Effect of body size on multiple blood feeding and egg retention of Aedes aegypti (L.) and Aedes albopictus (Skuse) (Diptera: Culicidae)

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Effect of body size on multiple blood feeding and egg retention of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae)

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Abstract: Multiple blood feeding (MBF) in a gonotrophic cycle in vector mosquitoes influences pathogen transmission by increasing host-vector contact. Multiple blood meals can be caused by malnutrition in the larval stage, a harsh environment in the adult stage, or interrupted feeding due to host defense. We focused on the effect of body size on MBF in two vector mosquitoes, *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), in the laboratory, using small and large adults of both species. Most females (94.3% of *Ae. aegypti* and 88.2% of *Ae. albopictus*) oviposited with the first blood meal. There was no relationship between body size and MBF proportion in either species. However, there was a negative relationship between body size and egg retention ratio in *Ae. albopictus* ovaries and between body size and the ratio of immature follicles in both species. Small *Ae. albopictus* laid some eggs but retained the rest in their ovaries, as did 5.3% of *Ae. aegypti*. Large *Ae. albopictus* developed 68.4–81.7% of follicles, whereas, small ones developed about 50%. Large *Ae. aegypti* developed 98.0–99.8% of follicles, whereas small females developed only 83.5–88.4%. These results suggest that oviposition was incomplete in small females with low energy reserves, and that females emerging under subpar-diet conditions may perform MBF to improve fecundity.

Key words: *Aedes aegypti*, *Ae. albopictus*, body size, multiple blood feeding

Introduction

Multiple feeding is important in epidemiology, as it may increase the frequency of host contact (Garrett-Jones, 1964; Garrett-Jones and Shidrawi, 1969; Dye, 1986). Two forms of multiple blood feeding in one gonotrophic cycle have been recognized: supplementary feeding due to nutritional reserve scarcity in teneral females and interrupted feeding, possibly due to host defense (Clements, 1999). Supplementary feeding has been reported in some *Anopheles* species (Senior White, 1952; Smith and Weitz, 1959; Edman and Downe, 1964; Boreham and Garrett-Jones, 1973; Burkot et al., 1988) and in *Aedes aegypti* (L.) (Scott et al., 1993a, 1993b; Xue et al., 1995; Scott et al., 2000). *Anopheles* often requires supplementary feeding of two or more blood meals to produce the initial batch of eggs (Briegel and Horler, 1993). In anophelines, the nutritive state, rather than interrupted blood meals, forces repeated blood feeding (Briegel and Horler, 1993). Midgut cells of anophelines are capable of continuously synthesizing high titers of trypsin with the intake of multiple blood meals (Horler and Briegel, 1995). In anophelines, the ovaries reach the late resting stage only with 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 anopheline females and an important component of their reproductive strategy (Briegel and Horler,

1993).

Interrupted feeding, the other type of multiple feeding, may 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). The physiological mechanism of multiple feeding as interrupted feeding has been studied in *Ae. aegypti*: host-seeking after a blood meal is inhibited by two distinct physiological mechanisms, abdominal distension and egg development (Klowden and Lea, 1978, 1979a, 1979b). Moreover, the initial size of the blood meal and the nutritional state of the female limits feeding frequency at the beginning of each gonotrophic cycle (Klowden and Briegel, 1994).

The relationship of mosquito body size to blood feeding behavior has been well studied (Klowden and Lea, 1978; Nasci, 1986; Briegel, 1990; Chambers and Klowden, 1990; Nasci, 1990; Xue et al., 1995; Scott et al., 2000). Although large mosquitoes are reported to be more involved with multiple blood feeding (Klowden and Lea, 1978; Xue et al., 1995), small mosquitoes have also been found to feed more frequently than large mosquitoes because they have lower teneral energy reserves (Nasci, 1986, 1990; Briegel, 1990; Chambers and Klowden, 1990). Small females need more than one blood meal to initiate vitellogenesis, complete egg maturation, and perform maintenance activities (Briegel, 1985, 1990; Foster, 1995). As mosquito body size is governed by environmental factors such as the availability of nutrients and the temperature of the larval habitat, the relationship between body size and blood feeding can be modified by the larval environment. Teneral females raised under poor nutrition may require more than one blood meal for their first oviposition (Scott et al., 2000; Reyes-Villanueva, 2004). Hence, predictions differ as to whether small females perform more multiple blood feedings as supplementary feedings or large mosquitoes do more as a result of their larger blood meal size threshold.

