Reprinted from JOURNAL OF MORPHOLOGY
Vol. 153, No. 3, September 1977

Sense Organs on the Antennal Flagellum of Mayflies (Ephemeroptera)

ELEANOR H. SLIFER [1]
Department of Entomology, Academy of Natural Sciences, 19th and the Parkway, Philadelphia, Pennsylvania 19103

ABSTRACT A few very small sensory pegs with the characteristics of chemoreceptors are present in both males and females on the antennal flagellum of the mayflies, *Stenacron interpunctatum* (Say) [2] and *Cloeon* sp. They are situated near the proximal end of the flagellum on its ventro-lateral surface and have escaped the attention of earlier investigators.

Earlier workers have concluded that there are no sense organs on the antennal flagellum of the mayfly. Nagel (1894) reported for *Ephemera virgita* that "Der ganze Fühler trägt kein einziges Sinnesorgan, nicht einmal ein Fühlhaar, geschweige denn eine Geruchsgrube, stellt vielmehr einen kahlen kurzen Dorn dar." Handlirsch ('30) described the flagellum as a bristle "an welcher keine besonderen Riechorgane ausgebildet zu sein scheinen." According to Deegener ('28), "Die kahlen, kurzen Antennen tragen kein Sinnesorgan."

In 1973 Müller-Liebenau described bristles and scales on the surface of the scape and pedicel of the antenna of a mayfly, *Baetis* sp., and Schmidt, in 1974, published a detailed study of the fine structure of the internal mechanoreceptors in the pedicel of four ephemeropteran genera: *Baetis, Cloeon, Epeorus* and *Ephemera*. However, neither of these workers reported the presence of sense organs on the flagellum.

It will be shown here that a few extremely small sensory pegs are present on the antennal flagellum of some mayflies.

MATERIALS AND METHODS

The adult mayflies examined in the present study, *Stenacron interpunctatum* (Say), were collected in June 1975 in Iowa City, Iowa and *Cloeon* sp. caught in Haddonfield, New Jersey, in July 1976. All were fixed in Bouin's solution. Some antennae were prepared as whole mounts and examined unstained. Others were stained either with borax carmine, a 5% solution of crystal violet or with a 20% solution of $AgNO_3$. The last two were applied to the intact insect. Sections were cut of antennae embedded in Paraplast. These were stained with Heidenhain's iron-hemotoxylin or with Mallory's connective tissue stain. Scanning electron micrographs were made of fixed antennae that had been dehydrated slowly in alcohol, cleared in xylol and dried.

RESULTS AND DISCUSSION

In the mayflies studied here the antenna is composed of two well-developed basal segments—the scape and the pedicel—and a short, slender flagellum. The flagellum is about 750 μm long, 16-20 μm wide at its base and tapers to a diameter of 3 μm just below its tip (fig. 1). The surface sculpturing consists of many shallow, irregular depressions outlined by distinct ridges (figs. 6-8). These depressions are larger in *Stenacron interpunctatum* than in *Cloeon* sp.

The flagellum in the species examined here is not divided into subsegments. Contrary to this, Klapálek ('09) and Schoenemund ('30) both described the mayfly flagellum as many-jointed. Needham et al. ('35) refer to the flagellum as having about 23 joints and Despax ('49) speaks of it as multiarticulate. One can only surmise that these observations were made at relatively low magnifications or else that the species described here differ from other members of the order that were studied earlier.

A short distance above its base the flagellum widens and then narrows again about 70 μm beyond this point (figs. 1, 2). All of the sense organs are located in this wider region.

[1] Mailing address: 308 Lismore Avenue, Glenside, Pennsylvania 19038.

[2] *Stenacron interpunctatum* (Say) is *Stenonema interpunctatum* (Say) of earlier literature.

Distal to it the cellular elements in the lumen consist only of a low epidermal layer and an occasional hemocyte.

In most species of insects examined previously by the writer the flagellar lumen contains a well-developed blood vessel, trachea and one or more nerves. In the mayflies studied here no blood vessel or trachea was seen and the nerve entering the lower end of the flagellum is 3 μm or less in diameter and difficult to identify.

