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Oviposition Behavior in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Response to the Presence of Heterospecific and Conspecific Larvae

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Abstract

In mosquitoes, location of suitable sites for oviposition requires a set of visual, tactile, and olfactory cues that influences females before laying their eggs. The ability of gravid females to distinguish among potential oviposition sites that will or will not support the growth, development, and survival of their progeny is critical. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) share ecological niches, being highly competitive in larval stage. We studied the oviposition behavior of both species in the presence of larvae of one or the other species (heterospecific or conspecific larvae). The number of eggs laid by gravid females on oviposition sites (water with different or the same species of *Aedes* larvae) were compared. The presence and density of heterospecific or conspecific larvae had a positive or negative effect on the ovipositional responses, measured as an oviposition activity index. For both species, the oviposition was not affected by heterospecific larvae with densities between 10 and 100 larvae in water, but a strong attractant behavior was observed for a density of 500 larvae in water. For *Ae. albopictus* in the presence of larvae of the same species (conspecific oviposition), we observed an attractant effect for larvae density of 10 but not for 100 or 500 larvae in water. Instead, for *Ae. aegypti*, we observed attraction only for 100 larvae, not for 10 or 500 larvae. Results presented here provide an additional insight about oviposition behavior responses of gravid females in the presence of conspecific and heterospecific larvae in breeding sites.

Key words: *Aedes aegypti*, *Aedes albopictus*, oviposition, attractant, larva

Aedes aegypti (L.) and *Aedes albopictus* (Skuse) are container-breeding mosquitoes that commonly inhabit urban and wooded suburban areas throughout the world (Ho et al. 1973, Welch and Long 1984). Both species are diurnally active, highly anthropophilic, and potential vectors of three of the most important arboviral diseases in humans: dengue, yellow fever, and chikungunya. Considering that they have ecological similarities, it is likely that an interspecific competition could arise between these two *Aedes* species during the larval stage (Lounibos et al. 2002).

Ae. albopictus has been a successful introduced species in North America, Pacific Island, South America, Africa, and Europe. It potentially interacts with a number of other container-dwelling mosquitoes (Juliano 1998).

The larvae of *Ae. aegypti* and *Ae. albopictus* have been found coexisting in the same habitats, in particular in tires and artificial containers (Gould et al. 1968, Chan et al. 1971). Sprenger and Wuithiranyagool (1986) found larvae of three species of *Aedes* in artificial containers in Houston, TX: *Ae. albopictus* was the most abundant (53.0%), and *Ae. aegypti* and *Aedes triseriatus* represented the 17.8 and 2.6%, respectively. The relative distribution and abundance for sympatric populations of these species could be given by an interspecific larval competition. The interactions of *Ae. albopictus* with *Ae. aegypti* larvae were the most interesting and the best studied matter, particularly at laboratory level. The impact of competition on larval growth has been thoroughly examined, and findings that larval competition can lead to density-dependent effects on population growth, individual growth, individual fecundity, survival to adulthood, and developmental time were found (Juliano 1998, Lounibos et al. 2001, Reiskind and Lounibos 2009).

The study of intraspecific interactions seeks to determine how these coexistent species are responsible for their respective

distribution and abundance. Larvae competition studies between *Ae. albopictus* and *Ae. aegypti* showed that the latter grows faster and survives better than *Ae. albopictus* in mixed cultures (Moore and Fisher 1969, Ho et al. 1989). Similarly *Ae. aegypti* and *Ae. albopictus* were better competitors than *Ae. triseriatus*, a species that uses tree holes and artificial containers as breeding sites (Ho et al. 1989, Novak et al. 1993). However, there are negative interactions arising from the larval competition between these species, such as inhibition of *Ae. aegypti* and *Ae. triseriatus* eggs eclosion in the presence of *Ae. albopictus* larvae (Edgerly et al. 1993).

The decision where to oviposit is essential to maternal fitness in species in which the immature stages are unable to move to a more suitable habitat if conditions become adverse (Onyabe and Roitberg 1997, Spencer et al. 2002). Other studies indicate that physical attributes of oviposition sites, such as size, light–dark contrasts, and spectral reflectance from water surfaces, play a significant role in oviposition site selection (Harrington et al. 2008).

