INTERSPECIFIC MATING BETWEEN LOUISIANA STRAINS OF *AEDES ALBOPICTUS* AND *AEDES AEGYPTI* IN THE FIELD AND LABORATORY[1]

ROGER S. NASCI, S. GAY HARE AND F. SCOTT WILLIS

Department of Biological and Environmental Sciences, McNeese State University, Lake Charles, LA 70609

ABSTRACT. Interspecific mating between *Aedes albopictus* males and *Ae. aegypti* females was detected in the field using mark-release-recapture techniques. By 3 days after the release of virgin *Ae. aegypti* females into a field site containing only *Ae. albopictus*, 100% of the captured females were inseminated. Laboratory investigations indicated that male *Ae. albopictus* were very proficient at inseminating *Ae. aegypti* females and that *Ae. aegypti* males rarely inseminated *Ae. albopictus* females, especially if *Ae. aegypti* females were available. Most of the *Ae. aegypti* females inseminated by *Ae. albopictus* males contained only small amounts of dead sperm in their spermathecae, while inseminated females from the converse interspecific mating and from intraspecific matings contained only large amounts of live sperm. The results are discussed in relation to the decline in *Ae. aegypti* densities observed since the introduction of *Ae. albopictus* into the southern USA.

INTRODUCTION

In the four to five year period following its introduction into the Houston, Texas area (Sprenger and Wuithiranyagool 1986), *Aedes albopictus* (Skuse) has become well established in the continental USA. By 1988, large breeding populations were common in Texas, Louisiana and the southern states east of the Mississippi River. Scattered infestations have been discovered in discarded tires in states as far north as Ohio, Indiana and Illinois (Centers for Disease Control 1987, Moore et al. 1988).

The rapid expansion and large population densities achieved by this species have resulted in *Ae. albopictus* becoming a significant potential vector and a serious nuisance in several areas. By midsummer of 1988, 70% of the mosquito nuisance complaints reported to the Calcasieu Parish Mosquito Control Program by urban residents of Lake Charles, LA were caused by *Ae. albopictus* biting, and containers holding *Ae. albopictus* larvae were found at 50% of urban residences sampled (L. G. Terracina, personal communication). This species has become one of the major urban nuisance species in Houston, TX, and New Orleans, LA (D. Sprenger and E. Bordes, personal communication).

The establishment of *Ae. albopictus* throughout the southern USA has been accompanied by a marked reduction in the densities of *Aedes aegypti* (Linn.) populations in areas where their distributions overlap. Simultaneous establishment of *Ae. albopictus* and decrease in *Ae. aegypti* populations has been observed in Houston, TX (D. Sprenger, personal communication), and New Orleans, LA (E. Bordes, personal communication). In Lake Charles, LA, two tire dumps were being sampled for pupal and host-seeking female *Ae. aegypti*. Within 9 months of finding the first *Ae. albopictus* in the area, no *Ae. aegypti* pupae or host-seeking females could be found (R. S. Nasci, unpublished observations).

The close association of the decline in *Ae. aegypti* populations with the arrival and establishment of *Ae. albopictus* suggests that the decline in *Ae. aegypti* was caused by some type of competitive interaction between the species. Larval competition for food and mating interference are the most likely causes for the observed decline in *Ae. aegypti*.

Laboratory experiments investigating larval competition between *Ae. aegypti* and *Ae. albopictus* indicate that *Ae. aegypti* is superior to *Ae. albopictus* (Macdonald 1956, Moore and Fisher 1969, Sucharit et al. 1978). These results suggest that *Ae. albopictus* should not be able to establish in areas inhabited by *Ae. aegypti*. However, laboratory investigations often oversimplify larval interactions, and these predictions are not supported by field observations (see discussion of competitive interactions in Hawley 1988). Experiments examining larval competition between North American strains of *Ae. aegypti* and *Ae. albopictus* indicate that larval competition is not an adequate explanation for the decline of *Ae. aegypti* (Black et al. 1989).

