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Running head: Multiple blood feeding of *Aedes aegypti* and *Ae. albopictus*

Multiple blood feeding and host seeking behavior in *Aedes aegypti* and *Ae. albopictus* (Diptera:  
Culicidae)

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## ABSTRACT

The body size of mosquitoes can influence a number of bionomic factors, such as their blood feeding ability, host attack rate, and fecundity. All of these traits are important determinants of their potential to transmit diseases. Among abiotic and biotic factors, high temperature and low nutrition in the developing stages of mosquitoes generally result in small adults. We studied the relationship between body size and multiple feeding in a gonotrophic cycle and some fecundity attributes using three strains of two competent vector species, *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse). We raised small and large mosquitoes under low and high food conditions in the laboratory to measure parameters of fecundity and blood feeding behavior. Fecundity was positively correlated with body size in both species, while the number of blood meals, the frequency of host-seeking behavior, and egg retention were negatively correlated with body size in the *A. albopictus* Nagasaki strain. We found that multiple feeding and host-seeking behavior were negatively correlated with body size; i.e., small mosquitoes tended to have more contact with hosts. We found that two mechanisms that inhibit engorged mosquitoes from seeking out hosts, distension-induced and oocyte-induced inhibition, were not strong enough to limit host-seeking behavior, and multiple feeding increased fecundity. Size-dependent multiple feeding and host-seeking behavior affect contact frequency with hosts and should be considered when predicting how changes in mosquito body size affect disease transmission.

Keyword: body size, biting behavior, mosquitoes

## INTRODUCTION

The environmental factors that most affect mosquito population growth are temperature (Clements 1992, Atkinson 1994) and nutrition (Merritt et al. 1992). Two common mosquito species, *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), are competent vectors of several infectious disease in Asian countries. Temperature and nutrition also affect body size in mosquito species (Farjana et al. 2012). Body size is thought to influence several attributes of vector ecology, including fecundity and multiple blood feeding (Klowden and Lea 1978, Xue et al. 1995, Farjana and Tuno 2012), although these correlations are controversial (Klowden and Lea 1978, Nasci 1986, Briegel 1990, Chambers and Klowden 1990, Nasci 1990, Xue et al. 1995, Scott et al. 2000, Farjana and Tuno 2012).

Multiple feeding in a gonotrophic cycle can increase the risk of disease transmission by increasing the frequency of contact with hosts (Garrett-Jones 1964, Garrett-Jones and Shidrawi 1969, Dye 1986). Two types of multiple feeding have been recognized: supplementary feeding due to nutritional reserve depletion in teneral females and interrupted feeding due mainly to host defense (Clements 1999). Supplementary feeding has been reported in some *Anopheles* species (Senior White 1951, Smith and Weitz 1959, Edman and Downe 1964, Boreham and Garrett-Jones 1973, Burkot et al. 1988) and in *Ae. aegypti* (Reyes-Villanueva 2004, Scott et al. 1993a, 1993b, Xue et al. 1995, Scott et al. 2000). *Anopheles* females often require two or more blood meals for their first oviposition (Briegel and Horler 1993). In anophelines, the ovaries reach the late resting stage only after the first blood meal, and a second meal is required for complete maturation (Gillies 1954, Reisen et al. 1986). Multiple blood meals as supplementary feeding appear to be a widespread requirement in anophelines (Briegel and Horler 1993).

Interrupted feeding can occur when blood feeding is disrupted by the defensive responses of the host. Interrupted feeding has been observed in a wide range of species and a second partial meal is often taken within minutes of the first (Clements 1999) or within 2 days (Xue et al. 1995). Interrupted feeding has also been reported in *Ae. albopictus* (Delatte et al. 2010). The physiological mechanisms that take place during interrupted feeding have been studied in *Ae. aegypti*; host seeking after a blood meal is inhibited by multiple factors, including abdominal distension and egg development (Klowden and Lea 1978, 1979a, 1979b), as well as the initial size of the blood meal and the nutritional state of the female (Klowden and Briegel 1994).

The relationship between mosquito body size and blood feeding behavior has been examined by several researchers (Klowden and Lea 1978, Nasci 1986, Briegel 1990, Chambers and Klowden 1990, Nasci 1990, Xue et al. 1995, Scott et al. 2000, Reyes-Villanueva 2004). Although large mosquitoes may be more apt to engage in multiple blood feeding because of their larger food requirements (Klowden and Lea 1978, Xue et al. 1995), small mosquitoes also engage in multiple feeding because of their lower teneral energy reserves (Nasci 1986, 1990, Briegel 1990, Chambers and Klowden 1990, Reyes-Villanueva 2004). Hence, predictions differ as to whether small females perform more multiple feeding as supplementary feeding or large mosquitoes perform more as a result of their larger meal size threshold.

Multiple feeding has been studied in *Ae. aegypti* (Macdonald 1956, Yasuno and Tonn 1970, McClelland and Conway 1971, Pant and Yasuno 1973, Scott et al. 1993a, Xue et al. 1995, Scott et al. 2000) and has been described in terms of interrupted feeding rather than nutritional requirements. However, these studies did not adequately separate the two types of multiple feeding because the mosquitoes were offered multiple blood meals after interruption without determining whether they were able to lay eggs without the additional meals. To clarify this

debate, we studied the relationship between body size and multiple feeding in *Ae. aegypti* Singapore strain, *Ae. albopictus* Nagasaki (Japan) strain, and *Ae. albopictus* Ho chi Minh (Vietnam) strain in the laboratory. We did not study multiple feeding caused by host interruption but focused on clarifying whether the two aedine species need multiple feedings to lay eggs, even when they were fully engorged, and how host feeding behavior in engorged mosquitoes can vary according to body size.

