

# Age and Body Size Influence Male Sperm Capacity of the Dengue Vector *Aedes aegypti* (Diptera: Culicidae)

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**ABSTRACT** Understanding mosquito mating biology is essential for studies of mosquito behavior, gene flow, population structure, and genetic control. In the current study, we examine the effect of age and body size on spermatozoa number in two laboratory strains of the dengue vector, *Aedes aegypti* (L.), Thailand and Rockefeller (ROCK), and in wild-collected mosquitoes from Thailand. Body size was a major predictor of total spermatozoa number, with significantly greater sperm numbers in large (2.27-mm wing length) versus small males (1.85-mm wing length) within the same age group. Total sperm capacity also varied by male age. Spermatozoa numbers in virgin *Ae. aegypti* males increased significantly up to 10 d after emergence and then leveled off until 20 d. Significant variations in sperm number were detected among *Ae. aegypti* strains, with wild-collected mosquitoes having the greatest total number of sperm. Our study provides the first evidence of spermatogenesis in adult mosquitoes and indicates high rates of spermatogenesis in male mosquitoes up to 10 d of age (3.3 degree-days). Our results emphasize the potential role of body size and age on the mating capacity of this important vector of dengue and yellow fever viruses.

**KEY WORDS** *Aedes aegypti*, sperm capacity, mating, age and body size effect

Reproduction is one of the important characteristics of evolution and life history for all organisms, including insects (Emlen and Orings 1977, Thornhill and Alcock 1983). However, not all males experience equal reproductive success. For mosquitoes, many factors could potentially influence male reproductive success, including age, body size, sperm quantity, and sperm quality. Previous studies have reported an age-related effect on male mating capacity as measured by the presence of spermatozoa in cohabited females. For example, peak *Anopheles culicifacies* Giles male mating behavior occurred when males were 5–7 d old, and mating ability decreased by age 10–12 d (Mahmood and Reisen 1994). Mating success influenced by the maturation of accessory glands and testes morphology of male *Anopheles stephensi* Liston reached a peak 3–7 d after eclosion (Mahmood and Reisen 1982). Female *Anopheles gambiae* Giles exposed to 2-d-old males were more likely to oviposit than those held with 6-d-old males (Chambers and Klowden 2001). Hausermann and Nijhout (1975) demonstrated an influence of male age of *Aedes aegypti* (L.) on successful mating by comparing morphological traits of testes and seminal vesicles from 0- to 30-d-old males.

Mosquito insemination studies conducted with *Ae. aegypti* (performed by bouncing cages to stimulate flight and induce mating) demonstrated that male insemination rates were dependent on female age,

with virgin *Ae. aegypti* at 4 d postemergence most likely to be inseminated (Williams and Berger 1980).

Sperm capacity of male mosquitoes can be used to estimate reproductive potential. Spermatogenesis in mosquitoes is thought to peak during the pupal stage (Clements 1999). Jones (1967) reported the number of spermatocysts in male *Ae. aegypti* (Bangkok strain) decreased from the time of eclosion through the sixth week, but the length and size of testes were not significantly different. Furthermore, the number of spermatocysts decreased and the length of the sperm reservoir increased with age in *An. stephensi* (Mahmood and Reisen 1982) and *Culex tritaeniorhynchus* Giles (Mahmood et al. 1986).

Another parameter linked to mating success and fitness of insects is body size. Morphologically, body size should be positively correlated with reproductive organ size, total number of gametes, and reproductive success. In a study of *Anopheles freeborni* Aitken collected in California, larger males mated with more females than smaller males (Yuval et al. 1993). This positive correlation was detected based on altered morphology (color and size) of male accessory glands and testes after mating. Body size-dependent reproductive success determined from offspring sired per female encountered has been reported for the pitcher plant mosquito, *Wyeomyia smithii* Coquillett (Benjamin and Bradshaw 1994). Another study based on laboratory observations indicated that male *Anopheles gambiae* Giles preferred to mate with large females

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(Okanda et al. 2002). Larval crowding affected body size of adult *An. gambiae* and influenced their mating competitiveness (Ng'habi et al. 2005). Moreover, the body size of *An. freeborni* influenced by nutritional conditions during larval rearing affected accessory gland protein content (Dickinson and Klowden 1997).

