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INFLUENCE OF BODY SIZE AND AGE OF *AEDES ALBOPICTUS* ON HUMAN HOST ATTACK RATES AND THE REPELLENCY OF DEET[1]

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ABSTRACT. Human host attack rates and the repellency of deet (25% in ethanol) to nulliparous *Aedes albopictus* in the laboratory were significantly influenced ($P = 0.05$) by mosquito age and body size and by the interaction of age and body size. Host attack rates were higher for 20-day- (24.5%) and 15-day-old females (22.9%) than for 10- (15.1%) and 5-day-old females (11.0%), regardless of body size, and for large females (22.2%) compared with small females (16.6%), regardless of mosquito age. Deet on human skin repelled small-bodied females longer (3.87 h) than large females (2.31 h); 15-day-old females were repelled longer (3.75 h) than 5- (2.33 h), 10- (3.08 h), or 20-day-old females (3.07 h), regardless of body size. Host attack rates and deet repellency among 15-day-old parous and 15-day-old nulliparous large- and small-bodied female *Ae. albopictus* were similar but deet repellency was less (by ≈2 h) against large females compared with small-bodied females.

Host attack rates in mosquitoes depend on many factors, including mosquito age and body size (Gouck and Smith 1962, Klowden and Lea 1984, Klowden 1988, Klowden et al. 1988), blood meal size (Klowden and Lea 1978), biting persistence of some species (Nasci 1986, 1991), and the presence of repellents (Khan et al. 1975). Although Schreck and McGovern (1989) characterized the repellency of deet against 5- and 6-day-old nulliparous *Aedes albopictus* (Skuse), additional information on the biologic factors that influence host attack rates and repellent efficacy is needed to define personal protection strategies against this mosquito. This study determined if chronological age, parity, or body size in *Ae. albopictus* influence mosquito attack rates on human hosts or the repellency of deet (*N,N*-diethyl-3-methylbenzamide).

Aedes albopictus was colonized from wild females collected in 1992 at Gainesville, FL. Adults were maintained indoors in screened cages (45 cm high × 38 cm wide × 35 cm diam) at 27°C, 70% RH, and a photoperiod of 14L:10D. Adults were fed on a 10% sucrose solution and bloodfeeding was on a restrained chicken. Large- and small-bodied female *Ae. albopictus* were obtained by placing 250 or 500 1st-instar larvae, respectively, in 1 liter of well water in an enamel tray (30 × 19 × 5 cm) and providing them food according to the regimen outlined in Table 1.

The effect of diet, larval density, and time on adult size was verified by examining sizes of recently emerged ($n = 30$) and 15-day-old mosquitoes ($n = 108$) from each rearing regimen. Size was determined by measuring the distance from the axillary incision to the tip of the wing, and excluded the wing fringe (Nasci 1986). Average wing length (±SD) in recently emerged small and large female mosquitoes was 2.30 ± 0.16 mm and 3.13 ± 0.10 mm, respectively. Average wing length was unchanged in 15-day-old females ($\bar{x}_{small} = 2.38 \pm 0.08$ mm; $\bar{x}_{large} = 3.13 \pm 0.09$ mm).

We determined host attack rates and the repellency of deet to *Ae. albopictus* in 5-, 10-, 15-, and 20-day-old nulliparous females and in 15-day-old parous females. Parous females were obtained by feeding 5-day-old mosquitoes on a restrained chicken and allowing them to oviposit. Mosquito age was calculated as the elapsed time (in days) from the median emergence time for all females in each body size cohort until the time of testing.

Host attack rates and the effectiveness of deet were assessed in the laboratory between 3 and 5 h after simulated sunrise. The experimental design for studying these factors in nulliparous females was a split plot in time (Steele and Torrie 1980). Main units were mosquito body size (large and small) and subunits were mosquito age (5, 10, 15, 20 day postemergence). The time required to complete observations on all mosquitoes in each subunit was considered one observation period. For nulliparous *Ae. albopictus* there were 4 observation periods each for the experiments on host attack rates and repellent effects (one each for 5-, 10-, 15-, and 20-day-old females). Responses for parous and nulliparous 15-day-old females were compared using a 2 × 2 factorial design (Steele and Torrie 1980) with body size and parity as factors.

To determine the host attack rate in nulliparous mosquitoes, a human volunteer inserted a

[1] Research was conducted in compliance with principles stated in the *Guide for the Care and Use of Laboratory Animals.* Volunteers gave informed consent to participate in this study.

