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Interspecific interactions tested: two species of malarial parasite in a West African lizard

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Abstract *Plasmodium giganteum* and *P. agamae*, parasites of the rainbow lizard, *Agama agama*, in West Africa were studied to determine the nature of any interspecific interactions between the two malaria species. The plasmodia are distributed in *A. agama* throughout the mesic zone of Africa; *P. agamae* is sometimes found as a solitary malaria species in populations of the lizard, but *P. giganteum* has not been found alone. In 3170 lizards from Sierra Leone the prevalence of lizard malaria at 22 sites varied considerably (8–90% of lizards were infected), but the ratio of the two species was similar among sites (52–91% *P. agamae*). Larger lizards were more often infected. Mixed infections occurred 2–5 times more often than expected by chance. Parasite density within individual hosts, or parasitemia, was similar for each species when alone or in mixed infection. Natural infections followed in laboratory lizards stayed at constant levels for as long as 211 days. The two species use different classes of host cells (*P. giganteum* in immature cells and *P. agamae* in mature erythrocytes) and may have different periods of peak transmission. Analysis of the data does not support a neutral relationship between *P. giganteum* and *P. agamae*, nor ongoing competition for resources or heterologous immunity. The data best support facilitation in which *P. agamae* alters the host in a way that allows more successful establishment of *P. giganteum*.

Key words Parasite · Malaria · Lizards · Africa *Plasmodium*

Introduction

A central goal of ecological parasitology is to understand the forces that shape parasite communities within individual hosts. These forces may be similar to those affecting communities of free-living species (competition for resources, interference, mutualism, historical chance events), but must also include the influence of the host's immune system which is unique to parasites. The literature on community ecology of free-living organisms has long included debate on the relative importance of species interactions (especially competition) and random events. This discussion is mirrored in the parasitological literature. Some workers hold that parasite communities are structured primarily by competition and there are "assembly rules" governing coexisting parasite species (Holmes 1961; Esch et al. 1990), whereas others present evidence that these communities are noninteractive groupings of species (Price 1980; Simberloff 1990). Such dichotomous thinking is useful in designing field and laboratory studies, but probably does not reflect the complex situation in nature (Holmes and Price 1986). Particular pairs of ecologically similar species may interact (through heterologous immunity, for example). Otherwise, the final structure of some parasite assemblages may be "determined by the independent activities of individual species" (Simberloff 1990).

Most work on parasite communities examines the biology of helminths in the vertebrate alimentary tract (Esch et al. 1990). Another interesting and challenging group, the microparasites of vertebrate blood, sometimes forms complex assemblages. In our survey of blood parasites of the rainbow lizard, *Agama agama*, in west Africa, we found two species of malarial parasites (*Plasmodium*), perhaps five species of haemogregarines, two microfilarial worms, and a virus that forms huge assembly pools visible under the light microscope (Schall and Bromwich, unpubl.). Such coexisting blood parasites may experience the influence of the host's immune system in a more intense and prolonged fashion than gut helminths, and as such offer more complex potential interactions among species.

Our study concerns the relationship between species of *Plasmodium* within their vertebrate host. Over 170 *Plasmodium* species have been described (Schall 1990a), and some vertebrate hosts are exploited by two, three,

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or even four species of malaria (Richie 1988). Richie (1988) lists the kinds of interactions that might shape assemblages of plasmodia species: (1) Neutralism, in which coexisting species behave independently of one another [the "null hypothesis" of Connor and Simberloff (1984)]. (2) Competition for resources such as nutrients or particular blood cell classes; such competition may be reduced if the parasites coevolve to partition resources (different cell classes, for example), an example of the "ghost of competition past" (Connell 1980). (3) Interference via heterologous immunity such that one species causes an immune response that might hinder other species. (4) Facilitation, in which the presence of one species may aid the establishment of another either by host immune suppression or by causing an increase in the other parasite's preferred cell class (immature red blood cells, for example). This is not a kind of mutualism because the facilitation would be a fortuitous result; rather, this interaction would more closely resemble succession in free-living communities.

We present here a study of two species of malarial parasite of the African rainbow lizard, *Agama agama*. Our research examined distribution records from surveys of malaria in these lizards in Africa, the prevalence patterns of the two *Plasmodium* species among sites in Sierra Leone, the relative parasitemia of the two species within hosts at the Sierra Leone sites, the cell classes exploited by the two parasites, and the course of infection in naturally infected, laboratory-maintained lizards. These data are used to evaluate the importance in this system of the four kinds of interspecific relations that could occur in malarial parasites.

