Effect of body size and sugar meals on oviposition of the yellow fever mosquito, *Aedes aegypti* (Diptera: Culicidae)

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Received 3 August 2009; Accepted 24 March 2010

ABSTRACT: The effects of dietary sugar and body size on the oviposition of *Ae. aegypti* were studied under laboratory conditions. In female mosquitoes provided with sugar, the start of maximum fecundity was significantly delayed and the oviposition period was longer than in females provided with water. The peak of oviposition was also delayed in sugar-fed females. Large females oviposited more eggs per day than small females at maximum fecundity and during eight days of observations. Large females also visited significantly more water-containing cups in their cages per day than small females at maximum fecundity. During the eight days of observations, large females and sugar-fed females visited more water-containing cups in their cages than water-fed small females. Both large females and sugar-fed females oviposited their eggs at sites higher above the water line than water-fed small females. These results suggested that large and sugar-fed female *Ae. aegypti* mosquitoes had more energy reserves and oviposited their eggs at higher sites, which would lead to a time lag in hatching. ***Journal of Vector Ecology*** **35 (1): 56-60. 2010.**

Keyword Index: Oviposition, sugar meal, body size, *Aedes aegypti.*

INTRODUCTION

Aedes aegypti (L.) mosquitoes are the principal vectors of dengue virus (Barrett and Higgs 2007, Halstead 2008). Since a dengue fever vaccine has yet to be developed, the best way of preventing dengue fever at present is to reduce the habitats of *Ae. aegypti* larvae. *Ae. aegypti* females oviposit on damp substrates just above the water line in human-made containers in urban environments and in tree holes in their natural habitat (Christophers 1960). Females visit a number of sites to lay egg batches, a behavior called “skip oviposition” (Reiter 2007). Reiter (2007) suggested that skip oviposition is a strategy to avoid overcrowding in sites where larval nutrition is limited and to minimize risks associated with temporary sites.

The skip oviposition strategy requires females to fly longer distances to oviposit their eggs, leading to greater energy consumption, which suggests that energy reserves may have an effect on this behavior. Large *Ae. aegypti* females store more energy reserves both at adult emergence and after reaching their maximum size with sugar feeding (Briegel 1990). Body size and sugar availability may influence *Ae. aegypti* oviposition behavior, with larger females or females with easy to access to sugar able to disperse their eggs more widely. In other words, female mosquitoes with more energy reserves may be able to better disperse their eggs so as to minimize risks to the eggs.

The period between oviposition and hatching of mosquito eggs varies among tree holes, a strategy referred to as “germ banking,” which may have evolved in response to environmental factors such as increased predator abundance, reduced resource availability, competition, and cohort structure (Evans and Dennehy 2005). Using tree holes as oviposition sites also seems to be important for the hatching of mosquito eggs, because immersion in water is critical for hatching.

In this study, we examined the effect of sugar meals and body size of *Ae. aegypti* females on oviposition in the laboratory. The location of oviposited eggs, (i.e., height above the water surface) was also investigated.

MATERIALS AND METHODS

Mosquitoes

Ae. aegypti females used in this study were collected in Ho Chi Min City, Vietnam in August, 2007. Larvae were reared to the adult stage in pans containing tap water and yeast tablets, maintained at 27° C, with a 16-h photoperiod per 24 h. F2 and F3 generation mosquitoes were used in these experiments. Large and small mosquitoes were reared as described by Naksathit et al. (1999). Wing length was used as a measure of body size, because there is a high correlation between *Ae. aegypti* wing length and body weight (Christophers 1960). One wing of each female was removed and placed on a microscopic slide, covered with a cover glass, and measured at 20X magnification under a dissecting microscope equipped with an ocular micrometer. Measurements were taken from the subepaulet to the apical margin, not including the fringe of scales.

Effect of sugar feeding and body size on oviposition behavior

Adult mosquitoes were provided with 0.3% sucrose solution for one week after emergence. After feeding on a mouse, females were housed separately in 40 × 40 × 40 cm cages. Each cage contained four polyvinyl cups (8 cm diameter, 4.3 cm height). Each cup contained 40 ml water at 1 cm depth. A sheet of filter paper 30 cm wide and 5 cm high was rolled and inserted into the cup. The cups and papers were changed daily. The number of eggs laid on the paper was counted. Gravid females were divided into four groups after the blood meal: (a) small females provided with 0.3% sucrose, (b) small females provided with water, (c) large females provided with 0.3% sucrose, (d) large females provided with water. Preliminary observations showed that most females laid all of their eggs within one week. Therefore, the oviposition starting date, length of the period of pre-peak fecundity (the number of days to maximum fecundity after the blood meal), length of the fecundity period, and the number of cups with oviposited eggs (over eight days) were observed. Experiments were replicated twice for each treatment.

