

Physical characteristics and reproductive performance in *Aedes* (Diptera: Culicidae)

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Abstract

Body size is a physical factor of crucial importance underlying important traits of the reproductive dynamics of both sexes in mosquitoes. Most studies on the influence of body size in mating success of dengue vectors addressed sperm transfer to females and did not con-

sider egg production, a prerequisite for population maintenance; male body size impact on reproduction has attracted little research interest with respect to sterile insect technique. In experiments involving differently sized adults, we examined whether the body size of the mates is a source of variation in reproductive outcome in *Aedes aegypti*. In the absence of male partners, large females (LF) showed better fecundity than small females (SF). In intraclass mating trials, egg production was much greater in largesized than small-sized pairs. There were comparable fecundities in large females mated with small males and large pairs. [SF•SM] and [SF•LM] pairs showed equivalent fecundity. Nonmating did not result in the production of viable eggs by either small or large females. We also observed that eggs produced by large-sized females mated with small males had better hatching success than those from either small or large pairs. Mating between small females and large males resulted in poor egg viability.

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Introduction

Originating in Africa (Gubler, 2008), *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) is now found in many urbanized areas worldwide (Womack, 1993). This mosquito species is a vector of three important viral diseases-yellow fever, chikungunya, dengue (Morrison *et al.*, 2008) and Zika virus (Marcondes & Ximenes, 2016). This latter disease causes more human morbidity and kills more people than any other mosquito-borne diseases globally (Farrar *et al.*, 2007) and the World Health Organisation (WHO) rated dengue as the most important mosquito-borne viral disease in the world (WHO, 2013). No vaccines (Sabchareon *et al.*, 2012) or specific therapeutic agents have yet been made available for dengue, and prevention is currently limited to vector control measures (WHO, 2014). Insecticide use - the main strategy to combat dengue vectors (WHO, 2010) - has been ineffectual due to the development of resistance (Whalon *et al.*, 2008). Indeed, *Ae. aegypti* has developed resistance to nearly all insecticide classes (Dia *et al.*, 2012).

There are several currently active fields of research to develop effective means of control of dengue vectors, *i.e.*, the sterile insect technique (SIT), incompatible insect technique (IIT), and genetically modified mosquito (GMM) technologies (O'Connor *et al.*, 2012; Bellini *et al.*, 2007). The Food and Agriculture Organization (FAO/IAEA/USDA, 2003) reported that the triumph or failure of these control strategies is directly related to the ability of the released laboratory produced insects to effectively mate with their wild counterparts. For example,

SIT has been used successfully to control a number of dipteran pests (Schetelig & Handler, 2012; Ogaugwu *et al.*, 2013). However, most of these programs were associated with reduced sexual competitiveness of the released insects (Oliva *et al.*, 2012). Shelly and colleagues (2007) reported that the mass rearing inherent to SIT causes a reduction in the mating ability of the released individuals. The application of these technologies requires the rearing of substantial numbers of target insect larvae (Helinski *et al.*, 2008; Alphey *et al.*, 2010). However, larval competition is common in container-breeding mosquitoes (Reiskind *et al.*, 2009), and high larval density has been reported to have a negative effect on body size at emergence (Yoshioka *et al.*, 2012). Under highly crowded conditions, larvae are exposed to increased competition for food (Muturi *et al.*, 2011). Density-dependent competition for food during larval life is associated with a number of adult physical and physiological traits, including reduced feeding rate per individual, lower quantities of stored nutrients (Alto *et al.*, 2008), and decreased adult size (Briegel, 2003).

In insects, size at emergence has a potentially crucial effect on important traits of reproductive biology in both sexes. Large-sized drosophilid males rarely fecundate smaller females (Partridge *et al.*, 1987). In *Ceratitis capitata* (Wiedemann), the number of copulations, amount of sperm stored, and reproductive success are positively associated with female body size (Joachim-Bravo *et al.*, 2009). Many reproductive traits in mosquitoes favor large-bodied adults. For example, a large body size is often associated with an increased number of mature oocytes (Briegel & Timmermann, 2001), while small-bodied mosquitoes display delayed sperm cell development (Smith & Hartberg, 1974), reduced sperm capacity (Renshaw *et al.*, 1994), slow ovarian development, and low mating competitiveness (Ng'habi *et al.*, 2005). In dengue vectors, large-sized males produce more sperm and experience less rapid seminal depletion than small males (Helinski & Harrington, 2011). In addition, females are inseminated with more sperm by larger than smaller males (Ponlawat & Harrington, 2009). Thus, the size of insect mates seems central to their mating capacity in nature and is presumably an important source of variation in mating ability and reproductive success. In support of this suggestion, it has been reported that large males often have better copulation and mating success in nature (Sawadogo *et al.*, 2013).

In container-breeding mosquitoes, including dengue vectors, male and female larvae that pupate first are conceivably smaller than those that pupate later. In SIT, IIT, and GM, laboratory produced insects are released into the wild (Dobson, 2003), so they can mate with wild individuals of the target populations. Such populations are generally assemblages of males and females of different body sizes (Schneider *et al.*, 2004). This diversity in adults of different body sizes may affect the interactions between males and females of different sizes as well as variability in mating success. There have been some recent attempts to study male mating ability in dengue vectors (Oliva *et al.*, 2012; Dieng *et al.*, 2013), but these studies did not involve *Ae. aegypti*, a species that is currently the target of SIT and GMM operations in many parts of the world (Harris *et al.*, 2012; Carvalho *et al.*, 2014). Another recent study examined mating success in this species, taking into account body size (Ponlawat & Harrington, 2009), but the authors did not focus on male

size and reproductive outcome, *i.e.*, egg production, a prerequisite for the production of subsequent generations and a determinant of population maintenance.

The present study was performed to examine whether body size affects the mating ability of *Ae. aegypti* and the reproductive outcomes of sexual cross-affinity between small- and large-bodied adults. In addition, we also investigated whether egg production and fertility of unmated females differ according to body size.

Materials and methods

Mosquito colony

The *Ae. aegypti* mosquitoes used in this study were derived from a colony established at the Vector Control and Research Unit (VCRU), University Sains Malaysia. Samples of eggs acquired from the Entomology Laboratory of VCRU were submerged in dechlorinated water. Larvae that hatched within 24 hours were raised in quintuplicate at a density of 100 - 150 per metallic enamel container (diameter=12 cm, depth=2 cm) half-filled with dechlorinated water. They were supplied a mixture of dog biscuits, beef liver, yeast, and milk powder (2:1:1:1) according to the feeding regimen and procedures described previously (0.15 g on day 1 of development, 0.30 g on day 4, with replacement of fresh water medium before the second feeding) (Dieng *et al.*, 2014). Upon pupation, individuals were transferred into plastic cups (diameter=7.6 cm, depth=5.2 cm) the interior edge of which was lined with moist tissue paper. Pupal cups were placed in mosquito cages (cubic metallic wire covered with mesh net, 30×30×30 cm; length × breadth × height). Upon emergence, adults were dispensed a 10% sucrose solution through cotton wicks. Three to five days after emergence, females were given blood meals from restrained mice placed inside cages for 30-60 minutes. Three days after blood meal uptake, females were given opportunities to oviposit in plastic cups with a capacity of 250 mL, lined with a piece of filter paper as an oviposition substrate and half-filled with dechlorinated water. Eggs were allowed to dry under laboratory conditions (temperature 29°C±3.0°C, relative humidity 75%±1% RH, and photoperiod 13 D: 10 L, 1 h dusk). After three days of drying, eggs were stored in plastic desiccation vessels and used as egg banks.

