

REDUCING THE OVERWINTERING ABILITY OF *Aedes albopictus* BY MALE RELEASE

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ABSTRACT. Eggs of temperate *Aedes albopictus* populations are cold hardy and can diapause, but tropical populations are not cold hardy and cannot diapause. Heterozygotes possess intermediate diapause and cold hardiness. Males of a tropical strain from Malaysia with a distinctive genetic marker were released into an existing temperate population in East St. Louis, Illinois. Subsequent egg samples from the release site had genetic marker frequency of up to 24%. Reduced cold hardiness and decreased diapause incidence were also observed in the release site population. No such changes occurred at a nearby control site. The rank order of overwintering survival of eggs at the release site was: *Aedes triseriatus* > temperate *Ae. albopictus* > hybrid temperate/tropical *Ae. albopictus* > tropical *Ae. albopictus*. Eggs collected from the release population the next summer showed total absence of the genetic marker; presumably carriers were removed by the winter.

INTRODUCTION

Aedes albopictus (Skuse) inhabits all of south-east Asia and parts of temperate Asia, where it transmits dengue fever virus, *Dirofilaria immitis* (dog heartworm) and other pathogens. Sprenger and Wuithiranyagool (1986) discovered an established population of *Ae. albopictus* in Houston, Texas in 1985. It has since spread throughout the eastern U.S.A., presumably by used tire commerce (Hawley et al. 1987, Craven et al. 1988). In South America, it has been found in the São Paulo area of Brazil (Forrattini 1986). Its spread to the Western Hemisphere is of interest because, in addition to Asian viruses, it can transmit eastern equine encephalomyelitis virus (EEE) (Scott et al. 1990), La Crosse encephalitis virus, and many New World viruses (Shroyer 1986). Mitchell et al. (1992) reported EEE virus was found in field populations of *Ae. albopictus* in Polk County, Florida; the minimum infection rate was 1:600.

Aedes albopictus shows interesting geographic differences in diapause and cold hardiness. Temperate *Ae. albopictus* overwinter as eggs in photoperiodically induced diapause (Wang 1966, Imai and Maeda 1976, Mori et al. 1981). In contrast, tropical populations cannot diapause (Hawley et al. 1987). In addition, temperate *Ae. albopictus* populations are much more cold hardy than tropical populations (Hawley et al. 1987, 1989). The diapause (GBC, unpublished data) and cold hardiness (SMH, unpublished data) characteristics of F₁ progeny of crosses between

tropical and temperate *Ae. albopictus* are intermediate when compared with the parental stock. We predict that heterozygote eggs will not survive a temperate winter as readily as eggs from the temperate population.

The goals of the present investigation were: 1) to observe whether released tropical males will mate with temperate females in the field and produce offspring, 2) to determine if such offspring have a lower percent diapause and reduced cold hardiness relative to the original temperate population, and 3) to assess the extent to which a field population's overwintering ability can be reduced by released males.

MATERIALS AND METHODS

Release site: A site for field release was selected in East St. Louis, Illinois (Fig. 1). *Aedes albopictus* has been abundant in scrap tires throughout the area at least since 1987. The release site had about 30,000 discarded tires. Most of the tires were in full sun. Some were located in peripheral shrub vegetation, where most of the adult *Ae. albopictus* were found. A second similar pile of scrap tires about a kilometer away was chosen as control site. A strain of *Ae. albopictus* from the site was colonized in the laboratory in August 1990, under the designation ESL2. Earlier preliminary experiments had shown that *Ae. albopictus* from East St. Louis area (ESL1) had freeze-resistant eggs and showed egg diapause when adults were reared at photoperiods below 13.5L:11.5D. In this respect they resembled all other U.S.A. populations. As Hawley et al. (1987) pointed out, the U.S.A. infestation probably originated in Japan and was adapted to a temperate climate before it arrived.

Tropical strain for release: The tropical strain used originated in Kotah Kinablu, Sabah, East Malaysia. It was collected by Leonard Munster-

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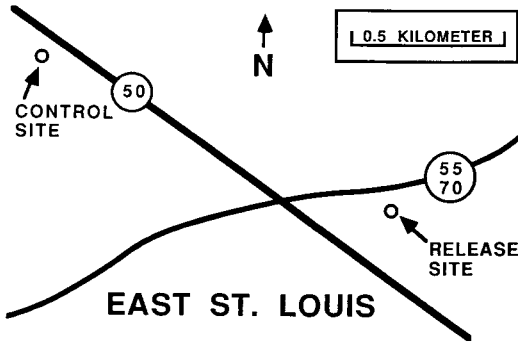