For the dengue vector, *Ae. aegypti*, the importance of sperm quantity in males has rarely been considered, despite its likely importance in reproductive success. In the current study, we test the hypothesis that sperm quantity in males is a significant factor influencing male fecundity and reproductive fitness by first defining the basic parameters of male sperm capacity in laboratory strains. To relate our laboratory findings to nature, we determined sperm capacity among field-collected *Ae. aegypti* in Thailand. In addition, for the benefit of vector control programs based on modified male releases, we wanted to determine at what age males would be most reproductively successful.

### Materials and Methods

**Mosquito Laboratory Strains.** Wild-caught *Ae. aegypti* (Thai strain) were collected from breeding sites in Mae Sot district, Tak Province, Thailand, in 2004. The Rockefeller (ROCK) strain of *Ae. aegypti* has been in colony for at least 40 yr. The Thai laboratory strain is augmented annually with eggs from field-collected mosquitoes. Both strains were maintained in an environmental chamber set with a simulated fluctuating daily temperature regime from 22 to 30°C to simulate real field temperatures from Mae Sot, Thailand, and 80% RH with a photoperiod of 14:10 (L:D) h. The light phase began and ended with a 2-h period of twilight.

**Mosquito Rearing and Preparation.** Eggs of *Ae. aegypti* (Thai and ROCK strains) were hatched in a vacuum flask for 30 min. A pinch of *Aedes* food (1:1 ratio of lactalbumin/brewers yeast) was added to the flask and held overnight at 27°C until larvae were large enough to sort into rearing trays. Preliminary experiments were conducted to determine optimal crowding regimes and diet for obtaining large- and small body-size mosquitoes. For large body-size mosquitoes, 75 larvae were placed into a 27- × 20- × 7.5-cm (10.5- × 8.0- × 3.0-in.) plastic tray (Lock&Lock, New South Wales, Australia) containing 1 liter of distilled water. To yield small adults, 750 first instars were placed in each tray. *Aedes* food (75, 0, 38, 75, 113, and 150 mg) was added into trays on day 1, 2, 3, 4, 5, and 6, respectively. To obtain virgin mosquitoes, pupae were separately distributed into 15-ml vials containing 3 ml of distilled water and plugged with cotton wool. Emerged adults were sorted by sex and maintained with 20% sucrose solution. Male sperm capacity was examined in males 1, 5, 10, 15, 20, and 29 d after emergence.

**Wild Mosquito Collection.** *Ae. aegypti* were collected from May to August 2006 from dengue endemic areas in Nong Suang subdistrict (15° 05' N, 101° 54' E), located in Kham Thale Sor District, Nakhon Ratchasima Province ≈260 km northeast of Bangkok,

Thailand. *Ae. aegypti* pupae were collected from water-holding containers and then transferred to 15-ml vials containing 3 ml of water from the breeding site, and the vials were plugged with cotton wool. As adult mosquitoes emerged, they were gently aspirated from vials, anesthetized on wet ice, and identified to species by morphological characteristics according to Rattanarithikul and Panthusiri (1994). *Ae. aegypti* adults were sorted by sex and separately placed in 30-cm<sup>3</sup> plastic cages (Megaview Science Education Service Co., Ltd., Taichung, Taiwan) provided with 20% sucrose solution. Adults and pupae were maintained at the field site.

**Sperm Quantitation.** Male reproductive organs (testes and seminal vesicles) were dissected using small insect pins (0.35 mm in diameter) under a dissecting microscope. Laboratory strains were dissected at 1, 5, 10, 15, 20, and 29 d after eclosion, whereas wild strain males were dissected at 1, 5, 10, and 15 d after eclosion. Approximately 10–16 males were dissected per age cohort. Testes and seminal vesicles were placed into a glass chamber containing 50 μl of phosphate-buffered saline (PBS). Samples were torn gently with pins followed by washing of pins with 150 μl of PBS to obtain the final stock volume of 200 μl. Sample solutions were mixed with a P10 pipettor, and 5 μl of sample from the stock was spotted on multiwell slides (MP Biomedicals, Aurora, OH). Slides were air-dried and fixed with 70% ethanol. After fixing, slides were stained with Giemsa dye (Sigma-Aldrich, St. Louis, MO) for 1 h, and then they were rinsed with distilled water and allowed to air dry. Mosquito sperm heads (stained pink by Giemsa) were counted for using a phase-contrast microscope. To optimize our sperm quantitation method, we conducted total counts of all spermatozoa (40 aliquots totaling 200 μl) in *Ae. aegypti* males. Afterward, 10 aliquots (50 μl total) were randomly selected and counted. The total counts for 10 aliquots were averaged, multiplied by 40, and compared with overall counts. Using this approach, the estimated sperm number closely represented the accurate total sperm population. Wing length was measured microscopically from the axillary incision to the wing tip as an index of mosquito body size (Briegleb 1990, Nasci 1990).