Production of body size cohorts

Two populations of *Ae. Aegypti* - small and large adults - were then produced using the stored eggs and used for the experiments. To obtain two different size classes, two larval densities and two feeding regimens were used. The first group (Group 1), which consisted of 100 newly hatched larvae (L1) and the second group (Group 2) consisted of 200 L1. The larval feeding schedule and amounts of food supplied are shown in Table 1. Virgin experimental adults were obtained according to the procedures described elsewhere (Dieng *et al.*, 2013). Pupae were placed individually into 1.5-mL Eppendorf tubes containing 0.05 mL of dechlorinated water to separate males from females. Pupae were

Table 1. Amounts of food supplied to *Aedes aegypti* larvae nurtured at different numbers to obtain adults of two different size classes.

Number of newly hatched larvae (L1)	Food supply schedule and amounts					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
100 (Group 1)	3 mL of 0.15 g	No food given	6 mL of 0.15 g	0.3 g*	0.3 g*	0.3 g*
200 (Group 2)	3 mL of 0.15 g	No food given	3 mL of 0.15 g	0.1 g*	0.1 g*	0.1 g*

Day 1: the first day of the occurrence of newly hatched larvae (L1) after eggs were submerged in water for 24 hours; Day 3: third day after the appearance of L1; Day 5: fifth day after the appearance of L1; Day 6: sixth day after the appearance of L1. *Supplied as powder.

checked every day, and adults that emerged were sexed. All males from Group 1 were put together in a cage labeled LM; Group 1 females, Group 2 males, and Group 2 females were also placed in cages labeled LF, SM, and SF, respectively. Experimental mosquitoes were provided 10% sucrose solution. Males and females from Group 1 were designated as large males (LM) and large females (LF), respectively, and those from Group 2 were designated as small males (SM) and small females (SF), respectively.

Body size

The wing lengths of adult specimens (16 males and 14 females) from Group 1 (LM and LF) and those of adults from Group 2 (10 SMs and 10 SFs) were measured as reported previously (Xue *et al.*, 2010; Dieng *et al.*, 2013). The length of one wing from each dead specimen was measured using a dissecting microscope (Olympus CX41; Olympus, Tokyo, Japan).

Egg production and viability in non-mated females

The first experiment examined fertility of eggs from unmated females of both sizes. Five virgin SFs (3-5 days old) were placed in a cage (18×18×24 cm; length × breadth × height) and provided 10% sucrose solution. After one day of sugar feeding, females were provided with blood meals from a restrained white mouse for 30 minutes. The same treatment was performed for five virgin LFs. Three days after blood feeding, gravid females were placed in oviposition tubes. Laid eggs were permitted to dry for three days under laboratory conditions (temperature 29°C±3.0°C, relative humidity 75%±1% RH, and photoperiod 13 D: 10 L, 1 h dusk). Dried eggs were immersed in 20 mL of dechlorinated water.

Fecundity of small and large females in intra-size class matings

The fecundities of SF and LF mated with males from their own size class were investigated as follows. One SM (2-5 days old) and ten blood-fed SFs (3-5 days old) were placed in a cage with 10% sucrose solution. In addition, one LM (2-5 days old) and ten blood-fed LFs (3-5 days old) were placed in another cage and treated as above. Both the cage with [SF•SM] and the cage with [LF•LM] were quadruplicated. Three days after blood feeding, females were singly placed in oviposition tubes.

Fecundity of small and large females in inter-size class matings

The following experiment was performed to investigate the fecundity of LF when mated with SM. One SM (2-5 days old) and ten blood-fed LFs (3-5 days old) were allowed to cohabit in a cage with access to sucrose solution as described in the experiment on *Fecundity of SFs and LFs in intra-size class matings*. The number of replicates for the [SF•LM] cage was 4. Egg production resulting from [LFs•LMs] in the experiment on *Fecundity of SFs and LFs in intra size class matings* served as a control. Three days after blood feeding, gravid females were placed in oviposition tubes. The numbers of eggs laid were recorded at the end of the oviposition period of 2 days. Four replicates of one LM (2-5 days old) and ten blood-fed SFs (3-5 days old) were caged, fed sucrose solution, and treated as described above. Here, the egg production resulting from [SFs•SMs] in Experiment 2 served as a control.

Larval eclosion

To examine whether the body sizes of adults in cross-mating pairs influence egg hatching success, samples of air-dried eggs from: i) 30 LFs cross-mated with one SM; ii) 34 LFs cross mated with one LM; iii) 20 SFs cross-mated with one LM; and iv) 15 SFs cross-mated with one

SM, were placed in 250-mL plastic vessels where they were flooded in 20 mL of hatching medium composed of 2 mL of dechlorinated water with 1 mL of larval food solution [0.003 g of mixture of dog biscuits, beef liver, yeast, and milk powder (2:1:1:1) in 100 mL of dechlorinated water]. All eggs were dried under the same conditions (temperature 29°C±3.0°C, relative humidity 75%±1% RH, and photoperiod 13 D:10 L, 1 h dusk). Hatching responses were monitored after a 24-h flooding time. Flooding was repeated two more times with a 4-day drying period between two flooding sessions.

Collection and analysis of data

In the fertility and size class fecundity studies, the numbers of eggs laid were counted under an Olympus CX41 dissecting microscope at the end of the 3-day oviposition period. The mean values were used as measures of fecundity. In the larval eclosion study, the numbers of eggs that hatched were determined by enumerating the numbers of first-instar larvae after each of the three flooding events. These numbers were used to compute the egg hatching rates, used as an indicator of fertility, as the number of hatched eggs/total number of eggs × 100. Wing length was used as an index of adult size, and was considered to be the distance in mm from the apical notch to the axillary margin, excluding the wing fringe, as reported previously (Xue *et al.*, 2010). Statistical analyses were carried-out as described previously (Dieng *et al.*, 2013). Briefly, the dissimilarities in fertility, fecundity, and egg hatching responses between the different crosses as well as differences in body size were examined by analysis of variance (ANOVA) using the Systat v.11 statistical software package (Systat Software Inc., 2004). In the egg hatching comparisons, the separation of means ± SE was performed using Fisher's LSD (least-significant difference) test. In all analyses, P<0.05 was taken to indicate statistical significance.

Ethical considerations

This study was carried out in accordance with the principles expressed in the Declaration of Helsinki. The study was approved by the Biological Research Ethics Committee at Universiti Sains Malaysia (USM).

Results

Body size patterns

The mean wing length of LMs was 1.96±0.14 mm and ranged between 1.28 and 2.54 mm. SMs had a mean wing length of 1.12±0.02 mm with a range of 0.98-1.22 mm. There was a significant difference between the two male size (ANOVA: F=19.53; P<0.001). Mean wing length also differed significantly between LFs (2.29±0.15 mm, range: 1.48-2.99 mm) and SFs (1.38±0.024 mm, range: 1.25-1.48 mm) (ANOVA: F=21.88; P<0.001), indicating that the former were larger than the latter (Figure 1).