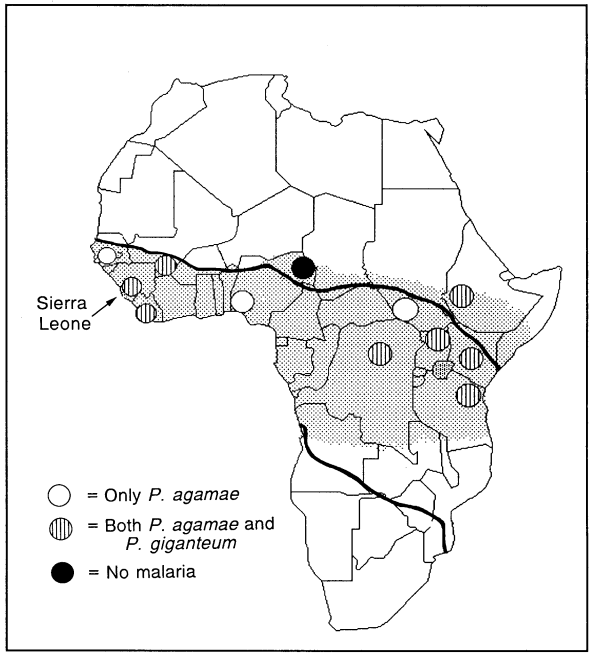

Fig. 1 Map of Africa showing sites and results of surveys for malaria in the rainbow lizard, *Agama agama*. Distribution of the lizard is indicated by the *shaded area* and range of mesic habitats by *dark lines*. The site in Ethiopia where malaria has been found in *Agama*, although outside the greater mesic range, is a highland moist patch of habitat. Data for study sites, distribution of *A. agama*, and range of moist habitats taken from many literature sources and authors' data

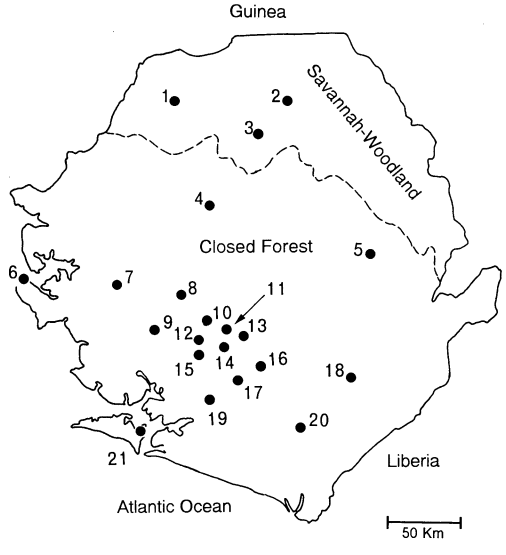

Fig. 2 Map of Sierra Leone, West Africa, showing study sites listed in Table 1

Methods

We studied *Plasmodium agamae* and *P. giganteum* in *Agama agama* in Sierra Leone, west Africa. The range of *A. agama* is shown in Fig. 1. The natural history of the lizard is given by Daniel (1960) and Harris (1964) and the effects of the parasites on rainbow lizards are presented by Schall (1990b). Both parasites are common in *A. agama* in Sierra Leone and are the only known malarial species infecting the lizards. We collected lizards at 22 sites scattered throughout the country; these are shown in Fig. 2 and Table 1. We had sites in a variety of habitat types: higher elevation savannah-woodland (sites 1, 2, 3), coastal swamp (site 21), and a variety of towns, riverside woodlands, and closed forest at the lower elevations. Lizards were collected by knocking them from their perching locations with long poles or by shooting them with a 0.22 calibre airgun. All lizards were sexed, snout-to-vent lengths (SVL) taken, and a blood smear made (immediately from shot lizards). Lizards captured alive were returned to their point of capture the next day. Lizards were collected in both the rainy (late April to late August) and dry (early January to early April) seasons.

Some lizards were brought into an indoor cage, 2.5 × 5.2 × 2.4 m high, outfitted with walls covered with wooden slats, many cross struts of wood, numerous plants, and full-spectrum Vitalights fluorescent lights set at a 12:12 LD cycle. Temperature and relative humidity in this cage were maintained at 25–32° C and 70%. Heat lamps around the cage allowed the lizards to thermoregulate, and they were fed with crickets, mealworms, and other insects. Each animal was individually marked with toe clips and blood smears made each week by nicking a clipped toe.

