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VARIATION IN SUSCEPTIBILITY OF AEDES AEGYPTI (L.)  
STRAINS TO WUCHERERIA BANCROFTI (COBBOLD)

by

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## INTRODUCTION

From a conversation with Professor Craig, I learned that Dr. P. Rodriguez of the Vector Biology Laboratory, University of Notre Dame, has recently demonstrated that Aedes aegypti formosus strains are more susceptible to infection with Brugia pahangi than the type form Aedes aegypti aegypti.

Thinking that the same phenomenon might hold for W. bancrofti, this short project was therefore undertaken to study the susceptibility of Aedes aegypti to W. bancrofti, emphasis being placed on comparisons of the type form strains with the feral strains.

## MATERIALS AND METHODS

The strains of Aedes aegypti mosquitoes used are shown in Table I. They were all obtained from colonies maintained by the Mosquito Biology Unit of the International Centre of Insect Physiology and Ecology, at Mombasa, Kenya. The mosquitoes were reared in an insectary maintained at a tropical ambient temperature of about 25°C and about 70% relative humidity.

When approximately five days old, the adult female mosquitoes were deprived of sugar and water for a period of about 14 hours, then they were fed between 21 and 23 hours (local time) on the arm of a man naturally infected with periodic W. bancrofti. The enclosed photograph shows Mr. Jefa Wawara of Garashi Location, Malindi District who kindly supplied the microfilariae. Mr. Waware did not have a previously recorded history of treatment for filariasis. All mosquito feeding proceeded inside a laboratory room, just adjacent to the insectary.

Before the mosquito feeding began, a temporary wet blood film was made by pricking the tip of a finger and examining the blood for microfilariae. At the same time, thick blood films were made for future references. These thick films were later stained with Weigert's Homa-toxylin in the Department of Parasitology and Medical Entomology Laboratory, Faculty of Medicine, University of Dar-es-Salaam. (It was not necessary to stain the thick smears at Mombasa since in this part of the world live W. bancrofti microfilariae are easily recognized on site). No attempt was made at determining the microfilarial densities, this can be estimated from the accompanying stained blood films.

The mosquitoes were allowed to engorge on the donor's arm, which was introduced through the sleeve of a 'gallon' cage. In all the three feedings, the two strains being compared were engorged within thirty minutes, (two attempts at feeding Culex pipiens fatigans which we previously collected as immatures from tyres with residual rain water, completely failed after exposing an arm for at least 30 minutes on each occasion).

Because of unforeseen timing difficulties the infected mosquitoes were held on sugar and water for 18 to 22 days (instead of 9 to 11 days) inside the insectary, after which the surviving ones were dissected, the abdomens examined immediately and the thoraxes and heads fixed in 85% alcohol, then shipped to Dar-es-Salaam. In the Dar-es-Salaam laboratory, the fixed mosquitoes were brought down to water, then stained for 24 hours in Mater's Acid Hemalum, after which they were washed in tapewater to "blue" the stain, and then stored in glycerol for subsequent dissections (Shute and Maryon, 1966).

### RESULTS

Three of the enclosed microscopic slides show W. bancrofti microfilariae on the three days of mosquito feeding. On these occasions, an average thick drop would contain more than 50 microfilariae.

Two of the enclosed microscopic slides show infective larvae of W. bancrofti.

Table II shows the susceptibility of four strains of Aedes aegypti to W. bancrofti. Two of the strains (Ganga and Tree-Hole) did not show any larvae. The Ganga strain did not show any larvae in two separate infection attempts. However, two other strains (Mkwaja and Mnazi) developed infective larvae. These infective larvae were found mainly in the head, although a few were still in the thoraxes. From abdomen examinations of all these mosquitoes, Dr. Hausermann recorded two infective larvae from the haemocoel of one Mnazi female.

Each infected mosquito had one to four infective larvae, the majority having two.

Neither first nor second - stage larvae were observed in any of the mosquitoes.

### DISCUSSION

Two of the strains showed no filariae at all. One of these strains (Ganga) was Aedes aegypti var. queenslandensis, the other (Tree-Hole) was Aedes aegypti formosus. Therefore susceptibility like refractoriness is not restricted to one subspecies.

Nelson *et al.* (1962) site workers in West Africa, the Congo and in the U.S.A. who succeeded in infecting Aedes aegypti with periodic W. bancrofti. However, Nelson found no natural infections of W. bancrofti in Aedes aegypti in Kenya; he also failed to infect Aedes aegypti with W. bancrofti in the laboratory. Those apparently contradictory results are not surprising since different mosquito populations differ in their vectorial capacity (Ramachandran *et al.* 1960; Kilama, in press). The dichotomous results presented in this report therefore confirm the view that different mosquito populations differ in their vectorial capabilities. As Kilama and Craig (1969) said, "the occurrence of simple monofactorial

factors controlling vectorial capacity should give pause to those medical entomologists who, on the basis of a few transmission experiments with insects from a single laboratory colony, quickly decide that a given species is or is not a potential vector of a given pathogen." It is very likely that the observed differences in the susceptibility of Aedes aegypti strains to W. bancrofti are not due to environmental factors, but are genetic.