**Temperature Recording.** Dataloggers (HOBO, Onset Corporation, Bourne MA) were used for laboratory and field experiments. The loggers were set to measure hourly temperature for the entire experimental period. Data were converted to degree-days to compare hourly intervals from laboratory and field studies as described below.

**Data Analysis.** Data for each experiment were tested initially for conformation to the assumptions of normality and homoscedasticity. Analysis of variance was used to test that low and high larval densities yielded significantly different size classes with MINITAB software (Minitab Inc., State College, PA). The age effect on sperm number was analyzed using general linear model (GLM). Differences in sperm numbers among age-group males were determined using Tukey pairwise comparisons with MINITAB. To

**Table 1.** Comparison estimates of spermatozoa per male and actual total counts

Avg sperm no./ aliquot $\pm$ SE	No. aliquots	Total estimated no. $\pm$ SE	Total counted no. of sperm
228 $\pm$ 2.12	40	9,120 $\pm$ 44.10	8,976
155 $\pm$ 4.36	40	6,200 $\pm$ 187.57	5,465
125 $\pm$ 2.57	40	4,918 $\pm$ 132.26	4,909
234 $\pm$ 0.89	40	9,360 $\pm$ 65.02	8,843
97 $\pm$ 3.13	40	3,786 $\pm$ 127.98	3,562
83 $\pm$ 2.68	40	3,320 $\pm$ 165.24	3,236
179 $\pm$ 3.01	40	7,160 $\pm$ 165.89	6,968
186 $\pm$ 1.79	40	7,288 $\pm$ 221.93	7,175
177 $\pm$ 2.02	40	7,080 $\pm$ 210.07	7,112
221 $\pm$ 2.12	40	8,840 $\pm$ 197.67	8,906

compare physiological age of our field and laboratory strains, we converted our temperature data to degree-days by calculating cumulative hourly degrees above the developmental threshold for *Ae. aegypti* (17°C) over the range of the experimental period and by dividing by 24 (Gerade et al. 2004).

## Results

### Optimization of Sperm Quantitation Methods.

Methods to estimate spermatozoa numbers in mosquitoes have been suggested to lead to a high level of errors (Clements 1999). In our current study, we developed a new and reliable sperm quantitation technique. By repeating counts with different aliquot subsamples and comparing our results, we found no statistically significant difference between estimates of total sperm capacity. Data on average counts for 5  $\mu$ l and total numbers of sperm are presented in Table 1.

**Effect of Body Size on Sperm Capacity.** Significantly different large ( $2.27 \pm 0.02$ -mm wing length) and small body sizes ( $1.85 \pm 0.02$ -mm wing length) were obtained when Thai laboratory strain males were reared under high and low crowding regimes ( $F = 261.82$ ;  $df = 1, 54$ ;  $P < 0.001$ ). The total number of spermatozoa in testes and seminal vesicles was significantly greater in large versus small males within the same age groups (Table 2). The average number of sperm produced by small 1-d-old males was 2,843, and large 1-d old males produced an average of 3,714 spermatozoa ( $F = 11.77$ ;  $df = 1, 31$ ;  $P = 0.002$ ). In Thai 10-d-old male *Ae. aegypti* small males had 7,306 spermatozoa, and large males had an average of 9,704 ( $F = 10.46$ ;  $df = 1, 22$ ;  $P = 0.004$ ).

