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CROSS-MATING BETWEEN *AEDES (S.) POLYNESIENSIS* MARKS AND *AEDES (S.) ALBOPICTUS* SKUSE IN A LARGE CAGE[1]

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INTRODUCTION

In small cage competition experiments, Gubler (1970a, b) observed a rapid displacement of *A. polynesiensis* by *A. albopictus,* in which cross insemination sterility of the *A. polynesiensis* females was thought to be an important factor. In small cages, the females are unable to avoid the extremely aggressive *A. albopictus* males. If cross insemination is to be effective in practical control of *A. polynesiensis,* it must occur where the females are not confined in a small area. The object of this investigation was to determine whether cross mating occurs readily in a large cage, which would more nearly simulate a natural environment, and in which there is ample space for maneuverability. Also, we attempted to find evidence that free association of the two species in this large cage was accompanied by a lower fertility in the *A. polynesiensis* females.

MATERIALS AND METHODS

The mosquitoes were taken from stock colonies. The *A. albopictus* originated in Poona, India, and had been maintained in our insectary since 1966. The *A. polynesiensis* was obtained from Samoa in 1950. Pupae were separated according to size and were placed in groups of 5 or less in test tubes. Most adults emerging in these tubes were of one sex; groups with both sexes were discarded. The adult males and females were kept temporarily in separate holding cages before they were liberated in the large experimental cage. This cage consisted of a screened enclosure in one of the insectary rooms, and had the following dimensions: length, 9 ft. 10 in., width, 5 ft.; height, 9 ft. 3 in. Temperature was maintained at about 80° F. and the relative humidity at about 80 percent. Bottles equipped with dental-dam wicks served as a source of 10 percent sucrose solution. Blood meals for the female mosquitoes were supplied by anaesthetized guinea pigs and the experimenters. A numbr of potted plants, bobbinet hangings, the undersides of shelves, as well as the walls and ceilings, furnished resting places for the adults.

Eggs to be examined for embryonation were depigmented by submersion in a dilute solution of sodium hypochlorite.

EXPERIMENT 1. EMBRYONATION OF EGGS. The object of this experiment was to determine whether *A. albopictus* sperm will bring about embryonation of *A. polynesiensis* eggs.

One hundred newly emerged, virgin

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female *A. polynesiensis* and 200 newly emerged *A. albopictus* males were kept together for 5 days in a 1-cubic foot cage. At the end of this period, the females were given a meal of human blood, and the fully engorged ones were placed individually in shell vials containing a strip of moist paper toweling for oviposition. After another 5 days the females were dissected and examined for sperm, and the paper strips for eggs.

There were 16 inseminated females which produced a total of 604 eggs. These eggs were kept moist for 4 or 5 days for maturation; then immersed in water for 1 week and observed for hatching and embryonation. None of the eggs contained embryos. Thus *A. albopictus* sperm do not induce embryonation of *A. polynesiensis* eggs.

EXPERIMENT 2. RELATIVE FREQUENCIES OF MATING ASSOCIATIONS. Two- to 3-day old virgin adults of each sex of both species were released in the large cage. Twice each day, in the morning and late afternoon, the experimenter entered the cage. At this time, mating activity was especially noticeable, as the males exhibited the characteristic "following swarm." Mating pairs were caught by means of an aspirator while they were hovering in mid air, or after they had settled on the wall or some other convenient surface. They were transferred to test tubes and later identified as to sex and species. These captures were begun one day after the mosquitoes were released, and continued daily thereafter for a total of 5 days.

There were 2 tests; in the first, there were 400, and in the second, 300 males and females of each species. Most of the mating activity was observed to take place during the first 3 days. As shown in Table 1, 71.8 percent of the mating pairs captured were *A. albopictus*. Of a total of 222 *A. albopictus* females, only 6 were taken in association with *A. polynesiensis* males, and one of these males was competing with another male *A. albopictus*. What is of special interest is that of 78 mating *A. polynesiensis* females captured, 34, or 43.6 percent, were with their own males, while 44, or 56.4 percent, were associated with *A. albopictus* males. In addition, one female was being pursued by a competing pair of males of both species.

It should be noted also that equal numbers of males of the two species had been placed in the cage, of which 262 *A. albopictus,* but only 41 *A. polynesiensis,* were recaptured while engaged in mating activity. Since most recaptures were made within 3 days of release, the cause would not appear to be differential mortality.

EXPERIMENT 3. CROSS-INSEMINATION OF *A. polynesiensis* FEMALES BY *A. albopictus* MALES. The next question is whether

TABLE 1.—Frequency of mating associations between *A. polynesiensis* and *A. albopictus* in a large cage.

