Title

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Journal

Journal of medical entomology, 43(2)

ISSN

0022-2585

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Publication Date

2006-03-01

DOI

10.1603/0022-2585(2006)043[0221:eolcap]2.0.co;2

Peer reviewed

Effects of Larval Competitors and Predators on Oviposition Site Selection of *Anopheles gambiae* Sensu Stricto

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J. Med. Entomol. 43(2): 221–224 (2006)

ABSTRACT We examined whether predators and competitors influence selection of oviposition sites by *Anopheles gambiae* Giles. Mosquitoes in cages laid significantly fewer eggs in rainwater conditioned with a predator (backswimmers, *Notonecta* sp.) than in unconditioned rainwater. Rainwater conditioned with a putative competitor (tadpoles, *Xenopus* sp.) also had fewer eggs than unconditioned rainwater. Similarly, mosquitoes laid significantly fewer eggs in rainwater conditioned with five and 50 *An. gambiae* larvae than in unconditioned rainwater. When larvae were present, significantly more eggs were laid in containers with five larvae than in containers with higher densities, but the differences in number of eggs laid were not significant among the densities of 40, 70, and 100 larvae. This study demonstrated that caged *An. gambiae* females avoid oviposition in habitats with supposed competitors and predators.

KEY WORDS oviposition, site selection, *An. gambiae*, predators, competitors

In sub-Saharan Africa, *Anopheles gambiae* Giles is the primary vector of human malaria. Immatures of this mosquito occur in small, temporary, sunlit pools such as borrow pits, hoof prints, tire tracks, drainage ditches, and small puddles (Gillies and De Meillon 1968, Minakawa et al. 1999, Gimnig et al. 2001). *An. gambiae* is a pioneer species and is able to colonize these habitats within a few days after the habitats are created (Minakawa et al. 2005a). In general, predation on larvae is less prevalent in temporary habitats than it is in large, permanent habitats, and competition is less common in newly created habitats (Service 1977, Washburn 1995, Sunahara et al. 2002).

It has been suggested that *An. gambiae* actively selects habitats favorable for oviposition rather than randomly colonizing them (Minakawa et al. 2004). Some mosquito species are known to avoid ovipositing in habitats with predators and competitors (Kiflawi et al. 2003, Mokany and Shine 2003a, Blaustein et al. 2004). However, gravid mosquitoes may be attracted to habitats with conspecific larvae, because presence of conspecific larvae may indicate suitable habitats for the species (Blaustein and Kotler 1993, Allan and Kline 1998, Sumba et al. 2004). The issue reflects a trade-off between the risk of choosing an unsuitable habitat and the cost of intraspecific competition (Spencer et al. 2002, Kiflawi et al. 2003, Blaustein et al. 2004).

Understanding the oviposition behavior of *An. gambiae* is important for development of effective vector control techniques. Elucidating the cues governing oviposition behavior may provide a tool for behavioral manipulation of mosquitoes in the field (Kiflawi et al. 2003). Because development of a malaria vaccine is slow, and development of parasite resistance to antimalarial drugs is rapid, vector control is still the most practical method for reducing malaria transmission in developing countries (Trape et al. 2002, Killeen et al. 2004). However, our understanding of ecology of malaria vectors in Africa is still insufficient to design efficient vector control methods. In this study, we examined whether predators and competitors influence oviposition site selection of *An. gambiae.*

Materials and Methods

Mosquito Colony. We established a mosquito colony from 200 *An. gambiae* adults that were collected in 20 randomly selected houses in Iguhu, Kakamega District, western Kenya, in February 2004. The following experiments were conducted using the colony in a house in Iguhu (34° 45′ E and 0° 10′ N).

Experimental Design. First, we tested whether predators affect oviposition site selection of *An. gambiae.* We used the backswimmer (*Notonecta* sp.) as a potential predator, which was often observed in temporary pools in Iguhu. Although it has been well documented that backswimmers prey upon other mosquito species and affect oviposition site selection of the mosquitoes (Blaustein et al. 1995, 2004), we conducted a pilot study to confirm that the backswimmer preys upon *An. gambiae* larvae. An adult backswimmer

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Table 1. Average numbers (standard error) of eggs laid by *An. gambiae* in the unconditioned rainwater and in rainwater conditioned with tadpoles, backswimmers, five conspecific larvae, and 50 conspecific larvae

	Unconditioned rainwater (control)	Conditioned rainwater	t	df	P
Tadpoles	251.3 (16.9)	173.5 (12.2)	5.93	119	<0.001
Backswimmers	189.0 (14.3)	95.3 (8.8)	4.39	119	<0.001
Five larvae	172.7 (16.5)	103.5 (16.0)	2.90	47	0.003
Fifty larvae	140.0 (15.4)	79.4 (10.7)	3.26	47	0.002

and 12 *An. gambiae* larvae were placed in a plastic container (300 ml) with 200 ml of rainwater. We used second instars of *An. gambiae,* because they were large enough to recognize and were readily available. The container was covered with a screen to prevent the backswimmer from flying away. We observed the backswimmer and mosquito larvae for 24 h. The experiment had five replicates.