Height of eggs oviposited

The filter paper from each cup was collected daily, dried, and the heights above the water line at which eggs were oviposited were measured. Eggs laid at the water surface were not measured.

Statistical analysis

Two-way ANOVAs were used to analyze the effect of sugar meals and body size on various oviposition parameters. For the analysis of the heights of oviposited eggs, data were transformed ln(X+0.5) before analysis (Yamamura 1999). Spearman's rank test was used to analyze the correlation between the mean and the coefficient of variation for the height of oviposited eggs. All analyses were performed with the R 2.4.1 statistical package (http:// www.r-project.org/).

RESULTS

Body size of mosquitoes

The wing length of small females (3.37±0.03 mm (mean±SE), n = 41) was significantly shorter than that of large females (3.94±0.03 mm, n = 53) (t = 13.10, P < 0.001).

Effect of sugar feeding and body size on maximum oviposition per day

The onset of the period of pre-peak fecundity period (i.e., the time to maximum fecundity) was significantly delayed in sugar-fed females (Figure 1) compared to water-fed females (F = 16.42; df = 1, 80; P < 0.001), although there was no difference between body sizes (F = 0.23; df = 1, 80; P = 0.60) (Figure 1). At maximum fecundity, large females oviposited more eggs per day than small females (F = 9.19; df = 1, 80; P < 0.01), although there was no statistically significant difference between sugar- and water-fed large females or between sugar- and water-fed small females (F = 0.12; df = 1, 80; P = 0.73) (Figure 2). Similarly, large females visited more cups per day (maximum number possible per day = 4) than small females at maximum fecundity (F = 4.76; df = 1, 80; P < 0.05), although, again, there was no difference between sugar-and water-fed large females or between sugar- and water-fed small females (F = 0.16; df = 1, 80; P = 0.69) (Figure 3).

Effect of sugar feeding and body size on total oviposition

There was a statistically significant difference in the total length of the oviposition period between the two feeding regimes (F = 12.52; df = 1, 80; P < 0.001), with sugar-fed females having a longer fecundity period, but there was no difference in the oviposition period between the two body sizes (F = 0.42; df = 1, 80; P = 0.52) (Figure 4). The start of oviposition tended to be earlier in water- than in sugar-fed females, but this difference was not statistically significant (F = 3.16; df = 1, 80; P = 0.08) (Figure 5). Large females produced more eggs than small females during the eight-day observation period (F = 12.61; df = 1, 80; P = 0.001), although there was no significant difference between sugar- and water-fed females (F = 0.06; df = 1, 80; P = 0.80) (Figure 6). Large females visited significantly more cups during the eight days to oviposit eggs than small females (F = 5.11; df = 1, 80; P < 0.05), and both large and small sugar-fed females made significantly more visits to cups for oviposition during the eight-day observation than the corresponding water-fed females (F = 8.24, df = 1, 80; P < 0.01) (Figure 7).

Height at which eggs were oviposited

Sugar feeding and body size affected the height of eggs oviposited on the papers in each cage (Figure 8). Females fed on sugar oviposited their eggs at higher sites than water-fed females (F = 10.46, df = 1, 4660; P < 0.01). Large females also oviposited their eggs at higher sites (F = 14.51, df = 1, 4660; P < 0.001).

DISCUSSION

Here we studied the reproduction of *Ae. aegypti* females in terms of body size and nutrition. Although blood consumption of large females is more than twice that of small females, the fecundity of large females was about four times that of small females (Briegel 1990). We confirmed that body size had an effect on fecundity of *Ae. aegypti* but found that the fecundity of large females was only twice that of small females, which is attributed to the smaller difference of body size in this study compared to that of Briegel (1990). Sugar availability often delays the start and completion of oviposition in gravid females, effectively reducing fecundity (Foster 1995). However, Day et al. (1994) provide evidence that there were no significant differences among sugar and blood meal treatments in total fecundity and fecundity period. According to the results reported here, the onset of the period of pre-peak fecundity was delayed and the oviposition period was prolonged in female *Ae. aegypti* mosquitoes provided with sugar. However, the maximum fecundity and the total fecundity were not different between females provided with sugar and those with water.

Regarding oviposition behavior at the peak of oviposition, large females visited more containers than small

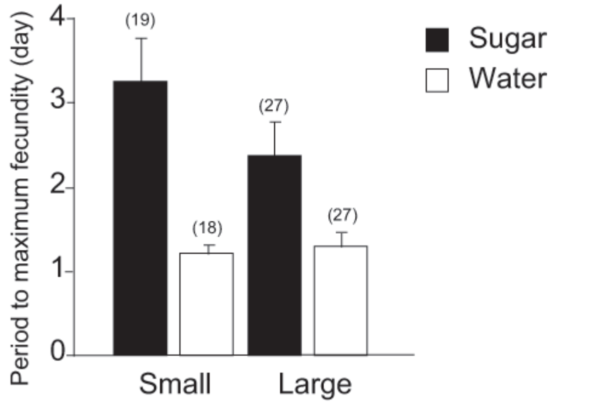

Figure 1. Time to maximum fecundity per day of small and large *Aedes aegypti* females provided with water or 0.3% sucrose. Numbers in parentheses = sample size.