**Sperm Capacity by Age. Thai Laboratory Strain.** Within the same age category, the total number of spermatozoa was significantly greater in large than

small Thai laboratory strain males, but the number of spermatozoa per 1 mm of wing length was not different among body size classes ( $P > 0.05$ ). GLM analysis revealed that age affected the number of spermatozoa per mm of wing length ( $F = 32.04$ ;  $df = 1, 92$ ;  $P < 0.001$ ). Total spermatozoa per millimeter of wing length in 1-d-old males were significantly less than those detected in 5-, 10-, 15-, 20-, and 29-d-old males ( $P < 0.001$ ). Total number of spermatozoa per 1 mm of wing length in 5-d-old males was significantly less than that in 10-, 15-, and 20-d-old males ( $P < 0.003$ ) but not different from that of 29-d-old males ( $P = 0.08$ ). There were no significant differences in mean total spermatozoa per millimeter of wing length among 10-, 15-, 20-, and 29-d-old Thai laboratory strain males ( $P > 0.05$ ) (Fig. 1).

**ROCK Laboratory Strain.** In the ROCK strain of *Ae. aegypti*, a significant difference in spermatozoa per millimeter of wing length also was detected between age groups ( $F = 4.47$ ,  $df = 49$ ,  $P = 0.004$ ). The number of spermatozoa per millimeter of wing length in 5-d-old males was lower than that in 10-, 15-, and 20-d-old males ( $P < 0.05$ ). As with the Thai strain, there were no significant differences in total spermatozoa number per millimeter of wing length among 10-, 15-, 20-, and 29-d-old ROCK males ( $P > 0.05$ ) (Fig. 1).

**Thai Field Strain.** The greatest number of sperm were produced by field-collected *Ae. aegypti* (Fig. 1). Consistent with results from the laboratory, an age effect on spermatozoa number per millimeter of wing length was detected ( $F = 129.56$ ;  $df = 1, 40$ ;  $P < 0.001$ ). The average sperm number for field strain males at age 1 d was significantly lower than that of subsequent age groups (5, 10, and 15 d) ( $P < 0.001$ ). There was no significant difference in mean total number of sperm between 5- and 10-d-old males ( $P = 0.09$ ). The number of spermatozoa per millimeter of wing length in 15-d-old males was higher than that in 10-d-old males ( $P = 0.01$ ; Tukey pairwise comparisons) (Fig. 1). All males died before the next time interval of 20 d, so no comparison could be made between the older age cohorts from the laboratory strains with our field strain. When comparing the degree-day ages of our field and laboratory strains, males that were 15 d old in the field were 8.7 degree-days old, which was closer to a 25-d-old male from the laboratory (Fig. 1). Despite strain variation, a similar trend in sperm count by age was found for all mosquitoes.

## Discussion

Our results demonstrate the role of mosquito age, body size, and strain on sperm capacity and potential reproductive fitness. The total number of spermatozoa in testes and seminal vesicles was greater in large body size males, indicating a greater reproductive capacity and fitness for large males. The ultimate significance of this effect will be determined after examining the number of viable progeny from matings with large males versus small males in studies currently underway.

**Table 2.** Average total sperm per body size class in Thai laboratory strain of *Ae. aegypti*

Age (d)	Size	Wing length (mm $\pm$ SE)	<i>n</i>	Total sperm/male $\pm$ SEM
1	Small	1.78 $\pm$ 0.01	16	2,843 $\pm$ 159.3
1	Large	2.23 $\pm$ 0.02	16	3,714 $\pm$ 197.8
10	Small	1.93 $\pm$ 0.02	13	7,306 $\pm$ 563.8
10	Large	2.32 $\pm$ 0.03	10	9,704 $\pm$ 416.8

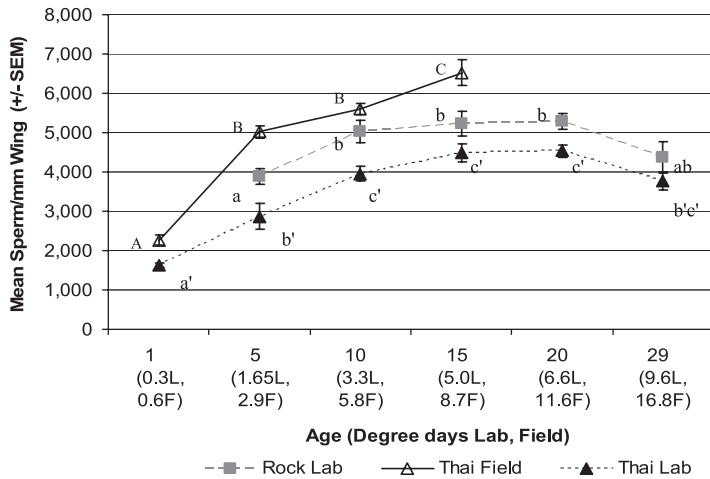


Fig. 1. Average total sperm per millimeter of wing length of two laboratory and one field-collected strain of *Ae. aegypti* by age groups ( $n = 213$ ). Data points with the same letter are not significantly different from each other.

