

Fine Structure of the Sense Organs on the Labella and Labium of the Mosquito *Aedes aegypti* (L.)

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Abstract: Fine structure of the sense organs on the labella and labium of male and female mosquito *Aedes aegypti* is described. Labellar hair on the outside of the two labellar lobes are consisted of long mechanoreceptive hairs, medium-sized chemoreceptive hairs containing 3-5 dendrites, and short papillae which are probably olfactory receptors. Two apical hairs each containing five dendrites not reported before are found deeply embedded inside each labellum. They emerged between folds at the tip of the labellum. These and other sensory hairs on the outside of the labella are probably involved in finding a suitable place for feeding after a mosquito has landed on a host. Six anteriorly directed papillae each containing 3-5 dendrites are found on the oral surface of each labellar lobe with no evidence of mechanoreceptors associated with these papillae. These papilla are probably chemosensory and are involved in detecting the food entering the food canal when a mosquito feed on water and other liquid diet such as nectar. A chordotonal organ with two sensory cells is found inside each labellum, and this organ has not been described on mosquito mouthparts before. These chordotonal organs probably function to monitor the spreading and closing of the labellar lobes during feeding. Mosquito spread their labellar lobes when feeding on water and sugar but these lobes are firmly pressed against each other when they feed on blood. Ligular hairs are definitely not sensory because of a lack any dendrites inside these hairs. Labial hairs proximal to the labella are probably mechanoreceptors because only one nerve cell is associated with each hair with nerve terminating at the base of the hair. Based on results from behavioral and functional studies, the function of these sensilla during feeding is described.

Keywords: Mosquito, mouthparts, sensilla, labellum, labium, sense organs, feeding.

INTRODUCTION

Because of the medical and economic importance of mosquitoes, the study of the sense organs and feeding behavior of mosquitoes has attracted the attention of many researchers. Gustatory discrimination after the mosquito has landed on a host may be a function of the tarsal hairs. Tarsal chemosensory hairs have been reported in different species of mosquitoes [1-6]. In probing to find a suitable spot for feeding, the mosquito uses the two labellar lobes located at the tip of the long, gutter-like labium [7-9]. The structure of the labellar sense organs in mosquitoes has been studied using light microscopy (LM) [1-4, 10, 11], scanning electron microscopy (SEM) [12], and transmission electron microscopy (TEM) [5, 13-15]. The limitation of these studies was that only some sense organs were studied, i.e. they were not comprehensive. Behavioral [1-4, 14-18] and electrophysiological studies [5, 12, 15, 19] were carried out to elucidate the function of the labellar hairs. However, functional studies of the labellar hairs were conducted mostly on limited types of hair. A thorough knowledge of the distribution and fine structure of the labellar sense organs is still lacking.

In this study, the structure of the sense organs on the labium of both sexes of *Aedes aegypti* (L.) was studied using

LM, SEM and TEM. These observations are integrated into the present body of knowledge on the sense organs on the mouthparts of mosquitoes in relation to their feeding behavior.

MATERIALS AND METHODS

A culture of *A. aegypti* was maintained in the insectary at 27°C and 65% R.H. with eggs kindly donated by Dr. A. S. West (Department of Biology, Queen's University, Kingston, Ontario, Canada). For LM, heads from newly emerged mosquitoes were preserved in alcoholic Bouin for 48 hours or more, double-embedded in 2% celloidin and paraplast, sectioned at 5 µm, stained with Gomori's trichrome, and mounted in DPX. Burgess and Remple's [20] method for vital methylene blue staining was used to observe innervation of the sensilla. Sections and whole mounts of the labella and labium were examined by conventional and phase contrast microscopy.

For SEM, heads of 1-3-day-old adult mosquitoes were fixed in 5% Formalin. The tip of the labium with the labella was removed from the head using a fine needle. The specimens were dehydrated through a graded series of ethanol, cleared in xylene, air-dried on a glass slide, coated with carbon and gold, and observed with a Cambridge Stereoscan S4 scanning electron microscope.

For TEM, newly emerged mosquitoes were anesthetized with chloroform. The tip of the labium with the labella was removed and fixed in 3% glutaraldehyde and 1% osmium

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tetroxide, following the procedure of Hooper *et al.* [21]. Specimens were embedded in Araldite or Spurr's low viscosity resin. Sections were cut on a Reichert Om-U2 ultramicrotome, mounted on single-hole grids supported with carbon-coated Formar film, stained with uranyl acetate and lead citrate, and examined in a Philips EM 300 electron microscope.

RESULTS

The following description applies to both sexes of *A. aegypti* as the distribution and the structure of the labellar and labial sense organs are similar in both sexes.

The two labellar lobes at the tip of the labium together form a pear-shaped structure (Figs. 1, 3). Each labellum is two segmented. The two segments abut obliquely to each other on the dorsal surface (Fig. 1), and horizontally on the ventral surface (Fig. 3). A ligula covered with hairs projects out between the two labellar lobes (Fig. 5), and contains the

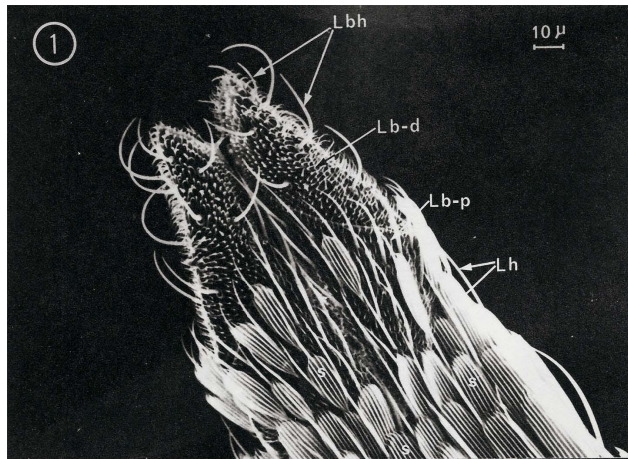


Fig. (1). Dorsal aspect of female *A. aegypti* showing labellar (Lbh) and labial (Lh) hairs. Each labellum is composed of a distal (Lb-d) and a proximal (Lb-p) segment. Scales (s) are found on the proximal labellar segment.

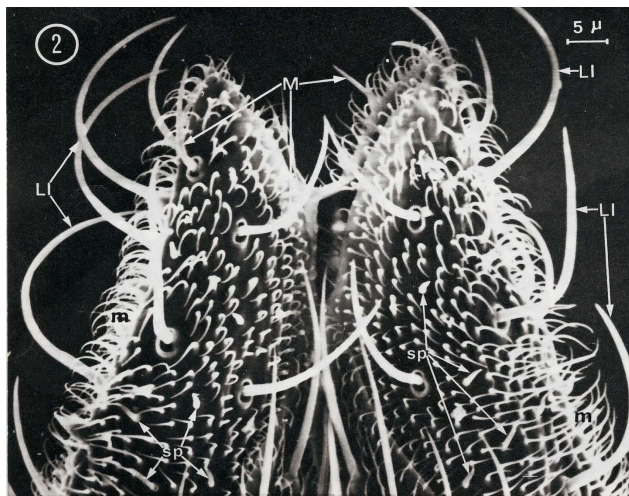


Fig. (2). Same at high magnification showing different types of labellar hairs. LI, long labellar hairs; M, medium-sized hairs; m, microtrichia; sp, short papillae. The short papillae are also socketed at the base.

