Mating Status and Body Size in Aedes albopictus (Diptera: Culicidae) Affect Host Finding and Deet Repellency

Authors: Xue, Rui-De, Ali, Arshad, and Barnard, Donald R.

Source: Florida Entomologist, 95(2) : 268-272

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.095.0204

MATING STATUS AND BODY SIZE IN *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE) AFFECT HOST FINDING AND DEET REPELLENCY

RUI-DE XUE[1], ARSHAD ALI[2] AND DONALD R. BARNARD[3]
[1]Anastasia Mosquito Control District, 500 Old Beach Road, St. Augustine, FL 32085, USA

[2](Retired) University of Florida, IFAS, Mid-Florida Research and Education Center, 2725 Binion Road, Apopka, FL 32703, USA

[3]USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

ABSTRACT

Variations in the conditions accompanying mosquito development and mating can result in females of variable size that have not been inseminated. In this study, we compared the host finding activity of mated and unmated large and small *Aedes albopictus (*Skuse) and the repellency to these mosquitoes of 25% DEET. The percentage of females seeking the source of human odor in an olfactometer was significantly influenced by body size (P = 0.007) but not mating status (P = 0.07). Most respondents were large (71%) but 40% of all host seeking females were not inseminated. Landing rates by *Ae. albopictus o*n human skin were influenced in screened cage tests by mating status (P < 0.0001) and body size (P = 0.004). Mated females exhibited the highest landing rates (17.3% [large] and 12.7% [small]) followed by unmated large females (7.3%) and unmated small females (6.4%). The Complete Protection Time (CPT) from mosquito landing provided by 25% DEET was significantly influenced by mating status (P = 0.002) and body size (P = 0.025). Unmated small females were repelled longer (7.5 h) than unmated large females (7.0 h) and longer than small and large mated females (6.3 h and 5.6 h, respectively). CPT using 25% DEET was inversely related to mosquito landing rate in all treatment groups with 75 min more protection on average from bites by unmated females compared with mated females.

Key Words: mosquito, host seeking, landing, probing, repellent, Complete Protection Time

RESUMEN

Variaciones en las condiciones que acompañan el desarrollo del mosquito y el apareamiento puede resultar en hembras de tamaños variables que no han sido inseminadas. En este estudio, se comparó la actividad de la búsqueda de hospederos de hembras de *Aedes albopictus* (Skuse) apareadas y no apareadas, grandes y pequeñas, y la repelencia a estos mosquitos al DEET de 25%. El porcentaje de las hembras que buscaron la fuente del olor humano en un olfatómetro fue influenciada significativamente por el tamaño del cuerpo (P = 0.007) pero no del estado de apareamiento (P = 0.07). La mayoría de las hembras que respondian fueron grandes (71%) pero el 40% de todas las hembras que buscaron hospederos no fueron inseminadas. La tasa de individuos de *Ae. albopictus* que pararon sobre la piel humana fue influenciada en las pruebas de jaulas seleccionadas por el estado de apareamiento (P < 0.0001) y el tamaño de cuerpo (P = 0.004). Las hembras apareadas exhiben la mayor tasa de paradas sobre la piel (17.3% [en las grandes] y el 12.7% [en las pequeñas]), seguido de las hembras no apareadas grandes (7.3%) y hembras no apareadas pequeñas (6.4%). El Tiempo de Protección Completa (TPC) de las paradas de los mosquitos debido al DEET de 25% fue significativamente influenciado por el estado de apareamiento (P < 0.0001) y el tamaño de cuerpo (P = 0.025). Las hembras pequeñas no apareadas fueron repeladas por más tiempo (7.5 horas) que las hembras no apareadas grandes (7.0 horas) y más largo que las hembras apareadas pequeñas y grandes (6.3 horas y 5.6 horas, respectivamente). El TPC usando DEET al 25% fue inversamente proporcional a la tasa de la parada de los mosquitos sobre la piel en todos los grupos de tratamiento con una mayor promedio de protección de 75 minutos de las picaduras de las hembras no apareadas en comparación con las hembras apareadas.