The numbers of infective larvae per mosquito was rather low (most positive mosquitoes showed only about two infective larvae). As Ramachandran et al. (1960) observed, "the optimum time for dissection was about the 9th - 11th days, for after that time there was a loss of larvae from mosquitoes the mortality of which also increased."

Moreover, Ramachandran (1966) also observed that "if infected mosquitoes are maintained after the larvae in them reach maturity a loss is likely to occur by the escape of infective larvae from the proboscis of the mosquitoes during the act of feeding on substances like apple, milk, sugar, water and raisins." Unfortunately in my experiments, the mosquitoes were held for 18 to 22 days. If Ramachandran's observations on Brugia malayi in Aedes aegypti also apply here, then larvae might have been lost and many mosquitoes may have died. The number of my recorded infective larvae is most probably short of the actual numbers, and also the percentage of infective mosquitoes may have decreased. Ramachandran (1966) could not find any infective larvae B. malayi, 18 days after an infectious blood meal. Because of this time limitation, may be it is not proper to compare my results with those of previous workers.

MacDonald (1962a and 1962b) observed that 17.1 per cent of parental generation Aedes aegypti showed mature larvae of semi-periodic B. malayi. In the same experiments he found an average of four infective larvae per infected mosquito. My results are therefore in line with Macdonald's.

Jordan and Goatly (1962) studied the infectivity of W. bancrofti to Culex fatigans in coastal Tanzania. In their extensive experiments, the infection rate of mosquitoes 14½ days after an infectious blood meal ranged between 52 and 100 per cent. When their results are compared with mine, the obvious conclusion is the C.o. fatigans is much more susceptible to W. bancrofti than any of the Aedes aegypti strains I tested. However the mean number of infective larvae per surviving C.o. fatigans compares favourably with that of my infected Aedes aegypti. However because of the greater proportion of infected C.o. fatigans and the overwhelming (still increasing) numbers of C.o. fatigans in urban East Africa, it is most probably that C.o. fatigans is a much more effective vector of urban W. bancrofti than is Aedes aegypti. In most rural East Africa, Anopheles gambiae complex and An. funestus are the major vectors. Since those two Anopheles species are also more susceptible to experimental infections than I have just shown for Aedes aegypti, then we may regard those Anopheles sp. to be more efficient vectors than Aedes aegypti.

Since Aedes aegypti is a diurnal feeder it would not be an efficient vector of nocturnal W. bancrofti. However, W. bancrofti periodicity in

in some parts of East Africa is relative, and E. Nnochiri (personal communication) maintains that it is subperiodic in certain areas of Uganda. Under such circumstances diurnal Aedes aegypti, that is relatively susceptible, would enhance opportunity of W. bancrofti transmission.

Epidemiologic investigations on W. bancrofti in East Africa (e.g. Nelson et al. 1962, Menu and Kilama 1972) have consistently shown higher infection rates and higher microfilarial densities in men than in women. There is not yet a fully plausible explanation for this observation; among the suggestions made is that hormones may influence the level of filaremia. However, if feral mosquitoes are proved to be efficient vectors, then this might account for the differences in exposure to infection among men and women. While men work in the 'forest', women stay at home; thus in addition to home acquired nocturnal infections, men would also acquire infections from diurnal biting exophagic Aedes aegypti.

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#### SUMMARY

The susceptibility of four strains of Aedes aegypti to W. bancrofti was studied.

Preliminary results indicate that susceptibility /refractoriness is/are not restricted to any one subspecies of Aedes aegypti.

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TABLE I. THE STRAINS OF A. AEGYPTI USED FOR INFECTION WITH W. BANCROFTI

NAME	SUBSPECIES, VARIETY, OR TYPE	STRAIN HISTORY AND COLLECTION SITE
MNAZI	FORMOSUS	Eggs collected with dirt samples from steps in coconut palms (Mnazi) in Rabai Location, August - November, 1971.
TREE-HOLE	FORMOSUS	Eggs collected with dirt samples from tree hole in mango tree at Mazeras, Rabai Location (3° 56'S, 39° 34'E), August 1971.
GANGA	QUEENSLANDENSIS	Adults caught in an indoor landing biting catch in various houses at Ganga, Rabai Location (3° 57' 10"S, 39° 33' 40"E), October 1971.
MKWAJA	TYPE FORM	Larvae collected from rain water drums at Mkwaja Village (Tanzania Coast) in backyards of several houses (5° 47' 30"S, 38° 51' 05"E) April 1971.

TABLE 2. THE SUSCEPTIBILITY OF FOUR STRAINS OF AE. AEGYPTI TO W. BANCROFTI.

TIME (HRS) OF EXPT.	DAYS BEFORE DISSECTION	MOSQUITO STRAIN	NO. ♀♀ DISSECTED	NO. ♀♀ WITH INFECTIVE LARVAE	NO. ♀♀ UNINFECTED
21:30 - 21:40	22	GANGA	92	0	92
21:20 - 21:30	18	TREE HOLE*	85	0	85
21:30 - 21:40	22	MKWAJA*	117	15	102
22:00 - 21:10	19	MNAZI**	41	4	37
22:10 - 22:15	19	GANGA**	127	0	127

\* FED JUNE 22ND, 1972

\*\* FED JUNE 23RD, 1972