The purpose of the present study was to clarify the relationship between body size and the proportion of multiple blood feedings as supplementary feedings in a single gonotrophic cycle in *Ae. aegypti* and *Ae. albopictus* under laboratory conditions. We varied female sizes by manipulating rearing temperature as well as food availability, as mosquito body size is regulated by larval rearing conditions, including temperature and nutrition (Briegel, 1990; Rae, 1990; Rueda et al., 1990; Tun-Lin et al., 2000; Farjana et al., 2011).

Materials and Methods

Mosquito source

Aedes aegypti (L.) were originally collected from Singapore about 5 years ago and *Aedes albopictus* (Skuse) were collected from Nagasaki, Japan (32°46′20.35″N and 129°52′9.86″E) about 10 years ago. Laboratory colonies were established at the Institute of Tropical Medicine, Nagasaki University, Japan. Both species were brought to Laboratory of Ecology in Kanazawa University and maintained for these experiments. Adults were maintained in cages at 25 ± 1°C and 70–90% relative humidity under 14L/10D photoperiod conditions. Adult mosquitoes were allowed to have access to 3% sucrose solution, and they were blood fed on rats once weekly.

Rearing larger and smaller mosquitoes

Combination of rearing temperatures (20°C or 30°C) and diet amount (low or high) in developing stage were controlled to yield larger and smaller females. Four groups of mosquitoes of each species were established from four treatment combinations (20°C × low diet, 30°C × low diet, 30°C × high diet, and 20°C × high diet). Larvae were reared in a plastic tray (40 × 30 × 7 cm) in the density of 200 larvae per tray. Larvae were fed with 0.05 and 0.1 mg/larvae/day of larval food to early stage (1st and 2nd instars) and later stage (3rd and 4th instars) larvae, respectively as low diet; whereas the rate was 0.4 and 0.8 mg/larvae/day for early stage and later stage larvae, respectively, as high diet. Larval food was made with mixture of rat food (CE-

2, CLEA Japan, Inc. Tokyo) and yeast extract powder (Ebios, Mitsubishi Tanabe Pharma Corporation, Osaka) (1 : 1 by weight). Emerged females were separated to monitor multiple blood feeding in a gonotrophic cycle, to count number of eggs laid, and to count number of ovarian follicles.

Multiple blood feeding and oviposition test

At eclosion, all adult mosquitoes were placed in rearing cages (20 × 20 × 30 cm) at 27℃, 70–90% RH under 14L/10D photoperiod conditions, and supplied with 3% sucrose solution. Males were kept together with females. At five to seven days post-eclosion, females were allowed to feed on human hand until they voluntarily finished their feeding. A total of seventy females were blood fed from each of four treatment groups. Every engorged female was kept separately after feeding in a plastic vial (3 cm diameter × 6 cm height) covered by mesh, with access to distilled water from a piece of soaked cotton through the mesh. A piece of wet cotton was placed at the bottom of the vial which was covered by a piece of filter paper to serve as ovipositioning substrate. Every female was checked daily for their oviposition. The starting date of oviposition was recorded. As mosquitoes did not lay eggs at once (based on observation in our preliminary study), they were kept to be observed for another 3 days after they lay eggs for the first time. Oviposited females were sacrificed for dissection if there are any retained eggs in their ovaries, to avoid the problem of retaining eggs in the females in the laboratory environments as unnatural artifacts (Packer and Corbet, 1989). The total number eggs laid or retained in her ovaries was considered as number of eggs by the female. Females those did not oviposited for 6 days in *Ae. aegypti* and for 8 days in *Ae. albopictus* after their first blood meal, were allowed to take the second blood meal. The mosquitoes transferred to rearing case from plastic vial to give 2nd/3rd blood meal. The timing of giving the second blood meal was determined by our observations in laboratory, considering the maximum time between blood intake and oviposition in the two species. This procedure was repeated up to the third meal. The experiment was terminated if the mosquitoes do not lay eggs after seven days of the third meal. Females those oviposited or did not even after the third meal were killed in freezers to measure wing length as an indicator of body size. The wing length was measured from the distal end of the axial inclusion to the apical margin, not including the fringe followed by Van Den Heuvel (1963) using a micrometer under a stereomicroscope.

