# Evolution of resistance to satyrization through reproductive character displacement in populations of invasive dengue vectors

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Recently, the highly invasive Asian tiger mosquito, *Aedes albopictus,* rapidly displaced resident populations of the yellow fever mosquito, *Aedes aegypti* in the southeastern United States and in Bermuda. Although multiple mechanisms of competitive displacement have been hypothesized, recent evidence of cross-insemination between these species in nature and the sterilizing effects of male accessory gland products asymmetrically favoring *A. albopictus* in interspecific matings support a role for satyrization (a form of reproductive interference) to explain the rapid displacements. Because of the drastic reproductive loss of *A. aegypti* females satyrized by *A. albopictus* males, we predicted selection for prezygotic isolation in populations of *A. aegypti* sympatric with *A. albopictus*. Exposures in cages demonstrated that female *A. aegypti* from populations in Florida sympatric with *A. albopictus* for the past 20 y were significantly less likely than nearby allopatric populations to mate with heterospecific males. Cross-inseminations of *A. albopictus* females by *A. aegypti* males were significantly less common, supporting the one-way direction of displacements observed in nature. Our results indicate rapid sexual selection leading to reproductive character displacement and the potential for satyr-resistant *A. aegypti* to recover from competitive displacements. These results have implications for increased risks of dengue transmission where these vector species meet worldwide.

Competitive displacement is based on the principle that two species cannot simultaneously occupy the same niche, leading to population reduction of one by interspecific competition (1). This phenomenon has been documented in nature, often in the context of biotic invasions or species introductions for biological control (2–4). Both exploitative and interference competition have been implicated in such displacements, which may be mediated by noncompetitive factors (3).

Among mosquitoes, a recent example of competitive displacement between vector species was the rapid reduction in range and abundance of *Aedes aegypti* (Linnaeus) (5, 6) following the invasion and spread of *Aedes albopictus* (Skuse) throughout most of the southeastern United States in the 1980s (7, 8). Despite the potential impacts for public health—*A. aegypti* being considered the primary vector of epidemic dengue (9), and *A. albopictus* recently emerging as the most important transmitter of chikungunya virus as well as frequently vectoring dengue (e.g., ref. 10)—our current understanding of the causative mechanisms involved in this species displacement has proven to be inadequate for explaining the patterns observed in nature. Among the possible mechanisms, the most widely cited is larval resource competition (11, 12). However, it is considered unlikely that larval competition alone could account for the rapid competitive reductions of *A. aegypti* within 1–3 y in the southeastern United States (7, 13, 14) or in Bermuda, where *A. albopictus* more recently displaced *A. aegypti* with comparable rapidity (15). In addition to larval competition, hypotheses to explain these displacements include greater reproductive efficiency in *A. albopictus* (16); apparent competition mediated by the intestinal gregarine protozoan *Ascogregarina taiwanesis* (12); and asymmetric

reproductive interference between *A. aegypti* and *A. albopictus* (4, 13). Greater reproductive efficiency in *A. albopictus*, although possibly beneficial in the long term, does not adequately explain the rapid declines of *A. aegypti*. Furthermore, surveys of larval habitats (17) and manipulative field experiments (11) did not support a substantial role for apparent competition as an explanation for the outcome of these species interactions. In contrast, reproductive interference or "satyrization," whereby males of one species mate with females of a related species, producing no viable offspring (18, 19), has been shown to be a strong possible mechanism for population suppression and under certain circumstances can lead to population extinctions (18). However, inconsistent results from cage experiments (13, 20) and the absence of evidence of cross-matings in nature led to waning confidence in satyrization as a plausible competitive displacement mechanism in this system.

Following recent findings that *A. aegypti* and *A. albopictus* mate bidirectionally in sites of sympatry in Florida (14) and that heterospecific male accessory gland products render *A. aegypti* but not *A. albopictus* females refractory to further insemination by conspecific males (14), we here provide evidence that satyrization has led to reproductive character displacement of *A. aegypti* by *A. albopictus*. In particular, we compare the frequency of interspecific mating between *A. aegypti* and *A. albopictus* from sympatric and allopatric populations, that is, populations where the two species either have been exposed to interspecific mating in the field or have not yet come in contact. Because the mistake of mating with an *A. albopictus* male is extremely costly for *A. aegypti* females [i.e., sterilization and loss of future reproductive potential (14)], evolution should favor females refractory to satyrization. *A. aegypti* populations that have been exposed to this pressure therefore are likely to show a certain amount of resistance, because avoidance mechanisms would be expected to evolve over time (e.g., refs. 21–26). Consequently, we hypothesized that rates of interspecific mating would be lower in *A. aegypti* females sympatric with *A. albopictus* than in geographically isolated populations of *A. aegypti* that would be more susceptible to cross-insemination.

