

## Scientific Note

**Oviposition habitat selection by a mosquito in response to a predator: Are predator-released kairomones air-borne cues?**

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Chemical cues play an important role in predator-prey interactions in aquatic environments (e.g., Petranka et al. 1987, Dodson et al. 1994, Wisenden 2000). Predator-released kairomones may induce morphological changes in prey (e.g., Laurila et al. 2004, Von-Bert and Stibor 2006), foraging changes by prey (e.g., McCarthy and Dickey 2002, Turner and Montgomery 2003), and behavioral responses of gravid prey females via oviposition habitat selection (e.g., Kiesecker and Skelly 2000, Orizaola and Brana 2003). There is a growing body of literature showing that a number of mosquito species detect some predators via chemical cues, causing them to avoid these predators when choosing an oviposition site (Petranka and Fakhoury 1991, Angelon and Petranka 2002, Eitam et al. 2002, McCall 2002, Blaustein et al. 2004, Munga et al. 2006). Although a number of chemicals have been identified that mosquitoes respond to when ovipositing (reviewed in Bentley and Day 1989, Clements 1992, McCall 2002), little is known about how mosquito females detect predator-released kairomones in breeding sites, and no such kairomone has been chemically identified. Mosquitoes may detect chemicals from the air when the chemical possesses sufficient volatility or, in the case of low volatility chemicals, by a gustatory mechanism involving direct contact with the water (Clements 1992).

Several studies show that mosquitoes chemically detect and avoid backswimmer species when ovipositing (e.g., *Anopheles* species: Munga et al. 2006, *Culex/Culiseta* species: Blaustein et al. 2004, Blaustein et al. 2005). Knowing whether the mosquito needs to touch the water or is able to detect the predator from the air will help narrow down the candidate chemicals that act as predator-released kairomones.

We have previously shown that the mosquito *Culiseta longiareolata* Macquart chemically detects and avoids the predatory backswimmer *Notonecta maculata* Fabricius (Blaustein et al. 2004). Here, we conduct an artificial pool experiment designed to test whether *C. longiareolata* can detect the chemical cues from *N. maculata*, without touching the water.

To assess whether *C. longiareolata* needs to make contact with the water to detect this predator, we used a behavioral assay in an outdoor experiment at the University of Haifa campus on Mount Carmel, Israel (32°44'N 35°01'E). Oviposition units consisted of a central, uncovered

oviposition pool dug into the ground surrounded by an adjacent, above-ground, water-filled channel that was screened at the top (Figure 1). Thus, ovipositing mosquitoes could land on the water surface in the central oviposition pool but could not reach the water of the surrounding screened channel. All oviposition pools were identical in size (LxWxH: 60x40x15 cm), water volume and type (30 liters of aged tap water), and food resource level (1 g Tetra Pond fish pellets). Water and fish food were added on 20 April 2004. The surrounding water channels (10 cm in width, 10 cm in height) along the periphery of the oviposition pool were one of two treatments: (1) control – aged tap water; (2) *Notonecta*-conditioned water (NCW). NCW was aged tap water that was first conditioned with adult *Notonecta maculata* in the laboratory at a density 0.75 per liter for four days prior to adding to water channels. Channel water was emptied and replaced every four days. We viewed this water change interval as sufficiently small because a previous study showed that *Notonecta*-conditioned water without predator replenishment continued to repel oviposition for about a week (Blaustein et al. 2004). Water in the oviposition pools was not renewed, except that aged tap water was added when needed to replace evaporated water and maintain a consistent water volume. We reasoned that mosquitoes searching for oviposition sites would first pass over the water channels before the central oviposition pool. If a predator-released kairomone that reduced oviposition was volatile, then we would expect mosquitoes detecting the NCW to be repelled and not continue on to the oviposition pool, resulting in fewer egg rafts in pools containing NCW channels compared to control pools with just tap water.

The experimental design consisted of five pairs of two oviposition units, with a control and NCW unit about 1 m apart (Figure 1). Mosquito egg rafts were collected and removed daily from all pools. They were brought to the laboratory and subsequent larvae were identified to the species level. Only *C. longiareolata* oviposited in sufficient numbers to assess treatment effects. Egg rafts were counted on 36 days. Because occasional disturbances resulted in our inability to use egg raft counts on some days for a specific pool, resulting in a variable number of days in which oviposition was counted for a specific unit, we used the average number of egg rafts per pool as our variable. We tested for a treatment effect using a paired t-test.

