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Functional morphology of antennal chemoreceptors of the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae)

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Abstract

The specialist parasitoid, *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), uses chemical cues from plants damaged by herbivore-feeding and also plant by-products in host location and acceptance. These chemicals are detected by the wasp's antennae. We conducted scanning and transmission electron microscopy studies of male and female *M. croceipes* antennae and detected no distinct morphological differences in the chemoreceptors between the sexes. Male antennae are approximately twice as long as female antennae. We found five morphological sensillar types in both sexes: sensilla (s) trichodea were the most abundant and distributed over the whole antenna; s. chaetica were present in low numbers only on the scape and pedicel; and s. basiconica, s. coeloconica and elongated s. placodea were found only on the flagellum. Ultrastructural investigations revealed pore systems on s. basiconica and s. placodea. In s. placodea, sensory neurons run parallel to the longitudinal axis of the sensilla with terminal pores. We recorded responses from single olfactory receptor neurons in s. placodea to two plant-emitted volatiles, *cis*-3-hexenol and ocimene, and two anthropogenic compounds, cyclohexanone and 2-diisopropylaminoethanol. Male receptor neurons were more sensitive than those of females with significantly higher spike frequency being registered from neurons in males. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Microplitis croceipes (Cresson) (Hymenoptera: Braconidae) is a specialist larval parasitoid of two major insect pests: corn earworm, *Helicoverpa zea* (Boddie) and tobacco budworm, *Heliothis virescens* (Fab.) (both Lepidoptera: Noctuidae). Adults of this wasp use both distant and close-range chemical cues to locate and discriminate hosts (Powell and King, 1984; Turlings et al., 1990; Tumlinson et al., 1993).

The success of a female *M. croceipes* as a biological control agent against herbivore pests depends on its ability to locate a host in a complex chemical and physical environment (Powell and King, 1984; Lewis et al., 1988; Whitman and Eller, 1992). When the host herbivore, a moth larva, feeds on a plant, the plant is induced to release volatile chemicals that attract parasitoids to the herbivore habitat and to the plant (Vet and Groenewold, 1990; Tumlinson et al., 1993). At a distance, the parasitoid uses chemical cues, especially green leaf volatiles, to determine that it is in a habitat that is likely to contain a suitable host larva. At close range, both host-specific synomones from damaged plants and host-produced kairomones, especially feces and other host by-products, are used by the parasitoid as reliable indicators of host presence and suitability (Eller et al., 1988; Whitman and Eller, 1992; Alborn et al., 1995; Turlings et al., 1995).

M. croceipes has demonstrated the ability to learn to respond to and locate different compounds or blends of compounds when its hosts are feeding on different species or varieties of plants (Lewis and Tumlinson, 1988; Lewis et al., 1991). This discrimination ability also becomes apparent after associative learning. Naive wasps do not differentiate between two very similar odor blends, but they subsequently prefer to fly upwind in response to one of the blends if they have experienced a reward in association with that blend (Lewis et al., 1991).

Like most other parasitoids, *M. croceipes* use antennal chemoreceptors to detect such blends and initiate habitat location, host location, and assessment. The antennae play an important role in host location in virtually all parasitic wasps, as demonstrated by various experiments involving partial and total antennal excisions (Hays and Vinson, 1971; Weselow, 1972). Although attention has been focused on the importance of potential olfactory receptor organs,

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mainly the elongated sensilla placodea that abound on nearly all species of parasitic wasps (Quicke, 1997), little consideration has been given to the role of other antennal chemosensilla in host-location, discrimination, and learning. For example contact chemoreceptor sensilla are numerous on the antennae and ovipositor of parasitic wasps and may be important in host discrimination and association (Alborn et al., 1995).

The aim of this study was to initiate a functional morphological investigation of the antennal sensory structures of *M. croceipes* involved in host location and acceptance behavior, to better understand the complex interactions between the parasitoid-herbivore-host-plant environment. The ultimate goal is to try to use the wasp's chemosensory capabilities to develop a useful biosensor for detecting and locating the sources of chemical agents of interest to defense and precision agriculture.

2. Materials and methods

2.1. Insects

Adult *M. croceipes* used in this study emerged from cocoons obtained from a colony maintained at the Insect Biology and Population Management Research Laboratory, USDA-ARS, Tifton, Georgia. Following emergence, wasps were segregated by sex and maintained in 25 × 25 × 30 cm cages at 23°C, 70% RH, and a 16:8 h light-dark photoperiod. Insects were fed with 10% sugar water and used 1–3 days after emergence.