Mating interference (i.e., interspecific mating between species) could lead to the observed decline in *Ae. aegypti* where the species overlap. If an *Ae. albopictus* male mates with an *Ae. aegypti* female (or if the converse occurs), sperm and seminal fluid are transferred to the female. However, the resulting eggs would not be viable. There are a few reports of viable offspring being produced from this cross (Downes and Baker 1949, Toumanoff 1950), but most crosses in this

[1] This project was funded by Louisiana Educational Quality Support Fund grant LEQSF-(86-88)-RD-A-06 and NIH Research Grant AI24088.

direction have produced no offspring (Leahy and Craig 1967). There appear to be numerous barriers to the production of viable hybrids by these crosses (Leahy and Craig 1967). If the *Ae. aegypti* females inseminated by *Ae. albopictus* males are refractory to subsequent matings, as they are after a conspecific insemination (Craig 1966), the females would be rendered sterile for life.

Theoretically, interspecific mating may result in the displacement of a species. The rate and degree of displacement depends upon the relative density, reproductive rates and dispersal rates of the two species, and on the degree of asymmetry in mating aggressiveness between the two species (Ribeiro 1988).

The tendency for *Ae. albopictus* to mate with other species is well established. In the laboratory, *Ae. albopictus* displaced *Ae. polynesiensis* Marks by mating interference (Gubler 1970, Ali and Rozeboom 1971a, 1971b, 1973). However, this failed to occur when laboratory-reared *Ae. albopictus* were released into a natural population of *Ae. polynesiensis* in the field (Rosen et al. 1976).

In a situation similar to that observed with *Ae. aegypti* and *Ae. albopictus* in the southern United States, the distribution and density of *Ae. guamensis* Farner and Bohart on Guam was reduced following the introduction of *Ae. albopictus* to the island. Though the data are incomplete, it has been suggested that mating interference played a role in the reduction of *Ae. guamensis* (Rozeboom and Bridges 1972).

The objective of this study was to determine if interspecific mating occurs between Louisiana strains of *Ae. aegypti* and *Ae. albopictus*. This paper describes interspecific mating in the field and laboratory, and provides a measure of the intra- and interspecific mating ability of the two species.

MATERIALS AND METHODS

Interspecific mating between *Ae. albopictus* males and *Ae. aegypti* females in the field was examined by using mark-release-recapture techniques. The releases were conducted in the vicinity of a pile of discarded automobile tires (approx. 1,500 tires) located between a woodlot and an old field. The study site was located in Lake Charles (Calcasieu Parish), LA. The woodlot and field were surrounded by a golf course and an airport runway. The mosquito population in the tire pile and in the general vicinity surrounding the pile had been sampled for the previous 3 years. The authors used the site to obtain pupae and host-seeking females for studies of body size and biting success. The area had also been sampled for species composition by the Calcasieu Parish Mosquito Control (L. G. Terracina, personal communication). *Aedes aegypti* was the primary mosquito species inhabiting the tire pile until *Ae. albopictus* pupae and biting adults were found in the area in September of 1987. The detection of *Ae. albopictus* in the tires was followed by a rapid decline in *Ae. aegypti* and an increase in *Ae. albopictus* densities. By spring of 1988 *Ae. albopictus* was well established. No *Ae. aegypti* eggs, larvae, pupae or adults were collected in the study area for 4 months prior to the releases or during the releases which were conducted in July and August of 1988. Another tire pile was located 300 m from the release sites. This tire pile was similar to the study area both in size and in that the arrival of *Ae. albopictus* in the site was accompanied by the eventual removal of *Ae. aegypti*. No other *Ae. aegypti* or *Ae. albopictus* habitats were found in the area.

Aedes aegypti used in the field releases and laboratory experiments were from a 4-year-old laboratory colony started from a population collected in Lake Charles, LA. *Aedes albopictus* used in the laboratory experiments were from a 3-year-old laboratory colony also started from a population collected in Lake Charles, LA. Both colonies are annually supplemented with field-collected males and females.