Differences in the conditions accompanying growth, development, and survival in mosquitoes can lead to adults of variable size and females that are unmated (uninseminated). Body size and mating status parameters in the mosquito population respond to many biotic and environmental factors including, in the former case, larval nutrition (Takken et al. 1998) and, in the later case, genetics (Olejnícek 1995), mosquito age (Iwanaga-Sawabe & Kanda 1990), adult energetics (Stone et al. 2009), and a complex of habitat variables that directly or indirectly regulate male and female accessibility for mating (Lea 1968; Corbet & Smith 1974; Verhoek & Takken 1994; Ameneshewa & Service 1996; Ponlawat & Harrington 2009).

In the laboratory, unmated female *Aedes aegypti* L. bite continuously whereas mated females bite less as ovarian development commences (Laviopierre 1958). The pattern in mated females is related to the rate of oocyte-induced inhibition of host-seeking. This rate is lower in unmated females when maintained on the same diet as mated individuals (Klowden & Lea 1979). In *Aedes albopictus* (Skuse), variations in host seeking and biting behavior cause different responses to DEET (Xue et al. 1995; Xue & Barnard 1996; Xue & Barnard 1999; Xue et al. 2008); however, the effects of mating status on DEET repellency in this mosquito species have not been reported.

We believe that differences in biting behavior related to mating status in female mosquitoes are likely to influence repellent protection time in laboratory bioassays. This study was made to quantify the effect of this factor on host seeking behavior in *Ae. albopictus* and on the protection time from mosquito bites provided by DEET. Given the influence of male body size on sperm transfer to females in *Ae. aegypti* (Ponlawat & Harrington 2009), we further differentiated the responses of mated and unmated females according to 2 adult body size categories (large, small) (Xue et al. 1995). Tests of host finding using the resulting 4 treatment groups comprised observation of the mosquito host seeking rate in an olfactometer and observation of the landing rate of female *Ae. albopictus* on the exposed forearm of a human subject when inserted into a screened test cage. In repellent tests we determined the mean protection time from landings by mosquitoes in each treatment group when exposed to skin treated with 25% DEET.

Materials and Methods

Mosquito Rearing, Body Size, Mating Status

The Gainesville (1992) strain of *Ae. albopictus* was used. Mosquitoes were reared and maintained in an insectary at 27 ± 2 °C and 70% RH under a 14:10 h L:D photoperiod using published methods (Gerberg et al. 1994). Adults emerged into screened stock cages, were provided continuous access to 10% sucrose/water solution, and were blood-fed (on restrained 5-7 wk-old chicks) as needed for colony maintenance.

Body size in female mosquitoes was indexed according to wing length, which was measured as the straight line distance between the anterior limit of the axillary incision and the end of the posterior branch of the 2nd longitudinal vein (Darsie & Ward 1981). Large females (mean wing length: 3.12 ± 0.11 mm) emerged from immatures (250 1st instars) reared in 1 L of well water in a 30 cm L × 19 cm W × 5 cm H plastic tray and fed 80 mg of food (3:2 liver powder: brewers yeast) daily. Small females (mean wing length: 2.31 ± 0.16 mm) emerged from immatures (500 1st instars) reared in 1 L of well water in the same-sized plastic trays and fed 30 mg of food daily. Unmated females were collected shortly after emergence and before contact with males. Insemination status in females was confirmed (after testing) by inspection of the spermathecae for sperm.

