DISTRIBUTION AND ABUNDANCE OF TWO MALARIAL PARASITES OF THE ENDEMIC *ANOLIS* LIZARD OF SABA ISLAND, NETHERLANDS ANTILLES

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ABSTRACT: The ecology of 2 parasites, *Plasmodium floridense* and *Plasmodium azurophilum*, was studied in the endemic lizard, *Anolis sabanus*, on Saba island in the eastern Caribbean. Prevalence of the two parasite species was similar, but prevalence varied among sites. Lowest prevalence occurred at dry, windy sites or cool, wet mountain peaks (0–20%); the parasites were more common at most other locations on the island (40–80% infected; overall prevalence = 47%). High and low prevalence sites sometimes were only a few hundred meters apart. Prevalence was similar for males and females but increased with body size except for a decline in the largest (=oldest) lizards. A surplus of mixed infections (*P. floridense* and *P. azurophilum* together in the same host) existed compared with chance proportions. Parasitemia generally was low; 70% of *P. floridense* infections were ≤100 parasites/10,000 erythrocytes, and 70% of *P. azurophilum* infections were ≤50/10,000. Parasitemia in solitary infections averaged more than twice that seen in mixed infections for both species.

We present here a study on the distribution and abundance of 2 malarial parasites (*Plasmodium azurophilum* and *Plasmodium floridense*) in the iguanid lizard *Anolis sabanus* on Saba, a small island in the eastern Caribbean. A substantial literature exists on the epidemiology of human malarial parasites. In contrast, similar data on the plasmodia of nonhuman vertebrate hosts are surprisingly scant. Lizard malaria has proven an excellent model for such studies because of the large number of species of *Plasmodium* available for study in lizard populations (77 have been described), their wide distribution in both temperate and tropical habitats, and the relative ease of working with small lizards in the field (review in Schall, 1996).

Our goals were to describe the distribution and abundance of the parasite at sites scattered throughout the island, to determine the prevalence and parasitemia of infections in males vs. females and in different size (=age) classes, and to search for evidence of any interactions between the 2 *Plasmodium* species. We compare our results with other studies of lizard malaria in California, U.S.A., west Africa, Colombia, Panama, and several other Caribbean islands.

MATERIALS AND METHODS

Saba, Netherlands Antilles, lies at 17°42′N latitude in the northeastern Caribbean. The 13-km² island is the tip of an underwater volcano. The topography is steep and complex, rising to Mt. Scenery at 887 m elevation (Westermann and Kiel, 1961). The leeward coastal areas are relatively hot and dry compared to the cooler and wetter valleys of the island, which support a rain forest or cloud forest at the summit of the mountain. Mean monthly temperature varies only from 26 to 28 C; total annual rainfall varies greatly, but monthly means show no seasonality (Netherlands Antilles Meteorological Service data).

The endemic anole *A. sabanus* is ubiquitous in nearly all habitats on the island, basking and scanning the habitat for insect prey while perching on warm rocks in drier habitats, fences, and houses in towns, and on trees, stems, and leaves in the forest. In our sample of collected lizards, males ranged from 29 to 72 mm (x = 54 mm; n = 1,420) in snout-to-vent length (SVL); females are smaller, ranging from 23 to 55 mm (x = 45 mm; n = 648). Gender was readily determined by the presence of enlarged postanal scales in the males.

Two species of *Plasmodium* exploit the Saban anole: *P. floridense* and *P. azurophilum*. Both parasite species occur widely in the Caribbean region (Staats and Schall, 1996). *Plasmodium azurophilum* is found

from Grenada north into the Greater Antilles. *Plasmodium floridense* is restricted to the northern islands of the archipelago, being found no further south than Montserrat, but also extending into the southeastern U.S. Lainson et al. (1974) erected the genus *Garnia* for malarial parasites that invade erythrocytes but do not produce hemozoin. Because *P. azurophilum* infects both red (RBCs) and white blood cells and rarely produces hemozoin, we follow Schall (1996) in maintaining this species in the genus *Plasmodium*.

