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EGG DEVELOPMENT MAY REQUIRE MULTIPLE BLOODMEALS AMONG SMALL *AEDES AEGYPTI* (DIPTERA: CULICIDAE) FIELD COLLECTED IN NORTHEASTERN MEXICO

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Aedes aegypti imbibes multiple blood meals in each gonotrophic cycle (Scott et al. 1993). It prefers human blood, and rarely feeds on sugar (Edman et al. 1992; Van Handel et al. 1994). Host-seeking *Ae. aegypti* females have bodies of widely differing sizes, and exhibit variable blood feeding frequencies (Nasci 1986). Smallest females often engage in more multiple feeding than largest ones (Nayar & Sauerman 1975). This metabolic adjustment is compensated by remaining previtellogenic in Christopher's stage I-IIb (Roubaud 1923; MacDonald 1956), hence they will require a subsequent blood meal to become vitellogenic beyond stage IIIa and produce mature eggs (Clements 1992).

Both wing-length and ovarian development of engorged mosquitoes have been examined only for laboratory *Ae. aegypti*, where the previtellogenic phase was found only in females ≤2.9 mm (Feinsod & Spielman 1982). Relationships between wing-length and egg maturation for neotropical *Anopheles* species was explored by using the logistic regression model (Lounibos 1994; Lounibos et al. 1998). This paper describes the wing-length distribution of wild *Ae. aegypti* host-seeking females, and a logistic regression model to estimate the probability for the previtellogenic phase occurrence in engorged females.

The study site was a dengue endemic neighborhood in Monterrey, Mexico. Climate in Monterrey is arid with an annual mean rainfall and temperature of 450 mm (range = 270 mm-620 mm), and 23°C (range = -2°C-44°C), respectively. The two rainy months are May and October, and the highest population densities of *Ae. aegypti* occur during these months (Salas-Luevano & Reyes-Villanueva 1994). Six human-biting collections (one in October 1994, May 1995, October 1995, and three in October 1996) were gathered from five houses between 17:00 to 20:00 h. Each collection consisted of 60 host-seeking female *Ae. aegypti*. Each collection was made within a 5-day period by a two-person team (the human bait and the collector catching mosquitoes on the human bait). Mosquitoes landing (i.e., posed) on the legs and arms of the human bait were immediately captured with a mouth aspirator by the collector. They were held in cardboard cages (25 × 15 cm) and transported to the laboratory immediately after their capture. Females were anesthetized by exposure to -2°C for 10 minutes and those with

abdomen completely empty were separated for blood feeding, whereas those with blood vestiges in their stomach and gravid females were removed. They had access to water and fed to repletion without interruption on the hand of a human volunteer 24 h after capture. Fed females were held 48 h before dissection to determine ovarian development under insectary conditions of 28°C and R. H. 80%. They were immobilized again by freezing, and ovaries removed and placed into a 0.5% saline solution. Christopher's stage of oocyte development was registered (Clements and Boocock 1984), and adult body size was estimated by measuring the left wing-length (Kelly & Edman 1992). If ovarian follicles had developed beyond stage IIIa, the mosquito was considered positive for egg development, otherwise, if the ovarian follicles were in stage I-IIb, the mosquito was previtellogenic (Clements 1992).

Mean ± SE wing length (mm) were calculated for all females. Wing-length of previtellogenic, and vitellogenic females among collections were analyzed by ANOVA (SAS 1995). The data set consisted of 360 scores made up of two variables: The dependent "previtellogenic" (a binary variable), and the covariate "wing-length" (WL). Dependent variable, which is the probability to be a previtellogenic female, was equal to 1 if the mosquito was previtellogenic and it was equal to 0 if the mosquito had follicles in stage IIIa. The logistic regression model was used (SAS 1995) to estimate the covariate WL influence on the probability to pass from previtellogenic to vitellogenic phase after engorgement.

One hundred and twenty three *Ae. aegypti* host-seeking females out of 360 (34%), remained previtellogenic in stage I-IIb. Of these, 58% were in Christophers stage IIb, while 48% exhibited previtellogenic stage I. Previtellogenic females exhibited a wing-length range from 1.8 to 3.1 mm, which was similar to the range observed for all mosquitoes in this survey (1.7-3.2 mm). There were 13 previtellogenic mosquitoes (2.92-3.15 mm range) with wings longer than 2.9 mm, which was 11% of total previtellogenic (13/123), and 4% of all captured individuals (13/360). Mean wing-length for all females was 2.52 ± 0.03 mm. A *t* test for unpaired means ($t = 3.20$, $df = 5$, $P < 0.001$) showed that previtellogenic females (2.45-0.02 mm, $n = 123$) were smaller than vitellogenic ones (2.56 ± 0.01 mm, $n = 237$). The biggest previtello-

TABLE 1. MEAN WING-LENGTH ± SE (STANDARD ERROR) (MM) FOR PREVITELLOGENIC AND VITELLOGENIC FEMALES OBSERVED IN SIX HOST-SEEKING *Aedes aegypti* COLLECTIONS FROM MONTERREY, MEXICO.

