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Report on Possible Relationships Between Reptilian Malaria and Aedes aegypti (L)*

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Refractoriness to Plasmodium gallinaceum has been found by Kilama (1970) to be genetically transmitted as a monofactorial gene, and of eight strains from around the world, this gene was found only in feral strains of African Aedes aegypti. Because P. gallinaceum has a deleterious effect on A. aegypti in lowering its fecundity (Hacker, 1971), this gene will therefore appear to offer an advantage to the mosquito in the face of possible malarial infection. Garnham (1966) states however that P. gallinaceum is found to occur naturally only in Asia, and therefore it is unlikely that this gene is maintained in the African A. aegypti populations through interaction with this malaria. Using precipitin testing of blood from wild-caught A. aegypti, McClelland and Weitz (1963) found that nearly 50% of the females from Ganda, near Malindi, Kenya, contained blood from reptiles. Therefore, Craig (1971) suggested that the selection for this gene occurred possibly as a protection against a reptilian malaria. Several species of lizard malaria are known from East Africa, including Plasmodium pitmani from skinks and P. acuminatum and P. fischeri from chameleons (Garnham, 1966).

In the light of these observations, it was felt that exploration of possible malaria-reptile-aegypti relationships would be useful. Therefore the following general lines of investigation were followed. First, a survey of reptilian populations for malarious individuals in selected areas was carried out. Secondly, a number of A. aegypti strains were tested for their ability to feed on these reptiles in the laboratory. The third step was to determine if the reptilian malariae can develop in individual mosquitoes blood-fed on the malarious lizards. If and when these factors can be established positively, then the relationship of the malaria and Kilama's susceptibility gene can be systematically investigated and compared to Plasmodium gallinaceum.

I. Collection of Lizards and Blood Smears

Materials and Methods

The lizards were collected over a three-month summer period from 1 June to 24 August from five areas in Kenya.

1. Mombasa. In and near the city of Mombasa on the southeast coast of Kenya.

2. Rabai. The Rabai area is 15-20 miles inland from Mombasa and is a comparatively hilly, farming area adjacent to an untilled forest area.

3. Malindi. This is a rolling farming area between the coastal city of Malindi and Ganda village, and is largely a cashew and coconut production area.

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4. Kodera. It is known alternately as Kodera Forest or Kodera Valley and is the site of a seedling nursery and a large, planted pine forest. It is located in South Nyanza Province about 25 miles from Lake Victoria.

5. Gembe Hills. This is an area of rocky hills and grass on the shores of Lake Victoria south and west of where Kivorongo Gulf opens into the lake. The collection area was a less rugged terrain between Gembe Hills and the lake shore.

The method of collection was either by stunning with a 1.5 x 30 cm rubber band "shooter" or by hand with the help of local people. The size range of the specimens was limited to those over 5 cm nose to anus length. Blood smears were occasionally taken on the spot, but usually were transported to the lab and caged before taking blood samples. Initially, the two blood smears were taken from each lizard by cutting the end of the tail. However, this method proved unsatisfactory and later mosquitoes were used as micropuncture needles. Since the bloodmeal coagulated within 5-10 minutes after ingestion by the mosquito, the smear was made immediately. The wings and legs were removed from the etherized mosquito, the thorax severed from the abdomen with a pair of minuten mounted on orangewood sticks, the bloodmeal forced from the abdomen on to the surface of the slide, and a thin smear was made in the normal manner.

All dried blood smears were fixed in methanol for three minutes, rinsed in distilled water and stained with Giemsa (George T. Gurr-R66) in 4% dilution for 30 minutes. Examinations of slides was with oil immersion at 1000x. The specimen was classified as negative if no parasites were found after four lengthwise sweeps.

Incidental to the blood smears, an inspection of the lizards for mite and tick ectoparasites was carried out. Those found were stored in 70% ethanol and later mounted in polyvinyl lactophenol or Turtox non-resinous mounting medium.

Results:

Tentative identifications of the lizards were made with the help of Dr. Alan McKay, at the Kenya National Museum, Nairobi and verified by Mr. Robert C. Drewes of the California Academy of Sciences. The total number of lizard specimens (n=97) and the number from which blood slides were collected (n=89) are listed in Table I. One of the Gekkonids collected in Mombasa, Lygodactylus angularis Gunther was found far north of its normal range--south of Southern Tanzania--and was probably brought in by the trading boats (dhows). The Latastia johnstoni Boul from Rabai was also found somewhat north of its recorded range (Drewes, pers. communic.).

With respect to the blood parasitism, only the lizards from Kodera were found to be positive. Among Mabuya striata striata Peters, a peridomestic skink, were found individuals infected with Plasmodium pitmani Hoare and Trypanosoma boueti Martin. Two of eleven specimens were found infected with the malaria in an initial collection, and in a subsequent collection, four malarious and one with trypanosomiasis was found among twenty individuals.