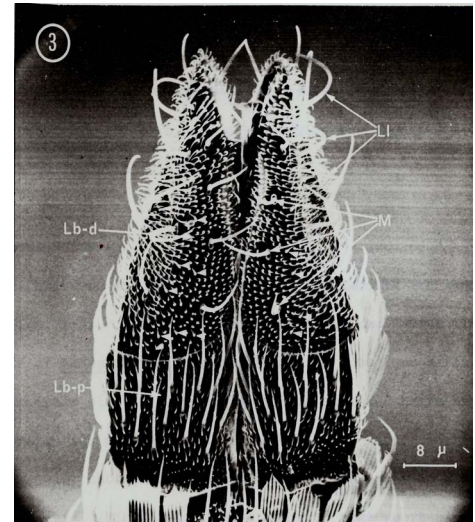


Fig. (3). Ventral aspect of female *A. aegypti* labella, showing the distal (Lb-d) and proximal (Lb-p) segments of the labella are joined transversely. Long labellar hairs (LI), medium-sized hairs (M), and short papillae (arrowheads) are also found here. Note scales are almost absent on the proximal labellar segment.

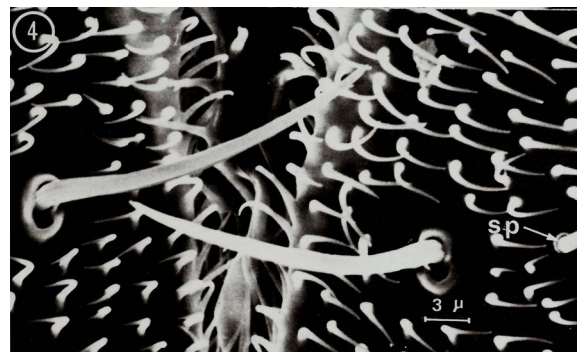


Fig. (4). Same at higher magnification showing hair socket at the base of a short papilla (sp).



Fig. (5). Anterior top aspect of the tip of female *A. aegypti* labium, showing ligula (Lg) situated between the two labellar lobes (Lb). Ligular hairs are not socketed at the base, and have smooth wall. Two apical hairs (Ap) extend out anteriorly through labellar folds at the tip.

tip of the fascicle on its trough-shaped dorsal surface. As the concave inner surfaces of the labella are facing the labral food canal, we will refer to the inner surface of the labellum as the oral surface, and the outer convex surface of the labellum as the aboral surface.

Aboral Hairs

Aboral hairs on the distal segment of the labella are symmetrically arranged (Figs. 1-4). As noted by Frings and Hamrum [2], aboral hairs of *A. aegypti* can be classified into four different types according to their sizes: (1) long, pointed, socketed hairs averaging 40 μm in length, (2) medium-sized, socketed, blunt-tipped hairs between 20-30 μm long, (3) short, blunt, socketed papillae 4-6 μm long and (4) short microtrichia (Figs. 1-4). They reported that short papillae are present only on the dorsal surface of the labella. However, we found that these papillae are also present on the ventral surface (Figs. 3, 4). Hairs on the proximal segment of the labellar lobes are all straight, socketed and have fine tips (Figs. 1, 3, 6).

Long Labellar Hairs

Longitudinal ridges are found on the hair shaft of the long labellar hairs, but only a single cavity is found inside the hair lumen (Figs. 6-9). Each longitudinal ridge is finely scalloped on its surface (Figs. 7, 8). A finely granulated substance is present inside the hair lumen. There is no evidence of any dendrites inside the lumen. Near the tip of the hair shaft, the lumen becomes smaller (Fig. 7), and it is very likely that these hairs do not have any opening to the outside. Crystal violet [22] did not stain the tips of these hairs indicating no opening at the tip. Structurally, these hairs are very similar to the thick-walled hairs found on the antennal flagel-

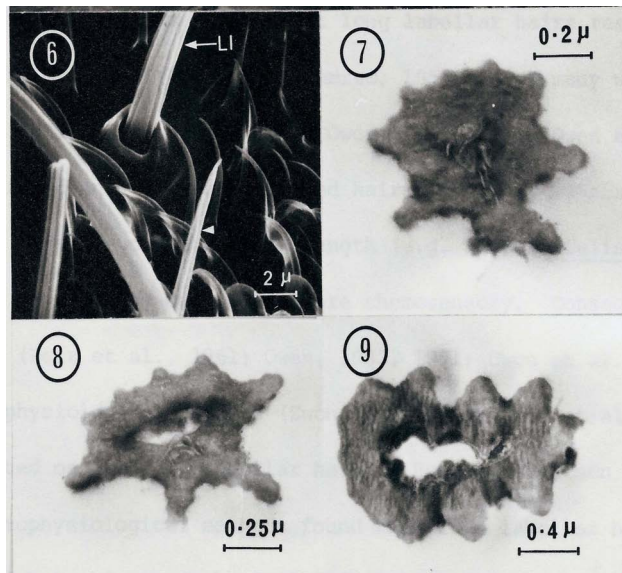


Fig. (6). Long labellar hair (L1) of male *A. aegypti* showing longitudinal ridges on the hair, and sharp pointed tip (arrowhead) of labial hair.

Figs. (7-9). Transverse sections of long labellar hairs of female *A. aegypti* Near the tip (Fig. 7), longitudinal ridges appear as points of a star. Proximal to the tip (Fig. 8) and near the base (Fig. 9), a single lumen appeared. Note the absence of dendrites inside the lumen.

lum of *A. aegypti* described by Slifer and Sekhon [23]. A mechanoreceptive dendrite is found at the base of the long labellar hairs (Fig. 10). Whether this dendrite is attached to the hair base, or enters into the hair lumen for a short distance is unclear.

Medium-Sized Hairs

These hairs are situated near the tip and on the dorsal and ventral aspects of the aboral surfaces of the labellar lobes. They are longitudinally grooved on the outside, and double-chambered inside. Three to five dendrites are present in one of the two chambers (Figs. 11-13). In some hairs containing three dendrites, three to four other dark, dendrite-like structures can be seen (Fig. 12).