2.2. Electron microscopy

The antennae for SEM were fixed in 70% ethanol for 3 days at 4°C before dehydration in graded ethanol series followed by critical point drying. Antennae were then mounted on aluminium stubs and sputter-coated with gold/palladium (40:60) in a Polaron E5400 high-resolution sputter coater. Specimens were examined in a JEOL T 330 SEM operated at either 10 or 15 kV.

For TEM, the first set of antennae ($n = 6$, 3 males and 3 females), were fixed in 2.5% glutaraldehyde in 0.1 μ cacodylate buffer overnight at 4°C. The specimens were rinsed in buffer, post-fixed in 1% osmium tetroxide for 2 h at room temperature and dehydrated in ethanol series. The antennae were then embedded in Epon and polymerized at 60°C for 48 h. A second set of antennae ($n = 5$, 1 male and 4 females), were fixed in a mixture of 2% paraformaldehyde: 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.4 for 36 h at 4°C. After post-fixation in 1% osmium tetroxide for 3 h, specimens were dehydrated through acetone series followed by overnight infiltration in 3:1 then 1:1 acetone:-Spurr's resin mixture, and finally polymerized in pure Spurr's resin at 60°C for 48 h. Ultrathin sections from both treatment series were cut with a diamond knife on a LKB ultratome and stained with uranyl acetate and lead citrate before examination in a JEOL 1200EX transmission electron microscope.