Mosquitoes for the release experiments were reared in the laboratory at 27°C and were given excess larval food (approximately 5.0 mg ground liver powder per larva). Using size as a criterion, pupae were separated by sex and allowed to emerge into separate containers to insure that the females used in the release experiments were virgins. The emergence cages contained fewer than 50 individuals, and were examined every 12 h during the emergence process. Males inadvertently placed in the emergence cages with the females were removed within 12 h postemergence, prior to the time that they are capable of mating.

Virgin female *Ae. aegypti* were marked with either Rocket Red or Saturn Yellow fluorescent dusts (Day Glo Corp., Cleveland, OH) prior to release. This was to identify mosquitoes that were part of the release experiment and to determine if there were any native (unmarked) *Ae. aegypti* females in the area. The mosquitoes were placed in a 0.3 m^2 screened cage with a cloth stockenette sleeve that provided access to the interior. The cage was placed in a large plastic bag. A medical insufflator (powder-type) was used to fill the atmosphere inside the cage with dust. All samples removed from the cage immediately after dusting were visibly marked with the dust.

Each day after the release, mosquitoes were collected coming to human bait. An 8-cm diam

battery-powered aspirator was used to collect all of the mosquitoes coming to the collector during a 1 h collecting period (0800–0900 h). The field-collected mosquitoes were examined for fluorescent marks using an ultraviolet light under a dissecting microscope. Marked mosquitoes were removed, identified to species and dissected as described below. Unmarked specimens were identified to species. No *Ae. aegypti* males or unmarked *Ae. aegypti* females were collected in any of the releases. In addition, no *Ae. aegypti* eggs (hatched and identified as larvae) were collected in 3 ovitraps placed in the area during period of the study.

Insemination of females was detected by dissecting the spermathecae from each female. The spermathecae were placed in a drop of saline on a glass slide, covered with a glass cover slip and examined for the presence of sperm using a compound microscope with Nomarski Interference illumination at 400× magnification. Sperm are also visible with brightfield or phase contrast illumination.

Four releases were conducted. In Release 1, approximately 750 virgin females were held in the laboratory with access to 5% sucrose on a cotton pad for 24 h after adult emergence. They were then marked with the fluorescent dust and released into the field. Host-seeking females were collected 24 h after the release. The effect of the marking on interspecific mating was examined by placing 20 marked, virgin *Ae. aegypti* females in a 0.3 m^2 screened cage with 20 *Ae. albopictus* males for 4 days with access to 5% sucrose. At the end of this period, the *Ae. aegypti* females were dissected to determine insemination.

In Release 2, approximately 1,800 virgin females were held in the lab with access to 5% sucrose for 3 days following adult emergence. They were then marked and released. Host-seeking females were collected 48 h after the release. It was hoped that the extended period of sucrose feeding would prevent the females from dispersing from the area and would increase recapture rates, and that insemination rates would be high after 2 days exposure to the males. The effect of marking on mating was also examined in conjunction with Release 2. Two 0.3 m^2 cages, each containing 20 virgin *Ae. aegypti* females and 40 *Ae. albopictus* males, were set up. The mosquitoes were held in the cages with access to 5% sucrose for 7 days prior to dissection.

In Release 3, approximately 2,000 virgin female *Ae. aegypti* were marked and released within 12 h of adult emergence. Host-seeking females were collected daily for 6 days following the release. In Release 4, approximately 1,000 virgin female *Ae. aegypti* were marked and released the day of adult emergence. Host-seeking females were collected daily for 3 days after the release.

Interspecific and intraspecific mating proficiencies of the 2 species of males were investigated in the laboratory using 2 experimental protocols. The first protocol paired the males with females of either one species or the other. The second protocol provided the males with a choice of mate species. *Aedes albopictus* and *Ae. aegypti* for these experiments were reared and separated by sex as described above. They were held in the laboratory for 2 days with access to 5% sucrose prior to setting up the experimental cages. All cages were held at ambient temperatures (20–25°C) and approximately 65% RH in the laboratory. A photoperiod of 16L:8D was maintained throughout the experiments. The cages used in all of the experiments were 0.3 m^2 and constructed of metal frames and screening.

