*Journal of the American Mosquito Control Association*, 13(2):205–207, 1997

# DIEL PATTERNS OF PUPATION, EMERGENCE, AND OVIPOSITION IN A LABORATORY POPULATION OF *AEDES ALBOPICTUS*

RUI-DE XUE AND DONALD R. BARNARD

*USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology, P. O. Box 14565, Gainesville, FL 32604*

ABSTRACT. There was no apparent daily pattern of pupation in *Aedes albopictus* in the laboratory [14:10 h (L:D); lights on: 0600 h, lights off: 2000 h], but diel patterns of emergence and oviposition were influenced by mosquito body size. Emergence rate was highest in large-bodied male mosquitoes at 1600 h and in small males at 1000 h but was lowest in large and small males, respectively, at 2400 h and 0200 h. Peak emergence of females was at 1600 h, regardless of body size; lowest emergence was at 0400 h. Half of all ovipositions by large females in their first gonotrophic cycle (GC1) were at 2000 and 2200 h but at 1800 to 2000 h in the second gonotrophic cycle (GC2). In small females, oviposition in GC1 and GC2 was highest at 1800 and 1600 h, respectively, and lowest at 0400 h. Half of all ovipositions in small females were at 1600 to 1800 h.

To our knowledge, there are no reports of diel patterns of pupation or emergence in laboratory populations of *Aedes albopictus* (Skuse). Cycles of oviposition are known for this mosquito in Asia (Chadee and Corbet 1989, Tsuda et al. 1989), but comparable data for North America do not exist. In the study reported here we observed daily patterns of pupation, emergence, and oviposition of *Ae. albopictus* in the laboratory and determined the influence of mosquito body size on these patterns.

Information from this study should be useful for improving mass rearing procedures for *Ae. albopictus*, for growing uniform populations of larvae and adult mosquitoes for use as test organisms in biological assays, and for avoiding the potential for development of laboratory ecotypes (Mackauer 1976). Data on oviposition patterns can be used to design tests of oviposition attractants and repellents.

*Mosquito rearing:* Mosquitoes were progeny of recently (1995) colonized adult *Ae. albopictus* collected at Gainesville, FL. Large and small mosquitoes (average wing length ± SD in newly emerged females: 3.13 ± 0.10 mm and 2.30 ± 0.16 mm, respectively) were reared and maintained separately (27°C, 14:10 h [L:D] photoperiod, photophase: 0600 to 2000 h) using the techniques described by Xue et al. (1995). Blood meals were obtained from restrained 3–4-wk-old chickens.

*Pupation pattern:* Shortly after feeding ceased, 4th instars in a randomly selected rearing pan (separate pans were selected for large and small larvae) were transferred in groups of approximately 70 large or 50 small individuals to new rearing pans containing 1 liter of well water. Beginning with the first even-numbered hour (e.g., 0800, 1200 h) following the appearance of the first pupa in the new pans, successive pupae were counted and removed from each pan every 2 h. Observations of pupation were replicated on 3 separate dates. Each replicate yielded a frequency distribution (over time) of the pupation responses for one cohort (i.e., rearing pan) of large mosquitoes and one cohort of small mosquitoes.

*Emergence pattern:* Groups of 100 large or 100 small pupae were removed from randomly selected rearing pans (separate pans were used for large and small pupae) and placed in 100 ml of well water in separate plastic cups (8 cm diam × 5 cm high) covered with nylon mesh. Following the emergence of the first adult, successive new adults were removed from each cup every 2 h (beginning with the first even-numbered hour) and counted. Observations of emergence were replicated on 3 separate dates. Each replicate yielded a frequency distribution (over time) of the emergence responses for one cohort (i.e., rearing pan) of large mosquitoes and one cohort of small mosquitoes.

*Oviposition pattern:* Groups of 5-day-old large and small nulliparous female *Ae. albopictus* were bloodfed on chicken. Three hundred mosquitoes were collected from each group (600 females total) and placed in one of 6 oviposition cages (45 × 38 × 35 cm) at the rate of 100 large or 100 small bloodfed females per cage. Sugar water (10%) was available at all times. The oviposition substrate was a 25 × 7.5-cm strip of white filter paper placed around the inside vertical surface of a 500-ml polystyrene cup (painted black) containing 250 ml of well water. Once oviposition started (3 days after blood feeding), and at 2 h intervals thereafter (beginning with an even-numbered hour), the oviposition cup, paper strip, and water were removed from each cage and replaced with a new cup, strip, and water. This procedure was repeated until oviposition stopped. Paper strips were air-dried, and the number of eggs on each strip was determined by visual inspection using a 20× hand lens. For females in the second gonotrophic cycle (GC2), the procedure used to determine oviposition patterns for females in the first gonotrophic cycle (GC1) was repeated except that parous mosquitoes were bloodfed when 13 days old.