The bulge in the lower part of the flagellum becomes conspicuous just below the most proximal sense organs and disappears above the most distal (figs. 1, 2). All of the sense organs are located on the ventro-lateral surface. Internal to them the cellular layer is from 7-8 μm deep while that on the remaining surface is closer to 5 μm in depth (fig. 5). At the point where the flagellum emerges from the pedicel its lumen is no more than 12-18 μm across. This narrow passage slows the entrance of fixatives and makes good cellular preservation difficult to obtain.

All species of insects from other orders examined previously by the writer have had more than one kind of sense organ on the flagellum. The sense organs on these two species of mayflies are all alike and consist of a few very small pegs from 2-2.5 μm long. Each is set in one of the depressions in the cuticle that is slightly deeper and more conspicuous than those surrounding it (figs. 3, 4, 7, 8). In *Stenacron interpunctatum* as many as 12 pegs are present on each flagellum while *Cloeon* sp. has four or five. The pegs may be arranged in an irregular group or in a longitudinal row. Sometimes two lie very close together (fig. 5). The number and position of the pegs on the right and left flagella of the same individual may differ. No differences were noted between those of males and females.

When an intact mayfly is treated with a 0.5% solution of crystal violet (Slifer, '60), the pegs stain brilliantly at their tip (fig. 3). This strongly suggests that a pore or pores is present at this point where the ends of the dendrites are exposed to the atmosphere and that the structure is a chemoreceptor. The tip of the peg shown in figure 8 suggests the presence of a single opening. Treatment of a whole mayfly with 20% $AgNO_3$ gives results similar to those obtained with crystal violet and, since the peg tips are blackened, makes the pegs easy to locate, especially if the species has a cuticle that is nearly colorless. A wide cuticular sheath that, we may assume, encloses the dendrites extends below the base of the pegs and is visible with an oil immersion lens both in sections and in whole mounts (figs. 3, 4).

In describing the chemoreceptor pegs and hairs of an insect it is customary to state that its cuticular wall is thick or thin; or, put in another way, whether the lumen is, relatively, large or small. Usually the cuticle of a thin-walled receptor is perforated by many extremely fine pores while a thick-walled sense organ has one or a few larger openings at its tip. The mayfly peg is so small that the relative thickness of the wall can not be determined with the light microscope. Electron micrographs of sections would be needed to make a decision. The matter is of some general interest for if the receptors are thick-walled the Ephemeroptera would be the first insect order so far examined in which species occurred that lacked thin-walled receptors.

The number of neurons associated with each peg could not be determined exactly but is probably close to three. This estimate is based on the measurement of the diameter of the projected images of all the nuclei in 47 consecutive cross sections, each cut at 6 μm, of the flagellum of a male that had 12 sense organs on it. Of the 258 nuclei present, four, at least, could be excluded as hemocytes. Among those remaining, 38 were rounded and had a diameter of 3 μm or more. All others were smaller and had an irregular profile. Since, in general, the nuclei of chemoreceptor neurons tend to be larger and more rounded than the nuclei of the epidermal cells and sheath cells that surround them, we may conclude that each of the pegs is innervated by about three neurons. It should be noted that none of the nuclei here interpreted as those of sensory neurons were located distal to the uppermost peg. The best evidence to check this estimate would be obtained with electron micrographs of cross sections through the cuticular sheath. A count could then be made of the number of dendrites enclosed within it. Since, however, the pegs are so small, few in number and scattered this would not be an easy task.

ACKNOWLEDGMENTS

The author is indebted to Doctor Barbara Stay of the University of Iowa for the specimens of *Stenacron interpunctatum* and to Mr. Gary Rosenberg of the Academy of Natural

Sciences for those of *Cloeon* sp.. Mr. Jay Richardson of the Stroud Water Research Center at Avondale, Pennsylvania, identified the *Stenacron,* Doctor C. F. Edmunds, Jr., of the University of Utah, identified *Cloeon* sp. and Doctor C. Y. Shih of the University of Iowa operated the scanning electron microscope in the Zoology Department.