Collection	No. previtellogenic females	Mean ± SE[1]	No. vitellogenic females	Mean ± SE[2]
Biting (Oct. 1994)	17	2.76 ± 0.05 a	43	2.69 ± 0.04 a
Biting (May 1995)	12	2.66 ± 0.08 ab	48	2.66 ± 0.04 a
Biting (Oct. 1995)	15	2.49 ± 0.05 bc	45	2.57 ± 0.03 ab
Biting (Oct. 1996)	23	2.37 ± 0.06 c	37	2.43 ± 0.04 b
Biting (Oct. 1996)	30	2.34 ± 0.04 c	30	2.45 ± 0.04 b
Biting (Oct. 1996)	26	2.32 ± 0.06 c	34	2.45 ± 0.04 b
Total	123	2.45 ± 0.06	237	2.56 ± 0.03

[1]Means for previtellotenic females with different letters were significantly different ($F = 7.94$, $df = 5$, $P < 0.0001$) among collections according to a REGWQ multiple comparison test.

[2]Means for vitellogenic females with different letters were significantly different ($F = 7.04$, $df = 5$, $P < 0.0001$) among collections according to a REGWQ multiple comparison test.

genic mosquitoes were collected in October 1994 (2.76 ± 0.05 mm) with a wing-length significantly higher ($F = 7.94$, $df = 5$, $P < 0.001$) than that of all *Ae. aegypti* mosquitoes collected in 1996 (Table 1).

The overall logistic model to calculate the occurrence probability for a previtellogenic female was significant ($P = 0.01$) according to a χ^2 distribution. The likelihood-ratio was -2 LOG L = 10.63 with 1 *df*, versus a $\chi^2 = 6.63$ from tables at $P = 0.01$. The model predicted 58.2% of the responses correctly. In addition, the Wald statistics ($\beta/S.E._{\beta}$)^2 which also has a χ^2 distribution with 1 *df*, was also significant ($P = 0.001$) with a value of 10.25. The coefficient for the "wing-length" covariate was negative and highly significant ($\beta = -1.2080$, $P = 0.001$), demonstrating an inverse relationship between wing-length and the likelihood for the previtellogenic female occurrence. The ratio $\pi/(1 - \pi)$ in the logit transformation is referred to as the Odds, and an important way of interpreting the logistic regression coefficient β is its effect on the Odds (Agresti & Finlay 1986). Particularly, when the antilog of β as e^{β} was calculated, an output of 0.2987 was obtained of which the reciprocal was 3.34. This means that after engorgement, smallest mosquitoes are 3.34 times more likely to remain as previtellogenic than largest mosquitoes.

In this survey, the previtellogenic phase was observed in small *Ae. aegypti* females as well as in large biting females (>2.9 mm). Herein, the results showed that 34% of the host-seeking females will need to have at least two blood meals to mature eggs, and three to complete a full gonotrophic cycle. Moreover, it is highly plausible that a human could be the host when a mosquito is imbibing the 2nd or 3rd blood meal, because this vector has acquired a noticeable synanthropic feeding habit to survive in domestic settings (Chow et al. 1993; Day et al. 1994).

Our results are not in line with one report, in which, *Ae. aegypti* exhibited a size-dependent divergence in host-seeking responses in favor of large individuals (Klowden et al. 1988). However, when previtellogenic mosquitoes were sorted by their wing-length in ascending order, the small ones located in the 2.2-2.8 mm classes were clearly predominant (64% = 79/123) in the collections. The prediction power of the logistic regression exploited here, to estimate the probability of being a previtellogenic *Ae. aegypti* mosquito, is acceptable according to the χ^2 distribution (Agresti & Finlay 1986). The model should be validated in further field surveys with the samplings including blood-deprived, and host-seeking females from human-landing collections. The high anthropophilism of *Ae. aegypti*, and the likely influence on oogenesis by the low isoleucine in human blood (Day et al. 1994) would need to be considered when constructing a more powerful model.

SUMMARY

Samplings of *Aedes aegypti* host-seeking females in Monterrey, Mexico, exhibited a 34% of previtellogenic phase in stage I-IIb after engorgement. Failure to reach the vitellogenic phase was present in females with a wing-length range from 1.8 to 3.1 mm. Logistic regression for a binary response (where Y = 1, previtellogenic, and Y = 0, non-previtellogenic), in function of wing-length as covariate, demonstrated that the likelihood of previtellogenic phase increases 3.34 times in smallest mosquitoes compared to the largest.

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