

Oviposition decreased in response to enriched water: a field study of the pitcher-plant mosquito, *Wyeomyia smithii*

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Abstract. 1. Environmental cues are known to influence oviposition behaviour in mosquitoes, with important consequences for larval survival and insect population dynamics. Enriched microhabitats have been shown to be preferred oviposition sites.

2. In a field experiment designed to determine whether ovipositing mosquitoes are sensitive to different levels of nutrient enrichment, new pitcher-plant (*Sarracenia purpurea*) leaves were opened and enriched with 0, 2, or 20 dead ants, and the number of pitcher-plant mosquito (*Wyeomyia smithii*) larvae resulting from subsequent oviposition were measured.

3. Oviposition rates were higher in leaves with low levels of enrichment (0 and 2 ants per leaf), although larval development was enhanced at the highest enrichment level.

4. Results suggest that, although these mosquito larvae are nutrient limited, ovipositing females preferentially avoid highly enriched leaves. This counterintuitive result may be due to low oxygen concentrations or a masked cue in enriched leaves, and contrasts with other oviposition studies.

Key words. Inquilines, mosquitoes, oviposition, oviposition behaviour, oviposition cues, pitcher-plants, *Sarracenia purpurea*, *Wyeomyia*.

Introduction

Oviposition behaviour is a plastic trait and can be affected by a variety of cues (Bentley & Day, 1989; Isoe & Millar, 1996; Yu *et al.*, 2004). The ability of the parent to distinguish among oviposition sites is important for many insects because microhabitat quality is often the major determinant of larval survival (Thompson, 1988). Oviposition behaviour is especially influential when larval movement is limited such that the habitat of larval development is determined wholly by oviposition site (Rausher, 1979; Reseterits, 1996). Consequently, oviposition behaviour can influence the density and distribution of insect populations.

Both abiotic and biotic factors affect mosquito oviposition behaviour (Bentley & Day, 1989). Abiotic factors known to influence oviposition behaviour include water temperature, depth, color, and salinity (Dhileepan, 1997). Biotic factors that can affect mosquito oviposition include the presence and density of conspecific larvae (Allan & Kline, 1998), predators (Tietze & Mulla, 1991; Fry *et al.*, 1994; Eitam & Blaustein, 2004), parasites (Lowenberger & Rau, 1994), pathogens (Reeves, 2004), bacteria (Nguyen *et al.*, 1999; Trexler *et al.*, 2003), and nutrients (Reiskind & Wilson, 2004). Multiple studies have demonstrated that mosquitoes preferentially oviposit in water enriched with organic matter and bacteria, presumably because these microhabitats provide more nutrients for developing larvae (Beehler & Mulla, 1995; Dhileepan, 1997; Du & Millar, 1999; Nguyen *et al.*, 1999). Many synthetic and naturally-occurring chemical cues have also been shown to influence mosquito oviposition (Millar *et al.*, 1994; Olagbemiro *et al.*, 1999; Geetha *et al.*, 2003).

The pitcher-plant mosquito, *Wyeomyia smithii* (Coq.), oviposits exclusively in the water-holding leaves of the

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pitcher-plant *Sarracenia purpurea* L., where in southern latitudes they develop through 4 instars to adults in approximately 14–20 days (Bradshaw & Holzapfel, 1990). The two species co-occur across a wide geographical range, along with an aquatic inquiline community composed of bacteria, protozoa, rotifers, mites, and two other specialist dipterans, the pitcher-plant midge, *Metriocnemus knabi* (Coq.), and the pitcher-plant flesh fly, *Fletcherimyia fletcheri* (Aldrich) (Buckley *et al.*, 2003; Miller & Kneitel, 2005). The inquiline community is fuelled by allochthonous energy from prey (primarily ants), which fall into the pitfall trap of the pitcher-plant and drown. The pitcher-plant mosquito is the omnivorous top predator in this community.