*Experimental design and data analysis:* The percentage response for pupation, emergence, and oviposition was calculated by dividing the mean response at each observation time by the sum of the mean responses (12) for the diel period. Percent-

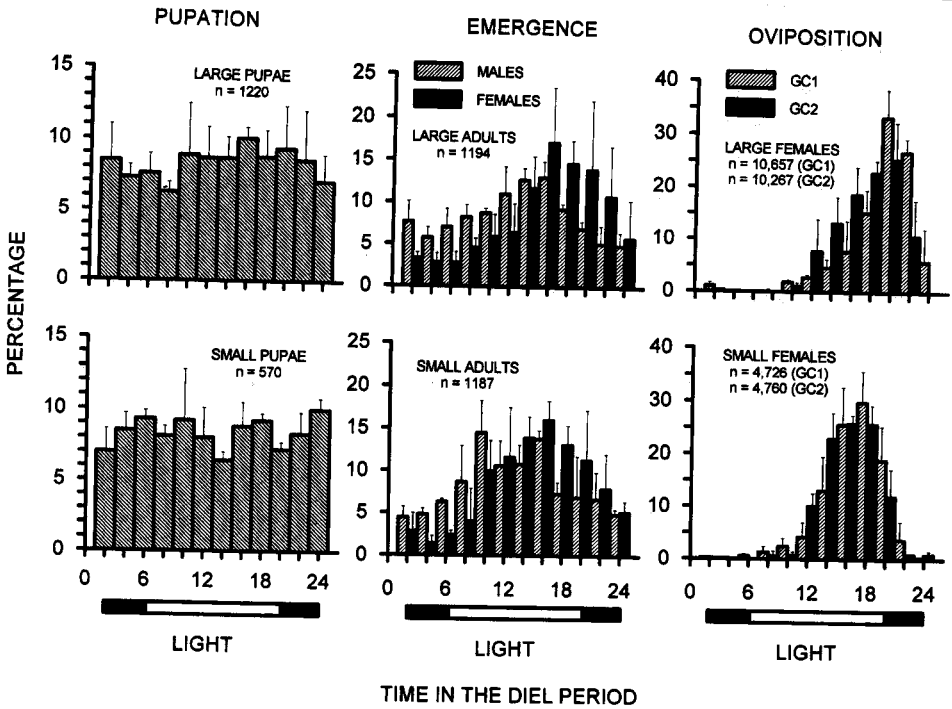


Fig. 1. Mean percentage pupation, male and female emergence, and oviposition during 2 gonotrophic cycles (GC1, GC2) in large and small *Aedes albopictus* by time of observation in the diel period. Vertical bar is one standard error of the mean.

ages were calculated separately, according to body size, for pupation, emergence, and oviposition and by sex and gonotrophic cycle for emergence and oviposition, respectively. A completely randomized design with replication in time was used in all studies. The experimental unit in pupation and emergence studies was a cohort (i.e., rearing pan) of larvae or pupae; for oviposition, the experimental unit was a cage of 100 bloodfed female mosquitoes. Percentage responses were transformed by arcsin and analyzed using analysis of variance (ANOVA) procedures (SAS Institute 1988). Duncan's new multiple range test ($P = 0.05$) was used for means separation.

*Pupation:* Differences in percentage pupation by time in the diel period were not significantly different when data were analyzed according to body size (Fig. 1). The comparison of pupation responses in large and small pupae by time in the diel period showed only one significant difference ($P = 0.04$): at 0800 h (large: 6.3%, small: 8.1%). In large mosquitoes, percentage pupation was lowest (21.1%) from 0400 to 0800 h. In small pupae, percentage pupation was lowest (35.2%) from 0400 to 1000 h.

