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The diel oviposition periodicity of *Aedes aegypti* (L.) (Diptera: Culicidae) in Trinidad, West Indies: effects of forced egg retention

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Abstract

The diel oviposition periodicity of two groups of *Aedes aegypti* females (Trinidad strain), (i) thoses allowed access to oviposition sites and (ii) females forced to retain-eggs for four days, were studied under laboratory conditions using one female per cage and monitoring by changing substrates every two hours. The individual females which were allowed access to oviposition sites showed peak oviposition between 16.00–18.00 h (50% of eggs), whereas individuals forced to retain eggs for four days showed a similar pattern but with a significantly ($P < 0.001$) larger peak oviposition between 16.00–18.00 h (94% of eggs). However, females forced to retain eggs laid most or all of their eggs in one container (84%), while females given access to oviposition sites distributed their eggs among 2–4 containers. The results of this study are discussed in the context of the strength of the circadian rhythms, oviposition strategies and its impact on vector control activities.

Keywords: *Aedes aegypti*, circadian rhythm, oviposition periodicity, laboratory, Trinidad

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Introduction

Recently, two new factors, density of *Aedes aegypti* (L.) females in an oviposition cage (Chadee, 2007) and physical interference at the oviposition substrate (Chadee, 2008), were added to the already known impacts of endogenous and exogenous factors which influence the shape and form of the diel oviposition periodicity of mosquitoes in the laboratory and field (Corbet, 1966; Clements, 1999). The density of mosquitoes in experimental cages was found to affect the peak in the diel oviposition periodicity by intraspecific competition among females at the oviposition site, delaying oviposition from 16.00–18.00 h to 18.00–20.00 h (Chadee, 2007), while the physical interference/movement caused by removing and replacing the oviposition substrate was also found to disturb ovipositing females, delaying the time of peak oviposition from 16.00–18.00 h to 18.00–20.00 h (Chadee, 2008).

Forced egg-retention has been reported to change the physiology and behaviour of *Ae. aegypti* mosquitoes (Woke, 1955; Clements, 1999). Dhileepan (1997) suggested that egg retention was possibly responsible for influencing physical and chemical factors, including visual, olfactory and tactile responses of mosquitoes (Bentley & Day, 1989). In addition, egg-retention has been found to affect vitellogenesis when these gravid females take a second blood meal (Elsie & Judson, 1972). Chadee (1997) reported that females forced to retain eggs modified their egg dispersal pattern, with most or their entire egg batch being laid in a single container. However, this pattern was not maintained in subsequent gonotrophic cycles, with females distributing their egg

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installments in two or more containers when not forced to retain eggs.

Nothing is known about the impact of forced egg retention on the diel oviposition periodicity of *Ae. aegypti*, especially as source reduction programs remove most or all oviposition sites from the field and females may have to search for long periods for suitable sites. Indeed, acquisition of such information, which is relevant to attempts to control the vectors and standardize laboratory methods, is long overdue.

In this paper, the effects of forced egg retention on the diel oviposition periodicity of *Ae. aegypti* in the laboratory is determined.

Materials and methods

The *Ae. aegypti* strain used during these studies originated in St Joseph, Trinidad, collected as eggs from June to August (1986) and designated the Trinidad strain. This strain was accommodated in two light-proof rooms at 26 ± 1°C and 70–75% relative humidity and a regime of 12 h light (06.00–18.00 h) and either 12 h dark (room1) or 11 h dark (room 2) with two 30 min 'twilight' periods immediately before and after the scotophase. Illumination was as described by Corbet & Ali (1987).

The colony was maintained in room 1 in accordance with regimes that standardized density and nutrition of larvae as described by Corbet & Chadee (1993). All *Ae. aegypti* used for experiments were transferred as eggs to room 2 and reared to adults. Female adults were selected so that the post-emergence age of each was the same and known to within one hour. On the third day post emergence, a sample of females (10–15) was confirmed as inseminated by *post-mortem* dissection. Thereafter, females were allowed to engorge on blood from an experimenter's arm within a 20 min period centered on 17.20 h, a time close to the main peak of landing and biting of *Ae. aegypti* in the field in Trinidad (Chadee & Martinez, 2000).

Experiment 1

On the fourth day post emergence, blood engorged females (assessed as such by eye) were placed individually, one per oviposition cage (30 × 30 × 30 cm) consisting of white cloth netting enclosing a wooden frame and containing a cube of white sugar in an uncovered Petri dish in the center of the cage. In each cage, eight numbered, small, white, polyethylene tubs (SWT) (diameter of tops 8.2 cm and bottoms 7 cm, height 5.8 cm, capacity 300 ml), painted black outside with the inside of each tub lined with a white paper towel and containing 200 ml of temperature-equilibrated tap water, were placed as described in the oviposition assay method developed by Corbet & Chadee (1993).

