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PREVALENCE OF MALARIA PARASITES (*PLASMODIUM FLORIDENSE* AND *PLASMODIUM AZUROPHILUM*) INFECTING A PUERTO RICAN LIZARD (*ANOLIS GUNDLACHI*): A NINE-YEAR STUDY

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ABSTRACT: The prevalence of malaria parasites was studied in the lizard *Anolis gundlachi* over a 9-yr period at a site in the wet evergreen forest of eastern Puerto Rico. Three forms of the parasite infected the lizards; these were *Plasmodium floridense*, *Plasmodium azurophilum* in erythrocytes, and *P. azurophilum* in white blood cells. Overall prevalence of infection for 8 samples during the study period was significantly higher for males than females (32% of 3,296 males and 22% of 1,439 females). During the study, the site experienced substantial climatic and physical disturbance including rising temperature, droughts, and hurricanes that severely damaged the forest. Parasite prevalence in the first sample, 8 mo after the massive hurricane Hugo, was slightly, though significantly, lower than for subsequent samples. However, overall prevalence was stable during the 9-yr period. The results show malaria prevalence is more constant at the site than found for 2 studies in temperate forests, and that the Puerto Rico system may be an example of the stable, endemic malaria described by standard models for human malaria epidemiology.

Among the first mathematical models in ecology were those of Ronald Ross (1911) who sought to explain the observed spatial and temporal variation in malaria prevalence among human populations in malarious regions. Indeed, Ross might well be regarded as a founder of mathematical ecology (Anderson and May, 1991). These models became classics in epidemiology when developed by Lotka (1923) and in modern form by Macdonald (1957). Macdonald recognized that at some sites malaria is absent or rare, at other sites prevalence is fairly low but prone to sudden epidemic outbreaks (epidemic malaria), and at others malaria is stable at high prevalence (endemic malaria). The model shows that the average number of blood meals taken by the vector(s) will determine if malaria is absent (few meals), epidemic (more meals), or endemic (many meals) (Aron and May, 1982). The outlook proposed by Ross, Lotka, and Macdonald has long been important in malaria epidemiology (Anderson and May, 1991) and continues to influence even sophisticated landscape analyses of malaria distribution (Craig et al., 1999).

Under conditions allowing endemic malaria, environmental perturbations can lead to changes in prevalence of the parasite in both human and vector hosts, but the system would rebound to its original, stable state (Aron and May, 1982). However, stability models in ecology typically assume only relatively minor variation over time in the model's terms (Yodzis, 1989). Greater intensity of disturbance can lead to unpredictable dynamics that fall outside the local stability predicted by the models. For example, endemic malaria might be expected in the wet tropics, but even these habitats can experience significant climatic disturbance, both acute due to tropical storms and secular if the regional climate is altered (Lugo and Scatena, 1996). This is the issue confronted here. Will prevalence of malaria parasites remain stable in a wet tropical system that experiences such climatic disturbances or long-term changes?

We have examined the prevalence of 2 species of malaria parasite, *Plasmodium floridense* and *Plasmodium azurophilum*, in their primary vertebrate host, the anole lizard *Anolis gundlachi* in the wet evergreen forest of eastern Puerto Rico. Eight samples were taken from June 1990 through March 1999. Although the tropical wet forest is generally regarded as a stable environment, the site in Puerto Rico has suffered regular disturbance by direct hurricane hits and periodic drought conditions and perhaps a long-term increase in temperature (below). This variation in environmental conditions has had a substantial impact on the forest (Reagan and Waide, 1996; Zimmerman et al., 1996) as well as the anoles (Reagan, 1991, 1996; Schall and Pearson, 2000). A preliminary study revealed that prevalence of *P. floridense* and *P. azurophilum* remained high in the anole over a 6-mo period (≈30%; Schall and Vogt, 1993) suggesting an endemic, stable condition for the parasite–host system. We asked if this is possible in the severely disturbed tropical environment in eastern Puerto Rico.

