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# MOSQUITO GENETICS IN RELATION TO FILARIAL INFECTIONS

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## INTRODUCTION

One of the most interesting complexes of mosquitoes for a genetic and evolutionary study is the subgroup of *Stegomyia* commonly known as the *Aedes scutellaris* complex. This complex, with about 30 species, extends from the Andaman Islands in the west to Okinawa in the north and to the Marquesas and the Tuamotu Archipelago in the eastern Pacific (Fig. 1). The systematics and species relationships are moderately well defined, but there are undoubtedly unknown island populations awaiting discovery and description. Marks (1954) gave a good introduction to the distribution and systematics of the complex, and, more recently, Belkin (1962) and Huang (1972) have described several new species and given detailed re-descriptions of many of those previously known. Table 1 lists the principal species within the complex and their distribution.

Besides their intrinsic interest, the members of the complex have attracted attention because they include a number of vectors of bancroftian filariasis, and many of the most gross forms of elephantiasis can be seen within the range of the *A. scutellaris* group vectors.

Some species, notably *A. polynesiensis*, have a wide distribution throughout several island groups, whereas others, such as *A. cooki*, are known only from one island. Such a distribution pattern, with island populations well separated by natural barriers, might suggest that control of vector populations may not be too difficult. However, owing to the wide range of natural and artificial larval habitats which are utilized by the species, larval control by chemicals is usually not practical, and the outdoor habits of the adults make control of adult populations expensive and short lasting.

In theory, island populations might be suppressed, or even eliminated, by genetic control techniques and the *A. scutellaris* complex has often been quoted as a group which merits study with this long-term aim. Two considerations have given support to this proposal. The first concerns the interspecific crossing relationships. Some species when intercrossed are almost fully fertile, whereas others, although they will mate more or less readily with one another, produce eggs which fail to hatch. This

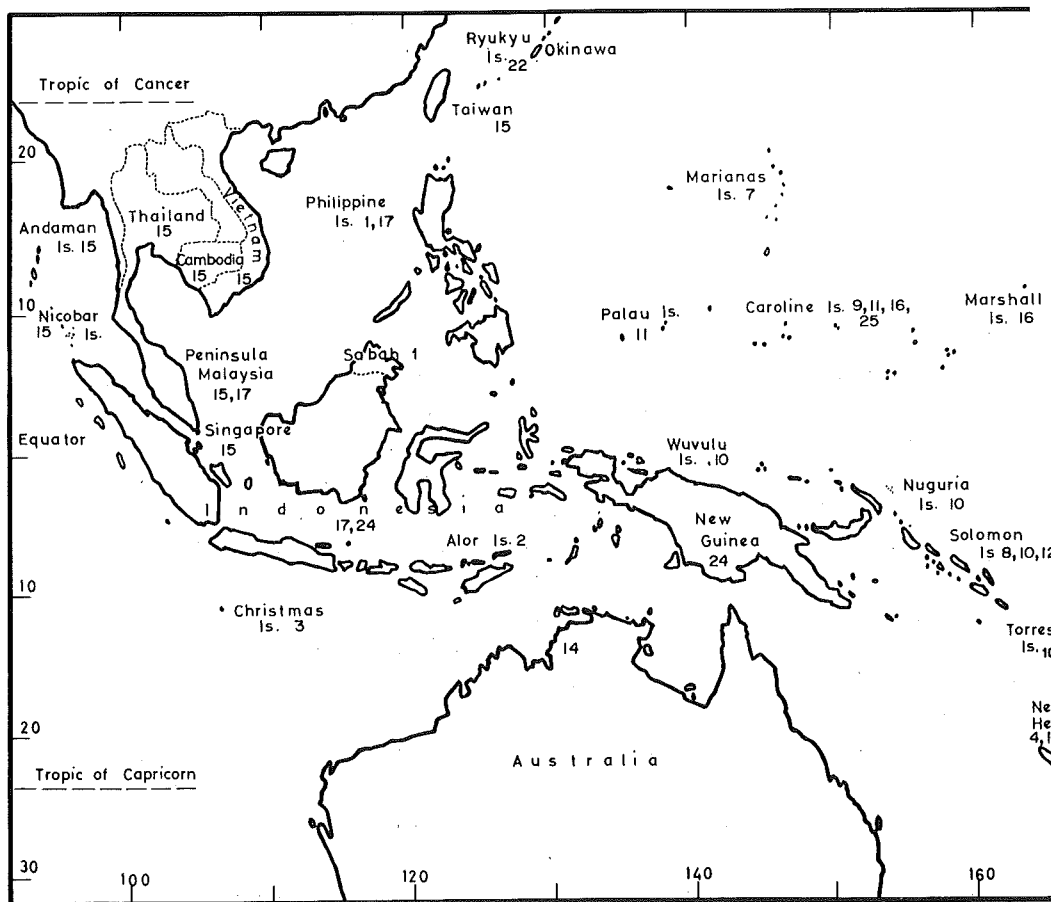
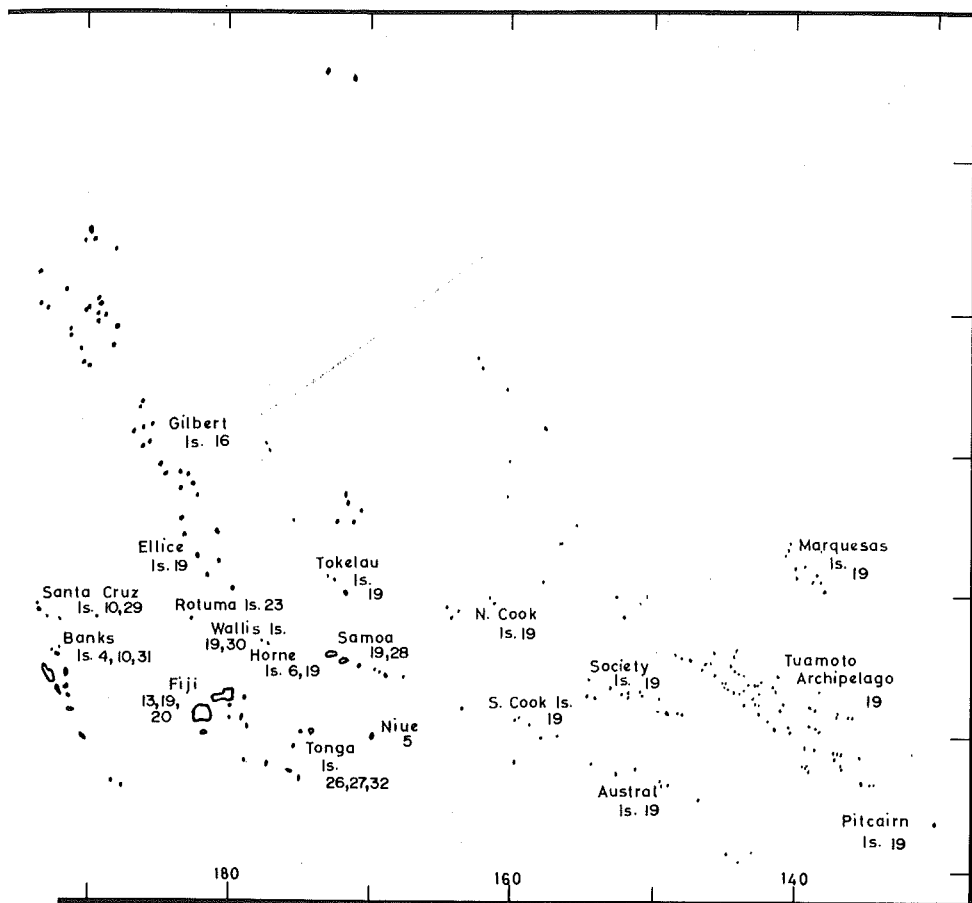


