VECTOR INCRIMINATION AND EXPERIMENTAL TRANSMISSION OF *PLASMODIUM FLORIDENSE* BY BITES OF INFECTED *CULEX (MELANOCONION) ERRATICUS*[1]

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ABSTRACT. A survey of mosquitoes which are attracted to and readily feed upon lizards in a northcentral Florida area where there is a relatively high prevalence of *Plasmodium floridense* in *Anolis carolinensis* was conducted. *Culex erraticus* and *Cx. territans*, collected in lizard-baited traps, readily fed on lizards in the traps and in the laboratory, and the former mosquito species is an experimental vector of *Plasmodium floridense*. Sporozoites were observed in the salivary glands from 11 to 14 days at 21–25°C following an infective blood meal. The prepatent period of *P. floridense* in *A. carolinensis* at 18–24°C was relatively long, 24–25 days (n = 2). At 32°C, the prepatent period was decreased to 13–17 days (n = 4). The transmission rate of *P. floridense* by bite of *Cx. erraticus* and intraperitoneal inoculation of sporozoites from the salivary glands of *Cx. erraticus*, was relatively low (16.2%). Peak parasitemias ranged from 912 to 4,280 parasites per 10,000 red blood cells. Sporogonic development of *P. floridense* in *Cx. territans* which fed on infected lizards was not observed.

INTRODUCTION

The first saurian malaria parasite was discovered in 1909 by Wenyon. Since then, 59 species of saurian *Plasmodium* have been described (Telford 1982, 1983, 1984a, 1984b; Garnham and Telford 1984). Mosquitoes, phlebotomine sand flies, and biting midges (Ceratopogonidae) have been suggested as possible vectors of saurian malaria but only mosquitoes have been incriminated as vectors of mammalian and avian malarias. A number of biting Diptera may be involved in saurian malaria transmission because previous studies have demonstrated that: (1) *Plasmodium mexicanum* Thompson and Huff is efficiently transmitted by bite of *Lutzomyia vexator* (Coquillett) (Ayala and Lee 1970, Klein et al. 1987), (2) sporogony of *Plasmodium agamae* (Wenyon) occurs in *Culicoides nubeculosus* (Meigen) (Petit et al. 1983), and (3) *Culex erraticus* (Dyar and Knab) transmits *Plasmodium floridense* Thompson and Huff (this paper). Differences observed in the ultrastructure of some saurian malaria sporozoites (Boulard et al. 1983, Klein et al. in preparation) and the ability of saurian malaria parasites to develop in several families of biting flies may indicate primitive characters (Mattingly 1965).

The criteria for vector incrimination were outlined by Barnett (1962). The mosquito vector-host relationships of many mammalian and avian malaria parasites have been described, but only two species of phlebotomine sand flies, *Lutzomyia vexator* and *Lutzomyia stewarti* (Mangabeira and Galindo), have been incriminated as vectors of a saurian malaria, *Plasmodium mexicanum* (Ayala and Lee 1970, Klein et al. 1987). Attempts to incriminate mosquitoes as a vector of this, or other, saurian *Plasmodium* have been unsuccessful. In some cases, a few mosquitoes developed small numbers of oocysts, but none had sporozoites (Petit et al. 1983, Jordan 1964, Huff 1941, Telford 1970).

This study describes the first successful laboratory transmission of a saurian malaria, *P. floridense*, by bite of a mosquito. Also, the incubation period and course of acute infection of *P. floridense* in *Anolis carolinensis* are reported for the first time following infected bites of *Cx. erraticus* and intraperitoneal (IP) inoculation of sporozoites from the salivary glands of *Cx. erraticus*.

MATERIALS AND METHODS

The Hatchet Creek study site, located near highway 26 approximately 15 km NE of Gainesville, Florida, was selected because of the relatively high prevalence of *Plasmodium floridense* in *Anolis carolinensis* Voight previously collected from this area (unpublished data). Hatchet Creek, a 2–7 m wide permanent stream, empties into Newnan's Lake. At the study site, Hatchet Creek separates into several channels which form pools during dry periods. Portions of Hatchet Creek consist of grassy margins and pools that intermittently flood during heavy rains. A combination of deciduous, deciduous-pine and pine forests occur along the margins and length of Hatchet Creek. The study site along the margin of the creek was heavily deforested in 1982, and during 1983–85, consisted primarily of young willows (*Salix* sp.). Adjacent

[1] The views of the authors do not purport to reflect the position of the Department of The Army or the Department of Defense (P. 4-3, AR 360-5). Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 85-23. Florida Agric. Exp. Station Journal Series No. 7925.

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to the study site, there are several permanent ponds of water covered, or nearly covered, with floating plants (*Lemna* sp.).

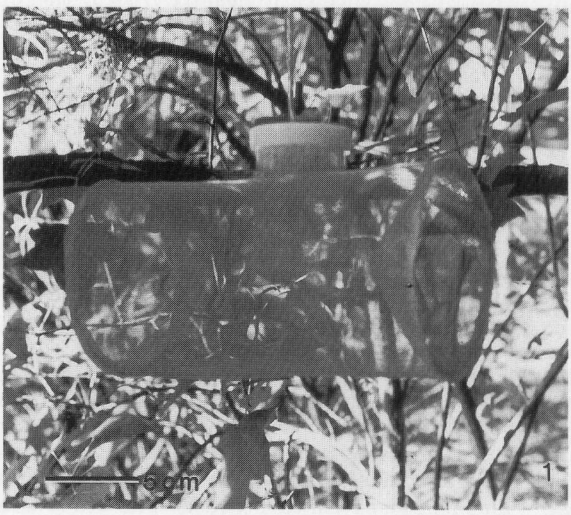
Vector attraction and blood-feeding propensity for feral biting arthropods were determined by the use of lizard-baited traps in which the host lizard was exposed to the arthropods (Fig. 1). These and CDC light traps were operated continuously for three nights per week at the same time from April 30, to September 6, 1984. Center for Disease Control light traps, operated from 1800 to 0800 hr the following morning, were used to determine seasonal changes in biting fly populations and to determine which species were present at the study site. Mosquitoes collected in the light traps were returned to the laboratory. Those that were dead were removed from the trap, counted and identified. Living mosquitoes were treated differently, as described below. Adult *Culex salinarius* Coquillett and *Culex nigripalpus* Theobald were often badly rubbed and could not be separated with certainty, and were considered together. Many *Aedes* spp. often were badly rubbed also, and were not identified to species.

