A BEHAVIORAL MECHANISM FOR RESTING SITE SELECTION BY PUPAE IN THREE MOSQUITO SPECIES

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ABSTRACT. Pupae of *Aedes aegypti, Ae. triseriatus* and *Culex restuans* dive less frequently when resting in a concave meniscus than when resting in open water. They also tend to terminate diving after contacting submerged vertical surfaces, increasing their chances of surfacing in a concave meniscus. As a result pupae tend to rest in concave menisci associated with emergent vertical surfaces, a behavioral adaptation by which they probably conserve energy and avoid predation.

INTRODUCTION

In addition to being anatomically distinct, the mosquito pupa is behaviorally different from both the larval and adult stages. It neither feeds nor displays behavior associated with reproduction, but simply rests motionless at the water surface or, if disturbed, dives. This paper deals with the relationship between diving and resting behavior.

Bates (1949) points out: "In general the pupae lie motionless, usually touching some emergent object (vegetation), an orientation that is probably governed by surface tension forces..." Likewise, laboratory-reared pupae typically rest where the water meets the vertical surface of the rearing container, i.e., in a concave meniscus. A concave meniscus is formed along any emergent hydrophilic surface where the surface film of water is pulled higher than the surrounding water. Since pupae are positively buoyant, it is physically impossible for them to rest in a convex meniscus (i.e., one produced along a hydrophobic surface where the surface film of water is pushed below the level of the surrounding water) since they are always buoyed to the highest water level.

Preliminary observation suggested that pupae resting in concave menisci are less likely to dive than those resting in open, flat-surfaced water and that pupae tend to ascend along a vertical surface when terminating a dive. Together these traits would constitute a behavioral mechanism which promotes resting in a concave meniscus associated with an emergent, hydrophobic surface. In this study, we present evidence for such a mechanism in pupae of *Aedes aegypti* (Linn.), *Ae. triseriatus* (Say) and *Culex restuans* Theobald. On the basis that pupae resting in concave menisci dive less frequently than those resting in open water, we predicted that pupae in concave menisci are less responsive to mechanical vibrations than those in open water. This hypothesis was tested using *Aedes aegypti.*

MATERIALS AND METHODS

Rearing procedures for *Aedes* spp. are described in Romoser (1975). *Aedes aegypti* has been maintained in our laboratory for at least 15 generations. *Aedes triseriatus* (Alabama strain) were reared from eggs obtained from The Ohio State University. *Culex restuans* pupae were reared from larvae collected in Athens County, Ohio. Specimens used in this study were post-teneral, but did not show signs of melanization characteristic of pharate adults approaching emergence. All observations were made in a clear plastic tank (diam., 10 cm; depth, 6.5 cm) under identical lighting conditions and at a temperature of approximately 21°C.

To test the hypothesis that pupae tend to rest in concave menisci associated with emergent surfaces, resting site preference in containers with and without concave menisci was observed. Concave menisci were formed by filling a container to within 5 mm of the top. Exposure of pupae to a situation where concave menisci were absent was achieved by filling the observation container in a larger tank in such a way that the top edge was barely covered with water. For each species, the resting positions of 10 pupae (edge versus open water) were determined after each of 12 dives. Resting site was not noted until all 10 pupae had come to rest since pupae commonly surfaced and dived several times before coming to rest. After each observation, pupae were stimulated to dive by tapping the top edge of the container with a pen until all dived.

To test the hypothesis that pupae dive less frequently when resting in concave menisci than when resting in open water, spontaneous diving frequency was determined for each resting site. Determination of the frequency of spontaneous diving from concave menisci was made by counting the number of dives made by a single pupa in a 3 minute interval. This was done for 15 pupae of each species in a slightly underfilled tank. To determine the diving frequency in open

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water, pupae were placed in a slightly overfilled tank. Determination of spontaneous diving rates (i.e., dives in response to random background stimuli or to possible endogenous stimuli) were made simultaneously to ensure that both were subject to identical stimuli. Further, tanks were placed on a cushioned marble slab which reduced vibrations.

To test the hypothesis that pupae tend to ascend along vertical surfaces, 10 consecutive dives of 36 pupae of each species were observed and the location of surfacing noted. Pupae surfacing within 3.0 mm of the container edge (i.e., the area within which pupae floated passively up into the concave meniscus) were scored as "edge" while all surfacing acts further than 3.0 mm from the edge were scored "open water".