Egg production by differently sized unmated females and viability

The mean number of eggs produced by unmated SFs was 29.60±6.23 and ranged from 10 to 43 eggs. For the LFs, the mean egg production averaged 55.40±8.16 eggs, with a range of 43 to 68 eggs. There was a significant difference in mean number of eggs produced between the two groups (ANOVA: F=10.64; P=0.011) (Figure 2). No eggs produced by unmated SFs or LFs hatched (Table 2). It was clear that the LFs had better fecundity than SFs, suggesting that larger females had greater larval-derived nutrient stores.

Egg production with intra-size class matings

When LFs were mixed with a single LM, 64.7% (34/40) of the females produced eggs. Egg production varied significantly between small and large pairs (ANOVA: $F=14.34$; $P<0.001$). For the large pair ([LF•LM]), the mean egg production was 54.52 ± 4.85 eggs and ranged between 8 and 117 eggs. When SFs were mixed with a single SM, 47.50% (19/40) of the females produced eggs. For the small pair ([SF•SM]), the mean number of eggs produced was 25.68 ± 5.29 and ranged between 1 and 73 eggs (Figure 3).

Egg production with inter-size class matings

When LFs were cohobated with one SM, 92.5% (37/40) of the females produced eggs. Egg production from [LF•SM] (58.21 ± 5.17 eggs, range: 8-100 eggs) tended to be slightly higher than that from [LF•LM] (54.52 ± 4.85 eggs, range: 8-117 eggs). However, there was no significant difference between the two means (ANOVA: $F=0.2$; $P=0.605$) (Figure 4A). The mean egg production of SF mated with LM ([SF•LM]) was 28.20 ± 5.91 eggs, and was similar to that of [SF•SM] (ANOVA: $F=0.10$; $P=0.754$) (Figure 4B).

Comparison of fecundity in relation to mating status

There was no significant difference in egg production between unmated and mated SFs (ANOVA: $F=0.1$; $P=0.707$) (Figure 5A) as well as between unmated and mated LFs (ANOVA: $F=0.005$; $P=0.947$) (Figure 5B).

Egg hatching responses

In the intra-class size mating involving large-sized adults, 67.6% (23/34) of females produced eggs that did not hatch. In contrast, all SFs mated with males of their own class size produced eggs that hatched. In the inter-class size mating, 40% (12/30) of LFs mated with SMs produced non-viable eggs, while 95% (19/20) of their small bodied counterparts mated with LMs produced eggs that did not hatch. Larval eclosion differed significantly between mating pairs (ANOVA: $F=3.03$; $P=0.033$). Egg hatching success gradually decreased when advancing from LF mated with SM ($15.16\pm 4.09\%$; range: 0%-81.66%) to large-sized pairs ($5.161\pm 1.88\%$; range: 0%-38.18%) to SF mated with LM (4.46 ± 4.46 eggs, range: 0%-89.36%) to small-sized pairs ($1.96\pm 1.96\%$; range: 0%-29.41%). For both LFs and SFs, egg hatching tendency to be greater among eggs derived from crosses with hetero-sized males. The mean egg hatching success in LFs mated with SMs was significantly higher than those from [LF•LM] [Tukey HSD: Matrix of pairwise mean differences (MPMD)= -9.99; $P=0.020$], [SF•LM] (Tukey HSD: MPMD= -10.69; $P=0.030$) and [SF•SM] (Tukey HSD: MPMD= -13.19; $P=0.015$). No significant differences in egg hatching success were observed between [LF•LM] and SFs mated with LM (Tukey HSD: MPMD= -0.69; $P=0.884$), and [SF•SM] (Tukey HSD: MPMD= -3.20; $P=0.542$). More eggs hatched among those produced by SFs mated with LM than among those laid by the [SF•SM] pairs, but the difference was not statistically significant (Tukey HSD: MPMD= -2.50; $P=0.664$) (Figure 6).

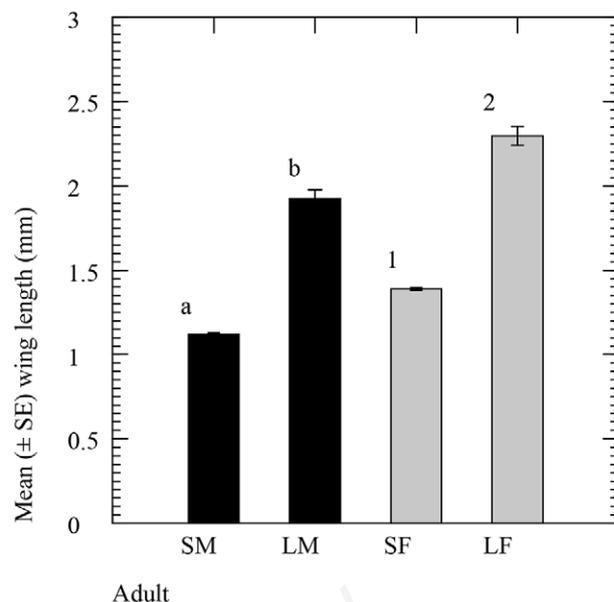


Figure 1. Mean wing length (\pm standard error) of experimental small and large *Aedes aegypti* adults. Bars of similar color and with the same letter or number are not significantly different ($P<0.05$).

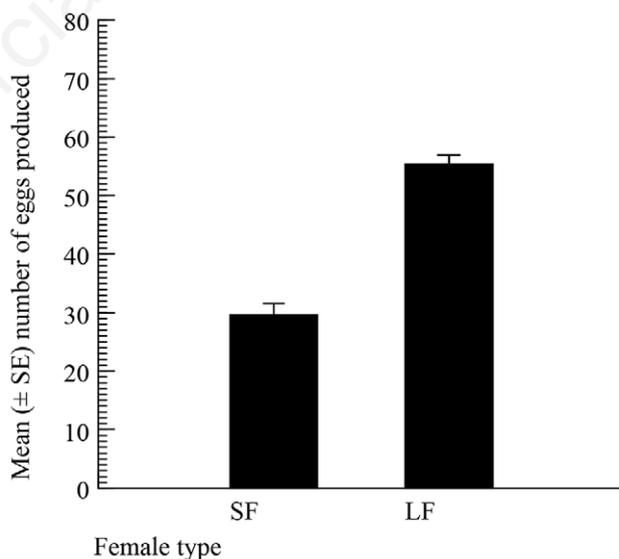


Figure 2. Numbers of eggs (mean \pm standard error) produced by unmated *Aedes aegypti* females.

Table 2. Hatching rates of *Aedes aegypti* eggs derived from unmated small and large females.

