

The Allee effect in site choice behaviour of egg-laying dengue vector mosquitoes

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Abstract. Surveillance and control of the dengue vector mosquito, *Aedes aegypti*, is commonly reliant on its egg-laying behaviour, which is affected by the presence of conspecific eggs. However, the influence of varying egg density and breeding site choice on *Ae. aegypti* egg-laying strategy is unclear. In this laboratory study *Ae. aegypti* demonstrated a strong oviposition preference for substrates with intermediate numbers of conspecific eggs, thus demonstrating an ‘Allee effect’. The withholding of some eggs, a trait required for skip oviposition, was almost non-existent when no site choice was available, regardless of egg density; indicating that skip oviposition behaviour is modulated according to the availability of suitable sites. These experiments have revealed a hierarchy of oviposition choices in *Ae. aegypti* that may thwart attempts to use semiochemicals from eggs to enhance oviposition-based surveillance and control methods.

INTRODUCTION

Dengue viruses (DENV) infect up to 50 million people each year, causing more than 20,000 deaths (World Health Organisation, 2008). The major vector is *Aedes aegypti* (L.), a mosquito well adapted to human environs through its ability to breed in artificial containers and its strong human blood feeding preference.

A lack of vaccine means mosquito control remains central to DENV management. Understanding of *Ae. aegypti* egg-laying behaviour is central to DENV control because it underpins the primary vector surveillance method, namely ovitrapping, involving the counting of eggs, or the capture of gravid females, in artificial containers (Fay & Eliason 1966; Ritchie *et al.* 2003). ‘Lethal’ ovitraps, in which insecticide is applied to artificial containers (Zeichner & Perich, 1999), are used to reduce *Ae. aegypti* population size (Perich *et al.*, 2003), and are also reliant upon egg-laying behaviour.

Aedes aegypti may lay eggs from a single gonotrophic cycle at several sites, termed

‘skip oviposition’ (Reiter, 2007). Such behaviour is considered a strategy for avoiding oviposition in sites where larval nutrition may be limiting, with individuals known to exhibit this behaviour over several days and several hundred metres (Reiter *et al.*, 1995). Furthermore, *Ae. aegypti* will oviposit on substrates already containing conspecific eggs, a behaviour termed ‘superoviposition’ (Chadee *et al.*, 1990).

Egg-laying in *Ae. aegypti* is affected by the presence of conspecifics in potential breeding sites. Existing larvae are attractive to gravid *Ae. aegypti* and enhance oviposition when at low densities, but deter oviposition at high densities (Benzon & Apperson, 1988). Conspecific eggs affect oviposition behaviour, with individually-caged gravid *Ae. aegypti* showing a preference for sites containing no conspecific eggs (Chadee *et al.*, 1990). Field studies offering substrates already containing conspecific eggs showed greater superoviposition when there were fewer than 25 already present (Chadee *et al.*, 1990), confirming this avoidance behaviour. These

mediators of oviposition behaviour have presumably evolved to minimise the adverse effects of larval crowding on fitness (Mulla, 1979).

Gravid *Ae. aegypti* respond differently to varying doses of semiochemicals identified from their eggs (Ganesan *et al.*, 2006). At particular doses, several fatty acids (C₁₂-C₁₈ length) are oviposition attractants, whereas several esters are deterrents (Ganesan *et al.*, 2006). However, such dose-specific responses to egg semiochemicals conflicts with the overall avoidance of conspecific eggs previously demonstrated (Chadee *et al.*, 1990).

Although individually-caged *Ae. aegypti* avoided superoviposition, the number of pre-existing eggs was not recorded in that study (Chadee *et al.*, 1990). While some avoidance of high conspecific egg densities would be expected to avoid crowding, we anticipated that conspecific eggs may be oviposition attractants at lower densities. It follows that there may be conspecific egg numbers that encourage or discourage oviposition.

There were two aims of our study. First, to determine whether conspecific eggs could be attractive to gravid *Ae. aegypti*, we experimentally characterised egg density preferences of individual mosquitoes. Secondly, to determine whether egg-laying decisions altered when no choice was offered (i.e., would *Ae. aegypti* withhold eggs in the presence of 'sub-optimal' egg densities?) individual gravid mosquitoes were offered no choice of site, with varying egg densities.