Days following release	Numbers of mating pairs or triplets[1]						
	♀ × ♂ A[2] A	♀ × ♂ A P	♀ × ♂ P P	♀ × ♂ P A	♀ × ♂ P AP	♀ × ♂ A AP	Totals ♀♀
1	91	1	5	10	0	0	107
2	102	3	24	28	1	0	158
3	18	1	5	5	0	1	30
4	3	0	0	1	0	0	4
5	2	0	0	0	0	0	2
Totals	216	5	34	44	1	1	301
% Total	71.8	1.7	11.3	14.6	0.3	0.3	100

[1] Combined results of 2 tests.
[2] A = *A. albopictus;* P = *A. polynesiensis.*

mating activities in the large cage culminate in the insemination of *A. polynesiensis* females by *A. albopictus* males. Four hundred virgin *A. polynesiensis* females, and 400 each of virgin *A. albopictus* females and males, were released in the cage. The mosquitoes were 1 day old, and were left in the cage for 5 days. At the end of this period of time, 223 *A. polynesiensis* females and 222 *A. albopictus* females were recaptured. All of a sample of 50 *A. albopictus* dissected were positive for sperm. Of 100 *A. polynesiensis* examined, 32 were positive. Thus, even in the presence of their own females, and at a male to female ratio of 1 to 2, the *A. albopictus* males sought out and inseminated a third of the *A. polynesiensis* females.

EXPERIMENT 4. FERTILE EGG PRODUCTION BY *A. polynesiensis* FEMALES IN COMPETING POPULATIONS. In the large cage, the 1200 or 1600 mosquitoes which were released in the above experiments did not create high population densities. Even so, cross mating and cross insemination took place readily. The object of this experiment was to determine whether, in freely competing populations in the large cage, *A. polynesiensis* females were being sterilized by the *A. albopictus* males.

These observations were made during the course of a competition experiment in the large cage over a 5-month period, when the total adult population was calculated to range from about 3,000 to 6,000 individuals, and the proportions of *A. polynesiensis* declined from about 40 to 5 percent (Rozeboom, 1971). Samples of *A. polynesiensis* and *A. albopictus* females which had just completed their blood meals and were resting on the walls of the cage, were captured, identified, and placed individually in shell vials. At each sampling, for each *A. polynesiensis* female, at least one *A. albopictus* female was also retained. A strip of moist paper toweling furnished a surface for oviposition. After the females had oviposited, or after 5 days in the case of those not ovipositing, they were examined for the presence of sperm in the spermathecae. The eggs were observed for hatching, and a female was considered to be normally fertile if 40 percent or more of the eggs she produced hatched. Egg batches with lower hatching rates were dried, reimmersed, and observed again for hatching. Those failing to hatch were examined for embryonation.

Cross mating and presumably insemination was taking place during the competition experiment. Mating pairs or triplets were caught and identified, and the relative species proportions in these mating groups were as follows: of 219 females, 19 or 8.6 percent were *A. polynesiensis*, and of 222 males, 5 or 2.3 percent were this species. The mating associations are shown in Table 2. Only 2 of the 19 *A. polynesiensis* females were taken in association with their own males, while 17 or 89.5 percent of these females were engaged in pre- or actual copulatory activity with *A. albopictus* males.

TABLE 2.—Cross mating of *A. polynesiensis* and *A. albopictus* in freely competing populations under simulated natural conditions.

	No. females		
Males	albo	poly	No. males
albo.	197	17	214
poly.		2	2
albo + poly	3		6[1]
Totals	200	19	222

[1] 3 *albopictus* + 3 *polynesiensis* males.

Table 3 presents the results of the observations on fertile egg production by females taken from the competing populations. Insemination rates for both species were high, and 84.5 percent of the inseminated *A. polynesiensis* produced egg batches of which 40 percent or more hatched. Based on embryonation, the fertility rate of these egg batches goes up to 95.7 percent. By both criteria, the fertility rate in *A. polynesiensis* females was not significantly lower than that in *A. albopictus* females.

TABLE 3.—Fecundity of females in freely competing populations of *A. polynesiensis* and *A. albopictus* under simulated natural conditions

	A. polynesiensis	*A. albopictus*
No. engorged ♀ ♀	144	165
No. inseminated	132	156
% inseminated	91.7	94.6
No. insem. ♀ ♀ ovipositing	116	141
No. fertile egg batches[1]	98	127
% fertile egg batches	84.5	90.1
No. egg batches embryonated[2]	111	138
% fertile or embryonated	95.7	97.9

[1] Fertile—40 percent or more of the eggs hatching.