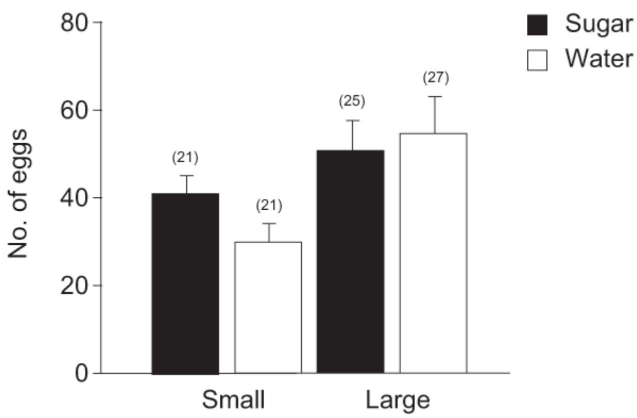

Figure 2. Number of eggs oviposited per day at maximum fecundity by small and large *Aedes aegypti* females provided with water or 0.3% sucrose. Numbers in parentheses = sample size.

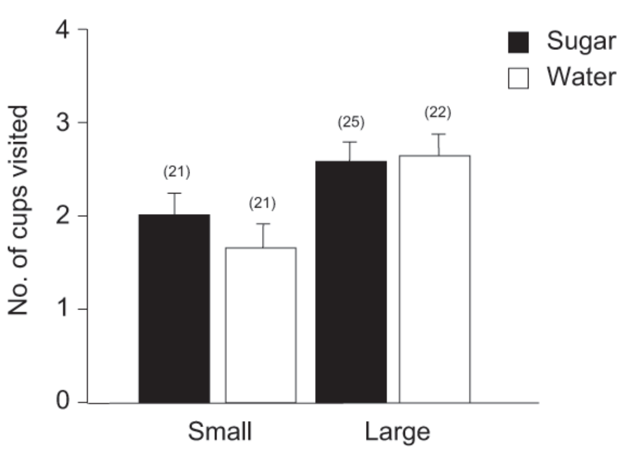

Figure 3. Number of cups visited per day at maximum fecundity by small and large *Aedes aegypti* females provided with water or 0.3% sucrose. Numbers in parentheses = sample size.

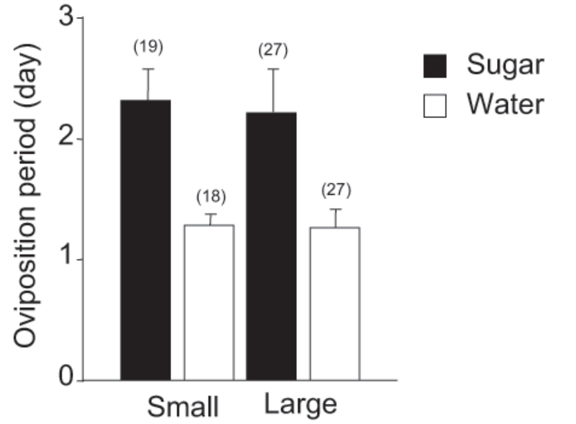

Figure 4. Oviposition period of small and large *Aedes aegypti* females provided with water or 0.3% sucrose. Numbers in parentheses = sample size.

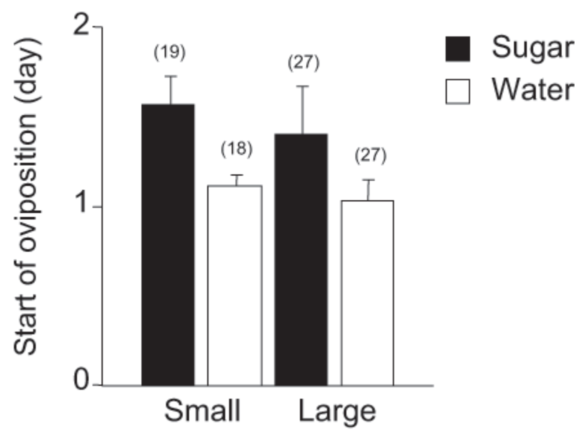

Figure 5. Number of days before the start of oviposition after blood meal by small and large *Aedes aegypti* females provided with water or 0.3% sucrose. Numbers in parentheses = sample size.