The oviposition periodicity was monitored by manually placing eight pre-prepared SWTs into each cage labeled in accordance with the cage number. The eight SWTs were exposed for intervals of two hours and removed and replaced with another set bearing the time of exposure and cage number. These females were monitored every two hours for 48 h.

Experiment 2

On the fourth day post emergence, engorged females (assessed as such by eye) were placed individually, one per oviposition cage as described above. The females were denied oviposition sites for four days after oviposition was due. That is, day 11 post emergence or seven days after blood engorgement.

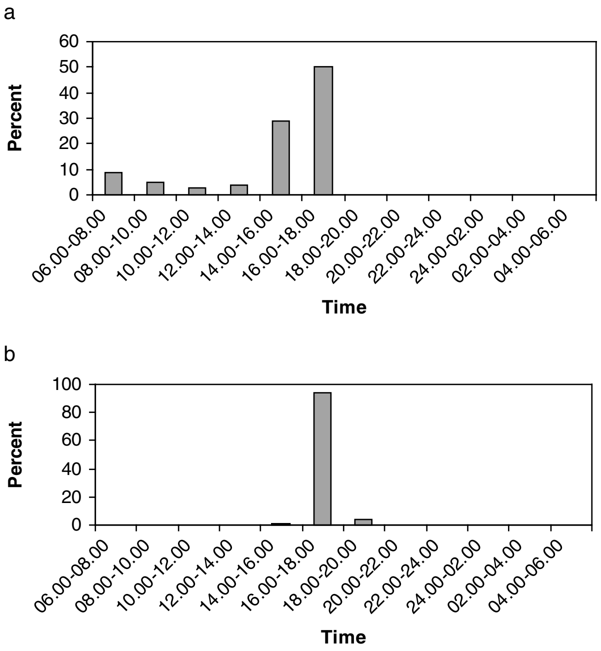

Fig. 1. *Aedes aegypti*. Showing the diel patterns of oviposition of females in the laboratory of (a) females allowed access to oviposition sites and (b) females forced to retain eggs for four days.

On the eight day post blood feeding, eight numbered SWTs were placed in each cage. The oviposition periodicity was monitored by manually placing eight pre-prepared SWTs into each cage labeled in accordance with the cage number. The SWTs were exposed for intervals of two hours and removed and replaced with others bearing the time of exposure and cage number. These females were monitored every two hours for 48 h.

Results of the number of eggs laid during each time interval were analyzed to determine the effect of forced egg retention on the oviposition periodicity and dispersal of eggs among various containers and are given as the percentages of the William's mean (Haddow, 1960). In addition, the temporal changes in the number of eggs laid were analyzed by transforming the data into contingency tables and a G-test applied (Sokal & Rohlf, 1981).

Results

The results of the oviposition periodicity are shown in fig. 1.

Individuals allowed access to oviposition sites

Twenty-five individuals of the Trinidad strain exhibited a distinct diel periodicity, with peak oviposition occurring between 16.00–18.00 h (fig. 1). A small morning peak (9%) was observed during the first two hours of the photophase,

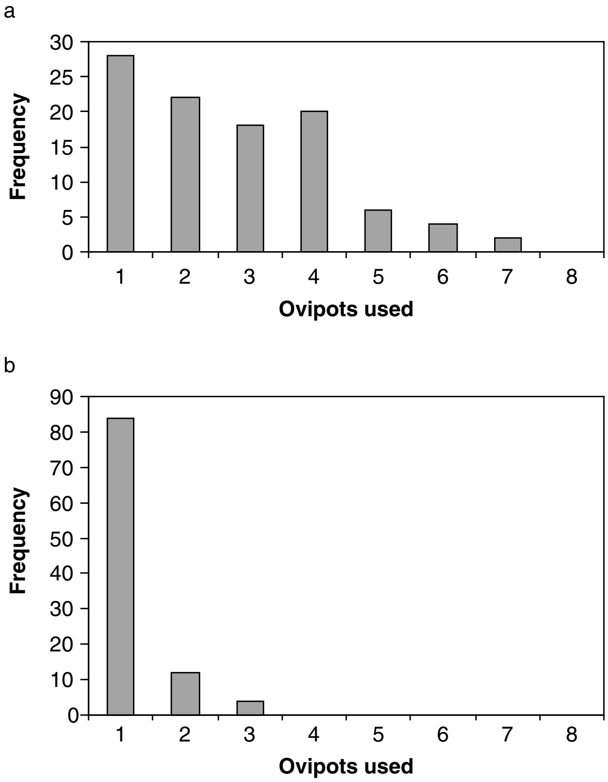

Fig. 2. *Aedes aegypti*. Frequency of ovipots usage (a) by females allowed access to oviposition sites and (b) of females forced to retain eggs for four days.

after which oviposition declined; during the second half of the photophase, oviposition increased, reaching a peak (comprising about 50% of eggs ($G = 58.3$; $P > 0.01$)) between 16.00–18.00 h (fig. 1a). No eggs were collected during the scotophase. This is regarded as the definitive baseline periodicity of the Trinidad strains and is similar to that described by Chadee (2007, 2008).