STUDY SITE AND METHODS

The study was conducted at the El Verde Field Station in the Luquillo Experimental Forest of eastern Puerto Rico (18°19′N, 65°45′W). The habitat is described by Waide and Reagan (1996). In brief, the site is a wet evergreen forest at approximately 400 m elevation, in rough terrain of steep slopes. The overall biodiversity is lower than mainland tropical sites; for example, only 8 species account for 75% of tree density. Eight species of *Anolis* occur at the site, but only *A. gundlachi* is commonly infected (prevalence was <1% in the other species; Schall and Vogt, 1993), so only *A. gundlachi* anoles were sampled in our long-term study. The parasite species that infect *A. gundlachi* are widespread throughout the eastern Caribbean (Staats and Schall, 1996).

Lizards were collected along trails cutting through a 36-ha portion of the field station. Samples were taken during 8 periods: (1) May–July 1990, (2) January 1991, (3) July and August 1996, (4) February 1997, (5) July 1997, (6) January 1998, (7) May 1998, and (8) March 1999. The animals were captured by hand or with a slip noose on the end of a pole. They were kept in mesh sacks until evening when a drop of blood was extracted from a toe clip to make a thin smear (thick smears are useless because the erythrocytes of lizards are nucleated). The smears were stained in Giemsa at pH 7.0 for 50 min. Each lizard was measured (snout-to-vent length in mm) and gender determined.

Smears were scanned under oil at 100× for 6 min during which approximately 10,000 erythrocytes were examined (Schall and Bromwich, 1994). For smears positive for infection, the species of parasite was recorded for 6 samples. *Plasmodium azurophilum* infects both erythrocytes and 2 classes of white cells (Telford, 1975; Schall, 1992). Ayala and Hertz (1981) suggested these could be 2 species of parasite using different cell classes, and ongoing gene-sequencing studies support this view (S. Perkins, unpubl. obs.). Therefore, *P. azurophilum* infections were characterized by cell class infected and each infection was scored as *P. floridense* and *P. azurophilum* in erythrocytes (RBC) or *P. azurophilum* in white blood cells (WBC).

There are 2 potential sources of error emerging from this protocol.

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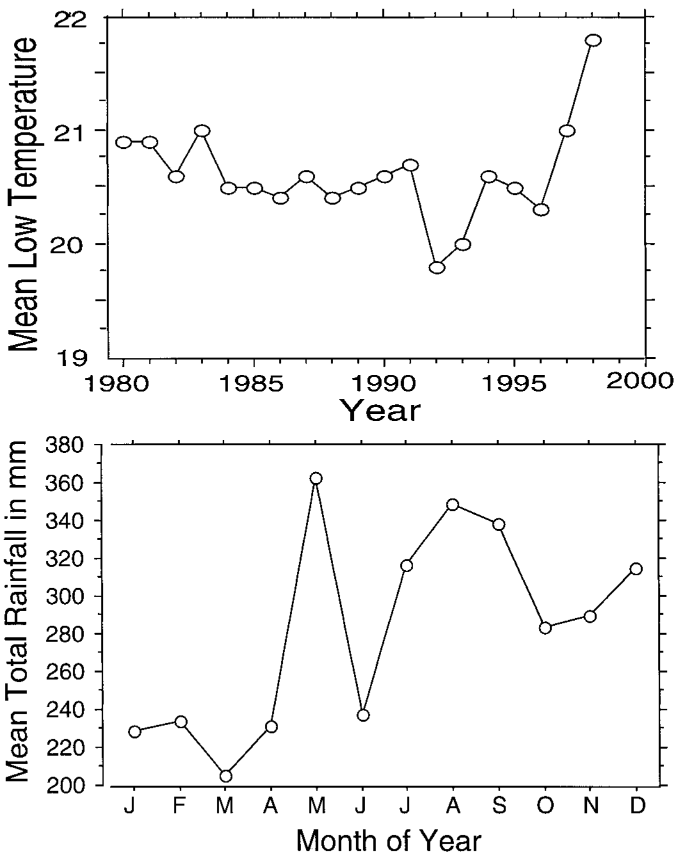

FIGURE 1. Top panel: Mean daily low temperature by year at El Verde, Puerto Rico site from 1980 to 1998 showing general warming trend during the period of the study from 1990 to the present. Bottom panel: Mean total rainfall per month for the period 1975–1998, revealing a seasonal rainy and drier periods.