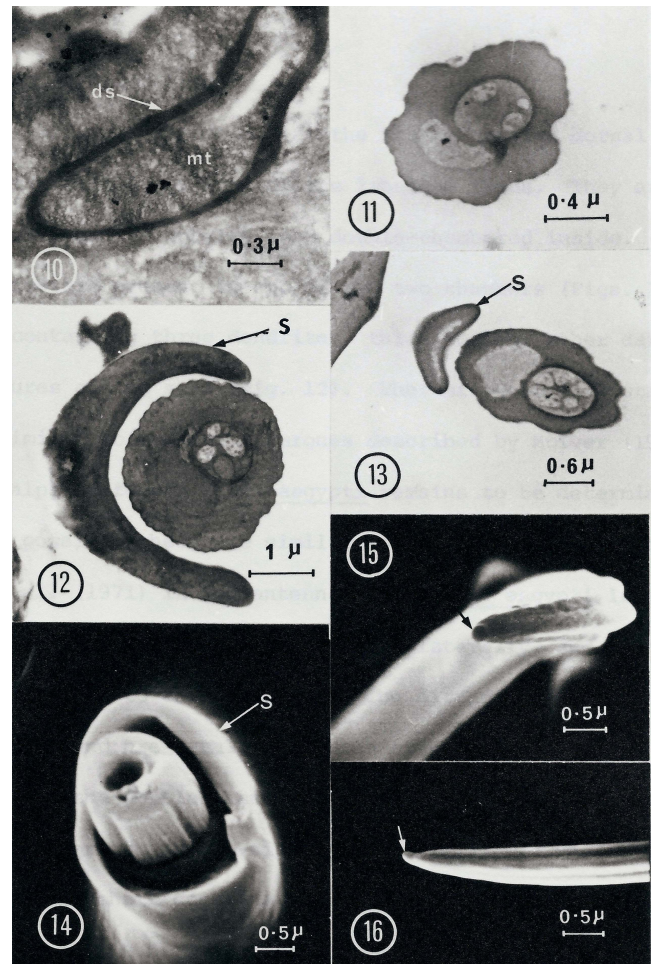


Fig. (10). Section near the base of a long labellar hair of female *A. aegypti* showing microtubules (mt) of the mechanoreceptive dendrite enclosed by a dendritic sheath (ds).

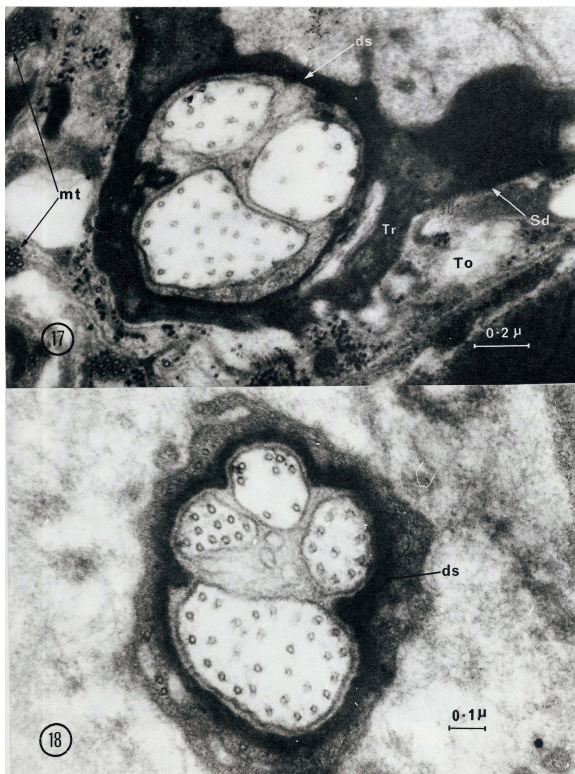
Figs. (11-13). Transverse sections of medium-sized labellar hairs of female *A. aegypti*. Each hair is double-chambered, with one chamber containing dendrites and the other a liquid with fine granules. Three to five dendrites are found in the circular chamber. S, hair socket.

Fig. (14). A medium-sized labellar hair broken near the base of a male *A. aegypti* labellum showing longitudinal ridges on the hair shaft and two chambers inside the hair shaft. S, hair socket.

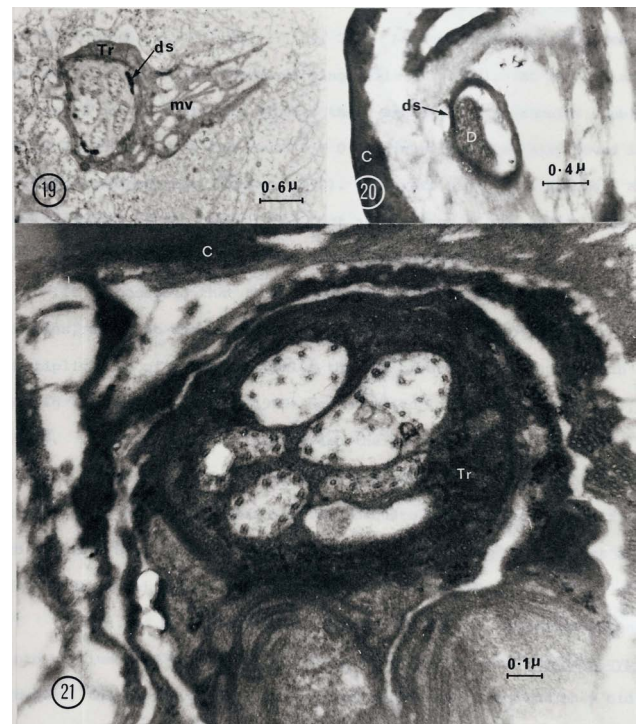
Figs. (15 and 16). Medium-sized labellar hairs of a female *A. aegypti* showing a drop of an unknown substance at the tip of the hair.

With SEM, the double-chambered structure of the hair shaft can also be seen in broken medium-sized hairs (Fig. 14). The dendrite-free lumen of the hair shaft contains remnants of the trichogen cell. A substance is found at the tip of some medium-sized hairs (Figs. 15, 16), which might be similar to the viscous droplets reported on the labellar and tarsal hairs of blowfly and stablefly [24, 25]. It is possible that this fluid is secreted through the dendrite-free chamber of the hair shaft.

At the base of the medium-sized hairs, three to five dendrites are found inside the dendritic sheath (Figs. 17-21). The dendritic sheath is surrounded by the trichogen cell, the latter in turn is enveloped by the tormogen cell (Figs. 17, 19, 21). Septate-desmosomes are found at the junction of the two enveloping cells (Fig. 17). At the ciliary region of one dendrite, it appears that there are 9 + 1 microtubular doublets (Fig. 18), instead of the usual 9 + 0 configuration generally found in insect chemoreceptors [26]. However, because some doublets at the periphery of the dendrite are not as distinct as the central one, it is difficult to interpret the micrograph with certainty. It is possible that this was due to the branching of the microtubules, and that one of the doublets was displaced into the center. Vesicles are found in between the dendrites, and microtubules are present in the extension of the trichogen cell that encloses the dendritic sheath (Fig. 18). Good fixation for mosquito labellar hairs is difficult to obtain. Similar difficulty was also encountered by Stürckow *et al.* [25] in studying the labellar hairs of the blowflies.