Few studies have examined the oviposition behaviour of pitcher-plant mosquitoes. Gravid pitcher-plant mosquitoes are thought to be drawn to pitcher-plant leaves by a water-soluble chemical cue (Istock *et al.*, 1983). Leaf characteristics may also act as cues. For example, younger leaves are strongly preferred over older leaves as oviposition sites (Bradshaw, 1983; Miller & Kneitel, 2005). In a manipulative experiment using newly opened leaves, Heard (1994) found that pitcher-plant mosquitoes prefer to oviposit in larger leaves containing either conspecific or midge larvae. Nastase *et al.* (1995) found that larval abundance was positively correlated with size and youth of leaves. Miller and Kneitel (2005) have also shown that younger leaves are preferred oviposition sites, that most oviposition probably occurs in the first few weeks, when the leaf also captures the majority of its prey (Fish & Hall, 1978), and that higher numbers of prey lead to higher abundances of bacteria and protozoa, which may be consumed by mosquitoes (Kneitel & Miller, 2002; Miller *et al.*, 2002; Hoekman, 2007). Taken together, these studies suggest that pitcher-plant mosquitoes choose oviposition sites on the basis of their potential for prey abundance, as higher prey abundance provides more nutrition for larval mosquitoes. Prey abundance, in turn, is affected primarily by leaf age; younger and, to a lesser degree, larger leaves attract more prey (Wolfe, 1981). The current study was conducted to determine the direct effects of prey number on oviposition behaviour in the pitcher-plant mosquito.

Gravid female mosquitoes should oviposit in what they perceive to be the best quality environment for larval development (Heard, 1994). Previous work has demonstrated that enriched environments, with higher bacterial levels, are favored oviposition sites for a variety of mosquito species (Dhilepan, 1997; Du & Millar, 1999; Nguyen *et al.*, 1999). Pitcher-plant leaves with more prey have been shown to have higher bacterial and protozoan densities (Kneitel & Miller, 2002; Hoekman, 2007), and mosquito larvae in enriched leaves (those with more per capita resources) have been shown to develop into larger and consequently more fecund adults (Bradshaw & Holzapfel, 1992). Furthermore, total mosquito biomass and number of pupae emerging have been positively correlated with prey capture (Bradshaw & Holzapfel, 1986). Female pitcher-plant mosquitoes might therefore be expected to oviposit preferentially in leaves containing higher densities of dead ants, on the expectation that such leaves will provide a better environment for larval development. This hypothesis was tested by manipulating ant density in naturally occurring pitcher-plant leaves.

Methods

The experiment was designed to determine whether mosquitoes were sensitive to both the presence and abundance of dead ants, which are the principal source of nutrients for the community. Three nutrient levels were created by adding either 2 or 20 ants to unopened pitcher-plant leaves and using leaves without ant additions as controls (0 ants added). The experiment was conducted at the Mud Swamp site, an open longleaf-pine *Pinus palustris* (Mill.) savanna in the Apalachicola National Forest, 6 km north-east of Sumatra, Florida (30°N, 84°W) in October 2004. New unopened leaves of similar size were located along an east–west transect across the field. The leaves were assigned randomly to one of the three treatments within 24 blocks. Unopened leaves were chosen to ensure that experimental pitchers of similar age and size were started with no prey.

Natural densities of dead ants in leaves open from one to two weeks range from 0 to 176, with an average of 11.14 (T. E. Miller, unpublished data), so our treatment levels reflect realistic natural variation. Each pitcher was carefully opened and the treatments were applied in 10 ml aliquots of water. To prevent additional ant capture by the pitcher-plant, Tanglefoot (The Tanglefoot Company, Grand Rapids, MI) was applied in a continuous line around the outside of each leaf.

After six days, the contents of each leaf were collected. A 2-day incubation of the samples allowed recently-laid mosquito eggs to hatch, after which the larvae were counted and categorised by larval stage (instar 1–4). Development time from egg to adult at this time of the year is normally greater than 20 days, so no mosquitoes could mature during the time period of the experiment. No dead larvae or unhatched eggs were found; natural larval mortality is low (T. E. Miller, pers. obs.).