From 15 May to 19 August 1992, 50 sites scattered in most habitats on Saba were surveyed for *Plasmodium* infections in the lizards (Fig. 1). Many of these sites were resampled in July 1993 and January 1994. At each site lizards were captured by hand or with a slip noose on a fishing pole. That evening, each lizard was sexed, its snout–vent length in mm (SVL) taken, a toe clipped, and a drop of blood obtained to make a thin blood smear. Smears were fixed in methanol and stained with Giemsa at pH 7.0, using standard techniques (Schall and Bromwich, 1994). Stained smears were examined under 1,000× for 6 min, sufficient time to inspect 10,000 red blood cells. Presence or absence of blood parasites was noted, and the species of any *Plasmodium* present was determined for infected lizards. Using this protocol, false positives are highly unlikely, but false negatives could occur if the parasite had not yet entered blood cells or had fallen to very low parasitemia. Such false negatives are not likely to influence the qualitative conclusions drawn in this study (see Schall [1996] for a review of this issue).

The 2 *Plasmodium* species are easily distinguished using morphological measures such as parasite size and shape and number of merozoites, presence of hemozoin, and blood cell type infected. *Plasmodium floridense* infects erythrocytes only, storing hemozoin that appears golden brown under the light microscope (Telford, 1974). The morphology of *P. floridense* is described in Telford (1974); it is small (5–32 merozoites produced by a mature schizont) and does not distort the nucleus of the RBC. In contrast, *P. azurophilum* is large (8–46 merozoites is typical, but often many more), it may distort the host cell nucleus, and stores no hemozoin. Most striking, *P. azurophilum* infects both erythrocytes and 2 classes of white blood cells (Telford, 1975; Schall, 1992).

To determine the frequency of mixed infections of the 2 species of *Plasmodium*, we examined data for 1,762 consecutive smears, which were studied to determine the species of any plasmodia present. Parasitemia in the peripheral blood was determined for a sample of infected lizards by counting approximately 1,000 RBCs in microscopic fields chosen at random on the smear to eliminate any possible bias as to location of the infected cells. Parasitemia is expressed as parasites per 10,000 RBCs.

RESULTS

Prevalence of *Plasmodium* in solitary and mixed infections

Smears from 2,086 lizards were examined for presence or absence of *Plasmodium* of either species; overall, 46.6% were
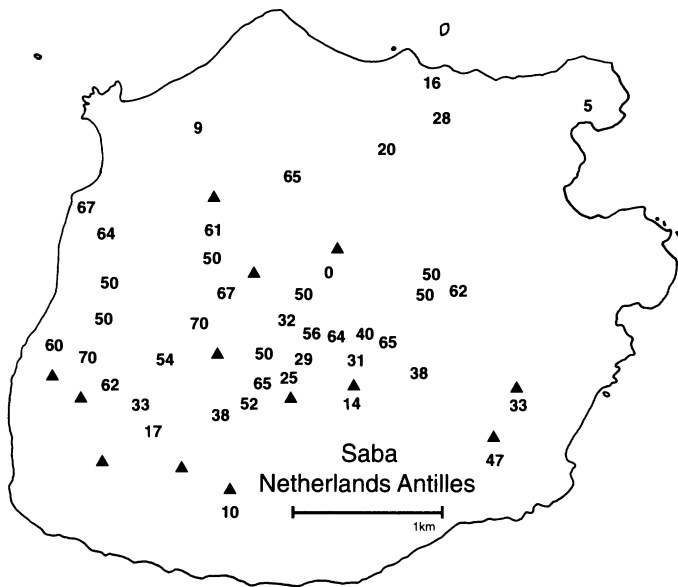
FIGURE 1.   Map of Saba island with study sites indicated. Numbers show percentage of the lizards at each site that were infected with *Plasmodium*. Major "peaks" are shown with a triangle. The complex topography of Saba is a result of multiple eruptions of the volcano leading to higher elevation domes, plugs, and dikes separated by steep valleys (Westermann and Kiel, 1961).
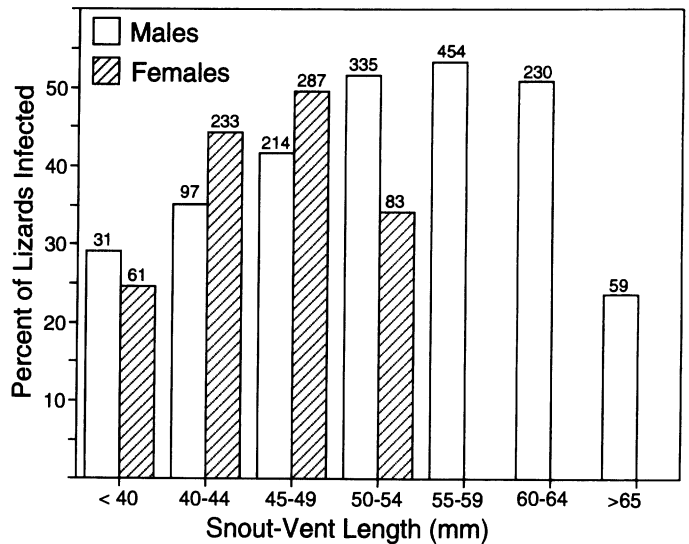