Oviposition is one of the most important events in the life cycle of mosquitoes, requiring the integration of internal and external stimuli. Gravid females of many species of mosquitoes show a high degree of preference in selecting oviposition sites. This preference may be due to the presence of oviposition pheromones or oviposition attractants and repellents in natural habitats (Hwang et al. 1980, Kramer et al. 1980). In recent years, a considerable attention was given to chemical cues that influence oviposition behavior (Takken and Knoles 1999, Guha et al. 2014). These chemical cues can be originated from mosquito larvae, their eggs, or both. *Aedes aegypti* females prefer oviposition sites containing conspecific eggs (Allan and Kline 1998). Wong et al. (2011) observed that free-ranging *Ae. aegypti* laid more eggs in sites that had recently held conspecifics compared with those that had not, suggesting that conspecific attraction is mediated by chemical cues. The preference for conspecific-conditioned water has been previously noted in the laboratory (Soman and Reuben 1970) and attributed to semiochemicals produced by larval-associated bacteria (Benzon and Apperson 1988). Semiochemicals may act as attractants to help females locate cryptic sites, and as stimulants to promote egg laying (Ponnusamy et al. 2008). Also, healthy larvae are associated with the presence of chemicals that render oviposition sites attractive to females, and conversely, starved larvae or infected with parasites render oviposition sites unacceptable to female mosquitoes (Bentley and Day 1989, Zahiri et al. 1997a). Other studies showed oviposition response of *Ae. aegypti* and *Ae. albopictus* to *n*-heneicosane, a C_{21} straight-chain hydrocarbon, identified in larval stages of both species (Seenivasagan et al. 2009, Gonzalez et al. 2014). In *Ae. aegypti*, when egg extracts were used in holding water different compounds were isolated, identified, and evaluated on the behavior of gravid females, finding sensitivity to all identified compounds (Ganesan et al. 2006). In *Ae. aegypti*, a strong oviposition preference for substrates with a intermediate number of conspecific eggs was demonstrated, leading to an "Allee effect" (Williams et al. 2008). Thus, the presence of conspecifics is attractive, and presumably beneficial to egg-laying *Ae. aegypti* up to a particular density.

Here, we attempt to obtain a better understanding of intra- and interspecific olfactory signals that mediate the effect of the oviposition behavior for both species; in this work, we evaluated the oviposition behavior of *Ae. aegypti* and *Ae. albopictus* mosquitoes in the presence of different larval densities (conspecific and heterospecific) in their breeding sites. We also studied the influence of an antibiotic and an antifungal compound on the oviposition behavior.

Materials and Methods

Biological Material

Aedes aegypti (derived from the Rockefeller strain from Venezuela) and *Ae. albopictus* (derived from the strain from Gainesville, FL) were used as reference strains. These colonies were reared since 1996 and 2010, respectively, in our insectary at 25 ± 2°C, 80–90% relative humidity, and a photoperiod of 12:12 (L:D) h and have been free of exposure to pathogens, insecticides, or repellents (Seccacini et al. 2006, Gómez et al. 2011). Larvae of both species were fed on a mixture of rabbit pellets and yeast. *Aedes albopictus* eggs were collected on a brown wet cardboard or paperboard and stored for at least 30 d in a Ziploc bag, with low moisture conditions, in a chamber at 18°C. *Aedes aegypti* eggs were collected on a white wet filter paper, dried at room temperature, and stored for at least 30 d. Stored eggs were submerged in dechlorinated water (500 eggs per 2 liter water) at 25 ± 2°C, and first-instar larvae were observed 24 h later.

Pupae were transferred to 250-ml plastic containers and allowed to hatch in 20- by 20- by 20-cm acrylic cages. Adults were offered ad libitum water and raisins and fed on pigeons.

The larvae stage and the age of females used in each study are described below.