## **MATERIALS AND METHODS**

### **Mosquito source**

*Aedes aegypti* were originally collected from Singapore (SG) city, Singapore (1°18'N, 103°51'E), in 2005 and *Ae. albopictus* were collected from Nagasaki (NG), Japan (32°46'20.35"N, 129°52'9.86"E), in 1990 (the colony declined once and was restarted with wild mosquitoes in 2005) and from Ho Chi Minh (HCM) city, Vietnam (10°45'N, 106°40'E), in 2006. Laboratory colonies were established at the Institute of Tropical Medicine, Nagasaki University, Japan. Both species were brought to the Laboratory of Ecology at Kanazawa University where they were maintained for these experiments. The generations used for the experiments were F40 and F41 in the three strains. Adults were maintained in cages at 25±1°C and 70–90% relative humidity (RH) under a light/dark photoperiod of 14/10 h. Adult mosquitoes had free access to 3% sucrose solution and they were allowed to feed on the blood of rats once per week.

### **Rearing large and small mosquitoes**

We performed two continuous trials in both species. In the first trial (from July to November, 2010), we examined multiple feeding, oviposition behavior, and the number of follicles in ovaries using *Ae. aegypti* SG and *Ae. albopictus* NG. Diet amount (low or high)

during developing stages was manipulated to produce large and small females. Two groups of mosquitoes of each species were established from the low and high diets at 30°C. Larvae were reared in plastic trays (40 × 30 × 7 cm) at a density of 200 larvae per tray. Larvae were fed 0.05 and 0.1 mg/larvae/day of larval food during the early stage (first and second instars) and the late stage (third and fourth instars), respectively, as a low diet. These rates were 0.4 and 0.8 mg/larvae/day for early-stage and late-stage larvae, respectively, in the high-diet treatment. Larval food was a mixture of rat food (CE-2, CLEA Japan, Inc. Tokyo) and yeast extract powder (Ebios, Mitsubishi Tanabe Pharma Corporation, Osaka) (1:1 by weight).

In the second trial (from July to September, 2011), we examined multiple feeding and host-seeking behavior in *Ae. aegypti* SG and *Ae. albopictus* HCM, which was not the same *Ae. albopictus* strain as in the first trial. Larvae were reared at a density of 100 larvae per tray, half of that in the first trial. They were fed the same rations as in the first trial for the low diet treatment, but fed 0.5 and 1.0 mg/larvae/day for early and late stage larvae, respectively, in the high-diet treatment.

In both trials, emerged females were monitored to record multiple feeding and host-seeking activity during a gonotrophic cycle, and to count the number of eggs and the number of ovarian follicles.

### **First blood feeding**

Newly emerged mosquitoes were placed in rearing cages (30 × 20 × 20 cm) at 27°C, 70–90% RH under a light/dark photoperiod of 14/10 h and supplied with 3% sucrose solution. Males and females were kept together. At 5-6 days after emergence, females were allowed to feed on blood from human hands until they voluntarily finished feeding, to make them fully engorged

mosquitoes. We paid attention to whether they looked fully engorged, as judged by their stretched abdomen when they had finished feeding. In cases where they were not fully engorged, we eliminated them from the data. In total, 70 females were blood fed from each of the two treatment groups (large and small). Every engorged female was kept separately in a plastic vial (3 cm diameter × 6 cm height) that was covered with mesh after feeding. A piece of wet cotton was placed at the bottom of the vial and was covered by a piece of filter paper to serve as an ovipositing substrate. Every female was observed daily to record host-seeking activity and was checked for eggs.

### **Host-seeking activity test**

Host-seeking activity was tested every day using *Ae. aegypti* SG and *Ae. albopictus* HCM from the second trial after blood feeding until oviposition. One at a time, the isolated females were released from their plastic vials into an empty rearing cage (30 × 20 × 20 cm). Host-seeking activity was induced by placing a human hand on the screen of the cage. The responses of the mosquitoes were divided into two categories depending on whether they flew towards the hand and tried to probe it with their proboscis (host seeking) or did not react to the hand within 15 min (non-responding).

### **Multiple feeding and ovipositing test**

Every isolated female was checked daily for evidence of ovipositing. The start date of ovipositing was recorded. Females were then kept for another 3 days of observation because mosquitoes often do not lay all of their eggs at once (Farjana and Tuno 2012). On the fourth day after first oviposition, a female was dissected to determine whether there any eggs were retained in her ovaries, as is common in mosquitoes (Farjana and Tuno 2012). The total number of eggs



laid and retained in the ovaries was considered the number of eggs produced by the female. Females who did not oviposit within 6 days in *Ae. aegypti* and within 8 days in *Ae. albopictus* after their first blood meal were allowed to take a second blood meal. Mosquitoes were transferred from their plastic vials to the rearing cage to receive further blood meals. The timing of the second blood meal was determined from our observations in the laboratory, considering the maximum time between blood intake and oviposition in the two species. This procedure was repeated up to a third meal. The experiment was terminated if a mosquito did not lay eggs within 7 days after the third meal. All females, regardless of whether they had oviposited, were killed in a freezer to measure their wing length as an indicator of body size. Wing length was measured from the distal end of the axial inclusion to the apical margin, not including the fringe, following Van Den Heuvel (1963), using a micrometer under a stereomicroscope.

### **Counting of ovarian follicles**

In addition to the number of eggs, we evaluated a mosquito's fecundity by counting the number of ovarian follicles. A subsample of newly emergent females from both the large and small treatments were raised with 3% sucrose solution and were killed within 5 to 6 days after emergence. Ovaries were dissected to count the number of ovarian follicles following the method described by Meadows (1968). We counted the number of primary follicles (Christophers' stage II); at this stage the follicles were about 100  $\mu\text{m}$  long and the ooplasm contained fine lipid droplets. Primary follicles usually remain in the previtellogenic resting stage until the female has taken a blood meal (Clements 1992). The number of follicles in one ovary was doubled to represent the number of follicles in the female. One wing from each female was measured using the method described above.

### **Statistical analyses:**

The relationship between wing length and the number of blood meals needed for the first oviposition was analyzed using logistic regression models for the two species. A total of 14 out of 280 females of *Ae. aegypti* (8 in the first trial and 6 in the second trial) did not lay eggs even after a third blood meal and were excluded from the analysis; they were considered developmental anomalies. We dissected their ovaries and found no follicles in them.

Linear regression models were used to analyze the relationship between wing length and the frequency of host-seeking behavior – *i.e.*, accumulated number of trials with host-seeking reactions per individual – in the two species. Relationships between wing length, the number of eggs, and the number of follicles in the ovaries were also analyzed using linear regression models. The statistical analyses were performed using JMP (ver. 5.0.1.2; SAS Institute, Cary, NC, USA).