FIGURE 2.   Percentage of *Anolis sabanus* infected with two species of *Plasmodium*. Data are divided by gender and body size, which is assumed correlated with age. Numbers above bars indicate sample sizes.

infected for all study sites combined. For the 1,762 smears used to identify parasites to species, 36.8% were infected. Prevalence of *P. azurophilum* and *P. floridense* was similar (approximately 21% for both). Table I shows the proportion of lizards infected with each species and the expected number of mixed infections if the 2 species coexist by chance (found by the product of the proportion of lizards infected with each species of *Plasmodium*). There was a surplus of mixed infections ($1.3\times$) than expected by chance. When the data are broken down by gender and body size (an index of age), a surplus of mixed infections is seen for

all analyses but was significant only for the sample of smaller males (Table I).

Prevalence of *Plasmodium* among sites

There was a significant difference in proportion of lizards infected among the sites ($\chi^2 = 771.7$; $P < 0.0001$). Sample sizes at the 50 sites ranged from 13 to 171; sites with n > 20 are shown on Figure 1. Figure 1 shows that sites only a few hundred meters apart differed substantially in prevalence of the parasites. This result remains when only sites with n > 80 are examined. For example, prevalence of 65–70% was found at several sites in the western part of the island near the town of The Bottom, and only 10% at a site near the town of St. John in the south. In part this variation is driven by the complex topography of the island. High prevalence tended to be in the west and central moist areas of the island and low prevalence tended to be in dry, windblown areas, or the very wet mountain top. There was no effect of elevation on percentage of lizards infected ($r = 0.14$, $P > 0.05$; arcsin transform of proportion infected).

Prevalence of *Plasmodium* by size classes and gender of lizard

Overall, there was no significant difference in percentage of males and females infected (43.8% of females and 48.0% of males; $\chi^2 = 3.26$, $P > 0.05$). Figure 2 shows the prevalence of *Plasmodium* in males and females for different size classes; large animals are assumed to be older. Prevalence of the parasites in the 3 smallest size classes did not differ for females and males ($\chi^2 = 0.21$–3.60; $P > 0.05$) but differed for the 50–54-mm SVL size class, with more males than females infected ($\chi^2 = 7.67$; $P < 0.01$). Prevalence dropped in higher SVL classes for both sexes (comparing largest size class with next largest for males, $\chi^2 = 15.1$, and females, $\chi^2 = 7.1$, $P < 0.05$). Among male lizards, smaller (and presumably younger) lizards tended to have higher percentages of *P. azurophilum* infections and larger, older lizards

TABLE I.   Prevalence of *Plasmodium azurophilum* (AZ) and *P. floridense* (FL) in solitary and mixed infections.*