Oviposition Bioassays

In choice binary assays, female mosquitoes of *Ae. aegypti* and *Ae. albopictus* were allowed to choose between water containers with the same larvae species versus a control container with water larvae free (conspecific oviposition) or between a water container with the other species of larvae versus a control container (heterospecific oviposition). Late third- or early fourth-instar larvae of *Ae. aegypti* and *Ae. albopictus* were carefully washed with dechlorinated water to eliminate any food particles, and 10, 100, or 500 larvae in 60 ml water, densities corresponding to 156.3, 1562.2, 7812.5 larvae per liter, respectively, were used in each assay. For reasons of readability, these will be hereafter referred to as 10, 100, and 500 larvae taking into account the densities specified.

According to Ganesan et al. (2006) two coated wire frame voile cages (750 by 600 by 600 mm^3) were used for each replicate of the oviposition bioassays. In each cage, one control jar and one treated jar (125-ml plastic jars, 10 cm in diameter) and 30 gravid *Ae. albopictus* and *Ae. aegypti* females 5–7 d old were used. The females were fed on pigeons twice: 2–3 d before and on the day the assay started. For each assay, three to four replicates were performed on different dates. Each assay lasted 4 d. As oviposition substrates, a brown cardboard (20 by 6 cm^2) was used for *Ae. albopictus,* while a white filter paper was used for *Ae. aegypti.* These substrates were chosen based on breeding protocol established in our laboratory (Seccacini et al. 2006, Gómez et al. 2011).

Heterospecific Oviposition Bioassays Performed in the Presence of an Antibiotic or an Antifungal Compound

Chloramphenicol was purchased from Anedra (Research AG S.A., Argentina). Nipagin (methyl *p*-hydroxybenzoate) was purchased from Parafarm (Saporiti, Buenos Aires, Argentina).

Aedes aegypti and *Ae. albopictus* heterospecific oviposition bioassays were performed by treating oviposition substrate with 100 µg/ml chloramphenicol and 10% v/v nipagin according to Zahiri and Rau (1998). Gravid females were allowed to lay eggs in jars with 60 ml water containing 500 late third- or early fourth-instar larvae of the other species versus control jars without larvae (heterospecific oviposition). We made this treatment only in high

density interspecific competition conditions to determine if the high attraction behavior was affected by water treatment with antibiotic and antifungal.

Statistical Analysis

The eggs laid in control and treated oviposition sites were manually counted to assess the oviposition preference of the mosquitoes. The oviposition attractant or repellent property of each treatment was expressed as the oviposition activity index (OAI) according to Kramer and Mulla (1979):

$$\mathrm{OAI} = \frac{Nt - Ns}{Nt + Ns}$$

where N denotes the mean number of eggs laid in treated (t) or control (s) water. Index values fall within the range of +1 to −1, with 0 indicating no preference. OAI values can be positive or negative. A positive value indicates that more eggs were laid in the treated substrate than in the control. Conversely, more eggs laid in the control than in the treated substrate result in a negative OAI value. According to Kramer and Mulla (1979) compounds with OAI of +0.30 and above are considered as attractants, while those with −0.30 and below are considered as repellents.

Data were analyzed statistically with STATISTICA '99 Edition for PC, StatSoft, Inc. The OAI was subjected to one-way ANOVA, then a Duncan's multiple range test was used to compare different larval densities.

Results

For the heterospecific oviposition competition bioassays using 10 and 100 larvae, the OAI values obtained were near zero both for *Ae. aegypti* and *Ae. albopictus* (Fig. 1A and B). Using 500 larvae, an OAI of 0.54 and 0.50 was obtained for *Ae. aegypti* and *Ae. albopictus*, *respectively* (Fig. 1).

For the conspecific oviposition competition bioassays using 10 and 500 larvae, OAI values of 0.177 and 0.137 were obtained, respectively, while for 100 larvae a significant attractant value of 0.58 was obtained for *Ae. aegypti* (Fig. 2A). For *Ae. albopictus*, using 10 and 100 larvae OAI values were 0.655 and 0.490 showing an attractant behavior; however, for a density of 500 larvae an OAI of 0.231 was obtained (Fig. 2B).