## Results

*A. aegypti* females from either allopatric or sympatric populations were exposed to *A. albopictus* males from allopatric or sympatric populations, and vice versa. Furthermore, intraspecific control crosses were conducted simultaneously for each species. After 3 wk of exposure to interspecific or intraspecific males, females were dissected for evidence of insemination.

Female survivorship did not differ significantly among treatments ($F = 0.70$, df = 15, $P = 0.76$) and averaged $76.11 \pm 11.9\%$ (throughout, data are expressed as SEM).

Among surviving females, intraspecific (control) crosses were 98–100% inseminated. On average, a significantly higher proportion ($0.43 \pm 0.29$) of *A. aegypti* females was inseminated by *A. albopictus* males than in the reverse cross (*A. albopictus* females × *A. aegypti* males) ($0.12 \pm 0.16$) ($F = 32.31$, df = 1, $P < 0.001$).

In crosses of *A. aegypti* females with *A. albopictus* males, the origin of the female had a significant effect [$\chi_{(1)}^2 = 42.29$, $P < 0.001$] on the likelihood of insemination, with females from allopatric populations more likely to be inseminated than females from sympatric populations (Fig. 1 *A* and *C*). This trend was consistent between group 1 and 2 [$\chi_{(1)}^2 = 0.15$, $P = 0.70$] of *A. aegypti* females (Key West, FL/Vero Beach, FL and Miami, FL/Fort Pierce, FL); however females of the second group (Miami/Fort Pierce) mated less readily overall (Fig. 1). The origin of the *A. albopictus* males had no effect on the insemination rates of females in the first group (Key West/Vero Beach) [$\chi_{(1)}^2 = 0.08$, $P = 0.77$] but did significantly affect the frequency of insemination in females of the second group (Miami/Fort Pierce) [$\chi_{(1)}^2 = 24.88$, $P < 0.001$], with sympatric males (Vero Beach) inseminating significantly more females than their allopatric (East St. Louis, IL) counterparts (Fig. 1 *A* and *C*). The significant group effect [$\chi_{(1)}^2 = 30.69$, $P < 0.001$] therefore can be attributed mainly to the origin of the males. The three-way interaction (female origin, male origin, and group) was not significant [$\chi_{(1)}^2 = 0.56$, $P = 0.45$].

In the reverse crosses of *A. albopictus* females with *A. aegypti* males, the origin of the females also had a significant effect [$\chi_{(1)}^2 = 110.47$, $P < 0.001$], with females from allopatric populations (East St. Louis) showing a lower insemination rate than those from sympatric populations (Vero Beach). This effect was consistent across groups [$\chi_{(1)}^2 = 0.80$, $P = 0.37$], although females in the second group (crossed to Miami/Fort Pierce males) showed slightly lower insemination rates (Fig. 1 *B* and *D*).

The origin of the *A. aegypti* male had no influence on the likelihood a female would be inseminated [$\chi_{(1)}^2 = 0.03$, $P = 0.86$]. The significant group effect [$\chi_{(1)}^2 = 7.32$, $P = 0.007$] in this set of crosses therefore is attributable mainly to the response of the females (Fig. 1 *B* and *D*). Again, the three-way interaction (female origin, male origin, and group) was not significant [$\chi_{(1)}^2 = 2.06$, $P = 0.15$].