### **RESULTS**

Large females were produced under high-diet conditions in both species. Wing lengths were  $2.82 \pm 0.09$  mm (mean  $\pm$  standard error [SE]) in the first trial and  $3.11 \pm 0.08$  in the second trial in *Ae. aegypti* SG, and  $2.53 \pm 0.09$  mm in *Ae. albopictus* NG and  $2.72 \pm 0.07$  mm in *Ae. albopictus* HCM strain (Table 1). Small females were produced under low-diet conditions; wing lengths were  $2.34 \pm 0.12$  mm (Mean  $\pm$  SE) in the first trial and  $2.39 \pm 0.04$  in the second trial in *Ae. aegypti* SG, and  $2.14 \pm 0.09$  mm in *Ae. albopictus* NG and  $2.31 \pm 0.07$  in *Ae. albopictus* HCM (Table 1). Larger females of *Aedes aegypti* were produced in the second trial under high-diet (10.5% increase, ANOVA test, F-value = 408.5;  $P < 0.001$ ) and low-diet conditions (2.0%

increase, ANOVA test, F-value = 9.5;  $P = 0.0025$ ), possibly caused by lower density in their larval rearing conditions (200 larvae per tray in the first trial vs. 100 larvae in the second). As a result, we could see bigger variation in the second trial. We did not compare the wing size of *Ae. albopictus* between the first and second trials statistically because different strains were used. However, the difference in wing sizes between low and high diet were almost the same in the two trials in *Ae. albopictus*.

Most *Ae. aegypti*, (93.6% in the first trial, 92.9% in the second trial) oviposited after the first blood meal (Table 1). Similarly, most of the *Ae. albopictus* (90% [n=140] in the NG strain and 87.9% [n=140] in the HCM strain) oviposited after the first blood meal (Table 1). A total of 4 out of 19 *Ae. aegypti* laid eggs after the second blood meal and 0 of 14 females took the third blood meal (Table 1). In *Ae. albopictus* NG strain, 12 out of 14 oviposited after the second meal and 1 of 2 oviposited after the third meal (Table 1), whereas all of the 17 *Ae. albopictus* HCM strain oviposited after the second blood meal (Table 1). There was no relationship between wing size and multiple feeding during a gonotrophic cycle (multiple feeding) in either trial in *Ae. aegypti* (logistic regression,  $r^2=0.014$ ,  $\chi^2=1.738$ ,  $P>0.1$  in the first trial;  $r^2=0.007$ ,  $\chi^2=0.487$ ,  $P>0.1$  in the second trial). In *Ae. albopictus* NG, there was no relationship between body size and multiple blood meals (logistic regression,  $r^2=0.00$ ,  $\chi^2=0.001$ ,  $P>0.1$ ), but a significant correlation was found in the HCM strain, suggesting that small *Ae. albopictus* had a greater tendency to perform multiple feeding than did large individuals (logistic regression,  $r^2=0.049$ ,  $\chi^2=5.099$ ,  $P<0.05$ ).

The period between the first blood feeding and oviposition was  $3.23\pm 0.95$  days (n=131) in the first trial and  $3.56\pm 0.92$  days (n=134) in the second trial in *Ae. aegypti*. This period was  $5.76\pm 2.44$  days (n=139) in *Ae. albopictus* NG strain and  $4.85\pm 2.22$  days (n=140) in *Ae.*

*albopictus* HCM strain. Large mosquitoes laid eggs earlier than did small mosquitoes in both species (Kendall's Tau-b correlation coefficients were -0.543,  $P < 0.0001$  in the first trial and -0.554,  $P < 0.0001$  in the second trial in *Ae. aegypti*; and -0.164,  $P < 0.001$  in the Nagasaki strain and -0.139,  $P = 0.029$  in the HCM strain in *Ae. albopictus*).

*Aedes aegypti* reacted to human hands from day 2 after taking a blood meal (on day 1), whereas *Ae. albopictus* HCM started to seek hosts on day 4 (Figure 1A and 1B). Although 92.9% (n=140) of *Ae. aegypti* laid eggs after their first blood meal, 36.2% (n=130) of them showed host-seeking behavior. Among them, 33% sought hosts on day 2, 36% on day 3, 23% on day 4, and 1% on day 5 (Figure 1A). Most (87.9%, n=140) *Ae. albopictus* HCM oviposited after a single blood meal, but 32.5% (n=123) showed host-seeking behavior before oviposition. In the host-seeking individuals, 27% did so on day 4, 29% on day 5, and 12% on day 6 (Figure 1B). All of the females that oviposited after the second blood meal (except for one *Ae. albopictus* HCM) showed host-seeking behavior.

Size-dependent host-seeking behavior was observed in *Ae. aegypti* SG but not in *Ae. albopictus* HCM. Small females showed more host-seeking reactions in *Ae. aegypti*. The regression equation for *Ae. aegypti* was as follows: frequency of host seeking =  $3.10 - 0.87 \times$  wing length (in mm) (N=130,  $F = 11.96$ ,  $r^2 = 0.09$ ,  $P < 0.001$ ). That for *Ae. albopictus* HCM was as follows: frequency of host seeking =  $1.70 - 0.47 \times$  wing size (N=123,  $F = 1.78$ ,  $r^2 = 0.02$ ,  $P > 0.05$ ).

The number of follicles was positively related to wing length in both species, with large females having more follicles (Figure 2A & 2B). Similarly, there was a positive relationship between the total number of eggs and wing size in both species, with large females laying more eggs than small females (Figures 3 and 4).