Figs. (17 and 18). Transverse section of medium-sized labellar hair sensilla proximal to the hair showing sensilla with three (Fig. 17) and four (Fig. 18) dendrites enclosed by a dendritic sheath (ds). Microtubules (mt) are found inside the trichogen cell (Tr) which enclosed the dendritic sheath and outer tormogen cell (To). Septate desmosomes (Sd) are found where these two cells come into contact.



Figs. (19-21). Transverse section of medium-sized labellar hair sensilla proximal to the hair base with five dendrites. Inside the labellum proximal to the hair socket, dendritic sheath (ds) surrounding the dendrites almost disappeared here (Fig. 19) and microvilli (mv) are found on one side of the trichogen cell (Tr). A mechanoreceptive dendrite (D) enclosed by a dendritic sheath (ds) is present at the base of the hair (Fig. 20). Microtubular doublets are found in some dendrites (Fig. 21). C, surface cuticle.

Oral Papillae

Six anteriorly directed, socketed papillae are found on the concave, oral surface of each labellar lobe (Fig. 22). On the dorsal and ventral oral surfaces, pseudotrachea-like structures are found, and oral papillae are sometimes found in between the microtrichia (Fig. 23). An opening approximately 0.15 μm in diameter is found at the tip of the papilla (Fig. 24). Vital methylene blue staining of the labella showed that these papillae have dendrites entering the lumen which extend to the tip (Fig. 25). TEM sections show that the papillae are double-chambered, with three to five dendrites inside the big chamber. The dendrites are enclosed in a dendritic sheath (Figs. 26-28). Proximal to the base of the papillae, three to five dendrites are enclosed in a dendritic sheath, the latter enveloped by the trichogen and tormogen cells (Figs. 29, 30). We found no evidence of mechanoreceptive dendrite terminating near the base of the papillae. Septate-desmosomes are found between the junction of the trichogen and tormogen cells (Fig. 29).

Chordotonal Organ

Inside each labellum, a chordotonal organ with two sensory cells associated with it is situated close to the oral papillae (Figs. 30, 31). At the ciliary region of the sensory cells, the cells are surrounded by six scolopale rods (Fig. 31). Desmosomes are found between the cell membrane of the

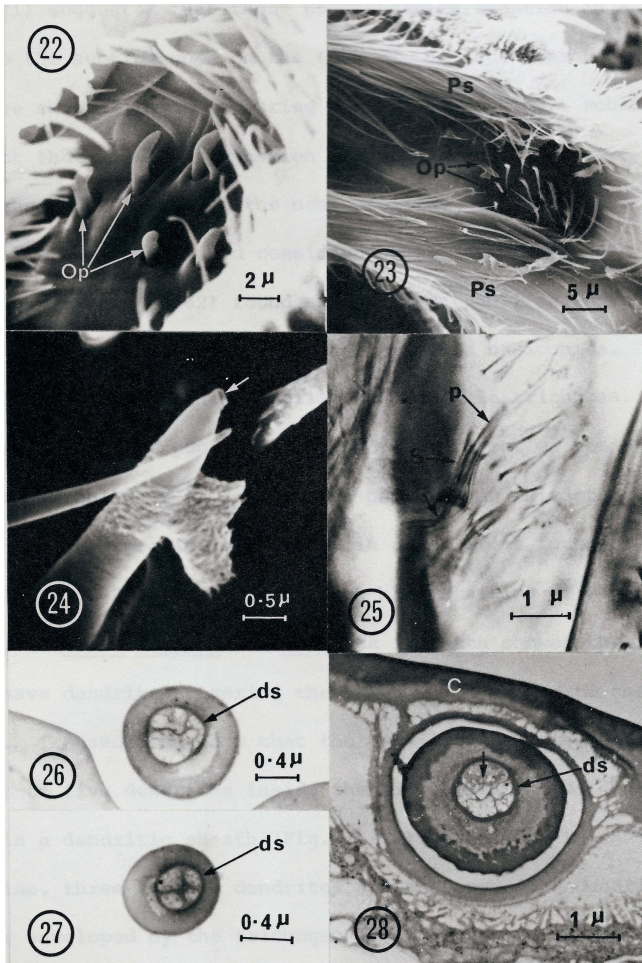


Fig. (22). Oral papillae (Op) on the oral surface of female *A. aegypti* labellum. Note the smooth surface of the papilla.

Fig. (23). Oral papillae (Op), and pseudotrachea-like structure (Ps) on the two lateral oral surfaces of a labellum from a male *A. aegypti*.

Fig. (24). Higher magnification of Fig. 23 showing an opening (arrow) at the tip of an oral papilla.

Fig. (25). Vital methylene blue staining of a female *Culiseta inornata* labellum, showing dendrites entering through hair socket (S) into the lumen of an oral papilla (p). The dendrites are constricted just below the socket (arrow), where the ciliary region of the dendrites is probably located.

Figs. (26-28). Transverse sections of oral papilla showing 3-5 dendrites inside a dendritic sheath (ds). One dendrite near the base (arrow, Fig. 28) is bigger than the others, probably a mechanoreceptive dendrite. C, surface cuticle.

sensory cells, and also between the sensory cell membrane and scolopale rods (Fig. 31). At the distal end of the chordotonal organ, only a single cap is present, which is surrounded by concentric layers of fibrous elements (Fig. 30). We were unable to determine the distal attachment of the chordotonal organ using TEM, because of a lack of serial sections. From LM sections, it seems that the cap is attached to the oral surface, at a region slightly anterior to the oral papillae.

Apical Hairs

Inside each labellum, there are usually two hairs deeply embedded in the lobe. Three apical hairs are found in some

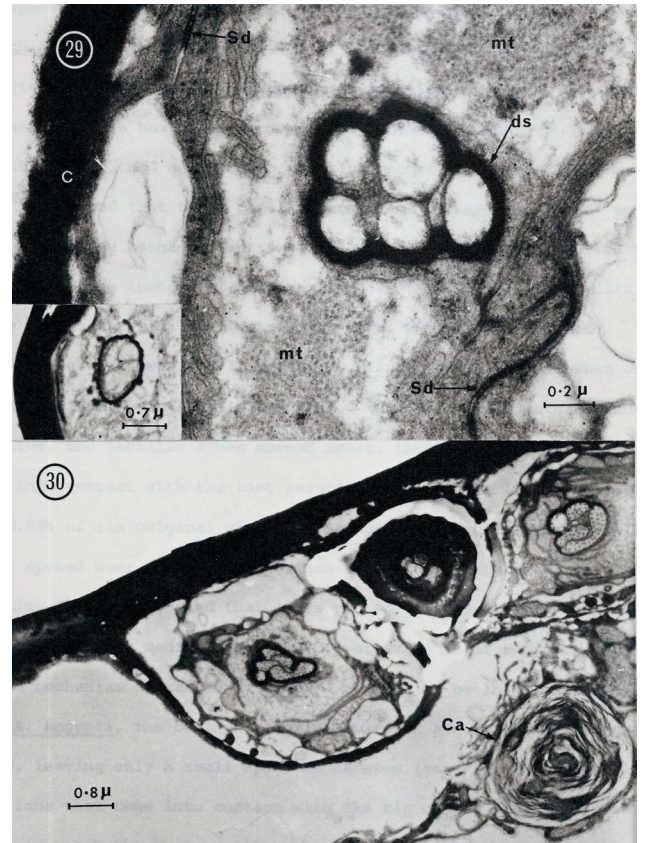


Fig. (29). Transverse section of an oral papilla sensillum proximal to the base in a female *A. aegypti* showing five dendrites. Microtubules are found in the trichogen cell surrounding the dendritic sheath (ds). Septate Desmosomes (Sd) are found at the junction between the trichogen and tormogen cells. C, surface cuticle; mt, microtubules. Inset shows one sensillum with three dendrites.

