

INCREASED FEMALE MORTALITY AS A BARRIER TO HYBRIDIZATION BETWEEN MEMBERS OF THE *Aedes scutellaris* COMPLEX OF MOSQUITOES

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ABSTRACT. Interspecific crosses between the mosquitoes *Aedes polynesiensis* and *Aedes malayensis* have shown a unidirectional pattern of compatibility. *Aedes polynesiensis* females inseminated by *Ae. malayensis* males fail to produce viable offspring while the reciprocal cross is viable. In both crosses, rates of insemination are comparable to control rates. The *Ae. polynesiensis* females fail to lay eggs. One apparent reason for this is that the *Ae. polynesiensis* females have a high rate of mortality after insemination by *Ae. malayensis* males. Such mortality is an effective barrier to hybridization in that cross, and is a new class of isolating mechanism.

INTRODUCTION

The *Aedes scutellaris* complex of mosquitoes is comprised of about 30 species distributed from the Andaman Islands in the west to the Marquesas and Tuamoto Archipelago in the east, and reaching as far north as Okinawa. Several members have been implicated as important vectors of filariasis in the South Pacific. *Aedes polynesiensis* Marks has been shown to be highly susceptible to both *Brugia pahangi* and *Brugia malayi* (Duhmkopf and Trpis 1980). It is distributed throughout the eastern South Pacific, reaching as far west as the Ellice Islands and as far east as the Marquesas, Tuamotos and Pitcairn Island (Macdonald 1976). *Aedes malayensis* Colless is refractory to filarial infection (Macdonald 1976 and Trpis et al. 1981). It is distributed throughout the western South Pacific and Southeast Asia. The limits of its distribution are the Malaysian Peninsula on the east, the Andaman Islands on the west, and Vietnam and Thailand on the north.

The factors involved in filarial susceptibility have been shown to follow a nonMendelian pattern of inheritance (Trpis et al. 1981a). It is possible that a rickettsial symbiont is associated with filarial susceptibility (Duhmkopf and Trpis 1981). During the analysis of the genetic system involved in susceptibility, several crosses were attempted between members of the complex. Most of these crosses showed a unidirectional pattern of compatibility. Of importance to this paper is the series of reciprocal crosses between *Aedes polynesiensis* and *Ae. malayensis*. When *Aedes malayensis* females are inseminated by *Ae. polynesiensis* males, viable hybrids result. When *Aedes polynesiensis* females are inseminated by *Ae. malayensis* males, no viable offspring are produced. Similar patterns have previously been reported in the *Aedes scutellaris* complex (Tesfa-Yohannes and Rozeboom 1974, Macdonald 1976), and in the *Culex pipiens* complex (Laven 1951). In both cases, a rickettsial symbiont has been reported as the cause of the

unidirectional pattern of compatibility (Yen and Barr 1973, Wright and Barr 1980, Wright and Wang 1980, Trpis et al. 1981b).

During attempts at these crosses it was observed that the *Aedes polynesiensis* females inseminated by *Ae. malayensis* males apparently had a higher rate of mortality than colony females. This was true in a variety of attempts involving several different laboratory strains. Even in attempted crosses of very large numbers (>500 females) mortality was so great that few, if any, eggs were laid. Such mortality could prove to be a substantial isolating mechanism, preventing interspecific hybridization in one direction through the post-fertilization death of the female prior to oviposition. This study is an investigation of the extent of that post-fertilization mortality.

MATERIALS AND METHODS

All mosquitoes were from colonies maintained in the Laboratories of Medical Entomology at The Johns Hopkins University School of Hygiene and Public Health. *Aedes malayensis* BANG strain was originally obtained from the SEATO Medical Research Laboratory in Bangkok in 1969. *Aedes polynesiensis* APIA strain was collected by Barry Engber in Apia, Western Samoa in 1977. All mosquitoes were reared in a controlled environment of 26°C and 80% RH on a 16:8 light:dark regimen.

Larvae were reared in 29 x 18 cm rectangular pans at a density of 100 per liter. Larvae were fed on diluted liver powder suspension. Upon pupation, the pupae were removed and sexed. The sexes were separated and held for emergence. Newly emerged adults were temporarily held in cylindrical paper containers, 18 cm high x 18 cm diameter. All crosses were set up with adults which were no more than 12 hours old.

