QUAESTIONES ENTOMOLOGICAE

A periodical record of entomological investigations, published at the Department of Entomology, University of Alberta, Edmonton, Alberta.

Volume 2 | Number 4 | 3 October 1966

CONTENTS

LABRAL AND CIBARIAL SENSE ORGANS OF SOME MOSQUITOES

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Quaestiones Entomologicae
2 : 259 - 270 1966

Sense organs on the labrum and in the cibarial pump of males and females of 22 species representing 8 genera of mosquitoes were investigated. Two types of sense organ were found on the labra of female mosquitoes, four hair sensilla at the tip and two subapical sensilla. The males of all species and the females of the non blood-sucking species Toxorhynchites splendens *lack the four sensilla at the tip. The labral sense organs are innervated by a branch of the labral nerve. The cibarial sense organs are also found in two groups. A dorsal group of four types of sensillum on and around the anterior hard palate is indirectly innervated by a second branch of the labral nerve. A ventral group at the posterior end of the cibarial pump appears to be innervated only by a small branch of the fronto-labral nerve. A suggestion concerning the neuro-muscular mechanisms of the food path and their control of the passage of food is offered.*

INTRODUCTION

In 1921 Vogel published a study of the mouth parts of Culicidae and Tabanidae. The mosquitoes that Vogel used were *Culex pipiens* L., *Anopheles maculipennis* Meigen, and *Anopheles claviger* (Meigen). In his summary he states (our translation):

> "In the ventrolateral edges of the labrum on each side there is a chitin canal which contains a protoplasmic thread and which is to be interpreted as a nerve. This thread or nerve ends in the labrum tip in a cell group which is located at the base of the fine chitin spines. The assumption that these are sense organs and apparently taste receptors is so much more probable since the other five stylets are completely chitinized at the tip."

In his text he also mentioned the number of observed spines:

> "Anterior to the cell group the labrum ends in two tips formed from

the labrum canals, each of which has two chitin spines laterally . ." Unfortunately Vogel's figures show only the cross-section of the labrum tip and therefore do not indicate the exact location of the sensory spines.

Robinson (1939) also described sense organs on the labrum of mosquitoes. He worked with the female of *Anopheles maculipennis*. Robinson referred to Vogel's work but may have misinterpreted it since his description differs from the original:

"At the tip, the stylet (labrum) is sharpened off ventrally like a quill pen and consequently the groove is open. Just below the point there is a pair of small pegs which may function as sense organs."

and,

"In a transverse section of the labrum the internal (haemocoelar) lumen can be seen to be occupied by a pair of protoplasmic strands lying laterally. Vogel (1920) interprets these as nerves which terminate distally in sense organs in the position of small pegs."

Robinson also referred to MacGregor (1931) who recorded:

"... the ability of *Aedes* and *Culex* to select a desired liquid by means of a sense located at the tip of the labrum."

Comparing Vogel's and Robinson's descriptions and illustrations, it becomes quite apparent that the two authors were describing two different sets of sense organs. Our study confirmed this.

Waldbauer (1962) examined the mouth parts of *Psorophora ciliata* (Fabricius), a culicid mosquito, under oil immersion. He did not record sense organs on the labrum, although the outer margins of apical setiform organs are shown in his fig. 17.

Snodgrass (1944) made a comprehensive study of the feeding apparatus of various groups of sucking insects and of generalized biting mouthparts. Unfortunately, although he referred to Vogel's and Robinson's work, he mentioned neither author in relation to taste receptors on the labrum. Snodgrass appears to have thought very little of sensory influence upon movements of the fascicle:

"Apparently however, the fascicle movements are entirely fortuitous, there being no evidence of a sensory influence, the fascicle often going close to a capillary without entering it, or sometimes penetrating clear through a blood vessel."

Snodgrass also mentioned MacGregor's work, but only in relation to the passage of food into the stomach or diverticula.