## Discussion

**Species Origins and Competitive Displacements.** *A. aegypti* originated in Africa and was introduced to the Americas between the 15th and 18th centuries (27), in all probability on ships involved in the slave trade, leading to its establishment across the southeastern United States. *A. albopictus* is of Asian origin and arrived in the New World relatively recently. It was first established in Houston, TX, in the mid 1980s (28) and spread rapidly across the southeastern United States (29, 30) into areas occupied by *A. aegypti*. The range expansion of *A. albopictus* coincided with
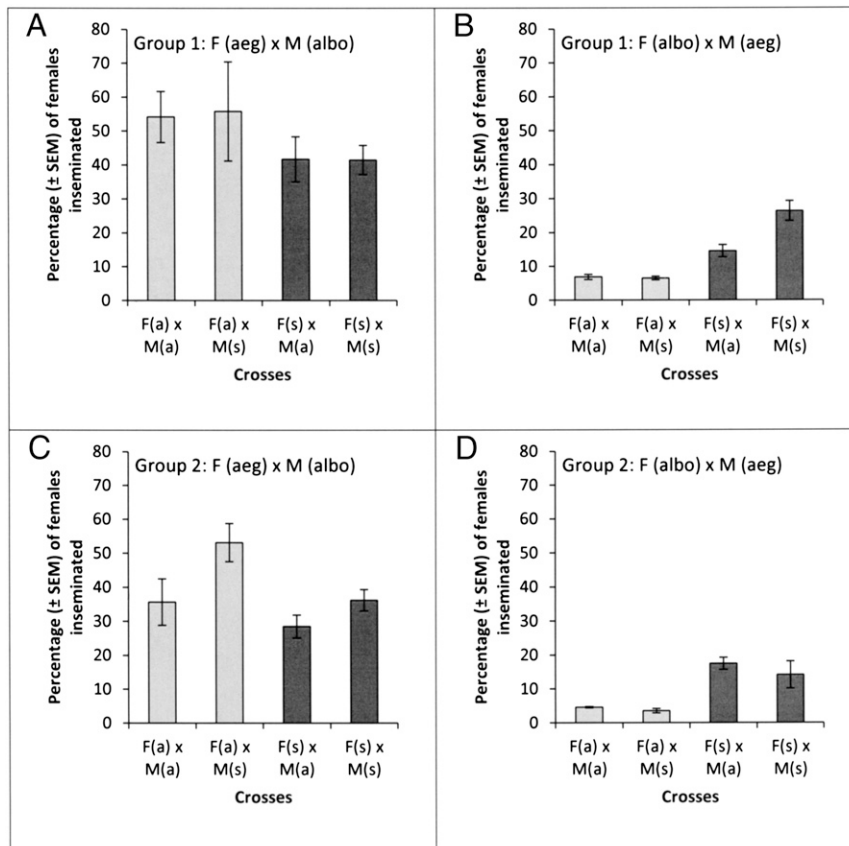


**Fig. 1.** Frequencies of insemination in interspecific exposures of *A. aegypti* (aeg) and *A. albopictus* (albo) mosquitoes from allopatric (a) and sympatric (s) populations. "F" (female) and "M" (male) denote the sex of the mosquitoes in a particular cross. Light gray columns represent exposures in which the female is from an allopatric population; dark gray columns represent crosses in which the female is from a sympatric population. (*A* and *B*) Group 1: *A. aegypti* strains are from Key West (a) and Vero Beach (s), and *A. albopictus* strains are from East St. Louis (a) and Vero Beach (s). (*C* and *D*) Group 2: *A. aegypti* strains are from Miami (a) and Fort Pierce (s), and *A. albopictus* strains are as in Group 1. See Table 1 for details of strain origins.

rapid declines and extinctions of *A. aegypti* populations (5, 6, 31–34). However, the mechanisms examined to date have not been adequate to account for the rapidity of *A. aegypti* extinctions.

We propose that asymmetric reproductive interference or satyrization has played an important role in these competitive displacements and could be key in explaining the rapid population declines of *A. aegypti*. Furthermore, our results provide an example of rapid directional evolution in nature which potentially could help explain the maintenance of distributions of *A. aegypti* and *A. albopictus* in places where they coexist around the world while shedding light on some of the discrepancies between results of earlier work that led to a decline in interest in satyrization as a driving mechanism for species displacement. We discuss our results with regard to their epidemiological implications as well as their significance for the evolutionary biology of *A. aegypti*.

**Mating Behavior and Reproductive Character Displacement.** Both *A. aegypti* and *A. albopictus* belong to the subgenus *Stegomyia* and share similar life histories and mating behavior; aggregating at vertebrate hosts (often humans) during similar diurnal peak activity periods (35, 36), where mating is initiated in flight, most likely following both visual and auditory cues (37, 38). Although the two species cannot produce viable hybrids (39), these similarities in mating strategy may increase the likelihood of errant interspecific mating when the species first come in contact.