To further test the hypothesis that pupae (*Ae. aegypti* and *Cx. restuans*) tend to ascend along vertical surfaces, surfacing behavior was compared in pupae exposed to concave menisci with and without submerged vertical surfaces immediately below. Concave menisci without submerged vertical surfaces were provided by touching 5 tongue depressors (12 x 1 cm) to the water surface within a barely submerged rearing container (Fig. 1). Concave menisci with submerged vertical surfaces below were provided by lowering the tongue depressors to the bottom of the container. Ten pupae were placed in the container and induced to dive by tapping. When all pupae had resurfaced, their positions were recorded. This procedure was replicated 12 times for both species tested.

To test the hypothesis that pupae resting in a concave meniscus are less responsive to mechanical stimuli than those in open water, responsiveness of pupae resting in each location was tested. Twenty-four pupae resting in a concave meniscus were compared to 24 pupae resting in open water. A mechanical stimulus was delivered to each pupa in each group 5 times. The number of times (out of 5) that a pupa responded by diving was recorded as a percent. The percent response for each pupa in the two groups was then used to calculate the mean percent response for each group. The mechanical stimulus (square wave) was generated by a General Radio Company unit R-C oscillator type no. 1210-C with a unit power supply type no. 1203-B, and a Heath Kit model AA-18 solid state amplifier. A stimulus was conveyed to the water by a tapering glass rod held in place by a clamp connected to a ring stand. The tip of the glass rod was submerged about 1 mm below the surface and placed about 2 cm from a test pupa. A hand-held switch was used to control the stimulus duration of about 1 second. Although frequency and amplitude were adjusted with reference to numerical values on the oscillator dials, it would be misleading to represent these values in Hertz or decibels because their accuracy was not determined, both with regard to the actual frequencies and amplitudes emanating from the oscillator and to the actual frequencies and amplitudes to which the pupae were exposed at some distance from the stimulus source. Preliminary testing revealed that stimuli delivered when the oscillator dials were set a 150 Hz and −10 dB with a distance of 2 cm between the glass rod and a test pupa in open water consistently stimulated diving. Therefore, this configuration of frequency, amplitude and stimulus source distance was used.

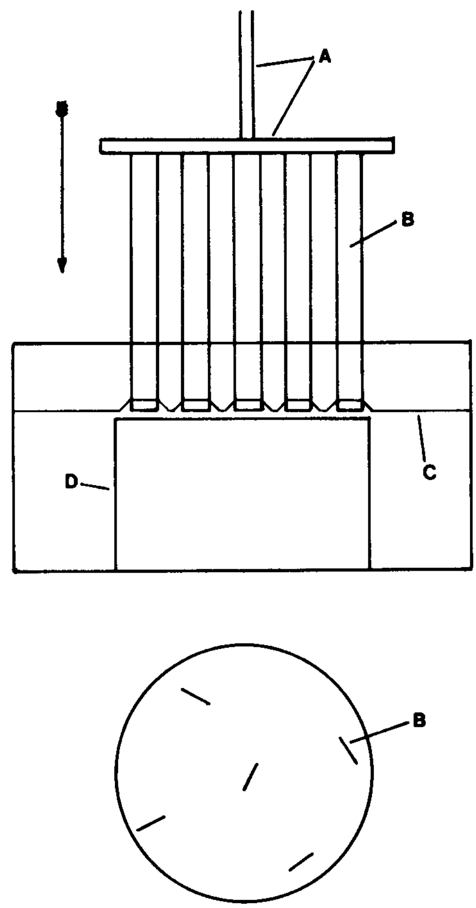

Fig. 1. Device for producing concave menisci with and without submerged vertical surfaces. As shown, concave menisci are present without submerged vertical surfaces. Concave menisci with submerged vertical surfaces are produced by lowering (in the direction of the arrow) the tongue depressors to the bottom of the observation tank. The lower diagram shows the tongue depressor arrangement. A, tongue depressor support; B, tongue depressor; C, water level; D, observation tank within accessory tank.

Data were analyzed according to methods outlined in Sokal and Rohlf (1969). Chi-square tests were used when observations could be tested against predicted values. One-way analysis of variance was used for the remaining data.

RESULTS

Table 1 summarizes the resting preferences of pupae in concave menisci versus no menisci. Pupae of all species studied rest significantly more often near the edge when a concave meniscus is available.

Table 2 summarizes the spontaneous diving rates in open water and concave menisci. Pupae resting in open water dive significantly more often than do those resting in concave menisci.

Observed surfacing in the "edge" area versus the "open water" area was compared to a predicted binomial frequency distribution based on the assumption that the location of a given surfacing act is random (Fig. 2). Thus the probability of a pupa surfacing in open water would be 0.88 (the relative surface area of "open water"), while the probability of surfacing within 3 mm of the edge would be 0.12 (the relative area of "edge"). When evaluated by the chi-square method all species studied differed significantly from the predicted frequency distribution ($P < 0.005$) and tended to surface more often within 3 mm of the edge than was predicted.