Day (1954) mentioned dorsal and palatal papillar sense organs in the cibarial pump. These had been described by Sinton and Covell (1927) and Barraud and Covell (1927) for their taxonomic value. Day described these sense organs in form and location and worked out some of the innervation. He believed that the sense organs are at least partially innervated by the frontal ganglion. Unfortunately, Day did not find any sense organs on the labra of his specimens. Day experimented with both males and females of *Aedes aegypti* L. He discovered that in males as well as in females blood was directed to the midgut, while sugar went to the diverticulum, although males do not usually imbibe blood.

Hosoi (1954) also worked on the food distribution mechanism in mosquitoes. He experimented with food stimulation of the entire fascicle and the labium and found that the fascicle is sensitive to whole blood and to the corpuscles separately. He also found that the partial amputation of the fascicle reduced its sensitivity to food stimuli considerably, but that the mosquito was still quite capable of imbibing food especially if the proboscis stump were pushed into it:

> "It is highly probable, therefore, that the sensory function of the fascicle originates in the labrum. It may be questioned, however, whether the receptors are located only on the tip of this organ, since mosquitoes imbibed blood into the stomach after the apical part of the fascicle had been amputated."

Hosoi suggested that the labrum should be sensitive along its entire length, and that food other than blood should also stimulate the labrum to some extent.

Christophers (1960) gave an excellent account of the fronto-labral nerve complex. He also described the gustatory papillae (see Robinson) on the labrum and both sensory groups of the cibarial pump. He did not however give the innervation of any of the sensory organs on the labrum or in the cibarial pump. Christophers was the first to mention that there are gustatory papillae on the labrum in both males and females. He also said that the cibarial sense organs are essentially the same in males and females.

Clements (1936) illustrated the labrum after Snodgrass but added sensory pegs (apparently taken from Robinson) without mentioning the change, despite the fact that Snodgrass found no sensory organs on the fascicle. He drew the subapical sensilla disproportionately large and omitted the apical bristle sensilla. Clements referred to Hosoi's work stating that the sense organs on the labrum and in the cibarial pump are at most only slightly sensitive to glucose, while they are sensitive to blood. Clements also took as fact assumptions made by Christophers and Day. Hosoi speculated that the labrum is sensitive over its entire length, but he did not work with this organ by itself. Furthermore, he made no reference to the cibarium or its sense organs.

Owen (1963) concluded that the fascicle of *Culiseta inornata* (Williston) bears no contact chemoreceptors. His statement is based upon experiments with feeding reactions of living mosquitoes after certain sensilla on the labium and tarsi were stimulated.

MATERIALS AND METHODS

For the study of the labral sense organs the species listed in table 1 were used. Males and females were compared whenever possible. The least number of specimens used to represent one sex of one species was three. Whole mounts of both unstained and stained labra were used; the stains were vital methylene blue and crystal violet. The mounting media were DePex or Farrant's medium and both conventional and phase microscopy were employed.

The cibarial pump sense organs were studied by two methods. Whole mounts were made of the cibarial and pharyngeal pumps of all the specimens used for the labral study. The whole head was treated in KOH and dissected before mounting. The location and shape of the cibarial sense organs could thus be determined.

Sectioned material was used to trace the innervation of the cibarial pump and the labrum. Males, females, and some pupae of *Aedes aegypti* were fixed in Masson's modification of Bouin's, washed and dehydrated via Zircle series, and embedded in Paraplast. Sections were cut at 3, 5, 7, and 10 μ. The stains used were Heidenhain's haematoxylin, aldehyde fuchsin, urea silver nitrate, and Novelli's nerve stain.

OBSERVATIONS

The labrum is the thickest and stiffest of the six stylets forming the fascicle. It forms a double walled, ventrally closed tube, the food channel. The dorsal surface of the labrum is attached to the clypeus at the base, where the lateral edges of the inner wall widen out and are continuous with the roof of the cibarial pump. The hypopharynx forming the closure of the food channel at this point is attached to the membranous floor of the cibarial pump. At the tip, the labrum is slightly curved and cut off, as Robinson (1939) says, like a quill pen.