In *Ae. aegypti* SG, most of the females (94.7% and 92.5% during the first and second trials, respectively) laid all of their eggs and had no eggs remaining in their ovaries, while the rest (5.3% and 7.5% during the first and second trials, respectively) had some remaining eggs (Figure 3A & 3B). In contrast, only 43.5% of *Ae. albopictus* NG laid all of their eggs while 66.5% retained some eggs in their ovaries, and 62.85% of *Ae. albopictus* HCM laid all of their eggs and 37.15% of them retained eggs (Figure 4A & 4B). There was no difference in wing size between the two *Ae. aegypti* groups in terms of egg retention (logistic regression,  $r^2=0.015$ ,  $\chi^2=0.74$ ,  $P>0.05$  and  $r^2=0.001$ ,  $\chi^2=0.05$ ,  $P>0.05$  in the first and second trials, respectively). *Aedes albopictus* NG females that retained eggs were smaller (logistic regression,  $r^2=0.02$ ,  $\chi^2=3.85$ ,  $P<0.05$ ), while no significant relationship between wing length and egg retention was found in *Ae. albopictus* HCM (logistic regression,  $r^2=0.001$ ,  $\chi^2=0.07$ ,  $P>0.05$ ).

We counted the number of follicles in nulliparous females for comparison with the number of eggs using the same cohort in both species because it is impossible to compare them within individuals (Table 2). Nearly all of the follicles (98.8–100%) underwent vitellogenesis in large *Ae. aegypti* under the high-diet condition, while 83.5–84.3% of the follicles did so in the low-diet treatment (Table 2). In contrast, the number of eggs was substantially lower than the number of follicles in *Ae. albopictus*, and this was most apparent in small mosquitoes in the low-diet treatment. Small *Ae. albopictus* NG in the low-diet treatment developed 55.3% of their follicles, while large individuals in the high-diet treatment developed 81.7%; *Ae. albopictus* HCM developed 71.6% of their follicles with the low diet and 83.6% with the high diet (Table 2).

In summary, fecundity, based on the numbers of eggs and follicles, was positively correlated with body size, while the period between first blood feeding and oviposition was

negatively correlated with body size in both species. The number of blood meals, host-seeking behavior, and egg retention were negatively correlated with body size in one of the tested strains.

## **DISCUSSION**

We studied the relationship between body size and multiple feeding in a gonotrophic cycle, and some fecundity attributes, using three strains of two species. Fecundity was positively related with body size but the other attributes were not. Most of the *Ae. aegypti* and *Ae. albopictus* individuals laid eggs after a single blood meal, although the *Ae. albopictus* strains showed a stronger tendency to multiple feeding compared to *Ae. aegypti*. Small *Ae. albopictus* HCM performed multiple feeding more often. Although the other two strains, *Ae. albopictus* NG and *Ae. aegypti*, did not show significant correlations, this might have been an artifact caused by colony maintenance. These two strains have been reared in the laboratory for many years, which may have affected their behavior. For example, as we maintain these colonies, when a strain does not grow well, we feed it for a couple of days on rats, which was the case with *Ae. albopictus* HCM. However, in most cases, we feed mosquitoes on rats for only 1 day to obtain eggs, which was the case for *Ae. aegypti* SG and *Ae. albopictus* NG. Therefore, individuals from strains that need multiple feeding are sometimes not able to produce offspring in the laboratory. Fortunately, our results did not find a positive correlation between mosquito body size and multiple feeding, but a negative correlation was found in some of the data. Hawley (1988) claimed that approximately 20% of wild *Ae. albopictus* perform multiple feeding in the field and suggested that this behavior is usually not necessary for egg maturation in *Ae. albopictus*. We found that approximately 90% of *Ae. aegypti* SG and *Ae. albopictus* HCM females oviposited after a single blood meal, but more than 30% of them showed host-seeking behavior, which means that

engorged mosquitoes performed multiple feeding even though they were able to oviposit without an additional meal. Moreover, a considerable number of follicles remained undeveloped following the first blood meal in small individuals in both species, indicating that females may need multiple feeding to develop all of their follicles and to enhance their fecundity, not only to enable ovipositing. Small *Ae. aegypti* SG showed more host-seeking behavior earlier, immediately after the day on which they were fully engorged. On the other hand, *Ae. albopictus* HCM were inhibited from host seeking for two consecutive days after their first blood meal and started host seeking only on day 4. Two mechanisms for inhibiting host-seeking behavior in mosquitoes have been reported, distention-induced inhibition and a humoral mechanism. Distention-induced inhibition begins with the activation of abdominal stretch receptors and acts until the blood meal has been digested and excreted, and the abdominal distention has been reduced (Klowden and Lea 1978, 1979b). The humoral mechanism starts when a blood meal initiates egg maturation and prevents host seeking from continuing (Klowden and Lea 1979a, Klowden 1981, Horler and Briegel 1995). Klowden and Briegel (1994) argued that the two mechanisms often overlap in *Ae. aegypti* to limit feeding frequency at the beginning of each gonotrophic cycle depending on the initial size of the blood meal and the nutritional state of the female. However, our results indicate that 36% of *Ae. aegypti* SG sought hosts starting on the day after they took a full blood meal, meaning that distention-induced inhibition lasted for a very short period or had no effect. On the other hand, 33% of *Ae. albopictus* HCM started to seek hosts on day 4 until day 6, indicating that distention-induced inhibition and humoral inhibition did not overlap and allowed mosquitoes to feed more than once. Therefore, multiple feeding may be realized more often than was previously predicted for two reasons. First, females that can lay eggs after a single blood meal still require additional blood meals to enhance their fecundity.

Second, the two mechanisms that limit host-seeking behavior do not substantially overlap. Also, the tendency for multiple feeding shows a more or less negative relationship with body size, suggesting that small mosquitoes have more contact with hosts.

The number of eggs that were retained in the ovaries varied with species and body size. Less than 10% of *Ae. aegypti* females retained eggs, whereas retention was more common in *Ae. albopictus*: 66.5% in the NG strain and 37.15% in the HCM strain. Small females retained more eggs than large individuals in both species. Mori (1979) reported that small *Ae. albopictus* tended to disperse further from their release points in a mark-release-recapture experiment. If small, nutritionally deprived females need to disperse further from their breeding sites, where crowding effects occur, they will need larger energy reserves for dispersal and may retain eggs as energy stocks for their own survival, assuming that egg absorption occurs (Magnarelli 1983). These possibilities may explain the higher egg retention in small *Ae. albopictus* individuals. The differences in egg retention among the species and strains may be due to the size of the species or strains. *Ae. aegypti* SG was the largest and *Ae. albopictus* NG was the smallest among the three strains that were used in this experiment.