Using pretreatment with chloramphenicol (antibiotic) and nipagin (antifungal) in an heterospecific oviposition bioassay in the presence of 500 larvae, a strong attractant behavior was observed with OAI values of 0.607 and 0.437 obtained for *Ae. aegypti* and *Ae. albopictus*, respectively (Fig. 3).

Discussion

The results obtained in this work show that the presence of larvae clearly influenced the oviposition site choice of both *Ae. aegypti* or *Ae. albopictus* females. In heterospecific oviposition bioassays for a density of 500 larvae, a strong attractant effect was observed with OAI values higher than 0.5. For both species of *Aedes*, 10 and 100 larvae oviposition behavior was not influenced by the presence of the other species. There are olfactory and visual signals that could act on the oviposition behavior involving displacement mechanisms and competition for breeding sites. In our work the gravid female would perceive that the crawed site by the other species eventually would lead to its extinction in that breeding place, but with the next water accumulation event it could trigger the successful emergence

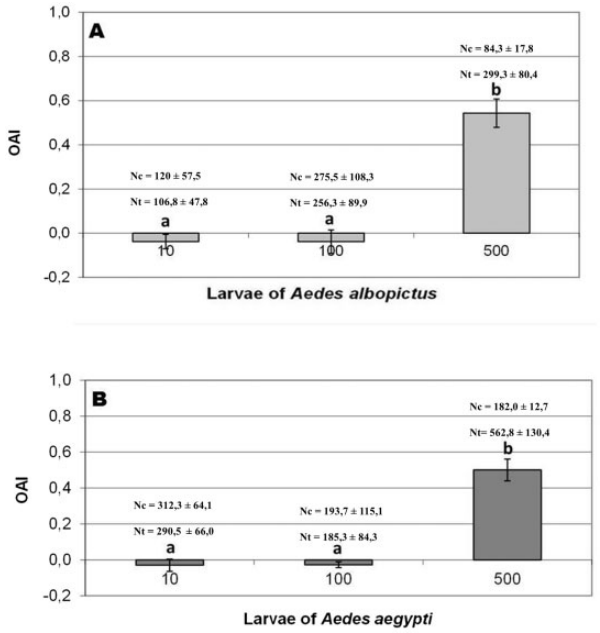

Fig. 1. Heterospecific assays. (**A**) Oviposition response of gravid *Ae. aegypti* female to different density of *Ae. albopictus* larvae (one-way ANOVA, $P < 0.001$, df = 10, $F = 41{,}372$, Duncan's test $P < 0.05$). (**B**) Oviposition response of gravid *Ae. albopictus* females to different density of *Ae. aegypti* larvae (one-way ANOVA, $P < 0.001$, df = 10, $F = 13{,}458$, Duncan's test $P < 0.05$). OAI—oviposition activity index. OAI (±SE) with the same letter are not significantly different. *N*c—mean number of egg in control (±SE); *N*t—mean number of eggs in treated (±SE).

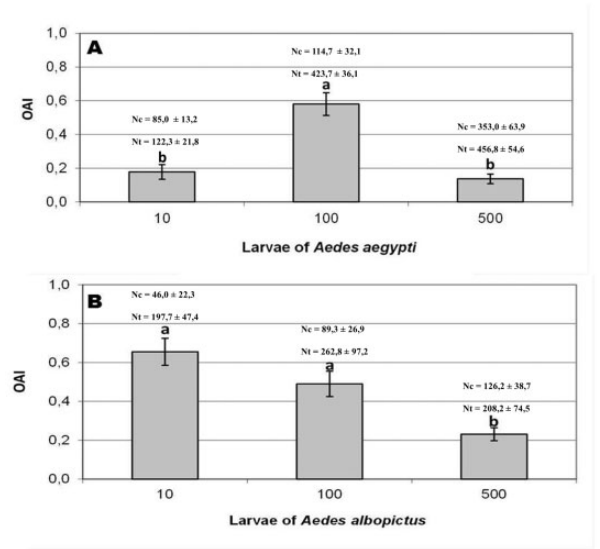

Fig. 2. Conspecific assays. (**A**) Oviposition response of gravid *Ae. aegypti* females to different density of *Ae. aegyptis* larvae (one-way ANOVA, $P < 0.001$, df = 10, $F = 26{,}043$, Duncan's test $P < 0.05$). (**B**) Oviposition response of gravid *Ae. albopictus* females to different density of *Ae. albopictus* larvae (one-way ANOVA, $P < 0.001$, df = 11, $F = 15{,}832$, Duncan's test $P < 0.05$). OAI—oviposition activity index. OAI (±SE) with the same letter are not significantly different. *N*c—mean number of egg in control (±SE); *N*t—mean number of eggs in treated (±SE).