The cibarial pump is tubular and flattened dorso-ventrally. It extends from the base of the proboscis to just beyond the posterior edge of the clypeus where it ends in two lateral processes or flanges. Between these it is linked to the pharyngeal pump by membranes. The division between the cibarium and pharynx is marked by the insertion of two precerebral dilators of the pharynx. These muscles lie between the frontal ganglion and the brain (Snodgrass 1944). The cibarial pump is mainly membranous but contains heavily sclerotized parts, the anterior hard palate, the posterior palate and the posterior sclerotized constriction which widens out into the lateral flanges. The two hard palates are dorsal, while the constriction is both ventral and dorsal. The pharyngeal dilator muscle inserts on the membrane just behind the constricted region of the cibarial pump.

Labral Receptors

In the generalized female mosquito, ventrolaterally, in the lumen of the labrum, there are two canals (Vogel's chitin canals) which contain cytoplasmic strands. Each of these strands ends in a small group of cells near the tip of the labrum. There are no other cells in the labral canals. At the point where the food channel opens completely, each canal bears one sensillum. In most specimens this sensillum is round or slightly oval, has a heavily sclerotized rim and is usually covered by a thin membrane. In surface view, it looks like a campaniform sensillum. The diameter varies from 3μ to 6μ according to species in both males and females. Very fine dendrites lead from the group of cells in the canal to the sensillum. However, some specimens of certain species have a short peg projecting from the center of the membrane. When pegs are present these organs resemble minute basiconic sensilla surrounded by a wide membranous socket, figs. 3-8. The diameter of the membranous area is at least three times the diameter of the peg. Table 1 shows the species in which these basicone-like sensilla were occasionally found.

TABLE 1. Sensilla on the labra of mosquitoes.

Species studied	Female: Apical setiform	Female: Peg in subapical*	Male: Apical setiform	Male: Peg in subapical*
Aedes aegypti (L.)	+	-	-	-
albonotatus (Coquillett)	+	-	-	-
dorsalis (Meigen)	+	+	-	-
excrucians (Walker)	+	-	-	-
fitchii (Felt & Young)	+	+	-	-
flavescens (Muller)	+	-	-	-
spencerii (Theobald)	+	+	-	-
trichurus (Dyar)	+	-	-	-
vexans (Meigen)	+	-	-	-
Armigeres subalbatus (Coquillett)	+	-	-	-
Anopheles earlei Vargas	+	-	-	-
gambiae Giles	+	-	-	-
Culex fuscanus Wiedemann	+	+	-	+
pipens fatigans Wiedemann	+	+	-	+
pipiens pipiens Linnaeus	+	+	-	+
pipiens molestus Forskal	+	-	-	-
tarsalis Coquillett	+	-	-	+
territans Walker	+	+	-	-
Culiseta alaskaensis (Ludlow)	+	+	-	-
inornata (Williston)	+	+	-	-
morsitans (Theobald)	+		-	-
Mansonia perturbans (Walker)	+	+	-	+
Toxorhynchites splendens (Wiedemann)	-	+ **	-	?
Wyeomyia smithii (Coquillett)	+	+	-	+

\+ present, - absent

* Socket-like subapical sensilla are present throughout.

** See text, p. 264

Beyond the subapical sensilla, the labrum quickly assumes the form of an I in cross-section, and finally draws out into a fine point above each canal. At the extreme tip, each side bears two fine bristle or setiform sensilla (Vogel's chitin spines), fig. 3. The distal spines vary in length from 9μ in *Culex territans* to 25μ in *Aedes excrucians*, *A. fitchii*, and *A. flavescens*. The proximal and lateral pair may be either longer or shorter than the distal and medial pair, and varies from 9μ in *C. territans* to 27μ in the larger *Aedes* species. These bristles are set into membranous bases surrounded by heavy chitinous rings. They are hollow, and fine dendrites from the cell groups innervate them.

Whole mounts stained with vital methylene blue show the cytoplasmic strands and nuclei of the cell groups stained, thus suggesting nervous tissue. This assumption finds further support in serial sections stained with Heidenhain's haematoxylin, aldehyde fuchsin, or urea silver nitrate, which revealed nerves at the base of the labrum. Vogel (1921), Robinson (1939), Christophers (1960), and Clements (1963) assume also that the cytoplasmic strand should be considered a nerve.