Fig. 1. Location of the field sites in East St. Louis, Illinois. The tropical SABAH SP2 males were released at the site labeled RELEASE SITE. The site labeled CONTROL SITE, where no release took place, was monitored simultaneously as a comparison.

mann in 1986. Eggs were killed by freezing, and the adults did not produce diapausing eggs at any light regime. This strain was selected for homozygosity for a rare fast migrating isozyme allele of GPD (glycerol-3-phosphate dehydrogenase). The selected allele frequency in the original population was below 0.01. The frequency in the selected strain, SABAH-SP2, was 1.0. The marker allele was not present in ESL2. The strain was put through 10 generations of single-pair brother-sister inbreeding, then expanded for 2 generations for field release.

Rearing: Mosquitoes were reared in an insectary at 27°C, 16L:8D and 80% RH. Larvae were reared in white enamel pans (300/pan) in about 2 liters of tap water. They were fed a solution of liver powder *ad libitum*. Pupation occurred on days 5–7 after hatching. The sexes were allowed to emerge into 15 liter plastic cages, 500 males/cage, with damp paper toweling as a moisture source. Sexes were checked again after emergence. Adults were fed on honey-soaked cellulose cotton.

Release: Virgin males were transported by car to the release site in East St. Louis (Fig. 1). They were no more than 3–4 days old. They were released into the shrub vegetation at the periphery. Males were released in 1990 as follows:

August 21—5,781
 September 14—7,420
 October 11—7,763
 Total 20,964

Field overwintering: Eggs of 3 strains (INDY, ESL2 and SABAH SP2) and one hybrid cross (ESL2 × SABAH SP2) of *Ae. albopictus* and *Ae. triseriatus* (Say) TRIS-ND were placed in a discarded tire at the study site on October 25, 1990. Egg samples of each strain were taken at

monthly intervals and transported to the laboratory where they were thawed and hatched. The resulting larvae were examined to determine survivorship.

Safety: We do not believe that release of males of this strain poses any hazard to anyone. The genome of the highly inbred strain for release was very different from the original field population from Malaysia. Knowledge of the genetics of vector competence for arboviruses is rudimentary; unlike malaria and filariasis, nobody has isolated single genes controlling transmission of arboviruses, albeit numerous laboratories have tried to find such genes. It seems probable that multiple polygenic factors are involved and that such complexes were broken up by the program of inbreeding.

It might be suggested that the strain as collected from Malaysia could have been carrying an arbovirus, such as Japanese B encephalitis, that could be maintained by transovarian transmission. Nobody has demonstrated a significant level of transovarian transmission of this virus by *Ae. albopictus*. The SABAH-SP2 was maintained for four years by laboratory feeding on human arms. All members of our laboratory have recently been checked for antibodies to Japanese B encephalitis and all were negative. Moreover, the long history of laboratory inbreeding makes accidental laboratory maintenance of feral viruses extremely improbable. In recent years, we have brought hundreds of newly collected field strains of *Ae. albopictus* into the laboratory, subsequently maintaining them for years on human arms. No viruses have been encountered in our laboratory colonies. The experience of other mosquito research laboratories is similar.

RESULTS

Field overwintering: The eggs of *Ae. triseriatus* TRIS-ND were unaffected by the winter cold conditions at the release site (Fig. 2). However, by January 31, 1991, the *Ae. albopictus* SABAH were all dead. On each sample date following December 6, 1990, the ESL2 × SABAH SP2 heterozygote survival rate was significantly greater than that of SABAH ($P < 0.05$, G test) but less than that of INDY and ESL2 ($P < 0.025$, G test). There was no significant difference between the INDY and ESL2 survivorship on any sample date with the exception ($P < 0.05$, G test) of January 31, 1991.

Diapause incidence: Figure 3A illustrates the diapause incidence of eggs produced by individuals collected as eggs at each site on each sample date. Since these eggs were obtained in diapause-inducing conditions, the eggs that did not hatch