Female type	Number eggs immersed	Hatching rate (%)
Unmated small female	148	0
Unmated large female	277	0

Discussion

The major finding of the present study was that small-sized *Ae. aegypti* can mate successfully with large-bodied partners, and that body size of the mates influenced reproductive success. Virgin SFs

cohabited with LMs produced viable eggs, as did LFs crossed with SMs. The degree to which these reproductive outcomes occurred exhibited size-dependent patterns in favor of large body size. Sugar- and blood-fed *Ae. aegypti* females developed eggs without ever mating with males. In mosquitoes, the female fat body undergoes structural modifications just after emergence, resulting in their fat tissues becoming

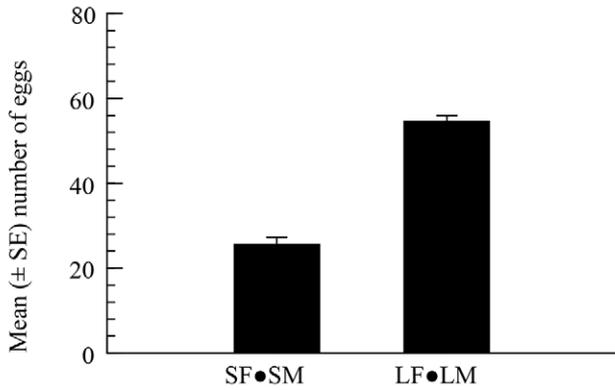


Figure 3. Numbers of eggs (mean±standard error) produced by small and large *Aedes aegypti* females (SFs and LFs, respectively) mated with corresponding males (SMs and LMs, respectively).

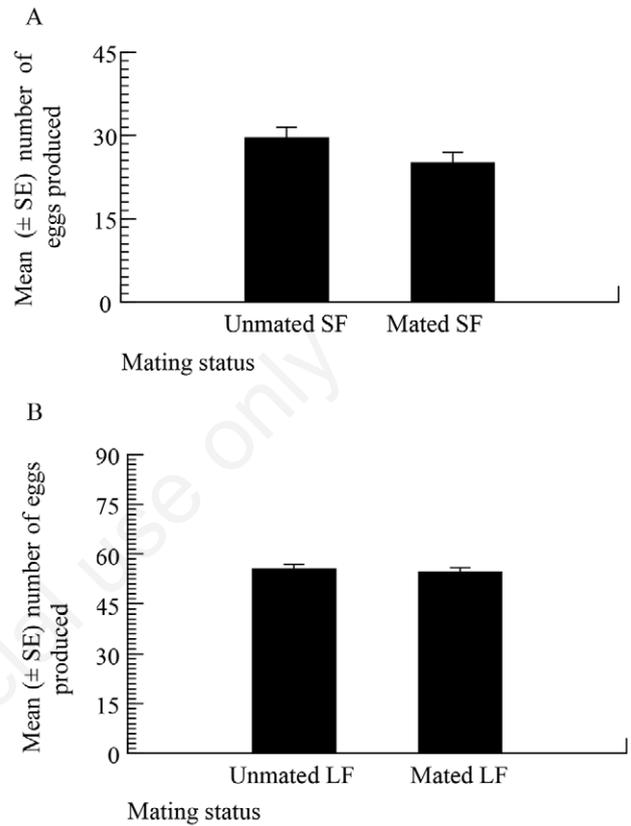


Figure 5. Comparisons of egg production between mated and unmated SFs and LFs of *Aedes aegypti*.

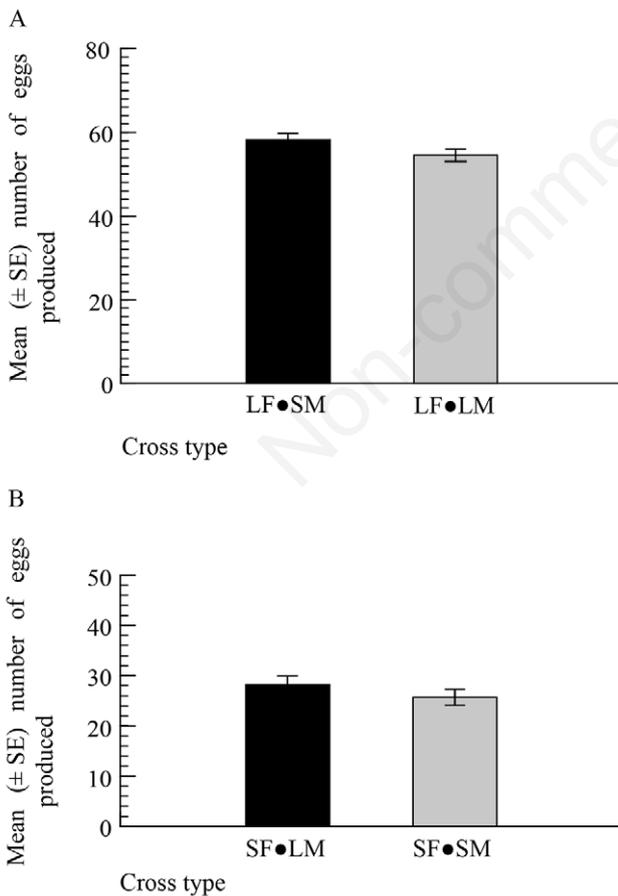


Figure 4. Numbers of eggs (mean±standard error) produced by LFs (A) of *Aedes aegypti* mated with SMs (A) and SFs (B) mated with LMs (B).

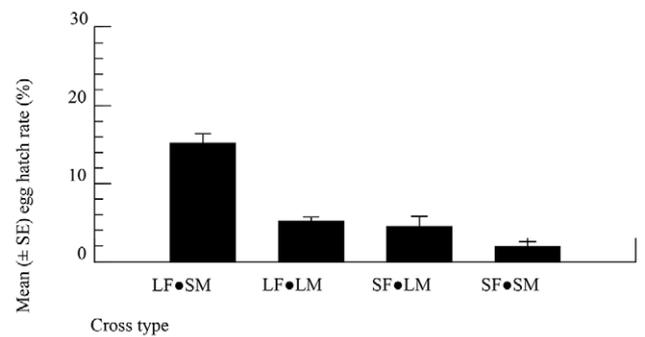


Figure 6. Mean (±standard error) hatch rates of *Aedes aegypti* eggs from mating pairs involving different male and female size classes.