The genera and species investigated differ little except in the size of the sensilla and in that the distance between the subapical and the setiform sensilla in *Culex* is relatively much longer than in the other genera, fig. 6.

One representative of the non blood-sucking genus *Toxorhynchites* Theobald was examined. The female of *T. splendens* (Wiedemann) has no apical setiform sensilla on the labrum. Subapical sensilla are present, and the labrum of *T. splendens* female (fig. 5) resembles the labra of males of other genera. The labrum of the male *T. splendens* differs a little in shape from that of the female, but bears only subapical sensilla as in males of other genera. Hairs project forwards from the membranous sockets of the subapical sensilla in both sexes and in this respect the subapical sensilla differ from those found in other genera, table 1. Two other autogenous species were examined *Wyeomyia smithii* (Coquillett) and *Aedes albonotatus* (Coquillett). The labra of both species were normal; the females having four well developed apical sensilla. Other members of the genera *Wyeomyia* and *Aedes* are blood-suckers and the blood-feeding habit may be recently lost in autogenous species.

The labra of both sexes terminate in two pronounced chitinous points which are obscured in most females by the apical sensilla. The membrane between these points may be drawn out into a third point; this is especially pronounced in *Anopheles earlei* Vargas.

The labra of all the male mosquitoes studied lacked the apical setiform sensilla, figs. 7 and 8. Subapical sensilla were present and basiconic projections could be seen in some species (table 1).

Cibarial Receptors

There are two groups of sense organs in the cibarial pump, a dorsal and ventral group (figs. 1 and 9). The dorsal group is situated at the anterior end of the cibarium on and around the anterior hard palate, and consists of four types (Day's terminology):

Palatal papillar - Two pairs. Heavy spines with membranous bases.

Campaniform sensilla - one pair. Heavily sclerotized rings around membranous bases.

Dorsal papillar - one pair. Heavy spines. Base as in the campaniform but otherwise very similar to the palatal papillar.

Hair-like sensilla - number variable but usually three pairs. Base as in the campaniform sensilla but the ring is much smaller. The ventral group is situated at the extreme posterior part of the cibarial pump, usually in the heavily sclerotized neck region. The sensilla are of the short spine type and occur in two closely associated pairs, one pair on each side of the median line.