Fig. (30). Section of a labellum showing three oral papillae with three to five dendrites, and the cap (Ca) of a labellar chordotonal organ.

specimens. These hairs emerge anteriorly through longitudinal "tubes", and project out between the folds at the tip of the labellum (Fig. 5). Near the distal end of the labellum, the two hairs share a common "tube" for a short distance (Fig. 34), but proximally, each hair is enclosed by a separate "tube" (Fig. 35). Vital methylene blue staining showed that these hairs are socketed at the base, with dendrites entering the hair lumen and extending to the tip of the hairs (Fig. 32). TEM sections of these hairs showed that these hairs are double-chambered, with the smaller chamber containing five dendrites (Fig. 33). Near the base of the hair besides the two lumina found at the distal end of the hair shaft, a third lumen appears (Fig. 35). This third lumen is probably the trichogen cell sinus. The dendritic sheath surrounding the dendrites becomes very distinct at this region. Proximal to the hair base, the dendritic sheath is enveloped by trichogen and tormogen cells, and the trichogen cell encloses the trichogen cell sinus (Fig. 36). The axons of these dendrites later join the labial nerve. As the number of dendrites at the hair tip is the same as that proximal to the hair base, and we could not find any evidence of a dendrite ending near the hair base, it is possible these apical hairs do not have a mechanoreceptive

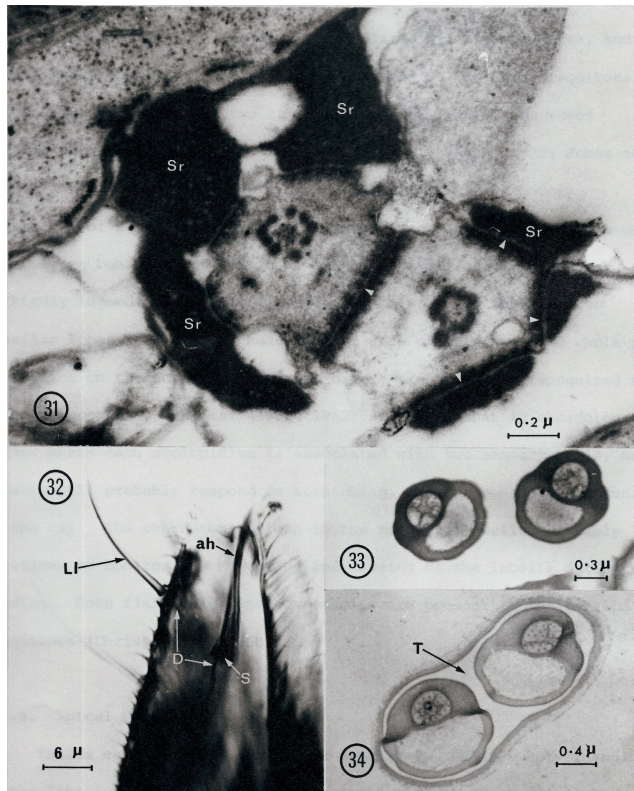


Fig. (31). Transverse section of a labellar chordotonal organ showing electron-dense scolopale rods (Sr) surrounding two sensory neurones. Desmosomes (arrowheads) are found at the junction between the two sensory neurones, and also between the scolopale rods and sensory neurones.

Fig. (32). Vital methylene blue staining showing dendrites (D) leading to the base of a long labellar hair (LI) and an apical hair (ah). S, socket.

Figs. (33 and 34). Transverse section of two apical hairs near the hair tip (Fig. 33) of a labellum and inside the labellum (Fig. 34), showing 4-5 dendrites inside the smaller round chamber. T, a common tubular channel shared by the two sensory hairs.

dendrite ending near the base of the hair. Not all the five dendrites found in the hair shaft extend to the tip of the hair. In some sections at the distal end of the hair, only four dendrites are found (Fig. 34).

Ligular Hairs

Cuticular hair-like projections covering the ligula in *A. aegypti* are not socketed at the base (Fig. 5). Each projection has a single lumen inside, but is devoid of any sensory structure. Transverse sections of the ligula also do not show any nervous tissue inside (data not shown). Therefore a non-sensory function can be assigned to them.

Labial Hairs

Proximal to the labella, the outer surface of the labium is covered with scales, hairs and microtrichia (Figs. 1, 3, 37, 38). Pearson [12] using LM, found that the labial hairs of female *A. aegypti* are innervated. Using vital methylene blue staining, we found that there is one nerve cell associated with each hair with a nerve extending to the base of the hair,

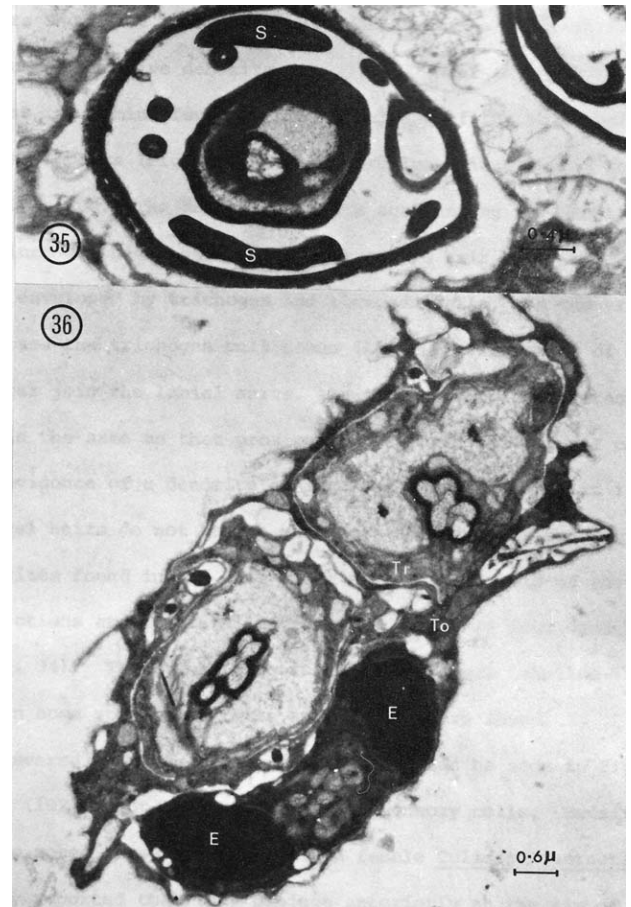


Fig. (35). Transverse section of an apical hair near the hair socket (S). A third lumen appears which is the extension of the trichogen cell sinus. Note five dendrites are found inside.

