

Function of the dendritic setae in *Aedes aegypti* mosquito pupae: float hairs don't float

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Purpose: Pupal behavior varies with buoyancy, which changes spontaneously at the water's surface and during diving. This behavioral regulation is energy conserving, which is a critical need in the nonfeeding but highly motile pupa. Although adult structures are apparent, there are uniquely pupal structures, including setae (most or all mechanoreceptors), ranging from hair-like to the complex, moveable, bilateral "dendritic setae" on the first abdominal tergum. Our aim has been to elucidate the function of the dendritic setae ("float hairs" in earlier studies). Based on the position and shape of dendritic setae plus ultrastructural evidence of mechanoreception, we hypothesized a buoyancy-sensing function related to bending of the setae as water currents flow over them during descent and ascent.

Methods: Using *Aedes aegypti*, we checked to see whether the dendritic setae are hydrophilic and tested the behavioral effects of their removal. In a preliminary closed-system experiment, a pupa was placed in water in a test tube and a syringe attached in continuity with the air space above the water. When pressure was increased by depressing the plunger, a pupa responded by swimming toward the surface. When negative pressure was applied by lifting the plunger, a pupa actively dove. Using a more sophisticated apparatus with a pressure transducer, we tested the effects of dendritic setae removal on behavior in response to pressure changes.

Results: The cuticular surface of the dendritic setae is hydrophilic. No significant differences were found in pupae with or without the dendritic setae relative to dive duration, applied pressure duration, or maximum pressure applied, but response time to pressure change in pupae (males and females) without the setae was significantly increased.

Conclusion: Hydrophilic dendritic seta cuticle is consistent with our hypothesis but not with a floatation function. Ablation experiments supported our hypothesis that the dendritic setae are involved with buoyancy sensing by bending in response to directional water currents.

Keywords: mosquito, pupa, dendritic setae, diving behavior

Introduction

During the pupal stage, the worm-like, aquatic, microplankton-feeding mosquito larva is transformed into the terrestrial-aerial, nectar- and blood-feeding (female) adult. Mosquito pupae are worthy subjects for study because they are a neglected stage in a very important family of insects and may be a weak link in the mosquito life-cycle.¹ Because they do not feed or reproduce, they display comparatively simple behavior patterns, making them ideal for behavioral/sensory studies.

Diving behavior in mosquito pupae varies in response to variations in buoyancy that can occur spontaneously at the surface and during diving when buoyancy is affected by several factors, including depth, time submerged, and temperature.² Lucas and Romoser³ provide evidence that this behavioral regulation is energy conserving,

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which is a critical need in the nonfeeding but highly motile pupa. When pupae are submerged, their buoyancy gradually decreases, and they eventually reach a state of neutral buoyancy at a given depth and “hover” briefly. They may then become negatively buoyant and begin to sink passively to the point where they are able to rest on the bottom, or they may actively swim to the surface.^{2,4} This behavior may be characteristic of many container-breeding mosquitoes as an adaptation that enables them to remain near the bottom of a container in response to mechanical stimuli at the water’s surface, such as raindrops. If they remained near or at the water’s surface during heavy rainfall, they could be washed out of the overflowing container.³ This hypothesis has recently been supported experimentally by Koenraadt and Harrington.⁵ Diving behavior may also minimize exposure to potentially lethal hits by heavy raindrops, which could easily disrupt the hydrostatic balance that depends on the integrity and spatial continuity of the ventral air space gas.¹

In association with the significant increase in buoyancy caused by the inflation of the adult tracheae, the appearance of air in the exuvial space, and inflation of the posterior midgut with air,⁶ pupal diving behavior changes dramatically with approaching pupal–adult ecdysis and is characterized by very shallow, brief dives and no hovering or bottom sitting.