Sample	n smears†	AZ only	FL only	Observed mixed	Expected mixed	χ^2
Total	1,762	270	276	101	80	5.51‡
♂	1,175	167	201	70	55	4.28‡
♀	583	104	74	31	24	2.25
♂ >50 mm	834	122	162	51	44	1.08
♂ ≤50 mm	340	45	38	19	11	6.40‡
♀ >42 mm	423	81	56	26	21	1.33
♀ ≤42 mm	160	23	18	5	4	—

* Expected proportion of mixed infections was found as the product of the prevalence of each malaria species (solitary infections + mixed infections = total number of infections with that species of parasite), and the expected number by multiplying this percentage by the number in the sample. The data are broken down by gender and SVL.
† The number of each kind of infection given based on a 1,762 consecutive smears examined for species identity of parasites (out of 2,086 smears total).
‡ $P < 0.05$.

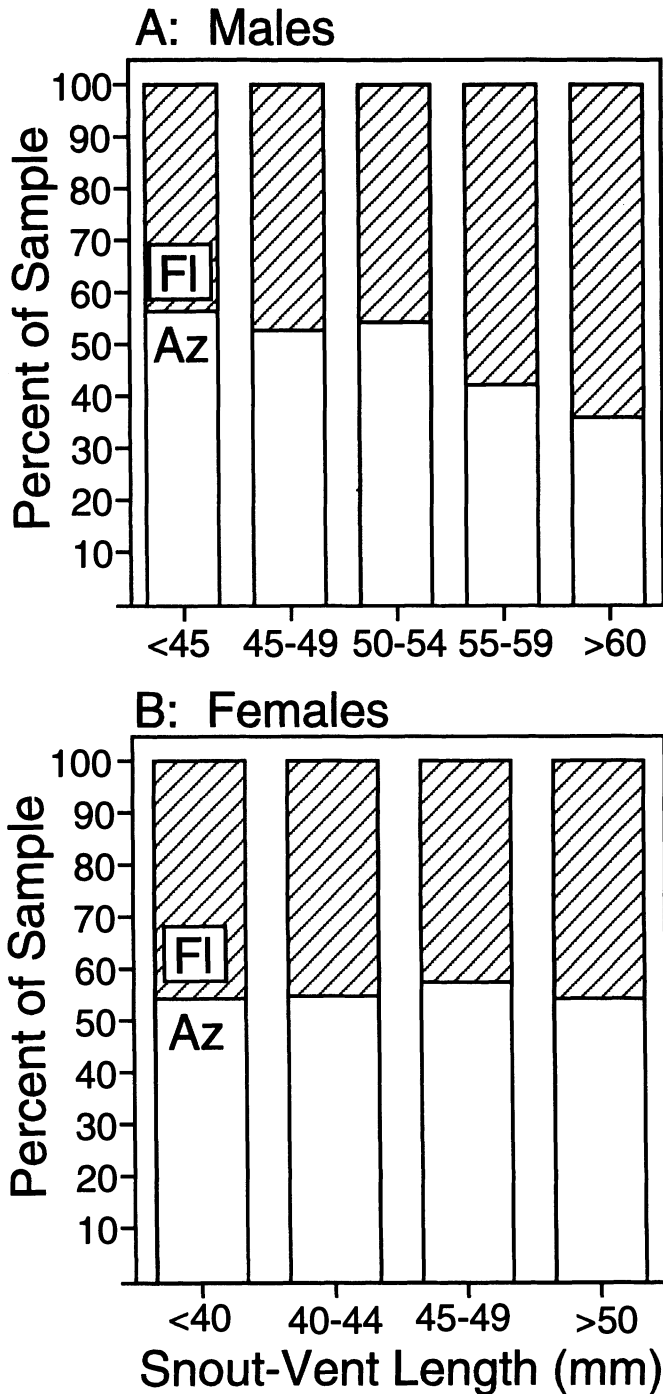## A: Males

B: Females

Snout-Vent Length (mm)

FIGURE 3. Percentage of infections of *Anolis sabanus* that were *Plasmodium azurophilum* and *P. floridense* by gender and body size. Male lizards above, females below.

more *P. floridense* infections (Fig. 3). No such trend appeared for female lizards.

