lizards followed via a mark-and-release program. The results of this study (Schall, 1989) showed that sex ratio of *P. mexicanum* infections observed in the laboratory varied significantly more than those followed under natural conditions. Figure 18 shows a sample of infections, and Figure 19 a summary of data for many infections (both figures show only infections in free-ranging animals). In some infections the sex ratio varied randomly over time, while in others the sex ratio changed over a steady trend. The most common situation, though,

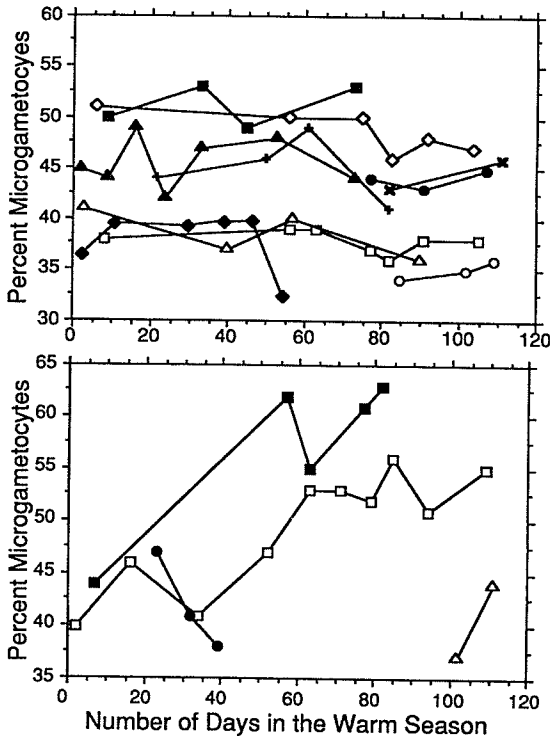


Figure 18 Gametocyte sex ratio of several infections of *P. mexicanum* over time. Upper panel shows some infections with a stable sex ratio, and lower panel shows changing sex ratio. The figures show that a stable sex ratio is more common and that sex ratio can change rapidly.

was a constant sex ratio (in some cases the sex ratio gradually changed to a final equilibrium value). Such constant sex ratios were observed in both infections that continued to grow (and produce more gametocytes) and those that had levelled off to constant parasitemia. Most striking was the variation in the sex ratio among those infections with a sex ratio that remained constant over time. The proportion of microgametocytes in such "constant sex ratio" infections varied among infections from about 35% to 55%. Thus, although there was an equilibrium sex ratio in many infections, the equilibrium ratio varied among infections. A smaller number of natural infections of *P. giganteum* and *P. agamae* were followed in captive rainbow lizards. Again, the sex ratios of infections in the laboratory often varied, but even in these infections the sex ratio differed substantially among infections. For example, in one infection the percentage males

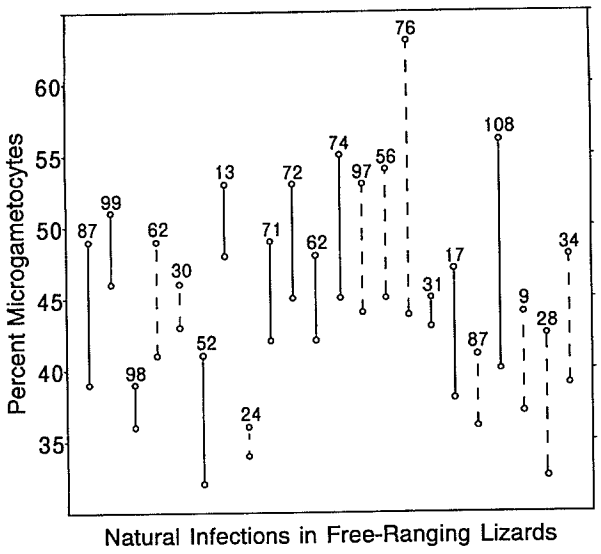


Figure 19 Range of gametocyte sex ratios of some infections of *P. mexicanum* followed over time in free-ranging lizards. Numbers indicate periods of time the infection was observed (in days). Infections in which parasitemia was rising or falling are indicated with dashed lines, and constant parasitemia with solid lines. There is no difference in tendency toward being stable vs changing sex ratio between infections with constant or changing parasitemia.

varied over time from 34% to 40%, while another infection varied from 39% to 48%.

These results could be explained if each infection can change to reach the sex ratio appropriate to the genetic diversity of parasites found in the infection (the kin selection model).

Both the group selection and kin selection models, but not the Fisher individual selection model, require that sex ratio and transmission success be correlated. That is, there is an optimal sex ratio for transmission of the parasite population to the insect vector. Intuition argues that sex ratio should be important for transmission, yet few data are available to test this assumption. Boyd *et al.* (1935) appear to have been the first to study the percentage of vectors infected when fed on infections with differing sex ratios of gametocytes. They concluded that sex ratio does influence transmission success in *P. vivax* but not in *P. falciparum*. Only a summary of the data are given in their paper and these data actually contain no hint that sex ratio influences transmission success of either parasite. For example, in *P.*

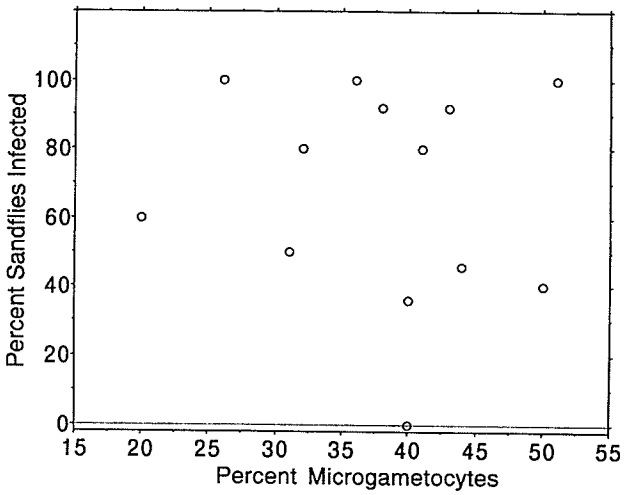


Figure 20 Effect of gametocyte sex ratio in *Plasmodium mexicanum* on its transmission success into its vector, *Lutzomyia vexator*. A similar pattern is seen when data on number of oocysts per sandfly midgut are plotted against sex ratio.

vivax the percentage of vectors becoming infected in different experiments ranged from about 51% to 73% for infections in which macrogametocytes were more common than microgametocytes, 55–57% when the sex ratio was 1:1, and 51% when macrogametocytes were less common than microgametocytes. Boudin *et al.* (1989) fed *Anopheles gambiae* on humans infected with *P. falciparum* with sex ratios ranging from females 1.7 to 5.6 times as abundant as males. In 72 experiments they found no correlation between percentage of the mosquitoes infected and sex ratio.

Using *P. mexicanum* and its vector *L. vexator*, I have done 16 experiments similar to those of Boudin *et al.* While this work is only preliminary (another 100 experiments were recently completed), I found no correlation between sex ratio and transmission success (Figure 20). Transmission success did not monotonically increase with increasing gametocyte density in the infections (Figure 21). Malariologists have known for many years that gametocyte density is not always a strong predictor of infectiveness to the vector (Pampana, 1969). There are many explanations for this phenomenon, but Pampana (1969) has proposed one that is relevant to hypotheses on sex ratio. The number of gametocytes in a blood meal from a heavy infection is much larger than the number of sites available for oocysts to develop. This predicts that transmission success will be nonlinearly related to gametocyte density: at low density the relationship

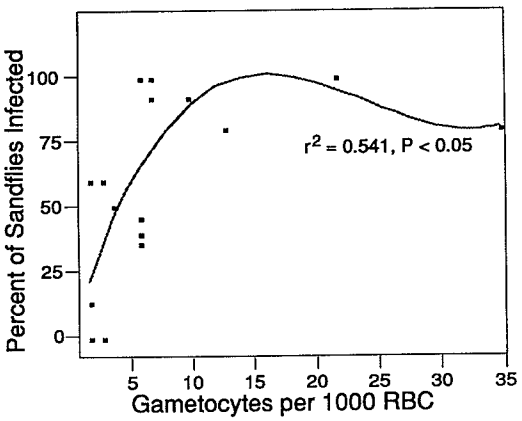


Figure 21 Effect of gametocyte density of *Plasmodium mexicanum* on transmission success into its vector, *Lutzomyia vexator*. Curve shown is a third degree polynomial fit to the data. The r^2 value shown was calculated for a similar fit to percentage data ARCSIN transformed. The complex relationship shown here is discussed in the text.

should be positive, but at higher densities there should be no effect. This is what is seen in Figure 21. Perhaps at high gametocyte densities in an infection, sex ratio is irrelevant to transmission success and group or kin selection could not mold the sex ratio and only Fisher individual selection would be functioning. In this context, it is interesting that the mean sex ratio of the three lizard malarias studied ranged from 37% to 47% (close to the 1:1 expected under the Fisher model), whereas the proportion for human malarias in the Read *et al.* (1992) study ranged from 14% to 18%, closer to what is expected under the group or kin selection models. Malaria parasitemia in lizards is typically of a much higher level than that seen in human infections (Bromwich and Schall, 1986).

7. INTERACTION BETWEEN MALARIA SPECIES

After we appreciate the great diversity of lizard malarias, and the coexistence of two to many species in one geographical region, we might expect that two or more plasmodia may infect the same species of lizard

host. We have found *P. azurophilum* and *P. floridense* infecting the same species of *Anolis* on a number of Caribbean islands, and *P. giganteum* and *P. agamae* in rainbow lizards in Sierra Leone. In Tanzania, Telford (1988b) found three species of malaria, *P. tanzaniae*, *P. uzungwiense* and *P. arachniformis*, all infecting the same individual chaemaeleon (*C. werneri*).