A simple preliminary experiment showed that if prior to the buoyancy increase associated with pupal–adult ecdysis a pupa is placed in water in a closed system and a syringe is attached in continuity with the air space above the water, the air pressure can be changed at the will of the researcher by depressing (positive pressure) or lifting (negative pressure) the plunger. These pressure changes are translated hydraulically (the Cartesian diver effect)⁷ to the pupal gas spaces (tracheal system and ventral air space) and cause changes in buoyancy to which a diving pupa responds. When pressure is increased by depressing the plunger, the pupa responds by actively swimming toward the surface. When pressure is decreased in the air space, ie, negative pressure is applied, a pupa responds by actively diving. Such behavior in a closed system is additional evidence that pupae sense their state of buoyancy and behave accordingly.

Although adult mosquito structures are clearly evident at various stages of development in the pupal stage, there are also several uniquely pupal structures. These include the ventral air space, an external cavity that is filled with gas at pupation and that enables a pupa to be positively buoyant at the water’s surface.⁸ Other uniquely pupal structures include the respiratory trumpets, paddles at the posterior end of the abdomen, and a variety of setae. Among the

setae are simple, hair-like structures located on the dorsal, ventral, and pleural regions of the abdominal segments; small setae that project from the distal edges of the paddles; and the dendritic setae on the dorsum of the first abdominal segment. Based on ultrastructural studies, most, if not all, of these setae are mechanoreceptors.⁹ The positions of the various setae are suggestive of their function. For example, the lateral segmental abdominal setae (Figure 1A) probably provides information with regard to lateral body contact. Such contact is associated with a decrease in spontaneous diving behavior, thus enabling a pupa to rest in a relatively safe location.¹⁰ Another example is the small seta at the distal end of each of the paddles (Figure 1A), which probably provides information regarding contact with the bottom and facilitates bottom sitting. Further, as a pupa bottom sits, its buoyancy continues to decrease, and increasing numbers of setae on the abdominal terga come into contact with the bottom, suggesting a proprioceptive tactile function.

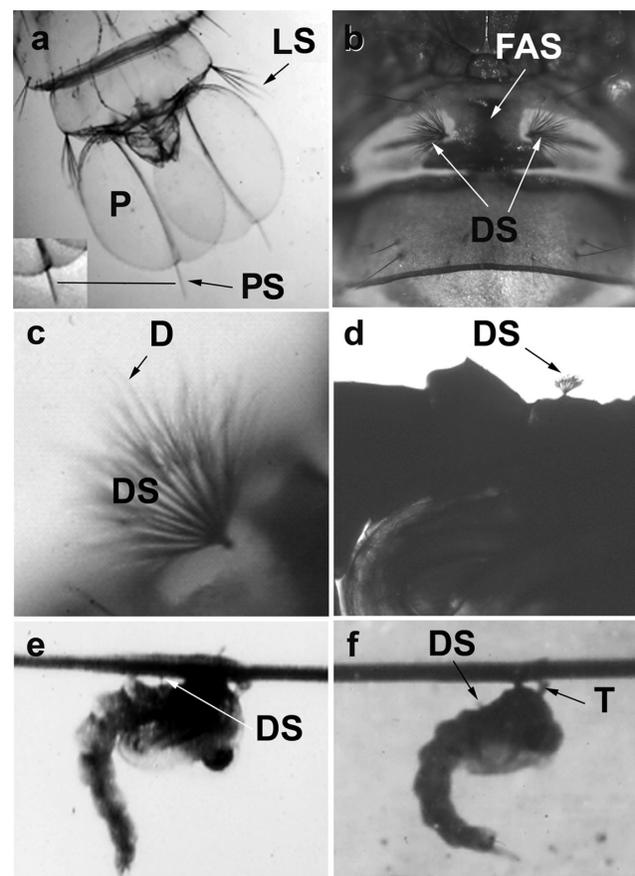


Figure 1 A) Ventral view of pupal paddles (high-contrast insert). B) Dorsal view in the region of the first abdominal segment. C) High magnification of dendritic seta. D) Lateral view of a backlit pupa showing the position of the dendritic setae on the body. E) Lateral view of pupa with the dendritic setae against the water’s surface. F) Lateral view of pupa with the dendritic setae out of contact.