Each species of malarial parasite is specialized to infect one or more vertebrate species and one or more insect vectors. Within the vertebrate, the parasite undergoes asexual reproduction in blood cells (usually erythrocytes), developing from feeding stages with a single nucleus (trophozoites) to multinucleated stages (schizonts) that eventually divide into many new infective cells (merozoites) that leave the blood cell to invade other cells of the

Table 1 Study sites in Sierra Leone, West Africa. Given are: elevation (m), sample size of *Agama agama* lizards collected, percent of lizards infected with malaria (either *Plasmodium giganteum* or *P. agamae*, or both), and percent of infections that were *P. agamae*. Site numbers are those seen in Fig. 2. Of 3170 *A. agama* collected, 184 could not be assigned to one of these sites, but are included in other analyses

Site #	Site	Elevation	*n*	% Infected	% *P. agamae*
1	Koto	68	1	100	–
2	Kabala	443	58	29.3	77.8
3	Fadugu	290	5	20.0	–
4	Makeni	91	48	47.8	70.0
5	Yengema	396	57	78.9	91.1
6	Freetown	4	19	26.3	66.7
7	Masiaka	61	36	77.8	58.3
8	Yonibana	61	60	8.3	57.1
9	Moyamba	15–76	39	51.3	85.7
10	Taiama	61	324	18.5	65.6
11	Senehun	46	54	40.7	70.4
12	Njala	46	1690	21.4	70.3
13	Gbaiima	61	11	27.3	66.7
14	Njama	30	10	20.0	–
15	Mano	61	168	40.0	52.0
16	Bo	76	218	40.4	69.3
17	Bumpe	30	21	19.0	66.7
18	Kenema	91	66	31.8	63.6
19	Serabu	46	6	33.3	–
20	Potoru	76	30	90.0	64.7
21	Bonthe	2	48	25.0	70.6
22	Bongeima	46	17	0	–

host's blood. Some of these merozoites do not develop into trophozoites, but instead mature into sex cells, or gametocytes, that are infective to the insect vector. Thus, infection of a vertebrate host, such as the rainbow lizard, can be determined by viewing parasites in the blood cells. Density of parasites in the blood is termed parasitemia.

Blood smears were dipped in absolute methanol, then stained with 1:10 Giemsa for 50 min at pH 7.2. This staining procedure made the parasites obvious. Schizonts and mature gametocytes differ greatly between the two species, immature gametocytes and trophozoites less so. Therefore, positive identification of each malarial speciesdepended on finding schizonts, mature gametocytes, or in the case of *P. giganteum*, large trophozoites. *P. agamae* produces only 8 merozoites per schizont, whereas *P. giganteum* produces at least 90 (Schall 1990b). *P. giganteum* is a much larger cell than *P. agamae*, and causes the infected erythrocyte to swell as it grows, finally displacing the host cell nucleus. Measurement of parasite cell size using a microscope drawing tube and digitizing planimeter gave mean cell size of schizonts of *P. giganteum* of 68.5 μm^2 (SD = 23.2; $n = 96$ from five infections) and for *P. agamae* of 17.6 μm^2 (SD = 11.4; $n = 100$ from five infections). Illustrations of the parasites are given in Theiler (1930).

Slides were scanned three times each for 6 min during which approximately 30,000 red blood cells were examined. Slides judged positive after these scans were then examined to identify the parasite species. If both species were seen, this scan ceased. Otherwise, scanning continued for at least 1 h. This modification of the "flexible stopping rule" as suggested by Richie (1988) decreased the likelihood of missing mixed infections. Even so, the scanning protocol could result in false negatives of very weakly infected lizards and a false record of only one species of malaria present if the other species had not yet entered the blood or was in very low densities. Parasitemia was determined by counting asexuals and gametocytes seen in 1–3000 red blood cells depending on the density of the parasites. Those infections in which only asexual stages were seen, and in which most of these were trophozoites, were judged to be new infections. Immature red blood cells were distinguished from mature cells by their larger nucleus, rounder shape, and more blue-staining cytoplasm; cells with this morphology contain less hemoglobin than mature erythrocytes (Schall 1990b). Immature red cells are not here termed reticulocytes because mature lizard erythrocytes retain considerable reticulum in the cytoplasm (Schall 1990b).