Counting of ovarian follicles

A part of unfed emergent females of respective 4 treatment groups (2 temperature × 2 diet) were raised with 3% sucrose solution and were killed within 5 to 8 days after emergence. Ovaries were dissected to count the number of ovarian follicles. We counted the primary follicles (Christophers' stage II), at this stage the follicles are about 100 μm long and ooplasm contain fine lipid droplets. The primary follicles usually remain previtellogenic resting stage until the female has taken blood meal (Clements, 1992). Number of follicles in one ovary was doubled to represent the number of follicles in the female. One of her wings was measured its length in the same way described above.

Statistical analysis

The relationship between the wing lengths of mosquitoes and the number of blood meals taken, 1 to 3 in a gonotrophic cycle were analyzed by logistic regression analysis. Linear regression was used to analyze the effect of wing size on the number of eggs and number of follicles in ovaries of the two species. Statistical analyses were performed using JMP version 5.0.1.2 (SAS Institute, Cary, NC, USA).

Results

The average wing size of *Ae. aegypti* (Singapore strain) was 2.75 ± 0.33 mm (mean ± SE) and that of *Ae. albopictus* (Nagasaki strain) was 2.46 ± 0.26 mm, with *Ae. aegypti* significantly larger than *Ae. albopictus* (*t*-test,

$P < 0.001$). Large females of both species emerged from adequate nutrient (high diet) conditions (average wing length: 3.21 ± 0.10 mm at 20℃ and 2.82 ± 0.09 mm at 30℃ for *Ae. aegypti* and 2.83 ± 0.10 mm at 20℃ and 2.53 ± 0.09 mm at 30℃ for *Ae. albopictus*), and small females emerged from low nutrient (low diet) conditions (average wing length: 2.67 ± 0.10 mm at 20℃ and 2.34 ± 0.12 mm at 30℃ for *Ae. aegypti*, and 2.36 ± 0.06 mm at 20℃ and 2.14 ± 0.08 mm at 30℃ for *Ae. albopictus*; Table 1). In all, 94.3% of *Ae. aegypti* ($n = 280$) and 88.2% of *Ae. albopictus* ($n = 280$) females oviposited with the first blood meal (Table 1). Of 16 *Ae. aegypti* females that did not oviposit with their first blood meal, 2 laid eggs with the second blood meal and 5 died, but none of the remaining 9 oviposited with the third blood meal (Table 1). In *Ae. albopictus*, 27 of 33 oviposited with the second meal, and 4 of the remaining 6 oviposited with the third meal but the other 2 did not oviposit (Table 1). Of those that oviposited with the first blood meal, wing lengths were 2.77 ± 0.33 mm ($n = 264$) and 2.47 ± 0.27 mm ($n = 247$) in *Ae. aegypti* and *Ae. albopictus*, respectively. Wing size did not differ between the two groups that laid or did not lay eggs with the first blood meal (logistic regression: *Ae. Aegypti*, $r^2 = 0.014$, $\chi^2 = 1.738$, $P > 0.1$; *Ae. albopictus*, $r^2 = 0.00$, $\chi^2 = 0.001$, $P > 0.1$). The number of days between emergence and oviposition was 9.23 ± 0.95 ($n = 266$) in *Ae. aegypti* and 11.76 ± 2.44 ($n = 278$) days in *Ae. albopictus*. The relationship between wing length and duration to first oviposition was significant in *Ae. aegypti* (one-way ANOVA, $F = 1.55$, $df = 1267$, $P < 0.05$) but not in *Ae. albopictus* (one-way ANOVA, $F = 1.03$, $df = 1174$, $P > 0.05$). In *Ae. aegypti*, 94.7% of females laid all of the eggs in their ovaries, with the remaining 5.3% retaining some eggs in their ovaries. In contrast, 43.5% of *Ae. albopictus* laid all of their eggs, whereas 66.5% retained some eggs in their ovaries. Egg retention did not vary in relation to wing size in *Ae. aegypti*′ (logistic regression: $r^2 = 0.035$, $\chi^2 = 3.7$, $P > 0.05$), whereas *Ae. albopictus* with retained eggs were smaller than those without (logistic regression: $r^2 = 0.052$, $\chi^2 = 18.5$, $P < 0.001$).

The relationship between total number of eggs (number of eggs laid and retained in the ovaries) and wing size was positive for both species (linear regression model; Fig. 1A & B). The regression equation for *Ae. aegypti* was $N_{egg} = 79.30 \times$ wing size (mm) $- 144.08$ ($n = 266$, $r^2 = 0.77$, $F = 882.25$, $P < 0.01$), and that for *Ae. albopictus* was $N_{egg} = 104.81 \times$ wing size (mm) $- 201.37$ ($n = 278$, $r^2 = 0.66$, $F = 540.29$, $P < 0.01$).