In conclusion, multiple feeding and attributes of host-seeking behavior were generally negatively correlated with body size. Small mosquitoes will generally have more contact with hosts. Host-defense-caused multiple feeding fell outside the scope of this research; however, we showed that two mechanisms that inhibit mosquitoes from host seeking are not powerful enough to limit host-seeking behavior throughout a gonotrophic cycle, and multiple feeding may be conducted to increase fecundity. Size-dependent multiple feeding and host-seeking characteristics will affect the frequency of vector host contact and should be considered when



predicting how changes in mosquitoes' body size will affect the dynamics of disease transmission by *Ae. aegypti* and *Ae. albopictus*.

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Figure 1. Daily proportions of host seeking females in engorged *Aedes aegypti* SG (A)(N=130) and *Aedes albopictus* HCM(B)(N=123) those laid eggs with the first blood meal (N=130).

Figure 1B. Seeking of blood meal in engorged those laid eggs with the first blood meal (N=123).

Figure 2A. The relationship of body size (wing length) and the number of follicles of two ovaries in *Ae. Aegypti* SG.

Figure 2B. The relationship of body size (wing length) and the number of follicles of two ovaries in *Ae. albopictus* HCM.

Figure 3. Body size (wing length) and number of eggs laid by *Ae. aegypti* SG Fig. 3A (the first trial) and Fig. 3B (the second trial). Open square indicate one female without eggs retained in her ovaries, and Dark triangle indicate one female with eggs retained in her ovaries.

Figure 4. Body size (wing length) and number of eggs laid by *Ae. albopictus* Fig. 4A (Nagasaki strain) and Fig. 4B (HCM strain). Open square indicate one female without eggs retained in her ovaries, and Dark triangle indicate one female with eggs retained in her ovaries.

**Table 1:** Number of blood meals needed for first oviposition and average wing size of the *Ae. aegypti* and *Ae. albopictus* under low and high diet conditions.

Species	Diet	n	1 <sup>st</sup> blood meal				2 <sup>nd</sup> blood meal				3 <sup>rd</sup> blood meal			
			Female laid eggs		Female unlaid		Female laid eggs		Female unlaid		Female laid eggs		Female unlaid	
			Wing size (mm)	n	Wing size(mm)	n	Wing size(mm)	n	Wing size(mm)	n	Wing size(mm)	n	Wing size(mm)	n
			Mean±SE		Mean±SE		Mean±SE		Mean±SE		Mean±SE		Mean±SE	
<i>Ae. aegypti</i>	Low	70	2.34±0.12	66	2.34±0.16	4	-	0	2.34±0.16	4	-	0	2.34±0.16	4
(first trial)	High	70	2.82±0.09	65	2.79±0.09	5	-	0	2.81±0.09	4	-	0	2.81±0.09	4
<i>Ae. aegypti</i>	Low	70	2.39±0.04	64	2.38±0.01	6	-	4	2.38±0.00	2	-	0	2.38±0.00	2
(second trial)	High	70	3.11 ±0.08	66	3.11±0.07	4	-	0	3.11±0.07	4	-	0	3.11±0.07	4
<i>Ae. albopictus</i>	Low	70	2.14±0.08	62	2.17±0.04	8	2.17±0.04	6	2.16±0.06	2	2.12	1	2.2	1
NG strain	High	70	2.53±0.09	64	2.56±0.13	6	2.56±0.13	6	-	0	-	0	-	0
<i>Ae. albopictus</i>	Low	70	2.31±0.07	56	2.34±0.05	14	2.34±0.05	14	-	0	-	0	-	0
HCM strain	High	70	2.72±0.07	67	2.72±0.07	3	2.72±0.07	3	-	0	-	0	-	0

n: Number of individual



**Table 2.** Wing size, number of follicles and number of eggs of *Ae. aegypti* and *Ae. albopictus* under low and high diet conditions.

Species	Diet	Wing size (mm) (mean±SE)	n	No. of follicles (mean±SE)	n	No. of eggs (mean±SE)	Ratio of follicles to eggs (%)
<i>Ae. aegypti</i>	Low	2.35±0.11	30	51.0±12.28	66	42.6±10.98	83.5
first trial	High	2.80±0.09	30	92.1±14.01	65	91.9±20.57	99.8
<i>Ae. aegypti</i>	Low	2.38±0.05	30	51.6±9.70	68	43.49±12.07	84.3
second trial	High	3.09±0.09	30	94.07±15.01	66	97.47±27.19	100
<i>Ae. albopictus</i>	Low	2.12±0.09	30	50.5±13.18	69	27.9±8.51	55.3
NG strain	High	2.49±0.10	30	84.3±7.38	70	68.9±20.12	81.7
<i>Ae. albopictus</i>	Low	2.30±0.06	30	49.4±11.85	70	35.37±8.99	71.6
HCM strain	High	2.71±0.05	30	84.3±13.11	70	70.46±19.81	83.6

