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BITING PERSISTENCE IN *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

By
ABEER S. YAMANY
Department of Zoology, Faculty of Science, Zagazig University, Zagazig, Egypt and Department of Biology, Faculty of Science and Arts, Hafr Al-Batin University, Saudi Arabia (Email: emarewan@yahoo.com)

Abstract

Mosquitoes' body size infuences a number of important factors determinants in disease transmission. Generally, high nutrition during developing stages results in large adults. The interrupted feeding of *Ae. albopictus* spreads zoonotic viruses.

Adult dry weight and total number of landings positively correlated with the larval nutrition represented by adult body size. Potentiality of *Ae. albopictus* to attack its hosts for blood meal required for egg development is an important factor in disease transmission. Although the biting persistence was described in the literature as the norm for mosquitoes, yet more study was needed. Measurement of the number of landings during a 60-min test for large and small females supplied different nutrients. Small females were significantly less persistent than larger ones. Supply of 10% sucrose solution or water ± 10% sucrose solution for 6 days significantly influenced biting persistence in large and small females. The pattern of persistence exhibited more increase in case of females fed 10% sucrose solution and 10% sucrose solution + water for 6 days than others fed for 4 days or 2 days, either from the high or low larval diet. This support the hypothesis that *Ae. albopictus* females body size influences bitting persistence.

Key words: Biting persistence, *Aedes albopictus,* larval nutrition, adult nutrition, body size

Introduction

Larval nutrition of mosquito controls adult body size (Fish 1985). Sugar and blood are two diet options for mosquitoes; the blood is essential for egg production, large female is more persistent to complete blood feeding successfully than small one in state of host defensive behavior (Walker and Edman, 1985) coupled with the decreased flying ability (Foster 1995), escape speed, and ability of a mosquito after a meal (Roitberg *et al,* 2003). Effects of blood feeding intermission and larvae food amount on growth rate of *Ae. albopictus* were studied in laboratory (Tsuda *et al,* 1992). Large females starved for 7 days caused significantly reduced persistence compared to *Ae. aegypti* starved for 3 days (Nasci, 1991). Brochero *et al.* (2006) and Xue *et al.* (2009) described the biting activity of *Anopheles* species and *Ae. albopictus* and found that body size affected disease transmission. Delatte *et al.* (2010) studied blood-feeding behavior of *Ae. albopictus* and found that interrupted feeding occurred when blood feeding was separated by defensive responses of host. Kamgang *et al.* (2012) assessed two elements of blood feeding behavior in *Ae. albopictus* females and found that species is extremely aggressive to humans and might be involved in man-man virus transmission. Farjana and Tuno (2013) reported that large mosquitoes have less contact with hosts, size-dependent multiple feeding influences contact frequency with hosts, and transmission was affected by body size. Manica *et al.* (2017) clarified possibility to foresee mean number of *Ae. albopictus* females biting and found that ovitrap information can be misused to gauge number of biting. Generally, *Aedes aegypti* and *Ae. albopictus* are vectors of Dengue fever, Yellow fever, Zika fever and Chikungunya fever, increasing in the amount of blood meal would increase prevalence and incidence of virus (El-Bahnasawy *et al,* 2015).

Materials and Methods

Ae. albopictus were given from colony previously kept up for 15 generations in the Zoomorphology Laboratory, Cell Biology and Parasitology Institute, Heinrich Heine

University, Düsseldorf, Germany.

Adults were reared in small wooden cages (30x30x30cm). Two levels of larval conditions were used to control body size at adult emergence: 1- 200 larvae, high larval diet or low larval density. 2- 600 larvae, low larval diet or high larval density. Before measuring biting persistence, emerged adults were reared for 2, 4 & 6 days at 25±1°C, 70-80% RH, photoperiod of 12:12 (L: D), and offered different adult diet (10% sucrose solution, water and 10% sucrose solution + water).

For blood-feeding persistence quantification, females were separated in small cages and given 15 minutes to be adapted. Via the cage opening, a human volunteer hand was introduced. Females were not allowed to feed after landing on the host by quivering the hand and obliging to leave the host. Times number of females landed on host every five consecutive minutes was recorded for 60 minutes. As an indicator of body size, wing length was measured from the root to apex at the test end using an ocular micrometer at 25X (Gillies and de Meillon, 1968). Killed females were air-dried for two days at 85°C and weighed. For each treated group, mosquitoes' persistence was quantified as the total number of landings in 60-minute tested period.