Host Attraction, Host Attack, and Repellency Responses

A triple cage olfactometer (Schreck et al. 1967) was used to determine the attraction responses of female *Ae. albopictus t*o odors from the human hand. Each test population of mosquitoes comprised 75, 5-7 day-old females. These were transferred to the olfactometer test chamber and allowed to rest for 1 h. To commence a test, a human subject (RDX) inserted their hand into the olfactometer. The olfactometer airstream was then redirected over the hand and through the test cage for 5 min. The number of mosquitoes that took flight, oriented to the odor source, and were trapped in the test cage assembly during the 5 min period was recorded. Responses were measured as percent attraction. Tests were replicated 5 times for each treatment group.

Landing responses were recorded for 5-7 day-old females. Each test comprised the assignment of 50 large/mated, 50 large/unmated, 50 small/mated, or 50 small/unmated females to 1 of 4 screened cages (45 cm W × 38 cm H × 35 cm D) where they were provided water and cubed sugar and allowed to rest for 12 h. At the start of a test, a human subject (RDX) inserted a latex glove-covered hand and forearm into a cage for 1 min. The number of mosquitoes that landed and remained on the skin surface exposed through a 10 cm × 5 cm opening in the glove was counted. Females that touched the proboscis to the skin were counted as they were removed using an aspirator; none was allowed to bite. This procedure was repeated for all 4 cages and the responses recorded as the landing rate (%) for each cage. Tests were replicated 9 times for each treatment group.

Repellency responses were observed by separately placing 50, 5-7 day-old females from each treatment group in 1 of 4 cages (45 cm W × 38 cm H × 35 cm D). Each cage had a cotton stockinette access sleeve on the front, clear acrylic sides (for viewing), a sheet aluminum bottom, and window screen on the top and backsides. Thirty min before a test began, the forearm of a test subject (RDX) was treated with 1 mL of 25% DEET in ethanol. The repellent was applied to the forearm between the elbow and the wrist using 1 mL of repellent solution on 650 cm^2 of skin surface area and allowed to dry for 30 min. A latex glove worn over the hand protected it from mosquito bites. A test was begun by placing the repellent-treated forearm into a cage for 3 min and observing for mosquitoes that landed on the skin surface. None was allowed to bite. This process was repeated at 30 min intervals until the test subject received 2 or more mosquito landings in the same observation period or 1 landing in each of 2 consecutive observation periods (a confirmed landing). The procedure was repeated for all 4 cages and the response in each case converted to Complete Protection Time (CPT), which was defined as the time elapsed between repellent application and the observation period immediately preceding that in which the confirmed landing was observed. Repellency tests were replicated 3 times.

Data Analysis

Analysis of variance procedures (SAS 2003) were used to assess mating status and body size effects in a split plot design with mating status assigned to main plots and body size to subplots. Tukey's Honestly Significant Difference (HSD) test was used for means separation. A significance level of 5% was used in all tests.

Results

Body size in female *Ae. albopictus w*as more important ($F_{1,19}$ = 12.9, P = 0.007) than mating status (P = 0.07) when determining the percentage of females responding to human host odor in an olfactometer (Table 1). A significantly higher percentage of large females (48.0 ± 6.5%) responded to host odor compared with small females (19.8 ± 7.4%) and mean responses in mated females (40.4 ± 9.6%) were higher than in unmated females (27.4 ± 6.3%). Within either body size category, mating status did not significantly influence host seeking responses.

Mating status ($F_{1,35}$ = 128.4, P < 0.0001), body size ($F_{1,35}$ = 11.1, P = 0.004), and the interaction effect significantly ($F_{1,35}$ = 5.1, P = 0.03) influenced *Ae. albopictus* landing rates in screened cage tests (Table 1). For mated females, the mean landing rate (15.0 ± 0.9%) was higher than for unmated females (6.8 ± 0.3%) and similarly for large females (12.3 ± 1.3%) compared with small females (9.5 ± 0.8%). A significant interaction effect indicated differences in the mean landing responses of large and small mated females (4.7%) compared with large and small unmated females (0.9%). When responses were separated according to mating status, mated large females had significantly higher ($F_{1,17}$ = 10.3, P = 0.0054) landing rates than mated small females, whereas unmated large and small females did not (P = 0.24) (Table 1). When responses were separated according to body size, landing rates in both large ($F_{1,17}$ = 52.9, P < 0.0001) and small ($F_{1,17}$ = 51.4, P < 0.0001) mated females were significantly higher than in the corresponding unmated female populations.