Fig. 1. The geographical distribution of the *Aedes (Stegomyia) scutellaris* mosquito complex. The list of species corresponding to the numbers on the map are given in Table 1.

incompatibility may occur in both parental crosses or only in one. Such bidirectional and unidirectional incompatibility has often been compared with the situation in the *Culex pipiens* complex, in which incompatibility between strains has been utilized for genetic control experiments. The second consideration concerns the genetic basis of susceptibility in the mosquitoes to infection with filariae. If a single gene controls susceptibility within the *A. scutellaris* complex, as is the case with *A. aegypti*, it may be feasible to construct a replacement population which would have most of the genome of one of the vector species but which would differ by being both refractory to infection and incompatible in both directions, or almost so, with the vector.

This review summarizes present knowledge of the relationships within the



*A. scutellaris* complex together with those aspects of the genetics of other mosquitoes, especially *C. pipiens*, which are relevant to the long-term aim of genetic control of a filariasis vector population.

### HYBRIDIZATION EXPERIMENTS

The members of the *A. scutellaris* complex can be fairly easily colonized in the laboratory, but because of their geographical isolation many species are difficult to collect and correspondingly few hybridization experiments have been carried out. However, those interspecific crosses that have been made have given most interesting results. In discussing them it is worth bearing in

Table 1

The principal members of the *Aedes (Stegomyia) scutellaris* complex and their distribution

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1. <i>A. alcasidi</i> Huang, 1972	Philippines, Sabah
2. <i>A. aloreensis</i> Bonne-Wepster and Brug, 1932	Alor Island
3. <i>A. andrewsi</i> Edwards, 1926	Christmas Island
4. <i>A. aobae</i> Belkin, 1962	Banks Islands, New Hebrides
5. <i>A. cooki</i> Belkin, 1962	Niue Island
6. <i>A. futunae</i> Belkin, 1962	Horne Islands
7. <i>A. guamensis</i> Farner and Bohart, 1944	Marianas Islands
8. <i>A. gurneyi</i> Stone and Bohart, 1944	Solomon Islands
9. <i>A. hakanssoni</i> Knight and Hurlbut, 1949	Caroline Islands
10. <i>A. hebrideus</i> Edwards, 1926	Wuvulu Is., Nuguria Is., Solomon Islands, Santa Cruz Islands, Torres Islands, Banks Islands, New Hebrides
11. <i>A. hensilli</i> Farner, 1945	Caroline Islands, Palau Islands
12. <i>A. hoguei</i> Belkin, 1962	Solomon Islands
13. <i>A. horrescens</i> Edwards, 1935	Fiji
14. <i>A. katherinensis</i> Woodhill, 1949	Northern Australia
15. <i>A. malayensis</i> Colless, 1962	Andaman and Nicobar Islands, Singapore, Peninsular Malaysia, Thailand, Cambodia, Viet Nam, Taiwan
16. <i>A. marshallensis</i> Stone and Bohart, 1944	Caroline Islands, Marshall Islands, Gilbert Islands
17. <i>A. paullusi</i> Stone and Farner, 1945	Philippines, Malaysia, Indonesia
18. <i>A. pernotatus</i> Farner and Bohart, 1944	New Hebrides
19. <i>A. polynesiensis</i> Marks, 1951	Fiji, Horne Islands, ? Wallis Islands, Ellice Islands, Tokelau Islands, Samoa, Cook Islands, Society Islands, Austral Islands, Marquesas Islands, Tuamotu Archipelago, Pitcairn Island
20. <i>A. pseudoscutellaris</i> (Theobald, 1910)	Fiji
21. <i>A. quasiscutellaris</i> Farner and Bohart, 1944	Solomon Islands
22. <i>A. riversi</i> Bohart and Ingram, 1946	Ryukyu Islands
23. <i>A. rotumae</i> Belkin, 1962	Rotuma Islands
24. <i>A. scutellaris</i> (Walker, 1858)	Indonesia, New Guinea
25. <i>A. scutoscriptus</i> Bohart and Ingram, 1946	Caroline Islands
26. <i>A. tabu</i> Ramalingam and Belkin, 1965	Tonga Islands
27. <i>A. tongae</i> Edwards, 1926	Tonga Islands
28. <i>A. upolensis</i> Marks, 1957	Samoa
29. <i>A. varuae</i> Belkin, 1962	Solomon Islands, Santa Cruz Islands
30. <i>Aedes</i> sp. Wallis form of Belkin, 1962	Wallis Islands
31. <i>Aedes</i> sp. Vanua Lava form of Belkin, 1962	Banks Islands
32. <i>Aedes</i> sp. Tafahi form of Hitchcock and Rozeboom, 1973	Tonga Islands

