Selection of *Culex Pipiens Fatigans* for vector ability to the rural strain of *Wuchereria Bancrofti* - a preliminary report

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INTRODUCTION

CULEX PIPIENS FATIGANS is the most important vector for **Wuchereria bancrofti** in many parts of the world. In West Malaysia, two biological varients of W. **bancrofti**, namely a rural and an urban strain, have been recognized (Wharton, 1960). The rural strain of W. **bancrofti** is mainly found among Orang Asli (aborigines) and rural Malays in West Malaysia, living away from the urban and semi-urban areas. This strain develops poorly in **Culex pipiens fatigans** and appears to be transmitted by anophelines in the few areas investigated so far (Wharton, 1960; Wharton **et al.**, 1963). The urban strain of W. **bancrofti**, which is found mainly in cities like Penang, Kuala Lumpur and Singapore, had been introduced since the turn of the century by immigrant Chinese and Indians (Poynton and Hodgkin, 1938; Wilson and Reid, 1951). This strain develops very well in **Culex pipiens fatigans** (Wharton, 1960) which has been shown to be the natural vector of the parasite in the city of Singapore (Danaraj **et al**, 1958).

Ramachandran **et al** (1964), in a filariasis survey among Orang Aslis living close to Kuala Lumpur, showed that 9 out of 43 people examined were positive for microfilariae and 6 among them were infected with the rural strain of **W. bancrofti.** They also confirmed Wharton's (1960) observation that although **Culex pipiens fatigans** was capable of supporting development of the microfilariae up to the infective third stage, it was however a poor host. The infectivity rates among five different lots of **Culex pipiens fatigans** obtained from different localities in West Malaysia and fed on a **W. bancrofti** – infected donor on the same occasion varied from 4 to 30 percent. In the present study, similar results were obtained (Table I) with five colonies of **Culex pipiens**

TABLE I

Infectivity rates of **Culex pipiens fatigans** obtained from various localities in West Malaysia to the rural strain of **Wuchereria bancrofti.**

Colony	Collected from	Generation No. of mosquitoes fed on the donor	Number of microfilariae per cmm of donor's blood at the time of mosquito feeding	Number of mosquitoes dissected	Number of mosquitoes with infective larvae	Percentage infective
'A'	Kuala Lumpur	P	3.0	75	5	6.6
'B'	Kuala Lumpur	P	2.7	40	8	20
'C'	Cameron Highlands	F_{11}	2.8	117	21	17.9
'D'	Port Dickson	F_1	3.1	48	8	16.6
'E'	Colony selected for dieldrin resistance	F_{17}	2.8	96	27	28.1

fatigans collected from different parts of the country. It was considered that these variations in vectorial capacity in the same species of mosquito collected from different parts of the country may be due to different gene frequencies.

Huff (1927) was the first to show that the susceptibility of a vector to a parasite could have a genetic basis. He also showed that the number of **Culex pipiens fatigans** susceptible to infection with **Plasmodium cathemerium** could be increased or decreased by selection (Huff, 1929). Huff (1931) suggested that the factor controlling susceptibility of **Culex pipiens fatigans** to infection with **P. cathemerium** was a simple Mendelian recessive. Macdonald (1962a) selected out a strain of **Aedes aegypti** highly susceptible to infection (mean susceptibility rate 84.8 percent) with the sub-periodic form of **Brugia malayi** from a colony which showed only 12 to 31 percent infectivity rate (Ramachandran **et al.**, 1960). Macdonald (1962b) also showed that the vectorial susceptibility to the parasite was controlled by a sex-linked recessive gene which he labelled f^n

The present work was undertaken to study the possibility of selecting out strains of **Culex pipiens fatigans** which are highly susceptible to the Malayan rural strain of **W. bancrofti.** Such a study could indicate if there was a possibility of populations of **Culex pipiens fatigans** in West Malaysia developing susceptibility to **W. bancrofti** as a result of changes in the gene frequency of the field population due to some natural or artificial selection pressure. If by laboratory selection studies, pure homozygous resistant and susceptible strains could be obtained, it may then be possible to work out the mode of inheritance of vector susceptibility of this species to the rural strain of **W. bancrofti.** Besides, it will provide the opportunity to study the possibility of replacing the indigenous parasite-susceptible strains with a parasite-resistant strain, involving a possible biological method of control for filariasis transmitted by **Culex pipiens fatigans.**

MATERIALS & METHODS

The colonies ('A' and 'B') of **Culex pipiens fatigans** reared from egg rafts collected in Kuala Lumpur were used. These field populations were used in order to get a high degree of genetic variability.

The larvae were reared under laboratory conditions at temperatures between 72° and 80° F. Adult mosquitoes were maintained at the same temperature and at a relative humidity of 70 to 80 per cent.

Female mosquitoes, 5 to 10 days old, were fed on **W. bancrofti**-infected donor between 2030 and 2230 hours. Estimates of microfilariae counts were made from the donor before and after a batch of mosqui-

TABLE II

Results of feeding six successive generations of two selected susceptible (+) strains ('A' and 'B') of **Culex pipiens fatigans** on a donor infected with the rural strain of **Wuchereria bancrofti.**

