

COMPARATIVE LABORATORY OBSERVATIONS ON SELECTIVE MATING OF *Aedes (Stegomyia) albopictus* Skuse AND *A. (S.) polynesiensis* Marks¹

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Induced sterility of female mosquitoes through insemination by incompatible sperm is a possible method of biological control. Incompatible sperm may be produced by irradiation, chemosterilants, and by closely related strains which are capable of cross-breeding but are nevertheless genetically or cytoplasmically incapable of producing viable offspring. Occasionally cross-insemination may even occur between distinct species. Gubler (1970) has demonstrated that males of *A. albopictus* (Poona strain) readily inseminate and so sterilize females of *A. polynesiensis* (Samoan strain). Ali and Rozeboom (1971a, b) observed that there are differences between strains of these species in their cross-mating activity. Furthermore, in a large cage under conditions presumably more nearly similar to those found in nature, cross-mating and cross-insemination took place readily; yet *A. polynesiensis* females continued to show normal fertility rates even in the presence of a great preponderance of *A. albopictus* males.

We do not know the mechanisms whereby the *A. polynesiensis* females are able to mate selectively with their own males under these conditions. One factor which may be associated with such selectivity is the age of the adults following emergence. Gwadz and Craig (1968) observed that *A. aegypti* females did not become inseminated during the first 24 hours of adult life. Only 12 percent of the ROCK strain females contained sperm after 36 hours. No *A. albopictus* females were positive for sperm after 24 hours,

and only 11 percent after 48 hours. On the other hand, Gubler (1970) found that the rate of insemination of *A. polynesiensis* females was as high during the first 24 hours of adult life as on the 5 succeeding days. Furthermore, this insemination was effected by either *A. polynesiensis* or *A. albopictus* males.

The objects of the experiments described below were to determine the extent to which *A. polynesiensis* females are able to discriminate between their own and *A. albopictus* males, and whether there is a difference in the time after emergence at which *A. polynesiensis* and *A. albopictus* females become inseminated.

MATERIALS AND METHODS

The mosquitoes were the Poona strain of *A. albopictus*, and the Samoan strain of *A. polynesiensis*. Both have been maintained in the laboratory for a number of years. In order to obtain virgin adults for most of the experiments, the pupae were sexed according to size and then distributed in groups of 5 to 10 in test tubes stoppered with a cotton plug. All adults from tubes in which both males and females were present were discarded; the others were placed in holding cages until needed for the experiments. In Experiment 3, adults were collected either from test tubes or from jars within 2 hours of emergence.

EXPERIMENTAL RESULTS

EXPERIMENT 1. INSEMINATION POTENTIAL OF *A. albopictus*. The object of this experiment was to ascertain how many females single males of *A. albopictus* and

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A. polynesiensis are able to inseminate. Pint-sized cardboard cylinders, covered at the top with bobbinette, were used as breeding cages. In a given test, there were three breeding cages, containing, respectively, the following combinations of mosquitoes: 1 male *A. albopictus* and 10 female *A. polynesiensis*; 1 male and 10 female *A. polynesiensis*; and 1 male and 10 female *A. albopictus*. The breeding period was 5 days, at the end of which the females were dissected and examined for the presence of sperm in the spermathecae. All the males survived the 5-day-breeding period.

The results of 6 replicate tests are given in Table 1. Each of the six *A. albopictus* males inseminated from 5 to 8 of their own females. Although *A. polynesiensis* males were far less effective under the conditions of this experiment, the results should not be interpreted as evidence that *A. polynesiensis* males are not able to inseminate larger numbers of females under more favorable conditions. In these small cages, as many as four females were inseminated by a single *A. polynesiensis* male, whereas the extremely active *A. albopictus* males were able to inseminate a total of only 2 *A. polynesiensis* females of the 59 examined. The *A. polynesiensis* females responded to a greater extent to their own males.

EXPERIMENT 2. THE EFFECT OF MALE DENSITY ON INSEMINATION RATES. The object of this experiment was to determine the effect of male density on insemination rates. There were six experimental 1-cubic foot cages, each with 50 *A. polynesiensis* females. *A. albopictus* males

were added to each cage respectively, in the following numbers: 10, 25, 50, 100, 200, and 300, so that the male to female ratios were 1:5, 1:2, 1:1, 2:1, 4:1, and 6:1. There were two sets of control cages, each with males and females in the same proportions as above, of either *A. polynesiensis* or *A. albopictus*. The mosquitoes were 1 to 2 days old at the start of the experiment, and the breeding period was 5 days.