Twenty-three (94%) of all females distributed their eggs among two or more SWTs during their peak oviposition period, which occurred at 16.00–18.00 h (fig. 2a).

Individuals forced to retain eggs

Twenty-five individuals of the Trinidad strain forced to retain eggs for four days (after oviposition was due) also exhibited a diel periodicity, with peak oviposition occurring between 16.00–18.00 h (fig. 1b). No morning oviposition activity was observed during the beginning of the photophase; but, during the second half of the photophase, oviposition increased significantly ($G = 101.23$; $P < 0.001$), reaching a peak (comprising about 94% of eggs) between 16.00–18.00 h (fig. 1b). Eighty-eight eggs (4.3%) were collected during the beginning of the scotophase but none thereafter.

Twenty-one females (84%) laid all their eggs in one container during the period 16.00–18.00 h (fig. 2b), while four females distributed their eggs in more than one container during the time intervals 14.00–18.00 h.

Discussion

The results of the present laboratory study showed that the oviposition periodicity of *Ae. aegypti* was diurnal with significant peaks in oviposition restricted to the last two hours of the photophase. This pattern was consistently observed during field oviposition periodicity studies in Kenya when peak oviposition occurred at 14.00–18.00 h and in Trinidad when peak oviposition occurred at 16.00–18.00 h when using four-hour (McClelland, 1968) and two-hour (Chadee & Corbet, 1987) monitoring intervals, respectively. In addition, these results are consistent with that of the pioneering work of A.J. Haddow, J.D. Gillett and P.S. Corbet, who demonstrated the influence of different light regimens on the oviposition periodicity of different mosquito species, including *Ae. aegypti* in the laboratory. It is clear that light influences oviposition by enabling *Ae. aegypti* females to search visually for oviposition sites (Beckel, 1955) and that the transition from light to dark (sunset or twilight) may set a time cue which positions egg laying on the following day (Gillett *et al*., 1961; Haddow *et al*., 1961).

It is well known that females frequently lay a single batch of eggs in installments, at 24-h intervals. Sometimes, however, a female which had oviposited at the end of one light stretch (06.00–18.00 h) would lay a few eggs of the same batch at the beginning of the next stretch. This pattern is clearly discernible from fig. 1a, which showed a large peak (50%) in the afternoon and a small (9%) peak in the morning. Gillett (1962) examined the contribution of individual females to the oviposition periodicity and found a similar pattern with both an evening and morning peak in oviposition in the laboratory. So, it is reasonable to assume that the morning peak is an extension of the previous evening's activity and not a new event.

In addition, this assumption is further supported by the fact that the morning peak reflects the activity of females which remained at the site during the night (when neglible oviposition occurs), probably waiting to complete the bout of oviposition begun the evening before (Chadee & Corbet, 1990). Consistent with this view is the knowledge that, in the laboratory (Gillett, 1962) and in the field (Chadee & Corbet, 1987), *Ae. aegypti* lay their eggs of one batch in installments; and that, in the first two gonotrophic cycles at least and regardless of the time of blood feeding, eggs are nearly always first laid in the evening, and a morning oviposition is nearly always preceded by oviposition the previous evening (Gillett, 1962).

Figure 1b shows an absence of a morning peak in oviposition among females forced to retain eggs; that is, there was only one major peak (94%) occurring between 16.00–18.00 h (fig. 1b). This pattern of oviposition by *Ae. aegypti* may reflect at least two important factors, the absence of suitable resting sites for gravid females during the night following oviposition (Corbet & Chadee, 1990) or when females are forced to retain eggs for over four days after oviposition was due (present study). During the present study, females were allowed access to oviposition sites overnight, so the change in oviposition patterns detected relate to forcing females to retain her eggs.

Studies by Chadee & Corbet (1990), Corbet & Chadee (1990) and the present study suggest that entry of gravid females into oviposition sites during the early afternoon is crucial for the initiation of the oviposition waves. This would suggest that visual cues (Beckel, 1955) and the highly

Table 1. Showing the possible sequence of activity of gravid *Ae. aegypti* females from searching for oviposition sites to completion of oviposition.