n: Number of individual tested

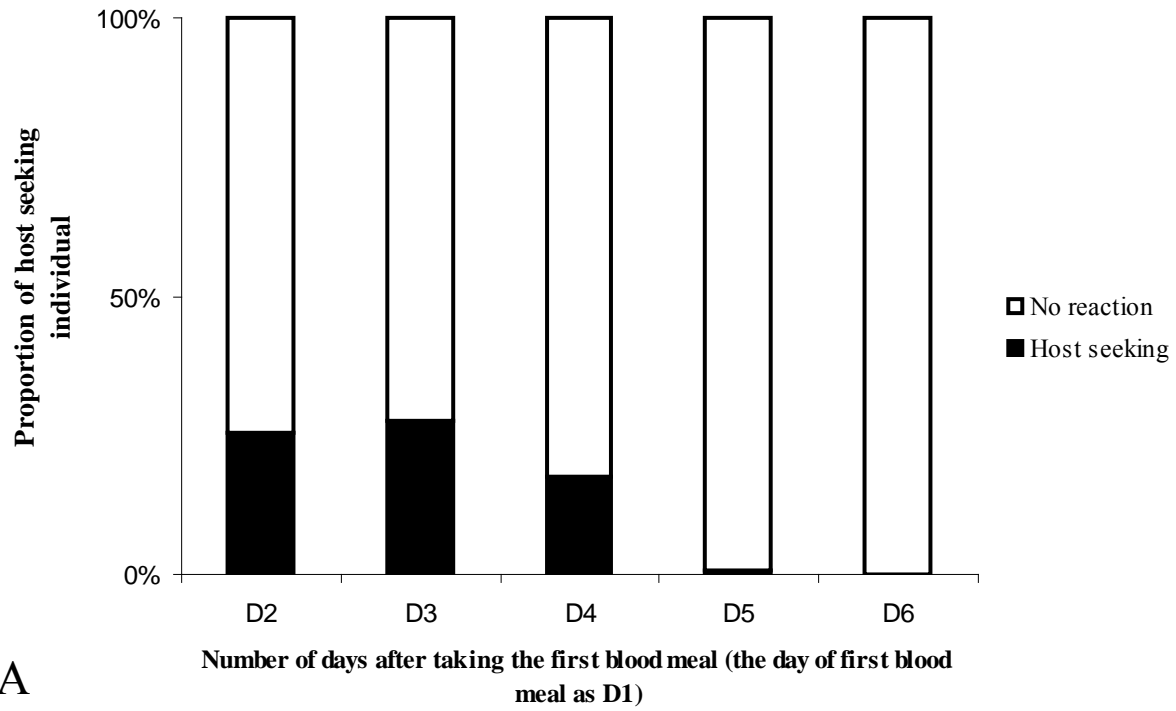


Figure: 1A

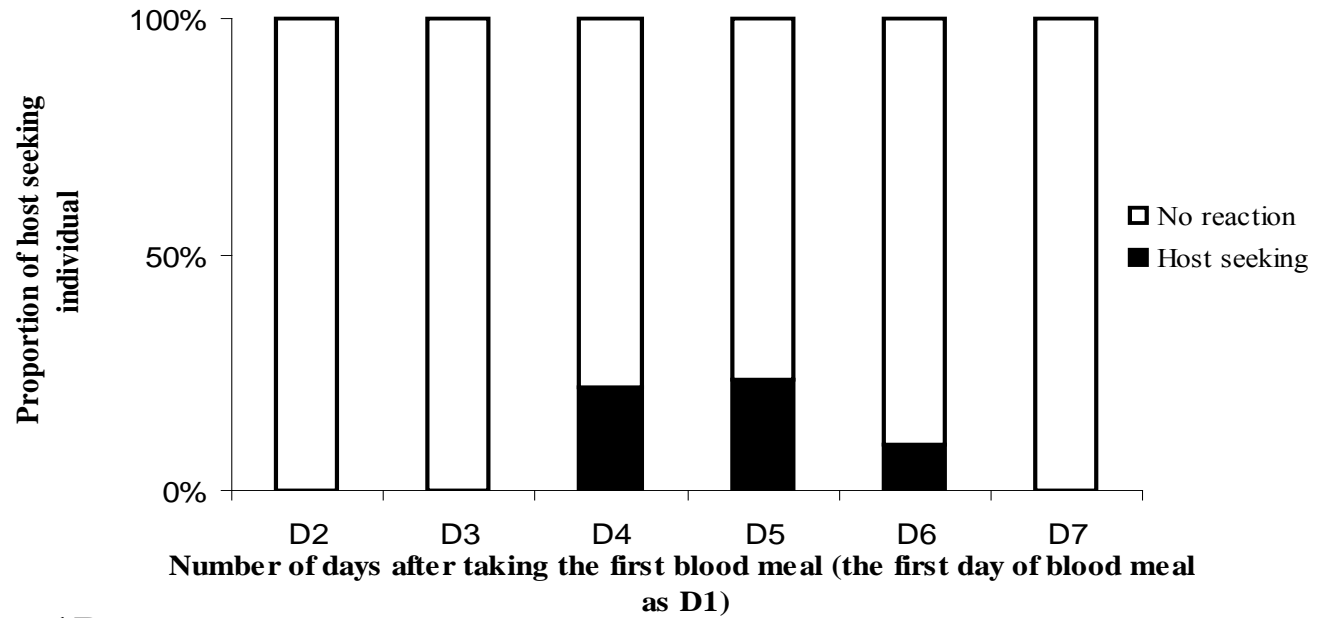


Figure: 1B

