

Lizard malaria: cost to vertebrate host's reproductive success

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SUMMARY

Plasmodium mexicanum is a common malarial parasite of the western fence lizard, *Sceloporus occidentalis*, in northern California, USA. Infected female lizards store substantially less fat during the summer activity season and produce smaller clutches of eggs than do non-infected animals. Stored fat is utilized in the production of eggs; the energy content of the decrement in stored fat is approximately equal to the energy content of the average reduction in number of eggs. Thus, there is ongoing strong selective pressure on the host to evolve appropriate anti-parasite measures.

INTRODUCTION

The malarial organisms (genus *Plasmodium*) parasitize a wide array of vertebrate taxa, including birds, mammals, snakes and especially lizards (Ayala, 1977). Approximately 125 species of *Plasmodium* have been described; only 4 are primarily parasites of humans, whereas about 65 are lizard-infecting forms. Malarial infection often has striking pathogenic effects on humans and experimentally infected laboratory animals. This suggests that plasmodial infection may have important consequences for natural, wild vertebrate hosts as well. However, the costs of non-human malarial parasites to wild host individuals and populations are very poorly known. For example, only very recently have the effects of any malarial species on wild lizards been assessed (Schall, 1982; Schall, Bennett & Putnam, 1982).

Since 1977 I have been studying the biology of *Plasmodium mexicanum* and its vertebrate host, the western fence lizard, *Sceloporus occidentalis*, in northern California, USA. One goal of these studies has been to assess possible costs of malarial infection to the lizard host's reproductive success (Darwinian fitness). Although histological and physiological pathologies observed in lizards infected with malaria can be striking (Schall, 1982), it is only ultimate effects on reproductive success that are important in the host's evolutionary response to parasitism. The components of lizard fitness being examined in the *Sceloporus-Plasmodium* system are clutch size, number of clutches produced during the activity season, growth rate, survival of eggs and hatchlings and differential mortality.

Here I report on the effect of malarial infection on reproductive output of western fence lizards. Also reported is the effect of infection on the host's ability to store lipids during the activity season. Lipids are accumulated by lizards after the reproductive season ends and before the onset of winter dormancy (Schall,

1978, 1982), and are utilized by female lizards to produce eggs the next season (Hahn & Tinkle, 1965). Therefore, any effect of infection on the host's lipid storage process should have an important impact on reproduction. I conclude by placing the effect of malarial infection on reproductive output in context with effects on other components of the host's fitness.

MATERIALS AND METHODS

The study site was the 24 km² University of California Hopland Field Station, an oak-woodland habitat 140 km north of San Francisco. At this study area lizards are abundant and are active from early May to late September. Approximately 25% of adult lizards are infected with *P. mexicanum* (Schall, 1982).

Each year lizards were collected and measured (snout to vent length) and blood smears made from blood extracted from a toe clip. Smears were stained with 10% Giemsa at pH 7.0 for 50 min and viewed at 1000× magnification. After viewing at least 20000 erythrocytes, lizards were scored as infected or non-infected with *Plasmodium*. Preliminary comparisons demonstrated that knowledge of the level of infection did not significantly improve the analysis (see Discussion section).

The clutch size of female lizards was determined by dissection and counting of oviducal shelled eggs or yolked follicles, measuring more than 5 mm in diameter, or by allowing females to deposit eggs in laboratory cages within a few days of capture. As lizards maintained in captivity often produce exceptionally large clutches (Schall, 1978), only clutches from very recently collected animals were used in this analysis.

Inguinal fat bodies from a sample of lizards collected in late September 1978 and 1979 were dissected out and weighed. Only adult lizards were utilized for this analysis and body sizes were matched for the infected and non-infected groups (body sizes did not differ between groups for either year; Mann-Whitney U-tests, *P* values > 0.05). Oviducal shelled eggs from dissected clutches were also weighed.

The energy content of fat bodies was determined by assuming that fat bodies are 90% fat (Hahn & Tinkle, 1965) and the average energy content of fat is 3.77×10^4 J/g (White, Handler & Smith, 1964). The energy content of oviducal lizard eggs can be estimated using literature estimates of (1) water content of eggs, which averages 51% (Hahn & Tinkle, 1965; Vitt & Ohmart, 1975; Ballinger & Clark, 1973; Tinkle & Hadley, 1973; Vitt, 1974); (2) ash content, which averages 5% (Vitt & Ohmart, 1975; Tinkle & Hadley, 1973; Congdon, Vitt & Hadley, 1978) and (3) J/mg of ash-free, dry lizard eggs, which averages 27.2 (Vitt & Ohmart, 1975; Ballinger & Clark, 1973; Tinkle & Hadley, 1973; Vitt, 1974; Congdon *et al.* 1978; Tinkle & Hadley, 1975).