This is the first report demonstrating an age-dependent relationship in the number of sperm produced by *Ae. aegypti* males. Older males ( $>10$  d old) were more likely to have more sperm in their reproductive organs than younger males ( $<10$  d old). Based on these results, sperm production in *Ae. aegypti* males from both laboratory strains increased significantly up to day 10 after eclosion and then gradually increased until 20 d old. After 20 d of age, the number of spermatozoa decreased. This result is in agreement with those published by Hausermann and Nijhout (1975). If males are mating daily with females through their lifetime, rapid increases in sperm capacity may reduce the chances of sperm depletion. It is not clear why sperm numbers would decrease (indicating sperm loss or cell death) in the oldest age groups, but declining male sperm counts with aging is a common biological phenomenon reported in organisms ranging from nematodes (Ward and Carrel 1979) to humans (Hellstrom et al. 2006).

Variation among laboratory and field strains was observed, with Thai wild strain males producing greater numbers of spermatozoa than laboratory strains. It is important to convert daily age comparisons to degree-day age when comparing field and laboratory experiments. In our study, due to much higher ambient temperatures, males from the field aged almost twice as fast as our laboratory strains. As a result, a 15-d-old male from the field was roughly equivalent to a 25-d-old male from our laboratory. Even considering equivalent degree-day ages, males from the field had significantly more spermatozoa than their laboratory counterparts. There may be factors such as better nutrients or more ideal environmental conditions for mosquitoes in the field that may have resulted in the increase in their spermatozoa numbers. Furthermore, we observed that *Ae. aegypti* males from the field mated with virgin females faster than laboratory strains of *Ae. aegypti* (unpublished data).

During spermatogenesis in insects, spermatozoa develop within spermatocysts of the testis. When the

spermatocysts mature and rupture, spermatozoa are released and enter seminal vesicles. For *Ae. aegypti*, only one other study has reported the number of spermatocysts in different age groups (0–6 wk old) of males. Using the Bangkok strain, Jones (1967) found a decrease in the number of spermatocysts in testes after it reached peak (22.5 cysts/testis) in newly emerged males ( $<24$  h after eclosion). In contrast to our findings, however, this study made no correlation between number of spermatocysts and total number of spermatozoa. We conclude that the spermatozoa maturation rate is faster but less predictable than spermatocyst formation. Moreover, the number of spermatocysts in older males ( $>20$  d) decreased steadily (Jones 1967), but this may not predict the number of spermatozoa, which dramatically decreased in our study. We did not measure changes in spermatocyst numbers over time, which is thought to be nominal; instead, we used sperm numbers as a more immediate measure of male reproductive capacity. Further studies should be conducted to determine whether initial increases in sperm number in *Ae. aegypti* are due to high rates of spermatogenesis in the adult stage or to increased rates of spermatocyst rupture releasing mature spermatozoa.

Our sperm quantity results contradict those of Hausermann and Nijhout (1975), who concluded (based on morphology of organs and sperm reservoir size) that total amount of spermatozoa produced by 5-d-old virgin *Ae. aegypti* (ROCK) males were greater than in 15-d-old males. Because sperm reservoirs in old males were tightly packed with sperm (Hausermann and Nijhout 1975), the total number of spermatozoa may be more difficult to quantitate using their methods than in younger males.

Our results suggest that larger and older virgin *Ae. aegypti* have the greatest reproductive potential. This capacity increases initially and then decreases over a virgin male's life span. In nature, adult males can mate with multiple females per day over several days. The ability to produce new sperm in the adult stage may

allow males to mate with greater numbers of females and reduce the chances of sperm depletion. Further investigations of mosquito mating biology will be especially important for genetic control programs that require an understanding of mosquito reproductive success.