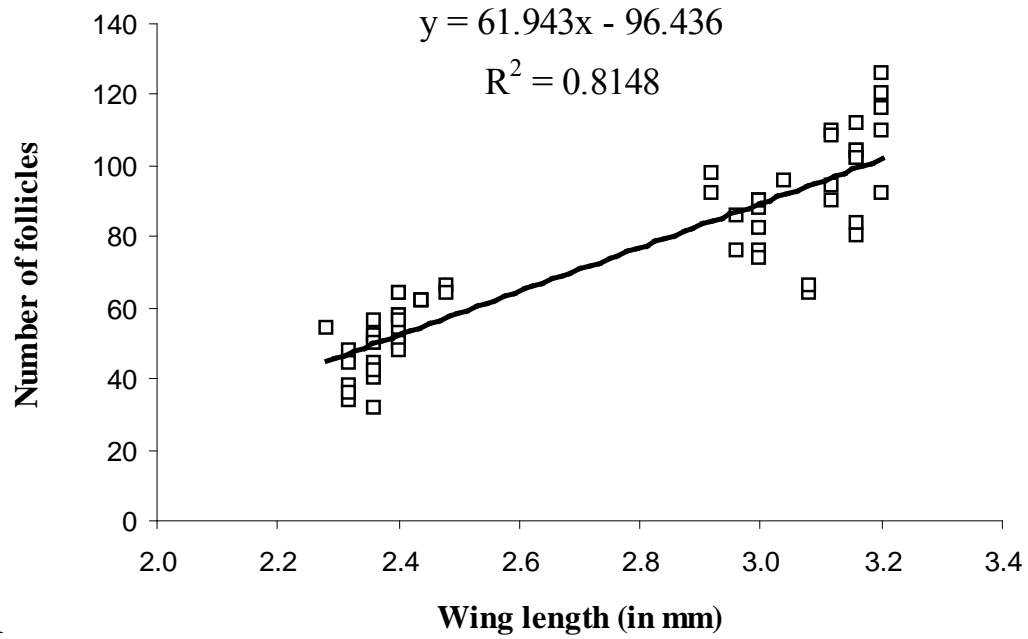


Figure: 2A

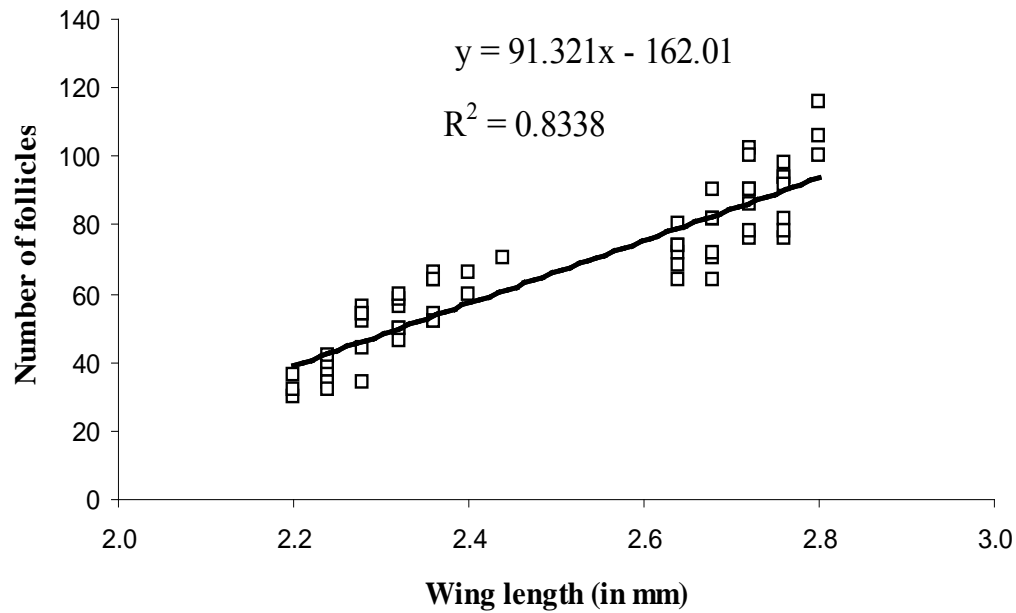


Figure: 2B

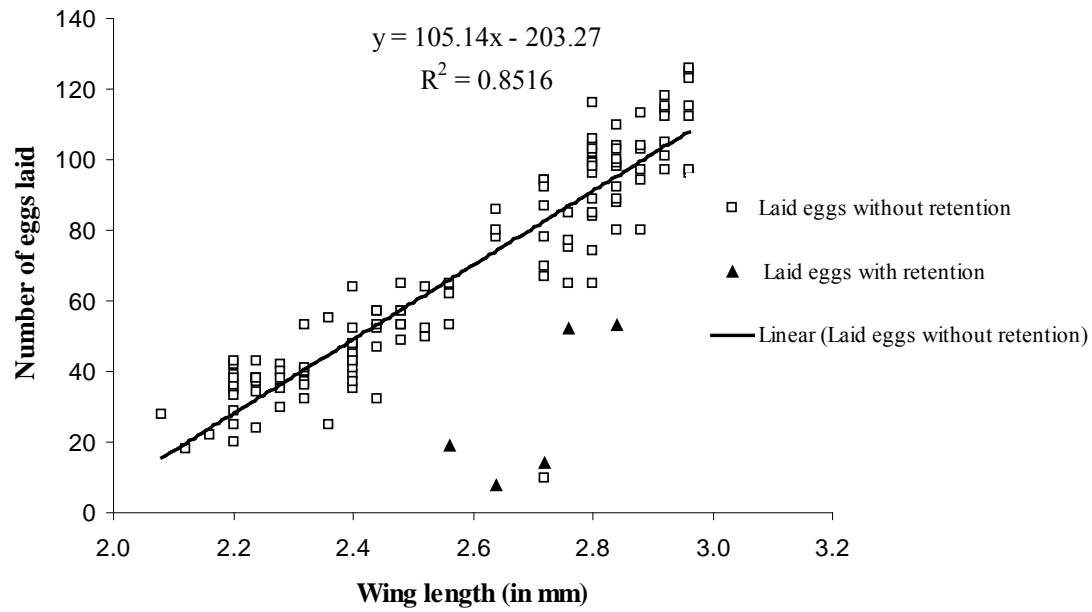


Figure: 3A