What kind of interactions might be expected when two or more plasmodia infect the same host? This is a special case of a more general issue, the debate over the factors that can shape parasite assemblages within their hosts (Esch *et al.*, 1990). Richie (1988) listed the kinds of interspecific interactions that might exist between species of co-existing plasmodia.

1. A neutral relationship with no effect of one species on the other.
2. Competition for resources (such competition could be reduced if the interacting species have evolved resource partitioning because of conflict in the past).
3. Interference via heterologous immunity.
4. One species may facilitate the establishment of the other by fortuitous suppression of the immune system or other alteration of the host.

To unravel possible interspecific interactions, the history of infections could be followed, either using natural infections brought into the laboratory or by observing experimentally induced infections. The usefulness of the first option is limited because the complete history of the infection cannot be known. The second option might appear the most desirable because presumed controlled infections are studied (and the order in which the species enter the host can be determined). However, the outcome of interspecific interactions in free-living species often has a strong stochastic component (the experiments of Park (1948) on competition between *Tribolium* beetles is a classic example), so very large sample sizes would be required to reveal the range of possible outcomes. Therefore, epidemiological surveys, with all of their problems, may best reveal patterns that suggest processes (Cohen, 1973; Molineaux *et al.*, 1980).

Schall and Bromwich (1994) studied *P. giganteum* and *P. agamae* in the rainbow lizard, *Agama agama*, in Africa in an attempt to understand what, if any, interactions occur between the two malaria species. The two plasmodia are found throughout the mesic tropics of Africa. Our own data and a search of the literature revealed that *P. agamae* sometimes occurs at a site alone. In no survey with a large sample size did *P. giganteum* exist alone; that is, it was always found with *P. agamae*. Malaria was found in the *Agama* at all of the 22 sites surveyed in Sierra Leone. However, a curious pattern emerged from this study. (Figure 22 illustrates the results for the 16 sites for which we had a large sample size of lizards.) Prevalence of malaria varied considerably among sites which suggests that

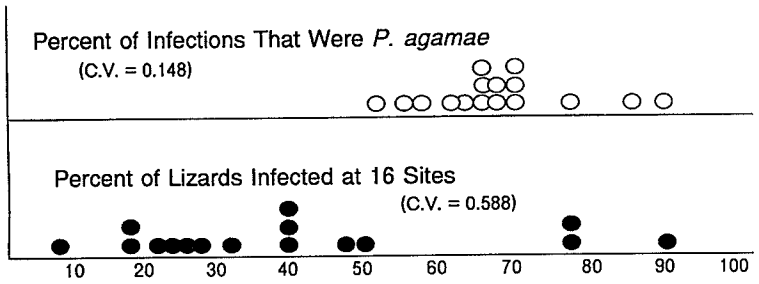


Figure 22 Natural history data for two species of malaria, *Plasmodium agamae* and *P. giganteum*, in *Agama agama* at 16 sites in Sierra Leone where sample sizes of lizards were large.

the various natural history factors that control abundance of malaria in lizards differ significantly at the sites. However, the relative abundance of *P. agamae* and *P. giganteum* was surprisingly similar at the sites. This hinted to us that the two *Plasmodium* species may not be ecologically independent. We next compared the number of mixed infections observed with the number expected if the two malaria species occur in hosts randomly. The percentage of mixed infections expected by chance is simply the product of the prevalence of each species (Cohen, 1973). Mixed infections were observed 2.3 times more often than expected in male lizards and 4.5 times more often than expected in females. Interspecific interactions that are mutually negative (competition for resources or interference) should result in negative association, and so these kinds of interaction are unlikely in this system.

Neutralism could result in an apparent surplus of mixed infections if the two malaria species share the same vector. Thus, *P. giganteum* and *P. agamae* would not be independent in transmission dynamics as assumed by the analysis. In this case the surplus of mixed infections should be found in adult hosts which have had a greater time to be bitten by vectors carrying both plasmodia. However, we found that the surplus of mixed infections was present for all age classes of *Agama* with no trend with age. A second possibility is that some individual lizards are more susceptible to malarial infection than others and the actual proportion of mixed infections expected by chance would be greater than that calculated using the entire sample of lizards examined (Cohen, 1973). That is, if some lizards are not susceptible to infection, the real host population of interest is smaller than the one sampled. This problem is present for any study of patterns of coexistence of parasites. We concluded that the positive association of *P. giganteum* and *P. agamae* represents the result of a real interaction between

the two species because the surplus of mixed infections remains significant even if we assume up to 85% of the non-infected animals are resistant to infection (that is, only 15% of non-infected lizards are assumed to be susceptible to infection). Recall that the surplus of mixed infections for the smallest size class of lizards is at least as great as for that seen in the overall population, yet the percentage of lizards infected approximately doubles from these small lizards to the largest, or oldest, class. Therefore, it is impossible that a very large proportion of the smaller lizards were immune to infection.

The similar ratios of *P. agamae* and *P. giganteum* at all sites, the positive association between the two species in individual hosts, and the lack of sites in Africa where only *P. giganteum* is found, all suggested to us that *P. agamae* may be acting to facilitate the initial establishment of *P. giganteum* in a rainbow lizard. We found that *P. giganteum* has a predilection for immature red blood cells (Garnham (1966) also noted this preference for such cells). Immature red cells are rare in non-infected lizards (see Section 9.1 below) but become more common after infection. *P. agamae* would always have a plentiful supply of its preferred cell host (99% of red blood cells are mature in hosts not yet infected and at least 50% for infected lizards). *P. giganteum*, however, will find relatively few of its preferred cell if it enters a non-infected *Agama*, but 10–50 times that number if it enters a lizard already infected with malaria. Thus, we concluded that *P. agamae* may be facilitating the entry of *P. giganteum*. The kind of facilitation proposed here concerns the chance of an infection becoming established, not its ultimate density. This was clear when we compared parasitemia for each species when alone in a host and when in a mixed infection (Figure 16). In 437 solitary infections of *P. agamae*, mean parasitemia was 85/10000 RBC. In 264 mixed infections the mean was 77.6. Mean parasitemia in 73 solitary infections of *P. giganteum* was 118.8 and in mixed infections, 75.4. (Note the rarity of solitary *P. giganteum* infections as expected when facilitation occurs — this would explain why *P. giganteum* may have difficulty existing at a site alone.) There was no significant difference between parasitemia of mixed and solitary infections for either species of *Plasmodium*. In fact, the total parasitemia in mixed infections (217/10000 RBC) approximated the sum of the mean parasitemia for solitary infections of the two species (204/10000 RBC).

Most studies done on interspecific interaction among parasites concern intestinal helminths (Esch *et al.*, 1990). The earlier studies by Cohen (1973) and Molineaux *et al.* (1980) argue that blood parasites, which elicit stronger host responses, can result in more complex, and even unexpected, interactions. The results on *P. agamae* and *P. giganteum* support this notion and suggest that additional studies on assemblages of malaria

species will provide results of great interest to general ecologists as well as malariologists.

8. VECTOR BIOLOGY

Ayala and Lee (1970) were the first to describe a vector of any lizard malaria: two sandflies, *Lutzomyia vexator* and *L. stewarti* in northern California. These workers discovered sandflies naturally carrying what appeared to be malaria oocysts on their midguts and were able to pass *P. mexicanum* to sandflies that fed on malarious fence lizards (Ayala, 1971). The competency of the sandflies as vectors for *P. mexicanum* has since been demonstrated in the laboratory (Klein *et al.*, 1987; Fialho and Schall, 1995). These sandflies became the first known non-mosquito insect vector of any *Plasmodium.* The California vectors of *P. mexicanum* were discovered by accident (the presence of malaria oocysts was noticed during research unrelated to lizard malaria; J. Anderson, personal communication). Other workers have systematically surveyed for the vectors of various common lizard malarias with only limited success.

Klein *et al.* (1987) sampled mosquitoes coming to lizard baits in traps and found one, *Culex erraticus*, to be the likely vector of *P. floridense* in Florida, USA. Oocysts developed to maturity in *Cx. erraticus* and the parasite was passed to non-infected lizards by bites of experimentally infected *Cx. erraticus*. In other studies, a small number of a potential vector developed oocysts, but complete development did not follow (*P. agamae* in Africa in the mosquito *Culicoides nubeculosus* (Petit *et al.*, 1983), *P. floridense* in *Aedes aegypti*, *Cx. territans* and *Cx. quinquefasciatus* (Jordan, 1964), and *P. azurophilum* in an allopatric sandfly (Schall, unpublished data)). Kimsey (1992) conducted an exhaustive survey for the vector(s) of *P. balli* and *P. fairchildi* in a common forest anole, *A. limifrons*, in Panama. *A. limifrons* is a short-lived lizard (probably < 1 year) and up to half of the lizards are infected at some sites. Therefore, malaria transmission must be intense and the vector common. Kimsey found that the only common lizard-feeding sandfly in his study area was *Lutzomyia trinidadensis*; mosquitoes were much more rare. Yet, the sandflies rarely feed on *A. limifrons*. Kimsey's study is another example, well known to malariologists, that finding vectors for even a common *Plasmodium* can be extremely difficult.

L. vexator and *L. stewarti* in northern California spend daytime hours in the burrows of the ground squirrel, *Spermophilus beecheyi* (Chaniotis and Anderson, 1967), where they also oviposit on ground squirrel feces. The summer daytime hours are too hot and dry for the sandflies to tolerate.

Therefore, the vectors are completely dependent on the rodent burrows for their hiding places as well as for reproduction. This dependence on the burrows makes the sandflies relatively easy to sample (compared to the effort required to quantify abundance of mosquitoes!). Also, the sandflies can be kept and cultured in the laboratory (methods described in Fialho and Schall (1995)).