Distribution throughout Africa of *A. agama* and its malarial parasites was determined from numerous literature records and from our own studies. If a study reported the presence of both *Plasmodium* species, we accepted the result even if the number of lizards sampled was small. However, if only one species of malaria was reported, but the sample size of lizards was small, we discounted the study because the other parasite could have been missed. We may have therefore biased our results toward locations where both species coexist.

Results

Figure 1 illustrates the distribution of the parasites in *A. agama* in Africa. The parasites appear to be distributed throughout the range of *A. agama* in the more mesic areas of southern Africa. The only site where the lizard was common, but the parasites missing, was at Maiduguri in northeastern Nigeria which is dry and at the periphery of the lizard's range.

Table 1 shows that both species of parasite were found at all sites in Sierra Leone where a substantial sample of lizards was examined. The prevalence of both species of malarial parasites increases with increasing lizard body size (Fig. 3). This could be a result of a larger cumulative chance of having been infected for larger, or older, animals, or perhaps larger animals simply provide a larger target for vectors seeking a blood meal. Overall, the percentage of lizards infected was higher for males than females (25.2% vs. 18.2%), but there is no difference between males and females within the range of overlapping SVL (χ^2 tests, $P > 0.05$). Therefore, for

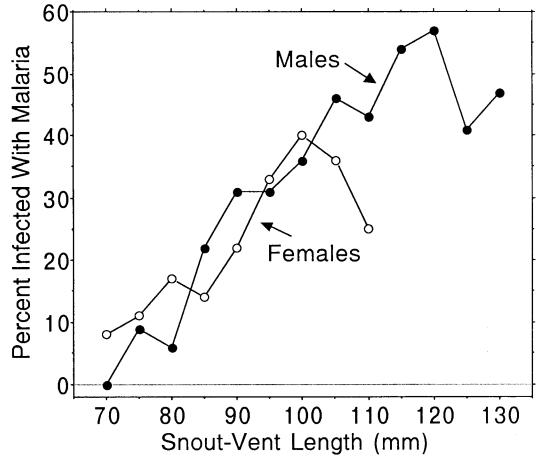

Fig. 3 Percent of individuals infected with malaria by body size for male and female *Agama agama* in Sierra Leone ($n = 2069$ lizards). Trends for *Plasmodium agamae* and *P. giganteum* are similar, and relatively few solitary infections of *P. giganteum* are included (Table 2), so the data for the two malaria species are combined in the figure

Table 2 *Plasmodium agamae* and *P. giganteum* in solitary and mixed infections in *Agama agama* lizards. Expected percentage of mixed infections is found as the product of the prevalence of each malarial species, and the expected number by multiplying this percentage by the total number of lizards sampled. Given are results for entire sample of lizards partitioned by gender and for different size classes (SVL = snout-to-vent length) for which the sample size was large enough to conduct a statistical test (indicated with *). All $P < 0.005$ (χ^2 tests). Total number of lizards examined = 2,870

SVL (mm)	Total smears	*P. agamae* only	*P. giganteum* only	Mixed infections (observed)	Expected number of mixed infections
Male *Agama*					
All	1549	316	57	187	79.3*
85–99	131	23	2	13	4.2
100–114	495	116	21	60	28.7*
115–138	418	129	28	58	38.3*
Female *Agama*					
All	1241	13	27	64	14.2*
70–84	303	26	8	9	2.0
85–99	579	77	12	21	5.1*
100–114	56	11	2	8	3.4

other analyses, data are merged for males and females, but are examined for possible effects of body size.

Body size distribution of lizards sampled among sites did not differ (χ^2 test, $P > 0.05$), so data are combined for subsequent analysis. Malaria prevalence varied considerably among sites (Table 1; CV = 0.588 for sites with sample sizes > 20 lizards), but the ratio of the two *Plasmodium* species was more similar among sites (CV = 0.148).