In the first protocol, 20 male *Ae. albopictus* were placed in a cage with either 20 virgin female *Ae. albopictus* or 20 virgin female *Ae. aegypti* on the third day after adult emergence. The same was done with *Ae. aegypti* males. The mosquitoes were given 5% sucrose on cotton pads in the cages and were held for 1, 3 or 5 days. Following the exposure period, all of the females in each cage were dissected to determine the percentage of insemination. Each species combination and time duration was replicated 3 times.

In the second protocol, the mosquitoes were reared and held as above. Twenty male *Ae. albopictus* were placed in a cage with 20 virgin *Ae. albopictus* females and 20 virgin *Ae. aegypti* females. The same was done with 20 male *Ae. aegypti.* The sexes were held with access to 5% sucrose for 5 days prior to dissection and determination of insemination. Each treatment was replicated 3 times.

Statistical comparison of percentages was performed by paired comparison or independent sample *t* test. Proportions were transformed prior to statistical analysis (arcsine $\sqrt{p}$) (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

In Release 1, 32 marked *Ae. aegypti* females were collected the day after release and were dissected. None of these females contained live sperm in their spermathecae. No marked females were collected on the second day after the release. In the laboratory experiments related to this release, 10% (2/20) of the marked female *Ae. aegypti* exposed to *Ae. albopictus* males for 4 days contained live sperm in their spermathecae, indicating that interspecific mating occurred between these species and that the marking tech-

nique did not prevent such matings. While doing the dissections for the laboratory experiments, masses of threadlike objects were observed floating inside the spermathecae of several females without live sperm. These objects were usually found in clumps floating inside the spermathecae. They were subsequently identified as dead sperm (H. C. Chapman, personal communication) and were evidence of insemination. Inactivation of sperm in the spermathecae has been commonly observed in crosses between these species (Leahy and Craig 1967).

In Release 2, no females were collected 48 h after the release. It was anticipated that the well-fed females might stay in the area longer or live longer and increase the recapture rate, and that the insemination rate might be higher after 2 days exposure to the males. However, this did not occur. In the laboratory experiments related to this release, 80% of the *Ae. aegypti* females were inseminated by the *Ae. albopictus* males (Table 1). Of these, 65% (19/29) contained only dead sperm. This indicated that numerous intraspecific inseminations may have been overlooked in Release 1.

The results for Releases 3 and 4 are shown in Table 2. Less than 3.2% of the marked *Ae. aegypti* females were inseminated 24 h after the releases, but the percentage increased markedly each day afterward. The insemination rates reached 100% by 4 days postrelease in Release 3 and by 3 days in Release 4. The low rate on day 1 after each release was most likely a result of the short exposure of the females to the males and because many of the newly-emerged females may have been too young to be receptive to males during the first day. These data indicate that the longer the *Ae. aegypti* females were exposed to the *Ae. albopictus* males, the higher the rate of interspecific insemination.

Table 1. Percent of virgin *Aedes aegypti* females inseminated by *Aedes albopictus* males after 4 days.

Replicate	No. dissected	No. (%) inseminated	No. (%) with dead sperm
1	20	16 (80)	13 (81)
2	16	13 (81)	6 (46)
Total	36	29 (80)	19 (65)

Live sperm was rarely found in the inseminated *Ae. aegypti* females. In 89.5% (17/19) of the field-collected inseminated *Ae. aegypti* females, dead sperm was the only evidence of insemination.

Results of the laboratory experiments examining the mating proficiency of *Ae. albopictus* males and *Ae. aegypti* males given either *Ae. albopictus* or *Ae. aegypti* females are shown in Table 3. When paired with conspecific females, males of both species inseminated almost all of the available females, and did so very quickly. There was no significant difference in intraspecific insemination rates between species ($P > 0.05$, t test).

The percentage inseminated increased with time in both of the interspecific crosses (Table 3). However, the albo male × gyp female insemination rates increased more rapidly, and were significantly higher than the gyp male × albo female insemination rates at each exposure duration ($P < 0.05$, t test). Therefore, *Ae. albopictus* males were more proficient at mating with *Ae. aegypti* females than were *Ae. aegypti* males at mating with *Ae. albopictus* females, at least under the conditions of the laboratory experiments. In fact, *Ae. albopictus* males were equally proficient with females of either species. There were no statistically significant differences in the percent of females inseminated by *Ae. albopictus* males within an exposure period, regardless of female species ($P > 0.05$, t test).