DEET repellency (mean CPT) in *Ae. albopictus* was influenced by mating status ($F_{1,11}$ = 56.3, P = 0.002). Body size effects were also significant ($F_{1,11}$ = 12.3, P = 0.025). Small females (mean CPT: 6.9 ± 0.3 h) and unmated females (mean CPT: 7.3 ± 0.1 h) were repelled longer by DEET than large females (mean CPT: 6.3 ± 0.3 h) or mated females (mean CPT: 6.0 ± 0.2 h). There was no significant interaction effect. When compared on the basis of body size (Table 1), unmated large females were repelled longer ($F_{1,5}$ = 64.0, P = 0.001) by DEET than mated large females as were unmated small females compared with mated small females ($F_{1,5}$ = 49.1, P = 0.002). When compared according to mating status, small mated females were repelled longer ($F_{1,5}$ = 8.0, P = 0.047) by DEET than large mated females and small unmated females were repelled longer ($F_{1,5}$ = 196.3, P < 0.0001) than large unmated females.

Table 1. Mean percent (±SE) of *Aedes albopictus* females attracted to human host odor in an olfactometer (Host seeking), landing on the skin of a human host in a screened cubic 35 cm cage (Landing), and the mean protection time (CPT) from mosquito bites provided by 25% DEET (in ethanol) applied to skin.

Response category	Body size	Mated[1]	Unmated
Host seeking (%)	Large	57.4 (18.5) aA	38.6 (19.8) aA
	Small	23.4 (31.8) aA	16.2 (13.3) aA
Landing (%)	Large	17.3 (4.0) aA	7.3 (1.0) bA
	Small	12.7 (1.7) aB	6.4 (1.9) bA
CPT (h)	Large	5.6 (0.3) aA	7.0 (0) bA
	Small	6.3 (0.3) aB	7.5 (0) bB

[1]Within each response category, row means followed by the same lower case letter and column means followed by the same upper case letter are not significantly different (Tukeys' HSD test, $P = 0.05$).

Discussion

There were measurable differences in the responses of large and small mated and unmated female *Ae. albopictus* to human host odor in the olfactometer, in the rates of landing by female mosquitoes on skin, and in the repellency of DEET. The mean host seeking response in large mated females was 3.5×, 2.5×, and 1.5× greater than in small unmated and mated females and large unmated females, respectively, although host seeking responses varied widely within some treatment groups but not others (the coefficient of variation ranged from 32% for large mated females to 135% for small mated females). Given these observations, olfactometric measurement of host seeking in an *Ae. albopictus* population comprising equal numbers of large and small mated and unmated females would favor representation by large mated females (42.3%) followed by large unmated (28.5%), small mated (17.3%), and small unmated females (11.9%).

Mating status affected landing rates by *Ae. albopictus*. Unmated females, regardless of size, were about half as likely to land on human skin as mated females, although differences between mated and unmated females varied significantly with mosquito body size as did differences in the landing rates for large and small mated females. About 40% of all landing females were large-bodied and mated; 29% were small/mated, 17% were large/unmated, and 14% were small/unmated.

Repellency responses were inversely related to landing rate in all treatment groups. Mean protection times provided by 25% DEET differed by 2 h, being shortest for large/mated *Ae. albopictus* and longest for small/unmated females. On average, mating status in *Ae. albopictus* accounted for 75 min more protection from bites by unmated females than from mated females (regardless of body size) when using 25% DEET.