responsive to vitellogenesis-inducing signals followed by a state of reproductive arrest (Attardo *et al.*, 2009). In general, an anautogenous female mosquito must digest a blood meal to produce eggs (Zhou & Miesfeld, 2009), and the ingestion of blood induces a series of events leading to egg production. Such an event cascade has been well documented in *Ae. aegypti*. Following blood meal uptake, the fat body is released from the state of reproductive arrest and thus initiates vitellogenesis (Baldini *et al.*, 2013). The female utilizes blood-derived amino acids to synthesize yolk precursor proteins, which are discharged into the hemolymph (Attardo *et al.*, 2009). Upon release, these proteins and their lipid transporters are incorporated into the ovaries (Cheon *et al.*, 2001) to produce oocytes (Attardo *et al.*, 2009). It is also widely believed that upon blood meal uptake, the brain of the female mosquito stimulates the ovaries to produce the steroid hormone ecdysone. In fact, the synthesis of 20-hydroxyecdysone (20E) breaks the state of reproductive arrest (Baldini *et al.*, 2013) and activates the expression of yolk protein genes (Chen *et al.*, 2005). That is why mosquito females that produce high levels of 20E themselves do not need to mate to complete egg development (Akst, 2013). As body dimensions are correlated with body size in *Ae. aegypti* (Timmermann & Briegel, 1999), larger females are expected to have greater fat contents than small females. Therefore, it is likely that the LFs had higher levels of lipid transporter proteins, vitellogenin synthesis activities, and thus greater oocyte production capabilities than SFs. Note that in *Aedes* mosquitoes, both larval-acquired teneral reserves and a blood meal can act as sources of yolk precursors and stimuli for egg production (Briegel, 1990b). The maximum number of eggs is closely related to the number of ovarioles in ovaries, known to depend on larval nutritional history (Mori, 1979). As body size reflects larval nutrition, a large female that developed as a larva in an environment with an abundant nutritional food supply will have more ovarioles than a small female from a food-scarce habitat (Shelton, 1972). In addition, there is a close association between blood meal size and the timing of ovariole initiation to egg development. In *Ae. aegypti* adults with small blood meals, the yolk is resorbed from most oocytes, thus retarding yolk formation (Lea *et al.*, 1978). In the present study, LFs and LMs were derived from well-nourished larvae, while SFs and SMs were from poorly fed larvae. Presumably, LFs had larger ovaries and consequently greater numbers of ovarioles than SFs. It is therefore likely that LMs had larger testes and thus greater sperm production potential than SMs. In support of this suggestion, it has been reported that in some dipterans the sizes of the ovary (Botto-Mahan & Medel, 2007) and testis (Pitnick, 1996) increase with body size. In mating experiments, we found that egg production varied considerably with body size of adults forming cross-mating pairs. Large-bodied females (LFs) produced far more eggs than their small-sized peers (SFs) when both mated with males of their respective sizes. The small-sized males and females (SMs and SFs) tested here were produced by rearing 200 newly hatched larvae with limited food supplies, whereas LMs and LFs were generated by raising 100 first instar larvae with an abundant food supply. Larvae maintained in a highly nutritious environment have more energy stores than those reared under crowded conditions with inadequate nutrition (Telang *et al.*, 2007). All had similar sugar feeding opportunities.

Both SFs and LFs had the same blood feeding time (30 min) and only fully blood-fed females were assessed for egg production. *Ae. aegypti* has been reported to display size-dependent variation in host-searching ability favoring larger individuals, which generally attack the host more than smaller individuals (Klowden, 1988). In this mosquito, the blood meal size threshold for egg development is high in small-sized females and low in larger females, and there is a close relationship between body size and blood meal mass (Xue *et al.*, 1995). Indeed, a large-bodied female will tend to ingest more blood than a smaller female. Although we did not determine blood meal volumes or teneral

reserves in the present study, the greater fecundity observed in LFs suggested that they had more yolk precursor sources than SFs.

Mating between [LF•LM] and [LF•SM] showed equivalent egg production, indicating comparable sperm transfer by both types of males. In nature, *Ae. aegypti* females exhibit polyandry, *i.e.*, one female mates with many males (Helinski *et al.*, 2012). This implies that a given female can receive sperm from one male, but will be receptive to additional inseminations. The males of dengue mosquitoes are known to be polygamous. A single *Ae. aegypti* male exposed to 20 females was shown to inseminate up eight of them within two days of cohabitation (Choochote *et al.*, 2001). These authors also reported that one female of either *Ae. aegypti* or *Ae. albopictus* (Skuse) kept with 10 males can successfully mate with four and three males, respectively within 1 h. They argued that repeated mating events with the same and/or different males occurred in the females of both species, and suggested that this multiple insemination behavior arose to ensure the transfer of adequate amounts of sperm to fertilize the eggs. Gwadz & Craig (1970) reported that such phenomena result from copulation with semen depleted males. In general, small dipterans, including mosquitoes, have small testes, less energy to invest in testicular tissue growth, and transfer small amounts of sperm per copulation (Ponlawat & Harrington, 2009). In the present study, SMs had a body size of 1.12 ± 0.02 mm, whereas LF had an average body size of 2.29 ± 0.15 mm; and one male (2–4 days old) was caged with 10 LFs. With reference to these reports and the experimental design used, it is tempting to suggest that SMs and LFs acted analogously to the adults in the study of Choochote and co-workers (2001).

It has been reported that *Ae. aegypti* males pass 2000 sperm to a female, only half of which is retained in the spermatheca to produce about 85 eggs (Jones, 1968). However, ejaculate size adjustment is another plausible explanation for the similarity in egg production between the small pairs and [SF•LM]. In many dipterans, males have been shown to gauge female quality (Martin & Hosken, 2002; Lupold *et al.*, 2011) based on a number of cues, including body size (Engqvist & Sauer, 2003). For example, male fruit flies supply significantly more sperm to larger than smaller females. It is possible that the LMs in the present study tuned the amounts of sperm delivered to SFs taking into account their small body size and associated tiny spermatheca.

The presence of males showed a clear-cut effect on egg fertility of *Ae. aegypti*. The eggs produced by either unmated SFs or LFs did not hatch, whereas those laid by females kept with males hatched in considerable numbers, thus confirming that insemination is necessary for successful fertilization and production of viable eggs in this species. During copulation, an *Ae. aegypti* female receives sperm and seminal fluid holding proteins in the bursa copulatrix (Jones & Wheeler, 1965), which are further stored in the spermatheca (Clements, 1999). Many studies have indicated a body size-associated effect on the amount of ejaculate transferred by a male to a female. For example, Ponlawat & Harrington (2009) investigated the factors associated with *Ae. aegypti* male mating success, and found significantly more sperm release by large males than their small counterparts. In a related study, Ponlawat & Harrington (2007) examined the impact of body size on sperm capacity in the same mosquito species and found this parameter to be a significant predictor of the total number of spermatozoa. They observed appreciably higher sperm counts in larger than small males. These discrepancies in sperm capacity could be explained by variations in the size of internal reproductive organs relative to body dimensions. In addressing this issue, Ponlawat and his colleague (2007) reasoned that, morphologically, body size is likely positively correlated with reproductive organ size. In drosophilids, testis size has been shown to increase with body size (Pitnick, 1996). In addition, ovary mass is heavier in larger than smaller females fruit flies (Botto-Mahan & Medel, 2007). Gonad size is positively correlated with sperm production and transfer rates (Baker *et al.*, 2003). The

eggs laid by LFs crossed with SMs were highly fertile, but the hatching success rate of eggs from SFs mated with LMs was relatively low. Many parameters play a role in the hatching success of mosquito eggs. The post-oviposition moisture conditions and bacteria present in the environment, which affect the levels of dissolved oxygen in the medium in which eggs are immersed, play central roles in hatching success (Borg & Horsfall, 1953; Novak & Shroyer, 1978). In the present study, all eggs were similarly preconditioned upon oviposition and the hatching medium used was identical in all egg hatch bioassays; therefore, it is unlikely that differences in egg hatching responses were due to discrepancies in egg preconditioning or immersion medium. Another parameter that may have impacted larval eclosion responses is the dormancy magnitude. All eggs tested remained immersed in the hatching medium for 24 h. In *Ae. aegypti*, only some eggs hatch during the first flooding, while the others require dehydration and additional flooding (Gillet *et al.*, 1977). The hatchability of dipteran eggs in relation to oocyte-sperm interactions has been determined in detail. Qazi & co-workers (2003) reported that ovulation begins when sperm is being stored. This non-coordination of oocyte release with the release of sperm from storage has been reported to result in low fertilization efficiency of the first ovulated eggs (Chapman *et al.*, 2001), which may explain the reduced hatchability of eggs from the [SF•LM] pairs. This has not yet been established in dengue mosquitoes and further investigations are required.

