

REPRODUCTIVE ISOLATION BETWEEN FLORIDA STRAINS OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS*

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ABSTRACT. The dynamics of heterospecific and conspecific mating between Florida strains of *Aedes aegypti* and *Aedes albopictus* was examined. In nonchoice experiments where conspecific males were not available, dissection of the spermathecae showed that heterospecific insemination was an infrequent event for both species combinations (10.6% for *Ae. albopictus* with *Ae. aegypti* males, 3.6% for the reciprocal cross). Few eggs were produced from heterospecific crosses and all were nonviable. Frequency of heterospecific mating was not increased when the hindtarsi of females were removed, eliminating a significant mechanism for fending off unwanted courtship. When held with males of both species, females mated with conspecifics and oviposited without regard to the presence of heterospecifics. In low density experiments, a single female of either species caged with an excess of heterospecific males, the conspecific male always located and inseminated the female. These results indicate that significant prezygotic and postzygotic reproductive isolation exists between *Ae. aegypti* and *Ae. albopictus*.

INTRODUCTION

The exotic mosquito, *Aedes albopictus* (Skuse), has become well established in the USA since its discovery in Houston, TX, in the mid-1980s (Sprenger and Wuithiranyagool 1986). It is believed that the North American infestation originated from the shipment of used tires from northern Asia (Hawley et al. 1987, Kambhampati et al. 1991). In addition to international movement of used tires, intra- and interstate transport of discarded tires has provided a means for the dissemination of *Ae. albopictus* (Reiter and Sprenger 1987); it is now widespread throughout the southern USA. In Florida, the recycling of plastic flower-holding containers has also contributed to the spread of exotic *Aedes* (O'Meara et al. 1992).

The establishment of *Ae. albopictus* in the southeastern USA is coincident with a decline in the density of *Ae. aegypti* (Linn.) in areas where their distributions overlap (Black et al. 1989). It has been suggested that a possible mechanism for this phenomenon is mating interference between the 2 species (Nasci et al. 1989). Working with Louisiana strains, they reported asymmetrical heterospecific mating, with male *Ae. albopictus* inseminating female *Ae. aegypti* at a high rate. However, researchers working with different strains of the 2 species have found no evidence of mating interference. In this paper, we describe the dynamics of conspecific and heterospecific mating between Florida strains of *Ae. aegypti* and *Ae. albopictus*.

MATERIALS AND METHODS

Origin of mosquito strains: Laboratory strains were established from larvae, pupae, and adults collected from the field. *Aedes aegypti* was collected in April 1990 from a tire dump located in Palm Beach County, FL, and *Ae. albopictus* was collected in February 1990 from a tire dump located in Polk County, FL. The 3rd through 5th generations were used in this study.

Rearing methods: All mosquitoes were reared in an insectary maintained at 21°C, 70% RH, and 18:6 LD photoperiod following the methods of Munstermann and Wasmuth (1985). Using size as the discriminating character, pupae were segregated by sex to produce virgin females. Twenty or fewer pupae were placed into emergence cups. After emergence, the cups were examined to ensure that no males were present with the females. If a male was present, the contents of the cup were discarded.

Rate of conspecific insemination: Twenty virgin males and females, 5-7 days postemergence, were placed in 4 1-liter bucket cages. At 24-h intervals, females were dissected to determine insemination status. The spermathecae were placed in a drop of saline on a glass slide, covered with a cover slip, and gentle pressure was applied. The slides were then examined for the presence of sperm using phase-contrast illumination at 100× magnification.

Heterospecific insemination: Three replicate sets of 20 virgin females of one species were caged with an equal number of heterospecific virgin males of the other species. All crosses employed adult mosquitoes 5-7 days old. After one week the spermathecae were removed to determine insemination status.

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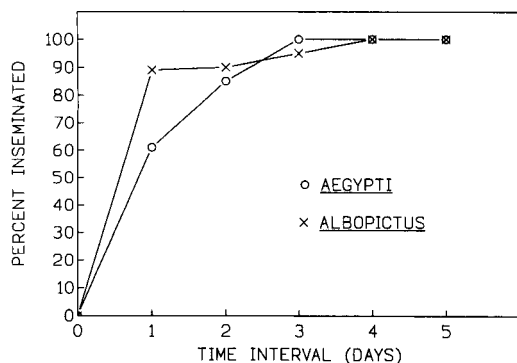


Fig. 1. Rate of conspecific mating for *Aedes aegypti* and *Aedes albopictus*. Each point represents the proportion of 20 females inseminated.

Egg production and viability: In these experiments, 10 virgin males and 10 females were placed in cages and bloodfed. After 14 days eggs were collected, counted, and held for 1 wk to ensure complete embryonation. Eggs were then hatched in a nutrient broth solution (Novak and Shroyer 1978). Control cages consisted of females with conspecific males. In nonchoice mating conditions, females of one species were housed with males of the other species. In choice conditions, females were caged with equal numbers of males of both species.