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### References Cited

- Benjamin, S. N., and W. E. Bradshaw. 1994. Body size and flight activity effects on male reproductive success in the pitcher plant mosquito (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* 83: 331–336.
- Briegel, H. 1990. Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *J. Med. Entomol.* 27: 839–850.
- Chambers, G. M., and M. J. Klowden. 2001. Age of *Anopheles gambiae* Giles male mosquitoes at time of mating influences female oviposition. *J. Vector Ecol.* 26: 196–201.
- Clements, A. N. 1999. The biology of mosquitoes, vol. 2. Sensory reception and behaviour, pp. 360–402. Cambridge University Press, Cambridge, United Kingdom.
- Dickinson, J. M., and M. J. Klowden. 1997. Reduced transfer of male accessory gland proteins and monandry in female *Aedes aegypti* mosquitoes. *J. Vector Ecol.* 22: 95–98.
- Emlen, S. T., and L. W. Orings. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science (Wash., D.C.)* 197: 215–223.
- Gerade, B. B., S. H. Lee, T. W. Scott, J. D. Edman, L. C. Harrington, S. Kitthawee, J. W. Jones, and J. M. Clark. 2004. Field validation of *Aedes aegypti* (Diptera: Culicidae) age estimation by analysis of cuticular hydrocarbons. *J. Med. Entomol.* 41: 231–238.
- Hausermann, W., and H. F. Nijhout. 1975. Permanent loss of male fecundity following sperm depletion in *Aedes aegypti* (L.). *J. Med. Entomol.* 11: 707–715.
- Hellstrom, W.J.G., J. W. Overstreet, S. C. Sikka, J. Denne, S. Ahuja, A. M. Hoover, G. D. Sides, W. H. Cordell, L. M. Harrison, and J. S. Whitaker. 2006. Semen and sperm reference ranges for men 45 years of age and older. *J. Androl.* 27: 421–428.
- Jones, J. C. 1967. Spermatocysts in *Aedes aegypti* (Linnaeus). *Biol. Bull.* 132: 23–33.
- Mahmood, F., and W. K. Reisen. 1982. *Anopheles stephensi* (Diptera: Culicidae): changes in male mating competence and reproductive system morphology associated with aging and mating. *J. Med. Entomol.* 19: 573–88.
- Mahmood, F., T. Parveen, and W. K. Reisen. 1986. *Culex tritaeniorhynchus* Giles: changes in male mating competence and reproductive system morphology associated with age and mating experience. *Pak. J. Zool.* 18: 273–296.
- Mahmood, F., and W. K. Reisen. 1994. *Anopheles culicifacies*: effects of age on the male reproductive system and mating ability of virgin adult mosquitoes. *Med. Vet. Entomol.* 8: 31–7.
- Nasci, R. S. 1990. Relationship of wing length of adult dry weight in several mosquito species (Diptera: Culicidae). *J. Med. Entomol.* 27: 716–719.
- Ng'habi, K. R., B. John, G. Nkwengulila, B.G.J. Knols, G. F. Killeen, and H. M. Ferguson. 2005. Effect of larval crowding on mating competitiveness of *Anopheles gambiae* mosquitoes. *Malar. J.* 4: 49.
- Okanda, F. M., A. Dao, B. N. Njiru, J. Arija, H. A. Akelo, Y. Toure, A. Odulaja, J. C. Beier, J. I. Githure, G. Yan, et al. 2002. Behavioural determinants of gene flow in malaria vector populations: *Anopheles gambiae* males select large females as mates. *Malar. J.* 1: 10.
- Rattananarithkul, R., and P. Panthusiri. 1994. Illustrated keys to the medically important mosquitoes of Thailand. *Southeast Asian J. Trop. Med. Publ. Health.* 25: 1–66.
- Thornhill, R., and J. Alcock. 1983. The evolution of insect mating systems. Harvard University Press, Cambridge, MA.
- Ward, S., and J. S. Carrel. 1979. Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. *Dev. Biol.* 73: 304–321.
- Williams, R. W., and A. Berger. 1980. The relation of female polygamy to gonotrophic activity in the ROCK strain of *Aedes aegypti*. *Mosq. News* 40: 597–604.
- Yuval, B., J. W. Wekesa, and R. K. Washino. 1993. Effect of body size on swarming behavior and mating success of male *Anopheles freeborni* (Diptera: Culicidae). *J. Insect Behav.* 6: 333–342.

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