Parasitemia

Plasmodium floridense had significantly higher parasitemia than infections of *P. azurophilum* (Table II; Wilcoxon sign-ranks tests; $P < 0.005$). Both species had significantly higher parasitemia in solitary than in mixed infections (Wilcoxon sign-ranks

TABLE II. Parasitemia of solitary and mixed infections by species of *Plasmodium*.*

Infection	Mean number of parasites/ 10,000 RBCs	Range	n
Overall infections	78.8	0–662	108
P. floridense in solitary infections	115	0–662	32
P. azurophilum in solitary infections	58	0–476	49
P. floridense in mixed infections	55	0–201	29
P. azurophilum in mixed infections	27	0–476	29

* If parasites were found during extensive scanning of the smear but none seen during the count of 1,000 erythrocytes, the parasitemia was recorded as 0.

tests, $P < 0.005$). Figure 4 shows parasitemia of most infections of both species is relatively low, with nearly 70% of *P. floridense* infections having 100 parasites/10,000 RBCs; *P. azurophilum* infections were even weaker, i.e., 70% of infections held only 50 parasites per 10,000 RBCs.

DISCUSSION

Plasmodium parasites are common in the Saban anole; infection prevalence was at least 40% at most sites. Prevalence is higher in *A. sabanus* than in other lizard malaria systems studied to date. Overall 47% of lizards are infected on Saba compared to approximately 25% of *Sceloporus occidentalis* (with *Plasmodium mexicanum*) in northern California (Schall, 1996), *Agama agama* (with *Plasmodium agamae* and *Plasmodium giganteum*) in west Africa (Schall and Bromwich, 1994; Schall, 1996), *Anolis limifrons* (with *Plasmodium balli* and *Plasmodium "tropiduri"* [actually probably more than one species]) in Panama (Rand et al., 1983), *Anolis auratus* (with *Plasmodium colombiense*) in Colombia (Ayala and Spain, 1976), *Anolis gundlachi* (with *P. azurophilum* and *P. floridense*) in Puerto Rico (Schall and Vogt, 1993), *Anolis gingivinus* (with *P. azurophilum*) on St. Martin (Schall, 1992), and *Anolis bimaculatus* (with *P. azurophilum* and *P. floridense*) on St. Kitts (Schall, 1996). The small Saban anole probably is a short-lived animal (Roughgarden, 1995), so the rate of transmission of the parasites must be very high. Unlike the temperate zone system (California), the lizards are active year-round on Saba. Also, unlike most of the other tropical systems, Saba has no real dry season. Transmission is therefore possible throughout the year.