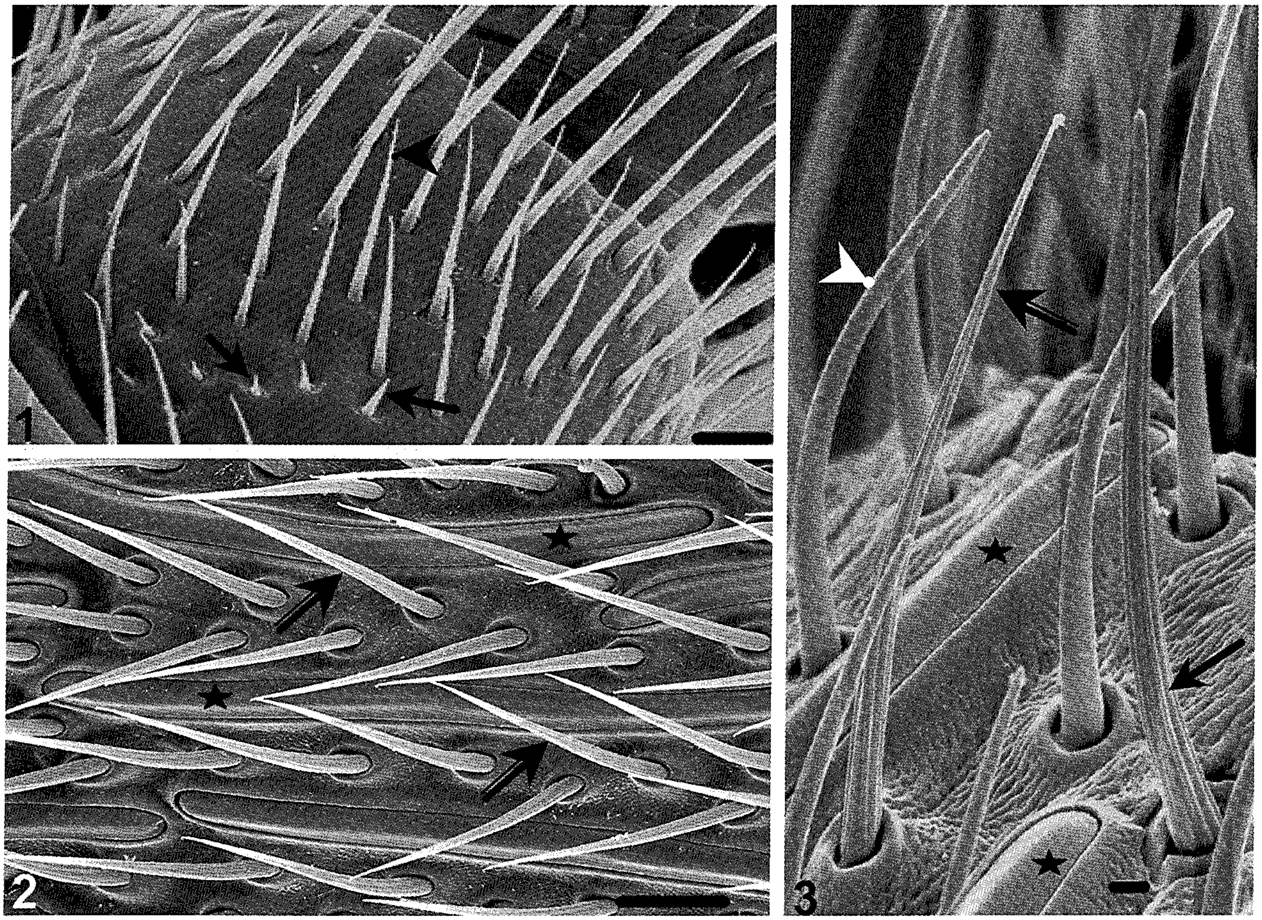
2.3. Electrophysiological methods

Single-sensillum recordings were made with tungsten electrodes (0.2 mm diameter) that were electrolytically sharpened with KNO_2 solution to a tip diameter of about 0.1 μm. The wasp was restrained on a dental wax with tungsten wire hooks. The preparation was mounted on a Syntech Portable Recording Unit, type INR-2 (Syntech, The Netherlands). The recording set-up was viewed at 280 × under an Olympus stereo microscope. At this magnification it was not possible to visualize the individual placoid sensilla from which recordings were made. Therefore, the recording electrode was inserted between the regular rows of antennal cuticular setae to establish contact with the receptor neurons. The indifferent electrode was implanted either into the body of the wasp or into the opposite antenna. The AC signal from the recording electrode was connected to the built-in amplifier of the portable recording unit and the AC output fed into a computer. We processed the data with Syntech AutoSpike version 4.0 software.

A stream of purified and humidified air continuously blew over the antenna (10 ml/s), and was directed through a 14-cm-long stainless steel tube (8 mm ID) whose outlet was positioned 2 cm from the preparation. For each compound, stimulus dilution in HPLC grade hexane was applied as a 10-μl aliquot on a Whatman No.1 filter paper (8 × 30 mm) that was inserted into a Pasteur pipette odor cartridge (14.5 cm in length). Serial dilution of each odorant was presented to the antenna in decadic steps from the lowest (1 μg) to the highest (1000 μg) dose. With a stimulus flow-controller device (Syntech), a 0.2-s air pulse at 10 ml/s-flow rate was injected through the odor cartridge and into the air stream through the pipette tip projecting into a hole in the stainless steel tube (8.5 cm from the outlet). Different odor stimuli were chosen at random, interspersed by 20–30 s.

We tested the responses of representative compounds that have been shown in other studies to be important in *M. croceipes*'s host location behavior. These compounds included *cis*-3-hexenol, a green leaf volatile that is normally released from damaged leaves immediately after commencement of host larval feeding, and ocimene, a representative of inducible monoterpenes that is released by plants several hours later in response to herbivore damage (Turlings et al., 1990, 1995; Röse et al., 1998). In addition, we tested responses to cyclohexanone and 2-diisopropylaminoethanol, anthropogenic compounds that have been shown to evoke electroantennogram (EAG) responses in *M. croceipes* antennae (Park et al., 2001). These compounds (chemical and isomeric purity >99%) were provided by Dr J. H. Tumlinson (USDA, Gainsville, Florida).

For dose-response curves, responses to the solvent controls were subtracted from responses to stimuli. When action potentials were elicited by a compound, their



Figs. 1–3. Fig. 1 Sensilla chaetica (arrows), on the proximal part of the pedicellus and s. trichodea (arrowhead) of various sizes. SEM, scale bar = 10 μm. Fig. 2 Flagellum sub-segment showing elongate s. placodea (asterisks), bounded by s. trichodea (double-stemmed arrows) that form a protective screen. SEM, scale bar = 10 μm. Fig. 3 Close-up view of a flagellum sub-segment showing s. placodea (asterisks), s. basiconica types 1 (arrow), s. basiconica type 2 (arrowhead), and s. trichodea (double-stemmed arrow). SEM, scale bar = 1 μm.

frequency was determined for 500ms following the commencement of excitation. Dose-response curves of individual compounds between the sexes were evaluated by analysis of variance followed by Duncan's multiple-range test.