of their own eggs with an immediate availability of organic matter for feeding. Nevertheless, it may happen that larvae feed on laid eggs or feed on newly hatched larvae of the other species. It would

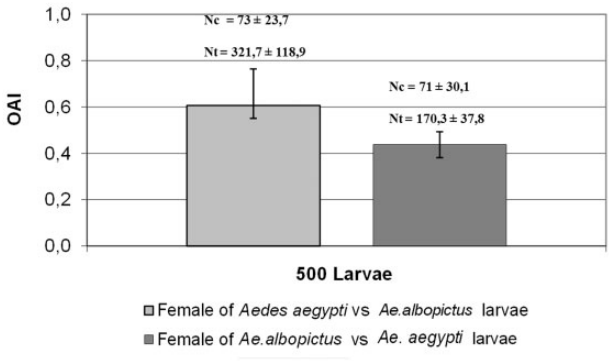

Fig. 3. Heterospecific oviposition response of *Ae aegypti* and *Ae. albopictus* with a substrate treatment with chloramphenicol and nipagin. OAI—oviposition activity index. OAI (±SE) one-way ANOVA, $P < 0.001$. *N*c—mean number of egg in control (±SE); *N*t—mean number of eggs in treated (±SE).

be interesting to study the success of that breeding place looking for a higher larvae mortality or if the eggs laid by the other species were eaten, etc.

The strong oviposition response by *Ae. aegypti* and *Ae. albopictus* females to heterospecific larvae (at high densities) is in agreement with a similar work from Allan and Kline (1998). They observed that the oviposition responses of *Ae. aegypti* females to *Ae. aegypti* larvae-rearing water were similar to the control, but were significantly higher in containers with *Ae. albopictus* larvae-rearing water, by comparison with control water containers. Similarly for *Ae. albopictus* females oviposition significantly increased in containers with larval-rearing water of *Ae. aegypti* in comparison with water controls (Allan and Kline 1998).

Our work also shows that in conspecific oviposition competition bioassays, in the treatment of 500 larvae for both species an OAI equal or lower than 0.2 was obtained. Thus, no attraction was observed. Maximal oviposition response was observed for treatment with 100 and 10 larvae for *Ae. aegypti* and *Ae. albopictus,* respectively. Other study shows that as the biomass of *Ae. aegypti* larvae increased in relation to the volume of rearing water, oviposition attraction of these water to conspecific gravid females first raised to a peak and then declined. Further increases in biomass rendered water strongly repellent (Zahiri and Rau 1998). A high density of larvae of the same species is supposed to affect negatively the survival index, the development, life expectancy, adult size, and fecundity (Juliano and Lounibos 2005). From this point of view, it would look as a positive matter to inhibit oviposition to avoid the competition with their own species. Maire (1985) showed that 900 larvae per liter was repellent to ovipositing *Aedes atropalpus* females reared under axenic conditions. In these cases a chemical that acts as an inhibitor and is produced by larvae could have evolved as a mechanism regulating oviposition, turning it less favorable or showing the site as overcrowded (Bentley et al. 1976, Kalpage and Brust 1973, Bentley and Day 1989).

In our work in conspecific conditions, the oviposition was inhibited with 500 larvae for both species; however, a high oviposition index was observed for heterospecific conditions at the same density. Therefore, in this study, we also analyzed the influence of the addition to the oviposition substrate of chloramphenicol and nipagin on the strong attractant effect obtained in this condition. No significant variation in the OAI values was observed for both species. This would indicate that the attraction observed in high larval density conditions is independent from the presence of bacteria or fungi susceptible to this treatment. However, it would be necessary to use other combinations of antibiotics and antifungals to confirm this statement.