LITERATURE CITED

Deegener, P. 1928 Sinnesorgane. In: Handbuch der Entomologie. C. Schröder, ed. Verlag von G. Fischer, Jena, *1:* 140-233.

Despax, R. 1949 Ordre des Éphéméroptères. In: Traité de Zoologie, Anatomie, Systématique Biologie. P.-P. Grassé, ed. Masson et Cie (Paris), *9:* 279-309.

Handlirsch, A. 1930 Erste Überordnung der Pterygogenea: Ephemeroidea. In: Handbuch der Zoologie. T. Krumbach, ed. Walter de Gruyter & Co., Berlin, *4:* 621-636.

Klapálek, F. 1909 Ephemerida, Plecoptera, Lepidoptera. In: Die Süsswasserfauna Deutschlands. Part 8. A. Brauer, ed. Gustav Fischer, Jena, pp. 1-32.

Müller-Liebenau, T. 1973 Morphological characters used in revising the European species of the genus *Baetis* Leach. In: Proc. 1st Internat. Confer. Ephemeroptera. W. L. and J. G. Peters, eds. E. J. Brill, Leiden, 312 pp.

Nagel, W. A. 1894 Vergleichend physiologische und anatomische Untersuchungen über den Geruchs- und Geschmackssinn und ihre Organe mit einleitenden Betrachtungen aus der allgemeinen vergleichenden Sinnesphysiologie. Bibliotheca Zoologica, *7:* 1-207.

Needham, J. G., J. R. Traver and Y.-C. Hsu 1935 The Biology of Mayflies. Comstock Publishing Co., Inc., Ithaca, New York, 759 pp.

Schmidt, K. 1974 Die Mechanorezeptoren im Pedicellus der Eintagsfliegen (Insecta, Ephemeroptera), Zeit. Morph. Tiere, *78:* 193-220.

Schoenemund, E. 1930 Eintagsfliegen oder Ephemeroptera. In: Die Tierwelt Deutschlands. Part 19. G. Fischer, Jena, pp. 1-106.

Slifer, E. H. 1960 A rapid and sensitive method for identifying permeable areas in the body wall of insects. Entom. News, *71:* 179-182.

PLATE 1

EXPLANATION OF FIGURES

All except figure 3 are of *Stenacron interpunctatum.*

1 Outline drawing of antennal flagellum of female. × 260.

2 Whole mount of basal end of flagellum of male showing ventro-lateral surface. Flagellum emerges from wide, distal end of pedicel below. Each of the nine spots indicates the location of a sensory peg. × 700.

3 Portion of whole mount of flagellum of *Cloeon* sp. to show three pegs in shallow cavities outlined by darker rims. Cuticular sheath visible below base of two of the pegs. Tips of pegs stained with crystal violet applied externally. Edge of flagellum at right shows surface sculpturing of cuticle in profile. Crystal violet, 15 minutes. × 2,000.

4 Longitudinal section through wall of flagellum of male showing a single peg lying in a shallow cavity; cuticular sheath extends below base. Larger, more rounded nuclei are probably those of neurons; others those of sheath and epidermal cells. Heidenhain's iron-hemotoxylin. × 2,000.

5 Cross section of flagellum of male including two sensory pegs on floor of recess in wall near edge at upper right. Compare with figures 4, 7, 8. Large shrinkage space between cuticle and cellular layer except in region of pegs. Note small central lumen. Heidenhain's iron-hemotoxylin. × 2,000.