The results of two replicate tests are presented in Table 2. The higher insemination rates for *A. polynesiensis* than those obtained in the previous experiment probably are the result of the larger cage size as well as higher mosquito densities. At all densities almost all of the *A. albopictus* females were inseminated by the *A. albopictus* males, whereas these males were unable to inseminate more than 80 percent of the *A. polynesiensis* females. At the 1:5 ratio, 76 percent of the *A. polynesiensis* females had been inseminated by their own males, but only 32.5 percent by the *A. albopictus* males. An increase in male to female ratios from 1:2 to 6:1 did not bring about more than a slight increase in insemination rates of the *A. polynesiensis* females by the *A. albopictus* males. Evidently about 20 percent of the females remained unreceptive in spite of the overwhelming numbers of males. The *A. polynesiensis* females were far more receptive to their own males, but again there was no significant increase in insemination rates as the male to female ratio increased from 1:2 to 6:1. About 10 percent of these females remained uninseminated even at the highest male densities.

TABLE 1.—Insemination potential of individual males of *A. albopictus* and *A. polynesiensis*.¹

Cross ²	Total ♀♀ dissected	Total ♀♀ insemin.	% ♀♀ insemin.	Insem. ♀♀ : Insem. ♀♀ / ♂ Range ³	Average
♀ A x ♂ A	59	40	67.8	5-8	6.7
♀ P x ♂ A	59	2	3.4	0-1	0.3
♀ P x ♂ P	58	14	24.1	0-4	2.3

¹ Combined results of 6 replicate tests, each with 10 ♀♀ and 1 ♂ of indicated species.

² A=*albopictus*; P=*polynesiensis*.

³ Range of numbers of positive ♀♀ per replicate.

TABLE 2.—Effect of male densities on insemination rates in 1-cubic foot breeding cages.

Densities Male: female		Percent insemination of females ¹					
		♀ poly x ♂ albo		poly x poly		albo x albo	
		♀ exam.	% pos.	♀ exam.	% pos.	♀ exam.	% pos.
10	50	80	32.5	87	75.9	99	98.0
25	50	89	63.0	88	88.6	97	99.0
50	50	69	65.3	85	89.4	100	99.0
100	50	60	80.0	73	93.2	98	100
200	50	76	76.4	71	91.5	95	100
300	50	48	72.9	86	90.9	91	98.9

¹ Combined results of 2 replicate tests.

EXPERIMENT 3. SELECTIVE INSEMINATION FREQUENCIES. In this experiment a comparison was made of insemination rates at different male to female ratios in single or mixed populations. A given test was set up with five 1-cubic foot cages with the following combination of adults: cage 1, females of both species plus *A. albopictus* males; cage 2, females of both species plus *A. polynesiensis* males (except in test 3); cage 3, *A. polynesiensis* females and *A. albopictus* males; cage 4, these females with their own males; and cage 5, *A. albopictus* females and males.

In the first two tests, the total number of adults was 100, with female to male ratios of 9:1 and 4:1; in the third test there were either 200 or 100 adults per cage in female to male ratios of 1:1. The breeding period was 5 days. Each test was repeated once.

The results are given in Table 3. Comparison of cages 1 and 3 in tests 1 and 2 shows that the proportions of *A. polynesiensis* females inseminated by *A. albopictus* males were about the same regardless of whether *A. albopictus* females were present. Comparison of cages 2 and 4 shows that the presence of *A. albopictus*

TABLE 3.—Mating preferences of *A. polynesiensis* and *A. albopictus*.

Test	Cage No.	Combinations ¹				Insemination Rates ²			
		poly		albo		poly ♀♀		albo ♀♀	
		♀♀	♂♂	♀♀	♂♂	No. diss.	% pos.	No. diss.	% +
1	1	45	..	45	10	64	23.4	81	84.0
	2	45	10	45	..	40	55.0	79	0
	3	90	10	93	34.5
	4	90	10	162	39.5
	5	90	10	169	47.9
2	1	40	..	40	20	67	43.3	79	100
	2	40	20	40	..	68	75.0	79	0
	3	80	20	124	35.5
	4	80	20	147	65.3
	5	80	20	80	92.5
3	1	50	..	50	100	84	70.2	97	99.0
	2	50	50	50	50	89	85.4	98	100
	3	50	50	78	75.6
	4	50	50	94	85.1
	5	50	50	98	100

¹ Numbers of mosquitoes in each replicate.