Results from this study suggest that response to DEET by *Ae. albopictus* is least variable in mated large-bodied females that manifest a positive landing response. This finding is consistent with other studies wherein DEET repellency has been related to the size, health, vigor, reproductive status, and blood feeding behavior of *Ae. albopictus* mosquitoes (Barnard 1998; Xue et al. 1995; Xue & Barnard 1996, 1999). However, a concern in this regard is the pre-selection of *Ae. albopictus* for repellent testing based solely on a positive attraction to host odor (Schreck & McGovern 1989). In this study, 71% of host seeking females were large bodied but only 42% were mated, whereas 56% of landing females were large bodied, 69% of which were mated. The unmated female component of the test population thus accounted for a 41% decline in (and a significant interaction effect for) landing rate responses when compared with the responses of unmated females in host seeking tests. This means that not all female *Ae. albopictus* that respond to host odor in an olfactometer will land on a human subject in a repellent test cage and that the host seeking responses of unmated females may be particularly misleading in this regard. Perhaps most important, in the absence of pre-selection, the proportion of the female test population that responds to host presence in a repellent test cage is low (17% to 57% [Table 1]). When the values for host seeking are compared with the proportion of the test population landing on exposed skin, the proportion of mosquitoes that would respond in a repellent test is even lower (6.4% to 17.3%). Given that DEET repellency in mosquitoes appears to be primarily a function of landing rate (Barnard et al. 1998), low *Ae. albopictus* landing rates in a repellent bioassay equate to overestimation of DEET CPT. In screened cage tests, this error will increase when using *Ae. albopictus* test populations comprising different adult body sizes and a mix of mated and unmated females.

Based on these findings, assessment of the effect of mating status on mosquito responses in field tests of DEET repellency is problematic. Our results suggest that *Ae. albopictus* populations initially attracted to and landing on human test subjects in the field will comprise mainly large bodied mated females. This group of mosquitoes is repelled by 25% DEET for the shortest time and their use in a repellent bioassay will result in a conservative estimate of CPT. Conversely, when using mosquito populations heterogeneous for mating status and/or age, size, and other factors, estimation of DEET protection time will be less precise and the CPT longer.

Knowledge of the different responses can be used in the design of field studies to improve the accuracy of estimated CPT. The potential for initial exposure of human subjects to primarily large mated female *Ae. albopictus* in a test, for example, suggests that each subject move continuously through a mosquito-infested field site while observing repellent-treated (or control) skin for mosquito landings, rather than observing for this response while positioned at a "station" or in the same location throughout a test.

Acknowledgment

V. Droit, and K. Posey provided technical assistance. This research was conducted according to protocol #445-96 approved by University of Florida, Institutional Review Board-01 (human subjects); volunteers gave informed consent prior to participation in the study. Animal care and use was in accordance with protocol

A057 approved by the University of Florida, Institutional Animal Care and Use Committee.

References Cited

AMENESHEWA, B., AND SERVICE, M. W. 1996. The relationship between female body size and survival rate of the malaria vector *Anopheles arabiensis* in Ethopia. Med. Vet. Entomol. 10: 170-172.

BARNARD, D. R. 1998. Mediation of deet repellency in mosquitoes (Diptera: Culicidae) by species, age, and parity. J. Med. Entomol. 35: 340-343.

BARNARD, D. R., POSEY, K. H., SMITH, D., AND SCHRECK, C. E. 1998. Mosquito density, biting rates and cage size effects on repellent tests. Med. Vet. Entomol. 12: 39-45.

CORBET, P. S., AND SMITH, S. M. 1974. Diel periodicities of landing of nulliparous and parous *Aedes aegypti* (L.) at Dar es Salaam, Tanzania (Diptera, Culicidae). Bull. Entomol. Res. 64: 111-121.

DARSIE JR., F. D. 1981. Identification and Geographic Distribution of the Mosquitoes of North America, North of Mexico. American Mosquito Control Association, Salt Lake City, UT

GERBERG, E. J., BARNARD, D. R., AND WARD, R. A. 1994. Manual for mosquito rearing and experimental techniques. J. Am. Mosq. Control Assoc. Bull. 5 (revised).