Of the female *Ae. aegypti* that were inseminated by *Ae. albopictus* in these experiments, 97% (100/103) contained only dead sperm in their spermathecae. The other 3 had sperma-

Table 2. Numbers of marked *Aedes aegypti* females captured, percent inseminated and percent with dead sperm in Releases 3 and 4.

Days postrelease	No. collected	No. (%) inseminated	No. (%) with dead sperm
Release 3			
1	95	3 (3.2)	1 (66.6)
2	27	3 (11.1)	3 (100)
3	9	6 (66.6)	6 (100)
4	2	2 (100)	2 (100)
5	Rain—no collection		
6	1	1 (100)	1 (100)
Release 4			
1	64	1 (1.6)	1 (100)
2	27	2 (7.4)	2 (100)
3	1	1 (100)	1 (100)

Table 3. Average percent of females inseminated in intraspecific and interspecific crosses between *Aedes albopictus* and *Aedes aegypti* (N = 3 replicates).

Species of		Average % inseminated (±SD) at each exposure duration (in days)		
Male	Female	1	3	5
*Intraspecific crosses**				
albo	albo	86.7 (8.5)	100 (0)	98.4 (2.3)
gyp	gyp	90.0 (4.1)	100 (0)	100 (0)
*Interspecific crosses***				
albo	gyp	41.1 (11.7)	68.2 (11.8)	88.7 (4.7)
gyp	albo	6.8 (4.8)	14.4 (8.9)	31.8 (10.0)

* Percent inseminated in intraspecific crosses not significantly different ($P > 0.05$, t test).
** Percent inseminated between crosses significantly different within exposure durations ($P < 0.05$, t test).

theca full of live sperm. None of the *Ae. albopictus* females inseminated by *Ae. aegypti* males and none of the females in either of the intraspecific inseminations contained dead sperm. They either contained live sperm or were empty. Similar observations of a high prevalence of dead sperm in crosses between *Ae. aegypti* females and *Ae. albopictus* males and low prevalence in the inverse interspecific cross and in the conspecific crosses were reported by Leahy and Craig (1967).

The mating proficiency of *Ae. albopictus* and *Ae. aegypti* males when given a choice of mate species is shown in Table 4. After 5 days, the percentage of *Ae. albopictus* and *Ae. aegypti* females inseminated by *Aedes albopictus* males did not differ significantly. Though *Ae. albopictus* males mated more quickly with conspecific females (Table 3), these results show that in a situation where conspecifics and *Ae. aegypti* females are available, these males mate equally well with both species. Conversely, *Ae. aegypti* males mated very well with *Ae. aegypti* females, but performed poorly with *Ae. albopictus* females.

As in the previous experiments, dead sperm were found in 98% of the *Ae. aegypti* females inseminated by *Ae. albopictus* males. The few *Ae. albopictus* females inseminated by *Ae. aegypti* males contained large amounts of live sperm, as did the intraspecifically inseminated females.

The disparity in interspecific mating proficiency of the males is probably due to a combination of differences in the ability of the males and females to discriminate between species and differences in the receptiveness of the females to interspecific mating advances. Regardless of the causes, interspecific mating between these species is asymmetrical. If the female *Ae. aegypti* inseminated by *Ae. albopictus* males in the field are refractory to further mating, mating interference, along with the high densities reached by *Ae. albopictus* populations, may be an important factor in the dramatic displacement of *Ae. aegypti* that has been observed since the introduction of *Ae. albopictus* into the southern USA.

Table 4. Average percent of females inseminated by *Aedes albopictus* or *Aedes aegypti* males exposed to females of both species simultaneously for 5 days (N = 3 replicates).

	Female average % inseminated (±SD)*	
Male	*Ae. albopictus*	*Ae. aegypti*
Ae. albopictus	100 (0)a	89.6 (3.8)a
Ae. aegypti	5.3 (4.3)b	100 (0)a

* Average percents with different letters are significantly different ($P < 0.05$, t test).