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mind that experiments of different workers, and even those of the same worker at different times, are not always in full agreement. This is partly because of strain differences among species and partly because of different laboratory conditions. Experience in Liverpool has shown that some interspecific crosses are sometimes relatively successful, at other times wholly unsuccessful. The reasons are not yet clear.

Table 2 summarises the compatibility relationships of those species which have been tested. There are two measurements to be taken into account in any cross: the insemination rate and the egg-hatching rate. Information is usually not available on both, and the table gives only an approximation of the relationships based on egg-hatching rates of inseminated females. From the data available, insemination rates of 20-60% are common, and it is noteworthy that *individual* egg batches from inseminated females either show a high hatching rate or all the eggs fail to hatch. In this important respect the hatching pattern differs from that of many *C. pipiens* crosses, in which no fully fertile egg-rafts are produced but in which small proportions of eggs of individual rafts may hatch.

*A. polynesiensis* x *A. polynesiensis*

Tesfa-Yohannes (1973) examined the relationships of three strains of *A. polynesiensis* from Samoa, Taiaro and Tahiti. Taiaro and Tahiti strains could be crossed easily in both directions giving normal insemination rates and egg-hatching rates. On the other hand, although the Samoan females inseminated by either of the other two strains laid average numbers of eggs, only 5% hatched. The resultant F<sub>1</sub> adults were fully compatible with all parental strains. Eggs from Tahiti females inseminated by Samoan males had a slightly reduced hatching rate.

*A. polynesiensis* x *A. pseudoscutellaris*

*A. polynesiensis* and *A. pseudoscutellaris* are closely related species difficult to separate morphologically. Both Woodhill (1954) and Rozeboom and Gilford (1954) showed that successful crosses could be made in both directions, although the egg-hatching rate was only between 5-37%. We have had similar results in Liverpool. The F<sub>1</sub> progeny are fertile and F<sub>2</sub> and F<sub>3</sub> progeny are readily obtainable.

*A. polynesiensis* x *A. malayensis*

Tesfa-Yohannes and Rozeboom (1974) found that although 10-15% of *A. polynesiensis* females were inseminated by males of *A. malayensis*, no fertile

eggs were laid. In Liverpool we have had almost the same experience but on one occasion a successful cross was made. In this experiment 9 of 11 females were inseminated and a small batch of eggs hatched. The  $F_1$  adults were backcrossed to *A. polynesiensis* males and females, but no progeny were obtained. Only 1 of 14 parental females was inseminated by the hybrid males.

The reciprocal parental cross using *A. malayensis* females and *A. polynesiensis* males is more likely to be successful. Tesfa-Yohannes and Rozeboom (1974) crossed three strains of *A. polynesiensis* with *A. malayensis*, and although the insemination rates were only 5-9% and none of the eggs hatched from two of the crosses, the eggs from the third cross, involving Samoan *A. polynesiensis*, gave a high hatching rate.

The hybrid  $F_1$  males were successfully mated to both parental females, but no viable eggs were obtained. The hybrid  $F_1$  females were also backcrossed to the parents and both crosses gave viable eggs. Successive backcrosses to *A. malayensis*, but not to *A. polynesiensis*, were achieved.

In Liverpool the *A. malayensis* female x *A. polynesiensis* male cross has been successful on several occasions, unsuccessful on others, but the backcrosses of the hybrids to *A. polynesiensis* parents have failed to produce progeny.

#### *A. polynesiensis* x *A. cooki*

In Liverpool experiments *A. polynesiensis* males readily inseminated *A. cooki* females, but only a single egg has hatched. In the reciprocal cross, on the other hand, most of the inseminated *A. polynesiensis* females laid fertile egg batches. The progeny of these crosses are being investigated.

#### *A. polynesiensis* x *A. tabu*

Reciprocal crosses between *A. polynesiensis* and *A. tabu* have been successful. The progeny are currently under investigation.

#### *A. polynesiensis* x "Tafahi" form

Hitchcock and Rozeboom (1973) have studied the Samoan population of *A. polynesiensis* and an autogenous unnamed species of the complex from the island of Tafahi, Tonga. Although *A. polynesiensis* females are readily mated by Tafahi males, no viable eggs were obtained, but the reciprocal cross was very successful and the hybrids were inbred for several generations. The  $F_1$  hybrid males could be backcrossed successfully to Tafahi females, but in the backcross to *A. polynesiensis* females, although the insemination rate was

high, less than 1% of the eggs hatched. The backcross to *A. polynesiensis* males gave a high hatching rate.