Fig. (36). Transverse section of two apical hair sensilla proximal to the hair base. Five dendrites are found inside the dendritic sheath enclosed by the trichogen cell (Tr). Two large vacuoles (E) filled with electron dense materials are associated with the tormogen cell (To).

suggesting that these hairs are probably mechanoreceptors. LM sections of the labium showed that these hairs have only a single lumen in the hair shaft (Fig. 37).

At the base of the labium, six to eight long socketed hairs are found on the ventral surface of the labium (Fig. 38). These hairs have sharp, pointed tips, with longitudinal grooves on the outer hair wall (Fig. 39), similar to the long labellar hairs. Whether these hairs are innervated has yet to be studied. However, their external morphology suggests that they are probably mechanoreceptors.

DISCUSSION

In the following, we will discuss the structure of the sensilla found on the labella and labium of *A. aegypti* in relation to previous reports; the probable functions of these receptors in the feeding behavior of mosquitoes are discussed based on the reports of other workers.

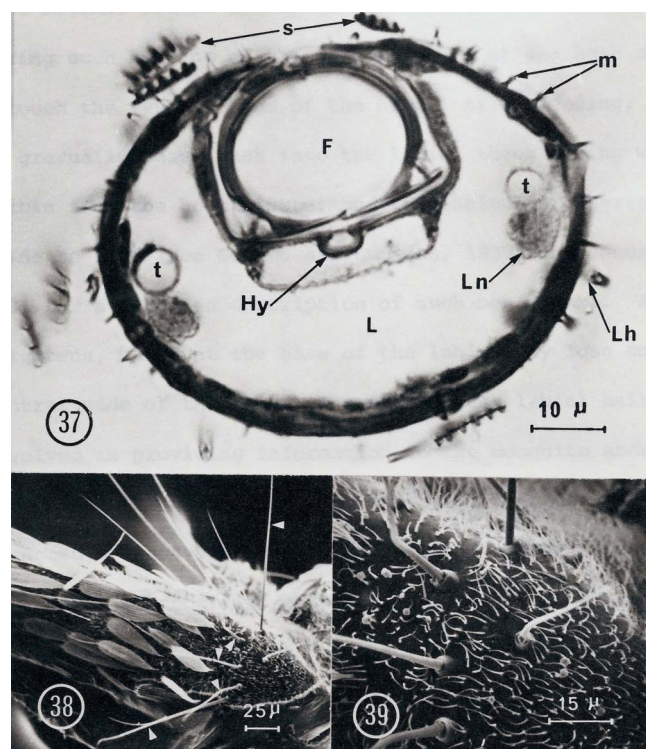


Fig. (37). Transverse section through the labium of a male *A. aegypti* showing the food canal (F) formed by the labrum. Hypopharynx (Hy) containing the salivary duct forms the ventral floor of the food canal. Two tracheal tubes (t) with labial nerve (Ln) close by are situated on the two lateral sides of the lumen (L). Long labellar hair (Lh), microtrichia (m) and scales (s) are found on the labial surface.

Fig. (38). Hairs (arrowheads) at the base of a labium from a male *A. aegypti*.

Fig. (39). Higher magnification of Fig. (38) showing socketed, longitudinally-grooved labial hairs among the microtrichia.

STRUCTURE OF SENSILLA

The labellar lobes of mosquitoes were considered by many workers as important in serving as a guide for the fascicle during piercing and sucking, but Robinson [27] found that mosquitoes with their labella removed were still able to feed on a host quite normally. Therefore he suggested that the labella serve to allow instant return of the stylets to the labial gutter after withdrawal, and that the theca of the labium is important in protecting the fascicle by conserving the fascicular fluid and preventing it from drying. Jones and Piliitt [28] however found that removal of the labella results in the failure of mosquitoes to penetrate the skin, thus showing the importance of the labella as a guide during piercing.

Long Labellar Hairs

Behavioral studies showed that long labellar hairs respond to mechanical stimulation [2]. But many workers [1, 3, 4, 11, 13, 15] have found only double-chambered hairs, and some of these workers have concluded that all aboral hairs beyond a certain length (e.g. 32 μm in *C. inornata* as reported by Owen [3]) are chemosensory. Consequently be-

havioral [1, 3, 4, 15] and electrophysiological studies [15, 19] were conducted on the long labellar hairs. However, Pearson [12] using electrophysiological methods found that long labellar hairs are very sensitive to minute mechanical deflections which normally result in proboscis extension. He also found that it is very difficult to apply a chemical to a long labellar hair without evoking a response from the mechanoreceptor, and cautioned the use of proboscis movement as the criterion for positive response towards chemical stimulation. Our morphological study supports his finding that the long labellar hairs are mechanoceptors.

Medium-Sized Hairs

Chaika and Elizarov [13] reported one to five dendrites ascending into the lumen of the aboral chemosensory hairs in female *A. aegypti*. However, the reproduction of their micrographs was poor, and they did not show any sections of hair shafts containing less than three dendrites. Here we found 3-5 dendrites inside the medium-sized hairs of *A. aegypti*. In female *C. inornata*, Zwonitzer [11] using LM found three to four neurones associated with each aboral hair. Owen *et al.* [15] using TEM found two types of sensory hairs on the aboral surfaces of *C. inornata*: one containing three dendrites and the other with five dendrites proximal to the base of the hairs, but only four dendrites in the hair shaft.

Results from behavioral studies have indicated that mosquito labellar hairs are sensitive to water, sugar solutions and unacceptable compounds [1-4, 15-18]. Electrophysiological studies have also shown that these hairs are stimulated by water, sugar, and NaCl [5, 15, 19]. Our study suggests that such responses may be mediated through the medium-sized hairs, which are double-chambered and innervated. However, many medium-sized hairs are located more proximally on the labellar lobes (Figs. 2, 3), and it is very likely that these hairs will never touch the substrate during probing and piercing. Mosquitoes often spread their labellar lobes when the labellar hairs are stimulated with sugar solutions [1-3, 14] and unacceptable compounds [18]. Such divarication will probably bring only some proximally-located hairs into contact with the substrate, thus raising an interesting question as to the probable function of the more proximally located medium-sized hairs.

Thick-walled chemoreceptors sensitive to strong odors have been reported on the labellar hairs of the stablefly *Stomoxys calcitrans* [29] and on the legs of grasshoppers [30]. Dethier [31] also found that chemoreceptors on the mouthparts and legs of the blowfly *Phorinia regina* that normally respond to aqueous solutions also respond to organic and inorganic acids, and various nonpolar compounds in gaseous state. It is possible that in mosquitoes, the more proximally located medium-sized hairs which do not normally come into contact with the substrate may respond to vapors.