Abbreviations: D, digit of dendritic seta; DS, dendritic setae; FAS, dorsum of first abdominal segment; LS, lateral seta; P, paddle; PS, paddle seta; T, respiratory trumpet.

Our specific aim has been to elucidate the function of the dendritic setae (sometimes called “float hairs” or “dendritic tufts”).¹¹ These paired, palmate, branching structures (seta 1-I, <http://mosquito-taxonomic-inventory.info/>) are attached by a single stalk to the first abdominal segment, one on either side of the dorsal midline (Figures 1B–D). Each of these setae is moveable at the base in an up and down direction and has a well-defined basal cuticular hinge. As suggested by the first listed name, some earlier investigators viewed these setae as flotation aids that enable a pupa to adhere at the water’s surface. Because the research reported here provides evidence that counters a flotation function, we will use the current terminology, “dendritic setae”, and refer to individual hairs fanning out from the stalk as “digits”.

Given that pupae respond to buoyancy changes, the shape and bilateral positioning of the paired dendritic setae on the dorsum of the first abdominal segment, the fact that these setae are often removed from proximity to the air–water interface (Figures 1E and F), and structural evidence that they are mechanoreceptors,⁹ we hypothesized that these structures bend in up and down directions and thereby provide information about buoyancy. That is, the dendritic setae respond to currents of water that flow over the pupa’s body as it descends (actively dives or passively sinks) or ascends (swims or floats to the surface).² Consistent with this hypothesis, the setae would be expected to be hydrophilic, as opposed to hydrophobic, which would be required to gain purchase at the water’s surface.

The objective of our research has been to test the buoyancy sensing hypotheses by determining i) whether the surface of the dendritic setae is hydrophilic or hydrophobic and ii) whether the dendritic setae provide information regarding directional (up and down) flow of water and thereby sensation of the state of buoyancy.

Materials and methods

Mosquito rearing

Aedes aegypti, the yellow fever mosquito, was studied. Rockefeller strain eggs were obtained from the US Army Medical Research Institute of Infectious Diseases (Fort Detrick, Frederick, MD, USA). Larvae and pupae were raised in a 16-hour light and 8-hour dark cycle at $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in 22×32 cm plastic pans containing 1000 mL of water. Larvae were fed a mixture (1:1:1) of ground lab chow, liver powder, and live brewer’s yeast. Approximately 100 mg of food was added daily to each pan of 150–160 larvae.

Experiments

All experiments were conducted at ambient room temperature, $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$. To determine whether the surface of the dendritic setae was hydrophilic or hydrophobic, eight setae from pupae of approximately the same age were tested. Using fine point tweezers, setae were plucked by their stalks while the pupa was held in place with another set of tweezers. Each seta was then subjected to the following two simple protocols. First, each seta was placed on the surface of a droplet of distilled water where it remained due to surface tension and its small size. A second drop of water was then added over the seta. Second, each seta was introduced to the surface of water in a test tube and then forced beneath the surface with fine tweezers. Setae that were submerged in this fashion were then carefully and slowly removed by their bases.

To test the effect of removal of the dendritic setae on diving behavior, individual pupae that did not yet show the darkening and buoyancy increase associated with impending ecdysis were placed in a closed-system apparatus, which was more sophisticated but similar to that described previously. This apparatus (Figure 2) consisted of a Kanetec[®] magnetic ringstand (Kanetec USA Corp., Bensenville, IL, USA), a test tube clamp, a Becton Dickinson and Company (Franklin Lakes, NJ, USA) 60 mL disposable Luer-lock syringe, and a pressure transducer connected via a transbridge to a Gateway[®] (Gateway, Inc., Irvine, CA, USA) equipped with the AcqKnowledge[®] data acquisition system for Windows 3.01 (BIOPAC Systems, Inc., Goleta, CA).

