Journal of Asia-Pacific Entomology 18 (2015) 507–513

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Changes in the biting activity of a dengue vector relative to larval and adult nutritional histories: Implications for preventive measures

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ARTICLE INFO

Article history:
Received 13 November 2014
Revised 13 June 2015
Accepted 16 June 2015
Available online 4 July 2015

Keywords:
Aedes albopictus
Host feeding patterns
Body size
Starvation

ABSTRACT

The magnitude of dengue transmission depends largely on the level of human–vector contact. Therefore, knowledge regarding the biting periodicity of mosquitoes is crucial to determine transmission periods' risk, and in planning personal protection measures. Dengue vectors are day-active and endure transitory periods of starvation overnight. However, it is unclear how their blood feeding activity pattern is related to body size when temporarily deprived of their main source of energy — sugar. We examined the changes in *Aedes albopictus* diurnal biting activity, taking into account larval nutritional history and adult starvation. Overall, large body size and non-starvation conditions were associated with better blood feeding success, but these parameters did not significantly modify the timing of first blood feeding attempt. Females of both sizes showed significant temporal variations in their blood feeding activities. Under conditions of starvation, blood meal uptake was much greater in large females from morning to evening. Similar variations of feeding activity were observed in small females, except in the morning. Under non-starvation conditions, the blood feeding activity of small mosquitoes tended to decrease over time, whereas blood meal uptake activity was high and remained almost constant from morning to evening for larger mosquitoes. This work emphasizes the importance of body size and hunger on the dynamics of vector–host interaction and has important implications for the development of novel strategies for the prevention of disease transmission. Knowing when dengue vectors actively bite during the day can help in timing effective personal protective measures.

Introduction

According to the World Health Organization this disease claims an estimated 20,000 victims per year and there may be 50–100 million dengue infections worldwide yearly, and more than 2.5 billion people (over 40% of the world's population) are now at risk (World Health Organization, WHO, 2012). The effectiveness of mosquitoes as disease vectors rests primarily on their host-seeking behavior and strong preference for human hosts. Blood feeding is required for reproduction as well as disease transmission. Therefore, understanding the factors regulating host-seeking behaviors is a priority in the search for effective vector intervention strategies.

Aedes albopictus, which is indigenous to the tropical forests of Southeast Asia (Gratz, 2004; Ponlawat and Harrington, 2005), has invaded most continents (Paupy et al., 2009). This mosquito species can be found in urban centers of tropical and temperate countries, where it is second to *Aedes aegypti* as a vector of dengue (Hawley, 1988). Similar to many other vectors, mosquitoes become infected by pathogens during feeding on the blood of infected hosts because they require blood protein for egg development (Foster, 1995), as well as to obtain energy (Nayar and Sauerman, 1975). Thus, female mosquitoes can pick up or transfer pathogens to the host during blood meals. Due to the importance of this physiological process, there have been a number of recent studies regarding the blood feeding behavior of mosquito vectors. Both host-seeking behavior (Klowden et al., 1988) and susceptibility to viral infection (Nasci and Mitchell, 1994) have been reported to be dependent on mosquito body size. For example, Sumanochitrapon et al. (1998) challenged *A. aegypti* of three distinct sizes with dengue viruses,

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http://dx.doi.org/10.1016/j.aspen.2015.06.006
1226-8615/

and reported that infection rates were much greater in smaller than larger mosquitoes. Another study (Grimstad and Haramis, 1984) demonstrated that small females of the related mosquito vector, *Aedes triseriatus*, are better vectors for La Crosse virus in suckling mice than large females.

Several factors are involved in successful blood meal uptake. In particular, the natural diurnal changes in light and dark phases markedly influence the success of blood feeding. There have been a number of studies regarding the correlation between circadian rhythm and the activity of organisms. Locomotion, flight, and feeding in mosquitoes are controlled by the circadian clock (Lima-Camara, 2010). A great deal of research effort has focused on gaining an understanding of the blood feeding patterns of *A. albopictus* throughout the day. Generally, blood feeding activity in this mosquito has been shown to occur during the day and at some times during the night with a bimodal distribution showing one peak when the sun rises and a major peak during the afternoon (Hawley, 1988). A study by Almeida et al. (2005) showed patterns in Asia similar to those reported by Hawley (1988), but the peak times were different. However, these studies did not take into account female size, which has been shown to markedly influence blood feeding success in dengue vectors (Nasci, 1986). An investigation of the biting activity of *A. albopictus* for 24 h showed that these mosquitoes do not feed on blood between 20:00 and 04:00 (Kawada et al., 2005). However, similar to a recent study in northern Malaysia (Dieng et al., 2010) female body size was not taken into account.