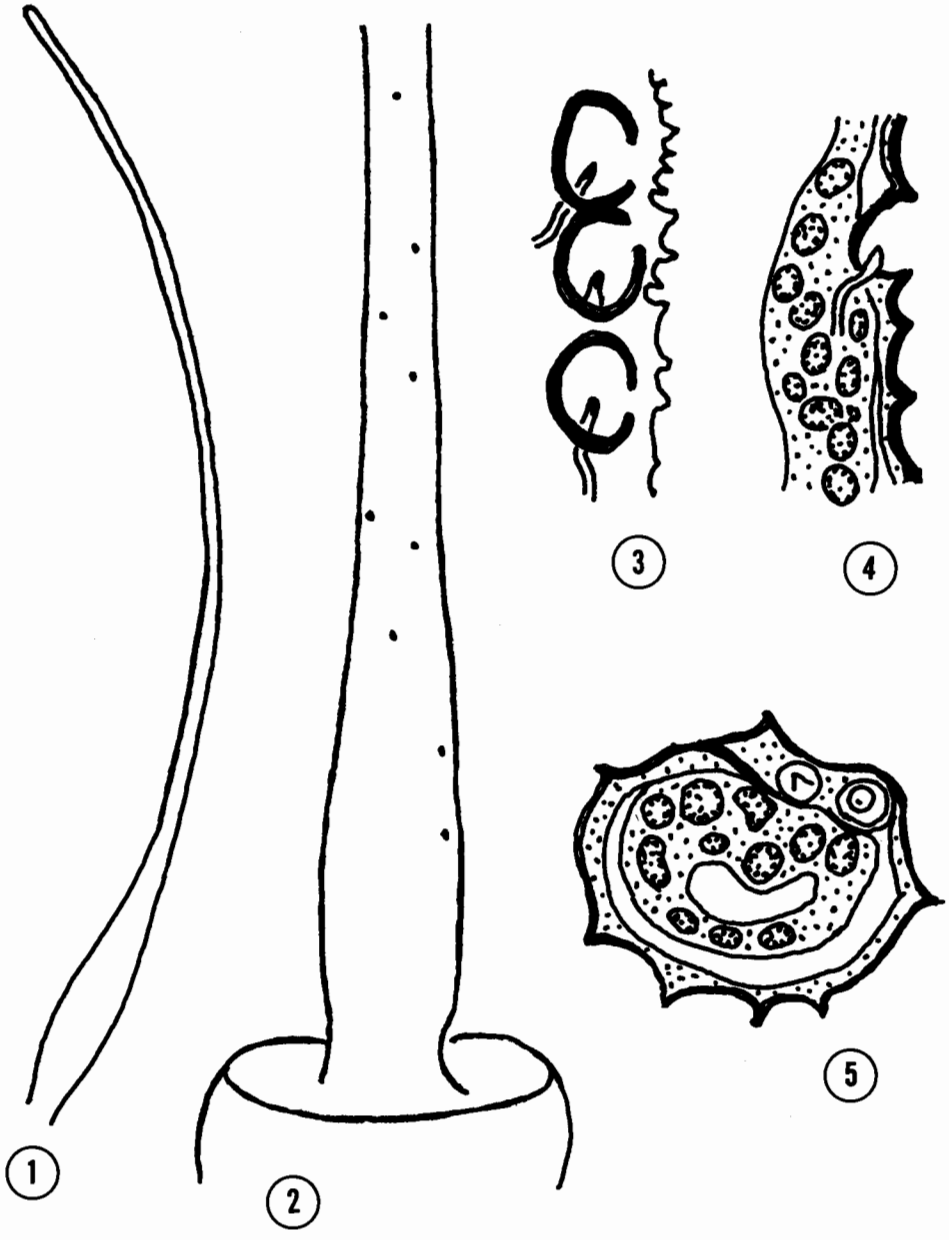

1
2
3
4
5

PLATE 2

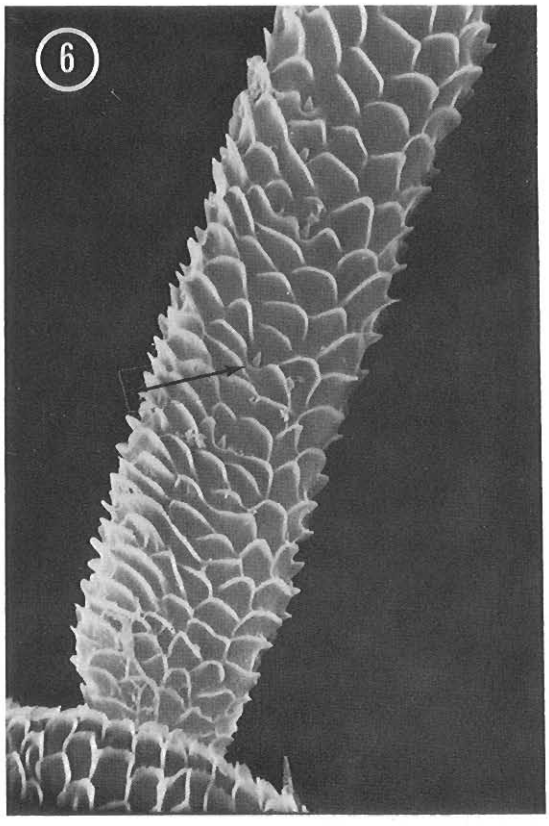
EXPLANATION OF FIGURES

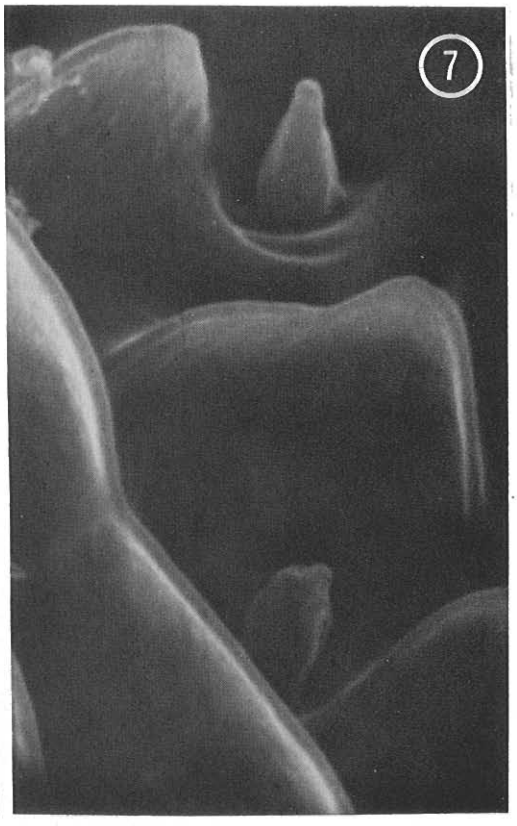
All of female *Stenacron interpunctatum*.

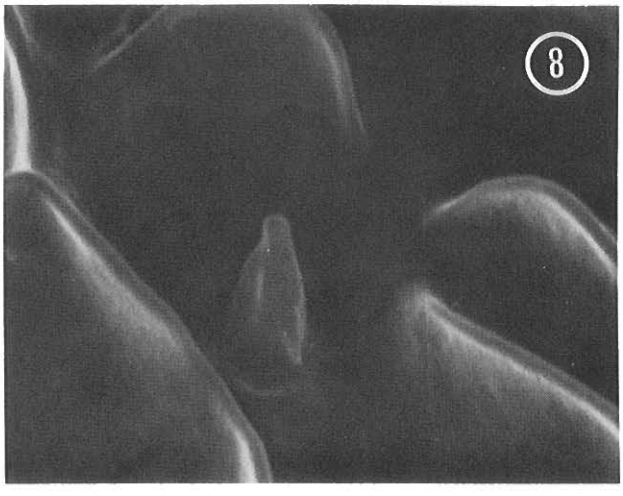
6 Scanning electron micrograph of basal portion of flagellum in ventro-lateral view. Note surface sculpturing. Eight pegs visible, one at arrow. × 1,040.

7 Scanning electron micrograph showing two pegs. × 10,500.

8 Scanning electron micrograph of peg. Depression at tip is probably a pore. × 10,500.


6


7


8