Table 1. Larval feeding schedule for obtaining small- and large-bodied adult *Aedes albopictus* in the laboratory.

Time (h)	Quantity of larval food (mg) provided[1] For large adults[2]	For small adults[3]
12	50	20
24	50	30
48	100	0
72	150	150
96	200	0
120	100	50
144	50	0
168	50	50 (water replaced)
192	50	0

[1] Food = 3 parts liver powder, 2 parts brewer's yeast.
[2] Two hundred fifty 1st-instar larvae per rearing tray.
[3] Five hundred 1st-instar larvae per rearing tray.

latex glove-covered arm into a cage with 100 female mosquitoes of known age selected at random from one of 6 cages; 3 of the cages contained 100 small-bodied females each and 3 contained 100 large-bodied females each. Females in each cage previously had been collected at random by aspirator from stock cages of large or small mosquitoes. During each observation period, mosquitoes were denied access to sugar or water. The number of female mosquitoes that landed on and probed the skin exposed in a 47-cm^2 window cut from the glove in the forearm section, in 1 min, was counted and recorded. After 5 min, a different cage was selected at random and the presentation of the gloved forearm repeated. This process was continued until 4 observations had been made for each cage. The average number of landing/probing responses for each cage in an observation period was used to define the host attack rate of large- and small-bodied mosquitoes in each observation period. We used the same procedure to determine host attack rates for parous 15-day-old *Ae. albopictus*.

The response of *Ae. albopictus* to repellent was determined by exposing mosquitoes to 0.5 ml of a 25% solution of deet in ethanol applied to the hand of a human volunteer. The same technique used to select cages in studies of host attack rates was used to select cages for testing of repellent except that the volunteer's treated hand was exposed to mosquitoes for 3 min at 30-min intervals until the first confirmed bite (i.e., one bite followed by another bite within 30 min). The mean duration of protection from mosquito bite (MDPB) for each cage was used to characterize the repellency of deet to large- and small-bodied mosquitoes. We used the same procedure to determine MDPB for deet for parous 15-day-old *Ae. albopictus*.

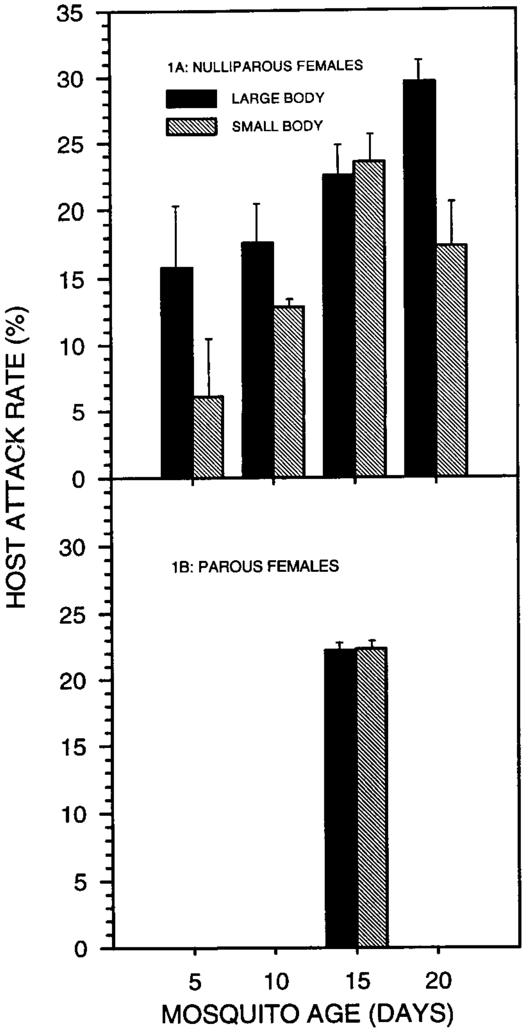

Fig. 1. Human host attack rates for nulliparous (1A) and parous (1B) female *Aedes albopictus*. Vertical bar represents one standard error of the mean.

Host attack rates and MDPB for nulliparous mosquitoes and for 15-day-old parous and nulliparous mosquitoes were analyzed using the general linear models (GLM) procedure (SAS Institute 1988). Means were separated using Tukey's ω procedure and a significance level of 0.05 (SAS Institute 1988).

Host attack rates in nulliparous female *Ae. albopictus* (Fig. 1A) were significantly influenced by body size and age ($P = 0.001$) and by interaction between age and body size ($P = 0.01$). On average, large-bodied females manifested a higher attack rate (22.2%) than small females (16.6%).