*A. pseudoscutellaris* x *A. scutellaris*

Woodhill (1950) made a few reciprocal crosses between *A. pseudoscutellaris* and *A. scutellaris*. He did not record whether insemination took place, but all the eggs failed to hatch.

*A. pseudoscutellaris* x *A. katherinensis*

Woodhill (1950) also tried to cross *A. pseudoscutellaris* and *A. katherinensis* but the eggs from reciprocal crosses failed to hatch.

*A. pseudoscutellaris* x *A. malayensis*

In Liverpool *A. malayensis* females were readily mated by *A. pseudoscutellaris* males and an insemination rate of 78% has been achieved. However, very few eggs hatched and only two hybrid females have been obtained. The backcross to *A. pseudoscutellaris* males was not successful. The reciprocal parental cross gave a low insemination rate of *A. pseudoscutellaris* females of 2%. One of two egg batches hatched but the backcrosses of the hybrid males and females to *A. pseudoscutellaris* were unsuccessful.

*A. scutellaris* x *A. malayensis*

Colless (1962) successfully crossed *A. scutellaris* and *A. malayensis* in both directions and both sets of progeny interbred and produced F<sub>2</sub> progeny. No backcrosses were attempted.

*A. scutellaris* x *A. katherinensis*

The earliest detailed studies of crossing relationships within the complex are by Woodhill (1949, 1950) and Smith-White and Woodhill (1954) using *A. scutellaris* and *A. katherinensis*. Woodhill was the first to observe that whereas the cross between *A. scutellaris* females and *A. katherinensis* males resulted in normal numbers of viable eggs, all the eggs from the reciprocal cross failed to hatch, although the female *katherinensis* had been inseminated. Of the four possible backcrosses of the hybrids to the parents three gave fertile eggs. The exception was the cross between hybrid males



Table 2  
Summary of interspecific crossing relationships, based on egg-hatching rates,  
within the *Aedes scutellaris* complex

♀ \ ♂	<i>cooki</i>	<i>hebrideus</i>	<i>katherinensis</i>	<i>malayensis</i>	<i>pernotatus</i>	<i>polynesiensis</i>	<i>pseudoscutellaris</i>	<i>scutellaris</i>	<i>tabu</i>	'Tafahi' form
<i>cooki</i>				+		—			++	
<i>hebrideus</i>					+					
<i>katherinensis</i>							—	—		
<i>malayensis</i>	++					+	+	+++	—	
<i>pernotatus</i>		—								
<i>polynesiensis</i>	+++			+			++		+++	—
<i>pseudoscutellaris</i>			—	+		++		—		
<i>scutellaris</i>			+++	+++			—			
<i>tabu</i>	+++			—		+++				
'Tafahi' form						+++				

+++ high compatibility  
 ++ medium compatibility  
 + low compatibility  
 — incompatible

and *A. katherinensis* females. Woodhill therefore postulated that there was some factor associated with *A. katherinensis* females which made them sterile unless mated with their own males. Smith-White and Woodhill then made a more extensive series of crosses and backcrosses and showed that even after successive backcrosses of (*A. scutellaris* ♀ x *A. katherinensis* ♂) female hybrids to *A. katherinensis* males the infertility of *A. katherinensis* females when mated with the backcross males was retained. The results were very similar to those reported by Laven (1953) from his crosses between strains of the *Culex pipiens* complex, for which a postulate was made that nuclear-independent cytoplasmic factors were responsible for the unidirectional fertility. This phenomenon of cytoplasmic incompatibility in strains is further discussed below.

#### *A. malayensis* x *A. cooki*

In reciprocal crosses made in Liverpool between *A. malayensis* and *A. cooki* 23-56% insemination rates were recorded, and small numbers of inseminated females laid eggs which hatched successfully.

#### *A. malayensis* x *A. tabu*

In reciprocal crosses made in Liverpool between *A. malayensis* and *A. tabu* insemination rates of 34-57% were recorded, but none of the eggs laid would hatch.

#### *A. cooki* x *A. tabu*

In reciprocal crosses in Liverpool between *A. cooki* and *A. tabu* high insemination rates of 64-88% were recorded. The cross between *A. tabu* females and *A. cooki* males produced large numbers of viable eggs, but the reciprocal cross was less successful and only 8 of 37 egg batches hatched.

#### *A. hebrideus* x *A. pernotatus*

*A. pernotatus* females mated readily with *A. hebrideus* males in the laboratory, but none of the eggs hatched (Perry, 1950). In the reciprocal cross, on the other hand, three of 37 *A. hebrideus* females produced eggs which hatched successfully. The 18 hybrid females which were reared were backcrossed to *A. pernotatus* males and 3 produced viable eggs. Most of the backcross progeny died, but 4 males and one imperfect female emerged.

## CYTOPLASMIC INCOMPATIBILITY

Incompatibility among populations of the *Culex pipiens* complex is very well documented. Laven (1957, 1967a), in particular, has made large numbers of inter-strain crosses and demonstrated both bidirectional and unidirectional incompatibility as well as bidirectional fertility between different strains. In a small field experiment in Burma Laven (1967b) also demonstrated that cytoplasmic incompatibility had potential as a means of genetic control. Subsequently it was proposed (Laven and Aslamkhan, 1970) that cytoplasmic incompatibility might be integrated with a male-linked translocation in order to provide strains for genetic control experiments, and such strains, with Paris cytoplasm, Delhi genome and male-linked translocations, have now been constructed (Krishnamurthy, 1974).