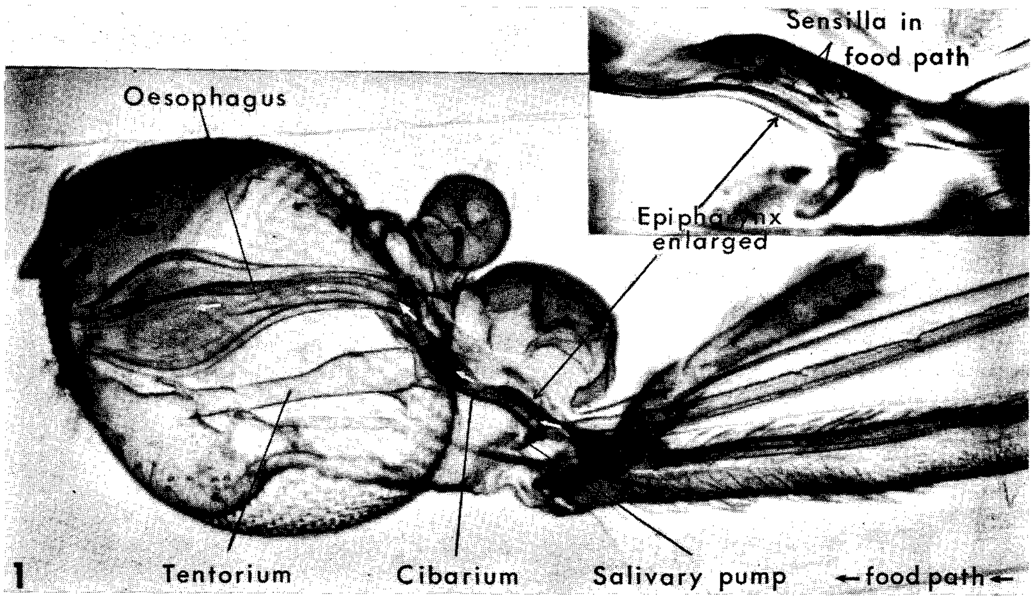
All sensilla in the cibarial pump are hollow and are innervated by fine dendrites originating from the sensory cells closely associated with them. Dendrites from the dorsal group lead to a larger group of loosely associated cells. This group lies dorsally to the cibarial pump and ventral to the retractors of the labrum, fig. 2. These muscles are innervated by the frontal nerve. The frontal ganglion lies dorsal to the junction between the cibarial and pharyngeal pump, and between the muscles leading to both these pumps. The frontal ganglion receives a branch of the fronto-labral nerve. This nerve arises on each side from the commissure and the suboesophageal ganglion. The nerve may branch immediately after leaving the commissure, but more often runs forward a little way before branching. The frontal ganglion connective passes dorsally anteriorly to the lateral flange of the cibarial pump to join the side of the frontal ganglion. The recurrent nerve issues from the posterior part of the frontal ganglion and runs just dorsal of the pharyngeal and suboesophageal pumps toward the neck region. The labral nerve branches soon after leaving the fronto-labral nerve. One branch (labral nerve I) passes forward and dorsally into the anterior and lower part of the clypeal dome, and splits up into fine neurons which supply the muscles and also the group of loosely associated cells previously described. The second branch (labral nerve II) passes forward along the side of and somewhat ventral to the cibarial pump and leads into the base of the labrum and into the labral canals (see fig. 2). In the generalized insect the labral nerve receives motor fibres from the frontal ganglion. We were unable to trace axons leading from the frontal ganglion to the labral nerve in any mosquito. It is quite plausible that axons lead directly from the sense organs to the tritocerebrum.

The ventral group of sensilla appears to be innervated by a small branch leading from the fronto-labral nerve to sensory cells associated with the sensilla.

The sense organs in the cibarial pump of male and female mosquitoes vary slightly in location, but no more than between specimens of the same sex and species. The general pattern does differ somewhat between different species and genera.

DISCUSSION AND CONCLUSION

The two types of labral sense organ described by Vogel and Robinson are both present. The apical bristles of Vogel have been overlooked



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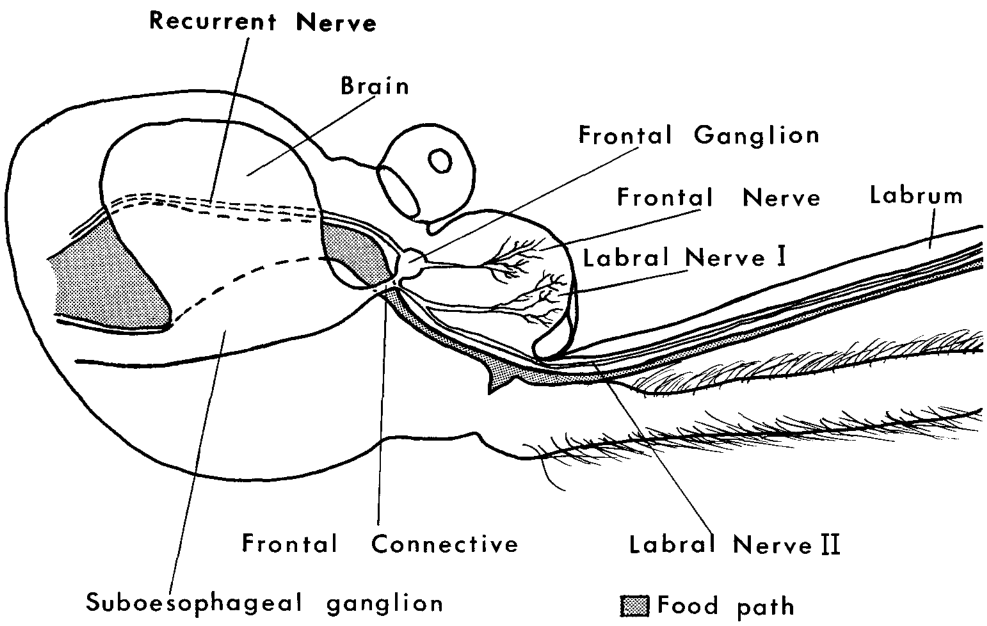

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Fig. 1. Cuticular structures in the head of *Culiseta inornata* X 118; cleared in 5% KOH, right side removed.