The number of sandflies leaving rodent burrows was monitored for two seasons at the Hopland Field Station. In the laboratory, generation time (egg to egg) for the sandflies is approximately 1 month which suggests that several generations are possible during the warm season in northern California (J. Schall and J. Bliss, unpublished data). This is supported by the capture data (Figure 23); there is a general increase in numbers of sandflies leaving rodent burrows over the course of the spring and summer. Figure 23 also shows that the number of sandflies leaving burrows fluctuates within this broad seasonal trend. We sought an explanation for these fluctuations (Schall and Marghoob, 1995). Each night the relative humidity, cloud cover, wind speed and temperature were measured at the time we emptied traps set over the entrances to the ground squirrel burrows. After holding date constant in the analysis, only temperature was correlated with number of sandflies active; few of the vectors were active when air temperature was below 16°C. This suggests an explanation for the absence of lizard malaria at higher elevations at Hopland if the vectors are able to be actively seeking blood meals on relatively few nights when air temperature is above 16°C. (Section 4.2).

Temperature could have another important impact on the parasite: cooler temperatures may prevent it from completing development before the vector takes its last blood meal. Anderson and May (1992) reviewed the literature on mosquito vectors and concluded that " . . . the life expectancy of mosquitoes under field conditions is often very short . . . and of not dissimilar magnitude to the latent period of infection [development within the vector]." Estimates of the probability of a mosquito living long enough after feeding on blood to be a successful vector for the parasite range from 80% (Macdonald, 1956) to as low as 0.001% (Macdonald, 1956; Rodriguez *et al.*, 1992). Malariologists have long suspected that some anopheline mosquitoes are poor vectors of malaria because of the delayed maturation of the parasite in those species and the low probability of the insect living long enough for the parasite to reach maturity (Pampana, 1969). Even for those species that are competent vectors of malaria because their survival is high, most individuals do not live long enough to take more than two blood meals, and natural selection should favor any parasite genotype that allows rapid development in the insect.

With this scenario in mind, Fialho and Schall (1995) examined the relationship between temperature and the transmission biology of *P.*

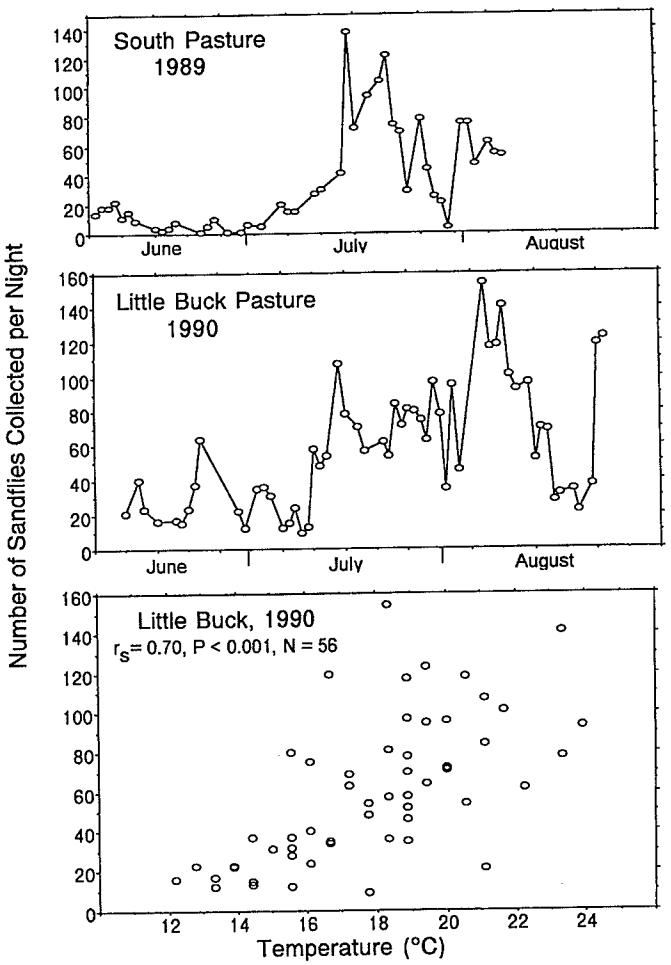


Figure 23 Top two panels: number of *Lutzomyia vexator*, the vector of *Plasmodium mexicanum* in California, collected each night from approximately 70 traps set over rodent burrows. The vectors become more abundant over the course of the warm season at the site, but considerable variation exists among sequential nights. Bottom panel: effect of temperature on number of vectors leaving the burrows. Temperature is the primary cause of the variation seen among nights in vector activity. Second panel from Schall and Marghoob (1995), © *Journal of Animal Ecology*, used with permission.

mexicanum in *L. vexator*. There is strong gonotrophic concordance in *L. vexator* in which each blood meal is used to produce a clutch of eggs with no supplementary feeding between reproductive episodes (Chaniotis and Anderson, 1967, 1968). Mortality of *L. vexator* after oviposition is very high; perhaps 98% of the sandflies do not live to take another blood meal. Thus, for successful transmission, *P. mexicanum* must complete its development in the sandfly before the insect takes its next blood meal because, to a first order of approximation, the next blood meal will be the vector's last. We determined the duration of parasite development in the vector, the rate of development of the insect's eggs, the sandfly's survival to egg laying, and percentage of sandflies becoming infected at a range of temperatures that mimicked the temperatures experienced by the insects in rodent burrows. Burrow temperatures were determined by constructing artificial burrow systems and by pushing a thermocouple probe into actual rodent burrows (the frequent presence of rattlesnakes in the natural burrows made this last type of measurement difficult to obtain!). Temperature preference of the sandflies was measured in a thermal gradient (temperature of the very small sandflies was assumed to equal that of the substrate on which they remained after exploratory flights about the gradient). We believe this was the first time the temperature preference of a small flying insect had been measured in the laboratory. Temperature preference was determined for unfed female sandflies, sandflies after feeding on blood from an non-infected lizard, and sandflies after feeding on infected blood.

Figure 24 (from Fialho and Schall, 1995) summarizes the results. The development time of the vector's eggs is constant over a broad range of temperatures (22–32°C in the experiments). However, temperature strongly influences the parasite's development time. Because the curves for the vector's egg development and the parasite's development differ in shape, an increase in temperature above 21°C would not be beneficial to the insect (nor harmful up to 32°C), but temperatures below about 25°C would prevent the parasite from being ready for transmission when the vector was ready for its next blood meal (provided the sandfly survived oviposition). Temperatures above 25°C would allow more rapid development of the parasite. Thus, if the sandflies behaviorally regulate their temperature to below 25°C, their eggs would mature at their maximal rate, but the parasite would not finish its development before its vector takes another blood meal.

Unfed sandflies chose a body temperature well below the minimum needed for the parasite to complete development. Sandflies feeding on a non-infected lizard raised their body temperature, presumably to increase the rate of digestion, but again the temperature chosen was below the minimum required by the parasite. Sandflies feeding on an infected lizard raised their temperature even more and this temperature just reached the

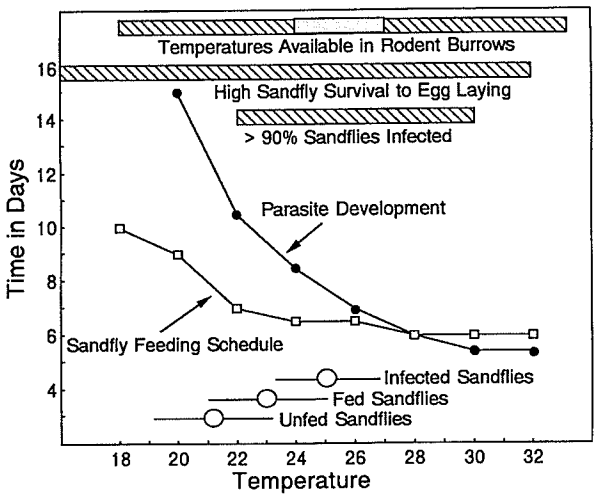


Figure 24 Effect of temperature on vector of *Plasmodium mexicanum* and the parasite's development. Sandfly feeding schedule is the estimated number of days between blood meals taken by the vector. Parasite development is the estimated number of days needed for *P. mexicanum* to produce mature sporozoites after the vector feeds on infected blood. Stippled area on top bar shows range of daily temperatures observed in burrows at least 0.5 m deep. Mean and SD shown for body temperatures chosen by the sandflies after three feeding treatments. Figure reproduced from Fialho and Schall (1995), © *Journal of Animal Ecology*, used with permission.

acceptable range for *P. mexicanum* to produce sporozoites in time for the vector to take its next blood meal. We suspect that the change in temperature preference by sandflies feeding on malarious blood represents an adaptive manipulation of its host by *P. mexicanum*. Published examples of proposed parasite manipulation of host behavior should be viewed skeptically (Yan *et al.*, 1994) and we are cautious in making strong claims for the effect seen in these experiments. If the change in host behavior really is driven by the malaria parasite, this would be the first record of a manipulation of its vector's thermoregulatory behavior by *Plasmodium*. Note that the body temperature chosen by the sandflies in the laboratory gradient matches the range found in the rodent burrows which suggests that the sandflies could readily thermoregulate in the complex burrow systems available to them in nature.

These results suggest that *P. mexicanum* uses a vector whose normal body temperature may be at the lower limit tolerated by *Plasmodium* in its insect host. The effect of temperature on the sporogonic cycle of 10 other species of *Plasmodium* are shown in Figure 25 with data for *P. mexicanum*

included for comparison. *P. mexicanum* has accelerated development compared to all other malaria species except for *P. berghei*, a parasite of mosquitoes that live in cool, closed forests. We assume that this rapid development of *P. mexicanum* has evolved to counter both the rapid development of eggs in sandflies at fairly low temperatures and the high mortality of sandflies after oviposition. The rapid development of *P. mexicanum* may be pushing the limit for *Plasmodium*. Only six of the 197 infections studied and represented in Figure 24 appeared to be mature earlier than indicated by the curve (and none were later). Four were mature 12 h early and two were mature 24 h early. Such low variation in phenotype suggests that little genetic variation for development rate exists in the insect population — exactly what would be expected if selection very strongly favors rapid development.