[2] Egg batches with 40 percent or more hatch or with most eggs embryonated.

In 18 of the 116 egg batches produced by *A. polynesiensis* females, few or none of the eggs hatched, but of these, in only 2 was there no embryonation, and in 3 others the embryonation rates were 33, 38, and 7 percent. It is possible that 2 of these females had been inseminated with *A. albopictus* sperm, and the other 3 with a mixture. However, there were also 3 inseminated *A. albopictus* females which produced only non-embryonated eggs. These females may have been inseminated by *A. polynesiensis* males, but neither Gubler (1970a) nor Ali (1970) have observed such cross insemination in small cage experiments.

DISCUSSION

Mating between *A. albopictus* males and *A. polynesiensis* females occurred readily in the large cages, and culminated in the insemination of the females. Cross mating occurred during the course of a long term competition experiment. Thus the ample space for flight did not prevent contact between the *A. polynesiensis* females and the *A. albopictus* males, and indeed, since about 90 percent of these females taken during the competition experiment were mating with *A. albopictus* males, they evidently were as ready to accept the attentions of the *A. albopictus* as they were their own males.

However, in spite of the frequency of cross mating, the freshly engorged females of *A. polynesiensis* taken from the cage, at a time when there was a progressive decline in the numbers of this species and a corresponding increase of *A. albopictus*, showed no apparent loss in fertility. For cross insemination to cause sterility of the *A. polynesiensis* females, it would have to take place before the females had been fertilized by their own males. There appears to have been enough differential sexual attraction in the large cage to enable most of the *A. polynesiensis* females, in the ample that desired blood meals, to be inseminated by their own males.

A possible difference in male activity is indicated by the strikingly lower recovery rate of male *A. polynesiensis* in Experiment 2. Mating pairs could be captured only by entering the cage. The presence of the experimenter stimulated the males to pursue the blood-seeking females. The junior author has observed such "following swarms" to occur almost invariably in coconut groves or bamboo thickets where *A. albopictus* is abundant. Males of other species of *Stegomyia* may be taken along with those of *A. albopictus* in such swarms. In coconut groves of Polynesia, male *A. polynesiensis* may be captured along with the females during adult biting catches. However, the junior author has the impression that these swarms are more diffuse and contain fewer individuals than those of *A. albopictus*. The lower response on the part of *A. polynesiensis* to the human host may have been the reason for the relatively few males that were captured.

In other experiments, Ali (1970) has observed that *A. albopictus* females were not inseminated before they were 30 or 36 hours old, although much copulatory activity on the part of both males and females began 12 hours after emergence.

The first sperm-positive *A. polynesiensis* female was only 18 hours old. Gwadz and Craig (1968) obtained an insemination rate of only 11 percent in *A. albopictus* females within 48 hours after emergence. *A. aegypti* females may attempt copulation during the first day of adult life, but insemination rarely occurs before these females are about 36 hours old (Gwadz and Craig, 1968; Spielman *et al.*, 1969). The suggestion of Gwadz and Craig that "the delay in insemination may have evolved as a mechanism to avoid inbreeding" is a possible clue to the reason for the apparent lack of cross insemination of *A. polynesiensis* by *A. albopictus* males in the freely breeding competing populations in the large cage.

In spite of the continued high fertility rates in the engorged samples of *A. polynesiensis,* cross insemination can not be ruled out as a factor in the displacement of *A. polynesiensis* in the competition experiment. The cross-inseminated females may not have engorged as readily as did the fertile females, and so would not be included in the samples taken from the cage. Harassment by *A. albopictus* males may have diverted the *A. polynesiensis* females from the host, and could even affect their longevity.

These observations emphasize the importance of an understanding of differential mating behavior of males that are released for the purpose of control of mosquito populations in nature.

SUMMARY

Cross mating and cross insemination between *A. polynesiensis* females and *A. albopictus* males were observed to take place readily under simulated natural conditions in a large cage. *A. polynesiensis* females, if inseminated by *A. albopictus,* are unable to produce fertile eggs. However, in the large cage in which *A. polynesiensis* and *A. albopictus* were competing freely, and in which there was ample room for maneuverability, *A. polynesiensis* females continued to lay fertile egg batches. This suggests that factors other than cross insemination sterility were responsible for the competitive displacement of *A. polynesiensis* by *A. albopictus* in the large cage. However, other means by which *A. albopictus* males may have exerted a deleterious effect on *A. polynesiensis* reproduction are by harassment, shortening the life span of these females, and by inhibiting their blood-seeking activity.

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