Analysis of variance (ANOVA) was used to compare differences in the total number of larvae between treatments. A priori contrasts were used to test for the effects of the presence of ants (0- vs. 2- and 20-ant treatments) and the abundance of ants (2- vs. 20-ant treatment). Multivariate analysis of variance (MANOVA), using the 4 mosquito larval stages as dependent variables, was used to compare differences in larval development between treatments. Correlations between the dependent variables and their canonical loadings were used to evaluate the relative importance of the 4 mosquito instars in determining treatment differences. All data were square-root transformed to improve normality and homoscedasticity.

Results

One block could not be relocated when the contents of the leaves were collected. Otherwise, all leaves were undisturbed after six days, and prey treatments appeared intact.

Oviposition rates were apparently high; more than 85% of leaves contained some larval mosquitoes. Leaves receiving 0 or 2 ants had over twice as many larvae as those receiving 20 ants (Fig. 1A). The overall variation among the three treatments was significant ($F_{2,69} = 4.94$, $P = 0.01$) (Fig. 1A). The quantity of ants (2 vs. 20 contrast) significantly affected the number of larvae found in each leaf ($F_{1,69} = 6.92$, $P = 0.01$), but the presence

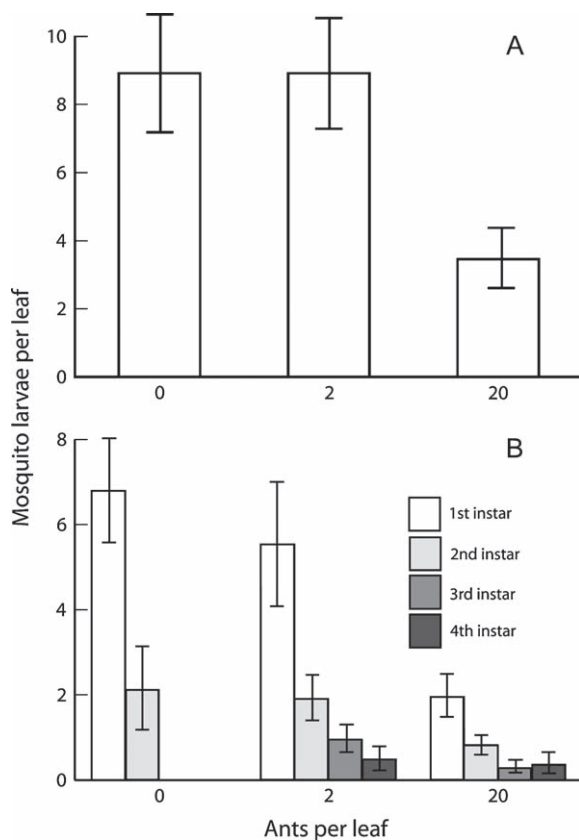


Fig. 1. Total number of mosquito larvae (A) and number per instar (B) found per leaf (\pm SE). Oviposition frequency depends on prey treatment (ants added per leaf).

of ants (0 vs. 2 + 20 contrast) did not have a significant effect ($F_{1,69} = 2.96$, $P = 0.09$).

Overall, ant treatment had a significant effect on the number of each instar in each leaf (Wilks' lambda $F_{8,132} = 2.58$, $P = 0.012$). As expected after recent oviposition, the majority of the mosquito larvae were 1st instar; in fact, the 0-ant treatment contained no 3rd- or 4th-instar larvae. The proportion of 1st- and 2nd-instar larvae declined with prey treatment (Fig. 1B). As indicated by product-moment correlations, the density of 1st instars was most influential in determining treatment differences (0.860), followed by the 3rd (−0.507), 4th (−0.365) and 2nd (0.184) instar density. The sign of these correlations reveal that high numbers of early instars are associated with low numbers of late instars.

Discussion

These results are contrary to the expectation that female pitcher-plant mosquitoes would oviposit preferentially in leaves with more ants. Instead, female mosquitoes preferred to oviposit in leaves with lower levels of enrichment, including leaves that had not received any added nutrients (Fig. 1A).