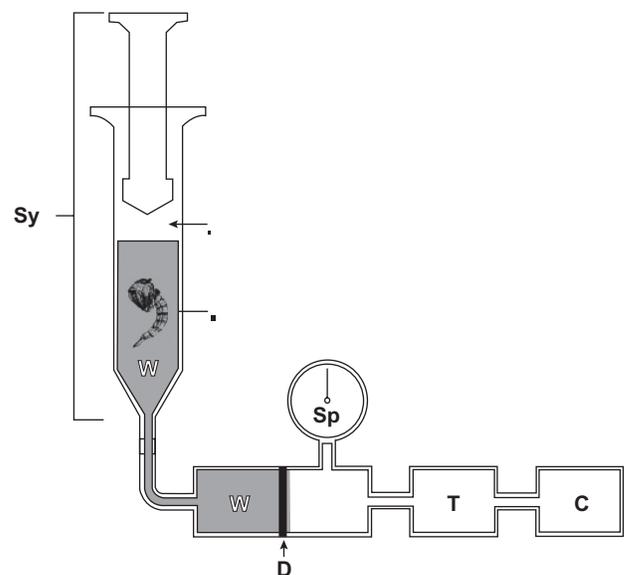


Figure 2 Experimental apparatus.

Abbreviations: C, computer; D, diaphragm; P, pupa; Sp, sphygmomanometer; Sy, syringe; T, transbridge; W, water.

Pressure changes brought about by depressing the syringe plunger were translated hydraulically through the water to a flexible diaphragm, which transduced the pressure changes to the air chamber on the other side of the diaphragm. The pressure changes in this air chamber were then transduced to voltage changes by the transbridge. To determine the voltage associated with a given pressure, the system was calibrated by attaching a sphygmomanometer to the pressure transducer.

Pupae were observed singly by placing them in the body of the syringe, which was about 75% filled with distilled water (at room temperature), leaving an air space above the water's surface. A pupa was stimulated to dive by tapping on the side of the syringe, and when it reached the state of hover (neutral buoyancy), the syringe air pressure was increased by continuing to slowly depress the plunger.

Two experimental groups were tested: i) pupae with the dendritic setae intact (80 males and 80 females) and ii) pupae from which the setae had been carefully removed with finely sharpened watchmakers forceps (65 males and 85 females). The following parameters were measured: i) dive duration, time from dive initiation to resurfacing, ii) pressure duration, the interval of time between application of pressure change and resurfacing, iii) maximum pressure applied during a given test, and iv) response time, the interval between the initiation of the pressure change and the first indication of active diving. For each test, effort was made to apply the same amount of pressure for the same interval of time.

Experiments involving the application of negative pressure were not carried out, because the application of negative pressure by lifting the syringe cylinder expanded the gases in the pupal spaces, resulting in passive floatation to the water's surface, which made it difficult to obtain valid measurements.

Statistical analysis

Data were analyzed using a Microsoft Excel 2000 data analysis tool. In addition to the generation of descriptive statistics,

hypothesis testing involved Levene's Test for Equality of Variances and Student's *t*-test. Because the pressure in the closed system was manually applied by the researcher, there was variation in the maximum pressure. In order to minimize this variation, we picked from each experimental group the 30 individual pupae tested that had been exposed to the most similar maximum pressures.

Results

Cuticular surface of the dendritic setae

When the dendritic setae were placed on the surface of a water droplet, they remained there due to surface tension, but when the second drop was added, the setae became submerged, did not float to the surface, the digits fanned out, and no adherent water bubbles were seen. The fanning out of the digits is shown in Figures 1B and C in which a submerged pupa was photographed. When setae were forced to submerge, they behaved in the same way as in the first experiment. When each seta was removed very slowly and carefully, water adhered and rose slightly above the otherwise flat surface. The adherent water then quickly broke away as the water molecules gradually released their adhesive grip on the cuticle surface.