The *Cx. erraticus* laboratory colony originated from wild caught females collected in lizard-baited and CDC light traps during June–August, 1984. Engorged *Cx. erraticus* and *Cx. territans* collected in the lizard-baited traps were returned to the laboratory, placed in screen-topped pint cardboard cartons (0.5 liter) and provided a 10% sucrose solution for 2–3 days. Subsequently, mosquitoes were removed from the cartons and placed in 15 ml oviposition vials half filled with tap water and plugged at the top with cotton. Circular, 14 mm diam sections of *Azalea* leaves were placed on the water surface to enhance oviposition of *Cx. erraticus*. Unengorged mosquitoes from both types of traps were placed in a screened cage (18 x 18 x 21 cm) with a lizard, *A. carolinensis* or *Sceloporus undulatus* (Latreille). Blood-fed *Cx. erraticus* and *Cx. territans* were removed after 24 hours and handled as above for laboratory colonization. The remaining mosquitoes were killed, identified, counted and the proportion of blood-fed females determined.

Eggs from each feral female of *Cx. erraticus* or *Cx. territans* were transferred to enameled larval rearing pans (18 x 30 cm) with deionized water. A small amount of larval food (2% suspension of Tetra® fish food) was added to the water of the larval rearing pans one day after oviposition (PO). Larvae hatched 2–3 days (PO) and were fed a mixture of the food daily. Approximately 100–150 larvae were reared in each pan. Pans were skimmed occasionally with a paper towel when scum appeared on the water surface.

Pupae were removed from the larval rearing pans and placed in 250 cc glass culture dishes (12 cm diam) containing water. All adults were provided a 10% sucrose solution soaked in cotton. Chicks were provided as a blood source. Culture dishes with *Azalea* leaf sections floating on the water surface were used for oviposition. Eggs were normally attached to the periphery of the leaf, but beginning with the third generation, adults would also oviposit on the water surface or the side of the culture dish, so the use of leaves was discontinued. The *Cx. erraticus* colony was maintained in an insectary at 27°C and 80% RH, and 16:8 LD photoperiod.

Anolis carolinensis and *Sc. undulatus* were hand-collected from Hatchet Creek, Austin Cary



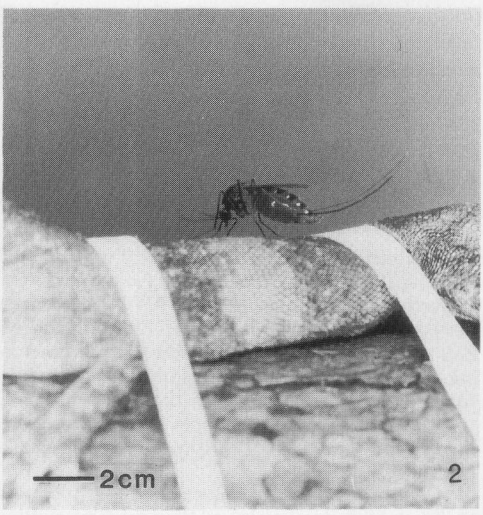


Fig. 1. Lizard-baited funnel trap used to capture biting Diptera.
Fig. 2. *Culex erraticus* feeding on a restrained *Anolis carolinensis*.

Table 1. Summary of *Plasmodium floridense* infections in *Anolis carolinensis* and *Sceloporus undulatus* collected from different localities in Florida (1983–85).

Locality	Species	No. collected	No. (%) infected
Alachua County			
Hatchet Creek	*A. carolinensis*	45	17 (37.8)
	Sc. undulatus	1	0
Gainesville	*A. carolinensis*	47	6 (12.8)
Cross Creek	*A. carolinensis*	18	3 (16.7)
	Sc. undulatus	62	0
Austin Cary Forest	*A. carolinensis*	33	13 (39.4)
	Sc. undulatus	51	6 (11.8)
San Felasco Park	*A. carolinensis*	6	0
Marion County			
Ocala Natl. Forest	*A. carolinensis*	2	0
Levy County			
Gulf Hammock	*A. carolinensis*	3	2 (66.7)
Total	*A. carolinensis*	154	41 (26.6)
	Sc. undulatus	114	6 (5.3)

Forest, and other localities near Gainesville (Alachua County), and from Gulf Hammock (Levy County), Florida (Table 1). In addition, *A. carolinensis* were collected from Manchac swamp, Tangipahoa Parish, Louisiana, where *P. floridense* infections have not been observed. Blood films were made from a clipped toe, air dried, fixed with absolute methyl alcohol, stained with Giemsa and examined for the presence of blood parasites. *Anolis carolinensis* were maintained in the laboratory as described elsewhere (Klein et al. in preparation) or in an environmental chamber at 32°C.

Laboratory reared *Cx. erraticus* females were placed in the afternoon in a plastic cylinder (4.5 x 15 cm) with a screened end, containing a lizard (*A. carolinensis* or *Sc. undulatus*) infected with *P. floridense*. The lizard was restrained on a tongue depressor with two thin pieces of tape, one over the shoulder, and the other over the pelvic girdle (Fig. 2). Blood-fed mosquitoes were removed the following morning, placed in 100 ml plastic urine specimen containers with a small amount of water, provided with a 10% sugar solution, and maintained in an incubator at 25°C and 80% RH. Later in the investigation, the sugar solution contained 0.1% Poly-vi-sol® multivitamin syrup. Midguts were dissected daily, beginning five days postfeeding (PF), and examined for oocysts. Ten days PF, the salivary glands were examined also, and the sporozoite rate determined (+1, 1–10; +2, 11–100; +3, 101–1000; +4, >1000 sporozoites.

In addition, F_1 progeny of wild caught *Cx. territans* (collected in lizard-baited traps) and *Lutzomyia vexator* (Klein et al. 1987) were fed also on infected *A. carolinensis*. Midguts and salivary glands were examined as for *Cx. erraticus*.