² Combined results of 2 replicate tests.

females had no effect on the insemination of *A. polynesiensis* females by their own males.

In cage 2 of test 3, in which there were equal numbers of both sexes of both species, there was no increase in the insemination rate of *A. polynesiensis* females over that of cage 4, which contained only this species. Although the 50 *A. albopictus* males were capable of inseminating three-fourths of these females (cage 3), the combined presence of both species of males in cage 4 did not increase the proportion of sperm-positive females. Some *A. polynesiensis* females remained non-receptive to their own males and to a somewhat greater extent to *A. albopictus* males.

EXPERIMENT 4. AGE OF ADULTS AT TIME OF INSEMINATION. The object of this experiment was to determine whether there is a difference between males and females of *A. polynesiensis* and *A. albopictus* in the length of time between emergence and the first inseminations. In the first two tests, adults were removed from the tubes within 6 hours of emergence. They were then distributed in five separate 1-cubic foot cages in the following combinations: 25 *A. polynesiensis* females and 25 *A. albopictus* males, 25 female and 25 male *A. polynesiensis*, and 25 female and 25 male *A. albopictus*. Thus there were three groups of five cages; the females from the first cage were removed and examined for sperm after 6 hours, and those from each of the remaining cages sequentially in 6-hour intervals. Because of the 6-hour intervals in the emergence

periods, the adults in the first cage were 6 to 12 hours old, those in the second cage, 12 to 18 hours old, and so on as indicated in Table 4. A second test was performed with only the unmixed mating groups of *A. polynesiensis* and *A. albopictus*.

The results are presented in Table 4. There does indeed appear to be a difference in the rapidity with which the first inseminations occur. There was a delay of 30 or more hours in the case of *A. albopictus*, whereas the first positive *A. polynesiensis* females were at most 18 hours old. Furthermore, 60 percent or more of the *A. polynesiensis* females were inseminated by their own males before they were 30 hours old, while none of the *A. albopictus* females in this age group was positive. Even after 30 to 36 hours, only 4 of a total of 50 *A. albopictus* females were inseminated.

The receptivity of the female again shows up as an important factor. Thus *A. polynesiensis* females were inseminated some 12 hours earlier by *A. albopictus* males than were the *A. albopictus* females.

One question concerning experimental procedure is whether the short 6-hour breeding period for the youngest group of mosquitoes could have been the reason for the lack of insemination, rather than the age of the mosquitoes. Therefore, another group of mosquitoes were allowed to age for 3 to 4 days after emergence, and then placed in three breeding cages in the same combinations of 25 males and 25 females as described above. They remained in the cages for 6 hours; then

TABLE 4.—Effects of age on insemination of *A. polynesiensis* and *A. albopictus*.

Test No.	Cross ¹	No. ♀♀ exam. per age group	No. ♀♀ inseminated at indicated no. of hours after emergence				
			6-12	12-18	18-24	24-30	30-36
1	♀ P x ♂ A	25	0	0	3	9	15
	♀ P x ♂ P	25	0	5	2	18	22
	♀ A x ♂ A	25	0	0	0	0	4
2	♀ P x ♂ P	25	0	4	3	15	17
	♀ A x ♂ A	25	0	0	0	0	0

A=*albopictus*; P=*polynesiensis*.

the females were removed and examined for sperm. The insemination rates of the females in these crosses were as follows: *A. polynesiensis* alone, 24 percent; female *A. polynesiensis* x male *A. albopictus*, 28 percent; *A. albopictus* alone, 88 percent. Thus the 6-hour breeding period was long enough for all but a few *A. albopictus* females to be inseminated, which is a marked contrast to the results obtained with the females which had spent the first 36 hours of adult life with their own males.

