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# Diel sugar feeding and reproductive behaviours of *Aedes aegypti* mosquitoes in Trinidad: With implications for mass release of sterile mosquitoes

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ABSTRACT

Studies on the diel sugar feeding periodicity of male and female *Aedes aegypti* were conducted under laboratory conditions and monitored in single cages using the polyphagometer device and examined every 2 h. Males mosquitoes displayed two peaks in sugar feeding, a small morning peak at 06.00–08.00 h (16% of sugar feeding) and a significant peak evening peak at 16.00–18.00 h (40% of sugar feeding). A similar pattern was observed among females: a small early morning peak (18% of sugar feeding) and a significant peak in the evening 16.00–18.00 h (42% of sugar feeding). Studies on the effects of sugar feeding on the excitation of males showed 100% erect antennal fibrillae after 36 h. In contrast, only 15% of the water-fed males responded. Laboratory studies on the effects of sugar feeding on the insemination rates of *A. aegypti* females showed similar inseminations rates among sugar and water fed males but after 4 days all water fed males died while the sugar fed males continued to survive and inseminate females. The synchronization of the male and female diel sugar feeding periodicity is discussed in the context of sterile insect techniques or genetic control methods.



## 1. Introduction

Various aspects of the circadian rhythm of *Aedes aegypti* (L.) (Diptera: Culicidae) mosquitoes have been studied in the laboratory and the field in Africa (Haddow and Gillett, 1957; McClelland, Q2 Q3 1968) and more recently in Trinidad (Chadee and Corbet, 1987; Chadee 2011; Chadee and Gilles, 2014). These studies have uncovered some new factors which influence the diel periodicity of oviposition in the laboratory such as the effects of density and disturbance/movement of the oviposition substrate (Chadee 2009, Chadee, 2010a,b) and have further demonstrated the strength of the circadian rhythms among females denied oviposition sites for 4 days (Chadee 2011). In addition studies on the diel landing or biting periodicity has been found to vary depending on changing light regimens in urban and rural communities in Trinidad but with the majority of the blood feeding occurring during 06–08 and 16–18 h (Chadee and Martinez, 2000).

Other mosquito behaviours which are controlled by circadian rhythms include the copulation periodicity which was recently studied (Chadee and Gilles, 2014) and sugar feeding periodicity of which little was known until recently about this behavior except for some preliminary work done on sugar feeding by Gillett et al. (1962). In contrast, over the last 150 years studies on nectar-feeding by mosquitoes have been reported (Larsen, 1948; Nielsen and Greve, 1950; Haeger, 1955), with the seminal work of VanHandel (1972), and that of Yuval (1992) and Foster (1995) laying the foundation for the current work on the role that flower nectar, honeydew and fruits play in nutrition and energy supply among mosquitoes. Recently, the role of nectar/sugar feeding in mosquito foraging behaviour has been used to develop intervention devices to reduce populations of *Anopheles sergentii* (Gu et al., 2011), *Culex quinquefasciatus* Say (Muller et al., 2010) and *Aedes albopictus* Skuse (Qualls et al., 2012). Promise has been shown by the use of these studies in attracting sugar-feeding mosquitoes to sites which contain baits with toxins which kill the adult mosquitoes and thus prevent transmission of disease pathogens (e.g. malaria, dengue, Western Nile) (Qualls et al., 2012).

With the increase in the global incidence of dengue fever (Bhatt et al., 2013) and the failure of current *A. aegypti* control programs (Chadee, 2010a,b), there has been renewed interest in the use of the sterile insect technique (SIT) and in understanding basic aspects of the mating success of *A. aegypti* mosquitoes (Chadee and Gilles, 2014). These studies include the effects of age (Ponlawat and Harrington, 2005), body size (Ponlawat and Harrington, 2009;

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Schneider et al., 2007, 2010), swarming (Hartberg, 1971), density (Ponlawat and Harrington, 2009) and copulation (Chadee and Gilles, 2014) on mating success and vector competence under laboratory and field conditions (Schneider et al., 2007, 2010). However, these studies did not include the peak times of sugar feeding although this information is vital to the successful implementation of SIT programs.

In 2008 the International Atomic Energy Agency (IAEA) awarded a research grant to the University of the West Indies, St Augustine, Trinidad to study the copulation vigor of sterile *A. aegypti* males in the field. Unfortunately very little evidence was available on the sugar feeding behaviour of *A. aegypti* male mosquitoes so it was important to undertake experiments to gain this information in order to maximize survival in the field.

The objectives of this study were to determine the diel sugar feeding periodicity of *A. aegypti* and to determine the effects of sugar feeding on insemination rates among *A. aegypti* in the laboratory. The results of these studies are required before effective implementation of vector control programs which utilize sterile males.