Seta ablation experiments

Using *t*-tests, dive duration, duration of applied pressure, maximum pressure applied, and pupal response time in females and males with and without dendritic setae (surgically removed) were compared. No significant differences were found in dive duration, pressure duration, or maximum pressure applied, but, in both females and males, response time was increased significantly by the removal of the dendritic setae (Table 1; Figure 3).

Discussion

The results of the experiments with individual dendritic setae removed indicate that the cuticular surface of the dendritic

Table 1 Measured parameters (mean \pm standard error) in females and males with dendritic setae present and setae surgically removed (absent)

	Dive duration (sec)	Pressure duration (sec)	Maximum pressure (mm Hg)	Response time (sec)
Females				
Present	15.45 \pm 0.64	9.96 \pm 0.42	337.9 \pm 3.64	2.86 \pm 0.17
Absent	14.9 \pm 0.91	9.97 \pm 0.59	341 \pm 1.8	8.54 \pm 0.18
Males				
Present	15.11 \pm 0.97	9.59 \pm 0.72	334.57 \pm 1.27	2.99 \pm 0.11
Absent	16.05 \pm 0.5	10.6 \pm 0.91	337.03 \pm 2.05	9.01 \pm 0.22

Notes: Females: present versus absent ($t = -22.747$, 58 degrees of freedom, $P < 0.01$). Males: present versus absent ($t = -24.694$, 58 degrees of freedom, $P < 0.01$). No significant differences were found with regard to the other parameters.

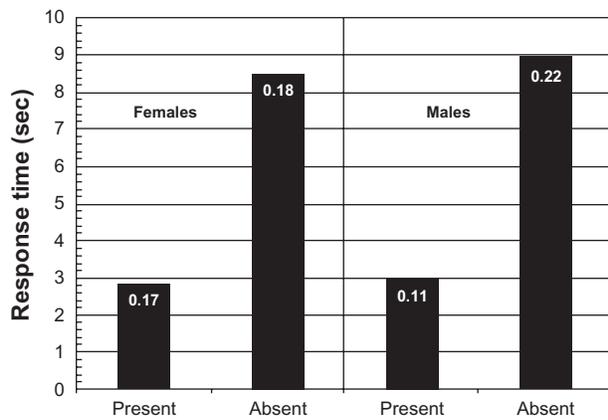


Figure 3 Mean response time in females and males with dendritic setae present versus setae surgically removed (absent).

Note: Standard error for each mean indicated in white on bars.

setae is hydrophilic (wetttable). Otherwise, if setae were unwettable, the digits would have become clumped. The fact that water adhered to the setae when they were carefully withdrawn from the water indicates that the bipolar water molecules are attracted to the cuticular surface of the hairs, which is further evidence that the surface is hydrophilic.

The results of the ablation experiments support our hypothesis that the dendritic setae are involved with sensing the state of buoyancy by bending in response to up or down water currents generated as a pupa descends or ascends. The fact that pupae respond to pressure changes even with the hairs removed suggests that other sensory structures are also involved and/or that hair bases remaining after removal still provide sufficient information to elicit a response. It is possible that in addition to simply providing information about up or down bending, the dendritic setae also provide information about the extent of bending and thereby information about the degree of buoyancy.

Our results provide a strong rationale for using the neutral term “dendritic setae” instead of the term “float hairs”, which inaccurately describes their function. When these hairs do contact the surface, it is because the ventral air space gas pushes the abdomen upwards, and the respiratory trumpets, which provide attachment at the air–water

interface (Figures 1E and F), act as hinges.¹² Given the periodic contact between the dendritic seta and the underside of the water surface film relative to spontaneous variations in buoyancy (Figures 1E and F), these setae probably also provide information about buoyancy when the pupa is at rest. The dendritic setae probably also sense surface contact at the end of a pupa’s ascent and are thereby involved with inhibiting any further swimming motion.

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Disclosure

The authors report no conflicts of interest in this work.

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