Fig. 2. Outline of head and food channel showing the fronto-labral nerve complex. *Culiseta inornata* X 118.

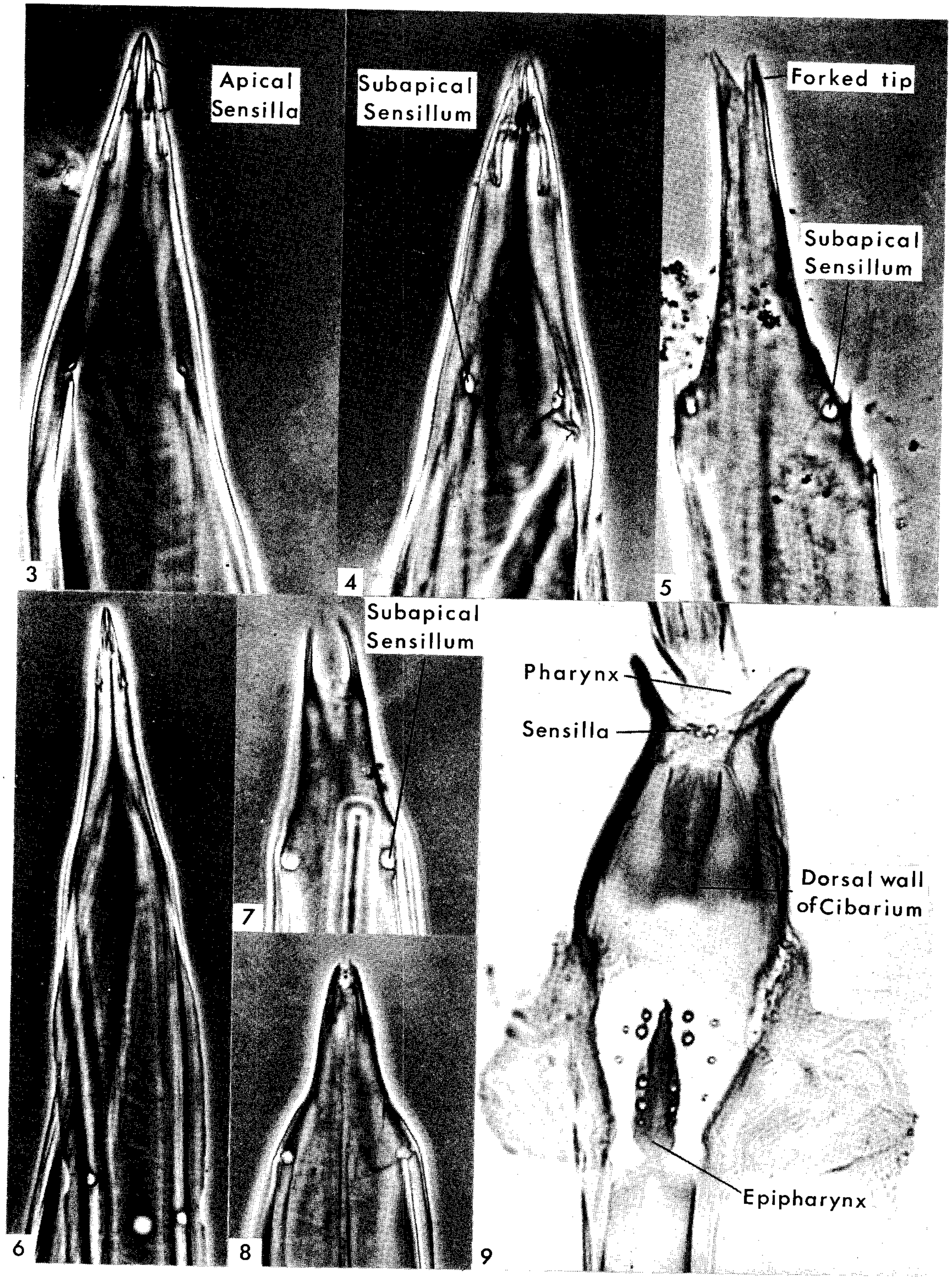

Fig. 3. Labrum of *Aedes vexans* ♀, X 590. Fig. 4. Labrum of *Aedes spencerii* ♀, X 590. Fig. 5. Labrum of *Toxorhynchites splendens* ♀, X 880. Fig. 6. Labrum of *Culex tarsalis* ♀, X 590. Fig. 7. Labrum of *Culiseta inornata* ♂, X 590. Fig. 8. Labrum of *Aedes vexans* ♂, X 590. Fig. 9. Dorsal wall of the cibarium of *Culiseta inornata*, X 265.

by recent workers because of their small size. Some species may lack Robinson's pegs, when only the thin socket is present. Both types are found on the labrum of female mosquitoes with the exception of *T. splendens*. They are indirectly innervated by one branch of the labral nerve and directly innervated by neurons leading from groups of sensory cells which lie in the anterior part of the labral canals. There are cytoplasmic strands but no other cells in the remaining length of the labral canals.

The cibarial pump sensilla are indirectly innervated by branches of the fronto-labral nerves. They are directly innervated by neurons leading from sensory cells closely associated with the sense organs and by those leading to a larger group of loosely associated cells just dorsal to the anterior end of the cibarial pump. The frontal ganglion appears to innervate only the various muscles of the pumps but may also innervate the large group of cells previously described. The ventral group of sensilla appears to be innervated only by a small branch of the fronto-labral nerve. Wenk (1953), in his paper describing the head of *Ctenocephalus canis*, describes a similar innervation of the cibarium and labrum. Day said that the cibarial sense organs are at least partially innervated by the frontal ganglion, but he was not able to trace the innervation with certainty.