Time	Activity	References
Early afternoon	Flight for finding oviposition sites	Corbet & Chadee (1990)
Late afternoon	Oviposition	Haddow & Gillett (1957), McClelland (1968), Chadee & Corbet (1987)
Twilight (dusk)	End of oviposition	Haddow & Gillett (1957), McClelland (1968), Chadee & Corbet (1987), Chadee & Corbet (1990)
Night	Resting at or close to oviposition site	Corbet & Chadee (1990), Chadee & Corbet (1990)
Twilight (dawn)	Re-initiate oviposition process	Chadee & Corbet (1987), present study
Early morning	Oviposition	Corbet & Chadee (1987), Corbet & Chadee (1990), Chadee & Corbet (1990), present study
Mid-morning	End of oviposition bout	Chadee & Corbet (1987)

sensitive ommatida to dim light (as low as >0.1 lx) (Kawada *et al*., 2005) in *Ae. aegypti* may contribute to females locating and staying at oviposition sites. Based on these results, it seems clear that oviposition follows the sequence summarized in table 1. Conversely, the sequence night-twilight-morning may not allow gravid females the opportunity to search and to find suitable oviposition sites because of the dark conditions at night and, consequently, may be unsuitable to initiate the oviposition wave.

Gillett *et al*. (1959) and Haddow *et al*. (1961) demonstrated the strength of the circadian rhythm, with females having matured eggs at night and during different times during the day but waiting until the afternoon period, 14.00–18.00 h, before laying her eggs. In the present study, similar results were observed; although females were forced to retain eggs for four days, oviposition did not occur until 14.00–18.00 h despite being offered oviposition sites from 06.00 h (fig. 1b). Similar oviposition periodicity was observed among females not forced to retain eggs and given access to oviposition sites from 06.00–08.00 h, 48 h post blood feeding (fig. 1a).

It is noteworthy that females forced to retain eggs for four days modified their oviposition patterns or egg dispersal patterns (figs 2 and 3). Buxton & Hopkins (1927) and Chadee *et al*. (1990) observed that gravid females dispersed their eggs over several sites with approximately 11–30 eggs per oviposition container, a behaviour often described as 'skip oviposition' (Corbet & Chadee, 1993). Recent studies using contemporary molecular markers (both DNA and RFLP) in Puerto Rico (Apostal *et al*., 1994) and Trinidad (Colton *et al*., 2003) also confirmed the skip oviposition behaviour described above. Using the assay method developed by Corbet & Chadee (1993), *Ae. aegypti* females forced to retain eggs laid most or the entire installment in one container, while females not forced to retain eggs dispersed their eggs among two or more containers and displayed the skip oviposition strategy (fig. 3a, b). These results demonstrate the usefulness of the assay method developed by Corbet & Chadee (1993) in determining not only oviposition preferences but the oviposition periodicity of females forced to retain eggs.

In light of these findings, vector control workers conducting source reduction programs may create conditions which may force females to retain eggs and to seek new oviposition sites. This searching behaviour can expand the geographical distribution of the vector, foster the transmission of dengue fever in new locations and, at the same time, females may lay most or all their eggs in one container in the new location(s).

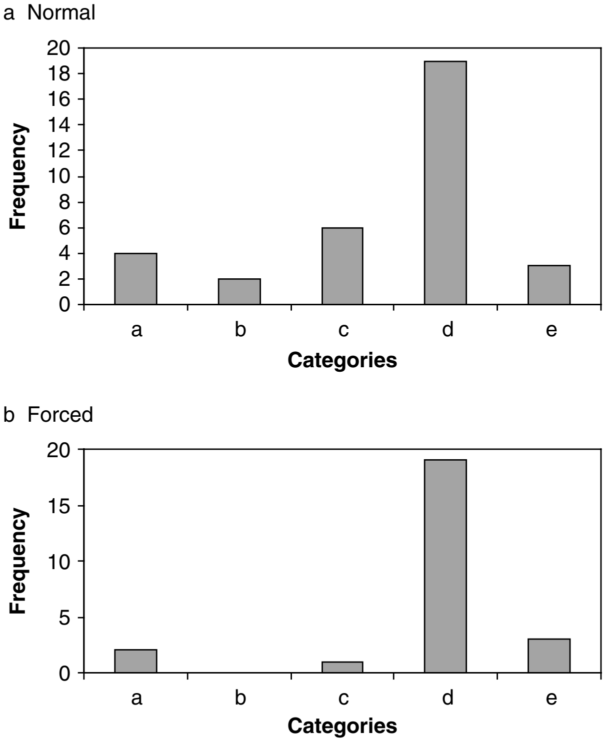

Fig. 3. *Aedes aegypti*. Frequency distribution of number of eggs laid during each occurrence (encompassing a two-hour period at each ovipot) recorded separately during all times of day for (a) females given access to ovipots and (b) females forced to retain-eggs for four days. Number grades: a, 1–2; b, 3–8; c, 9–30; d, 30–90; e, >91.

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