9. VIRULENCE IN THE VERTEBRATE HOST

The evolution of pathogen virulence is among the oldest problems in parasitology, yet it remains one of the most difficult and controversial. A classic argument holds that well-adapted parasites that have co-existed with

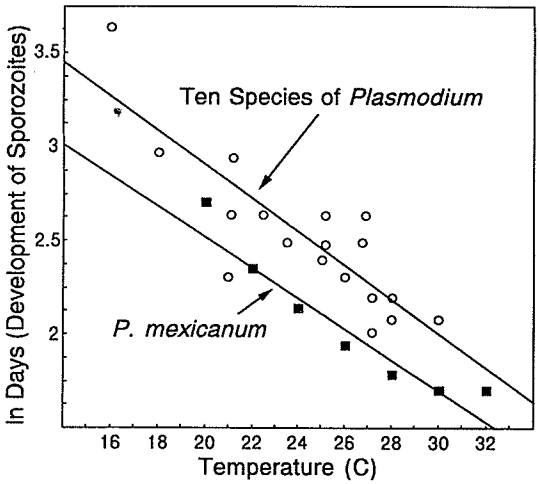


Figure 25 Development time of 10 species of *Plasmodium* of birds and mammals compared to development of *P. mexicanum*. Figure adapted from one in Fialho and Schall (1995), © *Journal of Animal Ecology*, used with permission.

their hosts for long periods should evolve toward low virulence either to keep the individual host alive and therefore to allow longer transmission (Burnet and White, 1972), or to maintain a healthy host population (Telford, 1971). This view requires that selection act at the group level rather than on individuals as envisioned by most evolutionary theory. More recent theory argues that virulence depends on the transmission biology of the parasite with vector-borne species being more virulent than those requiring the host itself to be mobile (Ewald, 1994).

A simple, general theory of the evolution of virulence may be impossible because of the great diversity of life cycles seen in parasites. For example, adult tapeworms in the alimentary tract of vertebrates do not reproduce in that host but cast eggs out in the feces. Therefore, the fitness of the parasite depends on its egg production which is dependent on lifespan. We might expect such parasites to evolve traits that reduce their cost to the host. Note that the kind of costs that are relevant to this discussion are those that reduce the host's lifespan, and not necessarily the host's fitness. A parasite might well evolve to reduce its host's fitness (by castration, for example) if this allows the host to live longer or provide more nutrients for the parasite to turn into eggs. The optimal phenotype for a malarial parasite within its vertebrate host is not as obvious. Selection acts on these organisms at two points in the life cycle: during asexual reproduction in the blood (more rapid or prolific reproduction would be favored) and to increase transmission (reduce asexual reproduction to produce gametocytes and also to keep the host alive for frequent transmission events). Parasite clones that reproduce rapidly in the vertebrate and produce few gametocytes would die with the host and rarely be transmitted to another host. However, clones that are "prudent" and reproduce slowly and produce many gametocytes could be quickly, and vastly, outnumbered by the fast reproducing clones. Intuition suggests that selection will balance out these two competing strategies and that the result will be parasites of intermediate virulence.

Another issue that is rarely considered in the theoretical discussions of parasite virulence is the actual origin of pathology induced by parasites. Malariologists have long known that the relationship between parasitemia and the symptoms of the disease in humans is weak at best (Covell, 1960).

A major goal of our work with lizard malaria has been to describe the costs of infection for the lizard host. Lizards might represent the original vertebrate host of *Plasmodium* (Manwell, 1955) and some existing lizard malaria parasite–host associations appear to be ancient. Recall that *P. mexicanum* occurs in a disjunct distribution in fence lizards in North America that was established in the Pleistocene. Likewise, the plasmodia of *Agama agama* are found throughout mesic tropical Africa including some disjunct mesic mountain sites that have been separated from the rest of the parasite's distribution since glacial times (Schall and Brom-

wich, 1994). Therefore, these systems can be examined to test the hypothesis that old parasite–host associations should evolve to a benign state. Also, the reproductive strategies of the plasmodia of lizards are very diverse (Figure 2) which might result in some species producing very rapidly growing infections compared to others. We have examined the cost of infection in detail for *P. mexicanum* in *S. occidentalis*, the western fence lizard, with comparative studies on *P. azurophilum* in the anole, *A. gingivinus*, on St Maarten island in the Caribbean, and two malaria species in the rainbow lizard, *A. agama*, in Sierra Leone. One of these, *P. giganteum*, produces about 100 merozoites per schizont and the other, *P. agamae*, yields about eight, thus placing them at opposite ends of the spectrum shown in Figure 2. We are now in the process of a major study of the costs of infection of *P. azurophilum* and *P. floridense* that both infect *Anolis sabanus* on the tiny island of Saba in the Netherlands Antilles. Again, *P. azurophilum* produces a large number of merozoites and *P. floridense* produces far fewer. The results of our studies are presented in this section.

9.1. Blood Pathologies and Exercise Physiology

A primary pathology in malarial infections in vertebrates is the destruction of erythrocytes and the concomitant production of immature RBC. In mammals and birds a significant fraction of the anemia associated with malarial infection seems to result from the host's immune system which destroys both infected and non-infected erythrocytes. The resulting influx of immature RBC occurs even in weak infections. This same effect occurs in lizard malaria infections. Scorza (1971a,b) and Scorza *et al.* (1971) showed that *P. tropiduri* infections are characterized by elevated numbers of immature RBC which differ biochemically from mature cells. This effect is now well documented for lizards infected with various species of *Plasmodium* (Ayala, 1970; Pienaar, 1962; Ayala and Spain, 1976; Schall *et al.*, 1982; Schall, 1990b, 1992). Counts of immature RBC show that non-infected lizards typically have 0–2% immature cells, whereas the mean for infected lizards ranges from 5% to 9%, with results for some individual infected animals reaching 50% (Schall, 1990b, 1992). *P. azurophilum* infections in *Anolis gingivinus* can exploit either RBC or two classes of white cells. When an infection consists only of parasites in RBC, the immature RBC count increases, but when in only WBC, no change in immature cell abundance is noted (Schall, 1992).

Hemoglobin concentration in the blood of infected animals is reduced compared to non-infected lizards. This reduction ranges from 11% to 45% depending on species of parasite and gender of lizards involved (Schall *et*

al., 1982; Schall, 1990a,b). This is not a result of any reduction in the number of RBC present (direct counts and hematocrit measures showed no difference between infected and non-infected fence lizards), but due to a lower amount of hemoglobin in the immature cells. The percentage of RBC that are immature is negatively correlated with blood hemoglobin concentration for *P. mexicanum* in fence lizards and *P. giganteum* and *P. agamae* in *Agama*. The percentage of immature RBC is not correlated with parasitemia (Schall, 1983a); as expected, parasitemia and hemoglobin levels are also not correlated.

Another interesting difference is noted in the blood chemistry of *A. gingivinus* infected with *P. azurophilum*. When the parasite infects monocytes and neutrophils, these cells contain reduced levels of acid phosphatase (Schall, 1992). Acid phosphatase is present in 68% of the non-infected white cells, but in only 38% of the infected ones. Perhaps the parasite manipulates the cells to cease production of this enzyme, or the parasite may enter primarily immature white cells that have not yet begun to produce the enzyme.

A reduction of 11–45% of hemoglobin in the blood of infected lizards should have important consequences for the host's ability to deliver oxygen to tissues. The activity of lizards during short bursts of effort is driven by anaerobic respiration, whereas longer bouts of locomotive effort are maintained by a combination of aerobic and anaerobic means and most lizards reach exhaustion after only a minute or two of maximal effort (Bennett, 1983). As might be expected, resting oxygen consumption was not measurably different for infected and non-infected lizards because oxygen use is very low for resting reptiles such as lizards (about 0.5 ml/g·h corrected to STP conditions). Maximal oxygen consumption of lizards was measured by inciting laboratory animals to continue running for 2 min periods in a chamber and then measuring their oxygen use. Maximal oxygen consumption was significantly reduced in *S. occidentalis* infected with *P. mexicanum* (39% reduction) and *A. agama* infected with *P. giganteum* and *P. agamae* (17% reduction). Blood hemoglobin concentration and maximal oxygen consumption of fence lizards is strongly positively correlated ($r = 0.68$, $P < 0.01$) and the data for both infected and non-infected lizards fall on the same regression line (Schall, 1990b). Therefore, the reduced maximal oxygen use by infected lizards is primarily a consequence of the reduction in hemoglobin carried in the blood.

Sprint running speed was determined by chasing individual fence lizards down a 2 m long track outfitted with electronically controlled timers (Schall *et al.*, 1982). As we expected (because sprint running is anaerobically maintained), no difference in sprint speed was seen in infected and non-infected lizards. Running stamina was measured for *S. occidentalis* and *A. agama* by placing them in an oval track in a constant temperature

room set at their preferred body temperature (determined from data from body temperatures of field animals discussed in Section 5.4) and chasing them around the track at their maximal running speed. Most lizards' running speed decreased during the 30 s of being chased and some ceased running before the period was completed. Distance run for infected animals was reduced for fence lizards (20% reduction) and the *Agama* infected with *P. agamae* (15%) or with mixed infections of *P. agamae* and *P. giganteum* (21%).

These results show a cascade of detrimental effects of malarial infection in lizards: infection initiates a destruction of mature erythrocytes and an influx of immature RBC into the peripheral blood that reduces the hemoglobin concentration of the blood. Oxygen transport is consequently disrupted which does not affect sprint running speed, but does reduce the running stamina of the infected lizards.

