Journal of the American Mosquito Control Association, 12(1):58–63, 1996

HUMAN HOST AVIDITY IN *AEDES ALBOPICTUS*: INFLUENCE OF MOSQUITO BODY SIZE, AGE, PARITY, AND TIME OF DAY

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ABSTRACT. Diel patterns of human host attack by *Aedes albopictus* in the laboratory were studied in relation to mosquito body size, age, parity, and time of day. Analysis of responses in 10-, 15-, and 20-day-old females indicated a significant main effect due to time of observation in the diel period, as well as significant time × parity and time × age interactions. The distribution of mean host attack responses during the diel period was bimodal with ≈70% of all activity during photophase (0800–2000 h); attack rates were highest in the morning (0800 h) and evening (1400–2000 h) and lowest between 0200 and 0600 h. The diel pattern of attack responses was bimodal for nulliparous and parous females, but parous females were more active than nulliparous females between 1400 and 2000 h. This pattern became increasingly bimodal during photophase, as mosquitoes aged, regardless of mosquito body size or parity. Variations in host avidity patterns between young and old females suggest that mosquito repellent bioassays initiated early in the day, that last ≥6 h, or that use young females (≈5 days old) overestimate the protection period of deet against mosquitoes >10 days old.

INTRODUCTION

Bioassays of mosquito repellents, whether using screened cages (Granett 1938, Gouck and Smith 1962, Schreck 1985), small cages (American Society for Testing and Materials 1983), or animal models (Kasman et al. 1953, Reifenrath and Rutledge 1983, Rutledge et al. 1994), require repeated assessment of repellent activity over long (≥8-h) periods or the repetition of tests at different times in the day. Methods for the analysis of bioassay data, as well as empirical models that describe the effectiveness of repellents on skin (Rutledge et al. 1985), assume that biologic factors that influence mosquito responses to a repellent are independent and normally distributed in the test population.

But mosquito biting rates change depending on mosquito age and time of day (Gouck and Smith 1962, Smith et al. 1963). Repellent effectiveness (i.e., protection time) in afternoon tests against *Aedes aegypti* (Linn.) can exceed that in morning tests by as much as 1,000% (Gouck and Smith 1962). And mosquito age by time of day interactions (Gouck and Smith 1962) suggest that, as mosquitoes age, diel patterns of host attack change.

In *Aedes albopictus* (Skuse), human host attack rates are influenced by the age and body size of the mosquito (Xue et al. 1995). Large, 15–20-day-old nulliparous females manifest higher host attack rates than do young or small females, whereas the average protection time against *Ae. albopictus* afforded by deet (*N,N*-diethyl-3-methylbenzamide, 25% in ethanol) applied to skin is ≈2 h less for large females than for small females. These facts suggest that the components of experimental error in mosquito repellent bioassays that can be attributed to differences in the time of day or age, body size, or parity in mosquitoes may not be independent and random.

The study presented here was made to characterize the influence of size, age, parity, and time of day on diel patterns of human host attack in *Ae. albopictus* in the laboratory. Correlation of the effect of these factors with the protection period provided by repellent, in a deet-sensitive species such as *Ae. albopictus* (Schreck and McGovern 1989), should provide information that can be used to define standard conditions for mosquito rearing and repellent bioassays.

MATERIALS AND METHODS

Mosquito rearing: Aedes albopictus were F_5 progeny of wild adult mosquitoes collected at Gainesville, FL. Mosquitoes were reared, maintained, and observed at 27°C and a photoperiod of 14:10 h L:D. Adults were held in screened cages (45 × 38 × 35 cm) and provided 10% sucrose in water. Blood meals were obtained from restrained 3–4-wk-old chickens.

To compare host attack cycles in female *Ae. albopictus* as a function of adult body size, mosquito larvae were reared in 30 × 20 × 5-cm white enamel pans using 2 feeding regimens (Xue et al. 1995). Small mosquitoes (mean female wing length: 2.31 ± 0.16 mm) were obtained by rearing larvae in a low-diet, high-density environment (1 liter H_2O, 500 larvae, 30 mg food [3:2 liver powder:brewers yeast] daily). Large mosquitoes (mean female wing length: 3.12 ± 0.11 mm) were obtained using a high-diet, low-density environment (1 liter H_2O, 250 larvae, 80 mg food daily).