This study was performed principally to determine the impact of body weight on reproductive success of the dengue vector *Ae. aegypti* with respect to potential implications for SIT and related genetic control approaches. The results of the present study indicated that reproductive outcome, and thus mating success, in *Ae. aegypti* is affected by the body sizes of the mating individuals-LFs mated with similarly sized males showed appreciably greater reproductive outcome than any other homologous or reciprocal crosses. Our study confirmed that small *Ae. aegypti* males can efficiently inseminate large-bodied females, which produce many eggs with appreciable levels of fertility. *Ae. aegypti* males are extremely sexually aggressive; a single female housed with 11 males received 50 copulation attempts by different males within 1 hour (Roth, 1948). This property may be especially pronounced in laboratory-reared males as they are acclimatized to living in small spaces. Such aggressiveness combined with their multiple mating behaviors in nature (Boyer *et al.*, 2012) suggest a similar capacity to inseminate large numbers of females as observed in the boll weevil by Haynes and his colleague (1977).

References

- AKST J., 2013 - Male mosquitoes trigger egg production. - The Scientist. Available from: <http://www.the-scientist.com/?articles.view/articleNo/38070/title/Male-Mosquitoes-Trigger-Egg-Production/>
- ALPHEY L., BENEDICT M.Q., BELLINI R., CLARK G.G., DAME D.A., SERVICE M.W., DOBSON S.L., 2010 - Sterile insect methods for control of mosquito-borne diseases: an analysis. - *Vector-Borne Zoonotic Dis.* 10: 295-311.
- ATTARDO G.M., HANSEN I.A., RAIKHEL A.S., 2005 - Nutritional regulation of vitellogenesis in mosquitoes: implications for anautogeny. - *Insect Biochem. Mol. Biol.* 35: 661-675.
- BAKER RH, DENNIFF M, FUTERMAN P, FOWLER K, POMIANKOWSKI A, CHAPMAN T, 2003 - Accessory gland size influences time to sexual maturity and mating frequency in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. - *Behav. Ecol.* 14: 607-611.
- BALDINI F, GABRIELI P, SOUTH A, VALIM C, MANCINI F, CATTERUCCIA F, 2013 - The interaction between a sexually transferred steroid hormone and a female protein regulates oogenesis in the malaria mosquito *Anopheles gambiae*. - *PLOS Biol.* 11: e1001695.
- BELLINI R., CALVITTI M., MEDICI A., CARRIERI M., CELLI G.S.M., 2007 - Use of the sterile insect technique against *Aedes albopictus* in Italy: first results of a pilot trial. - In: VREYSEN M.J.B., ROBINSON A.S., HENDRICH S. J. (eds) *Area-Wide Control of Insect Pests: From Research to Field Implementation*. - Springer, Dordrecht: 505-515.
- BRIEGEL H., 2003 - Physiological bases of mosquito ecology. - *J. Vector Ecol.* 28: 1-11.
- BRIEGEL H., 1990b - Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. - *J. Insect Physiol.* 36: 65-172.
- BRIEGEL H., TIMMERMANN S.E., 2001 - *Aedes albopictus* (Diptera: Culicidae): physiological aspects of development and reproduction. - *J. Med. Entomol.* 38: 566-571.
- BORG A., HORSFALL W.R., 1953 - Eggs of floodwater mosquitoes. II. Hatching stimulus. - *Ann. Entomol. Soc. Am.* 46: 472-478.
- BOTTO-MAHAN C., MEDEL R., 2007 - What does determine gonad weight in the wild kissing bug *Mepraia spinolai*? - *Mem. Inst. Oswaldo Cruz.* 102: 233-235.
- BOYER S, TOTY C, JACQUET M., LEMPÉRIÈRE G., FONTENILLE D., 2012 - Evidence of multiple inseminations in the field in *Aedes albopictus*. - *PLoS One.* 7: e42040.
- CARVALHO D.O., NIMMO D., NAISH N., MCKEMEY A.R., GRAY P., WILKE A.B.B., MARRELLI M.T., VIRGINIO J.F., ALPHEY L., CAPURRO M.L., 2014 - Mass production of genetically modified *Aedes aegypti* for field releases in Brazil. - *J. Visualized Exp.* 83: e3579.
- CATOR L.J., NG'HABI K.R., HOY R.R., HARRINGTON L.C., 2010 - Sizing up a mate: variation in production and response to acoustic signals in *Anopheles gambiae*. - *Behav. Ecol.* 21: 1033-1039.
- CHAPMAN T., HERNDON L.A., HEIFETZ Y., PARTRIDGE L., WOLFNER M.F., 2001 - The Acp26Aa seminal fluid protein is a modulator of early egg-hatchability in *Drosophila melanogaster*. - *Proc. Royal Soc. Lond. Ser. B: Biol. Sci.* 268: 1647-1654.
- CHEN L., ZHU J.S., SUN G.Q., RAIKHEL A.S., 2005 - The early gene Broad is involved in the ecdysteroid hierarchy governing vitellogenesis of the mosquito *Aedes aegypti*. - *J. Mol. Endocrinol.* 34: 262-262.
- CHEON HM, SEO SJ, SUN J., SAPPINGTON T.W., RAIKHEL A.S., 2001 - Molecular characterization of the VLDL receptor homolog mediating binding of lipophorin in oocyte of the mosquito *Aedes aegypti*. - *Insect Biochem. Mol. Biol.* 31: 753-760.
- CHOOCHOTE W., TIPPAWANGKOSOL P., JITPAKDI A., SUKONTASON K.L., PITASAWAT B., SUKONTASON K., JARIYAPAN N., 2001 - Polygamy: the possibly significant behavior of *Aedes aegypti* and *Aedes albopictus* in relation to the efficient transmission of dengue virus. - *Southeast Asian J. Trop. Med. Pub. Health.* 32: 745-748.
- CLEMENTS A.N., 1999 - The Biology of Mosquitoes. Sensory Reception and Behavior, Vol. 2. - CAB International Publication, London.
- DIA I, DIAGNE CT, BA Y., DIALLO D., KONATE L., DIALLO M., 2012 - Insecticide susceptibility of *Aedes aegypti* populations from Senegal and Cape Verde Archipelago. - *Parasit. Vectors.* 5: 238.
- Dieng H., Rajasaygar S., Ahmad A.H., Satho T., Miake F., Zuharah W.F., Fukumitsu Y., Saad A.R., Abdul Hamid S., Vargas R.E., Ab Majid A.H., Fadzly N., Abu Kassim N.F., Hashim N.A., Ghani I.A., Abang F.B., AbuBakar S., 2014 - Indirect effects of cigarette butt waste on the dengue vector *Aedes aegypti* (Diptera: Culicidae). - *Acta Tropica* 130C: 123-130.
- Dieng H., Ruslan N.B., Ahmad A.H., Rawi C.S., Ahmad H., Satho T., Miake F., Zuharah W.F., Fukumitsu Y., Saad A.R., Rajasaygar S., Vargas R.E., Majid A.H., Fadzly N., Ghani I.A., AbuBakar S., 2013 - Colonized *Aedes albopictus* and its sexual performance in the wild: implications for SIT technology and containment. - *Parasit. Vectors* 6: 206.
- DOBSON S., 2003 - Reversing Wolbachia-based population replacement. *Trends Parasitol.* 19: 128-133.
- ENGQVIST L., SAUER K.P., 2003 - Determinants of sperm transfer in the