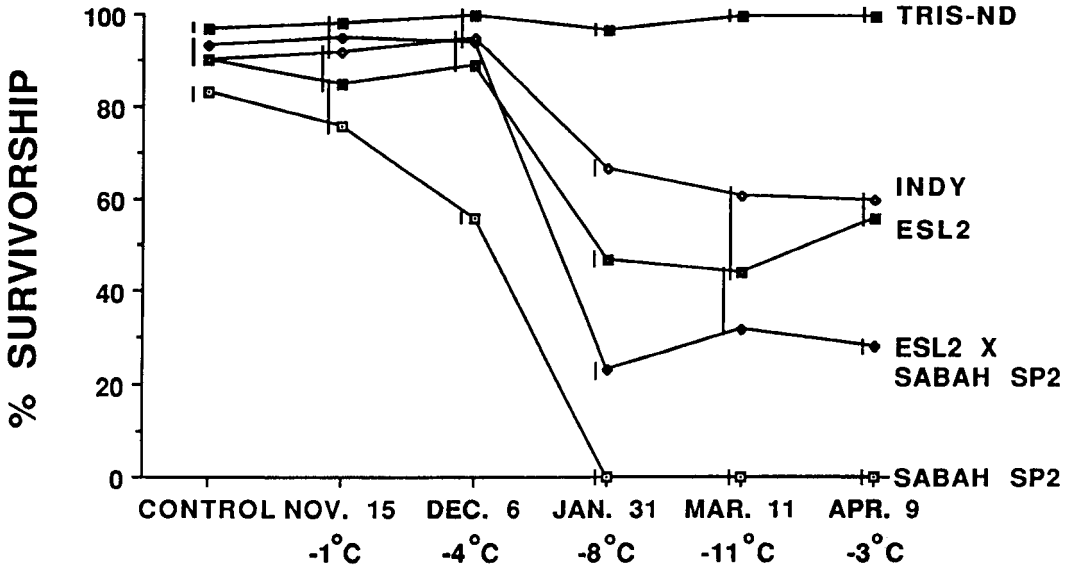


Fig. 2. Survivorship of eggs placed in discarded tire in East St. Louis, Illinois on October 21, 1990. The *Aedes triseriatus* TRIS-ND eggs were collected in ovi-traps at Notre Dame, Indiana in September 1990. The other strains (INDY, ESL2 and SABAH SP2) were *Ae. albopictus* eggs oviposited in the laboratory under diapause-inducing conditions. The minimum temperatures recorded below each sample data. The vertical lines to the right of each set of data points indicate statistical differences, and points associated with the same line are not statistically different ($P > 0.05$, G test). The sample sizes ranged from 33 to 351.

were assumed to be in diapause. Typical tropical and temperate strains were included also (Fig. 3B). The control site eggs hatched at a significantly lower rate than those of from the release site on August 30 ($P < 0.001$, G test), September 11 ($P < 0.025$, G test) and October 11, 1990 ($P < 0.001$, G test). The INDY and ESL2 strains (Fig. 3B), as expected, had a high diapause incidence (low percent hatch). The SABAH (Fig. 3B) had a predictably low diapause incidence (high percent hatch).

Cold hardiness: Figure 4A illustrates the cold hardiness of eggs produced by individuals collected as eggs at the release and control site on each sample date. The control site eggs' survivorship was significantly higher than that of the release site eggs on August 30 ($P < 0.05$, G test), September 11 ($P < 0.05$, G test) and October 11, 1990 ($P < 0.001$, G test). The INDY and ESL2 survived well at -10°C , as expected (Fig. 4B). The SABAH, as all tropical strains, did not survive at this temperature (Fig. 4B).

Genetic composition: Electrophoretic assay of 30 SABAH SP2 individuals revealed that the frequency of the tropical GPD allele was 0.98 in that strain. Similar assay of 30 ESL2 individuals showed that they were fixed for the temperate allele. Figure 5 shows that the tropical allele was detected in individuals collected at the release site on all sampling dates, with the exception of September 26, 1990. The frequency of tropical

genes in the October 11, 1990 release site sample was significantly higher than that of all other samples ($P < 0.05$, G test). The tropical gene frequencies varied from 0 to 0.24 among release site samples.

In July of 1991, eggs of *Ae. albopictus* were collected from the release site. We tested 92 individuals for the presence of the isozyme marker. All were negative. In subsequent collections, no marker allele was ever found at the release site or elsewhere in East St. Louis.

DISCUSSION

Release of males with genetic markers: An allozyme was chosen as a genetic marker because morphological markers are often associated with decreased survivorship and/or reduced mating competitiveness (Lorimer et al. 1976). Neither the allozyme marker nor artificial selection appeared to decrease the competitiveness of the SABAH SP2 males, because our estimate of the competitiveness was not significantly different from 1.0.

Bohart (1956) and Chan (1985) discovered that, during the day, most adult *Ae. albopictus* are found in vegetation. Inspection of our release site confirmed this observation. For this reason, the SABAH SP2 males were released beside scrub vegetation to ensure maximum contact with feral females.