Strain	Gene-ration	Number of micro-filariae per cmm of donor's blood at the time of mos-quito feeding	Number of mos-quitoes fed	Number of mos-quitoes that died before egg laying	Number of mos-quitoes that died after egg laying	Percentage of mos-quitoes that died	Number of mos-quitoes dissected	Number of mosqui-toes (+) with infec-tive larvae	Percentage of infec-tive (+) mos-quitoes	Average number of mature larvae per infec-tive mos-quito
'A' (+)	P	3	92	4	13	18.4	75	5	6.6	3.4
	F_1	2.2	1	—	1	100	—	—	unknown	—
	F_2	2.2	17	3	2	29.4	12	5	41.6	1.6
	F_3	2.7	21	4	3	33.3	14	9	64.3	4.4
	F_4	2.8	60	14	7	35	39	13	33.3	2.4
	F_5	2.7	24	8	2	41.7	14	10	71.4	2.7
	F_6	2.8	20	3	4	35	13	7	53.8	2.8
'B' (+)	P	2.7	92	26	26	56.5	40	8	20	2.4
	F_1	2.6	19	4	4	42.1	11	2	18.1	2.5
	F_2	2.8	14	4	1	35.9	9	2	22.2	1.5
	F_3	2.8	16	3	1	25	12	4	33.3	2.0
	F_4	1.7	6	2	1	50	3	2	66.6	1.5
	F_5	2.8	4	2	2	100	—	—	unknown	—
	F_6	2.3	4	—	2	50	2	2	100	1.0

toes had fed. The average microfilariae count was between 1.7 and 3 per c.mm of peripheral blood (Table II). Wharton (1960) has estimated that a female **Culex pipiens fatigans** would take in about 4 c.mm of blood during a single feed.

The blood-fed mosquitoes were kept individually in 9 cm x 4.5 cm tubes which were closed with a pad of wet cotton wool. This kept the humidity in the tube high. No sugar solution was given until eggs were laid. On the 4th day after the initial blood meal, water was given to each mosquito for egg laying. As soon as a mosquito laid eggs, it was given a serial number and was then removed into a numbered tube and maintained on sugar solution until dissected. The egg raft laid was also given the same serial number. Many of the females laid eggs on the 4th or 5th day after the blood meal; some delayed egg laying for varying periods while a few never laid eggs at all. Irrespective of whether they laid eggs or not, all mosquitoes were dissected 14 to 16 days after the infective blood meal.

Each numbered egg raft was placed in water, in individual rearing bowls and the larvae which hatched from the raft were reared together. The mosquito larvae obtained were separated into two groups, depending upon the results of the dissections. The larvae derived from mosquitoes which had supported the development of the parasite to the infective third stage were pooled together as the susceptible (+) group or strain. Those derived from mosquitoes which had not supported the development of the parasite were pooled together as the resistant (-) group or strain. The adult mosquitoes which emerged from each of the strains were fed separately on the donor. In subsequent breeding operations, susceptible strains were maintained by using the progeny of susceptible parents only. Whenever sufficient number of males and females were available from a single susceptible parent, strict brother-sister matings were carried out. However, it was extremely difficult to

maintain a strictly inbred line for more than two generations. In-breeding depressions, resulting in heavy mortality of larvae as well as of adults, was observed. Out-breeding was done only with virgin mosquitoes taken from closely related lines.

RESULTS

Results of feeding five different colonies of **Culex pipiens fatigans** on a donor harbouring the rural strain of **W. bancrofti**, showed that their infectivity rates varied from 6.6 to 28.1 percent (Table I). The highest degree of infectivity rate was noticed among mosquitoes from a colony 'E' which has been previously selected out for dieldrin resistance for seven generations. It is difficult to say whether selection for dieldrin-resistance has had any concurrent effect on the selection of individual mosquitoes subsceptible to **W. bancrofti**, as well.

In the two strains ('A' (+) and 'B' (+)) which have been selected for susceptibility to infection with **W. bancrofti**, it was found difficult to get sufficiently large numbers of female mosquitoes to feed on the donor. This was perhaps due to the selection and inbreeding pressure in successive generations. The number of female mosquitoes which fed on the donor in successive generations varied from 1 to 92 (Table II). Among those that fed, there was a high mortality rate varying from 18.4 per cent to 100 per cent before dissection. As a result, the number of mosquitoes which were dissected were small and varied from 12 to 75 in 'A' (+) strain and 2 to 40 in 'B' (+) strain in the various generations.

Inspite of the small number of mosquitoes available for study, it was possible to select out from both strains of **Culex pipiens fatigans** a high degree of susceptibles to infections with the rural strain of **W. bancrofti**. The original susceptibility levels of 'A' and 'B' colonies were 6.6 percent and 20 percent respectively. In strain 'A' (+), the susceptibility rates had increased to 64 percent in the 3rd generation and, except in the 4th generation, this high level of susceptibility was maintained.

Similarly in strain 'B' (+), the susceptibility rates had increased to 66.6 per cent by the 4th generation. The degree of susceptibility was not measured in the 5th generation as all the 4 fed mosquitoes died before dissection. The susceptibility rate was 100 per cent in the 6th generation when only 2 mosquitoes were available for dissection. As the number of mosquitoes was only two, this 100 per cent susceptibility rate may not be as significant as it might have been if a larger number of mosquitoes had been dissected.

CONCLUSIONS

This preliminary work, in general, has shown that there is a low gene frequency in Malaysian **Culex pipiens fatigans** for susceptibility to infection with the rural strain of **W. bancrofti**. By careful isolation and selection, the susceptibility rates could be increased to a high degree over a few generations. If the gene frequency of the field population of mosquitoes changes due to some selection pressure in nature, the possibility exists that **Culex pipiens fatigans** may become good vectors for the rural strain of **W. bancrofti**.

It is hoped that further experiments will lead to isolation of pure homozygous lines which are susceptible and resistant to infection with the rural strain of **W. bancrofti** and that this would lead to the study of inheritance of vector ability of **Culex pipiens fatigans**.

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