Culex erraticus that were potentially infective, i.e., those from lots of mosquitoes which developed oocysts and/or sporozoites, were placed in the screened feeding cylinders and provided a second bloodmeal on wild caught, uninfected *A. carolinensis*, restrained as above. These lizards were considered to be uninfected if they were negative for blood stages of *P. floridense* for more than 30–90 days. Following the second bloodmeal, mosquitoes were removed, dissected and the salivary glands examined for sporozoites. Salivary glands with sporozoites from mosquitoes that had taken second bloodmeals on uninfected lizards were injected intraperitoneally (IP) into other uninfected lizards.

Blood films of *A. carolinensis* previously fed on by infected *Cx. erraticus*, or that were injected IP with sporozoites, were made at day 0 and at 2–4 day intervals 10 days after exposure to infected bites or IP inoculation of sporozoites. Parasitemias were expressed as the number of parasites per 10,000 red blood cells (RBC). Sufficient RBCs were counted to keep the probable error within 10% (Gingrich 1932).

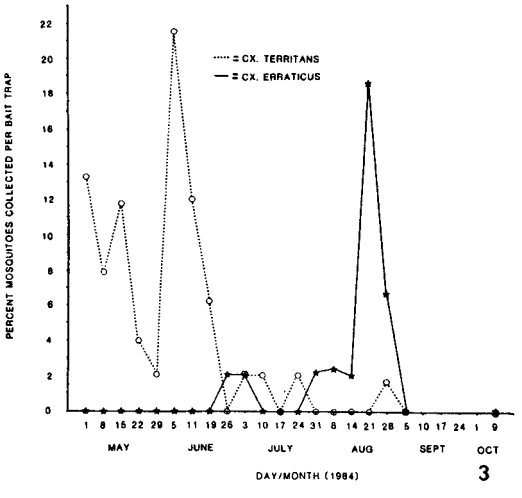
RESULTS

The *P. floridense* infection rate in wild caught *A. carolinensis* and *Sc. undulatus* from collecting sites near Gainesville, Florida is shown in Table 1. During 1983–84, 17 of 45 (37.8%) of *A. carolinensis* collected at Hatchet Creek were infected with *P. floridense*. The average infection rate for all anoles collected was 26.6% (41/154). Only 5.3% (6/114) of the bloodfilms of *Sc. undulatus* demonstrated parasites of *P. floridense*. Although 16.7% (3/18) *A. carolinensis* from Cross Creek demonstrated parasites of *P. floridense*, none (0/62) of the *Sc. undulatus* were infected.

Table 2 summarizes the mosquito species collected in CDC light and lizard-baited traps at Hatchet Creek, demonstrating the blood feeding propensity of local mosquito species on lizards in the field and laboratory. Bait traps were op-

Table 2. Summary of CDC light and lizard-bait trap collections and blood-feeding of feral mosquitoes in the field and laboratory from April 30 to October 10, 1984.

Species	Trap type (n)	Number female/(male) collected	Number bloodfed in trap/%	Number bloodfed in lab/%
Cx. erraticus	bait (924)	19	7 (36.8)	11 (91.7)
	light (43)	750 (9)	—	226 (72.2)
Cx. territans	bait	43	14 (33.3)	12 (48.0)
	light	2	—	—
Cx. salinarius/nigripalpus	bait	2	2	—
	light	915 (10)	—	74 (40.0)
Cq. perturbans	bait	2	2	—
	light	428 (11)	—	30 (20.1)
Cs. melanura	light	995 (14)	—	5 (2.7)
Psorophora spp.	light	16 (12)	—	0
Ur. sapphirina	light	443 (131)	—	0
An. perplexens	light	2	—	0
An. crucians	light	1,527 (32)	—	3 (<0.1)
Ae. fulvus pallens	light	39	—	0
Aedes spp.	light	1,065 (54)	—	29 (21.2)



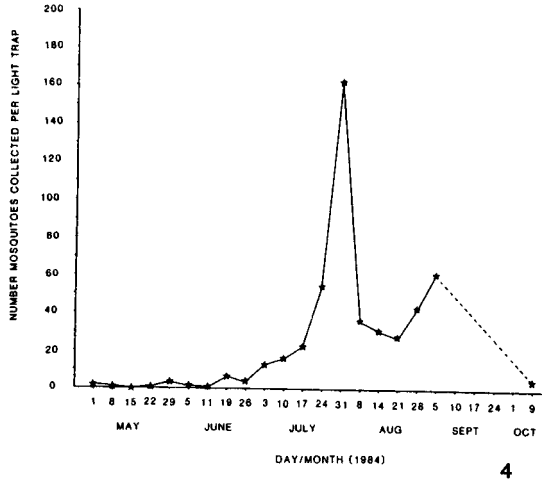


Fig. 3. Percent *Culex erraticus* and *Culex territans* collected per bait trap May 1–October 9, 1984.
Fig. 4. Number of *Culex erraticus* collected per light trap May 1–October 9, 1984.

erated from June through October 1983 and from May through October 1984, because Jordan (1964) indicated that *P. floridense* transmission occurred in late summer–early fall. During both trapping periods, mosquitoes were the only blood feeding arthropods collected in the lizard-baited traps. Excluding *Cx. territans*, *Cx. erraticus* accounted for more than 80% of the mosquitoes collected in the bait traps. Although *Cx. territans* were not collected during 1983, they accounted for more than 65% of the mosquitoes collected in 1984. Over 30% of the *Cx. territans* and *Cx. erraticus* fed on lizards in the bait trap. The other species [*Cx. salinarius/nigripalpus* and *Cq. perturbans* (Walker)] which readily fed on the lizards in the traps were infrequently collected (Table 2).

Two species of *Corethrella*, *C. brakleyi* Coquillett and *C. wirthi* Stone, and more than 14 species (7 genera) of mosquitoes were collected in the CDC light traps during 1984 (Table 2). *Culex territans*, the most frequently collected mosquito in the lizard-baited traps in 1984, was rarely collected in the light traps. A comparison of the frequency of *Cx. erraticus* in CDC light trap collections and percent of *Cx. erraticus* and *Cx. territans* collected in the lizard-baited traps is shown in Figs. 3 and 4. *Culex territans* was collected most frequently in the late spring–early summer: 71% of those captured were taken from May 1 to June 7. *Culex erraticus* was collected only from June 26 to August 30, and most often in the late summer.