The resting preferences of pupae in the presence or absence of submerged vertical surfaces under concave menisci are summarized in Table 3. Both species tested rested significantly more

Table 1. Number (± SD) of pupae out of 10 resting along tank edge: concave meniscus vs. no meniscus. Twelve trials were run for each specimen.

Species	Meniscus present	Meniscus absent	F_s
Ae. aegypti	9.75 ± 0.43	5.00 ± 2.12	52.95***
Ae. triseriatus	7.67 ± 1.03	3.66 ± 1.03	83.37***
Cx. restuans	8.92 ± 1.04	4.75 ± 1.30	69.43***

*** = significant difference at 0.001 level with 1 and 22 degrees of freedom.

Table 2. Rate of spontaneous diving per 3 minute interval: concave meniscus vs. open water. Fifteen different pupae of each species were tested.

	No. diving ± SD		
Species	Open water	Concave meniscus	F_s
Ae. aegypti	4.27 ± 2.14	0.20 ± 0.40	48.7***
Ae. triseriatus	5.27 ± 4.39	2.20 ± 2.97	4.7*
Cx. restuans	7.87 ± 5.94	1.27 ± 2.57	14.6**

*, **, *** = significantly different at 0.05, 0.005, and 0.001 levels respectively at 1 and 28 degrees of freedom.

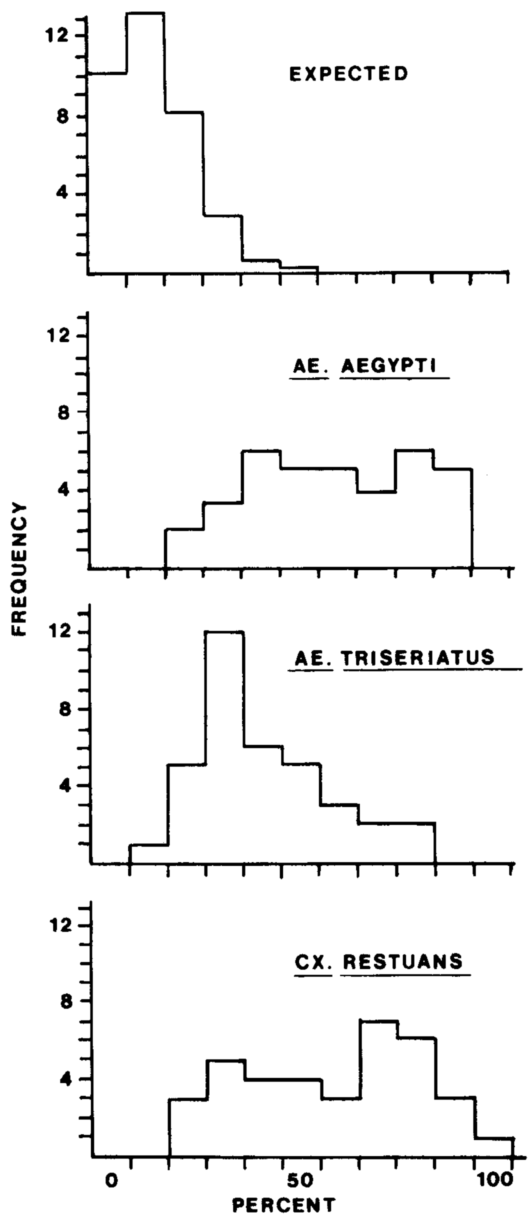

Fig. 2. Frequency (out of 10 consecutive dives) of surfacing within 3.0 mm of the edge of the observation tank: expected versus observed. Thirty-six pupae of each species were tested. ($P < 0.005$ for all species).

often in concave menisci when a submerged vertical surface was below each meniscus than when no vertical surface was below each meniscus.

Comparison of data from Fig. 2 and Table 1 provides further evidence that resting in a concave meniscus is associated with a decreased diving frequency (Table 4). Recall that for the data in Table 1, resting site was not recorded for a given trial until all 10 pupae had ceased diving. Resting site preference with no meniscus is not significantly different from surfacing location preference, indicating that the probability of diving from a resting site in open water versus edge water with no meniscus is the same. However, when a meniscus is present, pupae tend to rest more frequently within 3 mm of the edge than is suggested by the frequency of surfacing within 3 mm of the edge, indicating that the rate of spontaneous diving from concave menisci is less than from flat-surfaced, open water.