Statistical analysis: Mean wing length, dry weight and total number of landings in 60-minute test period (±SE) of treatment groups (larval and adult diets) were compared using one-way analysis of variance (ANOVA). Analysis was done using the SPSS software.

The patterns of females biting persistence rose from two larval diets and supplied different adult diets were compared using regression analysis (Sokal and Rolf, 1981).

Results

The results showed that the wing length of all large females raised from high larval diet whether starved or offered different adult diets (i.e. water, 10% sucrose solution, water +10% sucrose solution) was significantly ($P<0.001$) greater than small ones reared on low larval diet depicted (Tabs. 1 & 2).

Larval diet was significantly affected adult dry weight: females raised from high larval diet fed water, 10% sucrose or 10% sucrose + water for two days was ($P<0.001$) significantly greater than dry weight (0.667± 0.067 mg, n=16), (1.033±0.191 mg, n=16) and (1.15±0.126 mg, n=16) than females raised from low larval on same diet (0.239 ±0.0008 mg, n=16), (0.2447±0.001 mg, n=16) & (0.255± 0.001 mg, n=16), respectively.

Dry weight of small females from low larval diet offered 10% sucrose solution only or with water for 4 days after emergence showed no significant difference (water, 0.213±0.001mg, n=11; 10% sucrose soluteion, 0.231±0.001mg, n=14; water+10% sucrose solution, 0.249±0.001 mg, n=13). But, dry weight of large females from high larval diet fed 10% sucrose solution for 4 days after emergence (0.95±0.0456mg, n=14) was significantly more than those offered water for same period (0.433±0.0494mg, n=11). Large females offered 10% sucrose solution for 6 days weighed the same as others offered 10% sucrose solution for 4 days (0.817±0.145mg, n=14; 0.95±0.0456mg, n=14, respectively).

Adding water to 10% sucrose solution for 2 days, 4 days & 6 days did not significantly affect dry weight of large females (1.15± 0.1258mg, n=16; 1.033±0.133mg, n=16; 0.92±0.111mg, n=14, respectively for each day) starvation of large & small females for 2 days post emergence significantly reduced dry weight. Access to water for 4 days after emergence gave a significant reduction ($P< 0.01$) in dry weight (0.433±0.049mg, n=11) more than for 2 days (0.667±0.067mg n=16) of large females (Figs. 1 & 2). No significant differences were detected in the average dry weight of emerging females whether reared during larval stage on a high larval diet or low larval diet as an influence of the adult age have. While, adult diet have a high significant ($P< 0.01$) effect on average dry weight was between starved large females for 1 days & 2 days and large females off-

ered water +10% sucrose solution for 4 days & 6 days. Largest value of dry weight was detected when females were supplied with 10 % sucrose + water for 2 days. Larval diet has a highly significant (P<0.001) effect on the average dry weight regardless of adult diets. Large starved females for 1 days & 2 days were found significantly (P<0.001) heavier than small starved females. Statistical analysis showed no significant difference in average dry weight measured for large females supplied with water, 10% sucrose solution only and water +10% sucrose solution after 2, 4 or 6 days.

Statistically, total number of landings recorded for small females fed 10% sucrose for 2 days (272.143±0.487) was same as others fed water (300.33±1.171) for same period before test. Biting persistence positively correlated to adult age for all specimens supplied with 10% sucrose whether large or small. Access to 10% sucrose solution for 4 or 6day significantly increases the average total number of landings measured by 0.853 (4day 597.778 ±0.258, n=14; 6day, 625.833 ±0.149, n=14) (Y=315+52.5X, r=0.853, Slop=52.5, df=1, F= 98.3) for large females, and by 0.969 for small ones (4days, 321.167±0.723, n=14; 6days, 369.333± 0.871, n=13) (Y=187.5+ 31.25 X, r=0.969, Slop= 31.25, F= 591.093). Same results were obtained when large and small females were supplied 10% sucrose with water for 4 or 6days (4days, 579.143-±0.584, n=16; 6 days, 644.664±0.128, n=14) and (4days, 555.11±0.06, n=13; 6days, 627.83±1.342, n=13), respectively (Fig 3).