In any organism, the nutrient storage levels and requirements are dependent on body size; a large body has greater storage potential but also greater nutritional needs than a small body (Hahn and Denlinger, 2007). Therefore, any nutritional deprivation will likely have a greater effect on large-bodied organisms. Despite the importance of differences in adult body size in *A. albopictus* large females have higher human host attack rates and more frequent multiple blood meals than small females (Xue et al., 1995), little information is available regarding the combined effects of adult body size and circadian rhythm in this mosquito species. It is not yet known whether body size interacts or influences the circadian rhythm of host-seeking and feeding behaviors. This study was designed to examine the effects of larval rearing conditions and adult starvation on the host feeding patterns of female *A. albopictus* throughout the day.

Materials and methods

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 University Sains Malaysia.

Mosquitoes

The *A. albopictus* mosquitoes used in this study were obtained from egg samples from the Vector Control Research Unit of Universiti Sains Malaysia (VCRU). The eggs were flooded in a plastic container for 24 h using seasoned water supplemented with 0.10 g of larval food (2:1:1:1 mixture of dog biscuits, beef liver, yeast, and milk powder). Newly hatched larvae were reared at approximate densities of 150 in metallic enamel trays (diameter = 27.7 cm, depth = 3.3 cm) filled with 800 ml of dechlorinated water (seasoned water, prepared according to Chen et al. (2007)). They were fed daily according to the feeding regimen described previously (Dieng et al., 2007). Pupated individuals were transferred into plastic cups (diameter = 7.6 cm, depth = 5.2 cm) lined with moist tissue paper. Upon emergence, adults were placed in cages (cubic metallic wire covered with mesh net, 29 × 29 × 29 cm) and provided with cotton wicks saturated with a 10% sucrose solution. On days 3–5 after emergence, females were given blood meals by placing a restrained mouse inside the cages for 1–2 h. Mice were restrained within a wire mesh device secured to four corners by push pins (Deus, 2011). Oviposition cups consisting of paper or plastic containers (diameter = 11.5 cm and depth = 6.2 cm) lined with a piece of cardboard paper as an oviposition substrate were placed inside cages for egg collection 3 days after blood feeding. Eggs were dried under laboratory conditions (temperature: 31.3 °C ± 3 °C; relative humidity: 67% ± 15%; photoperiod L:D = 10:12, with 1 h of dusk and 1 h of dawn). Dried eggs were stored under laboratory conditions in small plastic containers and used as egg sources for colony maintenance.

Production of experimental mosquitoes

To obtain experimental mosquitoes, egg samples from the source were hatched as described above. To obtain adults from larval habitats of low and high qualities, we altered rearing density and feeding regime to produce low- and high-quality rearing habitats as described previously (Dieng et al., 2007). Group 1 consisted of 100 newly hatched larvae, while Group 2 consisted of 200 larvae. The rearing trays contained 800 ml of water. The larval feeding schedule and amounts of food supplied are shown in Table 1. Food was supplied according to slight modifications of the procedures described previously (Yee et al., 2004; Dieng et al., 2006). Food was given as a suspension prepared by mixing finely powdered larval food in 2 ml of deionized water. The mixed solutions were dispensed using pipettes (Juliano, 2002). Rearing water was replaced with fresh water prior to adding larval food on day 5. For convenience, we assigned the terms (i) high-quality rearing conditions (HQ) to Group 1 and low-quality rearing conditions (LQ) to Group 2.

Bioassays

We examined the effects of larval rearing conditions on biting activity throughout the day. Thirty 3–4-day-old females and ten 3–5-day-old males were randomly selected from each larval rearing condition. The strain of *A. albopictus* used in the present study is a reluctant blood feeder under laboratory conditions. Mather and Defoliart (1984) reported that incorporating mature males at adequate densities may help to increase feeding rates of caged *Aedes*. In *A. albopictus*, the presence of males has been suggested to stimulate feeding (Dieng et al., 2007). Therefore, we included males in the experimental cages. The adults were provided with a 10% sucrose solution for 3–4 days and used directly for the experiments. These mosquitoes were then placed in cages holding a restrained mouse and observed constantly to measure probing activity. We also examined the interaction between larval rearing conditions and adult starvation in determining biting activity. In this experiment, we created four treatment groups. We separated females and males from the LQ and HQ larval rearing conditions and maintained on a 10% glucose solution as before. An additional group was added from each larval rearing condition that was

Table 1
Larval feeding regimen for the production of adults in two distinct size classes. Day 1 (D1) = day of the appearance of larvae following egg flooding; day 3 (D3) = 3rd day after the appearance of L1 (newly hatched larvae); day 5 (D5) = 5th day after the appearance of L1 (newly hatched larvae); day 6 (D6) = 6th day after the appearance of L1 (newly hatched larvae).

Larval density	Food supply					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
100 newly hatched larvae (Group 1)	3 ml of 0.15 g	No food supply	6 ml of 0.15 g	0.3 g[a]	0.3 g[a]	0.3 g[a]
200 newly hatched larvae (Group 2)	3 ml of 0.15 g	No food supply	3 ml of 0.15 g	0.1 g[a]	0.1 g[a]	0.1 g[a]

[a] Dispersed evenly across the top of the water.