Efficiency of the cytoplasmic incompatible (D3) strain of culex pipiens fatigans to infection with the rural strain of wuchereria bancrofti

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INTRODUCTION

Two strains of *Wuchereria bancrofti* have been recognized in Malaysia (Wharton, 1960). The urban strain, restricted to the cities, has been introduced into Malaya over the last 100 years or so by immigrant Chinese and Indians (Poynton & Hodgkin, 1938; Wilson and Reid, 1951). At present this strain occurs in small foci in Singapore, Kuala Lumpur and Penang. This is transmitted by *Culex pipiens fatigans* as in other neighbouring countries. The second strain, the rural strain of *Wuchereria bancrofti,* is mostly found among the Orang Asli (aborigines) and to some extent in rural Malays and it seems to be the indigenous strain. The local strains of *C. p. fatigans* are considered to be poor vectors as they are mostly refractory to the development of the rural strain of *W. Bancrofti,* although a few will support the development of microfilariae up to the infective stage (Wharton, 1960; Ramachandran et al., 1964; Thomas & Ramachandran, 1970). The natural vectors of this strain in Malaysia are *Anopheles letifer* and *Anopheles* maculatus (Wharton, 1960).

Rapid urbanization in many developing countries, without the necessary sanitation has accelerated the rate of increase in numbers of *C. p. fatigans.* This poses the imminent threat of bancroftian filariasis transmission in these countries. *C. p. fatigans* is a very hardy mosquito which has developed in a high degree of resistance to many insecticides and has shown gene potentials for developing resistance rapidly to all types of synthetic organic compounds. Therefore, the insecticides which are now in use are not suffciently effective for the control of this species. This has necessitated the search for alternative or supplementary methods of control of this species.

Among the varius methods known at present, genetic control using cytoplasmic incompatibility seems to be the most advanced and a feasible method against this species as shown by Laven (1967) in Okpo, Burma. With this view in mind, studies were carried out on the cytoplasmic incompatible strain (D3). The males of this strian have been found to be imcompatible with females of 19 strains collected from various States in West Malaysia, Indonesia, Sabah, and Singapore (Thomas. 1971). Furthermore, recently conducted cage experiments indicated clearly that local females have no special preference for local males but would mate with incompatible males freely and would bring about 90% incompatibility among local females, if the ratio of the incompatible males to local males in the cages was about 10:1 (Thomes, 1972).

If incompatible males are released in the field daily in very large numbers over a period of time, it is then possible that some females may also inadvertently be released at the same time. Under such circumstances, this incompatible strain of

mosquitoes which is well adapted to local conditions, would sooner or later, partly or completely replace the local indigenous strain of *C. p. fatigans* or would establish themselves side by side with them. Therefore, it is absolutely essential that the incompatible strain which is used against local *C. p. fatigans* is not a vector of any mosquito-borne disease.

The most important prerequisite was, therefore, to study the efficiency of this species in transmitting the rural and the urban strains of *Wuchereria bancrofti* in West Malaysia. With this view in mind, studies on this strain were undertaken using a donor who was infected with urban strain of *W. bancrofti* (rural strain). It was not easy to find a donor who was infected with urban strain of *W. bancrofti* and therefore no studies were conducted with this strain.

Furthermore, selections were carried out over a few generations to obtain pure susceptible and resistant lines of the incompatible *C. p. fatigans* to rural *W. bancrofti* with a view to study the inheritnace of suceptibility of infection in this mosquito to the filarial parasites.

MATERIALS AND METHODS

The incompatible strain of *C. p fatigans* (D3) was obtained from Professor Laven, Director, Institut Fur Genetik, Mainz, Federal Republic of Germany in 1968. This strain has the cytoplasm of Paris strain and the genome of Fersno strain (Laven, 1967). This was the strain which Laven (1967) has successfully used to eradicate the inidgenous *C. p. fatigans* from Okpo. Burma.

The larvae were reared under laboratory conditions at room temperatures between 72° and 80°F. The adult mosquitos were maintained at the same room temperature and at a relative humidity of 70-80%. Female mosquitos which were about a week old were fed on a *W. bancrofti* donor between 20.30 and 22.30 hours. Microfilariae counts per Cu mm in the peripheral circulation of the donor were taken before and after a batch of mosquitos were fed. The average of these two were taken as the mean microfilariae count per cu mm. in the blood during experimental feeding.

After feeding, the blood-fed mosquitos were taken out and kept individually in 9 cm x 4.5 cm tubes provided with wet cottonwool. No sugar solution was supplied in the tube until after egg laying was completed. On the fourth day after the blood meal, water was given to each mosquito for egg laying. After a mosquito had laid eggs, both the adult and the egg raft were given the same code number. All mosquitos were dissected 12-14 days after the infective blood meal. The percentage of infectivity and the number of infective larvae per mosquito were determined.