This study also shows that even on an island as small as Saba, parasite prevalence differs substantially among sites. This small-scale patchy distribution of lizard malaria has been revealed in many other studies in both temperate and tropical systems (Ayala and Spain, 1976; Rand et al., 1983; Schall, 1992; Schall and Marghoob, 1995). For example, on nearby St. Martin Schall (1992) found abrupt changes in prevalence of *P. azurophilum* in *Anolis gingivinus* between sites separated by only 100 m. Examination of Figure 1 shows similar differences among sites over short distances. However, the topography of Saba is very complex; changes in habitat quality are apparent over short distances. The results suggest that movement of lizards from site to site is uncommon and that the vector density or feeding behavior varies among closely situated sites.
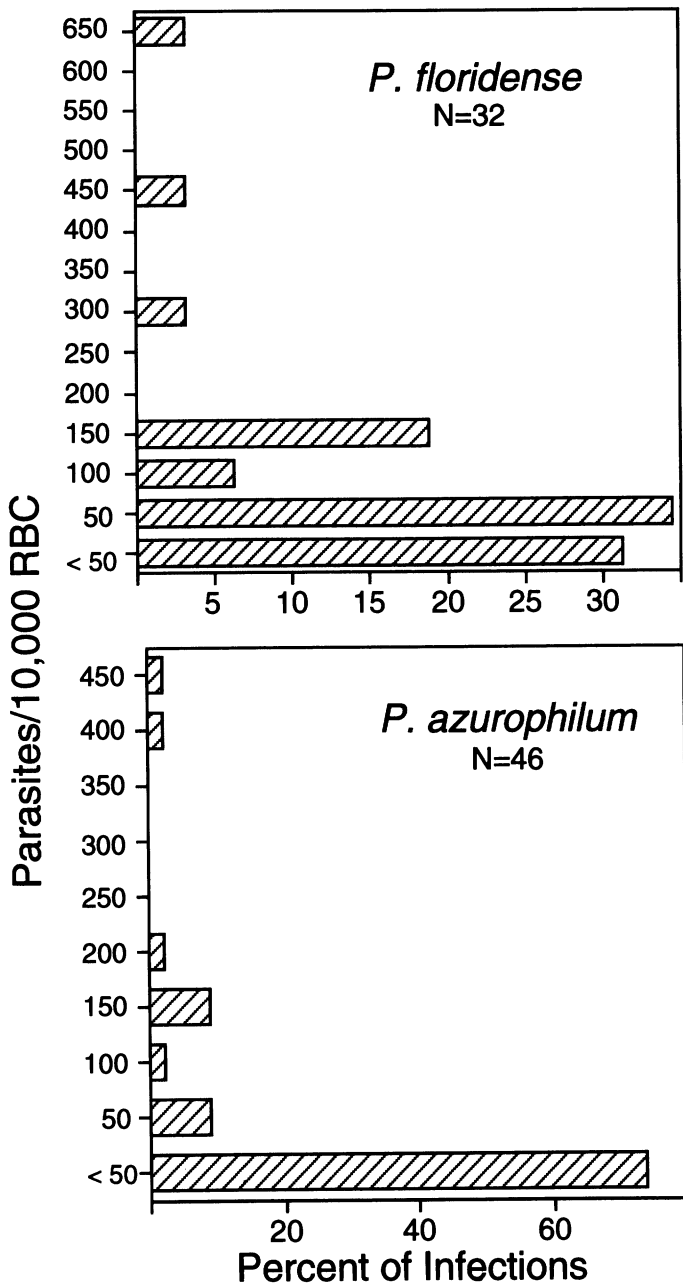
FIGURE 4. Parasitemia of 2 species of *Plasmodium* in *Anolis sabanus*.

Jordan (1964) and Schall and Marghoob (1995) found that temperature is an important influence in the prevalence of lizard malaria at temperate sites, with the parasite being common or absent where temperatures fall below some system-specific level. On Saba, however, the areas of lowest malarial prevalence are the cool, higher elevation sites on the highest peak, and the sunny, dry, and warmer coastal areas, so temperature alone cannot drive the variation in prevalence seen among sites.

Prevalence of the parasites on Saba did not differ significantly for male and female lizards of most body sizes. In some systems, malarial prevalence is higher in male lizards (California, west Africa, and Puerto Rico), but in others, there is no difference

(Saba, Sierra Leone, and St. Martin) (Schall, 1996). A current hypothesis argues that male vertebrates in general may suffer a handicap because testosterone impinges on functioning of the immune system, which allows higher parasite loads (Folstad and Karter, 1992). Lizard malaria provides an interesting system to pursue testing of this hypothesis because prevalence of *Plasmodium* infection in some systems differs with gender of host, and in others no gender-based difference is seen.

Malarial prevalence in Saban anoles first increases with size (=age) but drops for the largest (=oldest) size class. This pattern is the rule in lizard malaria systems (Schall, 1996). The increase in prevalence as lizards age is expected given that older lizards have had longer exposure to infection. The drop in prevalence for the oldest lizards may result when chronic infection eventually leads to a successful immune attack, which eliminates the infection or reduces it to undetectable levels. Alternatively, aging lizards may experience higher mortality when infected. For several lizard malaria systems, infections followed for long periods in the laboratory or field remained patent, leading to the conclusion that the drop in prevalence in older animals is more likely a result of higher mortality in infected lizards (Schall, 1996).