ACKNOWLEDGMENTS

The authors thank Harold C. Chapman and Jerome E. Freier for their assistance and advice.

REFERENCES CITED

Ali, S. R. and L. E. Rozeboom. 1971a. Cross-mating between *Aedes* (*S.*) *polynesiensis* and *Aedes* (*S.*) *albopictus* Skuse in a large cage. Mosq. News 31:80–84.

Ali, S. R. and L. E. Rozeboom. 1971b. Cross-insemination frequencies between strains of *Aedes albopictus* and members of the *Aedes scutellaris* group. J. Med. Entomol. 8:263–265.

Ali, S. R. and L. E. Rozeboom. 1973. Comparative laboratory observations on selective mating of *Aedes* (*Stegomyia*) *albopictus* Skuse and *A.* (*S.*) *polynesiensis* Marks. Mosq. News 33:23–28.

Black, W. C., IV, D. C. Arroyo, B. J. Turco and K. S. Rai. 1989. A laboratory study of competition between United States populations of *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.) (Diptera: Culicidae). J. Med. Entomol. 26:260–272.

Centers for Disease Control. 1987. Update: *Aedes albopictus* infestation—United States. Morb. Mort. Weekly Rept. 36:769–773.

Craig, C. B., Jr. 1966. Sterilization of female mosquitoes with male accessory gland substance. Bull. Entomol. Soc. Am. 12:300.

Downes, W. G. and R. H. Baker. 1949. Experiments

in crossing *Aedes* (*Stegomyia*) *aegypti* Linnaeus and *Aedes* (*Stegomyia*) *albopictus* Skuse. Science 109:200–201.

Gubler, D. J. 1970. Induced sterility in *Aedes* (*Stegomyia*) *polynesiensis* Marks by cross-insemination with *Aedes* (*Stegomyia*) *albopictus* Skuse. J. Med. Entomol. 7:65–70.

Hawley, W. A. 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 4:(Suppl. 1) 1–40.

Leahy, M. G. and G. B. Craig, Jr. 1967. Barriers to hybridization between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). Evolution 21:41–58.

Macdonald, W. W. 1956. *Aedes aegypti* in Malaya. II. Larval and adult biology. Ann. Trop. Med. Parasitol. 50:399–414.

Moore, C. G. and B. R. Fisher. 1969. Competition in mosquitoes. Density and species ratio effects on growth, mortality, fecundity and production of growth retardant. Ann. Entomol. Soc. Am. 62:1325–1331.

Moore, C. G., D. B. Francy, D. A. Eliason and T. P. Monath. 1988. *Aedes albopictus* in the United States: rapid spread of a potential disease vector. J. Am. Mosq. Control Assoc. 4:356–361.

Ribeiro, J. M. C. 1988. Can satyrs control pests and vectors? J. Med. Entomol. 25:431–440.

Rosen, L., L. E. Rozeboom, W. C. Reeves, J. Saugrain and D. J. Gubler. 1976. A field trial of competitive displacement of *Aedes polynesiensis* by *Aedes albopictus* on a Pacific atoll. Am. J. Trop. Med. Hyg. 25:906–913.

Rozeboom, L. E. and J. R. Bridges. 1972. Relative population densities of *Aedes albopictus* and *A. guamensis* on Guam. Bull. W. H. O. 46:477–483.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. 2nd ed. W. H. Freeman and Co., New York.

Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. J. Am. Mosq. Control Assoc. 2:217–219.

Sucharit, S., W. Tumrasvin, S. Vutikes and S. Viraboonchai. 1978. Interactions between larvae of *Aedes aegypti* and *Aedes albopictus* in mixed experimental populations. Southeast Asian J. Trop. Med. Pub. Health 9:93–97.

Toumanoff, C. 1950. L'intercroisement de l'*Aedes* (*Stegomyia*) *aegypti* L. et *Aedes* (*Stegomyia*) *albopictus*. Bull Soc. Pathol. Exot. 43:234–240.