For the main oviposition experiment, we conditioned 1 liter of rainwater by keeping 10 backswimmers in the water in the house for 3 d. The backswimmers were not fed during the conditioning period. We removed the backswimmers and then placed 100 ml of the conditioned rainwater in a plastic container (300 ml) with a filter paper (9 cm in diameter) as an oviposition substrate in a randomly selected corner of the experimental cage (30 by 30 by 30 cm) with 20 randomly selected gravid mosquitoes. A container with unconditioned rainwater and a filter paper was placed in the opposite corner of the cage. The next day, the number of mosquito eggs laid on the filter paper in each container was counted. The experiment had six replicates (cages) each time, and it was repeated 20 times between 23 March and 5 September 2004. The experiment had 120 replicates in total.

A similar experiment was designed to examine the effects of competitors on mosquito oviposition behavior. We used tadpoles of *Xenopus* sp., which co-occurred with *An. gambiae* larvae in Iguhu, as a potential competitor. Although there is no scientific evidence for competitive effects of *Xenopus* sp. on *An. gambiae,* it is known that tadpoles of frog species feed on algae that are consumed by mosquito larvae (Blaustein and Kotler 1993, Gimnig et al. 2002, Mokany and Shine 2003a). *Xenopus* sp. eggs were collected from temporary pools in Iguhu. Tadpoles that hatched were reared until they reached stage 25 (Gosner 1960). We conditioned 2 liters of rainwater by keeping 30 tadpoles in the water in the house for 3 d. The tadpoles were not fed during the conditioning period. Then, we followed the same procedure with the same number of replicates as for the experiment with backswimmers.

To test effects of intraspecific competition, we repeated the experiment using *An. gambiae* larvae. We designed two separate experiments by conditioning 1 liter of rainwater with five second instars or with 50 second instars for 3 d, and proceeded as described above. In addition, we tested whether larval density influenced mosquito oviposition behavior. In this experiment, we placed unconditioned rainwater, rainwater conditioned with five larvae, and rainwater conditioned with 50 larvae in the same cage, and proceeded as described above.

As a further experiment on intraspecific competition, we tested the effects of larval presence at different densities in rainwater that was not conditioned before the experiment. We examined the effects of four different larval densities: five, 40, 70, or 100 second instars per plastic container (300 ml). Each container was placed in a randomly selected corner of the cage. We set up four replicates each time and repeated the intraspecific competition experiments 10 times (40 replicates in total).

Statistical Analysis. We used paired t-tests to test for differences between the mean numbers of eggs laid in the conditioned rainwater and in unconditioned rainwater. For the experiment with multiple larval densities, we used one-way analysis of variance (ANOVA). When the effects were significant, Tukey–Kramer multiple comparison tests were used to compare mean values among the treatments. The significance level was 0.05 for all tests. We performed all statistical tests using SYSTAT version 10.2 (SYSTAT Software Inc., Richmond, CA).

Results

We confirmed that the backswimmer is a predator of *An. gambiae* larvae in the pilot study. The backswimmer preyed upon 10.2 ± 0.6 larvae (mean $\pm$ SE) in five trials.

In the oviposition experiment, mosquitoes laid significantly fewer eggs in the rainwater conditioned with backswimmers than in unconditioned rainwater (Table 1). Mosquitoes also laid significantly fewer eggs in rainwater conditioned with tadpoles than in the unconditioned rainwater. Similarly, the rainwater conditioned with five and 50 *An. gambiae* larvae had significantly fewer eggs than the unconditioned rainwater.

One-way ANOVA revealed significant effects of treatments when we placed unconditioned rainwater, rainwater conditioned with five larvae, and rainwater conditioned with 50 larvae in the same cage ($F = 4.35$; df = 2, 119; $P < 0.001$; Fig. 1A). A multiple comparison test revealed that the unconditioned rainwater contained significantly more eggs than the rainwater conditioned with 50 larvae. However, the differences were not significant between the unconditioned rainwater and the rainwater conditioned with five larvae, and between the rainwater conditioned with five larvae and with 50 larvae. When larvae were present, significantly more eggs were laid in the container with