The blood parasites and ectoparasites found, along with the specific designation of the hosts are recorded in Table II. Identifications to genus of the mites were based on Zumpt (1961) and the tick on Hoogstraal (1956). The Plasmodium was identified based on the descriptions of Garnham (1966) who had collected lizard blood smears in the same area (Kodera) in 1942 and again in 1962. The trypanosome was tentatively identified as Trypanosoma boueti Martin by Dr. F. G. Wallace at the University of Minnesota.

Table II. Blood and ectoparasites obtained from lizards in Kenya.

Lizard Host	Parasite	Frequency
	Blood Parasites	
Mabuya striata	Plasmodium pitmani	5/31
Mabuya striata	Trypanosoma boueti	1/31
	Ectoparasites	
Agama agricullis	Pterygosomid mites (Pterygosoma sp.)	1/1
Hemidactylus mabouia	Pterygosomid mites (Geckobia sp.)	1/11
Mabuya striata	Laelapid mites (Sauronyssus sp.)	several
Mabuya planifrons	Laelapid mites (Sauronyssus sp.)	2/18
Mabuya planifrons	Ixodid ticks (Aponomma sp.)	1/18

II. Bloodfeeding and Infection of Aedes aegypti

Materials and Methods:

A. First the ability of the mosquito to feed on lizards was checked. Examples from four families of lizards were chosen for the trials: Agamidae, Chameleontidae, Gekkonidae and Scincidae. All were collected in the Mombasa and Rabai area. The strains of Aedes aegypti tested were from laboratory colonies established for less than two years at the Mosquito Biology Unit/ International Centre for Insect Physiology and Ecology, Mombasa. The GANGA strain is a domestic, pale form of A. aegypti, TREEHOLE and MNAZI strains are feral dark forms, and MKWAJA an intermediate, peridomestic form.

The lizard was first immobilized by wrapping in a wire mesh and placed in the plastic "gallon" cage containing the mosquitoes. The mosquitoes had all been starved 24-36 hours previous to the trial. At termination of the trial, the number of mosquitoes which had blood-fed was recorded.

B. Infection of Aedes aegypti with malarious lizard blood was the final step attempted. The specimen (Mabuya striata) with the heaviest malarial infection was used in all trials. Even so, the level of infection was low, and counts made from smears taken on the infection trial days consistantly showed 0.8-1.0% of the red blood cells parasitized. As is apparently typical for Plasmodium pitmani, 45-50% of these cells contained mature gametocytes, while the remainder had all stages of trophozooites and schizonts represented.

Table I. Species identifications and numbers of specimens of lizards collected at five sites in Kenya.

Site	Lizard Family	Species Identification	Number Collected	Number Blood Smeared
MOMBASA	AGAMIDAE	*Agama agama lionotus* Boul	7	7
	GEKKONIDAE	*Lygodactylus angularis* Gunther	1	0
	GEKKONIDAE	*Lygodactylus picturatus mombasicus* Loveridge	5	4
	GEKKONIDAE	*Hemidactylus mabouia* (Jonnes)	8	8
	SCINCIDAE	*Mabuya maculilabris comorensis* (Peters)	2	2
	SCINCIDAE	*Mabuya planifrons* (Peters)	11	11
			34	32
RABAI	CHAMELEONTIDAE	*Chameleo dilepis* Leach	3	3
	CHAMELEONTIDAE	*Rhompholeon* sp.	1	1
	GEKKONIDAE	*Hemidactylus mabouia* (Jonnes)	1	1
	GEKKONIDAE	*Lygodactylus picturatus mombasicus* Loveridge	1	1
	LACERTIDAE	*Latastia johnstoni* Boul	1	0
	SCINCIDAE	*Mabuya striata striata* (Peters)	1	1
	SCINCIDAE	*Mabuya varia varia* (Peters)	2	1
			10	8
KODERA	GERRHOSAURIDAE	*Gerrhosaurus nigrolineatus nigrolineatus* Hallowell	2	1
	LACERTIDAE	*Lacerta johnstoni* Boul	3	0
	SCINCIDAE	*Mabuya striata striata* (Peters)	31	31
			36	32
GEMBE HILLS	AGAMIDAE	*Agama agricollis* (cf) *gregorii* Gunther	1	1
	SCINCIDAE	*Mabuya striata striata* (Peters)	3	3
			4	4
MALINDI	LACERTIDAE	*Eremias spekii sextaeniata* Stejneger	1	0
	SCINCIDAE	*Mabuya maculilabris comorensis* (Peters)	5	5
	SCINCIDAE	*Mabuya planifrons* (Peters)	7	7
	SCINCIDAE	*Riopa afer* (Peters)	1	1
			14	13

Preliminary trials were run on two strains of A. aegypti, MBALAMWENI and MOMSABU, but there was very high mortality due to the age of the mosquitoes when blood-fed. A second lot of 34, four-day-old adults of a marker stock (Si, s, blt) was then infected with very little mortality.

