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Presence of a predator image in potential breeding sites and oviposition responses of a dengue vector

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ABSTRACT

In dengue vector control, attempts to minimize or replace the use of pesticides have mostly involved use of predators, but success has been severely impeded by difficulties associated with financial and environmental costs, predator mass production, and persistence in target habitats. Visual deterrents have been used successfully to control animal pests, in some cases in an effort to replace pesticide use. Despite evidence that visual signals are crucial in site choice for egg deposition by dengue vectors, and that female mosquitoes respond to artificial predation, the role of predator intimidation as it affects the oviposition behavior of dengue vectors remains largely unexplored. Here, we examined the oviposition responses of *Aedes aegypti* exposed to various mosquito predator pictures. Gravid females were presented with equal opportunities to oviposit in two cups with predator images [*Toxorhynchites splendens*—TXI, Goldfish (*Carassius auratus*)—small (SFI) and large (LFI) and *Tx. splendens* + Goldfish—TXFI] and two others without pictures. Differences in egg deposition were examined between sites with and without these images. When given a chance to oviposit in cups with and without TXI, *Ae. aegypti* females were similarly attracted to both sites. When provided an opportunity to oviposit in cups displaying pictures of fish (SFI or LFI) and blank cups, egg deposition rates were much lower in the fish picture sites. Females showed a preference for blank cups over TXFI for egg deposition. They also equally avoided cups with pictures of fish, regardless of the size of the picture. Our results indicate that the presence of images of goldfish and their association with *Tx.* larvae significantly reduced egg deposition by *Ae. aegypti*, and this was not the case with the predatory larvae alone. The observations that the images of natural predators can repel gravid females of a dengue vector provide novel possibilities to develop effective and inexpensive alternative tools to harmful insecticides.

1. Introduction

The world has experienced unprecedented urban growth in recent decades, which has led to the extinction of many natural predators (McKinney, 2002) and also to the spread of mosquito vectors (WHO, 2014). And the current challenges for suppressing malaria (Benelli and Beier, 2017) are reminders of the unceasing menace associated with mosquitoes. These include, *Aedes* vectors that spread dengue and Zika disease (Benelli and Mehlhorn, 2016), which are major global public health threats (Hennessey et al., 2016; CDC, 2016; WHO, 2016). Vector control through the use of chemical insecticides was the main strategy for controlling these diseases (WHO/WPR, 2010a, 2010b), but the development of insecticide resistance (Whalon et al., 2008; Naqqash et al., 2016) together with environmental and human health concerns (Stahl, 2002) regarding these agents decreased the utility of this strategy (Karunamoorthi and Sabesan, 2013). In addition, the resulting reduction of the spectrum of effective agents (Dusfour et al., 2011) combined with the absence of effective vaccines and specific therapeutics

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(Laughlin et al., 2012) make it necessary to develop alternative vector control strategies, especially eco-friendly ones (Benelli, 2015).

The ability of *Aedes* vectors to transmit disease depends heavily on their persistence in nature, which in turn depends largely on the availability of breeding sites (Saleeza et al., 2011; Getachew et al., 2011). Although there have been reports of larvae in natural sites (Hawley 1988; Chadee et al., 1998), man-made containers are the primary breeding habitats of dengue vectors in cities (Townroe and Callaghan, 2014). In addressing dengue vector control, it has been recommended that containment strategies should take into consideration oviposition (Sarkar, 2010), the ecology of the immature stages (WHO, 2016), and eradication or reduction of breeding containers. However, ongoing urban development and the lifestyle of city dwellers severely restrict the feasibility of such approaches. Urbanization has led to an increase in availability of artificial containers (Townroe and Callaghan, 2014) providing new breeding sites (Silva de Mendonça et al., 2011) related to the anthropogenic use and abuse of day-to-day commodities (Dutta et al., 1999; Leisnham et al., 2004; Giusti, 2009). Dengue vectors typically breed in artificial and natural containers (Vezzani, 2007; Hiscox et al., 2013) and can colonize any container flooded naturally by rainfall or artificially by human water storage (WHO/PRO, 2013; Liehne, 1988). Carelessly discarded waste items, such as rubber, plastic, pottery, and porcelain containers, can hold rainwater and be exploited as larval habitats by dengue vectors (Dutta et al., 1999; Banerjee et al., 2013).

It has been suggested that programs targeting gravid females can maximize the disruption of disease transmission, while minimizing control effort (Le Menach et al., 2005; Gu et al., 2006). Host seeking and location by female mosquitoes are mediated by host-derived physical and chemical cues (Verhulst et al., 2011), but short-range cues become increasingly important only when a potential oviposition site has been identified (da Silva et al., 2015; Bezerra-Silva et al., 2016). Vision plays a crucial role in adult mosquito biology, including location of food sources and egg deposition sites (Allan et al., 1987). In dengue mosquitoes, which are diurnal and preferentially oviposit in the late afternoon (Tsuda et al., 1989), visual cues, in particular color, are crucial in container choice for egg deposition (Hoel et al., 2011). Their increased ability to accurately detect dark colors has been used as a framework for the development of standard black ovitraps routinely used in surveillance (Hoel et al., 2011).