*Emergence:* Emergence responses of large and small male and female mosquitoes differed significantly according to time in the diel period (Fig. 1). In large males, emergence was highest (13.1%) at 1600 h and lowest (4.9%) at 2400 h. In small males emergence was highest (14.6%) at 1000 h and lowest (4.4%) at 0200 h. Half of all large males emerged between 1000 and 1800 h, whereas half of all small males emerged between 1000 and 1600 h. In female *Ae. albopictus,* highest emergence of both large and small individuals (17.2 and 16.1%, respectively) was at 1600 h; lowest emergence (2.8 and 1.4%, respectively) was at 0400 h. Fifty percent of large females emerged between 1600 and 2000 h, compared to 1200 to 1800 h for small females. Differences in the emergence responses of large and small mosquitoes of the same sex, when compared by time in the diel period, were not significant.

*Oviposition:* For large females, Fifty percent of oviposition occurred at 2000 to 2200 h in GC1 but at 1800 to 2000 h in GC2. In both gonotrophic cycles, oviposition was highest (GC1: 34.1%, GC2: 25.8%) at 2000 h and lowest (GC1: 0.1%, GC2: 0.03%) at 0400 h (Fig. 1). For small females, in GC1, oviposition was highest at 1800 h and lowest (0.07%) at 0400 h; in GC2, oviposition was highest (26.1%) at 1600 h and lowest (0%) at 0400 h. Half of all oviposition in small females in both gonotrophic cycles was at 1600 to 1800 h. Oviposition responses of large and small females compared by time in the diel period were significantly different in GC1 at 1400 h (large: 4.6%, small: 13.1%), 1600 h (large: 7.8%, small: 25.9%), 1800 h (large: 15.3%, small: 30.4%), and 2000 h (large: 34.1%, small: 19.1%). Differences in GC2 females were

significant only at 2000 h (large: 25.7%, small: 11.9%). In females grouped by body size, differences in percentage oviposition compared on the basis of gonotrophic cycle and time in the diel period were significant in large females at 1400 h (GC1: 4.6%, GC2: 13.2%), 1600 h (GC1: 7.8%, GC2: 18.8%), 1800 h (GC1: 15.3%, GC2: 23.1%), and 2200 h (GC1: 27.1%, GC2: 10.7%). Differences in small females were significant at 1400 h (GC1: 4.6%, GC2: 13.2%).

The results of this study indicate that there is no diel pupation pattern in *Ae. albopictus*. In contrast, time in the diel period was an important factor for characterizing temporal patterns of oviposition activity and, to a lesser extent, adult emergence, both of which occurred in daily cycles. Emergence patterns of male and female mosquitoes were not influenced by body size; however, small males tended to emerge earlier than large males and large females later than small females during photophase. Emergence activity was lowest for all mosquitoes during late scotophase.

Oviposition activity was highly cyclical. In small females, the time of oviposition was not affected by gonotrophic cycle and was usually complete by the end of photophase. In large females, most ovipositions in GC1 were earlier in the photophase than in GC2.

The patterns of oviposition in *Ae. albopictus* observed in our study are similar to those for Asian strains of the mosquito, although no previous report has differentiated oviposition responses in *Ae. albopictus* on the basis of body size. Using a Singapore strain of *Ae. albopictus,* Chadee and Corbet (1989) observed that oviposition in the laboratory occurred mainly during photophase (98%) as well as during evening "twilight" and near the end of scotophase, and that about half (56%) of all eggs were laid 2 h before the end of photophase. In Japan, Tsuda et al. (1989) reported 79% of all oviposition by *Ae. albopictus* in the field to occur in a 2–3-h period each day; however, the temporal location of the oviposition peak changed each day and depended on environmental conditions. In Calcutta, Gubler (personal communication), showed oviposition activity in *Ae. albopictus* to increase after 1100 h and to reach a peak at 1700 h. Peak oviposition in his study coincided with peak times of biting activity, and the latter peak was thought to result, at least in part, from the biting activity of hungry females having recently oviposited.

## REFERENCES CITED

Chadee, D. D. and P. S. Corbet. 1989. Diel pattern of oviposition in the laboratory of the mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae). Ann. Trop. Med. Parasitol. 83:423–429.

Mackauer, M. 1976. Genetic problems in the production of biological control agents. Annu. Rev. Entomol. 21: 369–385.

SAS Institute. 1988. SAS/STAT user's guide, release 6.03 ed. SAS Institute, Cary, NC.

Tsuda, Y., M. Takagi and Y. Wada. 1989. Field observations on oviposition time of *Aedes albopictus*. Trop. Med. 31:161–165.

Xue, R. D., D. R. Barnard and C. E. Schreck. 1995. Influence of body size and age in *Aedes albopictus* on human host attack rates and the repellency of deet. J. Am. Mosq. Control Assoc. 11:50–53.