9.2. Reproduction

Many species of temperate zone lizards store fat during the warm activity season in the form of inguinal fat bodies. The stored energy is used by the lizards to survive extended periods of dormancy in the winter and, for females, to produce eggs the next spring (Hahn and Tinkle, 1965; Schall, 1978). Fence lizards at the California site infected with *P. mexicanum* stored less fat than non-infected animals by the end of the warm season (22–45% less for males for two sampling years, and 20–32% less for females; Schall, 1983a,b). For females the decrease in fat stored when infected translates to 6325 J one year and 4420 J the next which equals the energy in 1.46 and 1.02 eggs (Schall, 1983b). Thus, infected animals should produce smaller clutches of eggs the next spring.

Fence lizards produce one, and rarely two clutches of eggs during the spring and summer; clutch size increases with body size. Figure 26 shows the relationship between clutch size and body size for malarious and non-infected fence lizards. Infected females produce smaller clutches of eggs, and the reduction equals approximately 1–2 eggs as predicted by the fat body analysis. Thus, malaria in fence lizards reduces the fitness, or reproductive success, of female hosts by about 20%. Several measures of egg quality were also compared between infected and non-infected female fence lizards (egg mass, percentage of eggs hatched, size of young at hatching, and time taken to hatch in lab, all in laboratory trials). None of these measures differed for the two groups of lizards.

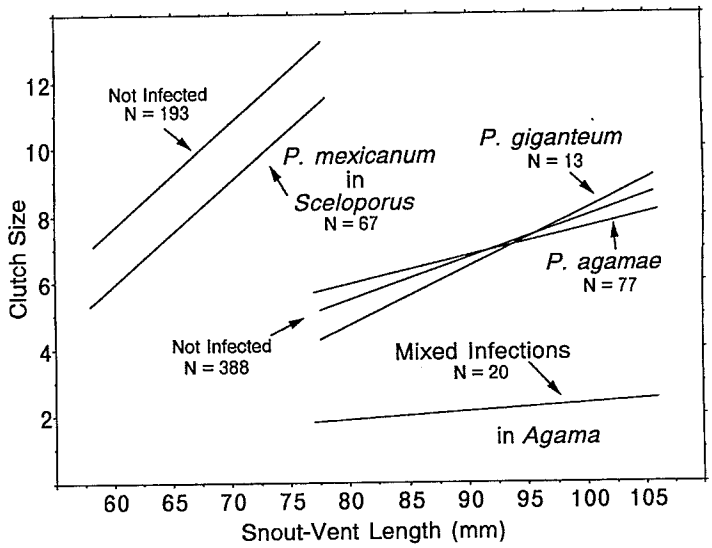


Figure 26 Clutch size of two species of lizards, comparing females infected and not infected with malarial parasites.

Agama agama in Sierra Leone store very little fat over any time of the year (Schall, 1990b) because of their tropical environment. Figure 26 shows that clutch size is not affected by infection with *P. agamae* and *P. giganteum* in these animals. *A. agama* in Sierra Leone produce multiple clutches of eggs throughout the year. I suspect that malarial infection in these animals also reduces energy and other resources needed for reproduction (as infection with *P. mexicanum* does in California fence lizards). If so, the effect would be a lengthening of the time between clutches (more time is required by the lizards to acquire the necessary resources to produce a clutch of eggs).

The effect of malaria on reproductive success in male lizards is more difficult to determine (in general, measuring reproductive output of any male animal is challenging). However, testis size of infected fence lizards in California was about 37% smaller than non-infected males (Schall, 1983b) for two annual samples in the late warm season. The reproductive consequences of this reduction are not known but it is reasonable to guess that larger testes may produce more sperm or reproductive hormones during the reproductive season. Again, a similar effect was not seen in *A. agama* in tropical Africa. Testis size did not differ between malarious and non-infected male lizards (Schall, 1990b).

9.3. Behavior

The disruptions of physiological processes and behavioral performance that were observed in the laboratory in malarious *S. occidentalis* suggested that infection may alter the behavior of fence lizards under natural conditions. For example, any vigorous activity that is supported aerobically, such as flight from predators or intense social interactions, including courtship, could be affected to the detriment of the host. (Despite the abundance of parasites, studies on animal behavior rarely consider the consequences of parasitic infection.) We conducted a study of the behavior of fence lizards at the Hopland site (Schall and Sarni, 1987). We had three questions in mind.

1. Do infected and non-infected lizards partition activity time in different ways? Perhaps infected animals are less likely to pursue prey or conduct other rapid movements.
2. How often do the fence lizards maintain vigorous activity for periods of time long enough for aerobic respiration to become important? Recall that short bursts of activity are anaerobically maintained and are not influenced by malarial infection.
3. Do sprint runs occur often enough so that the rate of recovery would become a limiting factor for the infected animals?

We collected lizards at Hopland, took a blood smear, then released the animals with an identifying number painted on the animal's dorsum. Infection status of the lizards was not determined until after the observation period (May through July). Each day, upon entering the study site, the observer would locate a lizard through binoculars, then watch the lizard for 5 min periods (maximum of three consecutive periods for any lizard that day). Behaviors were recorded continuously during the 5 min periods, then later transcribed into data books (146 lizards were eventually observed, with no bias in number of times infected vs non-infected animals were watched). *S. occidentalis* are "sit-and-wait" predators that spend over 90% of the daylight hours perching without moving while scanning the habitat (Schall and Sarni, 1987). During 1202 5 min observation periods (100 h total time), 599 runs were observed, or 6 h^{-1} observation. We were able to measure the length of 419 runs (by recording landmarks for the run that could later be located for measuring with a tape); a third of the runs were under 0.5 m in length, only 8% were over 3 m, and the longest was only 6 m. As lizards in the laboratory run at a velocity of 1.4 $m^{-1}s$ (Schall *et al.* 1982), most runs in the field lasted less than 1 s and the longest was only about 4 s.

Recovery from short bursts of activity was measured in the laboratory. Lizards were placed in small glass chambers in which oxygen content was

measured continuously. The animals were incited to run for short bursts (running was in place on the slippery glass surface), then the change in oxygen consumption was monitored to determine a return to normal levels. Oxygen consumption increased dramatically after runs lasting between 3 and 10 s in the laboratory apparatus (about a 3-fold increase; Schall and Sarni, 1987). Recovery time ranged from 4 min for a run of 4 s, to 6 min for a run of 10 s. Thus, recovery for any of the hundreds of runs observed in the field should have lasted for only a few minutes. As runs were rare, malarial infection is unlikely to be important in altering a lizard's ability to flee from predators or other lizards, or to capture food.

Because the fence lizards rarely move, the behavioral time budget for infected vs non-infected animals is best compared by examining only the "active" behaviors. As expected, behaviors that involve short bursts of activity (eat, walk, run to food, other runs, etc.) did not differ between the two groups of lizards. However, one kind of behavior that requires sustained activity, and would be aerobic, is social interaction. We observed some social behaviors that lasted over 8 min and this activity appeared very strenuous (head and body bobbing, spiral runs, etc.). Social behavior was seen during only 47% of the 5 min observation periods of infected male lizards, but 74% of observation periods of non-infected males revealed social activities. The mean length of the social activity was 25.6 s for infected males and 43.3 s for non-infected males. We concluded that the decreased aerobic abilities of infected animals results in their being less willing to engage in extended, vigorous social interaction. This should reduce the ability of male fence lizards to maintain territories, rebuff other males, and court females.

This suspicion was upheld in two subsequent studies. In one (Schall and Dearing, 1987), two adult male lizards, one infected and one not, and matched for size and ventral color pattern, were placed into a 4.9 × 4.9 m enclosure with an adult female. These pens were placed out of doors at Hopland. The pairs of lizards (N = 17) were watched for a total of 155 h (range for the 17 pairs was 3.9–11.0 h). The lizards were individually marked with dots of liquid paper; infection status of the lizards was unknown to the observer. A lizard in each pair was ranked as the "winner" (from 1 = weak win to 3 = strong win) based on number of times each lizard chased or was chased by the other, number of aggressive head bobs given, and number of times a female was approached. In our judgement of winning males, 12 were non-infected and five were infected with *P. mexicanum*. Of 10 wins judged "strong", nine were by non-infected lizards. Non-infected males were more likely to display to the other male or to the female. Parasitemia was correlated with the result of the outcome for infected males (ranking them as −3 for a strong loss to +3

for a strong win). Thus, infected males were less able to respond to the other male and less likely to court the female present in the enclosure.

A similar study was then conducted with free-ranging animals (Schall and Houle, 1992). Male lizards were collected, a blood sample taken, and they were individually marked with numbers, then released back at the point of capture. The lizards were then observed each day, without knowledge of their infection status. We observed 34 lizards during May–July. There was no difference for malaria-infected vs non-infected male lizards in the minimum number of days the lizards were known to remain at their original site, the number of days they were seen at the site, and home range size. However, non-infected animals were observed more often by the observers and were observed to chase other lizards more often. Thus, they were more active. At the end of the season, each lizard was subjectively ranked as submissive or dominant based on the number of times it was observed to chase other lizards, or be chased in turn. All 17 of the animals ranked as dominant were not infected. Ten of the 17 submissive lizards were infected. Again, this shows that infected animals have difficulty in maintaining territories and having access to females and this presumably reduces their reproductive fitness.

9.4. Showy Male Traits

In many species of animals, the males display some extravagant trait that is missing in females. Such traits include enlarged tail feathers in male swallows and peacocks, antlers in male deer, and bright colors shown by males of many species. Darwin argued that these showy traits evolved via sexual selection as signals between males and females in courtship. Why females should choose a mate based on showy traits has been the subject of a century-long controversy. One view holds that the trait provides information on the genetic fitness of the male; this is the "good genes" hypothesis (review in Møller, 1994). Hamilton and Zuk (1982) proposed an intriguing hypothesis that incorporates the effects of parasites on their hosts into sexual selection theory. They argued that showy traits in males allow the female to evaluate the parasite load of potential mates. The dimorphic trait in parasite-laden males would appear scrofulous, or simply less extravagant. Females that chose a male with the showiest trait would obtain a mate with a genotype that confers resistance to parasites. The Hamilton–Zuk hypothesis has provoked enormous controversy over the past decade, but whatever the merits of the hypothesis, the idea finally alerted ecologists to the importance of parasites for any study of animal behavior.