In the laboratory, more than 70% and nearly 50% of the wild caught *Cx. erraticus* and *Cx. territans*, respectively, fed on lizards (Table 2,

Fig. 2). Only 40% of the *Cx. salinarius/nigripalpus* and approximately 20% of *Cq. perturbans* and *Aedes* sp. fed on lizards in the laboratory. None or few of the females of the remaining mosquito species and no *Corethrella* females fed on lizards in the laboratory. *Lutzomyia vexator*, the suspected vector of *P. mexicanum*, was not collected at Hatchet Creek.

In view of their readiness to feed upon lizards, attempts were made to infect *Cx. erraticus*, *Cx. salinarius* and *Cx. territans* with *P. floridense*. *Culex erraticus* was the only mosquito in which sporogonic development of *P. floridense* was observed (Table 3). Efforts to infect *Cx. erraticus* with *P. mexicanum* and *Plasmodium hermani* Telford and Forrester (an avian malaria) (Nayar et al. 1981) were unsuccessful. Sporogonic development and oocyst frequency of *P. floridense* in *Cx. erraticus* were highly variable. The development of oocysts was asynchronous, whereas development within an oocyst was synchronous (Fig. 5). Sporozoites were observed in some oocysts 9 days after the initial bloodmeal (Fig. 6). These oocysts often were easily ruptured by pressure from the coverslip, releasing sporozoites and developing sporozoites still attached to the sporoblastoid. Beginning 9 days after the infecting feed, free sporozoites were observed from dissected midguts from mosquitoes maintained at 25°C on 10% sugar solution + multivitamin. However, oocysts may have ruptured during the dissection since sporozoites were not observed in the salivary glands until 11 days PF. Sporozoites were not observed in the salivary glands of mosquitoes until 13 days PF when maintained at 25°C on sugar water without multivitamins. In general, sporozoites were present in the salivary glands 11–14 days following a bloodmeal in mosquitoes that showed 20 or more oocysts (Figs. 7 and 8). However, sporozoites were not seen in the salivary glands of some mosquitoes more than 20 days PF when only a few oocysts were present.

Occasionally, melanization of some oocysts occurred, but usually was not observed (by light microscopy) until the later part of sporogonic development (Fig. 9). In one experiment, when the mosquitoes were maintained at 32°C, nearly all the oocysts were partially to completely melanized by 7 days PF. Fewer melanized oocysts were observed following the addition of a multivitamin solution to the sugar water during the first 14 days of development. However, many slower developing oocysts were melanized in heavily infected midguts after 20 days (Klein et al. in preparation).

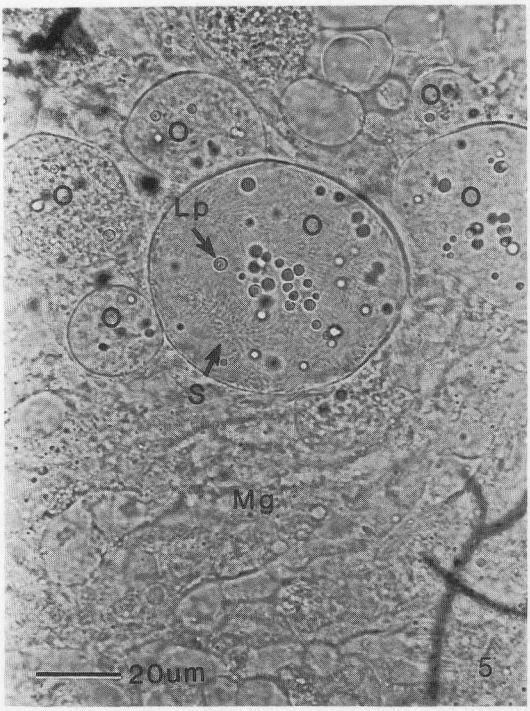
Attempts to transmit *P. floridense* were only partially successful. The rate of transmission by bite and IP inoculation of sporozoites was very low. Six of 37 (16.2%) of the *A. carolinensis* (Table 3), and none of the 7 *Sc. undulatus* became infected.

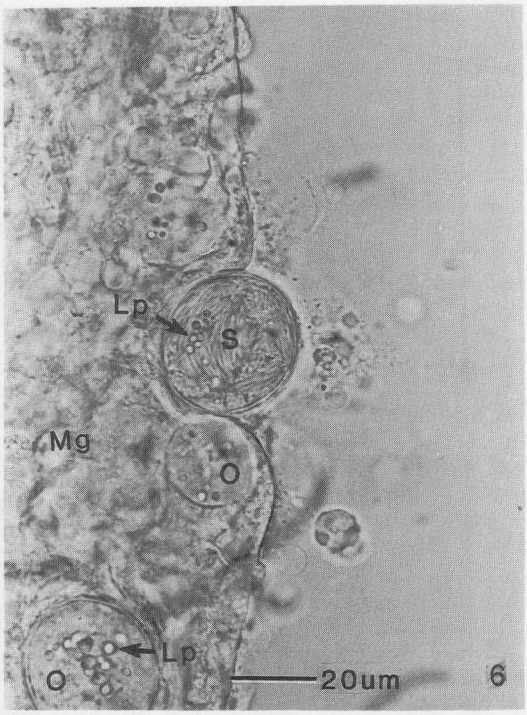
Although sporogonic development of *P. floridense* in *Lutzomyia vexator* occurred, only a few free sporozoites, which had similar gross morphological characteristics to those seen in *Cx. erraticus*, were observed from two dissected midguts (n = >50). These sporozoites may have been released by mechanical rupture during dissection. Sporozoites were never observed in the salivary glands of sand flies (n = >20) which survived more than 14 days PF.