The results of bioassays obtained in this work indicated the larvae density and the preexisting species affect the selection of the oviposition site. The effect of conspecific immature stages on the oviposition behavior of gravid mosquitoes changes according to the species. Aggregative oviposition has been well documented in culicines, such as *Culex quinquefasciatus* (Say) and *Culex tarsalis* (Coquillett) (Osgood 1971, Clements 1999) and volatile pheromones associated with the egg rafts mediate this behavior (Laurence and Pickett 1985, Blackwell et al. 1993). Among *aedine* mosquitoes, different studies have involved either larval-produced attractants (or stimulants; Zahiri et al. 1997b, Allan and Kline 1998, Zahiri and Rau 1998, Reisen and Siddiqui 1978) or repellents (or deterrents; Benzon and Apperson 1988, Chadee 1993). However, in the case of enhanced oviposition in water holding larvae, it is unclear whether the attraction or stimulation is caused by larval-produced chemical signals or by bacterial contamination of the water (Benzon and Apperson 1988). In addition, semiochemicals of egg origin and their effect on the ovipositional behavior of *Ae. aegypti* have been reported. Gravid females were found to be sensitive to all the identified compounds: 6-hexanolactone, methyl dodecanoate, dodecanoic acid, methyl tetradecanoate, tetradecanoic acid, methyl (Z)-9-hexadecenoate, methyl hexadecanoate (Z)-9-hexadecenoic acid, hexadecanoic acid, and methyl (Z)-9-octadecanoic (Ganesan et al. 2006).

Future studies of our laboratory will be directed to the evaluation of the olfactory cues found in the water with larvae of the same or different species and how they could modulate the oviposition behavior. It is implied from our results that the choice of the oviposition site could be not only affected by water soluble components of the cuticle, as shown by Allan and Kline (1998), but also by visual cues, volatiles, or both. These studies would allow us the identification of active components that generate an attractant effect in both species.

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References Cited

Allan, S. A., and D. L. Kline. 1998. Larval rearing water and preexisting eggs influence oviposition by *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 35: 943–947.

Bentley, M. D., and J. F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. Ann. Rev. Entomol. 34: 401–421.

Bentley, M. D., I. N. McDaniel, H. P. Lee, B. Stiehl, and M. Yatagai. 1976. Studies of *Aedes triseriatus* oviposition attractants produced by larvae of *Aedes triseriatus* and *Aedes atropalpus* (Diptera: Culicidae). J. Med. Entomol. 13: 112–115.

Benzon, G. L., and C. S. Apperson. 1988. Re-examination of chemically mediated oviposition behavior in *Aedes aegypti* (L.) (Diptera: Culicidae). J. Med. Entomol. 25: 158–164.

Blackwell, A., A. J. Mordue, B. S. Hansson, L. J. Wadhams, and J. A. Pickett. 1993. A behavioural and electrophysiological study of oviposition cues for *Culex quinquefasciatus*. Physiol. Entomol. 18: 343–348.

Chadee, D. D. 1993. Oviposition response of *Aedes aegypti* (L.) to the presence of conspecific eggs in the field in Trinidad, W.I. J. Fl. Med Assoc 64: 63–66.

Chan, Y. C., B. C. Ho, and K. L. Chan. 1971. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. 5. Observation in relation to dengue hemorrhagic fever. Bull. WHO. 44: 651–658.

Clements, A. N. 1999. The biology of mosquitoes: sensory, reception and behaviour, Vol. 2. CABI Publishing, Oxon, United Kingdom.

Edgerly, J. S., M. S. Willey, and T. P. Livdahl. 1993. The community ecology of *Aedes* egg hatching: implications for a mosquito invasion. Ecol. Entomol. 18: 123–128.

Ganesan, K., M. J. Mendki, M. V. S. Suryanarayana, S. Prakash, and R. C. Malhotra. 2006. Studies of *Aedes aegypti* (Diptera: Culicidae) ovipositional responses to newly identified semiochemicals from conspecific egg. J. Entomol. 45: 75–80.