For 781 infected lizards in which the identity of the malaria species could be positively determined, there was a significant overabundance of mixed infections (both malaria species present) compared to the number of mixed infections expected by chance (Table 2). The expected number of mixed infections under chance association of the two species was found as the product of the proportion of lizards infected by each species (= expected proportion of lizards with mixed infection) multiplied by the total number of lizards sampled (Cohen 1973). The surplus of mixed infections was also observed when data were broken down by season (rainy vs. dry) and for sites with large samples (Taima, Njala, Mano, and Bo) (χ^2 tests, $P < 0.01$). The pattern also exists when the sample was partitioned by size of the lizards. There was no trend for the proportions of mixed infections to increase or decrease with lizard body size (Table 2; χ^2 tests for three body size classes, $P = 0.15$ for females and $P = 0.56$ for males).

Parasitemia for both malaria species is similar for mixed and solitary infections (Table 3). The mean total parasitemia in mixed infections approximates the sum of mean parasitemia for solitary infections for both species.

We followed 61 natural infections in laboratory-maintained rainbow lizards for 7–211 days (67% were followed for > 30 days, 23% for > 50 days). Observations were stopped when a lizard appeared ill; the high mortality rates of laboratory animals suggests these animals were in far from "normal" conditions. All but three of the observed infections maintained constant parasitemia over as long as 211 days. Among the constant infections, parasitemia varied among individuals from barely detectable to infections greater than 500 parasites per 10,000 red blood cells. Of these infections, 38 were solitary infections of *P. agamae*, 10 were solitary *P. giganteum*, and 13 were mixed infections. As most infections remained at a constant level, similar to another species of lizard malaria studied in detail (Bromwich and Schall 1986), no difference was seen in the history of mixed compared to solitary infections. No replacement of one species by the other was observed in any case. No infection appeared to be cured spontaneously. In two mixed infections, parasitemia remained high for both species for 35 days before the animal died

Table 3 Parasitemia (parasites per 10,000 erythrocytes) of *P. agamae* and *P. giganteum* infections in *Agama agama* in Sierra Leone. Compared are solitary infections with a single malaria species and mixed infections. Mean, SD, range, and sample sizes are given along with results from a Mann-Whitney *U*-test comparing solitary and mixed infections

	Mean	SD	Range	*n*
P. agamae				
Alone	85.0	115.3	<1–840	437
Mixed	77.6	101.2	<1–750	264
	$P = 0.214$			
P. giganteum				
Alone	118.8	209.0	<1–1317	73
Mixed	75.4	139.8	<1–1436	264
	$P = 0.082$			
Total parasites in mixed infections	217.4	287.1	<1–2911	264

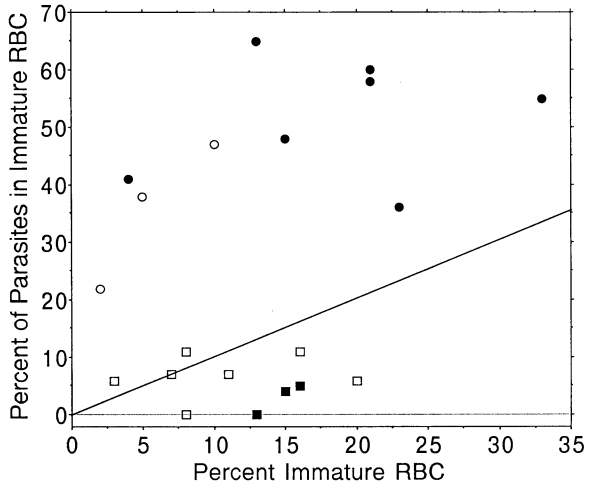

Fig. 4 Use of different cell classes by *Plasmodium giganteum* and *P. agamae* from infections with only one malaria species. Each *point* represents one infection; *circles* represent *P. giganteum* and *squares P. agamae*. Data show the percent of erythrocytes that were immature cells (*abscissa*), and percent of parasites that were seen in immature cells (*ordinate*). *Line* indicates random entry of parasites into cell classes (use of immature cells in their proportion in the cell population) and *filled points* are those significantly different from random use of cell classes (χ^2 tests). As the abundance of immature erythrocytes varied among infections, the number of cells counted was adjusted to give adequate sample size ($n = 638$–2332). Data show that *P. giganteum* uses immature cells and *P. agamae* uses primarily mature erythrocytes

(>300 parasites/10,000 red blood cells for *P. agamae*; and >200 for *P. giganteum*).