First, infections with very low parasitemia could be missed during the 6-min scan. This underestimation of percentage of hosts infected has long been known in malaria studies (Macdonald, 1926). This seems not to be a problem for most studies of lizard malaria because, in contrast to human malaria, lizard malaria parasites generally produce higher parasitemia in their vertebrate hosts that is readily detected during microscopic scanning (Bromwich and Schall, 1986). To test this conclusion, Perkins et al. (1998) used the polymerase chain reaction (PCR) to detect infections of another lizard malaria parasite, *Plasmodium mexicanum,* in fence lizards (*Sceloporus occidentalis*) in California. They were able to detect infections too weak to find during lengthy microscopic examination of blood smears but found that such infections were rare. That is, the highly sensitive PCR method was only marginally more effective in determining parasite prevalence. As the purpose of our study was to determine changes in prevalence over time, an underestimation of prevalence would be important only if the presence of very low parasitemia infections differed among sample periods. Second, the method will underestimate mixed infections when an infection reveals high parasitemia for only 1 or 2 of the parasite classes being scored. But again, this would be damaging to our study only if parasitemia varied in different ways over time for the different parasites.

The lifespan of *A. gundlachi* anoles is most likely only 1–2 yr. *Anolis stratulus,* another of the El Verde anoles, has a population turnover of about 1.4 yr (Reagan, 1996), and *A. gundlachi,* a larger lizard, probably lives slightly longer. The rapid turnover of the lizards means that point estimates of prevalence would reflect any changes in the transmission biology of the parasite.

Weather data were taken from records maintained by the field station staff. We used rainfall recorded for the previous 1–12 mo and mean daily lowest temperature for 1–12 mo prior to each sample period. The

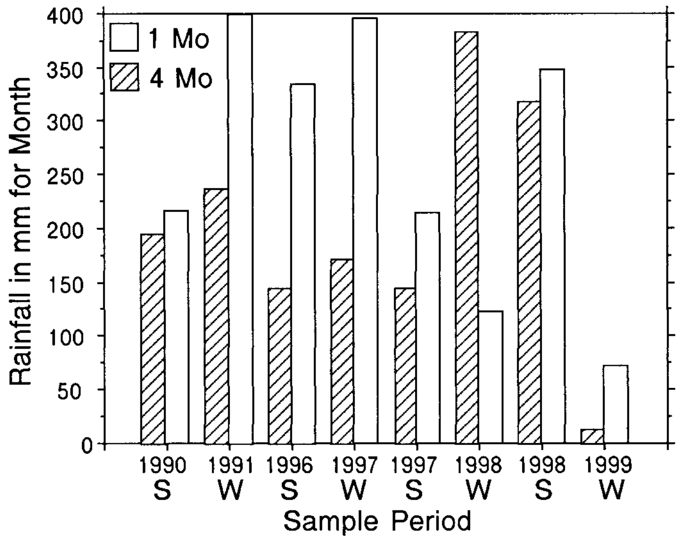

FIGURE 2. Total rainfall during the month (30 days) prior to the start of each sample at the El Verde, Puerto Rico site, and for the 30-day-period 4 mo prior to the sample. Summer (S) and winter (W) samples are indicated. Although the wet and dry seasons seen in Figure 1 were weakly present, there was substantial variation in rainfall during the study period.

daily low temperature was presumed relevant if the unknown vector(s) take blood meals at night. Records of hurricanes were extracted from the U.S. Weather Service web site.

RESULTS

Weather

As expected for a tropical forest, temperature varies only slightly during the year at the El Verde site. From 1975 to 1998, mean monthly low temperature differed only 3 C from January to July. However, there has been a slight warming trend since the study began in 1990 (Fig. 1). Although rainfall is generally high year-round at the site, there is a relatively dry period during January–April, and a shorter summer dry season in June (Fig. 1). Figure 2 shows the total rainfall for the month just prior to the beginning of each of our samples and for the fourth month prior to our samples. There was a weak seasonal trend in rainfall, but more striking is the variation in rainfall over the 9-yr period. Two of our samples came during droughts, July 1997 and March 1999. The study began in the summer of 1990 following the 17–19 September 1989 strike by Hurricane Hugo. Subsequent severe hurricanes included Luis and Marilyn in September 1995, Bertha in July 1996 during our third sample, and Georges in September 1998 (Fig. 3).