Unfortunately, incompatibility between strains is not as absolute as was at first thought. For example, Barr (1970) crossed a Californian population with the Italian Scauri strain of *C. pipiens*. The Scauri ♀ x Californian ♂ cross was fully compatible. In the reciprocal cross, however, although most of the egg-rafts failed to hatch, and could be classed as incompatible, a small proportion of rafts had a normal hatching rate and a larger proportion showed a reduced hatch.

A more recent study in Delhi of crossing relationships (Subbarao *et al.*, 1974) has revealed a polymorphism for mating type in wild populations of the *C. pipiens* group. Although the cytoplasm of the Paris and Delhi strains had been reported to show bidirectional incompatibility, Subbarao and her colleagues have now shown that although the majority of Delhi mosquitoes are incompatible with Paris stock in both directions, there is a minority component of the population with males which are fully compatible and females fully incompatible with Paris.

Ageing of the males has also been shown to have an effect on compatibility. Singh *et al.* (in press) in Delhi have demonstrated that with increasing age Paris males show increasing compatibility with Delhi strain females. When the females of these partially compatible matings were reared and successive backcrosses were made to ageing males of the Paris strain, the level of partial incompatibility increased significantly.

If these findings from Delhi, and those of Barr, have a wider application, the interpretation of other laboratory experiments will be made more difficult.

From his studies of incompatibility Laven concluded that the responsible factors were maternally transmitted through the cytoplasm, hence his term cytoplasmic incompatibility, and that the factors were inherited only from the female line through many generations of backcrossing. His hypothesis fits well the results of Smith-White and Woodhill (1954) from their crosses

of *A. scutellaris* and *A. katherinensis*. There have been alternative hypotheses to that of Laven, for example those of Smith-White and Woodhill (1954) and McClelland (1967), but the most recent development has been the hypothesis of Yen and Barr (1971, 1973) that incompatibility is related to the presence in the eggs of *C. pipiens* of large numbers of the rickettsia-like symbiont, *Wolbachia pipientis*. They postulated that males and females of a strain of *C. pipiens* carry the same strain of micro-organism and that individuals of different strains may have different organisms. In incompatible crosses the sperm of strain A, when they enter the eggs of females of strain B, are exposed to a foreign strain of organism and are prevented, or incapable, of forming normal diploid embryos. In such cases no progeny are produced except for an occasional parthenogenetic female. In the reciprocal cross the sperm of strain B may be unaffected by the micro-organisms of strain A and normal offspring would be produced. In some cases, where compatibility is said to be partial, a small number of normal offspring are produced from a cross, and Yen and Barr suggest that such a result may be obtained when the micro-organisms do not inhibit all the sperm of the other strain of mosquito and thus allow some survivors to unite with the female nuclei to form normal embryos.

Yen and Barr demonstrated that the micro-organisms could be eliminated by rearing the mosquito larvae in trays containing the antibiotic tetracycline hydrochloride. Owing to the effects of the drug on reproduction, the generation so treated produced few offspring, but the subsequent untreated generations, in which the micro-organisms were still absent, gave normal egg numbers and hatching rates. Mosquitoes in which the symbiont had been removed were termed aposymbiotic. When comparisons were made with formerly incompatible strains, with their symbionts and after their removal, the results were as follows. Males without symbionts became compatible with all females whether or not they had symbionts; and aposymbiotic females mated with males carrying symbionts, whatever their previous relationships, produced no offspring.

Analogies are often drawn between the *C. pipiens* complex and the *A. scutellaris* complex with regard to incompatibility between populations, but work with the latter group lags behind. However, light microscope studies in Liverpool by Dr. E.B. Beckett have shown that bodies which may be rickettsiae are present in the ovaries of some members of the *A. scutellaris* complex, and electron microscopy studies are now in progress to confirm their identity.

Attempts have been made to eliminate by means of antibiotics those putative rickettsiae. Preliminary efforts with tetracycline hydrochloride at concentrations used successfully by Yen and Barr (1973) with *C. pipiens* (0.025-0.05 mg/ml) were not very promising. A more extensive series of

experiments by my colleague, Dr. J.O. Wade, using streptomycin, ampicillin, tetracycline, erythromycin, chloramphenicol and rifampicin, produced no decisive improvement in the interspecific fertility of *A. polynesiensis* and *A. malayensis*, but in a few instances tetracycline seemed to show a beneficial effect.

Much remains to be accomplished before our understanding of incompatibility within the *C. pipiens* and *A. scutellaris* complexes is complete. And until we have such an understanding, proposals for manipulating this phenomenon for vector control programmes must remain tentative.

## GENETICS OF SUSCEPTIBILITY TO FILARIAE

### THE PARASITES

Five species of filariae have been used experimentally to study susceptibility of mosquitoes to infection, *Wuchereria bancrofti*, *Brugia malayi*, *B. pahangi*, *Dirofilaria immitis* and *D. repens*. By far the most important parasite to man is *W. bancrofti*, but since no host other than man is known, experimental work with this species has been restricted. This is the species which is widespread in the South Pacific, where the primary vectors are members of the *A. scutellaris* complex. By means of the freezing method developed for *B. pahangi* by Obiamiwe and Macdonald (1971), we have in Liverpool been preserving in liquid nitrogen the microfilariae of *W. bancrofti* imported from the tropics, and although the survival rate of the microfilariae following treatment is not as high as we should like, the stored material has been suitable for a number of susceptibility tests on mosquitoes.