Mean percent response to a mechanical stimulus among pupae resting in open water was 57.5 (± 34, SD; range 0–100). This value was significantly greater than the 25.8 (± 38; 0–100) mean percent response of pupae resting in a concave meniscus ($t = 3.027$, using arcsine transformed data; 46 degrees of freedom; $P \leq 0.01$).

DISCUSSION

Bates' (1949) suggestion that pupae are pulled into resting positions along emergent vegetation due to surface tension forces is reasonable since pupae are positively buoyant and would therefore tend to float passively to the highest water level. We have provided evidence that pupae also tend to terminate diving after contacting submerged vertical surfaces, increasing the probability of surfacing in concave menisci. This behavior, along with a decreased rate of spontaneous diving when resting in concave menisci, results in pupae tending to "find" and rest in concave menisci associated with emergent vertical surfaces. Such behavior fits the description of "object orientation" (Jander 1975), which involves two major orientation phases, search and approach (or avoidance). The search phase is based upon a motile organism having insufficient information about the spatial location of a resource and involves straight ongoing movements (transecting) or continual erratic turning movements (ranging) until the proximity of the resource is sensed. The approach phase is based upon precise information regarding the spatial location of a resource and typically involves a straight course guided by stimuli that pinpoint the resource. In mosquito pupae the spontaneous dives in open water would constitute the search phase, while ascent along a vertical surface would be the approach phase.

Table 3. Number (±SD) of pupae out of 10 resting in concave menisci: submerged vs. unsubmerged tongue blades. Twelve trials were run for each species.

	Tongue depressors		
Species	Submerged	Unsubmerged	F_s
Ae. aegypti	4.50 ± 1.80	1.67 ± 1.70	14.48***
Cx. restuans	7.25 ± 1.74	5.00 ± 1.68	9.51***

*** = significantly different at 0.001 level with 1 and 22 degrees of freedom.

Our results indicate that pupae are less responsive to mechanical stimuli when resting in a concave meniscus. This may be due to contact inhibition of diving, possibly due in turn to stimulation of mechanosensitive setae pressed against a vertical surface. McIver and Siemicki (1981) present ultrastructural evidence that several setae on the thorax and abdomen of *Ae. aegypti* are mechanosensilla and suggest that probably all body setae are mechanosensory. Another possibility is that the body orientation of a pupa resting in a concave meniscus may be such that sensilla sensitive to mechanical stimuli are not in a position to be stimulated. Still another possibility is an inhibitory influence due to differences in visual input in a meniscus versus open water. A combination of all these factors may be operating.

When considering the adaptive significance of pupal behavior, two factors are of particular importance, avoiding predation and conserving energy. Many organisms are known to prey on mosquito larvae and pupae (Chapman 1985). The following expresses the significance of energy conservation: "Since pupal mosquitoes do not feed, the time spent in this stage constitutes a considerable drain on the energy stores accumulated during the larval stage. In addition to the energy expended in maintenance of the life processes, energy must be expended during the profound tissue reorganization taking place. Further, mosquito pupae, unlike the pupae of most other holometabolous insects, are highly

Table 4. Frequency (out of 10 consecutive dives; $\bar{X}$ ± SD) of surfacing within 3 mm of tank edge versus number of pupae (out of 10) resting along edge with and without a concave meniscus.

Species	Surfacing[a]	Resting[b]	F_s	Surfacing[a]	Resting[b]	F_s
Ae. aegypti	5.92 ± 2.12	5.00 ± 2.21	0.23 ns	5.92 ± 2.12	9.75 ± 0.43	37.32***
Ae. triseriatus	4.00 ± 1.73	3.66 ± 1.03	0.38 ns	4.00 ± 1.73	7.67 ± 1.03	46.12***
Cx. restuans	5.81 ± 2.27	4.75 ± 1.30	2.24 ns	5.81 ± 2.27	8.92 ± 1.04	20.18***

*** = significantly different at 0.001 level with 1 and 46 degrees of freedom.

[a] Derived from data depicted in Fig. 2.

[b] Data from Table 1.

motile and, depending upon circumstances, may be quite active. Given these categories of energy drain and realizing that the newly emerged adult mosquito must fly to a sugar or blood source before there can be any renewal of energy stores, any energy-conserving mechanism during the pupal period would have survival value." (Romoser 1975).

The evidence presented in this paper indicates that diving ultimately promotes resting, in particular in concave menisci against emergent vertical surfaces. Furthermore, pupae in this situation are less responsive to mechanical stimuli and probably also to visual stimuli since their field of view would be somewhat attenuated by the emergent surface. Any behavior which promotes resting in mosquito pupae may be viewed as energy conserving. It is likely that pupae resting in concave menisci are less visible and accessible to predators.

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