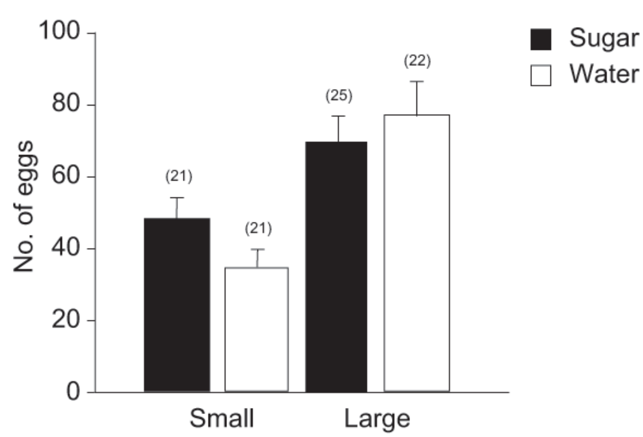

Figure 6. Number of eggs oviposited over eight days by small and large *Aedes aegypti* females provided with water or 0.3% sucrose. Numbers in parentheses = sample size.

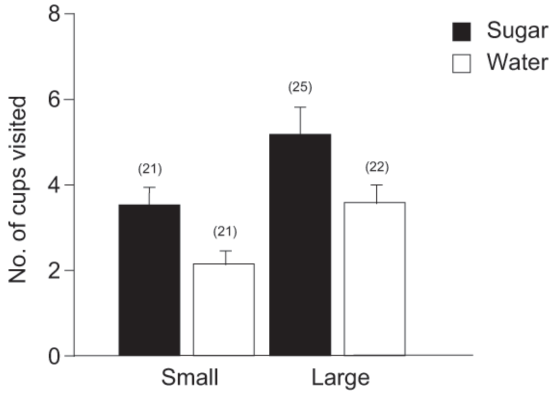

Figure 7. Number of cups visited over eight days by small and large *Ae. aegypti* females provided with water or 0.3% sucrose. Numbers in parentheses = sample size.

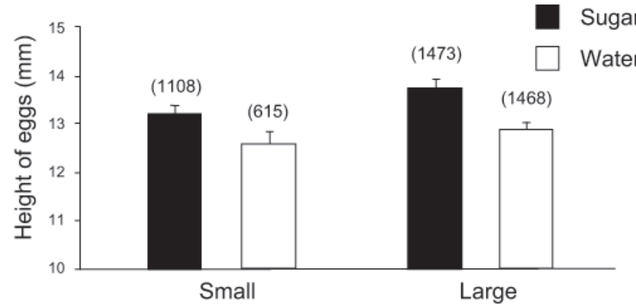

Figure 8. Height of eggs oviposited by small and large *Ae. aegypti* females provided with water or 0.3% sucrose. Values are before log transformation. Numbers in parentheses = sample size.

females. However, our results did not show a clear effect of dietary sugar on the number of sites visited at the peak of oviposition. It is likely that recent sugar feeding inhibits flight activity (Klowden and Dutro 1990). These results suggest that body size promotes "skip oviposition" in *Ae. aegypti* at the peak of oviposition. Our findings, that sugar availability and body size were important factors in female mosquitoes being able to visit containers, are consistent with de Meillon et al. (1967) that females, deprived of sugar, are close to death.

Reiter et al. (1995) speculated that individual gravid females in Puerto Rico laid their eggs over several days. However, domestic populations of *Ae. aegypti* in Thailand did not exhibit skip oviposition at the end of the rainy season (Harrington and Edman 2001). A strong positive relationship between container volume (and water surface area) and egg numbers of *Ae. aegypti* has been reported for both rainy and cool-dry seasons (Harrington et al. 2008). Our data showed that females visit more than two cups in laboratory. In Ho Chi Minh City, Vietnam, flower vases are extremely common in houses and a large proportion contained pre-adult *Ae. aegypti*, although the density of pupa was low (Tsuzuki et al. 2009). It is important to note that small containers such as flower vases promote "skip oviposition."

This study showed that the height of eggs oviposited on a paper by *Ae. aegypti* was influenced by sugar availability and body size. Eggs oviposited at higher sites would be submerged by rising water later than those at lower sites, leading to a time lag in hatching. Our results suggested that sugar-fed females had more energy reserves and oviposited eggs at higher sites. Dispersion of eggs among several containers and variation in the height of oviposited eggs may be examples of "germ banking," which can be seen as an adaptive variation in the timing of the emergence of offspring from a single clutch, leading to bet-hedging (Evans and Dennehy 2005). It is reasonable to suppose that *Ae. aegypti* females change oviposition sites for germ banking, depending on their energy reserves.

Acknowledgments

We are very grateful to C. Tsurukawa for her helpful support. We also thank T. Huynh (Ho Chi Minh Pasteur Institute) for kindness in offering the materials.

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