Ressel and Schall (1989) provided one of the first tests of the Hamilton–Zuk hypothesis. Male *S. occidentalis* display bright colors on their ventral

surface to females during courtship. These colors appear extravagant to the human eye and are visible from many meters away. We have seen females apparently studying the ventral surface of a male while he remained with his ventral surface elevated off the substrate. The hypothesis predicts that malarial infection should alter the color pattern of males and that females could use the appearance of the colors as a cue to infection status.

To test this idea we collected male lizards, then photographed their ventral surface under standard conditions (miniature photographic studio with strobe lights). The resulting photographs were of excellent quality as to focus and color accuracy. Each live lizard had its ventral surface pressed against a piece of glass and was held in place with a foam-backed gray cloth. All lizards were photographed in the evening after their body temperature was raised to 35°C for 1 h. Each animals was positioned so its ventral surface was evenly displayed on the glass. Black and white reproductions of three of these photographs are in Schall (1990a). The slides were projected onto a digitizing planimeter from a fixed distance (370 mm) from the projector lens to the planimeter tablet; lizards were magnified about 4-fold. The area of the entire ventral surface was measured, then the area of all black, yellow and blue spots. Infection status of the lizards was unknown during this measuring period, then later determined from blood smears. We eventually measured 827 male fence lizards, and 119 of them were infected. After measuring the colors on these lizards we realized that even males with very similar proportions of each color on their ventral surface could differ considerably in the pattern formed by the colors. Therefore, the next summer we made color prints of 35 "color pattern classes" that we picked by eye, and took a photo album of these pictures with us into the field. We collected 500 additional males over a 2 week period and matched each animal within a few minutes to one of the color pattern classes shown in the photos.

The proportion of all colors in the fence lizards differed by body size. That is, larger (= older) male lizards developed more black, blue and yellow on their ventral surface. Therefore, we had to correct for body size before comparing infected and non-infected lizards. There was no significant difference between the two groups of lizards for proportion of blue or yellow on the ventral surface. However, infected animals displayed significantly more black (Figure 27). Likewise, infected animals tended to fall into the color pattern classes with more black (Figure 28).

The results support the Hamilton–Zuk hypothesis in part because the infected animals display different ventral colors than non-infected males. The effect could also occur if infected animals grow more slowly, and if malarial infection does not alter color deposition as the animal ages. If so, infected animals of any size class shown in Figure 27 would be older than the non-infected lizards. This does not appear to be the case because

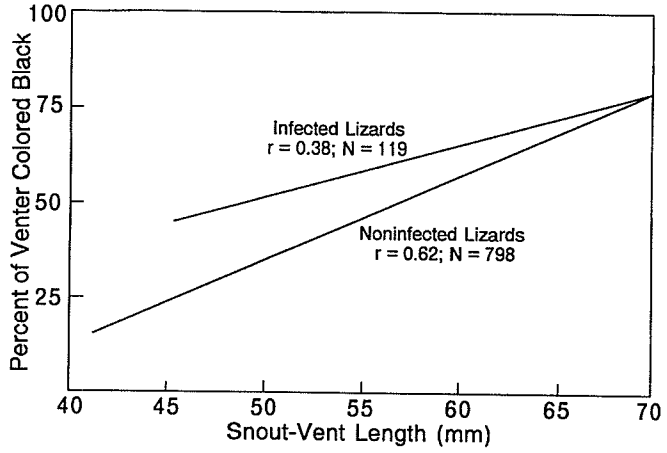


Figure 27 Color on ventral surface of fence lizards, *Sceloporus occidentalis*, infected or not infected with *Plasmodium mexicanum*. Figure shows that for most body sizes, infected animals tend to be darker. Figure adapted from one in Ressel and Schall (1989), © *Oecologia*, used with permission.

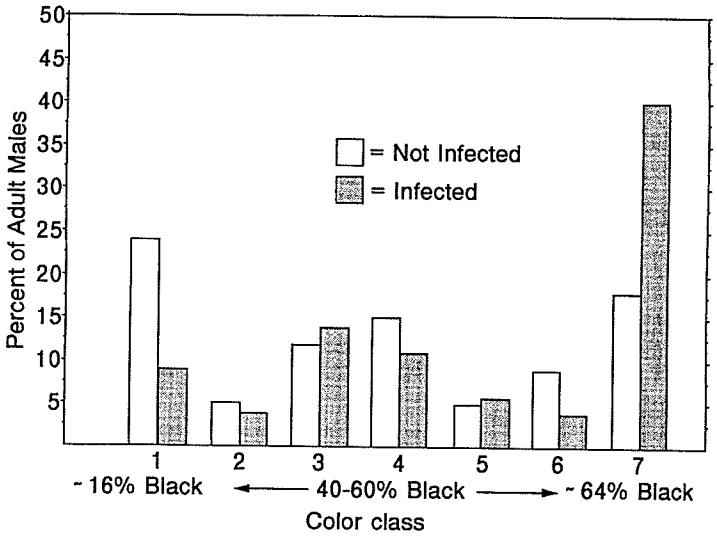


Figure 28 Body color classes of *Sceloporus occidentalis* infected or not infected with *Plasmodium mexicanum*. Figure redrawn from one in Ressel and Schall (1989), © *Oecologia*, used with permission.

infection does not alter growth rate in the lizards (Section 9.7). Thus, we concluded that *P. mexicanum* does alter an important sexually dimorphic trait in fence lizards, their ventral color.

Could the trend in color seen in fence lizards be used by females to aid in choosing a mate? Logistic regression revealed that male color was a weak cue of infection status and could improve a female's chances of selecting a non-infected male only marginally compared to random mate choice. If a female chose a mate randomly, she would have a 17.2% chance of picking an infected male (this was the percentage infected in our sample). The analysis showed that at best, the female could still inadvertently choose an infected male 16.9% of the time if she used the color of males as a weak cue to infection status. To improve her performance, and cut her errors to only 7%, she would have to reject 85% of non-infected males as well as the infected lizards. It is possible females would follow this conservative strategy if obtaining a mate with a genotype resistant to malaria is strongly favored by selection, but we doubt that this is likely. The courtship and territorial behaviors of infected males are greatly altered by infection (above) and are likely to be far more valuable to females in picking a mate.

Most perplexing about the results of this study is the fact that the infected animals appeared to be more extravagantly colored than non-infected males. That is, they displayed more black on the ventral surface, which is the most sexually dimorphic of the colors, seems most showy to the human eye on a bright day at Hopland when the lizards display, and which is the color that increases with age in males. This result is the opposite to what was originally proposed by Hamilton and Zuk. Other studies have shown similar results in lizards (Schall, 1986) and birds (Burley *et al.*, 1991).

9.5. Parasitism and Asymmetry

Environmental insults at any time during an animal's development, from earliest embryo to old age, may result in deviations from perfect symmetry in traits that are normally bilateral. If departures from perfect symmetry are random, a sample of individuals from a population will show no left or right bias (in contrast to the adaptive symmetry in some structures such as the hemispheric specialization in the human brain), and the distribution of measures will be normally distributed about a mean of zero. This is termed "fluctuating asymmetry" (FA) (Palmer and Strobeck, 1986). Recent studies indicated that some female insects and birds prefer symmetrical mates over those with relatively asymmetrical features (Møller, 1991; Thornhill, 1992). This suggests a general "good genes" hypothesis: females, and perhaps males, could use symmetry as a window into the quality of

potential mates. The window would be imperfect for any particular trait because if departures from symmetry are random, some individuals with poor genetic quality would also produce a symmetrical phenotype (random deviations from symmetry to the left side would sometimes be balanced by other random deviations to the right side).

Parasitism must be a common disturbance during development and limited data suggest infectious disease can cause increased FA in infected groups (Bailit *et al.*, 1970; Livshits and Kobyliansky, 1991; Møller, 1991, 1994; Polak, 1993). If so, individuals might favor symmetrical mates because this trait could indicate resistance to parasites (Thornhill, 1992). This "symmetry-favored" idea recalls the Hamilton–Zuk hypothesis. The two hypotheses would merge in those species in which showy traits are more likely to emphasize asymmetry in a parasitized individual compared to other, less extravagant, features. For example, in barn swallows, parasitized males have shorter tail feathers (the showy trait in this species) and the length of the tail feathers is less symmetrical. Females prefer both longer and more symmetrical tails (Møller, 1991, 1994). Small asymmetries in long tails would be more obvious to an inspecting potential mate than the same percentage difference in short tails. The Thornhill hypothesis is intriguing and suggests that an important kind of pathology induced by parasites, disruption of development and resulting asymmetry in form, has been almost ignored by parasitologists and animal behaviorists.

Do the symmetry and Hamilton–Zuk hypotheses converge on the same biological phenomenon? The Hamilton–Zuk hypothesis concerns genetically based resistance to specific parasites rather than overall genetic quality. Infection with a parasite might result in increased asymmetry, and thus the asymmetry would be a cue for animals to choose a mate with a genetically based resistance to parasitic attack. In contrast, individuals with overall poor genetic quality may suffer developmental errors, including malfunctions of the immune system and the consequent susceptibility to parasitic infection. In this case, both infection and greater asymmetry would co-vary with degree of genetic control over development, whereas in the first case they do not. If females, for example, prefer symmetrical males, they could either obtain a mate that has resistance to specific parasites (the Hamilton–Zuk image), or just overall better genes for development which could include resistance to pathogens.