The initial experiment was to compare the rates of insemination in control versus experimental populations. Four groups of 20 cages were set up. They were small, cylindrical paper

cages, 9 cm high x 9 cm in diameter. A vial of water was inserted through a hole in the bottom, and a piece of cotton soaked with 10% sucrose was kept at the top. Five males and five females were placed in each cage. In one set of 20 cages, all males and females were *Ae. polynesiensis*. In the second set of 20 cages, all males and females were *Ae. malayensis*. In the third set of 20 cages, the males were *Ae. polynesiensis* and the females were *Ae. malayensis*, and in the fourth set of cages, the males were *Ae. malayensis* and the females were *Ae. polynesiensis*. Every day, 10 females were randomly removed from various cages and dissected to inspect the spermathecae for the presence of sperm.

A similar procedure was used to investigate the rates of survival in the different populations. Eight groups of 20 small cages were set up as above. In the first two groups, all males and females were *Aedes polynesiensis*. In the second two groups, all males and females were *Ae. malayensis*. In the third two groups the males were *Ae. polynesiensis* and the females were *Ae. malayensis*, and in the final two groups, the males were *Ae. malayensis* and the females were *Ae. polynesiensis*. For each day over a 20 day period, the number of surviving females in each cage was recorded. In this way, there were two replicates for each group—*Ae. polynesiensis* control females (inseminated by *Ae. polynesiensis* males), *Ae. malayensis* control females (inseminated by *Ae. malayensis* males), *Ae. malayensis* experimental females (inseminated by *Ae. polynesiensis* males), and *Ae. polynesiensis* experimental females (inseminated by *Ae. malayensis* males). Analysis of the data was done using Log-rank Survival Analysis (Anderson et al. 1980).

RESULTS

The results of the insemination tests are shown in Table 1. The purpose of the insemination test was to determine whether females in interspecific crosses were being successfully fertilized. The results of the insemination tests show that, by the sixth day, all of the females had sperm in their spermathecae. Thus, the *Aedes malayensis* males were successfully inseminating the *Ae. polynesiensis* females, and *Ae. polynesiensis* males were successfully inseminating *Ae. malayensis* females.

The results of the mortality experiments are shown in Table 2 and Figs. 1 and 2. In Table 2, the daily survival rates are presented for the eight groups. From day 8 through day 20, the number of females in the *Aedes polynesiensis* experimental populations was less than the number in the control populations. The final results were many fewer females in the experimentals than the controls (46 and 36% in the

Table 1. Percentages of insemination in control and experimental females.

Day	<i>Aedes polynesiensis</i>		<i>Aedes malayensis</i>	
	Control	Experimental	Control	Experimental
1	0	0	0	0
2	0	0	0	0
3	10	0	10	10
4	40	20	50	40
5	80	70	90	90
6	100	100	100	100
7	100	100	100	100

experimentals and 78 and 71% in the controls). No such difference is seen in the data for the *Aedes malayensis* females (90 and 88% in the experiments and 92 and 96% in the controls).

In Figs. 1 and 2, the survival curves are shown over the 20 day period. Fig. 1 presents the survival curves for the four populations of *Aedes polynesiensis* females, and Fig. 2 presents the survival curves for the four populations of *Ae. malayensis* females. The curves demonstrate the differences between the populations which were presented in Table 2. It can be seen that the curves for the two *Ae. polynesiensis* experimental lines are different from those of the two control lines, while all four curves for the *Ae. malayensis* females are similar.

The Log-rank Survival Analysis results in a statistic which is distributed as a Chi-square. Differences between the *Aedes malayensis* groups were not significant ($\chi^2 = 3.93$, 3 d.f.). There were significant differences between the *Aedes polynesiensis* groups ($\chi^2 = 47.16$, 3 d.f., $P < 0.001$). Further comparisons of the *Aedes polynesiensis* groups showed no significant difference between the two control groups ($\chi^2 = 0.78$, 1 d.f.) and no significant difference between the two experimental groups ($\chi^2 = 3.17$, 1 d.f.). Thus, the experimental groups had a significantly reduced survival when compared to the control groups.