Gómez, A., E. Seccacini, E. Zerba, and S. Licastro. 2011. Comparison of the insecticide susceptibilities of laboratory strains of *Aedes aegypti* and *Aedes albopictus*. Mem. I. Oswaldo Cruz. 106: 993–996.

Gonzalez, P. V., P. A. Gonzalez Audino, and H. M. Masuh. 2014. Electrophysiological and behavioural response of *n*-heneicosane, oviposition pheromone of *Aedes aegypti*, on *Aedes albopictus*. Entomol. Exp. Appl. 151: 191–197.

Gould, D. J., T. M. Yuill, M. A. Moussa, A. Simasathien, and L. Rutledge. 1968. An insular outbreak of dengue haemorrhagic fever. III. Identification of vectors and observations on vector ecology. Am. J. Trop. Med. Hyg. 17: 609–618.

Guha, L., T. Seenivasagan, S. T. Iqbal, O. P. Agrawal, and B. D. Parashar. 2014. Behavioral and electrophysiological responses of *Aedes albopictus* to certain acids and alcohols present in human skin emanations. Parasitol. Res. 113: 3781–3787.

Harrington, L. C., A. Ponlawat, J. D. Edman, T. W. Scott, and F. Vermeylen. 2008. Influence of container size, location, and time of day on oviposition patterns of the dengue vector, *Aedes aegypti*, in Thailand. Vector-Borne Zoonot. 8: 415–424.

Ho, B. C., K. L. Chan, and Y. C. Chan. 1973. The biology and bionomics of *Aedes albopictus* (Skuse), pp 125–143. *In*. Vector Control in Southeast Asia, Proceedings of the First Southeast Asian Ministers for Education Organization Workshop.

Ho, B. C., A. Ewert, and A. L. M. Chew. 1989. Interspecific competition between *Aedes aegypti*, *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae): larval development in mixed cultures. J. Med. Entomol. 26: 615–623.

Hwang, Y. S., W. L. Kramer, and M. S. Mulla. 1980. Oviposition attractants and repellents of mosquitoes. J. Chem. Ecol. 6: 71–80.

Juliano, S. A. 1998. Species introduction and replacement among mosquitoes: Interspecific resource competition or apparent competition? Ecology 79: 255–268.

Juliano, S. A., and L. P. Lounibos. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. Ecol. Lett. 8: 558–574.

Kalpage, K. S. P., and R. A. Brust. 1973. Oviposition attractant produced by immature *Aedes atropalpus*. Environ. Entomol. 2: 729–30.

Kramer, W. L., and M. S. Mulla. 1979. Oviposition attractants and repellents of mosquitoes: Oviposition responses of *Culex* mosquitoes to organic infusions. Environ. Entomol. 8: 1111–1114.

Kramer, W. L., Y. S. Hwang, and M. S. Mulla. 1980. Oviposition repellents of mosquitoes: Negative responses elicited by lower aliphatic carboxylic acids. J. Chem. Ecol. 6: 415–424.

Laurence, B. R., and J. A. Pickett. 1985. An oviposition attractant pheromone in *Culex quinquefasciatus* Say (Diptera: Culicidae). Bull. Entomol. Res. 75: 283–290.

Maire, A. 1985. Effect of axenic larvae on the oviposition site selection by *Aedes atropalpus*. J. Am. Mosq. Control Assoc. 1: 320–323.

Lounibos, L. P., G. F. Omeara, R. L. Escher, N. Nishimura, M. Cutwa, T. Nelson, R. E. Campos, S. A. Juliano. 2001. Testing predictions of displacement of native *Aedes* by the invasive Asian Tiger Mosquito *Aedes albopictus* in Florida, U.S.A. Biol. Biol. Invasions. 3: 151–166.

Lounibos, L. P., S. Suárez, Z. Menéndez, N. Nishimura, R. L. Escher, S. M. O'Connell, and J. R. Rey. 2002. Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*?. J. Vector Ecol. 27: 86–95.