Selection of the susceptible (+) strain was carried out by isolating four to five egg-rafts which were laid by the females from F10 of D3 stock colony which supported the highest numbers of infective larvae. Similarly the resistant (−) strain was also selected by isolating a few (four to five) rafts deposited by resistant females of the same generation. Adults which emerged from each individual raft were kept together in individual cages and were allowed to be together till feeding to enable 100% brother-sister matings. Just before feeding, females from all selected susceptible strains were taken out and were put together in a single cage. Similarly females from all resistant lines were collected in one cage just before feeding. This method enabled strict sib-mating and as they were put together before feeding, sufficient numbers of adults were obtained. This would have been impossible if females from single rafts only were pooled together. However, in this method it was not possible to trace the exact parentage, unless some artificial markings were made on the females. Due to strict brother-sister mating, however, inbreeding depressions were observed and experimental feedings of one or two generations had to be interrupted. When such inbreeding depressions were noticed out-breedings were made with very closely related lines.

RESULTS

Females isolated from F_{10} to F_{13} generations of the D3 stock colony of *C. p. fatigans* were fed on the donor. The results of the experiments are given in Table 1. The mean number of microfilariae per cu mm of peripheral blood of thedonor at the time of these series of experimental feedings, varied form 1.8 to 2.3. The percentage of infectivity of these mosquitos to the rural strain *W. bancrofti* were 82.8% in F_{10}, 75% in F_{11}, 86.4% in F_{12} and 85.5% in F_{13}. The dissections of the mosquitos after 12-14 days showed that the number of infective larvae in susceptible mosquitos varied from a minimum of one to a maximum of 21 larvae per mosquito. The mean number of larvae per mosquito in F_{10} to F_{13}

generations were about 5.3, 6.6, 6.0 and 3.7 larvae respectivley. Wharton (1960) has shown that the intake of *W. bancrofti* microfilariae by *C. p. fatigans* was three times the number of microfilariae that would be expected in a full blood meal. This would explain the high rate of infective larvae in some of the suceptible mosquitos.

TABLE 1. SUSCEPTIBILITY OF D3 STRAIN OF CULEX PIPIENS FATIGANS TO INFECTION WITH A RURAL STRAIN OF WUCHERERIA BANCROFTI

Colony and generation	Mean No. mf/cumm of blood at the time of feeding	No. of mosquitos that took blood from the donor	No. of mosquitos that died before dissection	No. of mosquitos dissected	No. of mosquitos with infective larvae	Percentage of mosquitos which are susceptible to infection	No. of infective larvae per mosquito		
							Min.	Max.	Mean
D_3									
F_{10}	2.2	73	9	64	53	82.8	1	21	5.3
F_{11}	1.8	48	8	40	30	75.0	1	15	6.6
F_{12}	2.3	55	11	44	38	86.4	1	16	6.0
F_{13}	2.0	49	7	42	36	85.7	1	21	3.7

These results showed that the infectivity rates of the females were high and varied form 75% to 86.4%. In a previous study, the percentage of five local strains of *C.p.fatigans* to rural *W. bancrofti* varied only from 6.6% in the Kuala Lumpur strains to 28.1% in a dieldrin selected colony (Thomas & Ramachandran, 1970). These mosquitos were also fed on the same donor when the microfilariae counts ranged from 2.7 to 3.1 per cml of blood. Results of the present studies therefroe have shown clearly that the incompatible strain of *C. p. fatigans* is an extremely efficinet vector of the rural strain of *W. bancrofti.*

SELECTION OF INCOMPATIBLE COLONY OF *C.p. FATIGANS* TO INFECTION WITH RURAL STRAIN OF *W. BANCROFTI.*

Four or five egg rafts laid by the most susceptible females (F_{10}) of the D3 stock colony of C.P. fatigans were used as parents (P). The method of selecting, rearing and feeding have already been described in an earlier paper (Thomas & Ramachandran, 1970). Selection was continued for seven successive generations except in F4. The results are tabulated in Table 2.

TABLE 2. SELECTION OF SUSCEPTIBLE D3 STRAIN OF CULEX PIPIENS FATIGANS FOR FURTHER SUSCEPTIBILITY TO INFECTION WITH A RURAL STRAIN OF WUCHERERIA BANCROFTI