Typical parasitemia for *P. floridense* and *P. azurophilum* was low, probably representing chronic infections. A similar distribution of parasitemia among infections was seen in *P. agamae* and *P. giganteum* (Schall, 1996). In contrast, *P. mexicanum,* the temperate species infecting fence lizards in California, is much more variable in final parasitemia and produces many high-level infections that remain constant over time (chronic high parasitemia infections). Ewald (1994) proposes that temperate plasmodia experience a period of "impossible transmission" that leads to the evolution of reduced virulence, including reduced parasitemia in the vertebrate host. In contrast to this view, the results of study on the temperate vs. tropical lizard malaria systems reveal higher parasitemia in the temperate species (*P. mexicanum*).

A standing controversy in ecological parasitology concerns what, if any, interactions occur between parasite species within the same host (Schall and Bromwich, 1994). Richie (1988) proposed that blood parasites of vertebrates, including *Plasmodium,* might interact in complex ways, in part because they elicit strong immune responses from the host. Schall and Bromwich (1994) found a strong positive association between *P. giganteum* and *P. agamae* in populations of *A. agama* in west Africa. This and other evidence led them to conclude that the two plasmodia interact in a kind of succession in which *P. agamae* may alter the host to favor the establishment of *P. giganteum.*

Plasmodium azurophilum and *P. floridense* commonly coexist in the same species of local anole on many Caribbean islands (Staats and Schall, 1996); these parasites, therefore, provide a useful system for the study of interspecific interaction between plasmodia. On Puerto Rico, the two species were found in mixed infections in chance proportions, so Schall and Vogt (1993) suggested they may not interact within the host. *Plasmodium azurophilum* and *P. floridense* on Saba occur in proportions significantly higher than expected by chance. We doubt that this surplus of mixed infections reveals any positive interaction between the parasite species. First, a positive association is not found for most comparisons when the overall sample is broken down by gender and age. Second, positive associations can occur

if some fraction of the hosts are not susceptible to infection either because of their genotype or behavior (perching location, for example, in the case of *Anolis*). Such hosts are not available to the parasite and will deflate estimates of the real number of mixed infections that should occur by chance proportions (Cohen, 1973). We used the method of Schall and Bromwich (1994) to determine the smallest proportion of the *A. sabanus* on Saba that would need to be unavailable to the parasites for the observed positive association to vanish. If only 4% of the lizards (7% of the noninfected sample) were not available to the parasite, the expected number of mixed infections increases to a nonsignificant difference with the observed number. It is highly likely that a small fraction (such as 4%) of the lizard population is not prone to infection, so we conclude that the data on observed number of mixed infections points to a neutral association between the 2 species.

Other data, however, suggest otherwise. Parasitemia of *P. floridense* is higher than for *P. azurophilum* and for both species is suppressed in mixed infections compared to solitary infections. *Plasmodium azurophilum* is more common in younger male lizards and *P. floridense* in older animals. *Plasmodium azurophilum* can also infect white blood cells as well as erythrocytes. These results suggest some kind of complex interaction between the two plasmodia. Perhaps *P. floridense* is the stronger competitor when both species infect erythrocytes. *Plasmodium azurophilum* may enter white cells to avoid competition with *P. floridense,* similar to the way helminth parasites of passerine birds shift to use different portions of the small intestine to reduce interspecific competition (Moore and Simberloff, 1990). This speculation is challenged by the fact that *P. floridense* is rare relative to *P. azurophilum* in anoles of St. Martin and Puerto Rico (Schall and Vogt, 1993; Schall, 1996).

Some aspects of the distribution and abundance of plasmodia of lizards are similar among parasite–host systems. The differences seen among systems are more intriguing because we expect lizards to have similar physiology, immune response, and blood cell morphology, and this would be especially true in congeneric lizards on tropical islands (*Anolis* in the Caribbean).

ACKNOWLEDGMENTS

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