3. Results

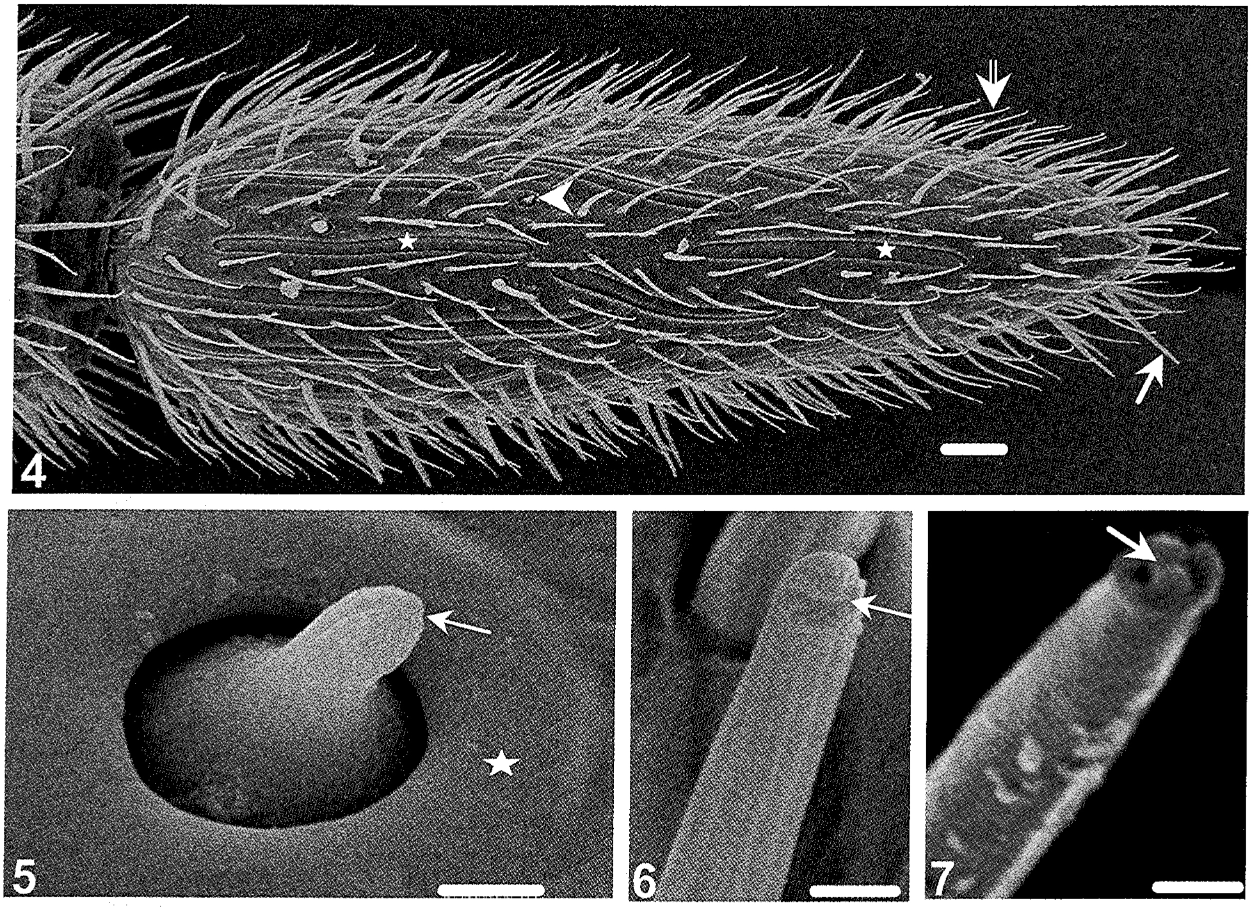
On *M. croceipes* antenna, the flagellum is composed of 16 sub-segments in both sexes. In males, each sub-segment is nearly twice as long as that of females, thus the male antennae are approximately double the length of female antennae. We identified five types of sensilla that are similar in morphology and ultrastructure in both sexes. Sensilla (s) chaetica, s. basiconica, s. trichodea, s. coeloconica, and elongated s. placodea, named according to nomenclature used in previous studies of this species (Norton and Vinson, 1974a; Navasero and Elzen, 1991). Sensilla trichodea, the most abundant type, are present on all antennal segments. Chemoreceptors such as s. basiconica and s. placodea are found on all flagellar sub-segments of both sexes but are lacking on the scape and pedicel, whereas s. chaetica are present only on the scape and pedicel.

Ultrastructural details of s. placodea were better revealed when fixed with glutaraldehyde alone, whereas the paraformaldehyde: glutaraldehyde mixture better preserved s. basiconica and s. trichodea.