Colony and generation	Mean No. of mf/cumm of blood at the time of feeding	No. of mosquitos that took blood from the donor	No. of mosquitos that died before dissection	No. of mosquitos dissected	No. of mosquitos with infective larvae	Percentage of mosquitos susceptible to infection	No. of infective larvae per mosquito		
							Min.	Max.	Mean
D3 F_{10} (P) Selected susceptible strain	2.2	73	9	64	53	82.8	1	21	5.3
F_1	2.7	41	3	38	33	86.8	1	24	7.2
F_2	1.5	42	8	38	29	76.3	1	12	3.0
F_3	2.2	45	12	33	28	84.8	1	8	3.5
F_4	-	-	No FEEDING		-	-	-	-	-
F_5	1.4	15	11	4	3	75.0	1	7	3.3
Microfilarial count in peripheral blood of donor fell to 0.3 larvae/cml blood									
F_6	0.3	50	14	36	6	16.7	1	1	1.0
F_7	0.3	30	11	19	2	10.5	1	1	1.0
EXPERIMENTS DISCARDED									

In the first three generations, the percentage of infectivity among adult mosquitos were high: 86.8% in F_1, 76.3% in F_2 and 84.8% in F_3. The microfilarial counts in the donor's blood at the time of feedings were 2.7, 1.5 and 2.2 larvae percumm espectivaly. The number of infective larvae in the susceptible mosquitos varied from 1-24 in F_1 with a mean of about 7.2 larvae; 1-12 (3.00 mean) in F_2 and 1-8 (mean 3.5) in F_3 respectively.

Due to the very small number of adults in F_4 the females were not fed on the donor but the experimental feedings were continued in F_5, F_6 and F_7. At the time when F_5 adults were fed on the donor, the microfilarial count in his peripheral blood dropped to 1.4 larvae per cumm The microfilarial level further dropped to 0 3 larvae per cml when F_6 and F_7 generations were offered blood meal.

Further experimental feedings on the donor had . to be discontinued due to the very low microfilarial count in the donor's peripheral blood (below 0.1 larvae per cml of blood). The percentage of infection among F_5 adults was 75%. The number of infective larvae per susceptible mosquito varied from 1-7 with a mean of about 3.3 (Table 2). The percentage of infectivity among F_6 and F_7 adults of the selected susceptible strain fell to a low level of 16.7% and 10.5% respectively. These rates were very much lower than those originally shown by the normal parental colony, and were most probably due to the low microfilarial rate in the donor's peripheral blood.

Similarly, the selection for resistance to infection with *W. bancrofti* was carried out on the

normally susceptible incompatible strain. For this, resistant parents were obtained from the females of F_{10} incompatible colony which were refractory to infections. The results are given in Table 3. All through the experiments, the two selected strains (susceptible and resistant strains) were fed simultaneously on the same night on a single donor.

TABLE 3. SELECTION OF SUSCEPTIBLE D3 STRAIN OF CULEX PIPIENS FATIGANS FOR RESISTANCE TO INFECTION WITH A RURAL STRAIN OF WUCHERERIA BANCROFTI

Colony and generation	No. (Mean) mf/cmm of blood at times of feeding	No. of mosquitos that took blood from the donor	No. of mosquitos that died before dissection	No. of mosquitos dissected	No. of mosquitos which are refractive to infection	Percentage of mosquitos resistant to infection	No. of infective larvae per susceptible mosquito		
							Min.	Max.	Mean
D3 F_{10} (P) Selected resistant strain	2.2	73	9	64	11	17.2	1	21	5.28
F_1	2.7	60	8	52	14	26.9	1	23	5.76
F_2	1.5	15	1	44	34	77.3	1	11	1.71
F_3	2.2	45	7	38	22	57.9	1	6	2.06
F_4	-	-	No feeding (due to insufficient number of adult mosquitos)						-
F_5	-	-	No feeding (due to insufficient number of adult mosquitos)						-
Microfilarial count in donor's peripheral blood fell to 0.3 larvae/cml blood									
F_6	0.3	105	62	43	40	93.1	1	1	1.00
F_7	0.3	45	22	33	30	90.0	1	1	1.00
Experiments discarded									

The resistance of the F_{10} females of D3 strain (P) was 17.2%and the microfilaria rate per cu mm blood of the donor was 2.2 (Table 3). Feeding was continued for three successive generations. The microfilarial count at the time of experimental feeding of these adults in these generations were the same as those when the suceptible strains were fed, i.e. 2.7 1.5 and 2.2 per cu mm respectively (Table 2 and Table 3). During these three generations, the resistance of the selected strain increased to 26.9% in F_1; 77.3% in F_2, and then dropped to 57.9% in F_3.

The F_4 and F_5 adults were not fed on the donor due to the shortage of females. The experimental feedings commenced again with F_6 adults and continue in the F_7 generation but later the feeding experiments were abandoned due to the sharp fall in the microfilarial count in the donor's peripheral circulation. The resistance in F_6 and F_7 generations, when the microfilarial count was 0.3 larvae per cu mm of blood was 93.1% and and 90.0% respectively.

As the rate of infectivity of the mosquitos of a given colony has been shown to be directly related to the rate of microfilaria (Wharton, 1960) it was not possible to compare the susceptibility/resistance of the F_6 and F_7 generations of the two selected colonies with their parent generations.