The structure of the short papillae (Figs. 2-4) in *A. aegypti* has yet to be studied. In female *C. inornata*, Zwonitzer [11] using LM called these sensilla basiconica, but was uncertain about the number of neurones associated with each papilla. Because of their small size, it is unlikely that they will get in touch with the host surface during probing by the mosquito. They are probably olfactory receptors. Short microtrichia on the aboral surfaces are clearly not sensory.

Oral Papillae

Vogel [10] had suggested that pseudotrachea on the oral surface of labella function in a manner similar to the suction cups on the toes of the gecko, by providing a strong hold on the skin of the host during biting and sucking. Robinson [27] pointed out that Vogel's suggestion has no backing. Still, Vogel was probably the first to notice the oral papillae in mosquitoes. He called them sensilla basiconica, and considered them to be shortened tactile bristles. Zwonitzer [11] found six of these papillae in female *C. inornata*, and also called them sensilla basiconica. Larsen and Owen [14] referred to these papillae as sensilla trichodea. Since these sense organs are papilla-like, and are present on the oral surfaces of the labella, we call them oral papillae in this study.

In *C. inornata*, Larsen and Owen [14] also found that the oral papillae are double-chambered. Their micrograph showed five dendrites enclosed in a dendritic sheath proximal to the base of the papilla. Pappas and Larsen [5] found 2-5 neurones associated with these papillae in *C. inornata*, but could not study their functions using electrophysiological method because of their small size and inaccessibility. In *A. aegypti*, we found 3-5 dendrites inside the big chamber of these papillae. If there is any mechanoreceptive dendrite associated with the oral papillae, such a dendrite instead of terminating near the base of the papilla probably enters the papillary lumen for a short distance (Fig. 28).

Larsen and Owen [14] found in *C. inornata* that when chemosensory hairs on the labella are placed in contact with water or sugar solution, the labellar lobes spread apart, thus permitting the ligula to come into contact with the test solution, causing the ligula to increase by 76.65% of its original size. Consequently the test solution will probably spread over the ligular surface and make contact with the oral papillae. They suggested that it is probably through this mechanism that the mosquito mediates sucking of water and sugar solution. Whether such a mechanism exists in *A. aegypti* remains to be investigated. In *A. aegypti*, the oral and ligular surfaces are very close to each other, leaving only a small space in between (see Fig. 10 of Lee, 1974)[32]. Solutions that come into contact with the tip of the labellar lobes can probably reach the oral papillae through capillary action.

Chordotonal Organ

This is the first report of chordotonal organs in the mosquito labellum. It is similar to the chordotonal organ described in the legs of the shore crab *Carcinus maenas* [33, 34], and also to the Johnston organ scolopidium of *Drosophila melanogaster* [35], in having two sensory cells associated with one chordotonal organ. However, in the mosquito, at the ciliary region of the sensory cells, the two cells are separated by cell membranes, whereas in the shore crab and fruitfly, the ciliary segments are inside the scolopale without any membrane separating them. Zacharuk and Blue [36] also found a chordotonal organ with a single nerve cell within the antennal cone of larval *A. aegypti*, and suggested that it functions either as a stretch receptor, or as a monitor for low frequency vibration in the adjacent aquatic environment.

Behavioral studies have shown that mosquitoes often spread their labella when the labellar hairs are stimulated with sugar, water, and unacceptable compounds, as dis-

cussed above. But when mosquitoes are feeding on blood, the two labellar lobes are firmly held against each other [27, 28, 37]. Dr. W. Horsfall of the Department of Entomology, University of Illinois at Urbana, Illinois, U.S.A., had made a film on the feeding behavior of female *A. aegypti* feeding on the foot-web of a frog, and he kindly loaned us the film for study. We also noted that the two labellar lobes were closely held against each other during the whole process of feeding on the host. Chordotonal organs are generally recognized as stretch receptors. Whitear [33, 34] suggested that in chordotonal organs where each scolopidium is associated with two sensory cells, one sensory cell probably respond to stretching, and the other to slackening of the cap. The chordotonal organ in the mosquito labellum probably functions to monitor the spreading and closing of the labella during feeding. Both flexor and extensor muscles are present in the labella of mosquitoes [38].

Apical Hairs

To our knowledge, this is the first time that the presence and location of the apical hairs in the labella of mosquitoes has been documented. Transverse sections of the apical hairs can be seen in Figs. (5a and 6) of Vogel [10], but he labeled them as sensory cells. Zwonitzer [11] also noted these apical hairs in female *C. inornata*, but incorrectly reported that they project anteriorly at the tip of the labellum in the same plane as the oral papillae. Structurally, the apical hairs can be classified as thick-walled chemoreceptors. They differ from the medium-sized, aboral labellar hair in having a smooth outer wall. Since the apical hairs are so located that they will come into contact with the substrate when the mosquito is probing on the host, these hairs may be involved in the discrimination of the host.

Ligular Hairs

Owen [3] reported from his behavioural studies using *C. inornata* and *Aedes dorsalis* that ligular hairs are chemosensory and respond to water and sucrose. This was later refuted by Larsen and Owen [14], who found that the ligular hairs in *C. inornata* are not chemosensory, and suggested that the behavioral response observed by Owen [3] was probably a result of the oral papillae coming into contact with the ligular surface which was coated with the test solution. Our results confirmed that the ligular hairs do not have a sensory function.

Labial Hairs

When a mosquito is feeding on a host, as the fascicle enters the host tissue, the labium becomes bent gradually, to a point where the labium almost becomes double under the head as the fascicle penetrates deeper. During such bending of the labium, hairs at the base of the labium may touch the ventral side of the head. After feeding, the fascicle is gradually eased back into the labial theca during withdrawal of the fascicle from the host tissue, and the labium is observed to rock from side to side [28, 37]. As the labium straightens, hairs at the base of the labium may lose contact with the ventral side of the head. Therefore these labial hairs are probably involved in providing information to the mosquito regarding the "state" of bending of the labium during and after feeding. Schiemenz [9] also noted a transverse row of seven hairs at the base of the labium in *Culiseta annulata*, and suggested

that these hairs probably play a role as tactile hairs in the bending of the labium during piercing and sucking. Christophers [38] found in *A. aegypti* that the number and arrangement of these hairs are more regular in the females than in the males. This may be related to the blood-sucking behavior of the females, which involves bending of the labium during insertion of the fascicle and feeding, whereas such a behavior is absent in the male mosquitoes.