DISCUSSION

Aedes polynesiensis has been the subject of intense study for the past 30 years because of its susceptibility to filarial parasites. As previously mentioned, crosses between *Ae. polynesiensis* and *Ae. malayensis* have demonstrated an unidirectional pattern of compatibility (Tesfa-Yohannes and Rozeboom 1974, Macdonald 1976, Trpis et al. 1981a). That pattern is believed to be due to the presence of a rickettsial symbiont (Wright and Wang 1980, Wright and Barr 1980, Trpis et al. 1981b). Unidirectional pattern of compatibility in members of the *Aedes scutellaris* complex are well known. Woodhill

Table 2. The percentage of females surviving on each day.

Day	<i>Aedes polynesiensis</i>				<i>Aedes malayensis</i>			
	CI ¹	CII ¹	EI ²	EII ²	CI ³	CII ³	EI ⁴	EII ⁴
1	97	100	99	99	100	100	100	99
2	97	100	99	99	100	100	100	99
3	95	99	98	97	100	100	100	99
4	94	99	98	90	100	100	100	98
5	92	99	97	90	100	100	100	97
6	92	97	96	85	99	99	100	97
7	91	95	93	71	97	99	100	96
8	91	92	87	69	96	99	100	96
9	91	89	84	67	95	99	100	96
10	89	88	77	67	95	99	97	96
11	88	85	74	51	95	99	97	95
12	86	83	71	51	95	99	97	95
13	86	81	69	51	95	99	97	95
14	83	79	66	49	95	99	95	93
15	81	77	54	46	93	97	93	93
16	80	76	51	44	93	96	93	93
17	78	73	51	44	93	96	93	90
18	78	72	46	36	93	96	93	88
19	78	72	46	36	92	96	93	88
20	78	71	46	36	92	96	90	88

¹ *Ae. polynesiensis* females X *Ae. polynesiensis* males.

² *Ae. polynesiensis* females X *Ae. malayensis* males.

³ *Ae. malayensis* females X *Ae. malayensis* males.

⁴ *Ae. malayensis* females X *Ae. polynesiensis* males.

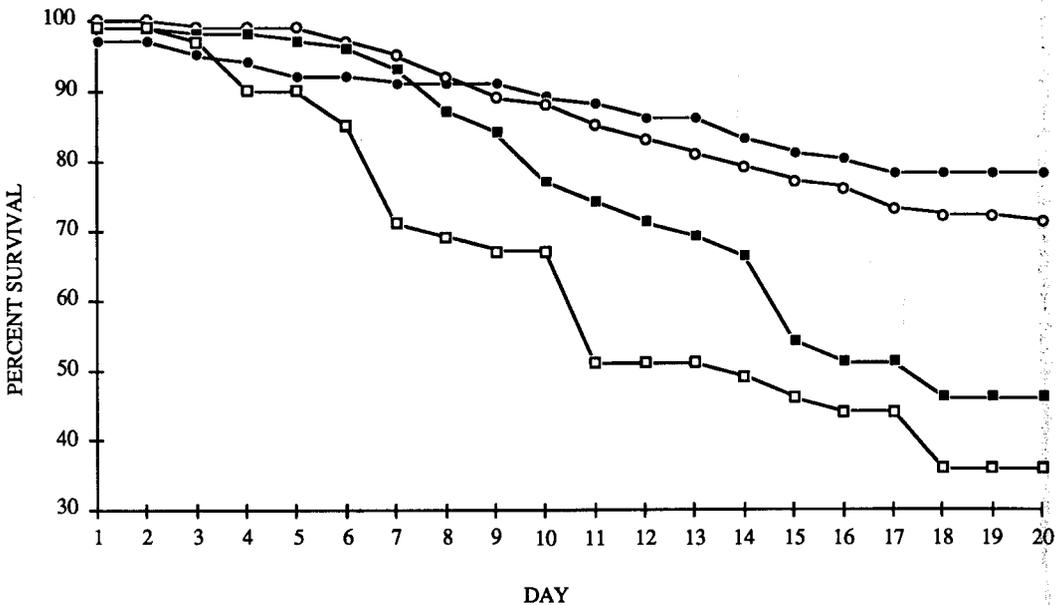


Fig. 1. The percent survival of *Aedes polynesiensis* females—● *Aedes polynesiensis* females X *Ae. polynesiensis* males (CI), ○ *Ae. polynesiensis* females X *Ae. polynesiensis* males (CII), ■ *Ae. polynesiensis* females X *Ae. malayensis* males (EI), □ *Ae. polynesiensis* females X *Ae. malayensis* males (EII).