Nutrient levels did affect larval development in an expected manner. In the low-nutrient treatment, larval abundance was

high, but no larvae developed past the 2nd instar (Fig. 1B). In the medium-nutrient treatment, larval abundance was equally high, but a small number of 3rd and 4th instars were able to develop. These results suggest that nutrient limitation prevented larvae from developing under low-nutrient conditions. Larvae in the high-nutrient treatment achieved levels of development similar to those of larvae in the medium-nutrient treatment (Fig. 1B). Though they were less abundant, eggs laid in the high-nutrient leaves appeared to develop normally and perhaps even more rapidly than eggs in other treatments. Their success can be attributed to high per-capita resources, due to high nutrients, low density, or their combination.

Although the pattern of larval development can be explained according to a nutrient or density effect, the abundance pattern is still counterintuitive: why were fewer eggs laid in high nutrient leaves? There may be an intermediate maximum in the relationship between prey availability and oviposition somewhere between 0 and 20 ants that was unable to be observed with our treatments. If so, we would have expected there to be an increase in oviposition between the 0- and 2-ant treatments; however, the possibility of an intermediate maximum cannot be excluded.

Another explanation is that the addition of 20 ants caused some pitcher microhabitats to become anoxic. Dissolved oxygen and number of ants are negatively correlated in leaf fluid (T. E. Miller, unpublished data). Extreme anoxic leaves are occasionally observed in the field and are characterised by a pungent odor that may repel gravid female mosquitoes. As mosquito larvae can breathe at the water surface, anoxia may not directly affect mosquito growth or survival but may affect the abundance of protozoa and bacteria, the primary mosquito prey.

One final possibility is that high densities of decomposing ants mask the olfactory cue that attracts ovipositing pitcher-plant mosquitoes. Because both oviposition rate and prey capture rate decline exponentially with leaf age (Bergland *et al.*, 2005), ovipositing females must frequently encounter young pitchers with high prey densities. Perhaps their apparent aversion to highly enriched pitchers helps them identify the youngest possible leaves, i.e. those that have just opened and have not yet accumulated prey. Pitcher-plant mosquitoes have been observed ovipositing in freshly opened leaves with no prey or standing water (Bradshaw & Holzapfel, 1986), displaying the anticipatory nature of their oviposition behaviour.

No study to the authors' knowledge has reported the unexpected pattern of lower oviposition in response to enriched water. In fact, previous studies have shown the opposite; other mosquito species preferentially oviposit in enriched environments (e.g., Beehler & Mulla, 1995; Isoe *et al.*, 1995; Lampman & Novak, 1996; Dhileepan, 1997; Du & Millar, 1999; Nguyen *et al.*, 1999; Reiskind & Wilson, 2004). Unfortunately, most of these studies included no measure of bacterial density and used only an enriched and an unenriched treatment rather than a gradient, so they could not document any threshold where attraction was gained or lost. In a lab bioassay experiment, however, Du and Millar (1999) did find both a monotonic and an intermediate response to enrichment by two different species of mosquitoes. In general, females preferred to oviposit in more enriched water, but *Culex quinquefasciatus* (Say) preferred the highest

concentration of bulrush infusion, whereas *Culex tarsalis* (Coq.) preferred intermediate concentrations.

One difference between prior studies and ours is the unique relationship between *W. smithii* and its host plant, *S. purpurea*. The leaf habitat may differ from the larval habitat of other mosquito species in that it goes through a predictable pattern of nutrient availability (Miller & Kneitel, 2005) and potential competition (Bradshaw & Creelman, 1984), which likely gives a very large benefit to oviposition in very young leaves. In a recent field experiment with pitcher-plant mosquitoes in a northern bog, however, Bergland *et al.* (2005) found that per leaf oviposition was positively correlated with larval resources (captured prey). It may be that latitude or seasonality also plays a role in the relationship between resources and oviposition behaviour.

A surprising, but ultimately unexplained, pattern is documented in this experiment. Clearly, oviposition rates strongly decline at high prey levels, although high prey levels appear to allow higher developmental rates. The consequence of this complex oviposition behaviour for the mosquitoes requires further study.

Acknowledgements

D.H. would like to thank the Arthur J. Schmitt fellowship at the University of Notre Dame for financial support. This research was funded in part by the National Science Foundation (DEB 0091776 to T.E.M.). The manuscript was improved by the careful editing of Anne Thistle and two anonymous reviewers.

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Accepted 11 August 2006