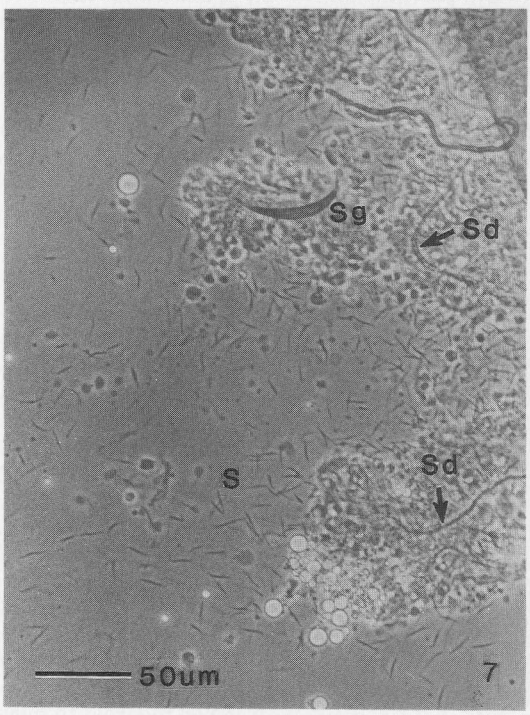
The number of parasites per 10,000 RBCs in lizards fed upon by an infected mosquito (A-85, AB-23, AB-9, and AB-321) or inoculated with sporozoites from the salivary glands of *Cx. erraticus* (AA-59, AB-271) was recorded for up to 120 days (Table 3, Fig. 10). Due to technical difficulties, blood films from lizard number A-85 were not made at 3–4 day intervals. The prepatent period in experimentally transmitted *P. floridense* (by bite and IP inoculation of sporozoites) ranged from 24 to 25 days and 13 to 17 days for *A. carolinensis* maintained at 18–24°C and 32°C, respectively. The parasitemia of the lizards rose rapidly, and peaked by 27 and 74 days postinfection. Peak parasitemias were 912 and 4,280/10,000 RBCs for each of the lizards (Table 3). The number of trophozoites and schizonts increased logarithmically until the peak parasitemia was reached. Thereafter, parasitemias became erratic. The gametocyte numbers fluctuated over the course of infection and gametocytes were not observed in several of the blood films.

DISCUSSION

The present study found that a large number of species of biting Diptera were present at the Hatchet Creek study site where anoles were commonly found infected with *P. floridense*. Although several species of mosquitoes fed on lizards in the laboratory, only a few were attracted to and fed on lizards in traps in the field. Only two species of mosquitoes, *Cx. erraticus* and *Cx. territans*, were collected in the lizard-baited traps with relatively high frequency. Both species readily fed on the lizards in the traps and laboratory. Jordan (1964) collected many of the same mosquito species at the Fargo-Okefenoke Swamp where *P. floridense* is relatively abundant, but *Cx. erraticus* was not reported to have been collected. *Culex erraticus* has a wide geographic distribution (from Michigan, USA to Bolivia) and probably occurs in the Fargo-Okefenoke Swamp. This species may have been represented by the three unidentified *Culex* sp. collected by Jordan, especially since one of them







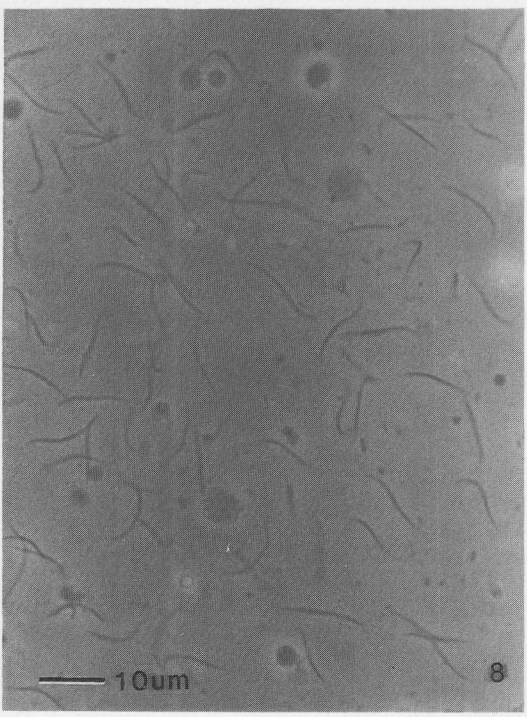


Fig. 5. Midgut (Mg) of *Culex erraticus*, (day 9 PF) with asynchronous development of *Plasmodium floridense* oocysts (0). Lipid-like globules (Lp) and budding sporozoites (S) are present.

Fig. 6. Oocysts (0) of *Plasmodium floridense* on the midgut (Mg) of *Culex erraticus* with many nearly mature sporozoites (S) (day 9 PF). Lipid-like globules (Lp) are present in mature oocysts.

Fig. 7. Salivary glands (Sg) of *Culex erraticus* with salivary ducts (Sd) and sporozoites (S) of *Plasmodium floridense*, day 14 PF.

Fig. 8. Sporozoites of *Plasmodium floridense* from the salivary glands of *Culex erraticus*, day 14 PF.

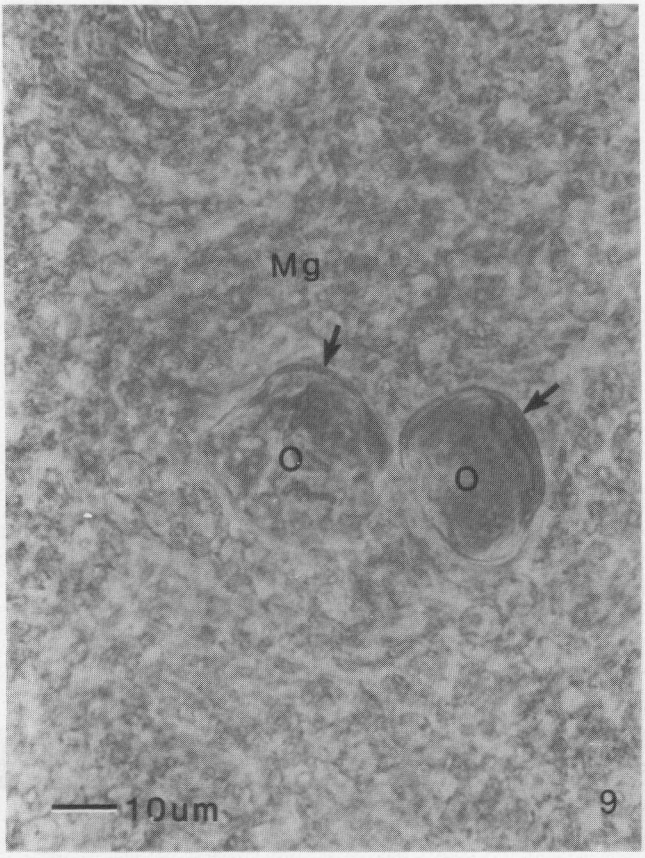


Fig. 9. Oocyst (O) of *Plasmodium floridense* on the midgut (Mg) of *Culex erraticus* on day 10 PF. Melanization (arrow) is developing along the oocyst capsule and spreading inward.