(1949, 1950, 1954) and Smith-White and Woodhill (1954) documented a series of unidirectional crosses between members of the complex.

In a series of papers directed towards using such findings in control of *Aedes polynesiensis*,

Gubler (1970a, 1970b, 1971) showed that once *Ae. polynesiensis* females are inseminated by *Aedes albopictus* (Skuse) males, they are essentially sterilized, and that presenting them with *Ae. polynesiensis* males after insemination by

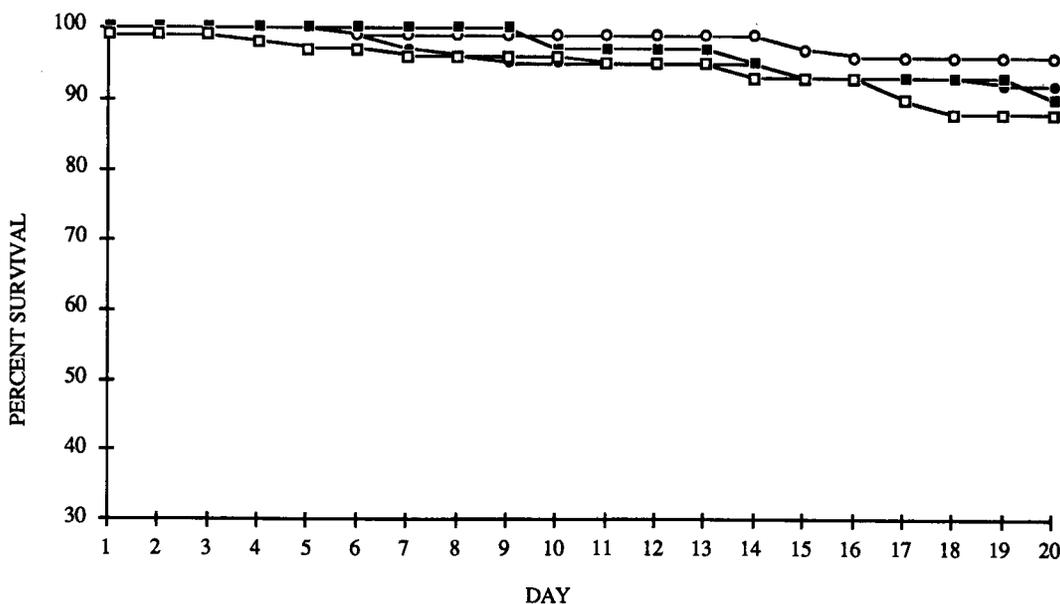


Fig. 2. The percent survival of *Aedes malayensis* females—● *Ae. malayensis* females X *Ae. malayensis* males (CI), ○ *Ae. malayensis* females X *Ae. malayensis* males (CII), ■ *Ae. malayensis* females X *Ae. polynesiensis* males (EI), □ *Ae. malayensis* females X *Ae. polynesiensis* males (EII).

Ae. albopictus males does not result in returned fertility. In addition, he showed that in a series of mixed populations, a population of *Ae. albopictus* will competitively displace a population of *Ae. polynesiensis*. Finally, this was related to oviposition behavior. He showed that the two species would most likely oviposit in the same places, resulting in larval competition, and because of the unidirectional nature of interspecific matings, in locations inhabited by both species, *Ae. albopictus* could competitively displace *Ae. polynesiensis*. However, none of these studies mention any increase in the mortality of the females of one species when inseminated by males of the other.