- scorpionfly *Panorpa cognata*: male variation, female condition and copulation duration. - J. Evol. Biol. 16: 1196-1204.
- FARRAR J., FOCKS D., GUBLER D., BARRERA R., GUZMÁN M.G., SIMMONS C., KALAYANAROJ S., LUM L., MCCALL P.J., LLOYD L., HORSTICK O., DAYAL-DRAGER R., NATHAN M.B., KROEGER A., 2007 - Toward a global dengue research agenda. Trop. Med. Intl. Health. 12: 695-699.
- Food and Agriculture Organization of the United Nations/International Atomic Energy Agency/United States Department of Agriculture, 2003 - Product quality control and shipping procedures for sterile mass-reared tephritid fruit flies. Manual, Version 5.0. IAEA, Vienna, Austria. Available from: <http://www.iaea.org/programmes/nafa/d4/index.html>.
- GILLET J.D., ROMAN E.A., PHILLIPS V., 1977 - Erratic hatching in *Aedes* eggs: a new interpretation. - Proc. R. Soc. Lond. B 196: 223-232.
- GUBLER D.J., 2008 - Dengue/Dengue haemorrhagic fever: history and current status, in new treatment strategies for dengue and other flaviviral diseases. In: BOCK G, GOODE J (eds) Novartis Foundation Symposium. John Wiley & Sons Ltd.
- GWADZ R.W., CRAIG G.B.JR., 1970 - Female polygamy due to inadequate semen transfer in *Aedes aegypti*. - Mosq. News. 30: 355-360.
- HARRIS AF, MCKEMEY AR, NIMMO D, CURTIS Z., BLACK I., MORGAN S.A., OVIEDO M.N., LACROIX R., NAISH N., MORRISON N.I., COLLADO A., STEVENSON J., SCAIFE S., DAFAL'ALLA T., FU G., PHILLIPS C., MILES A., RADUAN N., KELLY N., BEECH C., DONNELLY C.A., PETRIE W.D., ALPHEY L., 2012 - Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. - Nat. Biotechnol. 30: 828-830.
- HAYNES J.W., MITCHELL E.B., 1977 - Fractionated irradiation of boll weevils during pupal development: effect of sperm depletion and transfer as measured by female responsiveness. - J. Econ. Entomol. 70: 411-412.
- HELINSKI M., HARRINGTON L.C., 2011 - Male mating history and body size influence female fecundity and survival in the dengue vector mosquito *Aedes aegypti*. - J. Med. Entomol. 48: 202-211.
- HELINSKI M.E.H., HOOD R.C., KNOLS B.G.J., 2008 - A stable isotope dual-labelling approach to detect multiple insemination in un-irradiated and irradiated *Anopheles arabiensis* mosquitoes. - Parasit. Vectors. 1: 9.
- HELINSKI M.E.H., DEEWATTHANAWONG P., SIROT L.K., WOLFNER M.F., HARRINGTON L.C., 2012 - Duration and dose-dependency of female sexual receptivity responses to seminal fluid proteins in *Aedes albopictus* and *Ae. aegypti* mosquitoes. - J. Insect Physiol. 58: 1307-1313.
- JOACHIM-BRAVO I.S., ANJOS C.S., COSTA A.M., 2009 - The role of protein in the sexual behaviour of males of *Ceratitis capitata* (Diptera: Tephritidae): mating success, copula duration and number of copulations. - Zool. 26: 407-412.
- JOHANSSON A.S., 1964 - Feeding and nutrition in reproductive processes in insects. - Royal Entomol. Soc. Lond Symp. 2: 43-55.
- JONES J.C., 1968 - The sexual life of a mosquito. - Scientific Am. 218: 108-115.
- JONES J.C., WHEELER R.E., 1965 - An analytical study of coitus in *Aedes aegypti* (Linnaeus). - J. Morphol. 117: 401-424.
- KLOWDEN M.J., BLACKMER J.L., CHAMBERS G.M., 1988 - Effects of larval nutrition on the host-seeking behavior of adult *Aedes aegypti* mosquitoes. - J. Am. Mosq. Contr. Assoc. 4: 73-75.
- KLOWDEN M.J., CHAMBERS G.M., 1991 - Male accessory gland substances activate egg development in nutritionally stressed *Aedes aegypti* mosquitoes. - J. Insect Physiol. 37: 721-726.
- LEA A.O., BRIEGEL H., LEA H.M., 1978 - Arrest, resorption or maturation of oocytes in *Aedes aegypti*: dependence upon the quality of the blood meal and the interval between blood meals. - Physiol. Entomol. 3: 309-316.
- LUPOLD S, MANIER MK, ALA-HONKOLA O., BELOTE J.M., PITNICK S., 2011 - Male *Drosophila melanogaster* adjust ejaculate size based on female mating status, fecundity, and age. - Behav. Ecol. 22: 184-191.
- MARCONDES C.B, XIMENES M.D.F.F.D.M., 2016 - Zika virus in Brazil and the danger of infestation by *Aedes* (*Stegomyia*) mosquitoes. - Rev. Soc. Bras. Med. Trop. 49(1): 4-10.
- MARTIN O.Y., HOSKEN D.J., 2002 - Strategic ejaculation in the common dung fly *Sepsis cynipsea*. - Anim. Behav. 63, 541-546.
- MORI A., 1979 - Effects of larval density and nutrition on some immature and adults *Aedes albopictus*. - Trop. Med. 21: 85-103.
- MORRISON A., ZIELINSKI-GUTIERREZ E., SCOTT T., ROSENBERG R., 2008 - Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*. - PLOS Med. 5: 362.
- MUTURI E.J., KIM C.H., ALTO B.W., BERENBAUM M.R., SCHULER M.A., 2011 - Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. - Trop. Med. Intl. Health. 16: 955-964.
- NG'HABI K, JOHN B, NKWENGULILA G., KNOLS B.G.M., KILLEEN G.F., FERGUSON H.M., 2005 - Effect of larval crowding on mating competitiveness of *Anopheles gambiae* mosquitoes. - Malaria J. 4: 49.
- NOVAK R.J., SHROYER D.A., 1978 - Eggs of *Aedes triseriatus* and *Ae. hendersoni*: a method to stimulate optimal hatch. - Mosq. News. 38: 515-521.
- O'CONNOR L., PLICHART C., SANG A.C., BRELSFOARD C.L., BOSSIN H.C., DOBSON S.L., 2012 - Open release of male mosquitoes infected with a *Wolbachia* biopesticide: field performance and infection containment. - PLOS Negl. Trop. Dis. 6: e1797.
- OGAUGWU C.E., SCHETELIG M.F., WIMMER E.A., 2013 - Transgenic sexing system for *Ceratitis capitata* (Diptera: Tephritidae) based on female-specific embryonic lethality. - Insect Biochem. Mol. Biol. 43: 1-8.
- OLIVA C.F., JACQUET M., GILLES J., LEMPERIERE G., MAQUART P.O., QUILICI S., SCHOONEMAN F., VREYSEN M.J., BOYER S., 2012 - The sterile insect technique for controlling populations of *Aedes albopictus* (Diptera: Culicidae) on Reunion Island: mating vigour of sterilized males. - PLOS One. 7: e49414.
- OLIVA C.F., DAMIENS D., VREYSEN M.J.B., LEMPERIERE G., GILLES J., 2013 - Reproductive strategies of *Aedes albopictus* (Diptera: Culicidae) and implications for the sterile insect technique. - PLOS One. 8: e78884.
- PARTRIDGE L., EWING A., CHANDLER A., 1987 - Male size and mating success in *Drosophila melanogaster*: the roles of male and female behavior. - Anim. Behav. 35: 555-562.
- PITNICK S., 1996 - Investment in testes and the cost of making long sperm in *Drosophila*. - Am. Nat. 148: 57-80.
- PONLAWAT A., HARRINGTON L.C., 2007 - Age and body size influence male sperm capacity of the dengue vector, *Aedes aegypti* (Diptera: Culicidae). - J. Med. Entomol. 44: 422-426.
- PONLAWAT A., HARRINGTON L.C., 2009 - Factors associated with male mating success of the dengue vector mosquito, *Aedes aegypti*. - Am. J. Trop. Med. Hyg. 80: 395-400.
- QAZI M.C.B., HEIFETZ Y., WOLFNER M.F., 2003 - The developments between gametogenesis and fertilization: ovulation and female sperm storage in *Drosophila melanogaster*. - Dev. Biol. 256: 195-211.
- REISKIND M.H., GREENE K.L., LOUNIBOS L.P., 2009 - Leaf species identity and combination affect performance and oviposition choice of two container mosquito species. - Ecol. Entomol. 34, 447-456.
- RENSHAW M., SERVICE M.W., BIRLEY M.H., 1994 - Size variation and reproductive success in the mosquito *Aedes cantans*. - Med. Vet. Entomol. 8: 179-186.
- ROBINSON A.S., FRANZ G., ATKINSON P.W., 2004 - Insect transgenesis and its potential role in agriculture and human health. Insect Biochem. Mol. Biol. 34: 113-120.
- ROTH L.M., 1948 - A study of mosquito behavior — an experimental laboratory study on the sexual behavior of *Aedes aegypti* (Linnaeus). - Am. Midland Nat. J. 40: 265-352.
- Sabchareon A., Wallace D., Sirivichayakul C., Limkittikul K.,