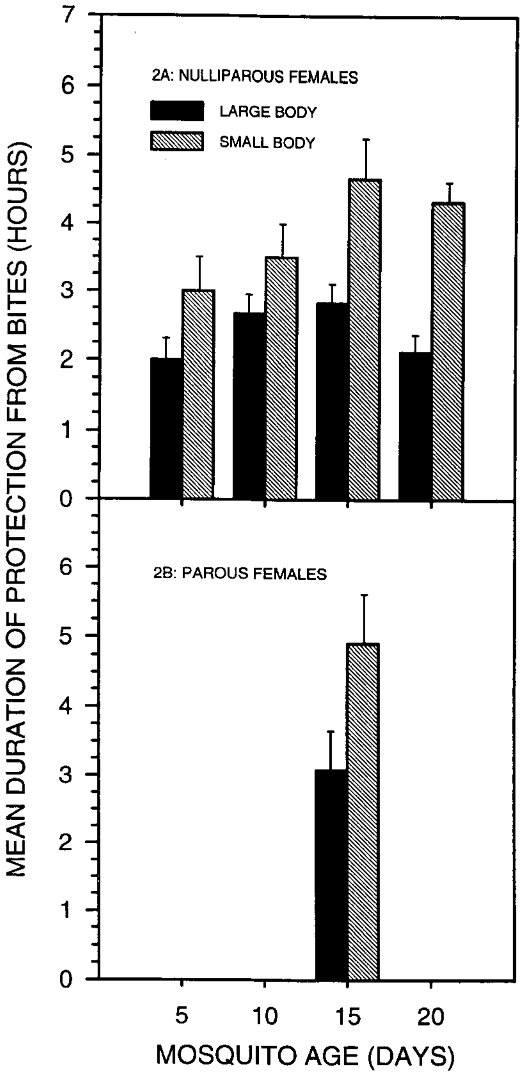

Fig. 2. Mean duration of protection from bites of nulliparous (2A) and parous (2B) female *Aedes albopictus* provided by 25% deet in ethanol applied to the skin. Vertical bar represents one standard error of the mean.

There was no difference in the attack rates of 15- (22.9%) and 20-day-old females (24.5%), which were higher than for 10-day-old females (15.1%), and rates for 5-day-old females (11.0%) were lower than for all other age groups. Increased attack rates in small-bodied nullipars on day 15, relative to the rates for large females, was the main source of interaction (Fig. 1A). The host attack rates of parous (Fig. 1B) and nulliparous 15-day-old females did not differ significantly on the basis of body size or parity status.

The mean duration of protection from bites of nulliparous *Ae. albopictus* provided by 25% deet applied to skin (Fig. 2A) was significantly influenced by mosquito body size ($P = 0.001$) and age ($P = 0.001$) and by interaction between age and body size ($P = 0.04$). Small-bodied nulliparous females were repelled longer (3.87 h) by deet than large females (2.31 h). The duration of deet repellency was longest (3.75 h) against 15-day-old females and shortest against 5-day-old females (2.33 h), but differences in the MDPB between 10-day- (3.08 h) and 20-day-old females (3.07 h) were not significant. Variations in the MDPB provided by deet against parous 15-day-old females (Fig. 2B) and nulliparous 15-day-old females were attributed to mosquito body size ($P = 0.001$); on average, small females (4.92 h) were repelled by deet nearly 2 h longer than large females (3.08 h).

Large-bodied nulliparous *Ae. albopictus* females have higher average attack rates on human hosts in the laboratory than small females and older females manifest higher attack rates than young females. Body size and parity do not appear to be factors that influence host attack rates in 15-day-old female mosquitoes.

The repellency of deet applied to skin is high against nulliparous and parous small-bodied female *Ae. albopictus* compared with large-bodied individuals, and is greatest against small-bodied 15-day-old females. There was no difference in the MDPB of deet against 15-day-old females when large and small individuals were compared on the basis of parity.

We observed a different average protection period for deet against small-bodied *Ae. albopictus* (3.87 h) compared with that for large-bodied females (2.31 h). This difference may be significant from the standpoint of disease transmission by mosquitoes because large-bodied females survive longer and have a greater infective life than small females (Mori 1979, Hawley 1988). Moreover, because large-bodied females have a higher host attack rate and are repelled by deet for shorter periods than small females, they are likely to comprise the majority in the human host-attacking mosquito population.

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