The results of this study show that both *Aedes polynesiensis* and *Ae. malayensis* females can be successfully inseminated by males of the other species. No attempt has been made here to judge competitiveness of the two species in inseminating females of the opposite species. It is likely that males of the opposite species would be at a competitive disadvantage when compared to males of the same species as the female. However, under the situations described in this study, *Ae. polynesiensis* males can successfully inseminate *Ae. malayensis* females, and *Ae. malayensis* males can successfully inseminate *Ae. polynesiensis* females. These results differ slightly from those of both Tesfa-Yohannes and Rozeboom (1974) and Macdonald (1976). Tesfa-Yohannes and Rozeboom reported that only 60% of the *Ae. polynesiensis* females were inse-

minated by *Ae. malayensis* males, and that only 8.6% of the *Ae. malayensis* females were inseminated by *Ae. polynesiensis* males. However, their dissections were on the second and third days, and it seems likely that more would be inseminated at a later time. Macdonald (1976) reported results similar to those of Tesfa-Yohannes and Rozeboom. However, the more crowded conditions in the present study could have promoted higher levels of insemination.

Of greater importance is the documentation that when *Aedes polynesiensis* females are inseminated by *Ae. malayensis* males, there is an increase in the mortality of the females. It should be noted that this study was done without providing the females with a blood meal. In the original crosses described in Trpis et al. (1981a), the mortality amongst the females was such that very large populations had to be used. In many cases, in excess of 500 females were necessary. Even with large numbers, few survived long enough to oviposit. Bloodfeeding seemed to be a confounding variable with reference to the present study, because, in the previous study (Trpis et al. 1981) it appeared that few of the *Aedes polynesiensis* females inseminated by *Aedes malayensis* males successfully took a blood meal. So, it was felt that bloodfeeding was a variable which was not necessary for showing the reduction in the numbers of females following interspecific insemination.

The results of the experiment carry with them at least two possible implications. The first is

that *Aedes malayensis* males might prove to be another means of possible control of *Ae. polynesiensis*. Since *Ae. malayensis* is not a vector of filariasis, use of males would not significantly add to the vector population. However, it should again be emphasized that no study has been done of the competitiveness of *Ae. malayensis* males compared to *Ae. polynesiensis* males. It seems likely that such a scheme of biological control would have little effect upon the *Ae. polynesiensis* population.

Secondly, this paper presents an apparently new post-copulatory isolating mechanism in mosquitoes. Classical post-copulatory isolating mechanisms involve such things as gametic and zygotic mortality and hybrid inviability or sterility. However, no record is known of the death of the female as a result of interspecific insemination in mosquitoes. Many secondary sources allude to this phenomenon. Dobzhansky (1951) briefly mentions experiments of Standfuss in 1896 with moths in which interspecific copulation caused damage to the female organs. Mayr (1963) mentions experiments by Sturtevant, Stalker and Spieth in which interspecific crosses in *Drosophila* lead to the death of the female. However, none of the papers referenced in the bibliography discuss such findings. Finally, Dobzhansky et al. (1977) refer to the death of the female in crosses between *Drosophila pseudoobscura* females and *D. melanogaster* males as a result of damage done to the female organs. However, again, no primary reference is given.

In some respects the importance of this is merely academic. *Aedes polynesiensis* and *Ae. malayensis* are not sympatric in their distributions, so the chances of interspecific matings in nature are very low. However, because of the great extent of unidirectional patterns of compatibility in crosses within the *Ae. scutellaris* complex, and between members of the *Culex pipiens* complex, if this pattern can be shown to hold in other crosses, it may very well be an important natural barrier to hybridization between closely related species.

The exact nature of the cause of mortality was not under investigation in this study. It would be of interest to investigate pathological changes in the ovaries of *Aedes polynesiensis* females inseminated by *Ae. malayensis* males. It is possible that the well documented rickettsial symbionts have something to do with this phenomenon. It might be possible that this is one of the reasons for the persistence of the symbionts. They may be a means by which the species maintains its integrity by preventing interspecific hybridization, and they may also be a means of speciation in this complex of mosquitoes.