Since the female labrum not only functions as a food channel but also in penetration and blood detection (Hosoi 1954) one would expect to find both mechano and chemoreceptors on the tip of the labrum. There are two types of sense organs present. Their functions could not be tested directly because of their very small size and close proximity. The apical setiform sensilla are hollow and innervated. One would not expect tactile hairs of this minute size to be hollow; also they are partially protected by the chitinous labrum tip (see Vogel 1921). They were never observed displaced and are therefore probably chemoreceptors rather than mechanoreceptors.

The male labrum has only the subapical sensilla; setiform sensilla are never present. The male normally feeds on exposed sugary fluids and does not have to pierce any tissue when feeding on nectar, but will penetrate fruit when kept in the laboratory. As the labrum of both sexes is only very slightly sensitive to sugar (Hosoi 1954), the subapical sensilla on the labra of males are unlikely to be chemoreceptors. The apical setiform sensilla in the female are most likely to be chemoreceptors which function in detection of blood or some component of it. This idea is supported by the fact that the female *T. splendens* which does not feed on blood lacks the apical setiform sensilla.

The subapical sensilla may be mechanoreceptors; in all except the genus *Culex* they are positioned at the end of the stiff side channels where campaniform sensilla could detect the bending of the tip. However they may be placoid or small basiconic chemoreceptors. Further behavioral studies involving micromanipulation and electron microscope work are required to resolve this question.