## 2. Materials and methods

### 2.1. Mosquito collection site

#### 2.1.1. Curepe

The Curepe study site (10° 42′N; 60° 24′W) is located along the Eastern Main Road to the north and the Southern Main Road to the west, 16 km east of Port of Spain, the capital of Trinidad. It includes approximately 3000 houses and 15,000 people. The wet season starts in May and ends in November while the dry season starts in December and ends by mid-May. The study area, meteorology, vegetation and population of *A. aegypti* have been previously described by Chadee (2004).

#### 2.1.2. Mosquito collection methods

This study was conducted from October 2010 to May 2011, two months in the wet and six months in dry season. Householders who had participated in previous studies were willing for us to conduct inspection and collection of immature *A. aegypti* populations for laboratory trials. A cluster of 100 houses was selected based on criteria which included the presence of *A. aegypti* breeding habitats, mainly water drums, and the householders willingness to allow access to workers conducting the study.

All outdoor containers were inspected using standard house to house inspection protocols (Focks and Chadee, 1997) and all pupae were collected from each positive container. The number of pupae in the pre-adult stage (pupae resting horizontally on the water surface and moving in a sluggish manner) were collected and recorded on standard work sheets. Samples of male and female pupae (based on size differences) were collected from containers including water drums and sent to the Parasitology Laboratory in the Department of Life Sciences, University of the West Indies, St. Augustine, Trinidad, where they were identified and segregated into batches of 100 males. These batches of 100 male pupae were transferred to rearing pans, placed into mosquito cages for emergence to the adult stage in the laboratory. Since pupae do not feed they were not provided with food but emerged adults were supplied with water soaked cotton wicks. Female pupae were treated in a similar manner and placed into different mosquito cages labeled appropriately and supplied with a water wick. These experiments were conducted in the laboratory at 28 ± 1.5 °C and 75–80% relative humidity and a regimen of 11.30 h light and dark with two 30 min "twilight" periods immediately before and after the scotophase.

### 2.2. Experiment 1.

#### 2.2.1. Male sugar feeding

Fifty large males were each placed into a 30 × 30 × 30 cm cage consisting of white cloth netting enclosing a wooden frame and containing cotton gauze soaked in a sugar solution as food (a mixture of 5% glucose and 5% sucrose) by means of the apparatus previously described by Gillett (1961) called a polyphagometer which consisted of a graduated tube, closed at the top and fitted with a perforated gauze at the lower end. This apparatus was placed on the tip of the gauze cover of the cage and the mosquitoes fed through the perforated lower end. Male abdominal distension was also scored every 2 h as empty, partial, semi-full or fully engorged. The polyphagometer results have previously been shown to be affected by evaporation so control cages without mosquitoes were used under the gauze cover and exposed for the same duration. This figure was subtracted from the experimental figure in order to determine feeding rates.

### 2.3. Experiment 11

#### 2.3.1. Female sugar feeding

Fifty females were each placed into 30 × 30 x30 cm cages and sugar fed as in experiment 1. Observations were made every 2 h as described above.

### 2.4. Experiment III.

#### 2.4.1. Reproductive behaviours

After emergence, males were transferred, 25 into one 30 × 30 × 30 cm cage supplied with four water soaked cotton wicks while in another cage (same size) 25 males were also supplied with four sugar-soaked wicks. Since *A. aegypti* copulation was diurnal with a small crepuscular component (Chadee and Gilles, 2014), observations were made at 10 min intervals throughout the diurnal and crepuscular periods. The number of males with extended antennal fibrillae were recorded prior to and after their flight activity. The number of males flying (total no of males, less the number at rest) were considered seeking copulation activity or "swarming" (Cabrera and Jaffe, 2007).

### 2.5. Experiment IV

#### 2.5.1. Insemination success

Two different experiments were conducted to determine the effects of sugar feeding on the copulation and insemination success of *A. aegypti* males. Two different sizes of cages were used which allowed greater opportunity for male and female encounters. A 2:1 ratio of females to males was used in keeping with physical interference observed during laboratory studies (Chadee, 2010a,b) and the recognized 55:45 male–female ratio observed in the field and laboratory (Clements, 1999). For the first experiment 25 newly emerged large males were each placed into 25 30 × 30 × 30 cm cages, supplied with two water soaked cotton wicks. Twenty-five cages were also supplied with two wicks soaked with a sugar solution. From a stock cage of one hundred (100), 3 to 4 day-old sugar-fed virgin females (supplied with sugar solution *ad libitum* from emergence), two females were removed and placed into each experimental cage.

To determine insemination rates over several days, different groups of females in separate cages with the newly emerged males from the stock cages were exposed for 3 days or until most males died, after which females were removed, dissected in saline, the spermathacae cracked and checked visually for the presence of sperm at 100× magnification. This experiment was repeated 2 times.



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