To examine these contrasting possibilities, my student, Jonathan Calos, and I scored two sexually dimorphic traits in male fence lizards and compared the degree of symmetry in males infected with *P. mexicanum* and males not infected. The first trait, number of enlarged pores on the femoral area of the hind legs, is set prior to hatching (Cole, 1966), and therefore cannot be altered by malaria. Ventral color, as described above, changes over the life of the lizard and is altered by *P. mexicanum* infection.

We reasoned that if infection results in asymmetry, infected lizards should show greater asymmetry in ventral color, but not in number of femoral pores. However, if the overall genotype results in reduced developmental homeostasis, including poor resistance to parasites, then both ventral color and femoral pores should show greater asymmetry in malarious lizards. In the first case, malarial infection causes asymmetry in those traits still developing; in the second, both infection and morphological asymmetry may result from poor genetic quality.

We used the same 35 mm photographic slides taken for the study described above (Ressel and Schall, 1989). The area of left and right ventral patches of blue was measured and number of femoral pores on the left and right hind legs counted. In studies of FA, measurement error can be close to typical estimates of asymmetry. It is therefore critical to demonstrate no bias in measurement error that inflates FA for one group. We rescored a random sample of the lizards for belly color to compare between measurements. There was no difference in measurement error for the infected (N = 11) and non-infected (N = 27) lizards (0.2% and 0.6% error in two remeasurings; U-test, P = 0.69). We then rescored 20 infected and 20 non-infected lizards for pore number; counts were different for only two lizards, by only one pore. A second kind of bias can occur if deviation from symmetry is correlated with body size (larger color patches could on the average be more asymmetrical), and if the two groups differ in size. Snout–vent length did not differ between the two groups of lizards used in this study. SVL also was not correlated with pore number, nor difference in pore count for left vs right side ($P > 0.05$). This is not surprising because pore number is determined at hatching and does not increase with body size. As might be expected, total ventral area was correlated with total area of the blue patches (r = 0.81, $P < 0.05$), but the absolute difference in size of the left and right patches was not correlated with ventral area, nor total color patch area ($P > 0.05$).

Both traits, femoral pore number and ventral color, were less symmetrical in lizards infected with malaria (Figure 29). Asymmetry for femoral pore area was measured as the absolute difference in number of pores for the left and right hind legs. Ventral color asymmetry is presented in Figure 29 as the ratio between the larger patch area and the smaller. A second way to view color asymmetry is to calculate the absolute difference in area of the left and right patches, then divide by total ventral area. In this analysis, infected lizards were also more asymmetrical (U-test, P = 0.015). Note that the difference between infected and non-infected lizards shown in Figure 29 is substantially greater than the measurement error reported above. There was also no correlation in deviation from symmetry in the two traits for both the infected and non-infected groups (both $r \approx 0$, $P >$ 0.05), suggesting they may be under independent control during develop-

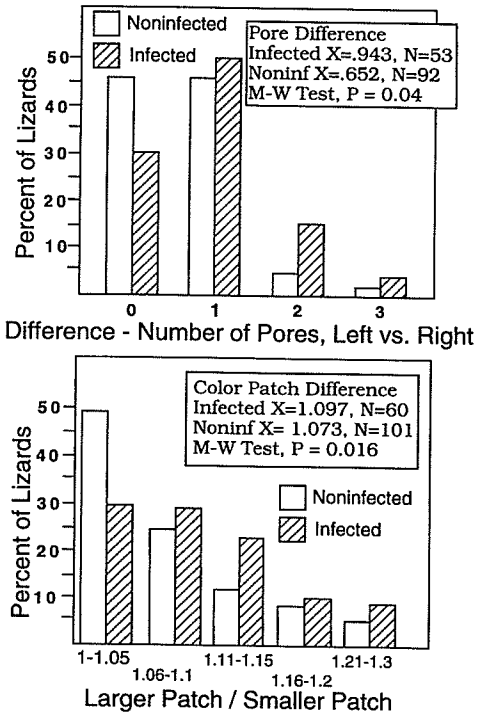


Figure 29 Frequency distribution of asymmetry in two traits of male fence lizards, *Sceloporus occidentalis*, comparing lizards infected with the malarial parasite, *Plasmodium mexicanum*, with those not infected. Means, sample sizes and results of Mann–Whitney (M-W) tests given. Top panel: difference in number of femoral pores on left and right legs. Bottom panel: ratio of larger vs smaller patch of paired blue patches on ventral surface of male lizards. Sample sizes differ because femoral pores were not clearly visible on all photographs examined.

ment. There was no side bias for greater number of pores or larger size of color patches (U-tests, $P > 0.05$). Also, the distribution of the departure from symmetry did not differ from normal for either pore number or blue patch size (goodness-of-fit test, $P > 0.05$, and test for kurtosis, $P > 0.05$). These last results suggest that deviations from symmetry seen here are a result of FA.

Our results provide further evidence of the association between parasitic infection and increased asymmetry. However, malarial infection cannot be considered as the cause of the increased asymmetry seen in the fence lizards. Instead, some lizards may have an overall genotype that results in reduced developmental homeostasis and multiple system deficiencies,

including reduced resistance to malaria. That is, reduced symmetry in color and pore number as well as likelihood of becoming infected with malaria all co-vary with genetic quality of the lizard. If female fence lizards selected mates based in part on symmetry (the difference between infected and non-infected males is slight, but natural selection may have provided female lizards with the sensory ability to detect such minor asymmetries), they could capture good genes for their offspring, including a generalized resistance to pathogens, but not the kind of resistance to specific parasites as envisioned by the Hamilton–Zuk model. A parenthetical result of this study is the first hint of genetical variation in susceptibility to a parasite for any reptile.

9.6. Hormones

As described above, when naturally infected with *P. mexicanum*, western fence lizards exhibit numerous reproductive pathologies. In summary, infected males display fewer courtship and territorial behaviors, have difficulty maintaining a territory, have altered sexually dimorphic coloration and have smaller testes. Infected animals store less fat by late summer which reduces clutch size in females in the spring. The reduction in blood hemoglobin in infected lizards, and the resulting reduction in aerobic abilities could explain why infected males display fewer social behaviors. Perhaps these behaviors require oxygen delivery to tissues in excess of that possible in the infected animals. Dunlap and Schall (1995) present data to support an alternative hypothesis, one that would explain all the reproductive pathologies observed in infected fence lizards.

In many vertebrates, glucocorticoid hormones from the adrenal gland coordinate physiological responses to diverse noxious stimuli, such as social aggression, disease, starvation and difficult weather conditions (Greenburg and Wingfield, 1987). Although these hormones are critical for surviving acute challenges, prolonged elevation of glucocorticoids can have important deleterious consequences, including inhibition of the reproductive system. In males this inhibition can be a direct effect of the stress hormones on the reproductive system or an indirect effect by reducing testosterone production (Dunlap and Schall, 1995). Malaria infection could act as a chronic stress that increases adrenal production of stress hormones and eventually reduces testosterone levels and changes reproductive behaviors and physiology.

We tested this hypothesis by capturing fence lizards; then for some, taking a blood sample at once, and for others only after the lizards had spent an hour in a cloth sack (blood samples were placed on ice immediately after collection). The confinement represented an acute stress to the

lizards. The infected male lizards revealed a 37% reduction in testosterone compared to non-infected males (23.5 vs 37.5 ng/ml). Infected and non-infected males had similar basal corticosterone (the major glucocorticoid hormone in reptiles), but under acute stress, the infected lizards produced more of the stress hormone (75% higher). We also found that the infected lizards carried significantly less glucose in their blood (10% lower) and that basal glucose correlated negatively with parasitemia determined by counting parasites seen on blood smears.

These results provoked a manipulative study. Exogenous corticosterone was supplied to experimental lizards by implanting plastic tubes filled with the hormone into experimental lizards. Control animals received an implant without the hormone. These animals were then housed in large outdoor enclosures. After 8 days, we found that the implanted animals (as expected) had elevated corticosterone levels, and also lower testosterone (by 47%, $P < 0.03$), smaller testis mass (by 34%, $P < 0.25$), and smaller fat bodies (although this effect was not significant, $P = 0.06$). Some of the effects seen in the corticosterone-implanted lizards were very similar to what is seen in malarious lizards. For example, testis mass dropped from 1.9% of body mass in the control males to 1.2% in the corticosterone-implanted males; non-infected free-ranging animals during the same time of year had a testis mass of 1.8% of body mass, and malarious lizards had testes of 1.3% of body mass. Fat body mass was 1.5% of control animals in the experiment as well as wild-caught non-infected males, but 1.0% in both the corticosterone-treated and naturally infected males.

These results support the following scenario. Malarial infection induces alterations in the response of the adrenal axis to acute challenges such as social conflict. These bursts of excess stress hormone eventually alter lipid metabolism and cause a decrease in testis size and concomitant reduction in testosterone production which inhibits reproductive behavior. These hormonal events may well also be responsible for the changes in the sexually dimorphic ventral color of infected male lizards.

9.7. Survival and Growth

When held in laboratory cages, fence lizards infected with *P. mexicanum* suffered increased mortality compared with non-infected lizards (Section 4). Data on field mortality are not available. However, during a mark–recapture program, multiple recaptures were actually more common for infected animals, suggesting they either were more easy to capture or enjoyed lower mortality than non-infected lizards (Bromwich and Schall, 1986). When 46 infected and 46 non-infected lizards were randomly chosen from the data set, and the number of days between first and last

recapture calculated for each animal, there was no difference for the two groups of lizards (58.9 days for infected animals and 57.8 days for non-infected lizards). This suggests there is no difference in mortality between the two groups, but this applies only to a single warm season study. Perhaps infected animals are more likely to die during the stressful winter dormancy period.