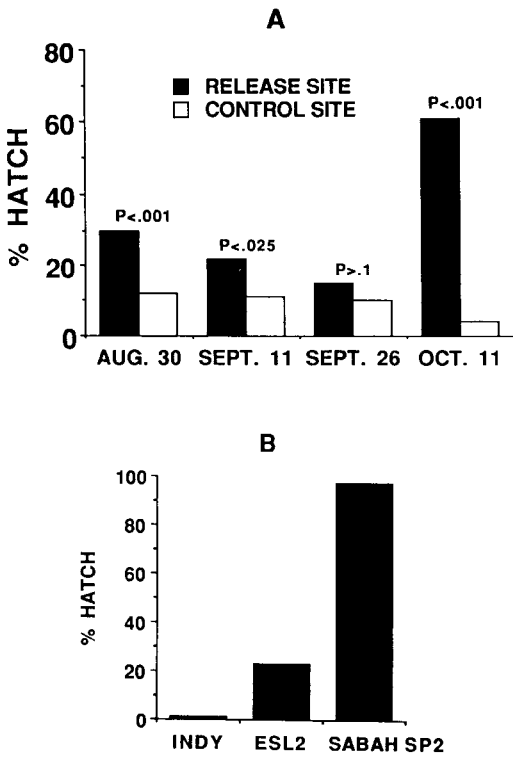


Fig. 3. A. Diapause incidence in F_1 eggs produced under diapause-inducing conditions by individuals collected as eggs on 4 sample dates in East St. Louis, Illinois. The data from both sites on each sample date were compared by G test. The probability that there is no difference between the 2 treatments is recorded above each pair of bars. The sample sizes of each group ranged from 102 to 284. B. Diapause incidence of INDY, ESL2 and SABAH SP2 eggs that were obtained under the same conditions at the same time as those in the figure above. The sample sizes of each group ranged from 105 to 353.

Genetic manipulation of field populations: Many researchers have attempted to genetically manipulate mosquito populations in the field. A number of these studies employed releases of genetically altered male *Ae. aegypti* into field populations. Fay and Craig (1969) released male *Ae. aegypti* with morphological genetic markers into a field population in Meridian, Mississippi. An ovitrap survey revealed that the marker genes were incorporated into the feral gene pool and were detectable for 9 wk after the releases ceased (Bond et al. 1970). Lorimer (1981) introduced morphological markers into a Kenyan population and demonstrated their presence in the field for 49 wk thereafter. McDonald et al. (1977) used translocation heterozygote males to decrease egg variability from a level of 97% to just 36%, though adult density decreased only

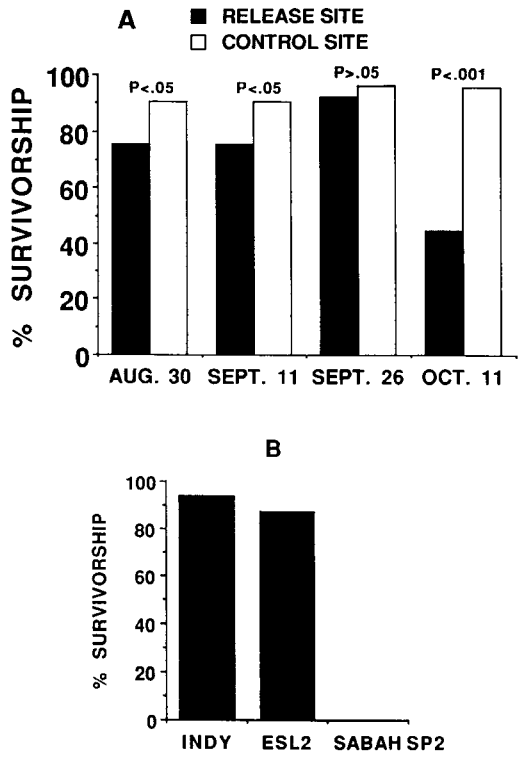


Fig. 4. A. Cold hardness of F_1 eggs produced under diapause-inducing conditions by individuals collected as eggs on 4 sample dates in East St. Louis, Illinois. The data from both sites on each sample date were compared by G tests. The probability that there is no difference between the 2 treatments is recorded above each pair of bars. The sample sizes of each group ranged from 170 to 478. B. Cold hardness of INDY, ESL2 and SABAH SP2 eggs that were obtained under the same conditions at the same time as those in the figure above. The sample sizes of each group ranged from 119 to 564.