Table 3. Laboratory transmission of *Plasmodium floridense* to *Anolis carolinensis* by bite of infected *Cx. erraticus* and by IP inoculation of sporozoites from the salivary glands of *Culex erraticus*.

Temp./ lizard no.	Lizard origin	Number of mosquitoes fed/IP inoc.	Sporozoite rate (day postfeed)	Day patent infection	Peak parasitemia day	No. parasites/ 10,000 rbcs at peak
18–24°C						
A-85	LA	1-F	+3 (16)	25	74	4,280
AA-59	LA	1-IP	+3 (18)	24	55	1,780
32°C						
AB-23	FL	2-F	+2, +3 (17)	13	43	1,480
AB-27-1	LA	1-IP[a]	+3 (17)	15	27	912
AB-9	FL	13-F[b]	+2, +3, +2, +3 (19–20)	17	46	2,310
AB-32-1	LA	2-F	+2, +3 (28)	16	50	2,380

[a] One salivary gland from *Cx. erraticus*, blood-fed on AB-23, inoculated.
[b] Only 4 mosquitoes were positive for sporozoites in the salivary glands.

developed 70 oocysts after feeding on an anole infected with *P. floridense*.

Early investigations by Huff (1941) and extensive studies by Jordan (1964) indicated that *P. floridense* is transmitted to lizards in the late summer–early fall, with new infections becoming more abundant in August, reaching a peak in November, then sharply declining in December. Although data are limited, most new infections in the present study were also observed in October and November. Based on this evidence and the bait trap data, it was concluded that *Cx. erraticus* was the most likely vector since its seasonal abundance corresponded well with the

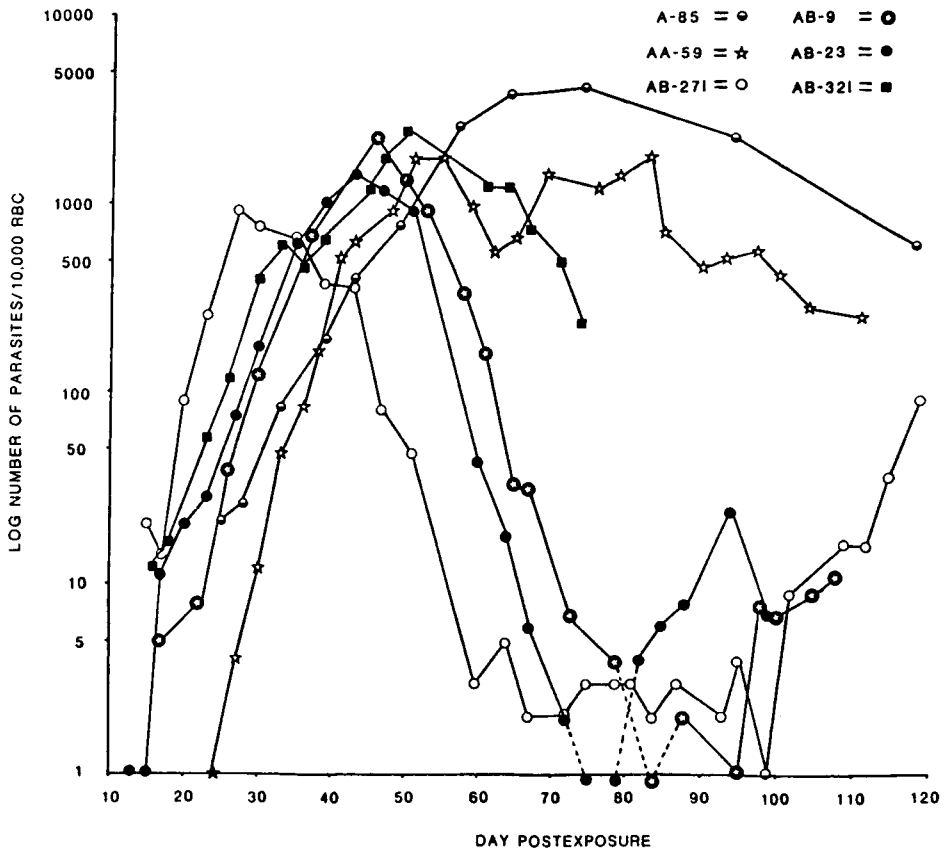


Fig. 10. Course of acute infection of *Plasmodium floridense* in four *Anolis carolinensis* (A-85, AB-23, AB-9, AB-32-1) infected by bite of *Culex erraticus* and in two (AA-59, AB-27-1) infected by IP inoculation of sporozoites from the salivary glands of *Cx. erraticus*.

period of suspected transmission of *P. floridense*. This mosquito is comparatively long-lived in the laboratory and is relatively abundant in the field when natural transmission is suspected to occur. Additional support for its role as vector is derived from its broad geographic distribution in North, Middle and South America, and the Caribbean, which includes within it the known range for *P. floridense* (Telford 1977).

While field data suggest that *Cx. erraticus* may be a natural vector of *P. floridense*, other arthropods cannot be ruled out. Based on evidence by Petit et al. (1983), some ceratopogonid species may be involved in the transmission of another saurian malaria, *P. agamae*. In addition, the psychodid flies *L. vexator* and probably *L. stewarti*, are natural vectors of *P. mexicanum* (Table 4). Present studies and those of Young and Perkins (1984) indicated that *L. vexator* readily develop oocysts of *P. floridense*. Approximately 50% of more than 600 sand flies which fed on infected lizards developed oocysts. Sporogonic development of *P. floridense* is relatively long, 11–14 days to produce mature sporozoites, and most of the laboratory reared sand flies (>90%) died within 14 days of feeding. Also, only a few (<10) sporozoites (probably as a result of mechanical damage to oocysts during dissection) were seen in the hemocoel in only two of the sand flies. Thus full development including salivary gland infections, was not proven. Since only a few sporozoites of *P. floridense* were observed in *L. vexator*, no attempt was made to determine if they were infective by inoculating them into a lizard. However, even if infective, sporozoites were never observed in the salivary glands, and therefore natural transmission would have to occur by some other mechanism other than blood feeding.