Another indirect measure of lizard survival is a comparison of the proportion of injuries to the tail seen in infected and non-infected lizards. Many species of lizards can lose their tail when attacked by a predator or aggressive conspecific, and later regrow a facsimile. The percentage of lizards with a broken or regenerated tail may be correlated with predation intensity (Schall and Pianka, 1980), so a higher proportion of broken tails observed for infected lizards could mean they are more often struck by predators (or by more aggressive conspecifics). Also, lizards that survive such attacks but lose their tails would be forced to channel resources into the regrowth of the facsimile tail. In most studies on tail injuries in lizards, males tend to have a higher proportion of breaks, perhaps because they are more prone to attack by both predators and other males. There is a trend in the lizard malaria systems I have studied for infected lizards to have a higher proportion of injured tails. At Hopland, 34.3% of non-infected adult male lizards (N = 2668) had an injured tail, compared with 38.3% of infected adult males (N = 1046). For adult females, 27.5% of non-infected animals (N = 2539) and 33.6% of infected animals (N = 614) had broken tails. The difference for both males and females was slight but statistically significant (G-tests, $P < 0.05$ for males and $P < 0.01$ for females). In a previous report (Schall, 1990b) I found a similar result, but the trend, with smaller sample sizes, was not significant. In Puerto Rico, both male and female infected *A. gundlachi* had a higher proportion of broken tails, but the difference was significant only for the sample of females (infected males = 30.2%, non-infected males = 22.8%, total N = 868; infected females = 29.8%, non-infected females = 18.0%, total N = 407). On St Maarten island, *A. gingivinus* adult males suffered high tail break frequencies when infected with *P. azurophilum* (45% (N = 91) vs 26% (N = 147), G-test, $P < 0.05$). There was no such difference for the female sample. No significant difference was seen in African male *Agama* when infected with *P. giganteum* (25.9%), *P. agamae* (17.6%) or in mixed infections (11.9%) when compared with non-infected males (15.2%) (N = 1322), nor in infected females (14.3%, 13.9%, 13.5% and 9.7%, respectively, N = 1015). A trend, however, emerges from the African data that might indicate a weak tendency for infected lizards to lose their tails to attackers.

Malarious lizards might be deficient at gathering resources; in Sierra Leone, infected *A. agama* had less food in their stomachs than healthy

lizards (food in the stomach equalled an average of 2.66% of total body mass for infected lizards, and 3.24% for non-infected animals; Schall, 1990b). Also, the disruption in lipid storage observed in infected California *Sceloporus* (above) suggests that overall metabolism might be affected by the parasite and that growth rate should suffer in infected lizards. This is not the case. Growth rate was monitored by mark–recapture programs at both the Hopland and Sierra Leone sites. Growth rate is highest in smaller lizards, so the analysis must correct for SVL. Two studies done at Hopland showed no difference in growth rate for lizards infected with *P. mexicanum* and non-infected animals (1979 sample at Hopland; about 700 animals marked and 60 later recaptured (Schall, 1983a); 1984 Hopland sample, 827 marked and 214 recaptured (Ressel and Schall, 1989); African sample of 38 recaptured *A. agama* (Schall, 1990b)).

Assimilated resources in any organism are partitioned into growth, maintenance and reproduction. The results presented here suggest that the "strategy" followed by infected lizards is to maintain growth (important for future reproduction because larger lizards produce larger clutches of eggs) and maintenance (important for survival), but to channel the losses incurred by infection to a reduction in clutch size and perhaps time between clutches. Thus, immediate reproduction suffers to assure future reproduction.

9.8. Parasite-mediated Competition

Park (1948) demonstrated that a protozoan parasite (*Adelina tribolii*) could alter the competitive relations between two species of *Tribolium* beetles in laboratory experiments. The competitively superior species of beetle was prone to infection with *Adelina* and when the parasite was present the normally competitively inferior species was able to survive or even dominate in mixed-species conditions. The possibility that this kind of parasite-mediated competition could be important in nature has elicited considerable interest (Haldane, 1949; Freeland, 1983; Price *et al.*, 1988; Minchella and Scott, 1991) but documented examples of parasite-mediated competition are few (review in Schall, 1992). I have investigated the effect of malarial infection on the distribution, and possible competitive interactions, of *Anolis* lizards in the Caribbean (Schall, 1992; Schall and Vogt, 1993).

Each of the small islands in the eastern Caribbean supports either one or two *Anolis* species (excluding very recent introductions). The anoles of single-species islands are all similar in body size suggesting there is an optimal body size for solitary species. On islands with two anoles, however, body size of the two species is usually very different which could

reduce competition for food and allow the two species to co-exist. On St Maarten two co-existing anoles are similar in body size, *A. gingivinus* and *A. wattsi*. Elegant field manipulative experiments have shown that there is ongoing severe competition between these two species (Roughgarden *et al.*, 1984).

A. gingivinus is distributed throughout the island in almost every habitat, whereas *A. wattsi* is found only in a patchy distribution in the wooded central hills. Although these woods are more mesic than other habitats, *A. wattsi* can occur in drier habitats on other islands. *A. gingivinus* appears to be the competitively superior species. What allows the two anoles to co-exist at some sites on St Maarten? *P. azurophilum* is a common malarial parasite of *A. gingivinus* at some sites on the island (23–46% of lizards infected), but *A. wattsi* is much less commonly infected (usually 0–4% infected). Parasitemia is also higher in *A. gingivinus* than *A. wattsi*. Infected *A. gingivinus* suffer an elevated number of immature erythrocytes and reduced blood hemoglobin; also, *P. azurophilum* frequently infects monocytes and neutrophils which, when infected, produce less acid phosphatase (Section 9.1).

I surveyed 17 sites on St Maarten for lizard malaria. At all sites where *A. gingivinus* exists alone, malaria is absent in these lizards. At all sites (except one location where construction of homes has greatly disturbed the habitat) where both anoles co-exist, *A. gingivinus* is often infected with *P. azurophilum*. This pattern exists even for sites only a few hundred meters apart. I conclude that the parasite mediates competition between the two species of anoles. The competitively inferior *A. wattsi* can co-exist with the normally superior *A. gingivinus* only where the parasite infects *A. gingivinus*. That is, sick lizards make poor competitors, allowing two similar sized anoles to live at the same site.

A similar situation may exist in the rainforest of eastern Puerto Rico (Schall and Vogt, 1993). Five species of anoles occur there in the lower parts of the trees and shrubs. In pair-wise comparisons, the species differ in body size or perching location. However, one pair, *A. evermanni* and *A. gundlachi* are similar in size and often are found in the same locations, and sometimes fighting bouts are seen. Normally, at other places in Puerto Rico, *A. evermanni* and *A. gundlachi* differ significantly in perching location. Although both species can be infected with malaria, only one of 386 *A. evermanni* collected was infected with *P. azurophilum*, but 22% of 1516 collected *A. gundlachi* were infected with one or both of *P. azurophilum* or *P. floridense*. *P. gundlachi* seems a normally very aggressive lizard, so perhaps the parasite, because it infects mostly *P. gundlachi*, allows both anoles to co-exist at that location.

9.9. Evolution of Virulence: Summary and Conclusions

Malarial infection initiates a spectrum of pathologies in the lizard host. Erythrocytes are destroyed, resulting in a cascade of effects from hematological to behavioral. The stress of infection changes the hormonal milieu, fat cycling is disrupted, and reproductive behaviors are changed. Fitness is clearly reduced: in males by the inability of infected lizards to hold territories and acquire mates, and in females by the reduction in number of eggs produced per clutch. These consequences of infection in individuals may reverberate to higher levels, including changes in interspecific interactions among host species.

Most of the data on the virulence of lizard malaria come from the California system (*P. mexicanum* in fence lizards), but comparative data from Africa and the Caribbean suggest that malaria is typically harmful to the lizard host. However, Rand *et al.* (1983) came to a different conclusion for two species of parasite, *P. balli* and a *P. tropiduri*-like form, in *A. limifrons* in Panama. They stated that infection is "without measurable effect on growth, reproduction, and survival of the lizards." When infected with malaria, *A. limifrons* showed an elevated proportion of immature erythrocytes. However, there was no difference between infected and non-infected *A. limifrons* in body mass/SVL (an estimate of overall condition of the lizards); mass of feces produced within 24 h after capture (an estimate of food intake); reproductive condition; proportion of tail injuries; or growth and survival. Therefore, malaria may vary in virulence among parasite and host species. However, it is intriguing that in some very ancient malaria–lizard associations, the parasite remains significantly virulent.

10. CONCLUSIONS

Parasitism was generally ignored by the majority of ecologists, evolutionary biologists and behaviorists until publication of Peter Price's classic volume, *The Evolutionary Biology of Parasites* (Price, 1980). Even the most influential general ecology textbook since 1950, *Evolutionary Ecology* (Pianka, 1974), regarded parasites as "weak predators" and devoted only a brief paragraph to their biology. In the past 10 years, a renaissance of interest in parasites has dominated evolutionary ecology, leading Richard Dawkins (1990) to note, "Eavesdrop morning coffee at any major centre of evolutionary theory today, and you will find 'parasite' to be one of the commonest words in the language."

If ecologists have long ignored parasites as subjects of study, then parasitologists are guilty of neglecting malaria in lizards as a productive system for general studies of parasite biology. The great diversity of known species of *Plasmodium* infecting lizards, their wide distribution, their ecologically important variation in reproductive traits, and the ease with which lizards can be collected, housed and observed all argue for the usefulness of lizard malaria for future studies in parasite ecology and evolution. Many kinds of studies that would be difficult in other kinds of vertebrate hosts of malaria are feasible for the lizard–plasmodia systems. Malaria plays a major role in every aspect of the lizard host that has been examined. Researchers in locations with small research budgets can conduct important studies at little expense. Parasitologically inclined biologists in North America need not travel far to study malaria in natural populations of hosts. I have collected malaria-infected lizards within sight of the Golden Gate Bridge and skyscrapers of San Francisco. Some of the conclusions drawn in this review have been deliberately provocative, in the hope that researchers will be drawn to studies of the general parasitology, evolution, ecology and effects on both vertebrate and insect hosts of these engrossing organisms.

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