3.1. Structure of sensilla

3.1.1. Sensilla trichodea

All antennal segments of both males and females are abundantly covered with s. trichodea (Figs. 1–4). These sharply pointed structures are inclined and slightly curved toward the apex of the segment. Each sensillum exhibits longitudinal grooves that spiral slightly around its surface. The sensillar cuticle is thick and non-porous as revealed by TEM (Fig. 10). The sensilla vary in length from approximately 20 μm on the scape and pedicel to approximately 60 μm on the flagellum. The base is inserted in a flexible socket, which is slightly elevated above the cuticle (Figs. 2 and 3).



Figs. 4–7. Fig. 4 Terminal flagellum sub-segment of a female wasp showing distributions of s. placodea (asterisks), s. basiconica type 1 that extend above other cuticular setae (arrow), s. trichodea (double-stemmed arrow), and s. coeloconica (arrowhead). SEM, scale bar = 10 µm. Fig. 5 Coeloconic sensillum with bulb-like terminal (arrow) and surrounded by a doughnut-shaped ring (asterisk) at the base. SEM, scale bar = 1 µm. Fig. 6 Basiconic sensillum type 1 with a nipple-like projection at the tip (arrow), and grooved cuticular surface. SEM, scale bar = 1 µm. Fig. 7 Terminal portion of a smooth-walled s. basiconicum type 2, showing finger-like pore slits (arrow). SEM, scale bar = 1 µm.

3.1.2. Sensilla chaetica

These sharply pointed setae are similar in shape to s. trichodea but much shorter in length, approximately 3–6 µm and possess blunt tips compared to s. trichodea (Fig. 1). They are found only in the proximal section of scape and pedicel in both sexes and are much fewer in number compared with other sensillar types. S. chaetica are inserted in cuticular depressions.

3.1.3. Sensilla basiconica type 1

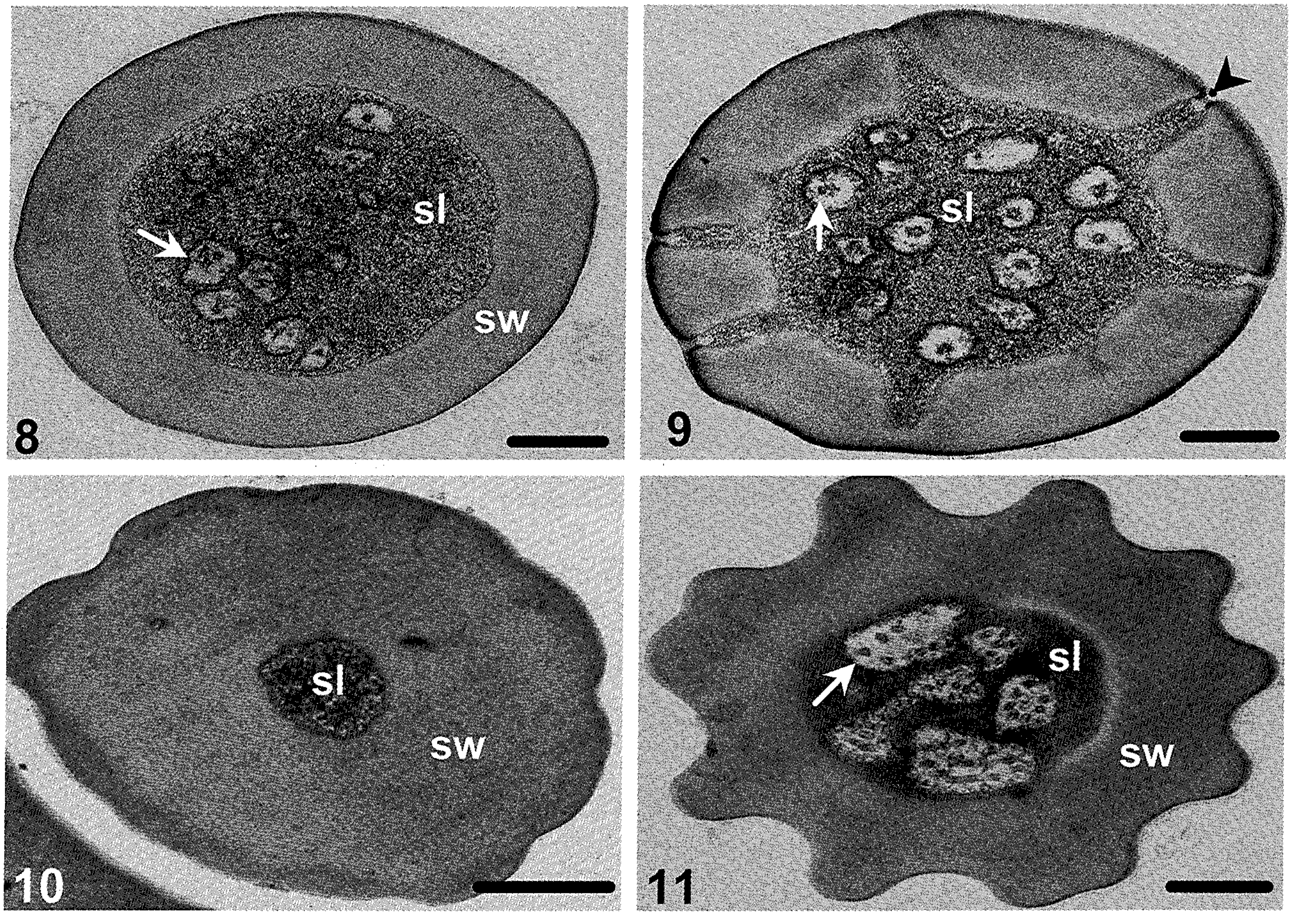
These sensilla are found on all flagellar sub-segments of both sexes and are especially evident at the distal portion of each flagellomere. They are characterized by a grooved surface and project slightly more perpendicularly with respect to the axis of the antenna than do s. trichodea and s. basiconica type 2 (Figs. 3 and 4). A thick nonporous cuticular wall surround an inner lumen innervated by up to six dendritic branches (Fig. 11). The length of these thick-walled sensilla varies from 15 to 25 µm, whereas their basal diameter ranges from 1.5 to 2 µm. Because of their length and orientation, the tips of s. basiconica type 1 are well above the level of other sensilla (Fig. 4). The tips of sensilla at the terminal flagellomere form a flap with slits, suggestive of contact chemoreception function (Fig. 6).