Prevalence

We sampled 4,735 *A. gundlachi* (1,439 females and 3,296 males). Prevalence of the parasites (all taxa combined) was higher for males ($\chi^2 = 53.8$, 1 df, $P < 0.0001$), so data are partitioned by gender for subsequent analyses. Prevalence of malaria for male lizards did not differ by season (winter vs. summer) ($\chi^2 = 3.68$, $P > 0.05$). An equivalent analysis for females is not possible because 2 of the winter samples had very small sample sizes, so we have only 2 useful winter samples for females.

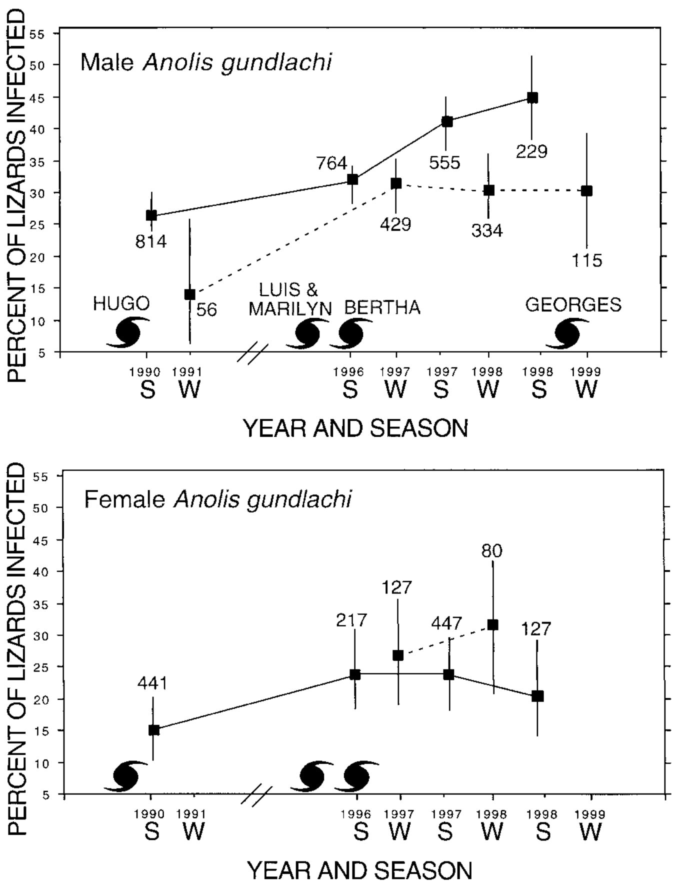

FIGURE 3. Total prevalence of malaria parasites for male (top panel) and female (bottom panel) *Anolis gundlachi* at the El Verde, Puerto Rico field site for 8 sample periods. Data combine 3 forms of malaria parasite, *Plasmodium floridense* and *P. azurophilum* in erythrocytes, and *P. azurophilum* in white blood cells. Summer samples (S) are connected by solid lines, and winter samples (W) by broken lines. Percentage of lizards infected is given with 95% confidence interval for each sample. Sample sizes, and timing and name of major hurricanes are also given. Results show stable prevalence of infection over a 9-yr-period.

For both males and females, the prevalence varied among samples (Fig. 3; Male $\chi^2 = 57.7$, 7 df, $P < 0.001$; Female $\chi^2 = 18.9$, 5 df, $P < 0.01$). Post hoc cell contribution tests showed that only the first 2 samples for males and the first sample for females (the size of the second sample of females was too small to add to the analysis) contributed to the variation among samples. Thus, only the samples immediately after Hurricane Hugo differed, with a lower proportion of the lizards infected.