Previous attempts to infect mosquitoes with *P. floridense* by feeding them on infected *A. carolinensis* and *Sc. undulatus* have been mostly unsuccessful (Jordan 1964, Telford 1970). It is unfortunate that one of the three unidentified *Culex* sp. which fed on an infected lizard and developed many oocysts (70) was not identified to species (Jordan 1964). Attempts to infect other mosquito species were negative or resulted

Table 4. Summary of sporogony and transmission of saurian malaria in bloodfeeding Diptera.

Vector family/species	*Plasmodium* species	Oocysts	Sporozoites	Spor. in glands	Transmission	Source
Psychodidae						
L. vexator	*mexicanum*	X	X	X	X	Ayala 1971, Klein et al. 1987
L. stewarti	*mexicanum*	X	X	X	—	Ayala 1971
Ceratopogonidae						
C. nubeculosus	*agamae*	X	X	—	—	Petit et al. 1983
Psychodidae						
L. vexator	*floridense*	X	X	—	—	present paper
Culicidae						
Cx. erraticus	*floridense*	X	X	X	X	present paper
Cx. territans	*floridense*	X	—	—	—	Jordan 1964
Cx. quinquefasciatus	*floridense*	X	—	—	—	Telford 1970
Ae. aegypti	*floridense*	X	—	—	—	Huff 1941

in a low percentage (5.7% or less) of infected mosquitoes with only a few oocysts (Jordan 1964, Huff 1941).

The relatively high frequency of *Cx. territans* collected in the lizard-baited traps, its feeding preference for cold blooded vertebrates, and the development of oocysts in four of 70 mosquitoes (Jordan 1964), suggested that they might be involved with transmission of *P. floridense*. However, attempts to infect F_1 *Cx. territans* in this laboratory were unsuccessful and the peak seasonal abundance appeared to be in early spring (Fig. 3), rather than late summer to early fall when transmission of *P. floridense* is suspected to occur (Jordan 1964).

Our study demonstrates conclusively that *Cx. erraticus* can transmit *P. floridense* to *A. carolinensis*. However, there are many unanswered questions regarding its role in the natural transmission of saurian malaria. Sporogonic development and oocyst frequency in *Cx. erraticus* were highly variable, for reasons not well understood (e.g., genetic, temperature or other factors). Ward (1963) demonstrated genetic selection for susceptibility to malaria parasites and derived a nonsusceptible colony of *Ae. aegypti* to *P. gallinaceum* from a susceptible colony. It is not known whether the colony of *Cx. erraticus* consisted of two genetically different populations, one susceptible and the other resistant to infection, or if other factors are involved.

Large numbers of oocysts (>100), that are sometimes seen in mosquitoes infected with mammalian and avian malarias, have not been observed in *Cx. erraticus* infected with *P. floridense*. This may be related to the smaller bloodmeal taken by *Cx. erraticus* and lower gametocyte levels in the saurian hosts. Furthermore, large numbers of gametocytes may have a detrimental effect on the mosquito since 63% (12/19) of the *Cx. erraticus* that fed on a lizard with numerous gametocytes died within three days after feeding. Those that did survive may have only taken partial bloodmeals. Mosquitoes were not sectioned or dissected to determine if the midgut was damaged by invading parasites, but since few mosquitoes died when fed on lizards with moderate numbers of gametocytes, parasite invasion of the midgut may have contributed to mosquito mortality. Therefore, the upper limit of infectivity may be approximately 100 oocysts. Klein et al. (1982) demonstrated that large numbers of oocysts (>100) significantly reduced longevity of *Anopheles dirus* Peyton and Harrison infected with *Plasmodium cynomolgi* Mayer after 11 days following the release of sporozoites. However, there was no significant difference in the survival of highly infected and noninfected mosquitoes from 0–3 days after a bloodmeal.

The low number of anoles infected by bite of *Cx. erraticus* infected with *P. floridense* cannot be explained when compared with the transmission rate (nearly 70%) of another saurian malaria, *P. mexicanum* by *L. vexator* to *Sc. undulatus* (Klein, et al. 1987). Nutritional requirements for proper maturation of the parasites are unknown. The addition of multivitamins to the sugar solution fed to mosquitoes during sporogonic development apparently shortened the period when sporozoites were first observed in the salivary glands by 2–3 days PF. The effects on parasite growth by multiple bloodmeals during the maturation period are unknown. Since the development of *P. floridense* is relatively long (11–14 days), the additional bloodmeals may provide nutritional requirements necessary for maturation.

Sporogonic development appeared to be normal in most *Cx. erraticus* maintained at 25°C, but melanization of some oocysts occurred in a few mosquitoes, usually beginning 10 or more days PF. When mosquitoes were maintained at 32°C, nearly all oocysts were melanized or were becoming melanized by 8 days PF. Melanization of oocysts and sporozoites (?) also occurred in slowly developing oocysts in heavily infected

mosquitoes (>30 oocysts) with diet supplemented with multivitamins. However, up until day 11, all oocysts appeared normal, no melanization could be detected, and at 22 days PF all mosquitoes dissected from this lot had sporozoites in the salivary glands. It is uncertain whether temperature or other factors are involved in this defensive reaction to parasite growth. Rearing *Cx. erraticus* at temperatures below 25°C may produce more infective/viable sporozoites than in the present study. More intensive studies on the temperature and nutritional requirements for development of *P. floridense* in *Cx. erraticus* and an extensive search for other potential vectors of *P. floridense* are indicated. Certainly, it will be important to find naturally infected mosquitoes.