DISCUSSION

These studies have shown very clearly that the D3 strain in *C.p. Fatigans* is an extremely efficient vector fo the rural strain of *W. bar crofti.* If the D3 strain were used in the control of *C. p. fatigans*, in Malaysia, it is possible that this strain would replace or coexist with the indigenous strain of *C. p. fatigans.* Being an excellent vector of the rural strain of *W. bancrofti* which is the more predominant of the two strains present in Malaysia the introduction of this strain would have very serious and far-reaching consequences. Therefore, this species should not be released into the field to control *C. p. fatigans* in Weast Malaysia.

No feeding experiments were carried out with the D3 strain of *C. p. fatigans* to estimate its susceptibility to the urban strain of *W. bancrofti.* It is therefore, difficult to predict its efficiency with accuracy. However, in most countries *C. p. fatigans* is an excellent vector of *W. bancrofti* and probably it would be so in Malaysia too.

Wharton (1960) estimated that a female *C. p. fatigans* would consume about 4 cml of blood during a single meal but the intake of *W. bancrofti* microfilaria by the mosquito would be equivalent to the number present in 12 cumm of blood. He also reported that many larvae were lost during development, and that many infective larvae left the mosquitoes even though no blood meal was taken. Therefore, although the mosquitos were susceptible to infection, unless the donor has a constant and an optimal number of microfilaria in the peripheral blood at the time of feeding, it would not be possible to select out susceptible strains or to estimate with certainty the vector susceptibility of the strain or compare its susceptibility with that of another strain. Therefore, when comparisons of vector suceptibilities were made, between strains, or between species or among various generations of a single strain, it is of prime importance to feed them on a single donor who supports a constant number of microfilaira per cumm of the peripheral blood. This factor was very clearly indicated in this series of experiments. When the mean microfilarial count was about 2 or more per cumm of peripheral blood the percentage of suceptibility of the incompatible strain was always above 80%. When the average microfilarial count fell to about 1.5larvae per cumm the infectivity rate inthe normally susceptible incompatible strain of *C. p. fatigans* showed a corresponding drop to 75%. The

same pattern of results was obtained in the substrain of *C. p. fatigans* which was selected for susceptibility to infection. In this selected substrain, the rate of susceptibility to infection after selection increased while the mean number of microfilaria was high (about two larvae per cumm of blood). When this count dropped to about 1.5 larvae per cumm of peripheral blood a corresponding fall in susceptibility to infectivity was also noticed among the adults. Subsequently, when the microfilarial count fell to 0.3 larvae per cumm of blood, the infectivity rate in the naturally susceptible strain which had already been selected dropped to about 16.6% and 10.5% in F_6 and F_7 respectively. Ramachandran et al. (1964) found a greater range of variation (12% to 53%) in infectivity rates among five strains of indigenous *C. p. fatigans* which have been collected form different localities in Malaysia and which were fed on the same donor at different times when the microfilarial counts per cumm of blood were not constant at the time of feedings. On the other hand, when these strains were fed simultaneously on the same donor when the microfilarial count in the blood was relatively constant, the rate of infectivity varied only from 11% to 30%.

The necessity of studying the vector ablity of all genetic strains to local parasites before they are considered as potential genetic weapons against the local strains of mosquitos should be emphasized here. It is extremely important that such studies are undertaken before rearing them in large numbers for release into the field. If such genetic tools are vectors of local strains of parasites, the release and their subsequent establishemnt would have far-reaching adverse effects.

SUMMARY

The feeding experiments with the D3 strain of *C. p. fatigans* on a donor showed that this strain, unlike the local strains of *C. p. fatigans,* is a very efficient vector of the rural strain of W. *bancrofti*. Therefore, it is not a suitable strain to use as a genetic weapon against local *C. p. fatigans*. The susceptibility of adults of four generations F_{10} to F_{13} of the D3 strain was above 80%.

Experiments were carried out to select out two substrains of *C.p. fatigans* one of which was susceptible and the other resistant to the rural strain of *W. bancrofti*. When the mean microfilarial counts in the peripheral circulation was about two larvae or more per cumm blood, susceptibility to infection in various generations remained high-around 85%. However, when the microfilarial count dropped to 0.3 larvae per cu mm blood, the susceptibility of the selected adutls in F_6 and F_7 also dropped to 16% and 10% respectively. Similar and comparable results were also obtained when experiments were carried out to select out resistant mosquitos to this infection.

These results showed that when a strain of *C. p. fatigans* adults is naturally susceptible to W. *bancrofti* infection, the number and percentage of mosquitos which become infected and the number of infective larvae that they carry are related directly to the number of microfilariae in the blood at the time of feeding.

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