Starvation and sugar deprivation were negatively influence significantly (P<0.001) in relation between biting persistence and adult age. Biting persistence of starved females decreased significantly by increas-ing adult age from one to two days (for large females, 1 day 255.571± 0.296, n=16; 2days 185.571±0.313, n=10 and for small females, 1 day, 193.5±0.214, n=16; 2 days 126.833-±0.154, n=9). Biting persistence of water fed females decreased significantly by increasing adult age from 2 to 4days (2days, 300.33±1.171, n=16; 4 days 247.5±1.997, n=11 for large females, 2days 236.167± 0.14, n=16; 4days 152.833± 0.179, n=11 for small females). No significant difference (P>0.05) was in total number of landings in water fed-large females for 4 days (247.5 ±1.997, n=11) as compared to starved large females for 1day (255.571± 0.296, n=16). A decrease total number of landings recorded significantly in females fed water only by increasing the female age from 2 to 4 days.

A significant increase in females fed 10% sucrose solution and water+10% sucrose solution increased female age from 2 to 4 to 6 days (P<0.001). Large *Ae. albopictus* fema les were significantly more persistent than small ones regardless of whether the females were starved or fed different types of food.

Table 1: Wing length, body dry weight & total number of landings/group in ***Ae***. *albopictus* (Skuse) raised from high level of larval diet during 60-min under laboratory conditions (25±1°C, 70-80% R.H.& D: L, 12: 12).

Adult diet	Age (days)	Number	Wing length (mm)	Dry weight (mg)	Total number of landings/group
Starved	1	16	3.614 ± 0.079	0.517 ± 0.017	255.571 ± 0.296
Starved	2	10	-	0.26 ±0.079	185.571 ±0.313
Water	2	16	3.617 ± 0.0946	0.667 ± 0.067	300.33 ± 1.171
Water	4	11	-	0.433 ± 0.049	247.5 ± 1.997
10% sucrose solution	2	16	3.675± 0.0796	1.033 ± 0.191	443. 3 ± 0.173
10% sucrose solution	4	14	-	0.95 ± 0.0456	597.778 ±0.258
10% sucrose solution	6	14	-	0.817 ±0.145	625.833 ± 0.149
Water + 10% sucrose	2	16	3.686 ± 0.079	1.15 ± 0.126	526.167 ±0.182
Water + 10% sucrose	4	16	-	1.033 ± 0.133	579.143 ± 0.584
Water + 10% sucrose	6	14	-	0.92 ± 0.111	644.664 ± 0.128

Discussion

Total number of landings during 60-minute test period was influenced significantly by larval diet, while pattern of biting persistence is affected significantly by adult diet in *Ae. albopictus*.

Larger females produced from high larval diet had more significant number of landings

Table 2: Wing length, body dry weight & total number of landings/group in *Ae. albopictus* (Skuse) raised from low level of larval diet during 60-min under laboratory conditions (25 ± 1°C, 70-80% R.H. & D: L, 12:12).

Adult diet	Age (days)	Number	Wing length (mm)	Dry weight (mg)	Total number of landings/group
Starved	1	16	2.7167± 0.0654	0.189 ± 0.001	193.5 ± 0.214
Starved	2	9	-	0.138 ± 0.001	126.833 ±0.154
Water	2	16	2.708 ± 0.0534	0.239 ± 0.001	236.1667 ± 0.14
Water	4	11	-	0.213 ± 0.001	152.833 ±0.1796
10% sucrose solution	2	16	2.785 ± 0.031	0.245 ± 0.001	272.143 ± 0.487
10% sucrose solution	4	14	-	0.231 ± 0.001	321.167 ±0.723
10% sucrose solution	6	13	-	0.228 ± 0.007	369.333 ±0.871
Water +0% sucrose	2	16	2.693 ± 0.03	0.255 ± 0.001	378 ± 0.556
Water+10% sucrose	4	13	-	0.249 ± 0.001	555.11 ± 0.06
Water+10% sucrose	6	13	-	0.234 ± 0.009	627.83 ± 1.342

than smaller and lighter females raised from low larval nutrition. Females offered access to water only or 10% sucrose solution only for 2days before testing had significantly the same biting persistence. Similar results were reported for *Ae. aegypti* (Klowden *et al,* 1988) and for *Ae. triseriatus* (Walker and Edman, 1985).