The third test consisted of a series of observations in which *A. polynesiensis* and *A. albopictus* adults were removed from the emergence tubes or jars at 2-hour intervals. They were obtained from larvae which were reared simultaneously in pans placed on a rack in a corner of an air-conditioned room. Thus environmental conditions were identical for both species. The females were placed in pint- or gallon-sized gauze-covered cartons. Males which had emerged 1 to 3 days earlier were added to the cartons, at a ratio of 2 males per female. Starting at 18 to 20 hours, the females were removed from the breeding cages and examined for the presence of sperm in the spermathecae.

The results, presented in Table 5, confirm those of the first test. Most of the *A. polynesiensis* females became inseminated during the first day of adult life,

whereas there was a delay of about 2 days before a significant proportion of the *A. albopictus* females became receptive. Also, as shown in the first test, as well as by Gubler, the day-old *A. polynesiensis* females can be inseminated by *A. albopictus* males. Thus it appears that the female determines whether there is to be a successful transfer of sperm. One should note also that the young adult *A. polynesiensis* males were able to inseminate the females.

DISCUSSION

Gubler (1970) has demonstrated that in caged competing populations, when *A. albopictus* males outnumber *A. polynesiensis* males by a ratio of 3 to 1 or more, there is a marked reduction in the fertility of the *A. polynesiensis* females as a result of cross-insemination. In our experiments also, *A. albopictus* males readily inseminated *A. polynesiensis* females in the presence of their own females. However, under the conditions of our experiments, *A. polynesiensis* females were less receptive to *A. albopictus* males than they were to their own males. Thus, although *A. polynesiensis* females may be overwhelmed by a preponderance of the highly aggressive *A. albopictus* males, our observations suggest that to some extent at least, they are capable of mating selectively with their own males.

TABLE 5. Age at insemination of *A. polynesiensis* and *A. albopictus* females.

Age of female (hours)	♀ <i>poly</i> x ♂ <i>poly</i>		♀ <i>albo</i> x ♂ <i>albo</i>		♀ <i>poly</i> x ♂ <i>albo</i>	
	No. diss.	% pos.	No. diss.	% pos.	No. diss.	% pos.
18-20	15	86.7
20-22	19	79.0
22-24	26	84.6
24-26	31	71.0	43	0	12	41.7
28-30	32	3.1
40-42	15	0
44-46	16	12.5
46-48	44	38.6
48-50	26	34.6
64-74	14	100	71	84.5

Our results are similar to those obtained by Gwadz and Craig (1968) in that there was a delay of about 48 hours after emergence before *A. albopictus* females became inseminated. These authors suggest that the delay in insemination is a mechanism whereby the sexes from different areas may become mixed, and so reduce the frequency of inbreeding of small local populations. We also confirmed Gubler's finding that a high percentage of *A. polynesiensis* females become inseminated within 24 hours after emergence. This still is enough time for some dissemination and mixing to occur, but the difference in time suggests the existence of age-dependent selection mechanisms. Thus, the partial selective ability noted above may be more effective in the young adults. Young *A. polynesiensis* adults may find each other before the females begin their host-seeking flight, during which they would encounter alien males.

Gwadz and Craig also point out that early insemination at the breeding site could adversely affect the ability of introduced males to inseminate females of the target species in a control program. Thus a knowledge of the factors involved in selective mating and insemination becomes of considerable importance.

SUMMARY AND CONCLUSIONS

Single *Aedes albopictus* males were shown to be capable of inseminating as many as eight *A. albopictus* females in pint-sized containers. In 1-cubic foot cages they also readily inseminated females of *A. polynesiensis*, whether their

own females were present or not. However, even in the presence of large numbers of *A. albopictus* males, a considerable proportion of the *A. polynesiensis* females were not receptive. These proportions of uninseminated females ranged from 20 to 27 percent when male to female ratios ranged from 2:1 to 6:1. *A. polynesiensis* females were more receptive to their own males; thus it appears that selective mating can occur even when large numbers of the aggressive *A. albopictus* males also are present.

Few adult *A. albopictus* females became inseminated until they were about 48 hours old, whereas 60 percent or more *A. polynesiensis* females became inseminated within 24 hours after emergence. This difference in the age at which insemination takes place suggests the existence of selection factors, which may in part be age dependent, in that the young adult *A. polynesiensis* females may be inseminated by their own males before extensive flight activity brings them into contact with alien males.

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