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REFERENCES CITED

- Anderson, S., A. Anquier, W. W. Hauk, D. Oakes, W. Vandaele and H. I. Weisberg. 1980. Statistical methods for comparative studies. John Wiley and Sons, New York.
- Dobzhansky, T. 1951. Genetics and the origin of species. Columbia Univ. Press, New York.
- Dobzhansky, T., F. J. Ayala, G. L. Stebbins and J. W. Valentine. 1977. Evolution. W. H. Freeman and Co., San Francisco.
- Duhrkopf, R. E. and M. Trpis. 1980. The degree of susceptibility and levels of infection in ten different strains of *Aedes polynesiensis* Marks infected with subperiodic *Brugia malayi* and *Brugia pahangi*. Am. J. Trop. Med. Hyg. 29:815-819.
- Duhrkopf, R. E. and M. Trpis. 1981. The effect of tetracycline treatment on filarial susceptibility in members of the *Aedes scutellaris* complex. Mosq. News 41:729-732.
- Gubler, D. J. 1970a. Induced sterility in *Aedes (Stegomyia) polynesiensis* Marks by cross-insemination with *Aedes (Stegomyia) albopictus* Skuse. J. Med. Entomol. 7:65-70.
- Gubler, D. J. 1970b. Competitive displacement of *Aedes (Stegomyia) polynesiensis* Marks by *Aedes (Stegomyia) albopictus* Skuse in laboratory populations. J. Med. Entomol. 7:229-235.
- Gubler, D. J. 1971. Studies on the comparative oviposition behavior of *Aedes (Stegomyia) albopictus* and *Aedes (Stegomyia) polynesiensis* Marks. J. Med. Entomol. 8:675-682.
- Laven, H. 1951. Crossing experiments with *Culex* strains. Evolution 5:370-375.
- Macdonald, W. W. 1976. Mosquito genetics in relation to filarial infections. In: A. Taylor, E. R. Muller and R. Muller (eds.). Genetic aspects of host-parasite relationships. Blackwell Scientific Publ., Oxford.
- Mayr, E. 1963. Animal species and evolution. Belknap Press. Cambridge, Mass.
- Smith-White, S., and A. R. Woodhill. 1954. The nature and significance of nonreciprocal fertility in the *Aedes scutellaris* and other mosquitoes. Proc. Linn. Soc. N. S. W. 79:163-176.
- Tesfa-Yohannes, Tesfa-Michael and L. E. Rozeboom. 1974. Experimental crossings of *Aedes (S.) polynesiensis* Marks and *A. scutellaris malayensis* Colless. J. Med. Entomol. 11:323-331.
- Trpis, M., R. E. Duhrkopf and K. L. Parker. 1981a. Non-Mendelian inheritance of mosquito susceptibility to infection with *Brugia malayi* and *Brugia pahangi*. Science 211:1435-1437.
- Trpis, M., J. B. Perrone, M. Reissig and K. L. Parker. 1981b. Control of cytoplasmic incompatibility in the *Aedes scutellaris* complex. J. Hered. 72:313-317.
- Woodhill, A. R. 1949. A note on experimental crossing of *Aedes (Stegomyia) scutellaris* Walker and *Aedes*

- (*Stegomyia*) *scutellaris katherensis* Woodhill (Diptera: Culicidae). Proc. Linn. Soc. N. S. W. 74:224-226.
- Woodhill, A. R. 1950. Further notes on experimental crossing within the *Aedes scutellaris* group of species. Proc. Linn. Soc. N. S. W. 75:251-253.
- Woodhill, A. R. 1954. Experimental crossing of *Aedes* (*Stegomyia*) *pseudoscutellaris* Theobald and *Aedes* (*Stegomyia*) *polynesiensis* Marks (Diptera: Culicidae). Proc. Linn. Soc. N. S. W. 79:19-20.
- Wright, J. D. and A. R. Barr. 1980. The ultrastructure and symbiotic relationships of *Wolbachia* of mosquitoes of the *Aedes scutellaris* group. J. Ultrastruct. Res. 72:52-64.
- Wright, J. D. and B. Wang. 1980. Observations on *Wolbachiae* in mosquitoes. J. Invertebr. Pathol. 35:200-208.
- Yen, J. H. and A. R. Barr. 1973. The etiological agent of cytoplasmic incompatibility in *Culex pipiens*. J. Invertebr. Pathol. 22:242-250.