Host attack patterns: Host attack responses were recorded for nulliparous females at 2-h intervals over a 24-h (diel) period. Mosquitoes were 5, 10, 15, and 20 days old when tested. Each time, the test population consisted of 3 groups of 100 large females and 3 groups of 100 small females aspirated from stock cages (45 × 38 × 35 cm) and placed into one of 6 cages of the same size (with water and cubed sugar) 12 h before observations began. To evoke mosquito attack, a human volunteer (the same volunteer was used in all tests) placed a latex glove-covered arm into a cage for 1 min. Mosquitoes that probed through a 9.8 × 4.8-cm, 1.7-mm-mesh-covered window in the distal forearm section of the gloved arm were categorized as host avid (counts during scotophase were made using red light). This procedure was repeated for all 6 cages (cage order selected at random) and the results were averaged to obtain the mean percentage of host avidity of large and small females in each observation period.

The same method was used to determine host avidity responses in parous females. These mosquitoes were obtained by bloodfeeding small and large nulliparous females from stock cages on day 5. Bloodfed individuals were maintained in separate oviposition cages (45 × 38 × 35 cm) and then tested for host avidity when 10, 15, and 20 days old.

Experimental design and data analysis: Direct comparison of host attack responses in large nulliparous and parous females with responses in small nulliparous and parous females was made for 10-, 15-, and 20-day-old mosquitoes. The experimental design used for this purpose was a split plot (Steele and Torrie 1980). Main plots were body size (small, large) and parity (nulliparous, parous), subplots were mosquito age (10, 15, 20 days), and sub-subplots were times in the diel period.

Host attack responses also were analyzed according to parity of females. One analysis comprised responses of 5-, 10-, 15-, and 20-day-old nulliparous females, a second, separate, analysis comprised the responses of 10-, 15-, and 20-day-old parous females. A split-plot experimental design was used in each case with size as main plots, age as subplots, and times in the diel period as sub-subplots.

Differences in percentage of host attack were tested using analysis of variance procedures (SAS Institute 1988). Percentage data were converted to proportions and transformed by arcsine before analysis. Tukey's HSD (at $P = 0.05$) was used as the means separation test.

RESULTS

Diel patterns of host attack for *Ae. albopictus* in the laboratory are shown in Fig. 1. Analysis of the data for 10-, 15-, and 20-day-old females indicates that the main effects of time are significant ($F = 34.48$, df = 11,355, $P < 0.0001$). The distribution of mean host attack responses according to this factor is given in Table 1. The pattern of these responses is bimodal during the diel period with ≈70% of all activity during photophase. Host attack rates were highest in the morning, at 0800 h, and in the evening between 1400 and 2000 h and were lowest between 0200 and 0600 h.

Significant interaction between time and parity ($F = 3.27$, df = 11,355, $P = 0.0003$) and between time and age factors ($F = 4.66$, df = 22,355, $P < 0.0001$) indicates that temporal differences in host attack responses depend on parity (when responses are averaged across body size and age factors) and on mosquito age (when responses are averaged across body size and parity factors). Time × parity interaction means (Table 2) indicate a bimodal diel host attack pattern for nulliparous and parous females but increased host attack activity by parous females in the evening (1400–2000 h) compared with nulliparous females. Time × age interaction means (Table 2) indicate that host attack patterns become increasingly bimodal as mosquitoes age (with peaks at 0800 h and between 1400 and 2000 h), regardless of mosquito body size or parity.

Analysis of host attack responses based on parity in females indicates that time in the diel period ($F = 28.53$, df = 11,217, $P < 0.0001$) is a critical source of variation for nulliparous females. Maximum activity by this group was at 0800 h and between 1400 and 2000 h (Table 2). Significant interaction between body size and time in nulliparous females ($F = 3.09$, df = 11,217, $P = 0.0007$) was manifest in small individuals, regardless of their age, by a lower average host attack rate and reduced activity in the evening compared with large females. Age × time interaction means ($F = 5.19$, df = 33,217, $P < 0.0001$) for nulliparous females indicate a slight bimodal periodicity of host attack in 5- and 10-day-old mosquitoes during photophase (maxima at 0800 and 2000 h) but a distinct bimodal diel periodicity in 15- and 20-day-old females (maxima at 0800 h and from 1400 to 2000 h).