RESULTS

The combined data for 5 years on clutch size and lizard body size are presented in Fig. 1. Since clutch size increases with body size, an analysis of covariance was performed. Residual variance and slopes did not differ between malarious and non-infected lizard samples (*F*-test, *P* > 0.05), but regression line elevations did differ (*F* = 78.9, D.F. = 1, 257, *P* < 0.001). Infected lizards produced clutches with 1–2 eggs less than non-infected animals, as much as a 20% reduction. Overall,

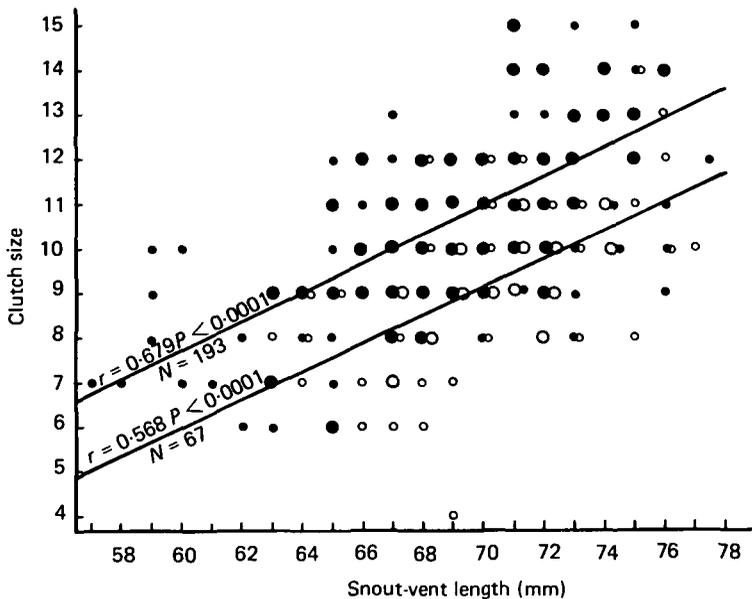


Fig. 1. Body size plotted against clutch size for western fence lizards infected with malaria (○, lower regression) and not infected (●, upper regression). Large points indicate 2–9 overlapping data. Data are fit to a linear regression model; various transformations of the data did not significantly improve the correlations.

infected animals produced an average clutch of 9.2 eggs, whereas non-infected animals produced 10.6 eggs (Mann-Whitney U-test, $P < 0.0001$).

There was some variation among years in average clutch size (Fig. 2) and such variation was significant for non-infected animals (Kruskal-Wallis H-test, $P < 0.01$). However, the infected females consistently produced smaller clutches. This decrement usually ranged from 1 to 2 eggs. Clutch size–body size regressions for the 2 years for which these regressions were significant, 1978 and 1979, were examined. For both years residual variance and slopes did not differ between infected and non-infected lizard samples (F -tests, $P > 0.05$), but elevations were significantly different (F -tests, 1978 $F = 36.8$, D.F. = 1, 62, $P < 0.001$; 1979 $F = 32.9$, D.F. = 1, 123, $P < 0.001$).

The etiology of the observed reduction in reproductive output by infected female fence lizards seems to involve the effect of malarial infection on the lizard's lipid economy. For both 1978 and 1979 samples, infected animals stored significantly less fat by the end of the season than did non-infected lizards (1978: \bar{X} infected = 0.412 g, $N = 12$, \bar{X} non-infected = 0.599 g, $N = 31$; 1979: \bar{X} infected = 0.529 g, $N = 21$, \bar{X} non-infected = 0.659 g, $N = 44$; U-tests, $P < 0.05$). On average, infected lizards stored only 68% as much fat as non-infected animals in 1978, and 80% in 1979.

In 1978 the average fat decrement of 0.168 g translates to 6325 J, and in 1979 a decrease of 0.130 g represents 4420 J. The mean egg mass of eggs from 78 weighed *Sceloporus* clutches was 0.362 g. An average *S. occidentalis* egg would contain about 4332 J. Therefore, the decrement in fat stored by the end of the activity season equals the equivalent of 1.46 eggs in 1978 and 1.02 eggs in 1979, close to the observed reduction of 1–2 eggs in infected lizards.

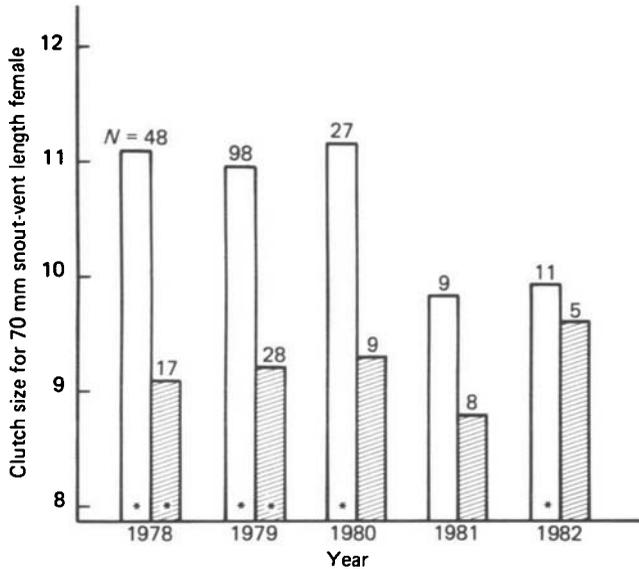


Fig. 2. Clutch size for a 70 mm fence lizard infected (▨) and non-infected (□) with malaria over a 5-year interval. Clutch size of average 70 mm lizard determined from regressions calculated from the clutch size–body size data for each year. Significant regressions are indicated by a star (*) at the base of the bar.