MATERIALS AND METHODS

Aedes aegypti mosquitoes from Cairns, far north Queensland, Australia, were held in colony for approximately 10 generations at 28°C and 80% relative humidity. Seven days after feeding on human blood for the first time, gravid females (10-20 d old) were used for bioassays. Eggs 3-10 d old were obtained from the stock colony on cardboard paddles (80 x 80 x 3 mm). Viable eggs from these paddles were counted. Non-viable eggs (hatched or dehydrated) were removed from

paddles using a needle. In general there were few such eggs, and paddles were only used if the non-viable egg portion was no more than 5% of the total, as we considered it possible that some semiochemical residue may be left from non-viable eggs.

Oviposition responses to conspecific eggs were observed in 510 x 510 x 510 mm cages. Glass beakers (155 mm diameter, 80 mm depth) holding cardboard oviposition paddles and 225 ml distilled water were used to provide gravid females with oviposition substrate options. Each bioassay was 72 h duration.

Experiment 1: Three equidistant oviposition beakers, each containing one paddle with 0 eggs, 11-38 eggs (median 20), or 39-74 eggs (median 53) were placed in the cage, along with a single gravid mosquito. These egg densities were chosen as they provided a range of *Ae. aegypti* eggs typical of small containers in Australia (Williams *et al.*, 2007). The beakers were rotated clockwise after 24 and 48 h to avoid any bias for site preference. Replicate assays were performed 29 times.

Experiment 2: This was identical to Experiment 1 except that individual gravid mosquitoes were offered no choice of beaker for oviposition. In the 28 replicate experiments, a range of egg densities (0 – 111) was offered. At the end of each assay the number of eggs laid in the beaker was determined, and mosquitoes dissected to determine what proportion of eggs was retained.

Statistical Analysis: the chi-square goodness of fit test was applied to the sum of eggs laid by 29 mosquitoes in Experiment 1 to test the hypothesis of equivalent distribution of eggs amongst beakers.

RESULTS

In Experiment 1, a significant preference for mid-range egg densities was observed ($\chi^2 = 276.46$, df. 2, $P < 0.0001$), with more than double the number of eggs laid in beakers containing 11-38 eggs, than those with 0 or > 39 eggs (Fig. 1). The mean number of eggs laid by each mosquito was $28.6 \pm \text{SE } 4.6$. Skip

oviposition (laying in one or more containers) was observed in 19 of 29 (65.5%) mosquitoes.

In Experiment 2, a range of egg densities (0 – 111) was offered. Only one mosquito retained any eggs after 72 h, meaning that 27/28 (96%) laid their entire clutch when given no choice of container. Thus there was no apparent influence of existing egg density on egg-laying behaviour when no choice was available. The mean number of eggs laid was $41.9 \pm \text{SE } 5.7$.

DISCUSSION

Conspecific eggs may encourage oviposition in *Ae. aegypti*, a finding in contrast to a previous report (Chadee *et al.*, 1990). An intermediate number of eggs was more attractive than higher egg densities and containers with no eggs. This finding is consistent with the dose-responsive oviposition behaviour of *Ae. aegypti* to egg semiochemicals. It is plausible that the phenomenon recorded here was mediated by previously identified semiochemicals: octadecanoic acid, an oviposition attractant

at low concentrations but a deterrent at high concentrations; methyl dodecanoate, being increasingly deterrent with rising concentration (Ganesan *et al.*, 2006).

The preference for oviposition in sites of intermediate egg density can be explained by the need to avoid overcrowding in offspring (Mulla, 1979). However, by this explanation the absence of all eggs should provide the most attractive option for gravid *Ae. aegypti*, something not observed here. It is possible that conspecific eggs, at low densities at least, provide an attractive cue for gravid *Ae. aegypti* because they are a signal of a suitable breeding site. We speculate that this may be an indirect signal for the absence of egg predators, or for the presence of non-sibling conspecifics that may serve as mates for offspring. We consider this evidence of an Allee effect (Allee, 1931; Stephens *et al.*, 1999), in which the presence of conspecifics is attractive, and presumably beneficial, to egg-laying *Ae. aegypti* up to a particular density.