Heavier females raised from high larval diet had a greater number of landings, a higher success rate of feeding on blood and more host seeking behavior than lighter females rose from low larval diet regardless of the adult diet. Besides, heavier females are more persistence than lighter ones and were more able to complete blood feeding successfully. Same results were reported for *Ae. triseriatus* (Walker and Edman, 1985), for *Ae. aegypti* (Klowden *et al,* 1988; Nasci, 1991) and for *Ae. albopictus* (Tsuda *et al,* 1992). On the contrary, Farjana and Tuno (2013) claimed that the biting persistence and host-seeking behavior were negatively correlated to body size and large females have less contact with hosts.

Biting persistence of all heavier and lighter females were influenced significantly by the access to water only, 10% sucrose solution only or adding both for 4 or 6 days, while the dry weight are not influenced. Severe starvation of mosquito females significantly reduced total number of landings and the body dry weight regardless of females' size of. Also, when females were offered water only for 4 days, they were less persistent than those that fed 10% sucrose solution and 10% sucrose solution plus water. Walker and Edman (1985) found that sugar fed female *Ae. triseriatus* was more persistent than starved ones. Nasci (1991) found that body dry weight and biting persistence of large females were not altered by access to sucrose for 3 or 7. Severe starved females *Ae. aegypti* significantly influenced total number of landings.

In the present study, pattern of landings was influenced mainly by adult diet regardless of its age or size. A high level of persistence maintained relatively for all mosquitoes offered water+10% sucrose solution for a longer time than others fed water or 10% sucrose solution alone. Adult body size was positively correlated with persistence and nutrient contents. Large females achieved a successful blood meal within the shortest time span. But, duration of starvation was negatively correlated with adult body size; this was consistent with the hypothesis that biting persistence controlled by stored nutrient levels for *Ae. aegypti* (Nasci, 1991). Van Handel and Day (1988) found that lipid and glycogen reserved affect positively on wing length of *Ae. vexans* (Meigen). Nasci (1991) discovered that large *Ae. aegypti* adults raised from high larval diets, emerged with higher caloric reserves than smaller raised from low larval diets. Van Handel (1971) stated that energy source for mosquito flight depended primary on stored glycogen in tissue during larval stage, adult size-related variation correlated with larval diet and nutrient reserves, a primary factor control biting persistence. Edman and Lynn (1975) and Nayer and Sauerman (1975a,b) showed that after a blood meal carbohydrate withdrawal reduced females number that gave mature

eggs and reduced number of subjects to behavioral inhibition that accompanied oocyte development. Steinwascher (1982) reported that large female body size correlated with increased biting persistence and increased female longevity and starvation resistance.

Conclusion

Biting persistence positively correlated with adult nutrition and body size. Large mosquitoes females have more contact with hosts and thus more prevalence of viral hemorragic fevers

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Explanation of figures

Fig 1: Adult dry weight (mg) of *Ae. albopictus* (Skuse) females fed different adult diets and raised from low and high levels of larval diet under laboratory conditions (25±1ºC, 70-80% R.H. &D: L, 12: 12).

Fig.2: Total number of landings/group during 60-min measurements of biting persistence in *Ae. albopictus* (Skuse) fed different adult diets and raised from low and high levels of larval diets under laboratory conditions (25 ± 1ºC, 70-80% R.H. & D: L, 12: 12).

Figs. 3-6: Total number of landings during 60-min period for large *Ae. albopictus* females tested for treatment combination of high larvel diet/adult diet (water + 10% sucrose solution), regression formula: Y = number of landings, X = adult age, and r = correlation coefficient, R Sq Linear= 0.959. Slopes of regression formula showed a significant difference (P<0.05). Triangles showed number of landings per adult age for each individual tested. Line = formula shown as solid line via data under laboratory conditions (25±1ºC, 70-80% R.H. and D: L, 12: 12).