Cage populations of laboratory strains of *A. aegypti* typically achieve 100% insemination within 72 h after teneral females and males are placed together (40). Despite the longer exposure times needed to achieve interspecific inseminations in our experiments, results showed a significantly higher interspecific insemination rate in female *A. aegypti* from allopatric (44.37–54.96%) populations than in females from populations with a history of sympatry (32.33–41.54%) with *A. albopictus*. This decrease in the frequency of interspecific mating in sympatric populations suggests that selection to avoid errant mating, which causes effective sterilization, is high enough for the development of prezygotic mating-avoidance mechanisms in *A. aegypti* females. Development of similar mating barriers forming within mosquito species complexes has been documented, e.g., in the *A. albopictus* subgroup of Southeast Asia (26), and was studied extensively in *Drosophila* species by Dobzhansky and his successors, who reported that sympatric populations were more refractory to interspecific mating than allopatric populations of the same species (41, 42).

**Asymmetric Satyrization and Mate Choice.** Therefore, acting in combination with previously documented mechanisms, such as larval competition, satyrization may be key to population reductions of *A. aegypti*, particularly when the two species first come in contact. A simulation model applicable to arthropod pests and vectors predicts reproductive interference to be much more powerful, even at low frequencies of interspecific mating, than Lotka–Volterra competition when the two mechanisms operate jointly to cause competitive displacement and extinction (18, 19).

Furthermore, *A. albopictus* females generally were far less likely to mate interspecifically in cages (mean = 11.74%) than *A. aegypti* females (mean = 43.30%), corroborating earlier reports of unequal bidirectional mating (13) and favoring the observed asymmetry of satyrization in this species pair.

In contrast, the origin of *A. aegypti* males had no influence on the likelihood that a female *A. albopictus* would be inseminated. This asymmetry in the response between the sexes is not surprising, because the pressure for female *A. aegypti* to protect their reproductive potential (against sterilization) will be stronger than the cost to males of an incompatible mating (time, energy, and gamete expenditure). Our results, however, do add to the growing evidence suggesting that the traditional assumption that the mating system of *A. aegypti* is driven predominantly by male scramble competition (i.e., the first male to seize a female

also will mate successfully with that female) may be too simplistic. Instead, the relatively rapid development of mating barriers suggests a more complex system in which female choice is exercised. Recent work on acoustic courtship in this species, which assesses the harmonic convergence of wing beat frequencies in courting mosquitoes (43), also supports this view.

**Rapid Evolution and Species Distributions.** The development of reproductive character displacement, evident from comparisons of allopatric strains of *A. aegypti* and strains that were colonized from sites of sympatry with *A. albopictus*, is an example of rapid evolutionary change, the sympatric strains in our study having first come in contact as recently as 20–22 y ago. Artificial selection experiments as well as numerous examples from the field (reviewed in refs. 44 and 45) demonstrate the wide-ranging potential for fast evolutionary change, and mathematical models predict that such rapid change in interspecific interactions significantly affects population structure and dynamics. As discussed by Thompson (45), many of the best-documented examples of such rapid directional evolution have involved introduced species and are informative examples of the rate at which populations can adapt to fluctuating environmental conditions and the speed at which evolution continually can reshape community structure. In the case of interactions between *A. aegypti* and *A. albopictus*, rapid rates of adaptation could affect the stability of distribution patterns of these two species in areas where they frequently co-occur around the world (4, 46). Satyrization may suppress *A. aegypti* populations, whereas the development of resistance to satyrization may allow recovery. In combination with other biotic and abiotic factors (47, 48), this interplay could account for the observed patchy distributions of these two species where they encounter one another (e.g., refs. 49 and 50).

Furthermore, the high variability in the rates of insemination may explain the discrepancies in past laboratory mating trials (13, 20). The conflicting results may be caused, in part, by the tested populations displaying varying levels of adaptation to satyrization pressure. However, differences in experimental protocol, such as cage size, exposure time, and sex ratio, also may have contributed to the variation among results.

**Vector Displacements and Arbovirus Transmission.** The epidemiological implications of the competitive displacement of *A. aegypti* by *A. albopictus* should be considered. Assessing the speed at which mate-choice preferences or avoidance mechanisms develop may help predict future changes in the distribution and abundance of vector populations and, by extension, the risks of arbovirus transmission. If, for example, strong mating barriers and therefore satyrization-resistant populations are established, *A. aegypti* may be able in the future to recolonize areas from which it was displaced by *A. albopictus*.