The effect of different temperatures on blood induced infections of *P. floridense* indicated that infections became patent earlier at 30°C ($\bar{x}$ = 3.2 days) than at 20°C ($\bar{x}$ = 10.0 days) and that parasitemia also increased at a much faster rate at 30°C (Thompson and Winder 1947). While the prepatent period for sporozoite induced infections was longer than for blood induced infections, the prepatent period for lizards maintained at 32°C was much shorter than in lizards maintained at room temperature, 18–24°C. Thompson and Winder (1947) did not determine if there were significant differences in the numbers of gametocytes produced at the two different temperatures. In the present study, gametocytes apparently appeared earlier in lizards maintained at 32°C. This corresponds to the results observed when wild caught infected lizards were placed in the incubator at 32°C. Generally, all wild caught infected lizards produced more gametocytes when maintained at 32°C. However, those with light infections generally remained light and produced few gametocytes. Those with moderate or heavy infections, although fluctuating, continued to show moderate to heavy parasitemias and produced many gametocytes. The lengthy prepatent period of *P. floridense* at 18–24°C corresponds to other studies on *P. floridense* in wild caught lizards (Goodwin and Stapleton 1952) and on *P. mexicanum* (Klein et al. 1987). Both prepatent period and course of infection also appear to be affected by host behavior in temperature regulation.

The initial course of infection of *P. floridense* (Fig. 10) is similar to that of *P. mexicanum* (Klein et al. 1987). However, unlike *P. mexicanum*, experimental infections of *P. floridense* were rarely fatal. Two experimentally infected lizards survived in the laboratory for one and two years, respectively, following infection. Both lizards were used in experimental *P. floridense* transmission studies and were good donor lizards for the demonstration of sporogonic development in *Cx. erraticus*.

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REFERENCES CITED

Ayala, S. C. and D. Lee. 1970. Development of sporozoites in two species of phlebotomine sandflies. Science 167:891–892.

Barnett, H. C. 1962. The incrimination of arthropods as vectors of disease. Proc. XI. Int. Congr. Entomol. (Vienna) 1:341–345.

Boulard, Y., G. Petit, I. Landau, A. F. Gomes and L. Touratier. 1983. Sporogonie de *Plasmodium agamae* chez *Culicoides nubeculosus*: II—Observations ultrastructurales. Protistologica 19:543–551.

Edman, J. D. 1979. Host-feeding patterns of Florida mosquitoes (Diptera: Culicidae) VI. *Culex* (*Melanoconion*). J. Med. Entomol. 15:521–525.

Garnham, P. C. C. and S. R. Telford, Jr. 1984. A new malaria parasite *Plasmodium* (*Sauramoeba*) *heischi* in skinks (*Mabuya striata*) from Nairobi, with a brief discussion of the distribution of malaria parasites in the family Scincidae. J. Protozool. 31:518–521.

Gingrich, W. D. 1932. Immunity to superinfection and cross immunity in malarial infections of birds. J. Prev. Med. 6:197–246.

Goodwin, M. H. and T. K. Stapleton. 1952. The course of natural and induced infections of *Plasmodium floridense* Thompson and Huff in *Sceloporus undulatus undulatus* (Latreille). Am. J. Trop. Med. Hyg. 1:773–783.

Huff, C. G. 1941. Saurian malaria. J. Parasitol. (Suppl.) 27:29 (Abstract).

Jordan, H. B. 1964. Lizard malaria in Georgia. J. Protozool. 11:562–566.

Klein, T. A., B. A. Harrison, R. G. Andre, R. E. Whitmire and I. Inlao. 1982. Detrimental effects of *Plasmodium cynomolgi* infections on the longevity of *Anopheles dirus*. Mosq. News 42:265–271.

Klein, T. A., D. G. Young, S. R. Telford, Jr. and R. Kimsey. 1987. Experimental transmission of *Plasmodium mexicanum* by bites of infected *Lutzomyia vexator* (Diptera: Psychodidae). J. Am. Mosq. Control Assoc. 3:154–164

Mattingly, P. F. 1965. The evolution of parasite-arthropod vector systems. *In*: Evolution of parasites. A. E. R. Taylor (ed.). 3rd Symposium of the British Society for Parasitology. Blackwell Scientific Publications, Oxford.

Nayar, J. K., M. D. Young and D. J. Forrester. 1981. *Culex restuans*: An experimental vector for wild turkey malaria, *Plasmodium hermani*. Mosq. News 41:748–750.

Petit, G., I. Landau, Y. Boulard, A. Gomes and L. Touratier. 1983. Sporogonie de *Plasmodium agamae*

chez *Culicoides nubeculosus*, au laboratorie: I—Experimentation et description du cycle. Protistologica 19:537–541.

Telford, S. R., Jr. 1970. Comments on the vector relationships of saurian malaria. J. Parasitol. 56:340.

Telford, S. R., Jr. 1977. The distribution, incidence and general ecology of saurian malaria in Middle America. Int. J. Parasitol. 7:299–314.

Telford, S. R. Jr., 1982. *Plasmodium lionatum* sp.n., a parasite of the flying gecko, *Ptychozoon lionatum*, in Thailand. J. Parasitol. 68:1154–1157.

Telford, S. R., Jr. 1983. *Plasmodium saurocaudatum* sp.n., a parasite of *Mabuya multifasciata* in southeast Asia. J. Parasitol. 69:1150–1155.

Telford, S. R., Jr. 1984a. Studies on African saurian malarias: Three *Plasmodium* species from gekkonid hosts. J. Parasitol. 70:343–354.

Telford, S. R., Jr. 1984b. Haemoparasites of reptiles. pp. 385–517. *In*: G. L. Huff, F. F. Frye and E. R. Jacobson (eds.). Diseases of amphibians and reptiles. Plenum Pub. Corp., New York.

Thompson, P. E. and C. V. Winder. 1947. Analysis of saurian infections as influenced by temperature. J. Infect. Dis. 81:84–95.

Ward, R. A. 1963. Genetic aspects of the susceptibility of mosquitoes to malarial infection. Exp. Parasitol. 13:328–341.

Wenyon, C. M. 1909. (dated 1908). Report of traveling pathologist and protozoologist. A. Balfour (ed.). Third Report, Wellcome Research Laboratory at the Gordon Memorial College, Khartoum. Bailliere, Tindall and Cox, London.

Young, D. G. and P. V. Perkins. 1984. Phlebotomine sand flies of North America (Diptera: Psychodidae). Mosq. News 44:263–304.