The number of follicles was positively related to wing length in both species (linear regression model, Fig. 2A & B). The regression model for *Ae. aegypti* was $N_{follicles} = 73.72 \times$ wing size (mm) $- 122.79$ ($n = 120$, $r^2 = 0.88$, $F = 846.75$, $P < 0.01$), and that for *Ae. albopictus* was $N_{follicles} = 129.99 \times$ wing size (mm) $- 222.76$ ($n = 120$, $r^2 = 0.89$, $F = 976.88$, $P < 0.01$).

The number of eggs was directly correlated with the number of follicles present before the first intake of blood meals (Table 2), thus demonstrating that nearly all follicles (98.0–99.8%) underwent vitellogenesis in large *Ae. aegypti* under high diet conditions, whereas 83.5–88.4% of them did under low diet conditions (Table 2). In contrast, the number of eggs was substantially lower than the number of follicles in *Ae. albopictus*, and more so in small mosquitoes under low-diet conditions. Small *Ae. albopictus* under low-diet conditions developed 45.3–55.3% of follicles, whereas large ones under high-diet conditions developed 68.4–81.7% of follicles (Table 2).

Discussion

We compared the body size variations that we created in two species of mosquitoes with relevant studies to determine whether our sample size was appropriate to clarify relationships between body size and MBF. Our *Ae. albopictus* specimens with wing lengths of 2.14–2.83 mm were similar in size to those reported by Mori (1979), who manipulated larval density and food supply to yield adults with wing lengths of 2.19–2.96 mm in experimental groups with less than 30% mortality. However, both our samples and those of

Table 1. Counts of multiple blood feeding and average wing size of the 4 rearing conditions in *Ae. aegypti* and *Ae. albopictus*.

Species	Temp (°C)	Diet	n	1st blood meal				2nd blood meal				3rd blood meal			
				Female laid eggs		Female unlaid		Female laid eggs		Female unlaid		Female laid eggs		Female unlaid	
				Wing size (mm) Mean ± SE	n	Wing size (mm) Mean ± SE	n	Wing size (mm) Mean ± SE	n	Wing size (mm) Mean ± SE	n	Wing size(mm) Mean ± SE	n	Wing size(mm) Mean ± SE	n
Ae. aegypti	30	Low	70	2.34 ± 0.12	66	2.34 ± 0.16	4	–	0	2.34 ± 0.16	4	–	0	2.34 ± 0.16	4
	20	Low	70	2.67 ± 0.10	64	2.69 ± 0.09	6	2.66 ± 0.2	2	2.71 ± 0.02	4	–	0	–	0
	30	High	70	2.82 ± 0.09	65	2.79 ± 0.09	5	–	0	2.81 ± 0.09	4	–	0	2.81 ± 0.09	4
	20	High	70	3.21 ± 0.10	69	3.00 ± 0.00	1	–	0	3.00 ± 0.00	1	–	0	3.00 ± 0.00	1
Ae. albopictus	30	Low	70	2.14 ± 0.08	62	2.17 ± 0.04	8	2.17 ± 0.04	6	2.16 ± 0.06	2	2.12 ± 0.00	1	2.2 ± 0.00	1
	20	Low	70	2.36 ± 0.06	60	2.38 ± 0.07	10	2.35 ± 0.05	7	2.43 ± 0.10	3	2.48 ± 0.06	2	2.32 ± 0.00	1
	30	High	70	2.53 ± 0.09	64	2.56 ± 0.13	6	2.56 ± 0.13	6	–	0	–	0	–	0
	20	High	70	2.83 ± 0.10	61	2.76 ± 0.09	9	2.77 ± 0.11	8	2.76 ± 0.07	1	2.8 ± 0.00	1	–	0

n: Number of females observed.

Table 2. Ratio of follicles and eggs of the 4 rearing conditions in *Ae. aegypti* and *Ae. albopictus*.