Mating discrimination: It is known that females push away unwanted soliciting males with their hindlegs. Amputation of the hindtarsi can reduce the ability of a female to exercise mate preference, but removal of foretarsi does not have an effect (McLain et al. 1985). To determine if females were terminating mating attempts, isolation tests were conducted using heterospecific males. Ten virgin females and 10 males were

housed together for 1 wk. In test cages females had their hindtarsi removed; in the controls the foretarsi were amputated. After 7 days the spermathecae were examined for the presence of sperm. To measure the ability of conspecifics to locate each other in low density situations, we adopted the protocol of Miyagi and Toma (1989), which they called "hide and seek." We separately placed one virgin conspecific male and female into a cage containing an excess (15–20) of heterospecific virgin females. As an experimental variation, one cage was set up with 5 pairs of conspecifics. Both the conspecific as well as interspecifics were dissected after 48 h to determine insemination status.

Statistical analysis: All statistical manipulations were performed using the InStat statistical package (GraphPad Software, Inc., 1993²). Prior to analysis by *t*-test or ANOVA, proportions were transformed to the arcsine of the square root of the proportion and counts were transformed to the square root of the count.

RESULTS

Insemination rates: The rates of conspecific insemination for *Ae. aegypti* and *Ae. albopictus* are shown in Fig. 1. *Aedes aegypti* females held with conspecifics reached 100% insemination by day 3, whereas *Ae. albopictus* required an additional 24 h for 100% insemination.

In nonchoice experiments, insemination was low for both heterospecific combinations. The mean insemination rate \pm SD of *Ae. aegypti* females by *Ae. albopictus* males was $10.6 \pm 10.1\%$

² GraphPad Software, 10855 Sorrento Valley Road #203, San Diego, CA 92121.

Table 1. Comparison of egg production and viability between several combinations of *Aedes aegypti* and *Aedes albopictus*.

Cross Female \times male	No. of replicates	Mean no. \pm SD of eggs	Percent hatch
Conspecifics			
<i>aegypti</i> \times <i>aegypti</i>	4 ¹	1,076 (127)	82
<i>albopictus</i> \times <i>albopictus</i>	4	1,119 (145)	78
Nonchoice			
<i>aegypti</i> \times <i>albopictus</i>	3	39 (24)	0
<i>albopictus</i> \times <i>aegypti</i>	3	16 (16)	0
Choice			
<i>aegypti</i> \times <i>albopictus</i> & <i>aegypti</i>	3	1,106 (35)	72
<i>albopictus</i> \times <i>aegypti</i> & <i>albopictus</i>	3	898 (75)	71

¹ Ten males and 10 females per replicate.

Table 2. Number of females inseminated by a conspecific male while in the presence of an excess of heterospecific males.

No. individuals				Percent inseminated	
<i>Aedes aegypti</i>		<i>Aedes albopictus</i>		<i>Ae. aegypti</i>	<i>Ae. albopictus</i>
Female	Male	Female	Male		
19	—	1	1	0	100
19	—	1	1	0	100
20	—	5	5	0	100
1	1	19	—	100	0
1	1	15	—	100	0
1	1	15	—	100	0
5	5	20	—	100	0

and $3.6 \pm 3.1\%$ for the reciprocal cross. The difference between these rates was not statistically significant (t -test, $P > 0.10$).

Egg production and viability: One measure of mating interference is egg production and viability (Leahy and Craig 1967). Table 1 shows the mean number of eggs oviposited and the percent hatch from several mating combinations. In the heterospecific, or nonchoice crosses virtually no eggs were produced and the eggs that were deposited were nonviable. Because the eggs were collected from groups of 10 females, the insemination status of the females that produced eggs could not be determined. When *Ae. aegypti* females were given a choice between their conspecific and *Ae. albopictus* males, a similar number of viable eggs were deposited as compared to conspecific crosses. *Aedes albopictus* females in a choice situation produced a slightly lower number of eggs than did controls, but this difference was not significant (ANOVA, $P > 0.05$).

Mating discrimination: Mate preference studies showed that heterospecific males rarely attempted to copulate. Shortening the hindlegs of virgin females had no effect on the rate of heterospecific mating. Examination of the spermathecae in *Ae. aegypti* females in which the hindtarsi were removed found sperm in only 1 of the 26 dissected, whereas all *Ae. albopictus* females remained uninseminated. As for the control group in which the foretarsi were shortened, only 2 female *Ae. aegypti* were inseminated by heterospecific males. These results suggest that the low rate of heterospecific matings was not due to female avoidance efforts.

In the "hide and seek" test, both *Ae. aegypti* and *Ae. albopictus* show a high degree of mate preference (Table 2). Males of either *Ae. aegypti* or *Ae. albopictus* readily found and inseminated only the conspecific female even in low-density situations.

DISCUSSION

These laboratory studies demonstrate that strong reproductive isolation exists between Florida strains of *Ae. aegypti* and *Ae. albopictus*. Although some insemination occurred in heterospecific crosses, the rate of mating between *Ae. aegypti* females and *Ae. albopictus* males was not significantly different than that seen for the reciprocal cross. These observations are consistent with the results obtained by several investigators using different strains of *Ae. aegypti* and *Ae. albopictus* (Leahy and Craig 1967, Black et al. 1989, Duhrkopf and Hartberg 1992).