IWANAGA-SAWABE, K., AND KANDA, T. 1990. Studies on the effective attractants of Anopheline mosquitoes, I. Blood-feeding, mating and flight activity of female *Anopheles balabacensis* (Diptera: Culicidae). Appl. Entomol. Zool. 25: 231-238.

KLOWDEN, M. J., AND LEA, O. A. 1979. Humoral inhibition of host-seeking in *Aedes aegypti during oocyte maturation.* J. Insect Physiol. 25: 231-235.

LAVOIPIERRE, M. M. J. 1958. Biting behavior of mated and unmated females of an African strain of *Aedes aegypt*i. Nature 181: 1781.

LEA, A. O. 1968. Mating without insemination in virgin *Aedes aegypti*. J. Insect Physiol. 14: 305-308.

OLEJNÍCEK, J. 1995. Influence of males on the blood-feeding behavior of female *Culex pipiens* complex mosquitoes during the first gonotrophic cycle. J. Vect. Ecol. 20: 147-152.

PONLAWAT, A., AND HARRINGTON, L. C. 2009. Factors associated with male mating success of the dengue vector mosquitoes, *Aedes aegypti*. Am. J. Trop. Med. Hyg. 80: 395-400.

SAS [STATISTICAL ANALYSIS SYSTEM INSTITUTE, INC.]. 2003 SAS for Windows, Release 9.1. SAS Institute, Inc., Cary, NC.

SCHRECK, C. E., GOUK, H. K., AND SMITH, C. N. 1967. An improved olfactometer for use in studying mosquito attractants and repellents. J. Econ. Entomol. 60: 1188-1190.

SCHRECK, C. E., AND MCGOVERN, T. P. 1989. Repellents and other personal protection strategies against *Aedes albopictus*. J. Am. Mosq. Control. Assoc. 5: 247-250.

STONE, C. M., TAYLOR, R. M., ROITBERG, B. D., AND FOSTER, W. A. 2009. Sugar deprivation reduces insemination of *Anopheles gambiae* (Diptera: Culicidae), despite daily recruitment of adults and predicts decline in model populations. J. Med. Entomol. 46: 1327-1337.

TAKKEN, W., KLOWDEN, M. J., AND CHAMBERS, G. M. 1998. The effect of body size on host seeking and blood meal utilization in *Anopheles gambiae* s. s. (Diptera: Culicidae): the disadvantages of being small. J. Med. Entomol. 35: 639-645.

VERHOEK, B. A., AND TAKKEN, W. 1994. Age effects on the insemination rate of *Anopheles gambiae s.l.* in the laboratory. Entomol. Exp. Appl. 72: 167-172.

YEH, C., AND KLOWDEN, M. J. 1990. Effects of male accessory gland substances on the pre-oviposition behavior of *Aedes aegypt*i mosquitoes. J. Insect Physiol. 36: 799-803.

XUE, R. D., BARNARD, D. R., AND SCHRECK, C. E. 1995. Influence of body size and age of *Aedes albopictus* on human host attack rates and the repellency of deet. J. Am. Mosq. Control Assoc. 11: 50-53.

XUE, R. D., AND BARNARD, D. R. 1996. Human host avidity in *Aedes albopictus*: influence of mosquito body size, age, parity, and time of day. J. Am. Mosq. Control Assoc. 12: 58-63.

XUE, R. D., AND BARNARD, D. R. 1999. Effects of partial blood engorgement and pretest carbohydrate availability on the repellency of Deet to *Aedes albopictus*. J. Vest. Ecol. 24: 111-114.

XUE, R. D., ALI, A., AND BARNARD, D. R. 2008. Host species diversity and post-blood feeding carbohydrate availability enhance survival of females and fecundity in *Aedes albopictus* (Diptera: Culicidae). Exp. Parasitol. 119: 225-228.