In parous females, age significantly influenced host attack responses ($F = 11.11$, df = 2,4, $P = 0.02$). Mean attack rates were highest in 20-day-old females (9.4 ± 2.9%), second highest in 15-day-old females (7.1 ± 3.5%), and lowest in 10-day-old females (5.1 ± 1.6%).

Parous females were most likely to attack human hosts in the laboratory at 0800 h and between 1400 and 2000 h (Table 3). Attack was least likely between 0200 and 0600 h. Time × size interaction means for parous females ($F =$

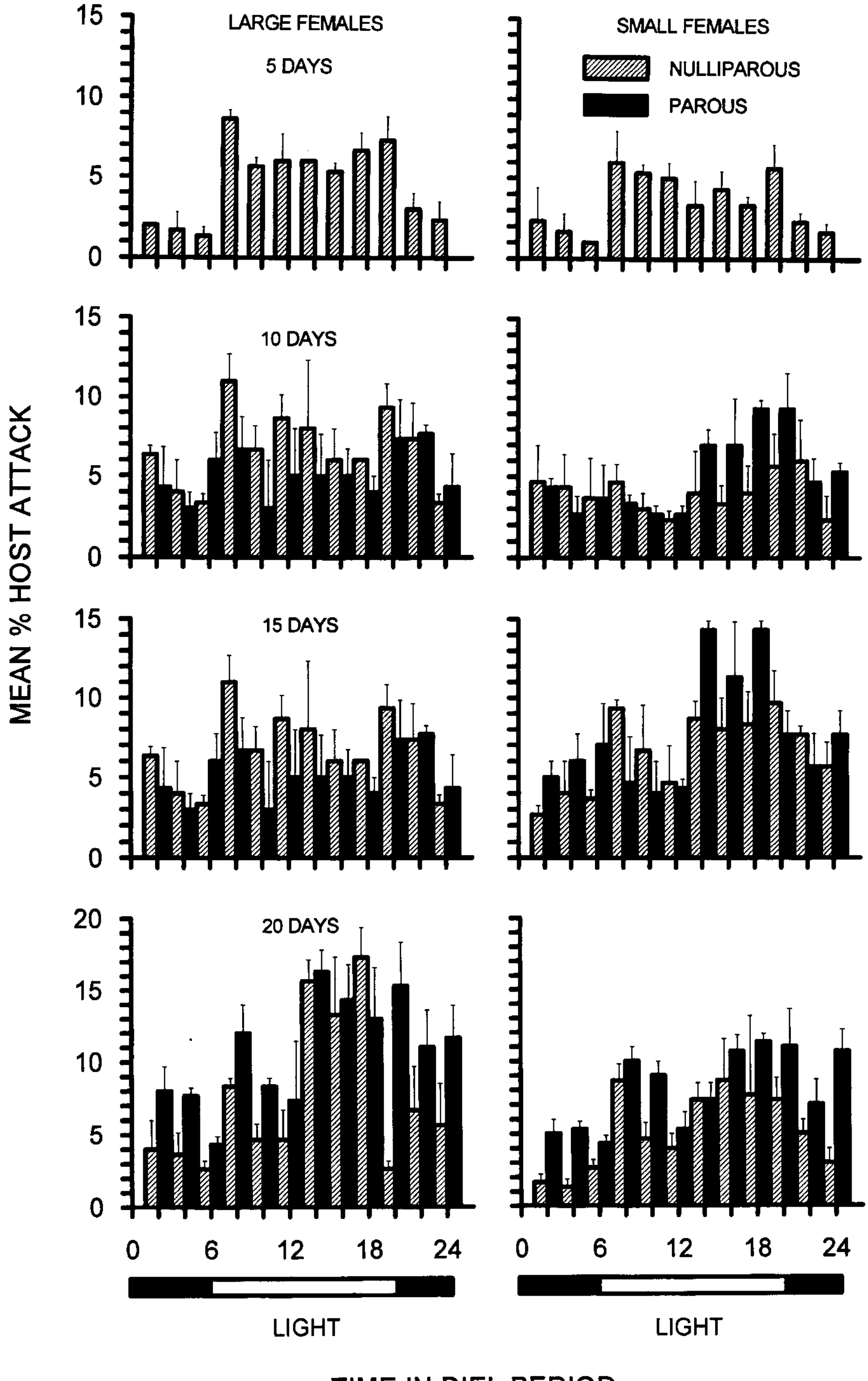

Fig. 1. Mean human host attack rates for female *Aedes albopictus* by time in the diel period. Vertical bar is one SD.

Table 1. Mean percentage (SD) of human host attack rates for 10-, 15-, and 20-day-old female *Aedes albopictus.*

Time (hour)	All females	Interaction of time with: Parity		Age (days)		
		Nulliparous	Parous	10	15	20
0200	4.4 (2.0)	3.7 (2.0)	5.1 (1.9)	4.0 (1.7)	3.6 (1.5)	4.6 (2.6)
0400	4.1 (2.1)	3.6 (2.0)	4.6 (2.1)	3.5 (1.5)	4.3 (2.1)	4.5 (2.5)
0600	4.2 (1.9)	3.4 (1.6)	5.1 (1.9)	4.2 (1.9)	5.0 (2.4)	3.5 (1.0)
0800	8.0 (2.9)	8.7 (2.3)	7.3 (3.4)	6.4 (3.2)	7.9 (2.7)	9.8 (1.9)
1000	5.1 (2.4)	5.2 (1.9)	5.1 (2.9)	3.8 (2.3)	4.8 (2.0)	6.6 (2.2)
1200	4.8 (2.3)	4.8 (2.4)	4.9 (2.3)	4.7 (3.0)	4.5 (1.3)	5.3 (2.4)
1400	9.5 (4.3)	8.6 (4.3)	10.4 (4.5)	6.0 (2.9)	10.8 (3.4)	11.6 (4.6)
1600	9.0 (3.7)	8.2 (3.9)	9.8 (3.7)	5.3 (2.2)	9.9 (2.4)	11.8 (3.4)
1800	9.9 (4.4)	8.9 (5.0)	10.7 (3.8)	5.8 (2.4)	11.4 (2.9)	12.3 (4.7)