- Chanthavanich P., Suvannadabba S., Jiwariyavej V., Dulyachai W., Pengsaa K., Wartel T.A., Moureau A., Saville M., Bouckennooghe A., Viviani S., Tornieporth N.G., Lang J., 2012 - Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomized, controlled phase 2b trial. - *Lancet*. 380: 1559-1567.
- Sawadogo SP, Diabate A, Toe HK., Sanon A., Lefevre T., Baldet T, Gilles J., Simard F, Gibson G., Sinkins S., Dabiré R.K., 2013 - Effects of age and size on *Anopheles gambiae* s.s. male mosquito mating success. - *J. Med. Entomol.* 50: 285-293.
- SCHETELIG M.F., HANDLER A.M., 2012 - A transgenic embryonic sexing system for *Anastrepha suspensa* (Diptera: Tephritidae). - *Insect Biochem. Mol. Biol.* 42: 790-795.
- SCHNEIDER J.R., MORRISON A.C., ASTETE H., SCOTT T.W., WILSON M.L., 2004 - Adult size and distribution of *Aedes aegypti* (Diptera: Culicidae) associated with larval habitats in Iquitos, Peru. - *J. Med. Entomol.* 41: 634-642.
- SHELLY T.E., MCINNIS D.O., RODD C., EDU J., PAHIO E., 2007 - Sterile insect technique and mediterranean fruit fly (Diptera: Tephritidae): assessing the utility of aromatherapy in a Hawaiian coffee field. - *J. Econ. Entomol.* 100: 273-282.
- SHELTON R., 1972 - The effects of blood source and quantity on production of eggs by *Culex salinarius* Coquillett (Diptera: Culicidae). - *Mosq. News.* 32: 31-37.
- SMITH R.P., HARTBERG W.K., 1974 - Spermatogenesis in *Aedes albopictus* (Skuse). - *Mosq. News.* 34: 42-47.
- STEINER L.F., MITCHELL W.C., HARRIS E.J., KOZUMA T.T., FUJIMOTO M.S., 1965 - Oriental fruit fly eradication by male annihilation. - *J. Econ. Entomol.* 58: 961-964.
- SYSTAT V. 11 STATISTICAL SOFTWARE PACKAGE, 2004 - Systat for Windows: statistics. Systat Software Inc., Richmond.
- TELANG A., FRAME L., BROWN M.R., 2007 - Larval feeding duration affects ecdysteroid levels and nutritional reserves regulating pupal commitment in the yellow fever mosquito *Aedes aegypti* (Diptera : Culicidae). - *J. Exp. Biol.* 210: 854-864.
- TIMMERMANN S.E., BRIEGEL H., 1999 - Larval growth and biosynthesis of reserves in mosquitoes. - *J. Insect Physiol.* 45: 461-470.
- WHALON M.E., MOTA-SANCHEZ D., HOLLINGWORTH R.M., 2008 - Global Pesticide Resistance in Arthropods. - Oxford University Press, London.
- WISHART G., RIORDAN C.F., 1959 - Flight responses to various sounds by adult males of *Aedes aegypti*. - *Canadian Entomol.* 23: 181-191.
- WHO, 2010 - Dengue and severe dengue. - World Health Organization, Geneva. Available from: <http://www.who.int/mediacentre/factsheets/fs117/en/>
- WHO, 2013 - Global strategy for dengue prevention and control 2012-2020. - World Health Organization, Geneva. Available from: http://apps.who.int/iris/bitstream/10665/75303/1/9789241504034_eng.pdf
- WHO, 2014 - Dengue vaccine research. - World Health Organization, Geneva. Available from: http://www.who.int/immunization/research/development/dengue_vaccines/en
- WOMACK M., 1993 - The yellow fever mosquito, *Aedes aegypti*. *Wing Beats.* 5: 4.
- XUE R.D., BARNARD D.R., SCHRECK C.E. 1995 - Influence of body size and age of *Aedes albopictus* on human host attack rates and the repellency of Deet. - *J. Am. Mosq. Contr. Assoc.* 11: 50-53.
- XUE R.D., BARNARD D.R., MULLER G.C., 2010 - Effects of body size and nutritional regimen on survival in adult *Aedes albopictus* (Diptera: Culicidae). - *J. Med. Entomol.* 47: 778-782.
- YOSHIOKA M., COURET J., KIM F., MCMILLAN J., BURKOT T.R., DOTSON E.M., KITRON U., VAZQUEZ-PROKOPEC G.M., 2012 - Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). - *Parasit. Vectors* 5: 225.
- ZHOU G., MIESFELD R., 2009 - Differential utilization of blood meal amino acids in mosquitoes. - *Open Access Insect Physiol.* 1: 1-12.