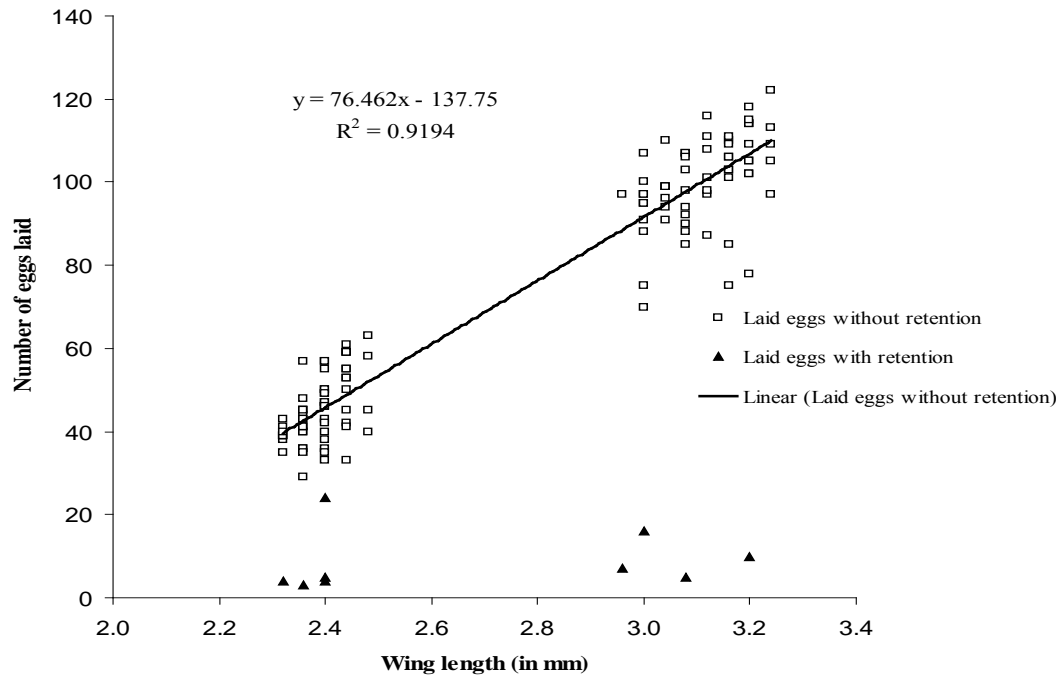


Figure: 3B

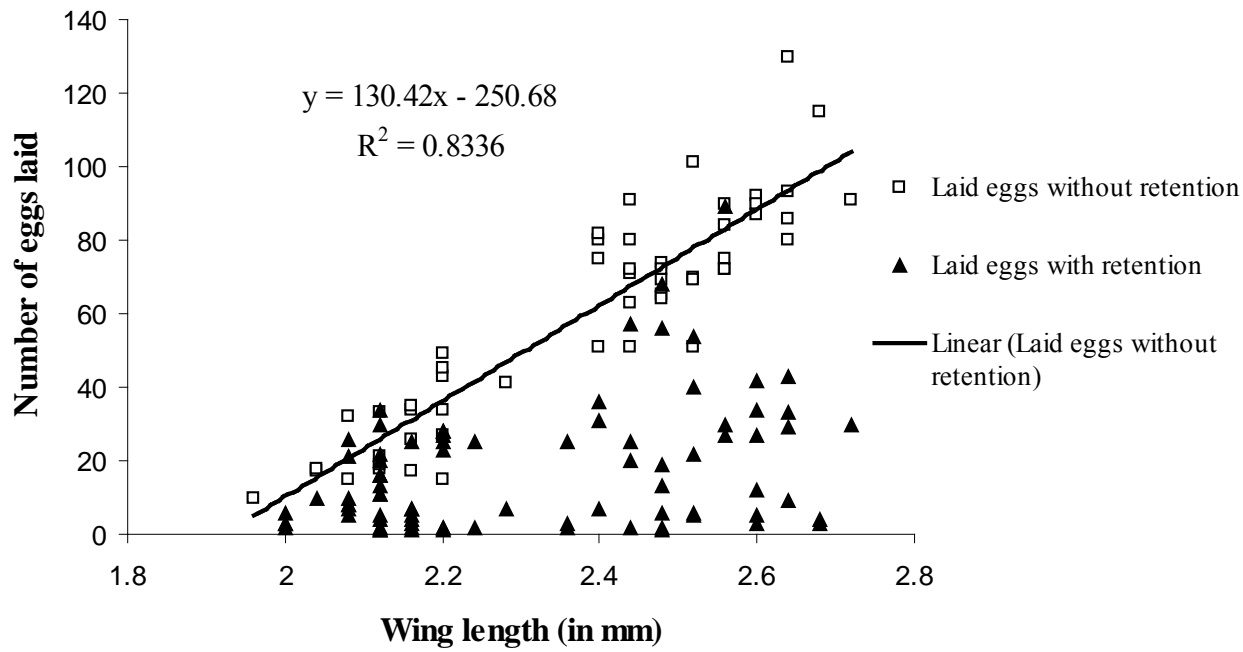


Figure: 4A

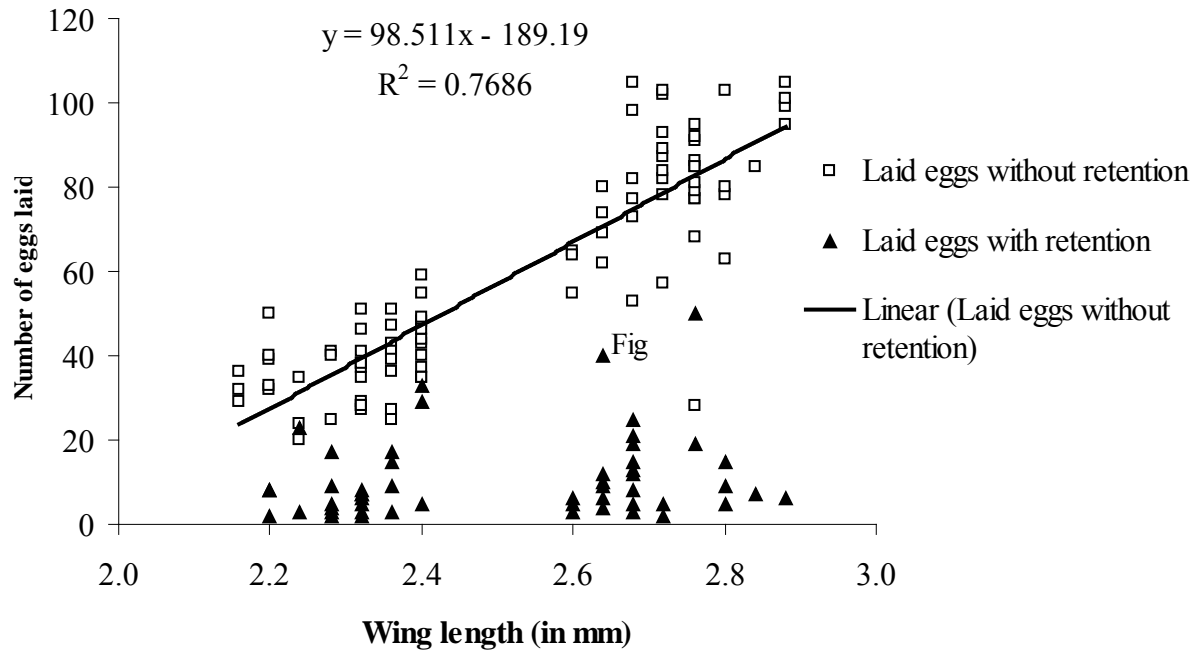


Figure: 4B