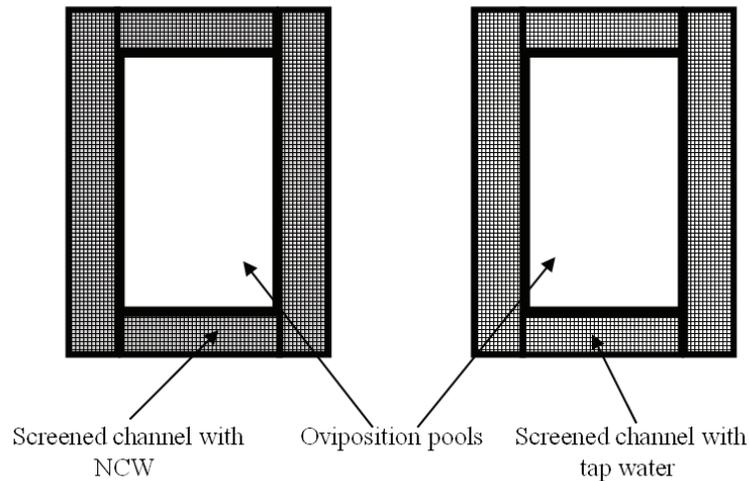


Figure 1. Schematic diagram of a pair of oviposition units. Each oviposition pool is dug into the ground and surrounded by a screen-covered water channel that is not dug into the ground. Two units are paired. For each pair, one oviposition pool is surrounded by a channel containing control water (aged tap water) and the second is surrounded by a *Notonecta*-conditioned water (NCW) channel.

*Culiseta longiareolata* oviposited significantly more in the central pools surrounded by channels containing control water than in NCW: 56 of 81 egg rafts (69%) were oviposited in the control pools (paired t-test;  $df=4$ ;  $t=3.55$ ;  $p=0.024$ ; Figure 2). As the experimental set-up prevented any direct contact by the ovipositing female with channel water, these results indicate that ovipositing *C. longiareolata* female were deterred from continuing to the central pool when predator-released volatile compound(s) emanated from the surrounding channel. However, past studies containing an equal number of control and *Notonecta* or NCW pools consistently showed that *C. longiareolata* oviposits ~90% of the egg rafts in predator-free pools when allowed to come in direct contact with the water surface (Blaustein et al. 1995, Blaustein 1998, Kiflawi et al. 2003,

Blaustein et al. 2004, Arav and Blaustein 2006). One simple explanation for a reduced repellency in this study is that some mosquitoes ventured past the narrow (10 cm width) NCW channels and discovered a suitable oviposition pool in the middle of the set-up. Indeed, we expected only a fraction of those mosquitoes deterred by a NCW channel to reverse course, rather than proceed forward in search of a suitable oviposition habitat. A second possible explanation for the relatively low proportion could be additional deterrent effects of other predator-released kairomones that are not sufficiently volatile. The combined effect of volatile and non-volatile cues is known to affect insects in host detection (Gikonyo et al. 2000, Foster et al. 2003, Dugravot and Thibout 2006, Blande et al. 2007). Similar kairomones are sometimes used to detect both host and oviposition

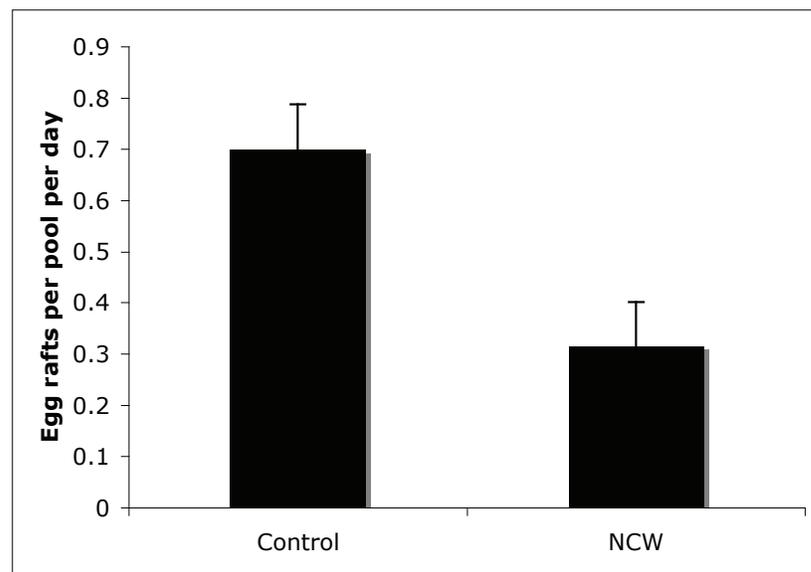


Figure 2. Number of egg rafts per pool per day in oviposition pools surrounded by control and *Notonecta*-conditioned water (NCW) channels. Error bars are one standard error.

site (Dougherty and Hamilton 1997, Takken 1999, Sunish et al. 2003, Qiu et al. 2006). If this is true in this case, then *C. longiareolata* females may detect a combination of volatile and nonvolatile compounds when avoiding larval predators.

*Notonecta maculata* has already been shown to reduce oviposition by three mosquitoes including *Anopheles gambiae* (Munga et al. 2006) and the predator-released kairomone(s) may be the same for all three mosquito species. The chemicals may be common to all backswimmers, as other backswimmer species (e.g. Eitam et al. 2002), but not other predator groups (Stav et al. 2000), elicit the response in some mosquitoes. Future studies should determine the specific volatile compound(s) that reduce mosquito oviposition, which may then be used in control efforts.

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