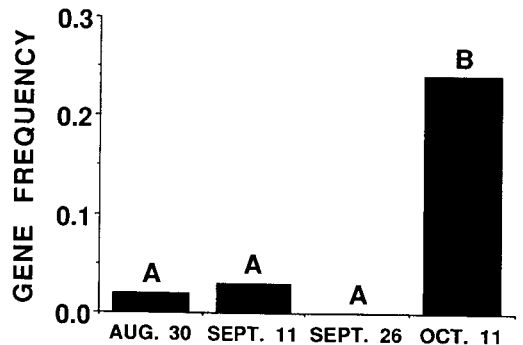


Fig. 5. Incorporation of tropical genes into the field population at East St. Louis, Illinois. Gene frequency of the SABAH SP2 gene marker was calculated for each sample. Bars with different letters are significantly different ($P < 0.05$) according to G test. The sample sizes of each group ranged from 25 to 46.

slightly. Unfortunately, the released males displayed low mating competitiveness, which was attributed to poor larval nutrition. Petersen et al. (1977) decreased egg viability from over 93% to under 40% using translocation heterozygote males. Egg viability remained low for 8 wk after the release ceased. The adult population density decreased significantly as well. Lorimer et al. (1976) attempted population replacement with male translocation homozygotes, but the released strains did not become established in the field, and no population decrease was detected.

Sterile male technique has been used to successfully alter field populations of other species as well. For example, an adult *Anopheles albimanus* Wied. population was reduced by 99% after introduction of chemosterilized males (Lofgren et al. 1974). In addition, Laven (1967) controlled an isolated population of *Culex quinquefasciatus* Say by releasing males that were cytoplasmically incompatible with the feral females. Grover et al. (1976) employed chemosterilized and cytoplasmically incompatible translocated males to cause a 77–94% egg raft sterility rate in *Cx. quinquefasciatus*. *Culex quinquefasciatus* males were chemosterilized by a sound trap treated with hempa (hexamethylphosphoric triamide), causing an egg raft sterility rate increase of 13% (Ikeshoji and Yap 1987).

Other techniques remove males from the population. Ikeshoji et al. (1985) reduced the insemination rate of a field population of *Cx. tarsalis* Coq. by 14–36% by sound trapping males. In addition, the proportion of male *Ae. albopictus* in a Tokyo temple compound was reduced from 0.85 to 0.04 through male sound trapping (Ikeshoji and Ogawa 1988).

In contrast, we sought to reduce the overwintering ability of a temperate *Ae. albopictus* population by introducing tropical genes. The diapause and cold hardiness data (Figs. 3 and 4, respectively) corroborated the gene frequency data (Fig. 5), because the release site sample with higher tropical genes frequencies had lower diapause incidence and were less cold hardy, which are tropical characteristics. In contrast, the diapause incidence and cold hardiness levels of the control site samples did not change. These data indicate that the incorporation of tropical genes into the release site gene pool did indeed reduce the overwintering ability of that population. The absence of tropical genes at the release site during the summer following the releases suggests that all progeny of the tropical males failed to survive the winter.

Unequal marker gene frequency between samples (Fig. 5) indicated that the rate of incorporation of tropical genetic material into temperate gene pool varied. This rate assumedly

depended on factors such as temperature, humidity, virgin female population density, feral male population density, and survivorship of SABAH SP2 males. In addition, tropical and temperate males may react differently to identical sets of biotic and abiotic environmental factors. Unfortunately, the paucity of information about *Ae. albopictus* mating behavior in the field does not allow for anything more than speculation about what took place at the release site during the experiment.

Our data show that the hybrid tropical/temperate heterozygote eggs survived at a lower rate than the temperate eggs in the field (Fig. 2). The heterozygote eggs produced by matings between the released tropical males and feral females would have an even lower overwintering survivorship for these reasons: 1) these eggs would not be prevented from hatching, unlike the eggs of Fig. 2, which were placed in a plastic bag; 2) about 50% of these eggs would not be in diapause (GBC unpublished data); and 3) a significant proportion of these nondiapause eggs would hatch if submerged on a warm day even in late autumn, and the resulting larvae would not survive a temperate winter (Ishii et al. 1954, Mori et al. 1981).

This tropical male release method may be feasible as a component of an integrated control program in northern areas. Its effectiveness would obviously depend upon the rate of incorporation of tropical genes into the existing gene pool and winter mortality rate of the heterozygotes. One possible way to maximize the proportion of tropical genes in a temperate gene pool would be a springtime release of SABAH SP2 males. The adult *Ae. albopictus* population is relatively low in the spring, so this may be the best time to attempt to overwhelm the feral population with the released males.

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