Prezygotic isolation is evident from behavioral observations. Courtship, the grasping of a female by a male, is rarely seen between these species, although *Ae. aegypti* males are more sexually aggressive than *Ae. albopictus* males (Black et al. 1989, Duhrkopf and Hartberg 1992). The same pattern of behavior also was exhibited by the Florida strains (J. Harper, unpublished data). Removal of the hindtarsi of females had no effect on insemination rate, further demonstrating that heterospecific males are not attempting to copulate to any significant degree.

The egg production and viability study show that in a choice situation, females of either species mate and oviposit without regard to the presence of heterospecifics (Table 1). Similar results were reported by Black et al. (1989) for other USA strains of *Ae. aegypti* and *Ae. albopictus*. Thus, heterospecific male aggressiveness does not interfere with conspecific mating. When heterospecific mating does occur, nonviable eggs result, indicating postzygotic isolation.

Duhrkopf and Hartberg (1992) also found differences in male mating response between the 2 species, with males unable to find heterospecific females for mating attempts. In our studies, both species are very adept at locating conspecific fe-

males, even in a low density situation. Although a low rate of heterospecific mating occurred in nonchoice conditions, in the presence of a single conspecific female no crossmating occurred. Therefore, heterospecific mating between these species is probably a laboratory aberration.

Nasci et al. (1989), working with Louisiana strains of *Ae. albopictus* and *Ae. aegypti*, reported asymmetrical rates of heterospecific insemination, with *Ae. albopictus* males inseminating *Ae. aegypti* females at a much higher rate than the reciprocal cross. This is in contrast to several published reports (Leahy and Craig 1967, Black et al. 1989, Duhrkopf and Hartberg 1992) and results described herein. It is possible that this reflects a strain difference in mating discrimination, although we were unable to duplicate their results when working with the same Louisiana strains in our laboratory (J. Harper, unpublished data).

The critical question, however, is not whether crossmating occurs, but rather, do heterospecifics interfere with conspecific mating and oviposition? Once mated with a conspecific, a female mosquito is rendered refractory to subsequent insemination through the action of a male accessory gland substance, matrone, but heterologous effects of matrone are less pronounced (Craig 1967, Fuchs et al. 1968). Nasci et al. (1989) suggested that if *Ae. aegypti* inseminated by *Ae. albopictus* are also refractory to further inseminations, the females would, in effect, be made sterile because viable hybrids are not produced. Unfortunately, although they reported high rates of heterospecific insemination for the *Ae. aegypti* female-*Ae. albopictus* male cross, they did not test for mating interference. However, Black et al. (1989) found no decrease in oviposition rate and egg viability when females were placed with increasing densities of heterospecific males and a constant number of conspecific males. We also saw no effect of the presence of heterospecific males on reproductive success for either species.

If mating interference is not the mechanism for the apparent displacement of the resident species by the exotic, other hypotheses must be considered. Numerous laboratory studies of larval interactions have failed to find a clear-cut competitive advantage for either *Ae. albopictus* or *Ae. aegypti* (Black et al. 1989, Ho et al. 1989). In field situations, the species rarely share the same habitat, with urban development favoring *Ae. aegypti* (Pant et al. 1973). It is possible that the species fluctuate independently of each other. For instance, the spread of *Ae. aegypti* throughout southeast Asia during this century while *Ae. albopictus* declined in some areas may have been the result of urbanization rather than competi-

tive displacement (Hawley 1988). Also, the *Ae. aegypti* density in the southern USA has previously undergone unexplained declines. During the 1950s and 1960s, *Ae. aegypti* disappeared from coastal areas around the Gulf of Mexico (Tinker and Hayes 1959, Morlan and Tinker 1965, Hayes and Ritter 1966). It was proposed then that *Ae. triseriatus* (Say) was displacing it (Wills and Hayes 1977), but the *Ae. aegypti* populations eventually rebounded.

Another hypothesis is that mosquito parasites mediate the outcome of competition. Both species carry protozoan gut parasites of the genus *Ascogregarina*. However, the species infecting *Ae. albopictus* causes severe pathology in *Ae. aegypti*, but *Ae. albopictus* is refractory to infection by the *Ae. aegypti* parasite (Paulson et al., unpublished data). Because the parasites are ubiquitous in field populations, this asymmetric pathology may be an important factor in deciding the outcome of competition between the 2 species.

In conclusion, a clear understanding of the nature of the interaction between *Ae. aegypti* and *Ae. albopictus* remains elusive. Laboratory experiments can be useful, but often exclude important aspects of natural field conditions such as temperature fluctuations, limited nutritional resources, and the presence of parasites and predators. Simple field observations of relative densities may suggest a causal relationship that does not exist. Additional research, especially in the field, is necessary to better define this phenomenon.

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