2000	8.8 (3.4)	7.3 (3.1)	10.2 (3.4)	7.9 (2.4)	9.4 (2.3)	9.1 (5.2)
2200	6.9 (2.3)	6.7 (2.2)	7.1 (2.5)	6.4 (2.1)	6.8 (1.9)	7.4 (2.9)
2400	5.9 (3.1)	4.1 (2.1)	7.7 (3.0)	3.8 (1.6)	6.2 (1.9)	7.8 (4.1)

2.09, df = 11,160, P = 0.02) show increased activity for large females at 0800 h compared with small females and maximum host attack responses for small females at 1800 h. Age × time interaction means (F = 4.47, df = 22,160, P < 0.0001) reveal bimodal diel periodicity of host attack in 20- and 15-day-old parous mosquitoes (maximum activity in the evening for 15-day-old mosquitoes) but 4 activity maxima (i.e., at 0600, 0800, 1400 and 2000 h) for 10-day-old females.

DISCUSSION

In laboratory populations of *Ae. albopictus* in which body size, parity, and age factors were equally represented, differences in the host attack responses of mosquitoes were related to time in the diel period. In populations comprising equal representation by body size and age groups, but differing by parity status, nulliparous females showed higher host attack responses in the morning compared with parous females, and parous females showed higher host attack responses in the evening compared with nulliparous females. In groups of mosquitoes with equal representation by body size and parity groups, but separable by chronological age, the diel pattern of host attack is strongly bimodal for females ≥15 days old compared with females <15 days old.