However, color is not the only physical parameter that can modulate oviposition responses. Avoidance of sites holding predators is a common behavior in some mosquitoes (Albeny-Simões et al., 2014). This potential for predation of vector populations has led to the use of many types of predator, some of which are effective in controlling dengue vectors in diverse container habitats (Kay et al., 2002; Moore et al., 2010). Predation-based control technology is based on the introduction of native or exotic predators into the habitat of the target species (Moore et al., 2010). However, there have been many cases of unintended consequences, e.g., the displacement of native species (Economidis, 1995; Pyke, 2008), side effects on biodiversity (Follett and Duan, 2000; US National Research Council, 2000), and access to alternative prey (Symondson et al., 2006; Kumar et al., 2015). Other problems include difficulties associated with the mass breeding (van Driesche and Bellows, 1996), storage, transport, and distribution of such predators, as well as economic costs (Sørenson et al., 2012; Pascacio-Villafán et al.,2017). Thus, efficient and sustainable vector control using predators remains challenging and innovative strategies are needed.

Female dengue mosquitoes preferentially oviposit in habitats that highly reduce exposure to predators (Pamplona et al., 2009; Torres-Estrada et al., 2001). They can perceive the presence of live (Wong et al., 2011) and dead (Albeny-Simões et al., 2014) immature conspecifics, immature allospecific predators (Albeny-Simões et al., 2014), and the act of predation (Wasserberg et al., 2013). Recently, it has been shown that the mere presence of a predator can cause sufficient stress to kill dragonflies, even when there is no chance of them being eaten (Shannon et al., 2011). A similar response to predatory intimidation has also been reported in dengue vectors (Alto et al., 2012). There have been a number of reports of cases where the populations of a pest species have been successfully repelled using visual intimidation. Visual deterrent products, such as eye balloons or plastic owls, were reported to be effective in preventing infestation by pest birds (Rensel and Wilder, 2011). An electric female decoy replicating the surface structures and colors on beetles has been highly effective to prevent successful invasion by emerald ash borers (*Agrilus planipennis Fairmaire*) by attracting its males (Domingue et al., 2014). Many threatening images have been used to chase away animal pests (Koehler et al., 1990). Despite evidence that female dengue mosquitoes can accurately perceive the presence/absence of predators (Torres-Estrada et al., 2001; Wasserberg et al., 2013; Albeny-Simões et al., 2014) and that they use visual signals to select sites with dark-colored materials (Hoel et al., 2011), the impacts of predator intimidation on the oviposition behavior of dengue vectors remains unexplored. The present study was performed to examine whether *Aedes aegypti* Linnaeus changes its oviposition activity in response to the presence of predator images in potential breeding sites.

2. Materials and methods

2.1. Mosquito source

The *Ae. aegypti* mosquitoes used in this research were obtained from a colony maintained under conditions of 28 °C ± 2.0 °C, 75% ± 5% (relative humidity), 14-h light/10-h dark (photoperiod) at the Entomology Unit (External Laboratories of the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Kota Samarahan, Malaysia). For maintenance, larvae were reared at densities ranging between 100 and 150 in plastic trays (As One Corporation, Osaka, Japan) with 1 L of tap water and fed with powdered cat food (ProDiet Cat Food, Malaysia). Food was supplied every 2 days and rearing media were renewed at least once before pupation. Emerging adults were gathered in cages (30 × 30 × 30 cm, BugDorm; MegaView Science Co., Ltd., Taichung, Taiwan) where sugar diet (10% sucrose solution) was permanently available. Four to five days after emergence, females were given blood meals from restrained hamsters for egg production. Eggs collected on paper substrates were air-dried under laboratory conditions and stored as stock colonies.

2.2. Experimental mosquitoes

To obtain experimental mosquitoes, papers with eggs from the stores were submerged in tap water. Newly eclosed larvae were reared in quadruplet at a density of 150 per plastic tray holding 1 L of 2-day-old tap water. Food (0.15 g of powdered cat food pellets; ProDiet Cat Food) was given every 48 h and the media were renewed once before pupation. Pupae were transferred singly into BugDorm cages (30 × 30 × 30 cm) where emerging adults had access to 10% sucrose solution. Females were allowed to take blood meals for 30 min from immobilized hamsters, 4 – 5 days post-emergence. Fully blood-fed females were transferred to a new cage with access to a sugar diet (10% sucrose solution). After a 3-day blood meal digestion period, these individuals were considered as gravid females and were used in the oviposition bioassays. When necessary, additional gravid females were produced following the larval rearing and adult feeding procedures outlined above.

2.3. Experimental predators and pictures

Two predators of mosquito larvae—*Toxorhynchites splendens* Wiedemann (Diptera: Culicidae) and the goldfish (*Carassius auratus* Linnaeus: Cypriniformes: Cyprinidae)—were used in this study. Larvae