Separating the 3 parasites reveals that *P. azurophilum* in RBC remained the dominant parasite over the 9-yr period (60–80% of infections), whereas *P. azurophilum* in WBC and *P. floridense* remained at about 10–30% of infections (Fig. 4). For each parasite, data were cast into a contingency table, and results show no significant difference among samples for *P. azurophilum* in WBC ($\chi^2 = 10.1$, 5 df, $P > 0.05$). The other 2 parasites did vary over time (*P. azurophilum* in RBC $\chi^2 = 15.6$, 5 df, $P = 0.009$; *P. floridense* $\chi^2 = 26.8$, 5 df, $P = 0.0002$). Post hoc tests show that it is the first sample that leads to this

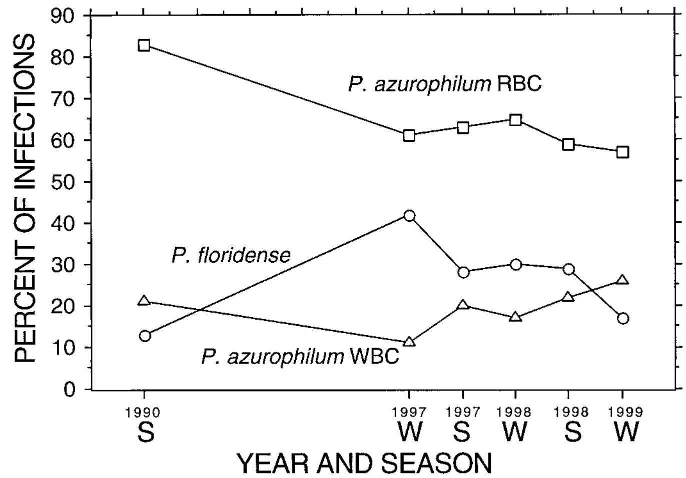

FIGURE 4. Percentage of infected *Anolis gundlachi* lizards with each of 3 forms of malaria parasite (*Plasmodium floridense* and *P. azurophilum* in erythrocytes [RBC], and *P. azurophilum* in white blood cells [WBC]). Percentage of infections for the 3 parasite forms will sum to >100 because many infections contained more than 1 form of the parasites. Results show stable prevalence of each of the parasite forms over a 9-yr-period.

result; subsequent to that sample, *P. floridense* became more common and *P. azurophilum* less common.

The little difference in malaria prevalence among samples suggests that neither rainfall nor temperature would be correlated with prevalence of the parasites. This proved to be correct. No correlation existed for cumulative rainfall from 1 to 12 mo before the sample and prevalence of the parasites in either male or female lizards ($P > 0.05$) or for total rainfall in a single month from 1 to 12 mo prior to the sample ($P > 0.05$). Likewise, mean low temperature by month or for cumulative months for 1–12 mo before the sample was not correlated with prevalence ($P > 0.05$).

DISCUSSION

The El Verde site, although a wet evergreen tropical forest, has experienced substantial environmental variation over the past decade. Our study began 8 mo after Hurricane Hugo devastated the forest, knocking down most of the larger trees and stripping away the canopy. Reagan (1996) describes the landscape after Hugo as resembling "a forest of telephone poles and 3–5 m deep piles of leaf and branch debris." During our first sample, the remaining trees had grown new leaves, but the canopy still appeared bare and the forest floor was well lit. Two anole species normally abundant in the canopy (Reagan, 1996), *A. stratulus* (21,500/ha) and *Anolis evermanni* (1,500/ha), but normally almost absent near the forest floor, were commonly seen at the base of trees and on fallen branches (Schall and Vogt, 1993). The density of *A. gundlachi* reached only 18% of prehurricane numbers and recovered to only 35% 13 mo later (Reagan, 1991). By our third sample in July and August 1996, much of the canopy had recovered. The situation was once again reversed after Georges that knocked down few trees but removed almost all leaves at canopy level. Our last sample was taken 6 mo after that storm. The trees had cast out new leaves, but the canopy still appeared open and the forest floor was again well lit.