No morphological support could be found for Hosoi's suggestion that the labrum might be sensitive along its entire length. On the contrary, it was found that the labrum is not permeable to crystal violet (Slifer 1960) except at its very tip, and there only slightly. Some sensitivity of the lower part of the labrum would be expected after cutting off the tip and thus exposing the nerves in the labral canals. It is probable that food is detected by the apical labral sense organs and the pumping action is initiated by impulses received by the cibarial muscles from the labral sense organs via the frontal ganglion. A preliminary distinction between blood and sugar solutions may be made by the labrum. After the food enters the cibarial pump, the various sensilla there are excited and send out impulses to the muscles controlling the openings of the stomach and diverticula, thus setting the food-directing mechanism in action. This would explain Hosoi's finding that blood is sent into the stomach and sugar solutions into the diverticula even after the fascicle had been cut off. Further investigation is required to verify this.

ACKNOWLEDGEMENTS

The authors wish to acknowledge with gratitude the assistance of those who contributed to the preparation of this paper. Special thanks are due to Dr. Janet Sharplin for her excellent guidance and supervision throughout the project, to Dr. B. Hocking for his suggestions and comments and to Dr. Andrew Spielman, Department of Tropical Public Health, Harvard University who provided material of several species. Thanks are also due to Mr. N. Belur for providing specimens and to Mr. J. S. Scott for assistance with the illustrations. Finally the authors wish to acknowledge the financial assistance of the U.S. Army Grant No. 63-G83 (Hocking Trust) which made this study possible.

REFERENCES

Barraud, P. J. and G. Covell. 1927. The morphology of the buccal cavity in anopheline and culicine mosquitoes. Indian J. Med. Res. 15 : 671-680. Cited from Day.

Cameron, M. L. and J. E. Steele. 1927. Simplified aldehyde fuchsin staining of neurosecretory cells. Stain Technol. 1959, 34 : 265-266.

Christophers, S. R. 1960. *Aedes aegypti* (L.). The yellow fever mosquito: its life history, bionomics and structure. Cambridge University Press : London. xii + 739 pp.

Clements, A. N. 1963. The physiology of mosquitoes. Pergamon Press : London. ix + 393 pp.

Day, M. F. 1954. The mechanism of food distribution to midgut or diverticula in the mosquito. Aust. J. biol. Sci. 7 : 515 - 524.

Gurr, E. 1962. Staining animal tissues. Practical and theoretical. Leonard Hill : London.

Hosoi, T. 1954. Mechanism enabling the mosquito to ingest blood into the stomach and sugary fluids into the oesophageal diverticula. Annotnes zool. jap. 27(2) : 82-90.

Kuvana, Z. 1935. The innervation of the alimentary canal of the silkworm larva. Annotnes zool. jap. 15 : 257-260.

MacGregor, M. E. 1931. The nutrition of adult mosquitoes. Trans. R. R. Soc. trop. Med. Hyg. 24 : 465. Cited from Robinson.

Novelli, A. 1952. A new and easy rapid method of staining nervous tissue. Experientia 8 : 357-358.

Owen, W. B. 1963. The contact chemoreceptor organs of the mosquito and their function in feeding behavior. J. ins. Physiol. 9 : 73-87.

Robinson, G. G. 1939. The mouth parts and their function in the female mosquito, *Anopheles maculipennis*. Parasitology 31 : 212-242.

Sinton, J. A. and G. Covell. 1927. The relation of the morphology of the buccal cavity to the classification of anopheline mosquitoes. Indian J. Med. Res. 15 : 301-308. Cited from Robinson.

Slifer, E. H. and V. T. Brescia. 1960. Permeable sense organs on the antennae of the yellow fever mosquito, *Aedes aegypti* (L.). Ent. News 71 : 221-225.

Snodgrass, R. E. 1944. The feeding apparatus of biting and sucking insects affecting man and animals. Smithsonian Misc. Coll. 104(7) : 1-113.

Vogel, R. 1921. Kritische und erganzende Mitteilungen zur Anatomie des Stechapparats der Culiciden und Tabaniden. Zool. Jb. 42 : 259-282.

Waldbauer, G. P. 1962. The mouth parts of female *Psorophora ciliata* (Diptera, Culididae) with a new interpretation of the functions of the labral muscles. J. Morph. 3 : 201-216.

Wenk, P. 1953. Der Kopf von *Ctenocephalus canis* (Curt.). Zool. Jb. (Anat.) 73(1) : 103-164.