When presented with three oviposition sites in close proximity, nearly two-thirds of *Ae. aegypti* exhibited skip oviposition. However, egg withholding behaviour

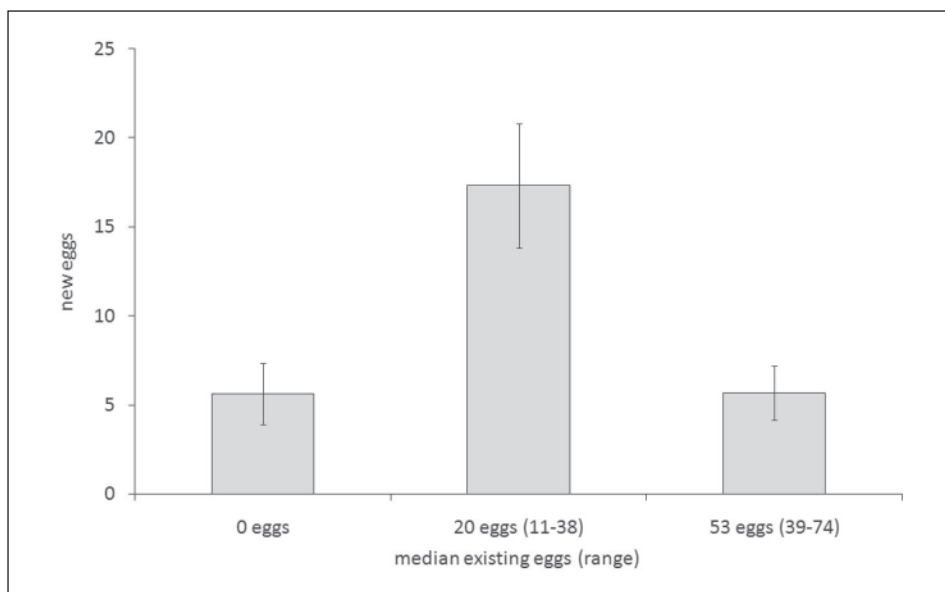


Figure 1. Choice experiments in which *Ae. aegypti* mosquitoes laid eggs on paddles with a variable number of existing eggs already present. Mean (\pm SE) of eggs laid by 29 gravid females in 72h.

(required for skip oviposition), disappears when no choice is available. This strongly supports the idea that the presence of available oviposition sites will determine the extent of skip oviposition, hence the extent of dispersal.

Thus there is a hierarchy of egg laying behaviour choices in *Ae. aegypti*. The foremost need is to lay eggs quickly, demonstrated here by the laying of all eggs within 72 h despite high numbers of eggs already present. If a choice of sites is available, then the Allee effect becomes apparent as secondary choices based on conspecific egg density are made. We contend that the presence of alternate breeding sites will be a stronger influence on *Ae. aegypti* oviposition behaviour than existing egg number, as this is higher in the hierarchy.

The findings presented here have implications for attempts to improve dengue control and *Ae. aegypti* surveillance by making breeding sites more or less attractive. Given that breeding sites with intermediate amounts of eggs are most attractive to *Ae. aegypti*, it may be argued that semiochemicals may be applied to ovitraps to enhance their attractiveness, and in turn their effectiveness. However, given that as more eggs are laid the attractiveness of the container will decrease, it is hard to see how egg semiochemicals may be applied in this manner. Furthermore, organic infusions are already being successfully used to enhance ovitrap attractiveness (Reiter *et al.*, 1991; Ritchie, 2001). Attempts to make potential breeding containers less attractive by adding large amounts of deterrent egg semiochemicals is also unlikely to work, as we have demonstrated here that when no oviposition choice is available, *Ae. aegypti* will almost always lay its entire egg load within a few days.

For those seeking to use semiochemicals to enhance surveillance and control of mosquitoes (e.g., Zwiebel & Takken, 2004), we recommend that the Allee effect and hierarchical oviposition choice behaviour demonstrated here be considered. We contend that the use of egg semiochemicals

is unlikely to provide reliable and strong attraction or repulsion of gravid *Ae. aegypti* in the field for the purposes of surveillance and control.

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