*B. pahangi* is a parasite of a variety of mammals in Malaysia, whereas *B. malayi*, which includes both a periodic and a subperiodic form, is a parasite of man and animals in Southeast Asia. Both can be maintained in the laboratory in cats and dogs, but, of the two, *B. pahangi* is the less difficult. Most experimental work on the genetics of infections has therefore been based on *B. pahangi*, but since there is evidence, given below, that susceptibility of *Aedes* to *Wuchereria* is controlled by the same gene as that for *Brugia* it is hoped that the conclusions based on *B. pahangi* will be applicable to *W. bancrofti*.

*D. immitis* and *D. repens* are parasites of dogs and the work on these species is correspondingly less relevant to the solution of public health problems. Nevertheless, the experimental work on the susceptibility of mosquitoes to these infections supplements our knowledge of vector genetics.

## THE MOSQUITO VECTORS

There are many natural vectors of *W. bancrofti*, of which the most important are in the *C. pipiens* complex (for the periodic form of the parasite) and the *A. scutellaris* complex (for the subperiodic form). The principal vector in the latter group is *A. polynesiensis*; several other members of the group have been reported as vectors of local importance on island groups. Most experimental work on genetic aspects of vector susceptibility, however, has been carried out with a mosquito which is not a vector in nature, *A. aegypti*, but which because of its convenience in the laboratory and its genetic variability provides a valuable model for work with the *A. scutellaris* complex.

*Aedes aegypti*

A single sex-linked recessive gene, designated  $f^m$ , controls the susceptibility of *A. aegypti* to *B. malayi*, *B. pahangi* and *W. bancrofti* (Macdonald, 1962; Macdonald and Ramachandran, 1965). In tests of 43 geographic strains of *A. aegypti* Rodriguez and Craig (1973) found that  $f^m$  was absent from 30 of the strains, and the estimated frequency in the remainder varied from 0.10 to 0.73. Most of the strains from East Africa, but none of those from West Africa, carried  $f^m$ . However, since many of the strains tested had been maintained in the laboratory for a number of years and may have originated from only a few wild-caught mosquitoes, the reported frequencies may not reflect accurately the true frequencies in the field. In this context it is notable that the strain which originated from Brazzaville in the Congo showed no evidence of  $f^m$ , whereas an earlier investigation in the former province of Leopoldville, Belgian Congo, by Henrard *et al.* (1946) showed that 7 of 25 colony *A. aegypti* (presumably of a local strain) were susceptible to *W. bancrofti*.

The position of  $f^m$  on the sex chromosomes has been located at  $3.4 \pm 1.1$  crossover units from the sex locus (Macdonald and Sheppard, 1965), and the gene can be transferred between *A. aegypti* strains. The manipulation of the gene would be much easier if male mosquitoes could be scored for susceptibility. This is now possible with the methods of Terwedow and Rodriguez (1973) and Townson (1974, 1975), who have shown by the inoculation of exsheathed microfilariae that males of a susceptible strain of *A. aegypti* are fully susceptible. Unfortunately, a complication which has not yet been resolved has arisen from the experiments of Townson (1974). He has shown that in an apparently fully refractory strain, as determined by tests on a large series of females, a significant proportion of males are susceptible. Work is still in progress to attempt to explain this apparent anomaly.

Macdonald and Ramachandran (1965) showed that susceptibility of *A. aegypti* to *Dirofilaria* infections was not controlled by *f<sup>m</sup>*. Raghavan *et al.* (1967) showed, however, that susceptibility to *D. immitis* infections is heritable and, more recently, Zielke (1973) and McGreevy *et al.* (1974) reported independently that a sex-linked recessive gene, designated *ft* by the latter workers, controlled susceptibility. The location of the gene on the sex chromosomes has not yet been established.

The mode of action of the genes *f<sup>m</sup>* and *ft* are not understood but a study in India dealing with *A. aegypti* susceptible or refractory to *D. immitis* and *D. repens* (National Institute of Communicable Diseases, 1973) reports that susceptible mosquitoes are characterised by high levels of activity of the enzyme alkaline phosphatase. There were no marked differences in the level of acid phosphatase activity in susceptible and refractory mosquitoes.

#### *Culex pipiens* complex

Unfortunately the gene controlling susceptibility to *Brugia* in the *C. pipiens* complex does not control susceptibility to *W. bancrofti*. Whereas all *C.p. fatigans* populations tested are, so far as can be established, very susceptible to urban *W. bancrofti*, several tests have shown them to be either refractory to *B. pahangi* (Ogunba, 1969), or to have low infective rates of 4% (Ewert, 1965) or 8.5-25.4% (Desowitz and Chellappah, 1962). *C.p. molestus*, on the other hand, has shown infective rates of 14-19% (Schacher and Khalil, 1965) and 36.3% (Ogunba, 1969). Dr. B.A. Obiamiwe selected a highly susceptible strain from a *C.p. molestus* x *C.p. fatigans* hybrid and made a series of crosses and backcrosses with this stock and refractory *C.p. fatigans*. In a preliminary note, Obiamiwe and Macdonald (1973) reported that susceptibility to *B. pahangi* in the *C. pipiens* complex was controlled by a sex-linked recessive gene which they designated *sb*.

There have been a few unsuccessful attempts to select from vector populations a strain refractory to *W. bancrofti*. For example, Partono and Oemijati (1970) in Jakarta selected for three generations from phenotypically refractory individuals, but although there was a small and perhaps significant reduction in the proportion of susceptible mosquitoes at each generation, there was no evidence of the presence of a major gene for resistance to infection. Singh and Curtis (1974) in Delhi also tried to select a refractory strain from a vector population, but after five generations of selection they concluded that there was no evidence of a gene for non-susceptibility in the original strain. Thomas and Ramachandran (1970) tackled a reciprocal problem. Their *C.p. fatigans* populations in Malaysia had low susceptibility rates of 7-28% to a Malaysian rural periodic strain of *W. bancrofti*, and they attempted to select highly susceptible lines from two of

their stocks. Although they appeared to have some success in increasing the susceptibility rates, the numbers of mosquitoes dissected were relatively low. A statistical analysis of the results suggests that additional tests are required to confirm their conclusions.