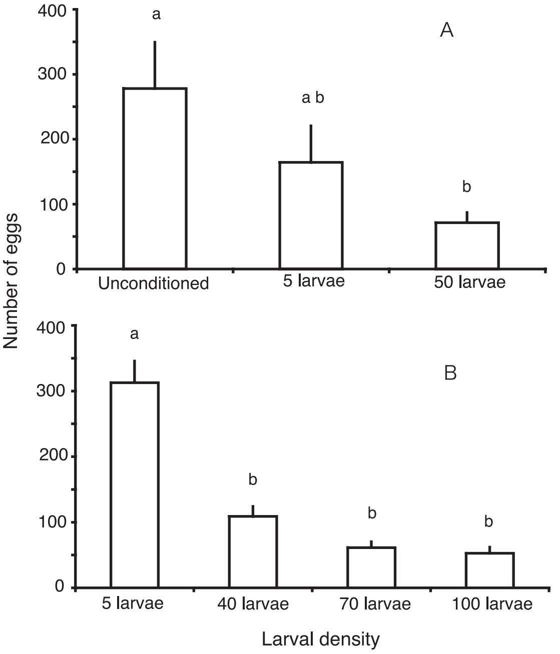

Fig. 1. Average numbers (SE) of eggs laid by *An. gambiae* when unconditioned rainwater and in rainwater conditioned with five or 50 conspecific larvae were placed in the same cage (A), and average numbers of eggs laid when larvae were present in water (B). The alphabetical letters indicate results of Tukey-Kramer multiple comparison tests. The values with the same letter were not statistically significant at $P = 0.05$ level.

five larvae than in the containers with the other larval densities, but the differences among the densities of 40, 70, and 100 larvae were not significant ($F = 39.69$; df = 3, 159; $P < 0.001$; Fig. 1B).

Discussion

This study demonstrated that cues from backswimmers and tadpoles influence selection of oviposition site by gravid *An. gambiae* in cages. Gravid mosquitoes laid significantly fewer eggs in rainwater conditioned with backswimmers and tadpoles than in unconditioned rainwater. These results are similar to previous findings on mosquitoes of other genera (Edgerly et al. 1998, Mokany and Shine 2003a, Blaustein et al. 2004), and they suggest that gravid mosquitoes avoid habitats containing competitors and predators to reduce the risk of mortality of offspring. This behavior is probably one of the mechanisms responsible for the heterogeneous distribution of *An. gambiae* larvae (Minakawa et al. 2005b).

The results from this study in cages also suggest that *An. gambiae* females might select habitats with fewer conspecific larvae to avoid intraspecific competition. Gravid *Aedes aegypti* (L.) also are known to avoid habitats crowded with conspecific larvae (Zahiri and Rau 1998). Intraspecific competition prolongs larval development and reduces adult body size of *An. gambiae* (Gimnig et al. 2002). Prolonged larval development and reduced adult body size reduce productivity (Lyimo and Takken 1993, Ameneshewa and Service 1996). Thus, gravid mosquitoes oviposit in the habitats that will maximize their fitness.

However, Sumba et al. (2004) reported that *An. gambiae* laid more eggs in water from natural anopheline habitats than in distilled water. Their results and our results suggest that *An. gambiae* females are not attracted by presence of conspecific larvae, but they are attracted by other habitat characteristics that probably enhance immature stage survivorship and development. The characteristics of preferable habitats may be high quality and/or quantity of food sources such as algae and microbes (Gimnig et al. 2002, Mokany and Shine 2003a). This reflects a trade-off between the risk of choosing an unsuitable habitat and the risk of intraspecific competition (Spencer et al. 2002, Kiflawi et al. 2003, Blaustein et al. 2004). Probably, gravid *An. gambiae* disregard the presence of conspecific larvae as long as larval density is low enough to minimize intraspecific competition.

Because the predator and competitors had been removed before the oviposition experiments by using the conditioned rainwater in this study, the results suggest that *An. gambiae* females are able to detect a chemical substance released by the predator and competitors, or by microorganisms associated with them (Mokany and Shine 2003b). Various aquatic insects use chemical cues to detect predators (Petranka and Fakhoury 1991). A predator-released chemical is the cue for oviposition avoidance by the mosquito *Culiseta longiareolata* Macquart (Blaustein et al. 2004). Volatile substances released from microorganisms may strongly affect oviposition site selection of anopheline mosquitoes (Knols et al. 2004, Rejmankova et al. 2005). Thus, it is likely that *An. gambiae* also has evolved to use olfactory cues for selecting a better oviposition site, and it might be possible to isolate the substances that produce these olfactory cues, or to mimic the cues in some way to control mosquito behavior.

Acknowledgment

We thank M. Okonji, S. Ajuma, P. Lutiali, W. Miheso, and A. Wabwile for technical assistance, and S. Gage for helpful comments on the manuscript. This research was supported by National Institutes of Health grants R01 (AI) 50243 and D43 TW01505. This article is published with the permission from the Director of the Kenya Medical Research Institute.

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Received 6 July 2005; accepted 18 October 2005.