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

Novak, M. G., L. G. Higley, C. A. Christianssen, and W. A. Rowley. 1993. Evaluating larval competition between *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) through replacement series experiments. Environ. Entomol. 22: 311–318.

Onyabe, D., and B. D. Roitberg. 1997. The effect of conspecifics on the oviposition site selection and oviposition behaviour in *Aedes togi* (Theobald) (Diptera: Culicidae). Can. Entomol. 129: 1173–1176.

Osgood, C. E., and R. H. Kempster. 1971. An oviposition pheromone associated with the egg rafts of *Culex tarsalis*. J. Econ. Entomol. 64: 1038–1041.

Ponnusamy, L., N. Xu, S. Nojima, D. M. Wesson, C. Schal, and C. S. Apperson. 2008. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. Proc. Natl. Acad. Sci. U.S.A. 105: 9262–9267.

Reisen, W. K., and T. F. Siddiqui. 1978. The influence of conspecific immatures on the oviposition preferences of the mosquitoes *Anopheles stephensi* and *Culex tritaeniorhynchus*. Pak. J. Zool. 10: 31–41.

Reiskind, M. H., and L. P. Lounibos. 2009. Effects of intraspecific larval competition on adult longevity in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. J. Med. Vet. Entomol. 23: 62–68.

Seccacini, E., H. Masuh, S. Licastro, and E. Zerba. 2006. Laboratory and scaled up evaluation of *cis*-permethrin applied as a new ultra low volume formulation against *Aedes aegypti* (Diptera: Culicidae). Acta Trop. 97: 1–4.

Seenivasagan, T., R. S. Kavita, K. Sekhar, K. Ganesan, S. Prakash, and R. Vijayaraghavan. 2009. Electroantennogram, flight orientation, and oviposition responses of *Aedes aegypti* to the oviposition pheromone *n*-heneicosane. Parasitol. Res. 104: 827–833.

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.

Soman, R. S., and R. Reuben. 1970. Studies on preference shown by ovipositing females of *Aedes aegypti* for water containing immature stages of the same species. J. Med. Entomol. 7: 485–489.

Spencer, M., L. Blaustein, and J. E. Cohen. 2002. Oviposition habitat selection by mosquitoes (Culiseta: longiareolata) and consequences for population size. Ecology 83: 669–679.

Takken, W., and B. G. J. Knols. 1999. Odor-mediated behaviour of Afrotropical malaria mosquitoes. Ann. Rev. Entomol. 44: 131–157.

Welch, J. B., and J. D. Long. 1984. *Aedes aegypti* collections in rural southeast Texas. Mosq. News 44: 544–547.

Williams, C. R., K. J. Leach, N. J. Wilson, and V. R. Swart. 2008. The Allee effect in site choice behaviour of egg-laying dengue vector mosquitoes. Trop. Biomed. 25: 140–144.

Wong, J., S. T. Stoddard, H. Astete, A. C. Morrison, and T. W. Scott. 2011. Oviposition site selection by the dengue vector *Aedes aegypti* and its implications for dengue control. PLoS Neglect Trop. Dis. 5: e1015.

Zahiri, N., and M. E. Rau. 1998. Oviposition attraction and repellency of *Aedes aegypti* (Diptera: Culicidae) to waters from conspecific larvae subjected to crowding, confinement, starvation, or infection. J. Med. Entomol. 35: 782–787.

Zahiri, N, M. E. Rau, and D. J. Lewis. 1997a. Oviposition responses of *Aedes aegypti* and *Ae. atropalpus* (Diptera: Culicidae) females to waters from conspecific and heterospecific normal larvae and from larvae infected with *Plagiorchis elegans* (Trematoda: Plagiorchiidae). J. Med. Entomol. 34: 565–568.

Zahiri, N., M. E. Rau, D. J. Lewis, and S. Khanizadeh. 1997b. Intensity and site of *Plagiorchis elegans* (Trematoda: Plagiorchiidae) infections in *Aedes aegypti* (Diptera: Culicidae) larvae affect the attractiveness of their waters to ovipositing, conspecific females. Environ. Entomol. 26: 920–923.