The results from the work on the *C. pipiens* complex contrast therefore in two main respects with those reported with *A. aegypti*. First, whereas a major gene controls susceptibility to *B. pahangi*, the same gene seems to have no influence on susceptibility to *W. bancrofti*. Second, members of the complex can be highly susceptible to the widespread urban periodic form of *W. bancrofti* but relatively refractory to the rural periodic strain. Therefore, at least two genes, perhaps three, are concerned with susceptibility to *Brugia* and *Wuchereria* infections.

#### *Aedes scutellaris* complex

Progress with the genetics of the *A. scutellaris* complex in relation to filarial infections has been slow. The most promising finding has been that *A. malayensis* is refractory to infection with *B. pahangi*, whereas *A. polynesiensis*, *A. pseudoscutellaris*, *A. cooki* and *A. tabu*, all of which are vectors of subperiodic *W. bancrofti*, are highly susceptible. Coupled with this finding has been the demonstration that *A. polynesiensis* and *A. pseudoscutellaris* are also susceptible to urban periodic *W. bancrofti* and that *A. malayensis* is refractory to this strain. We have not yet confirmed that *A. malayensis* is refractory to subperiodic *W. bancrofti*. There is a subperiodic strain of *W. bancrofti* on the Nicobar Islands, where *A. malayensis* occurs, but so far only the *Aedes* (*Finlaya*) *niveus* group has been incriminated in its transmission (Kalra, 1974). It is of course desirable that the various mosquitoes be tested against several strains of *W. bancrofti* and it is hoped that in the future this will be possible, but for the time being *B. pahangi* must serve as a laboratory model.

The hybrid females of crosses between *A. polynesiensis* and *A. malayensis* have been tested for susceptibility to *B. pahangi* and shown to be refractory. Susceptibility is therefore a recessive trait. Both male and female hybrids have been backcrossed to the *A. malayensis* parents. The backcross progeny from the parental female were tested and 47 out of 47 were refractory. Only four of the progeny of the backcross to the *A. malayensis* male could be tested and these also were refractory. The much more informative backcrosses are those to the *A. polynesiensis* parents but, unfortunately, these we have not yet achieved, although insemination of an *A. polynesiensis* female by a hybrid male was recorded once, and Tesfa-Yohannes and Rozeboom (1974) obtained backcross progeny from their crosses of hybrid females to parental males. The solution of the genetics of susceptibility in



the *A. scutellaris* complex must therefore await successful backcrossing. Several promising lines are being followed using *A. cooki* and *A. malayensis*.

The same perplexing problem as was experienced with males of a 'refractory' stock of *A. aegypti* has been experienced with *A. malayensis*, and Townson (1975) has reported susceptible male *A. malayensis* following inoculation with exsheathed microfilariae of *B. pahangi*. This problem is currently being investigated.

## GENETIC CONTROL OF FILARIASIS VECTORS

There are several recent reviews of the various alternative genetic control methods which have been proposed for mosquitoes (see, for example, Davidson, 1974; Pal and LaChance, 1974; Pal and Whitten, 1974). In the case of the *C. pipiens* complex, which has been given most attention, five alternatives which may have an application to the *A. scutellaris* complex can be considered. These alternatives involve three components, either alone or in combination: cytoplasmic incompatibility, chromosomal translocations, and genes responsible for resistance to filarial infection.

### CYTOPLASMIC INCOMPATIBILITY

In 1967 successful suppression of a small population of *C.p. fatigans* in a village near Rangoon was achieved by the release of males of a strain incompatible with the native mosquitoes (Laven, 1967b). Less successful trials were made in 1972 in India, recorded briefly by Rao (1974) and Pal (1974), and there is no doubt that by itself cytoplasmic incompatibility is not an economic means of suppressing large vector populations. The chief reason is that only males may be released, and the difficulty and the cost of ensuring 100% efficiency in the sexing of the large numbers of mosquitoes which are required for a release programme make the method impractical. The same problem would arise if cytoplasmic incompatibility were considered by itself for suppression of any member of the *A. scutellaris* complex.

### CHROMOSOMAL TRANSLOCATIONS

Population suppression by the use of chromosomal translocations, which give a degree of egg sterility in the mosquito progeny, has been postulated by several authors, and Laven (1969) has described the production of translocations in *C. pipiens* and the promising results of some of his cage experiments. There are many advantages in using male-linked translocations,

and, with his co-workers, Laven has described the steps leading to a village experiment in Montpellier which was designed to test the effects of introducing such a translocation into a *C. pipiens* population (Laven and Jost 1971; Laven *et al.* 1971; Laven *et al.* 1971a,b). The translocated males were released for two months in 1970 and the frequency of the translocation was monitored during 1971-73 (Cousserans and Guille, 1972, 1974), during which period it declined from 80% in April to less than 1% in September 1973. The results did not fit the earlier predictions of Laven *et al.* (1971a,b) who had expected the frequency to increase. However, Curtis (1975) has since shown that their reasoning had been faulty and that the observed decline agreed with the theoretical expectation.

• There was no evidence that the *C. pipiens* population in Montpellier was suppressed. The reduced egg-raft collections in 1971, as compared with 1970, can be explained by a change in the accessibility to the mosquitoes of the primary larval habitat. Furthermore, the egg sterility which was achieved may well have been counterbalanced by higher survival of the larvae owing to density dependent regulation.