Although a recent meta-analysis confirmed *A. aegypti* to be the primary outbreak vector of epidemics of severe dengue (51), *A. albopictus* has been documented as the principal vector of dengue where *A. aegypti* is rare or uncommon [e.g., in China (52–54), Bangladesh (55), and South India (56, 57)] as well as in recently invaded areas of Africa in the native range of *A. aegypti* (10). With regard to dengue fever, if *A. albopictus* is less of a public health threat than *A. aegypti* (51), then in dengue-endemic regions the displacement of *A. aegypti* by *A. albopictus* may lower transmission rates. In turn, a reduced prevalence of dengue may result in lower herd immunity in the resident human population. In this case, the reinvasion of satyrization-resistant *A. aegypti*, after a period of absence, could cause a resurgence of disease.

## Methods

Sympatric and allopatric *A. aegypti* and *A. albopictus* were obtained in 2011 from field collections of aquatic immatures from artificial containers, such as discarded tires or cemetery vases, using a turkey baster (Table 1). Individuals

**Table 1. Laboratory strains established from field populations**

| | A. aegypti | | A. albopictus | |
| Group | Allopatric | Sympatric | Allopatric | Sympatric |
|---|---|---|---|---|
| Group 1 | Old Town, Key West; population probably never exposed to *A. albopictus* (59) | M&K salvage yard, Vero Beach; populations of *A. aegypti* and *A. albopictus* have coexisted at this site for 20–22 y (60) | East St. Louis; populations never exposed, because the location lies outside the range of *A. aegypti* before the arrival of *A. albopictus* in the United States (61). | M&K salvage yard, Vero Beach; Populations of *A. aegypti* and *A. albopictus* have coexisted at this site for 20–22 y (60) |
| Group 2 | Woodlawn Cemetery, Miami; *A. albopictus* has not been detected in yearly surveillance in this cemetery since 1994–1995 (62) | White City Cemetery, Fort Pierce; populations of *A. aegypti* and *A. albopictus* have coexisted at this site for 20–22 y (60) | East St. Louis (see above) | M&K salvage yard, Vero Beach (see above) |

were identified to species, sorted, and reared separately to adulthood in insectaries at the Florida Medical Entomology Laboratory. Each colony was established from no fewer than 100 individuals, except for the *A. aegypti* line collected from White City Cemetery, Fort Pierce, which was established from 30–40 individuals.

Adults used in the experiments were second generation ($F_2$), except for the allopatric strain of *A. albopictus*, which was $F_5$. Second-generation mosquitoes were used to avoid maternal effects (58). Experiments were carried out in screened, plastic BugDorm (BioQuip Products Inc.) cages (30 × 30 × 30 cm) in an insectary maintained at 27 ± 0.62 °C and 89 ± 5.28% relative humidity under a 14-h light:10-h dark photoperiod.

Larvae were reared from hatch to pupation in pans containing 1 L of tap water (100 larvae per pan) and were provided with 0.6 g of a 1:1 brewer's yeast/egg albumin mix on day 1. Pupae were sexed according to morphological differences in their external genitalia and segregated by species and sex in small containers (10–20 pupae per container) for emergence. If a mistake in sexing was detected after emergence, the container was discarded. Three days after emergence, adults were transferred to BugDorm cages (150 females crossed with 150 males per cage) and left to cohabit for 3 wk.

Experiments were conducted in two sequential rounds (groups 1 and 2; Table 1). The groupings of the allopatric/sympatric lines of *A. aegypti* (Key West/Vero Beach and Miami/Fort Pierce) were arbitrary (based on strain availability) and therefore were treated as a blocking effect in the statistical analysis.

In each group (Table 1), *A. aegypti* females from either allopatric or sympatric populations were exposed to *A. albopictus* males from allopatric or sympatric populations. The reciprocal crosses between *A. albopictus* females and *A. aegypti* males also were performed, giving a total of eight combinations. Each cross combination was replicated in three sequential repeats. Additionally, three conspecific cages containing males and females of either *A. aegypti* or *A. albopictus* were set up as controls.

Females were dissected 3 wk after initial exposure, and the presence of sperm in one or more spermathecae was recorded as an insemination. Mosquitoes were provided a 10% sugar solution throughout the exposure periods.

**Statistical Analyses.** Data were analyzed with JMP version 7.0 (www.jmpdiscovery.com).

To detect variations in insemination frequency between *A. aegypti* and *A. albopictus* females, the proportions of females inseminated were Arcsine transformed and analyzed by ANOVA. The effect of population origin (sympatric vs. allopatric) of males and females on the likelihood of cross-mating was analyzed with a nominal logistic model, including "group" as a blocking effect. Differences between crosses in adult survivorship were analyzed by ANOVA.

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