Transmission may be seasonal for *P. agamae*. New infections were more common in the dry compared to rainy season (33% of 487 lizards vs. 22% of 55 lizards; *G*-test, $P < 0.01$). No such seasonality was detected for *P. giganteum*, although sample size for the wet season was small (48% of 139 dry season lizards and 48% of 25 lizards in the wet season; *G*-test, $P > 0.05$).

Plasmodium giganteum and *P. agamae* differ in the cell class utilized; *P. giganteum* has a predilection for immature red blood cells and *P. agamae* is found primarily in mature cells (Fig. 4). Garnham (1966) stated that *P. giganteum* has an affinity for immature red cells, but provided no data to support this correct statement, and incorrectly suggested that *P. giganteum*-like cells seen in mature erythrocytes are most likely misidentified *P. agamae*.

Discussion

Using the data presented here, we now evaluate the kinds of interspecific interactions that could occur between malaria species.

Neutralism

The best evidence for a noninteractive association between the two malaria species is that the total parasitemia in mixed infections approximates the sum of mean parasitemia seen in solitary infections of *P. giganteum* and *P. agamae*. However, although prevalence of malaria varies considerably among sites, suggesting different transmission dynamics or other ecologically important forces, the ratio of the two species of parasites in the population is similar among sites. This hints that the two species are not independent parasites. Also, the significant surplus of mixed infections compared to that expected by chance association argues against neutralism.

Two possibilities could explain the surplus of mixed infections if the there was no interaction between the two malaria species. First, the two malaria species could share the same vector, which would mean they are not independent in transmission dynamics as assumed by the χ^2 analyses performed here. The vectors of *P. agamae* and *P. giganteum* are unknown, but are likely to be in the same groups of insects that are known to be the vectors of other lizard malarias, mosquitoes and sandflies (Klein et al. 1987a, b). The data on seasonality of newly established infections are equivocal, but weakly suggest timing of peak transmission for the two species differs, and thus two vectors may be involved. Also, if mixed infections commonly occur because of the nonindependent transmission of the *P. agamae* and *P. giganteum*, we expect the chance of mixed infection to increase over the lifetime of the lizard, if all age classes are equally likely to be bitten by a vector. The prevalence of mixed infections, in contrast to this prediction, does not increase with size, and presumably age, of the lizards.

Second, if some individual lizards are more susceptible to malarial infection than others, 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 here. The percentage of lizards infected with each species would then be larger than calculated above and the true expected number of mixed infections under chance association would be higher. We have no data on any tendency for some individual lizards to be resistant to the parasite, let alone data on the proportion of the population that may be resistant. However, Fig. 5 shows the expected number of mixed infections under chance association of *P. giganteum* and *P. agamae* for different proportions of the noninfected lizards that are assumed to be susceptible to the parasite. The results show that there is a significant surplus of observed mixed infections until approximately 85% of the noninfected group are assumed to be resistant to infection (only 15% of noninfected lizards assumed to be susceptible to infection). We find it difficult to believe that almost all of the noninfected lizards in the population are resistant to infection. Recall that the surplus of mixed infections for the smallest size class of lizards is at least as great as that seen for the overall population (Table 2), yet the percentage of lizards infected approximately doubles from these

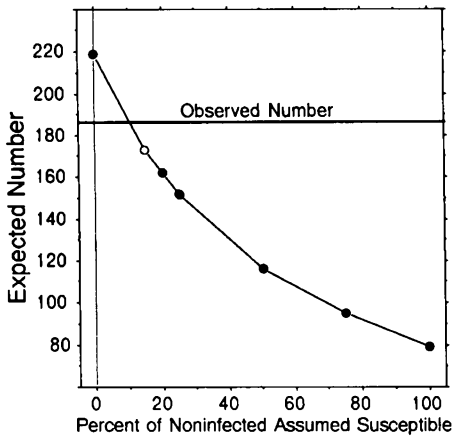

Fig. 5 Observed number of mixed infections of two malaria species (*horizontal line*) compared with number of mixed infections expected if the two species associated independently. *Curve* shows expected number with various percentages of the noninfected sample assumed to be susceptible to the infection; 100% assumes all noninfected lizards could become infected, and 0% that all are immune to the parasites. *Filled points* indicate those significantly different from the observed number (χ^2 tests) (see text for discussion)

small lizards to the largest (oldest) class (Table 2; Fig. 3). Therefore, it is impossible that at least 85% of the smaller lizards were immune to infection.