Eight days post-infection, the midguts of the surviving MBALAMWENI and MOMSABU strains were dissected out in a drop of saline and mounted permanently in polyvinyl lacto-phenol medium. The midguts of the marker stock were dissected five days post-infection and were similarly mounted. All were examined immediately after dissection for presence of oocysts at 250x with phase contrast microscopy.

Results:

The mechanical obstacles offered by the lizard can be overcome, although in the case of the skinks (Scincidae) the scales are an effective barrier. In these lizards, almost all blood-feeding occurred in the soft membranes around the eyes, with additional biting at the leg-body juncture and occasionally under the larger scales dorsal and posterior to the hind legs. Although all the lizards tested proved to be susceptible to blood-feeding, the Agama agama and the Chameleo dilepis were particularly so.

(Table III).

Table III. Blood-feeding ability of A. aegypti strains among four families of lizards.

Lizard Family	Lizard species	Strain	No. of aegypti tested	Exposure time (min.)	Percent blood-fed
AGA	A. agama	GANGA	20	75	85
CHA	C. dilepis	GANGA	16	45	81
CHA	C. dilepis	TREEHOLE	10	30	20
CHA	C. dilepis	MNAZI	20	30	85
GEK	H. mabouia	MKWAJA	8	45	50
GEK	L. picturatus	GANGA	15	120	20
SCI	M. planifrons	GANGA	20	90	25
SCI	M. planifrons	GANGA	110	20	15
SCI	M. planifrons	MNAZI	20	45	40

In the 39 midguts dissected and inspected, no oocysts development was seen. The mortality among blood-fed individuals in the first three trials was 63-92%. The A. aegypti used in these trials were relatively old (10-20 days). However mortality among the marker stock which was four days old at the time of infection was much lower, around 6% (Table V).

Table V. Infection of Aedes aegypti with Plasmodium pitmani from the skink lizard, Mabuya striata.

Mosquito Strain	Strain Age (days)	No. Infected	Days After Infec.	Mortality	No. Dissected
MBALAMWENI	18	8	8	5 (63%)	3
MOMSABU	18	24	8	22 (92%)	2
MOMSABU	20	9	8	7 (78%)	2
SI-S-BLT	4	34	5	2 (6%)	32
		Total dissections:	39		
		Total with oocysts:	0		

Discussion:

The data collected here do not provide definitive answers to the questions asked, but at least they begin to put the problem in a working perspective. The lizard collections in each of the five areas provide a rough index of species commonly seen and most easily caught. The choice of Kodera and Gembe Hills as collection sites was not random. Garnham (1966) had found Plasmodium pitmani in Kodera twice previously, as well as at other locations along the shores of Lake Victoria. However, he noted that the malarial distribution was spotty and highly localized. In view of this, it is perhaps not surprising that infections were not found in areas other than Kodera since 1) the other localities were chosen as a matter of convenience rather than as known sites of lizard malaria occurrence, and 2) the number of lizard specimens per species collected was small.

The relative susceptibilities to A. aegypti blood-feeding of the four families of lizards indicate that the more heavily scaled Scincidae are more resistant to mosquito feeding than the others. Further investigations comparing preferences of the biting abilities of domestic versus feral strains on these lizards are also indicated.

The failure to produce oocysts in the mosquito gut walls under the conditions described herein, while certainly not precluding its possibility, may have been expected for the following reasons. First of all, the blood parasitemia was fairly low and the infection not new (as reticulocytes were common in the smears). At least in Plasmodium gallinaceum these factors have a large effect on the infectivity of blood to the mosquito (Kilama, 1970). Secondly, the number of mosquitoes blood-fed was far too few, and it is possible that the time allowed for oocyst development before dissection (5 days) may have been too short. Thirdly, attempts to infect A. aegypti with lizard malariae have been positive only in the case of the presence of a single oocyst after infection with Plasmodium agamae (Bray, 1959). Jordan (1964), though unable to infect A. aegypti found as many as 70 oocysts in Culex pipiens after infection with Plasmodium floridense. Finally, the discovery of a phlebotomine vector for Plasmodium mexicanum in California (Ayala and Lee, 1970; Ayala, 1971) gives the first convincing evidence that at least some lizard malariae are not vectored by mosquitoes at all.

Summary:

1) The striped skink, Mabuya striata, in Kodera Valley, Kenya, are found to be frequently infected with Plasmodium pitmani.

2) Aedes aegypti are found capable of feeding on selected members of four commonly found, East African families of lizards, with the chameleons and agamids being most frequently bitten.

3) Positive infections of Aedes aegypti with the lizard malaria, Plasmodium pitmani were not found.

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