Species	Temp (°C)	Diet	Wing size (mm) (mean ± SE)	n[1]	No. of follicles (mean ± SE)	n[2]	No. of eggs (mean ± SE)	% of follicles developed to eggs
Ae. aegypti	30	Low	2.35 ± 0.11	30	51.0 ± 12.28	66	42.6 ± 10.98	83.5
	20	Low	2.64 ± 0.11	30	65.2 ± 10.80	66	57.6 ± 11.2	88.4
	30	High	2.80 ± 0.09	30	92.1 ± 14.01	65	91.9 ± 20.57	99.8
	20	High	3.21 ± 0.10	30	109.3 ± 16.87	69	107.0 ± 15.47	98.0
Ae. albopictus	30	Low	2.12 ± 0.09	30	50.5 ± 13.18	69	27.9 ± 8.51	55.3
	20	Low	2.35 ± 0.08	30	73.1 ± 9.97	69	33.2 ± 11.52	45.3
	30	High	2.49 ± 0.10	30	84.3 ± 7.38	70	68.9 ± 20.12	81.7
	20	High	2.83 ± 0.09	30	151.1 ± 19.05	70	103.3 ± 22.66	68.4

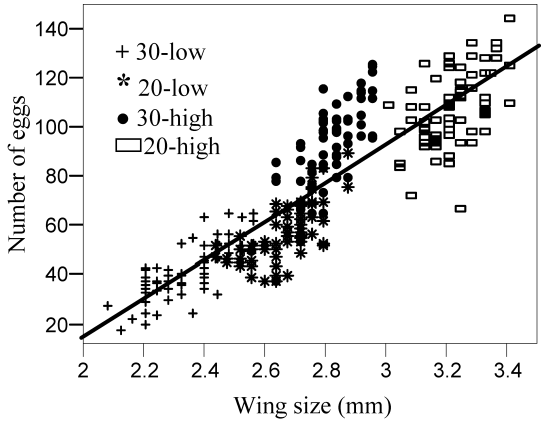
n[1]: number of females observed for counting the number of follicles.
n[2]: number of females observed for counting the number of eggs.

Mori (1979) seemed to be less variable than wild-type *Ae. albopictus*, lacking the large individuals with wing lengths of 2.2–3.7 mm recorded by Suzuki et al. (1993), who collected wild *Ae. albopictus* females in Nagasaki in June. Our samples of *Ae. aegypti*, ranging in wing length from 2.34 mm to 3.21 mm, were similar to those of Schneider et al. (2004), who recorded wild *Ae. aegypti* wing lengths of 2.1–3.05 m in Iquitos, Peru, and to those of Tun-Lin et al. (2000), who raised *Ae. aegypti* with wing lengths of 2.43 mm (35℃) to 3.26 mm (20℃) in the laboratory. On the other hand, these samples appear to be less variable than wild *Ae. aegypti* in Australia with wing lengths of 2.69–3.94 mm (Tun-Lin et al., 2000). Hence, we judged our specimens to be both small and large enough in size to demonstrate relationships between body size and MBF.

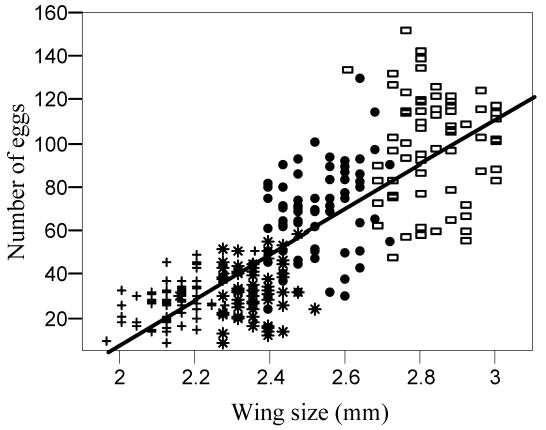
We gave a complete blood meal to the mosquitoes, allowing them to feed until they voluntarily withdrew their proboscis from the host. No size-dependent MBF was observed in the two species in our study. In the field, blood feeding can be interrupted by defensive behavior by the host. Although Shemanchuk et al. (1963) observed that mosquitoes, particularly *Aedes* species, are not easily disturbed once they have started the feeding process, a complete blood feeding in the wild is not thought to be frequent due to host defenses (Klowden and Lea, 1979c). Much higher MBF ratios in *Ae. aegypti* have been reported in the field (Scott et al., 1993a, 1993b; Scott et al., 2000). This suggests that in *Ae. aegypti* MBF is mainly caused by host defenses, not supplementary feeding.

In our study, *Ae. albopictus* showed comparatively higher tendencies to perform MBF. Hawley (1988) mentioned that MBF is usually not necessary for egg maturation and estimated that very few to perhaps 20% of wild *Ae. albopictus* perform MBF in the field. Moreover, in this species, a considerable number of follicles were undeveloped following the first blood meal, indicating that females might need MBF to develop all follicles to enhance their fecundity. Mori and Wada (1977) found that 9 of 1170 released engorged female *Ae. albopictus* sought hosts while still having undigested blood meals. As only 449 of the mosquitoes were recaptured, the relevant proportion is higher than the sample number of 1170 suggests.