Competition

Several lines of evidence argue against the likelihood of competition being important now in this two species system. Parasitemia of each species in mixed infections is not lower than when the species of malaria occur alone in a lizard host as would be expected if competition for resources was ongoing. The history of mixed infections observed in the laboratory did not reveal the replacement of one species of malaria by the other. There is also a surplus of mixed infections compared to what is expected by random association, rather than the scarcity of mixed infections expected if the two species were competitors. Perhaps competition was important in the past evolution of these species, because the use of different cell classes could be viewed as resource partitioning that evolved specifically to reduce competition.

Interference via heterologous immunity

No evidence supports this kind of interaction in this system and the results refuting ongoing competition likewise refute heterologous immunity.

Facilitation

Plasmodium giganteum may be facilitated in its establishment in a lizard host if *P. agamae* is already present, and *P. giganteum* may not be able to persist in a population of lizards unless the other species is also present. *Plasmodium giganteum* is a very striking species and is unlikely to be missed even in casual surveys; *P. agamae*, in contrast, could be mistaken for immature *P. giganteum* by inexperienced workers. Despite this bias, the known distribution of the two malaria species shown in Fig. 1 indicates that *P. giganteum* has not been found as a solitary species, whereas *P. agamae* has been discovered alone at some sites. Also supporting the facilitation hypothesis is the positive association of the two species (surplus of mixed infections) and the fairly similar ratio of *P. agamae* and *P. giganteum* infections among lizards in a sample.

How would presence of *P. agamae* facilitate the establishment success of *P. giganteum*? *Plasmodium giganteum* has a predilection for immature erythrocytes, whereas *P. agamae* was seen primarily in mature cells. Circulating immature erythrocytes are rare in noninfected lizards but increase dramatically in lizards with malaria (Schall 1990a). This is typical for lizards and other vertebrates infected with malaria and probably results when the immune system discards any even slightly aberrant red cells in an effort to eliminate the parasite. Some malaria species that favor use of immature erythrocytes may even actively elicit production of these cells by the host (Roth and Herman 1979). *Plasmodium agamae* will usually find a plentiful supply of its preferred cell host when it first enters a lizard (mature red cells >99% in hosts not yet infected and at least 50% for lizards already infected). However, *P. giganteum* will find relatively few of its preferred cell if it enters a noninfected lizard (<1% of erythrocytes are immature), but 10–50 times that number if it enters a lizard already infected by malaria. Thus, *P. agamae* could alter the blood environment to give *P. giganteum* a better chance of becoming established. At sites where *P. agamae* is absent, the probability of successful establishment of *P. giganteum* infections may be too low to permit the species to remain in the lizard population. This would account for the distribution of the two lizard malaria species in Africa (*P. giganteum* not found alone, but *P. agamae* sometimes the solitary species in rainbow lizards).

The data show that parasitemia of solitary *P. giganteum* infections is not significantly lower than that seen in mixed infections. The course of infection of *P. giganteum* observed in the laboratory also did not differ for solitary versus mixed infections. These data might argue against facilitation. However, if a solitary infection of *P. giganteum* is established, the host will ultimately produce an increased number of immature RBC in response to the infection. The environment for a successfully established solitary infection of *P. giganteum* would not differ from that for a mixed infection. The kind of facilitation proposed here concerns the chance of an infection becoming established, not its ultimate density.

We conclude that most of the data do not support neutral association of the two species or ongoing competition. However, coevolution of *P. giganteum* and *P.*

agamae may have occurred leading to present resource partitioning by their using different blood cell classes. Interference competition via heterologous immunity is unlikely. The data best support facilitation in which the presence of *P. agamae* aids the establishment of *P. gigan-teum* infections. Examples of possible facilitation of one malaria species by another via host immune system effects are reviewed by Richie (1988), but the kind of facilitation suggested here, in which the preferred cell type of one species is increased by another, has not been reported before for malarial parasites.

Obtaining unequivocal evidence for interaction between parasite species in a community presents a daunting challenge (Simberloff 1990). The diverse evolutionary histories of malarial parasites, plus the complexity of possible parasite-immune system effects, should give rise to a variety of kinds of interspecific interactions among species of *Plasmodium*, exactly as described in the literature (Richie 1988). This study adds to the evidence that *Plasmodium* species form interactive assemblages and demonstrates that studies on the community ecology of vertebrate blood parasites will provide interesting, complex, and productive models for investigation of parasite community organization.

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