

**FACTORS AFFECTING THE TRANSMISSION OF FILARIAE :
THE EFFECT OF PARASITIZATION BY *BRUGIA MALAYI* IN
LABORATORY POPULATIONS OF *AEDES AEGYPTI***

by

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Several recent articles have reviewed factors affecting the transmission of filariae (for example, LAVOPIERRE, 1958b - HAWKING and WORMS, 1961 - EDESON and WILSON, 1964). The general features of transmission are well-known, and the hosts, parasites and vectors are for the most part defined. Little is known, however, of the effects of a parasite on the individual vector, or of the effects, if any, of parasitization on the vector population. This paper is an introductory account of experiments designed to follow the effects of parasitization by *Brugia Malayi* in laboratory populations of *Aedes aegypti*.

There is experimental evidence that a filarial parasite may have harmful effects on its insect host. In the case of filariae and mosquitoes the first effect may be seen within a day or two, or even an hour or two, of microfilariae being ingested. When large numbers of microfilariae are present in the blood of the donor a high proportion of the mosquitoes die, and ESSLINGER (1962) showed that in the case of *Anopheles quadrimaculatus* and *Brugia pahangi* death was related to the extensive damage done to the developing peritrophic membrane and the midgut epithelial cells by the microfilariae. There are no records of the surviving mosquitoes being adversely affected during the subsequent development of the parasites until the latter are nearly mature.

At the time when the filarial larvae are mature, or nearly so, there may be a harmful effect on the mosquitoes. KERSHAW *et al* (1953, 1955) recorded an increased mortality rate in *Ae. aegypti* infected with *Dirofilaria immitis* when the filarial larvae became mature. WHARTON (1957) found that *Mansonia bonnea/dives* fed on a carrier of *B. malayi* with a microfilarial density of 10.7 mf. per c. mm. showed an increased mortality when the filarial larvae were near maturity, although this effect was absent when the mosquitoes were fed on carriers with microfilarial densities of 2.3 mf. per c. mm. or less. The explanations of these results are not yet clear. In the case of *Ae. aegypti* infected with *B. malayi* little or no damage is caused to the muscles in light or moderate infections (E. B. BECKETT and W. W. MACDONALD, unpublished). LAVOPIERRE (1958a), discussing *Chrysops silacea* and *Loa Loa*, suggested that mature larvae may cause injury to the mouth-parts during their emergence when the fly is feeding; this suggestion has not yet been confirmed.

Since there is evidence suggesting that infected mosquitoes are at a disadvantage to those uninfected, it may follow that in a population which includes two kinds of mosquitoes, one refractory to infection and the other susceptible, those which are refractory might have a selective advantage over those which are susceptible when the population is exposed to the parasite.

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In Liverpool we have a colony, maintained for nearly 30 years, which includes mosquitoes of both kinds. In about 20 per cent. of individuals the microfilariae of *B. malayi* will develop to become mature larvae. In the larger proportion the microfilariae die in the thoracic muscles within a day or two of being ingested with the blood meal. From this colony a highly susceptible sub-colony was selected (MACDONALD, 1962a), and with this and a refractory strain it was shown that susceptibility of *Ae. aegypti* to *B. malayi* was controlled by a sex-linked recessive gene f^m (MACDONALD, 1962b). Further experiments provided a preliminary estimate of the situation of the gene (MACDONALD, 1963), and unpublished data confirm a preliminary report (MACDONALD and WHARTON, 1963) that mosquitoes homozygous for f^m are not only susceptible to sub-periodic *B. malayi* but also to two other strains of *Brugia* and to two strains of *Wuchereria*. Conversely, mosquitoes which are heterozygous or homozygous for the dominant allele-morph are refractory to each infection.

The stock colony of the Liverpool *Ae. aegypti* has never been exposed to *Brugia* infections, and the selective forces which maintain the balanced polymorphism with respect to the gene for susceptibility can not be defined. It may be that the gene has some other effect of which we are at present ignorant.

To investigate the stability of the polymorphism when the mosquitoes were exposed to a filarial parasite, four populations were made up from two parent strains, one of which was a pure susceptible strain, and the other was an almost pure refractory strain. In each population the frequency of the gene was either high or low. One population with a high gene frequency and one with a low gene frequency were fed on a cat infected with sub-periodic *B. malayi*. The remaining two populations, one with a high and one with a low gene frequency, were fed on an uninfected cat. In each generation the populations were fed twice an interval of 4-7 days between feedings. The eggs laid after each blood-meal produced the next generation. The experiment was run for 14 generations and a sample of mosquitoes from each population of each generation was tested for susceptibility to infection.

In the two uninfected populations, one had the gene f^m at an initial frequency of more than 0.70. The value fell for five generations and from F_6 to F_{14} it fluctuated slightly between 0.42-0.53. The other population had an initial gene frequency of about 0.30. After several fluctuations, the value remained between 0.20 and 0.35.

The difference between the two populations fed on infected cats was also maintained throughout the experiment. The population with a high gene frequency remained between 0.70-0.80 for four generations, then fell and fluctuated slightly between 0.56 and 0.70 until F_{14} . The remaining population showed a frequency of 0.45-0.55 for seven generations then fluctuated at a lower level and settled between 0.33-0.39 from F_{10} to F_{14} .

The feeding regimen, therefore, had apparently little effect on the equilibrium frequency of the gene. The two populations with initial high frequencies remained at a higher level than the low gene frequency populations. This may be the result of differences in genetic background of the two original parent stocks, the former two populations being constituted mainly from the highly susceptible strain with a small proportion of mosquitoes from the refractory stock, and the other two populations being made up with reciprocal proportions.

Comparing the two high level populations one with the other, it may be significant that the frequency of the gene f^m was consistently higher in the population fed on the infected cat. To a less marked extent this was also true for the low level populations. The results show, therefore, that, during the period of its early development, the filarial parasite did not have a harmful effect on the genotypically susceptible mosquitoes, at least so far as feeding and egg-laying were concerned.

The microfilarial densities in the donor cats used to infect the mosquitoes averaged 2.3 mf. per c. mm throughout the experiment. WHARTON (1957) found that *Mansonia bonneae/dives* infected with similar densities showed no difference in mortality when compared with uninfected controls.

At the F_{14} generation a change was made in the experiment. The adults of this and subsequent generations were fed three times over a period of 11 days, each population being fed on

an infected or uninfected host as before. The eggs resulting from the first two meals were discarded, and those laid after the third meal, i.e. about 15 days after the first and 11 days after the second meal, were used for the next generation. If the susceptible mosquitoes were at a disadvantage at the time the filarial larvae became mature, and their mortality rate increased, then fewer eggs would be laid by them and in the next generation there would be a lower proportion of susceptible mosquitoes.

This part of the work is still in progress, but after four generations the relative gene frequencies in the populations have not changed, and there is no indication that genotypically susceptible mosquitoes are at a selective disadvantage under the conditions of the experiment.

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