Body size, age, and parity factors interact

Table 2. Mean percentage (SD) of human host attack rates for 5-, 10-, 15-, and 20-day-old nulliparous female *Aedes albopictus.*

Time (hour)	All females	Interaction of time with: Body size		Age (days)			
		Large	Small	5	10	15	20
0200	3.4 (1.9)	3.9 (2.1)	2.8 (1.8)	2.2 (1.3)	5.5 (1.7)	3.0 (1.5)	2.8 (1.8)
0400	3.1 (1.9)	3.4 (2.1)	2.8 (1.9)	1.7 (1.0)	4.2 (1.8)	4.2 (2.4)	2.5 (1.6)
0600	2.8 (1.6)	2.9 (2.9)	2.7 (1.6)	1.2 (0.4)	3.5 (1.6)	4.0 (2.1)	2.7 (0.5)
0800	8.4 (2.2)	9.5 (1.6)	7.2 (2.2)	7.3 (1.9)	7.8 (3.7)	9.8 (1.1)	8.5 (0.8)
1000	5.3 (1.6)	5.6 (1.1)	4.9 (1.9)	5.5 (0.5)	4.8 (2.3)	6.0 (2.0)	4.7 (1.0)
1200	4.9 (2.1)	5.9 (2.3)	4.0 (1.5)	5.5 (1.3)	5.5 (3.6)	4.5 (1.7)	4.3 (1.5)
1400	7.6 (4.0)	9.3 (4.7)	5.8 (2.7)	4.7 (1.7)	6.0 (3.8)	8.2 (2.5)	11.5 (4.7)
1600	7.3 (3.6)	8.6 (4.0)	6.1 (2.9)	4.8 (0.9)	4.7 (2.0)	8.8 (2.2)	11.0 (4.0)
1800	7.9 (4.6)	10.1 (4.9)	5.8 (3.4)	5.0 (2.0)	5.0 (1.5)	9.3 (2.6)	12.5 (6.4)
2000	7.1 (2.7)	7.2 (3.3)	7.1 (2.3)	6.5 (0.8)	7.5 (2.5)	9.5 (2.4)	5.0 (2.7)
2200	5.7 (2.6)	6.2 (2.9)	5.3 (2.3)	2.7 (0.9)	6.7 (2.3)	7.7 (2.0)	5.8 (2.2)
2400	3.6 (2.0)	4.0 (2.2)	3.2 (1.8)	2.0 (0.9)	2.8 (1.1)	5.2 (2.1)	4.3 (2.4)

Table 3. Mean percentage (SD) of human host attack rates for 10-, 15-, and 20-day-old parous female *Aedes albopictus*.

Time (hour)	All females	Interaction of time with: Body size, Large	Body size, Small	Age (days), 10	Age (days), 15	Age (days), 20
0200	5.1 (2.7)	5.3 (2.6)	4.7 (0.8)	4.3 (1.6)	4.3 (1.3)	6.5 (2.0)
0400	4.6 (2.6)	4.6 (2.5)	4.6 (1.8)	2.8 (0.9)	4.5 (2.0)	6.5 (1.3)
0600	5.1 (2.8)	5.1 (1.7)	5.0 (2.2)	4.8 (2.1)	6.0 (2.6)	4.3 (0.5)
0800	7.3 (4.2)	8.6 (3.0)	6.0 (3.4)	5.0 (2.2)	6.0 (2.5)	11.0 (1.7)
1000	5.1 (3.3)	4.8 (3.0)	5.2 (3.1)	2.8 (1.9)	3.6 (1.3)	8.6 (0.8)
1200	4.9 (2.9)	5.6 (2.9)	4.1 (1.3)	3.8 (2.3)	4.5 (0.8)	6.3 (2.9)
1400	10.4 (5.9)	11.3 (5.2)	9.5 (3.6)	6.0 (2.0)	13.5 (1.2)	11.8 (5.0)
1600	9.8 (5.3)	10.0 (4.3)	9.6 (3.1)	6.0 (2.4)	11.0 (2.2)	12.5 (2.6)
1800	10.8 (5.6)	9.9 (4.8)	11.6 (2.2)	6.6 (3.0)	13.6 (1.2)	12.1 (2.4)
2000	10.2 (5.3)	11.2 (4.1)	9.3 (2.3)	8.3 (2.4)	9.3 (2.4)	13.1 (3.4)
2200	7.1 (3.7)	8.3 (2.5)	5.7 (1.8)	6.1 (1.9)	6.0 (1.5)	9.0 (2.9)
2400	7.7 (4.2)	7.5 (3.6)	7.8 (2.5)	4.8 (1.4)	7.1 (1.1)	11.1 (1.8)