In addition to these physical disturbances to the forest, rainfall and temperature also differed among our sample periods. Monthly rainfall during our study varied from almost none (1–2 cm) to nearly a meter. Rainfall in the months prior to the samples varied substantially, even when season is held constant (Fig. 2). As a last indication of environmental change, the mean low temperature at El Verde has been rising over the past 8 yr (Fig. 1). These differences in rainfall and temperature had an effect on the anoles; Schall and Pearson (1999) found that a measure of body condition (relative body mass) declined during cooler and drier periods.

Despite these objective and subjective indications that the environment at El Verde is unstable, prevalence of *Plasmodium* infection in both male and female lizards showed little difference among the samples. The higher prevalence of malaria parasites observed in male *A. gundlachi* is similar to the pattern seen in many populations of lizards harboring such parasites, although the cause is unknown (Schall, 1996).

The relative proportions of the 3 kinds of parasite (*P. floridense* and *P. azurophilum* in RBC and WBC) remained constant over the 10-yr period. The prevailing difference in prevalence of *P. floridense* and *P. azurophilum* in RBC suggests the 2 parasites may exploit different vectors. If so, then the stability of prevalence of both species over time would be even more remarkable, because 2 independent parasite–host–vector systems could be involved.

The stability of malaria prevalence at El Verde contrasts with findings from the 2 other long-term studies conducted on lizard malaria, both from temperate environments with mild winters. In Georgia, Jordan and Friend (1971) followed *P. floridense* in *Sceloporus undulatus* for 13 yr (1958–1970). Prevalence appeared to have followed a cycle over that time falling from 53% infected, to 12%, and rising again to 47%. Jordan suggested a secular trend in rainfall drove the changes. In California, Schall and Marghoob (1995) during a 13-yr-study found an apparent cycle of about 10 yr duration of *P. mexicanum* in *S. occidentalis.* Subsequent additional data for a total of 20 yr of observations support this conclusion (Schall, 1996; J. Schall, unpubl. obs.). Prevalence at the California site varied among years from 10% to 37% but was not correlated with any environmental measure. Schall and Marghoob (1995) suggested the prevalence of *P. mexicanum* was following a stable limit cycle that would result if nonlinearities exist in the relationships between the parasite, vectors, and lizard.

The high and constant prevalence of lizard malaria at El Verde suggests that the parasite–host system there would match the stable, endemic condition pictured by the Macdonald (1957) epidemiological model. This model is general in its application (Aron and May, 1982), such that even with likely differences in vector biology and immune response in lizard versus human malaria, its conclusions would be relevant for the El Verde lizard malaria system. What allows the important terms of the model (such as number of lifetime blood meals taken by the vectors and the density of those vectors) to remain within the boundaries necessary to allow such stability even in the face of major habitat changes as observed at the Puerto Rico site? The Macdonald (1957) epidemiology model suggests that the answer lies in the ecology of the vector(s). The identity of the vector(s) of lizard malaria at El Verde is unknown, but perhaps the variable environment has selected for vectors that are unaffected by changing rainfall patterns or damage to the forest structure. The vectors of *P. mexicanum* in California are psychodid sandflies that spend most of their lives in rodent burrows where the environment is constant and emerge only on nights when temperature and humidity are appropriate (Fialho and Schall, 1995; Schall and Marghoob, 1995). Environmental conditions above ground are therefore disconnected from the transmission biology of the parasite. The vectors of *P. floridense* and *P. azurophilum* may have behaviors that buffer them from droughts or hurricanes and that allow them to rebound in density very soon after acute habitat disturbance. Our sampling protocol would thus have missed short-term disruptions in transmission and the resulting drop in infection prevalence.

Some authors argue that large scale climate changes will alter the distribution and abundance of parasites of both veterinary (Baylis et al., 1999) and human public health importance (Rogers and Packer, 1993; Linthicum et al., 1999), including changes in prevalence of human malaria (Martens et al., 1995). El Niño events have been associated with increase in malaria prevalence in South America (Nicholls, 1993; Bouma and Dye, 1997), and a warming trend has been correlated with increase in malaria in Africa (Loevinsohn, 1994) and Sri Lanka (Patz et al., 1996). In contrast, the results from El Verde suggest that substantial climatic disturbances do not always lead to changes in *Plasmodium* prevalence. The causes of this difference in response to environmental changes presents an interesting problem in ecological parasitology.

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