Comparable studies to those on *C. pipiens* have been made with *A. aegypti*, and Rai and McDonald (1971), for example, have described the production by irradiation of sex-linked and autosomal translocations and the synthesis and genetics of double translocation heterozygous males. In a small field experiment near Delhi, Rai *et al.* (1973) released male-linked translocation heterozygotes into an *A. aegypti* population and gave evidence that the translocation was successfully introduced into and maintained by the field population. Their experiment was not designed to suppress the population. Some of the advantages of using double translocation heterozygotes for population suppression have been given by Uppal *et al.* (1974) but no field trials have yet been reported with this system.

No attempts have yet been made to induce translocations in the *A. scutellaris* complex but the relative ease with which they can be produced by irradiation in *C. pipiens* and *A. aegypti* suggests that the work should present no great difficulty.

#### CYTOPLASMIC INCOMPATIBILITY + TRANSLOCATIONS

Laven and Aslamkhan (1970) proposed the integration within *C. pipiens* of cytoplasmic incompatibility and a translocation. It can readily be shown that such an integrated strain would have theoretical advantages. Laven and Aslamkhan concluded that the release of a strain having bidirectional incompatibility with a target population and having, as a result of the translocation, a reproductive potential of only 15% of the normal would lead to eradication of the target population in 3 or 4 generations when the release

ratio was only 1:1. The released strain would replace the original population and, it was reasoned, the population size would only reach 15% of the original. Such reasoning is undoubtedly too simplistic since it takes no account of density dependent regulation in the larval populations, nor of the natural selection which would favour individuals with lower rates of sterility than 85%. Laboratory observations have shown that individuals of translocated strains may vary significantly in their sterility and that in unselected stocks the sterility rate may decline (Krishnamurthy, 1974).

An integrated strain of *C.p. fatigans* with Delhi genome and Paris cytoplasm which is bidirectionally incompatible with Delhi mosquitoes (but see p. 10) and which carries a male-linked translocation has been constructed by Krishnamurthy and Laven (in press). This strain, designated IS-31B, was used in trials in three villages in the Delhi area (Rao, 1974) and egg-raft sterility of 50-68% was recorded. A detailed account of this experiment has not yet been seen.

In the future it may be desirable to investigate the possibility of constructing such an integrated strain within the *A. scutellaris* complex. Since there is no incompatibility between strains of *A. aegypti* no comparable work on integrated strains is possible.

#### TRANSLOCATIONS + GENES FOR RESISTANCE TO FILARIAL INFECTION

Curtis (1968) has theorized on the merits of linking genes for resistance to infections with translocations. The proposal would then be to replace a normal vector population with translocation homozygotes carrying a selected gene. If the translocation could be introduced at a frequency higher than the equilibrium frequency, which would be a function of the relative fertility of the introduced strain and the wild-type, replacement would proceed to fixation for the translocation and the selected gene. Unfortunately, since genes for resistance to infection with *W. bancrofti* have not yet been identified in the *C. pipiens* complex and since no work is being done at present on translocations within the *A. scutellaris* group, this system is unlikely to be evaluated in the near future.

#### CYTOPLASMIC INCOMPATIBILITY + GENES FOR RESISTANCE TO FILARIAL INFECTION

Bidirectional cytoplasmic incompatibility might serve as a means of replacing a vector population with a population refractory to filarial infection. The principle is the same as for replacement with a cytoplasmic incompatible strain which carries a translocation but whereas the translocation strain

would be partially sterile, the non-translocated strain would be fully fertile. Curtis and Adak (1974) have described cage experiments with non-overlapping generations and different release ratios in which they demonstrated the replacement of one or other of two mating types of the *C. pipiens* complex according to the relative frequencies of the two types at the start of the experiments. Although for convenience they used for replacement an incompatible strain with a translocation, they cancelled the effect of the partial sterility by starting each new generation with the same number of larvae from partially sterile rafts as from normal rafts. In this way the translocation was used as a neutral marker which could be considered equivalent to a gene for resistance to infection.

The main advantage of population replacement by means of cytoplasmic incompatibility is that after the new population is established natural selection will operate against wild-type immigrants, provided their number is not too large (see, for example, Laven 1967b). Furthermore, whereas proposals for population suppression have to take account not only of immigrants but of density dependent regulation of larvae, replacement with a fully fertile strain, whose genome will be very largely the same as that of the strain being replaced, does not present this problem. In a number of respects this system looks promising for the *A. scutellaris* complex: incompatibility between species has been established; there is a good chance that a single gene may be responsible for susceptibility to filariae; and the isolated island populations provide ideal targets for genetic control trials. At the present time it would be premature to draw analogies with *C. pipiens* and *A. aegypti* too far, and confirmation of the nature of incompatibility and of monofactorial inheritance of susceptibility are most urgent, but the results and experience derived from both these difficult species encourage the belief that the problems posed by the *A. scutellaris* complex can be overcome.

## CONCLUSION

The genetics of the *A. scutellaris* complex promise to be even more interesting and rewarding than those of *C. pipiens* or *A. aegypti*, and the prospects for genetic control of some of the island populations are promising. It is nevertheless too early to present an unduly optimistic picture of these prospects. Apart from the great deal that remains to be done on interspecific incompatibility and on the genetics of filarial susceptibility, account must be taken of the ecology of the vector populations; and, owing to the relative inaccessibility of many of the islands where filariasis is endemic, relatively few ecological studies have been made. If the laboratory experiments during the next year or two confirm the possibility of

producing a mosquito with a vector genome minus susceptibility genes within a selected cytoplasm which is incompatible with a vector population, more extensive and intensive field studies will be warranted.

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