with time in the diel period to shape patterns of host attack in *Ae. albopictus*. Several profiles of host attack activity result. Responses delineated according to parity, for example, are influenced by the body size of the mosquito. The average attack rates and diel patterns of attack are, respectively, higher and bimodal in large nulliparous females compared with small females. In parous females, age and time effects determine host attack rate, as does interaction between age and body size factors. Females ≥15 days old were more likely to attack human hosts than were younger females (<15 days old), although both groups were most active in the evening. Highest attack rates overall were manifested by old, large-bodied parous females.

Host attack in *Ae. albopictus* varies significantly during the diel period but particularly during photophase when bioassay of repellents is performed. Similar observations have been made for many other species of mosquitoes. Gouck and Smith (1962) concluded that maximum uniformity of results in repellent studies with *Ae. aegypti* could be obtained using 7–8-day-old mosquitoes, provided dosages of repellent were low enough to permit the completion of testing in the morning or an afternoon, but that a comparison of morning and afternoon test results should not be made.

The results of our study indicate that nulliparous, 5-day-old, large-bodied *Ae. albopictus* provide the most consistent, albeit lowest, mean host attack responses of any of the combinations of mosquito size, age, or parity factors tested. To date, this age group has been used as a standard in laboratory bioassays of repellents against *Ae. albopictus* (Schreck and McGovern 1989). However, mosquitoes categorized in other ways, e.g., by parity or age, can provide a more rigorous test of repellent effectiveness (because of higher mean host attack rates) than is possible with 5-day-old nullipars. Fifteen- and 20-day-old females tested between 1400 and 2000 h are one example. The host avidity differential for this group (Table 4), when calculated as a percentage of the responses for 5-day-old females, ranges from −18 to +148%. The differential is negative only between 1000 and 1200 h (in 15-day-old females) and at 1200 h (in 20-day-old females). Age-dependent differences such as these suggest that for repellent bioassays initiated early in the day and performed for ≥6 h using 5-day-old nulliparous females, the protection period extrapolated for 15- and 20-day-old females may be too long.

Under actual use conditions, overestimation of the repellent protection period is unlikely to

Table 4. Human host avidity differential (%) for 10-, 15-, and 20-day-old female *Aedes albopictus* using host attack rates for 5-day-old nulliparous females as the baseline.

Time (hour)	Mosquito age (days), 10	15	20
0800	−12.3	8.2	34.2
1000	−30.9	−12.7	21.8
1200	−14.5	−18.2	−3.3
1400	27.6	129.8	148.9
1600	10.4	106.3	128.0
1800	16.0	128.1	146.2
2000	21.5	44.0	40.3

result in a significant failure of personal protection measures targeted at nuisance-annoyance mosquito activity. However, in situations where the threat of disease transmission by mosquitoes is ongoing, particularly if the vectorial capacity of the mosquito population is high (as could be the case in a population of old, large females), the consequences of overestimation of the repellent protection period are potentially serious.

At least 2 techniques can be used to address time-of-observation-based systematic error in repellent bioassays that use laboratory-reared *Ae. albopictus* as the test organism. The first technique, which involves the continued use of ≈5-day-old nulliparous females, is to recognize the potential for negative host avidity when bioassays are initiated early in the day and to restrict the extrapolation of results to nulliparous females in the ≈5-day-old group. The second technique meets the requirement for equivalent host avidity during bioassay (Smith 1970); however, it requires that tests be performed only between 1400 and 2000 h. For repellents that require a bioassay time ≥6 h, a single test could comprise 2 phases (early and late), of 3–6 h duration each, with the order of dates of testing for replicates of each phase selected at random.

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