Functions of Labellar and Labial Sensilla During Feeding

From behavioural studies, mosquitoes are attracted by host odours, CO₂, warmth, humidity, and optical stimuli. Detection of odours, CO₂, warmth and chemo-attractants are the function of various receptors on the antennae of the mosquitoes [39-48]. Sensilla on the maxillary palps can also detect CO₂ and mosquito repellents [49, 50]. In nature, mosquitoes also feed on plant nectar, and such feeding affects the longevity and dispersal potential of mosquitoes [51]. In the following discussion, the probable chain of events regarding the feeding behaviour of mosquitoes after landing on a host is described based on the results from this study and the reports of other workers.

Mosquitoes often walk around soon after landing on a suitable host and probably detect the acceptability of the host using the chemoreceptors located on the tarsi of the pro- and mesothoracic legs. Tarsi of the metathoracic legs may not be important in host discrimination, as the hind legs are often raised when the mosquito is walking around. Behavioral studies have suggested that mosquito tarsal hairs are sensitive to sugar, salt and water [2-4]. Frings and Hamrum [2] found in *A. aegypti* that stimulation of the tarsal hairs with NH₄Cl only made them restless, but the mosquito did not look for a more suitable substrate. Jones and Pilit [28] found that when all the tarsi of female *A. aegypti* were removed, the mosquitoes were still able to pierce the skin and take a blood meal rapidly, indicating that the tarsi are not essential in providing the anchoring force for piercing.

Probing of the substrate using the two labellar lobes follows shortly after landing. The long labellar hairs probably monitor the positioning of the labellar lobes, with the medium-sized hairs near the labellar tip and the apical hairs detecting the suitability of the host. The medium-sized hairs posterior to the tip of the lobes probably detect the odour(s) from the host. When feeding on plant nectar, the presence of sugars may be detected by the labellar chemosensory hairs, so that the two lobes then spread apart, thus bringing the labral food canal opening to the solution [6]. The chordotonal organs in the labellar lobes may monitor the spreading and closing together of the lobes. The spreading of the labella also brings the ligula into contact with nectar, and this contact causes the ligula to increase in size. The function of this swelling of the ligula may be two fold. One is to spread the solution over the ligular surface, thus bringing the solution into contact with the oral papillae, thereby mediating the sucking of the solution, as suggested by Larsen and Owen [14]. The other is probably to hold the labral tip in place, and serve as a mechanical support, since the tip of the fascicle is situated in the dorsal groove of the ligula. Sucking activity of the cibarial and pharyngeal pumps is initiated when medium-sized hairs posterior to the tip of the labella and oral papillae

are simultaneously stimulated [6]. During nectar feeding, the mosquito shows discontinuous suction [52, 53]. The food passing over the labral campaniform sensilla may affect the pumping action of both cibarial and pharyngeal pumps. The above description applies to both sexes of mosquitoes.

In female mosquitoes when feeding on blood, secretion present on the host skin, and also host odour probably stimulate the labellar chemosensory hairs. Now the labellar lobes do not spread apart, but are held tightly together. The penetration of the fascicle into the host tissue is aided by the alternating cutting action of the two maxillary stylets (laciniae) [27]. The overlapping mandibles probably cover the opening of the labrum during penetration, to prevent the host tissue from entering the food canal. Similarly, interdigitating finger-like projections at the tip of the hypopharynx may prevent possible blockage of the apical salivary canal opening by the tissue. Mandibular teeth are found in some species of mosquitoes [54], but the main function of the mandibles is to separate the food canal from the hypopharynx, to form a two-channel system: one for sucking the blood during feeding, and the other for the injection of saliva [32, 54].

During the initial insertion, the substance blocking the opening of the labral sense organs may get rubbed off by friction with the tissue, thus exposing the receptor sites. Lateral teeth on the two maxillary stylets (laciniae) are important in piercing and withdrawal of the fascicle during feeding, and these teeth are absent in mosquitoes which do not feed on blood [54]. The fascicle is very flexible in the host tissue, as it often bends dorsally at almost a right angle to the plane of insertion after entering the skin, and the tip of the fascicle is capable of bending in different directions [37]. Muscles controlling the two walls of the labrum are responsible for the dorsal and ventral flexion of the fascicle, and the differential actions of the laciniae are responsible for lateral flexion [55]. The apical and subapical sensilla probably detect the presence of blood [18, 32] and the stimulating factor in the blood is probably the adenine nucleotides [16, 56-58]. Apical and subapical labral sensilla are absent in male mosquitoes which are not known to suck blood in nature, and in females of mosquitoes not known to suck blood [58]. Owen and Reinholz [59] found in *C. inornata* that water satiated mosquitoes refused 5-adenylic acid, ADP and ATP in Tris buffer, whereas thirsty mosquitoes imbibed these solutions. They therefore suggested that the acceptance of nucleotides was mediated by the water receptor.

As soon as a blood source is detected, the retractor muscles of the mandibles contract, exposing the opening of the food canal. Entry of food into the labral food canal may be detected by the labral campaniform sensilla, which may influence the action of the cibarial and pharyngeal pumps. The mosquito may feed by inserting the fascicle into a capillary (capillary feeding), or feed from the hemorrhage in the tissue caused by the puncture (pool feeding), with the average time for capillary feeding 3 minutes and 10 minutes for pool feeding [37, 60]. Capillary feeding is more frequent than pool feeding [61]. Saliva is injected at different stages of penetration as tiny "puffs" [37], and such injection probably continues even after a blood supply is tapped [60], and saliva injection is an important step in the transmission of diseases carried by the mosquitoes.

Palatal and dorsal papillae in the cibarium probably monitor the chemical nature of the food [62]. Indeed mosquitoes stop aspiration as soon as unacceptable compounds enter the cibarium [3, 18]. If the food is blood, then the discontinuous suction is changed into continuous suction until the mosquito is satiated [3]. The trichoid sensilla probably register the flow of the food into the pump and the cibarial campaniform sensilla may monitor the pumping action of the cibarium [62]. The ventral papillae probably detect the type of food thus providing the information for the initiation of the switching mechanism: sugar solution enters the ventral diverticulum and blood goes to the midgut [62]. The two small dorsal diverticula probably function as air separators, trapping air that comes in with the food [52, 63]. Sugar solution stored in the ventral diverticulum is gradually passed to the midgut for absorption [53]. Day [63] suggested that as a blood meal is required by a majority of female mosquitoes to mature their eggs, the ability to take a blood meal in spite of a recent nectar meal is of survival value. Another theory is that sugar solution in the diverticulum serves as a supply of water.

Termination of feeding is initiated by the intersegmental abdominal stretch receptors [64]. Withdrawal of the fascicle from the host tissue is aided by the laciniae, and Robinson [27] and Jones and Pilitt [28] had given detailed descriptions of this. The labellar lobes probably help the fascicle to return into the labial gutter after withdrawal [27].

CONCLUSION

Feeding behavior of mosquito is quite complex involving many types of receptors. Here we have provided a comprehensive description of the sense organs on the labella which are involved in host discrimination and initiation of feeding either on nectar or blood. Such knowledge is essential in our attempt to find more effective mosquito repellents in order to protect us from mosquito-borne diseases.

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