DISCUSSION

The data presented here provide the first demonstration of a reduction in reproductive output in a non-human vertebrate resulting from natural malarial infection. Most data on pathogenicity of non-human malarias are based on experimental infections, often of artificial hosts, and may say little about natural situations (Seed & Manwell, 1977). In the case described here, natural malarial infection clearly reduces the fitness of its vertebrate host by reducing the host's fecundity.

The effect of *P. mexicanum* on its vertebrate host's reproductive output appears to derive from disruption of infected lizards' normal lipid storage processes. Less fat is stored by infected lizards by the end of the activity season and this results in smaller clutches of eggs the next spring. The concordance between energy content of the fat decrement in infected animals and observed reduction in eggs produced is striking. Thus, the effect of malarial infection on host reproductive output is delayed from one activity season to the next. This would explain why the level of infection at the time when reproductive data were gathered is not a useful predictor of decrement in eggs produced. Also, if the 'infected' sample of lizards contained animals with new infections, this might lead to an underestimation of the effect of malarial infection on the reproductive output of the host. Any such error is probably minor. In spring, when samples were taken, almost all infections consisted in part of large, vacuolated gametocytes, indicating that the infection had overwintered from the previous year (Schall, 1982). In any case, infected lizards with unusually large clutches (outliers on Fig. 1) were not necessarily animals with apparently new infections.

It is unclear why malarious lizards are able to store less fat than non-infected

lizards. Perhaps infected animals are less able to catch insect prey, reducing their basic intake of resources. A more likely possibility is that a fraction of assimilated resources is simply lost to the parasite. Malarial infection has a substantial impact on the haematology and respiratory physiology of these lizards (Schall *et al.* 1982), suggesting that there is a cascade of effects leading from haematological pathology, to disruption of normal lipid metabolism, and ultimately to the observed reduction in the number of offspring. A similar series of effects are experienced by male fence lizards, leading to reduced lipid storage and decreased testis size, although the final effects on reproductive output are unknown (Schall, 1982).

The impact of malarial infection on important components of the lizard host's fitness can now be partially assessed. Clearly, clutch size of infected *S. occidentalis* is reduced, but a useful estimate of overall reduction in host fecundity requires knowledge of the number of clutches produced/activity season. *S. occidentalis* at Hopland produce 1 or 2 clutches, depending on body size (Schall, unpublished observations); perhaps large infected lizards might not produce a second clutch. However, the proportion of infected and non-infected females that are gravid at any time during the activity season does not differ (Schall, 1982), suggesting that complete reproductive failure is not common. Survival of eggs and the health of hatchlings must also be considered. Eggs produced by infected and non-infected *S. occidentalis* do not differ in size, incubation period, percentage hatching in laboratory-maintained clutches, or in initial size of hatchlings, a measure of hatchling health (Schall, 1982). Thus, offspring produced by malarious lizards differ in quantity, but not quality, compared to those of non-infected lizards.

The growth rate of lizards indirectly influences fitness because larger females produce larger clutches of eggs (Fig. 1), and larger males might establish more extensive or higher quality territories. Mark-recapture studies demonstrate that there is no substantial difference in the growth rate of infected and non-infected adult *S. occidentalis* (Schall, 1982). However, juvenile lizards, which vary much more in growth rate among individuals, may experience reduced growth when infected (C. Bromwich & J. Schall, unpublished observations). Unfortunately, we find very few infected juveniles, which prevents rigorous testing of this possibility.

A last component of fitness, differential mortality, is poorly known for this system. Infected *S. occidentalis* suffer increased mortality when maintained in the inherently stressful environment of captivity (Schall, 1982), but extensive mark-recapture data demonstrate no increase in mortality of free-living infected lizards (Bromwich & Schall, unpublished observations).

As malarial infection substantially reduces the fitness of fence lizard hosts, there must be strong natural selection presently acting on *S. occidentalis* to evolve mechanisms to reduce the impact of *Plasmodium* infection. Lizards may well have been the original vertebrate host of malaria (Manwell, 1955), and the *Sceloporus-Plasmodium* association in California probably has existed since at least the *Pleistocene* (Ayala, 1970). At Hopland the proportion of lizards infected has remained remarkably constant since at least 1969 (Ayala, 1970; Schall, 1982). The fact that infection still has such an impact on the lizard's fitness suggests that coevolution of parasite and host has led to an equilibrium in costs to both lizard and *Plasmodium*. The existence of such equilibria is occasionally proposed in the parasitological literature (for example, Burnet, 1962), but rigorous theoretical or empirical support for the idea is scanty.

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