We found that 66.5% of *Ae. albopictus* retained eggs in their ovaries. The phenomenon was size dependent, in that small females retained more eggs. Egg retention may be rare in nature (Packer and Corbet, 1989). The observed high proportion of egg retention may have been due to a limited choice of oviposi-

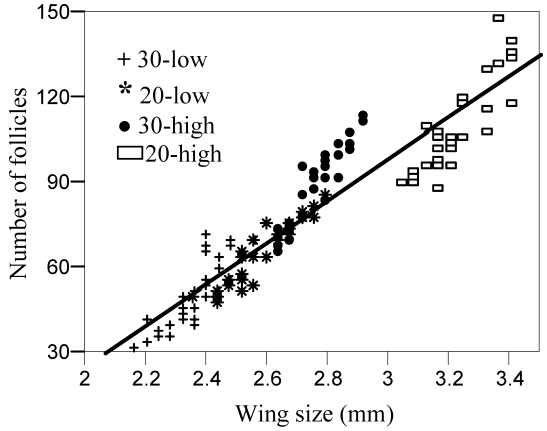


A. *Ae. aegypti*

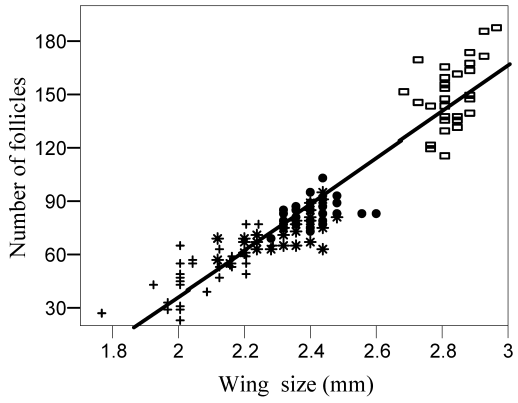


B. *Ae. albopictus*

Fig. 1. Wing size and number of eggs of 4 rearing conditions in Fig. 1A: *Ae. aegypti* and Fig. 1B: *Ae. albopictus.*



A. *Ae. aegypti*



B. *Ae. albopictus*

Fig. 2. Wing size and number of follicles of 4 rearing conditions in Fig. 2A: *Ae. aegypti* and Fig. 2B: *Ae. albopictus.*

tion sites, or restricted movement in the laboratory (Leisnham et al., 2008). However, we speculate that egg retention may reflect a real feature of *Ae. albopictus* ovipositioning. Mori (1979) studied the relationship between body size and adult behavior and found that small emergents (due to poor food supply or crowding in the larval stage) tended to disperse further from their release points. This may explain why small females showed a tendency to retain their eggs without laying in our experiment, in that they might need to disperse before ovipositioning. In the field in Nagasaki, Suzuki et al. (1993) found that 55 of 1250 host-seeking Ae. albopictus females (4.4% of the whole collection, 9.6% of the parous part) retained eggs. In contrast to our results, they found that egg retention was greater in large females. However, our results may not be contradictory, as Suzuki et al. (1993) also reported that large females survived longer. In our experiment we compared the relationship between size and egg retention within same-age females, whereas Suzuki et al. (1993) used groups of old (parous) and young (nulliparous) females. Thus, egg-retaining mosquitoes (older individuals) would be larger than the young females in their

study. We also suggest another possibility: small females may need more energy reserves for dispersal and may keep their eggs as energy stocks for their own survival, if we assume egg absorption by mothers (Magnarelli, 1983).

Follicle maturation was nutrition-dependent in both species. Mosquitoes reared under low diet conditions may need more than one blood meal to develop all follicles (Mori, 1979; Nasci, 1986, 1990; Briegel, 1990; Chambers and Klowden, 1990).

We suggest that females may perform MBF to increase the number of eggs oviposited, even when they can oviposit without MBF, since size-dependent fecundity was observed in variable steps in the two species. However, the generality of our observation should be tested using multiple strains of the two species. The Singapore strain of *Ae. aegypti* that we used was substantially larger than the Nagasaki strain of *Ae. albopictus*. Thus, investigating strains of small *Ae. aegypti* and large *Ae. albopictus* is desirable to determine the possibility of differences representing specific or size-dependent characters.

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