3.1.4. Sensilla basiconica type 2

These thin-walled sensilla are present in both sexes and are distributed more in the mid- and proximal-portions of each flagellum sub-segment. Each sensillum is gradually curved distally with a blunt tip and smooth cuticular surface (Fig. 3). They are olfactory hairs with branched dendritic processes and cuticular pores (Figs. 8 and 9). On the distal two flagellar sub-segments of females, s. basiconica type 2 with nipple-like pore-slits at the tip, suggestive of contact chemoreception function, were found (Fig. 7).

3.1.5. Sensilla coeloconica

These sensilla are located on the dorsal surface of the flagellar sub-segments of both sexes (Figs. 4 and 5). There are usually one and at most two s. coeloconica per segment. They are located in sunken depressions and surrounded by a doughnut-shaped ring (Fig. 5). These sensilla terminate in a



Figs. 8–11. Fig. 8 Transverse section of the proximal portion of s. basiconicum type 2. The sensillum wall (*sw*) is non-porous at this level. Dendritic branches (arrow) are present within the sensillum lymph (*sl*). TEM, scale bar = 200 nm. Fig. 9 Transverse section of the mid portion of s. basiconicum type 2 with porous sensillum wall (arrowhead) and dendritic branches (arrow), bathed in the sensillum lymph (*sl*). TEM, scale bar = 200 nm. Fig. 10 Transverse section of the base of trichoid sensillum with thick non-porous sensillum wall (*sw*), and sensillum lymph (*sl*) that is not innervated. TEM, scale bar = 500 nm. Fig. 11 Transverse section of the mid portion of s. basiconicum type 1 with non-porous sensillum wall (*sw*), and dendritic branches with tubules (arrow) within the sensillum lymph (*sl*). TEM, scale bar = 200 nm.

bulb-like tip; length varies from 2 to 2.5 μm, with the basal diameter ranging from 2 to 3 μm.

3.1.6. Elongated sensilla placodea

The placoid sensilla are numerous on all flagellar subsegments of both sexes. These sensilla are slightly elevated above the antennal surface and are located between the rows of s. trichodea and basiconica (Figs. 2–4). The sausage shaped sensillum is surrounded by a grooved gap and a cuticular ridge (Fig. 13). They are aligned generally in parallel with the antennal axis. Neuronal cell bodies (numbering 4–6, data not shown) are located proximally in each plate with their dendrites projecting distally within the medial channel along the sensillum axis. The dendrites branch profusely once they reach the distal end that contains a complex cuticular pore system (Figs. 12 and 13). The dimensions of these sensilla vary greatly, with lengths ranging from 60 to 100 μm, and a width of approximately 3 μm.

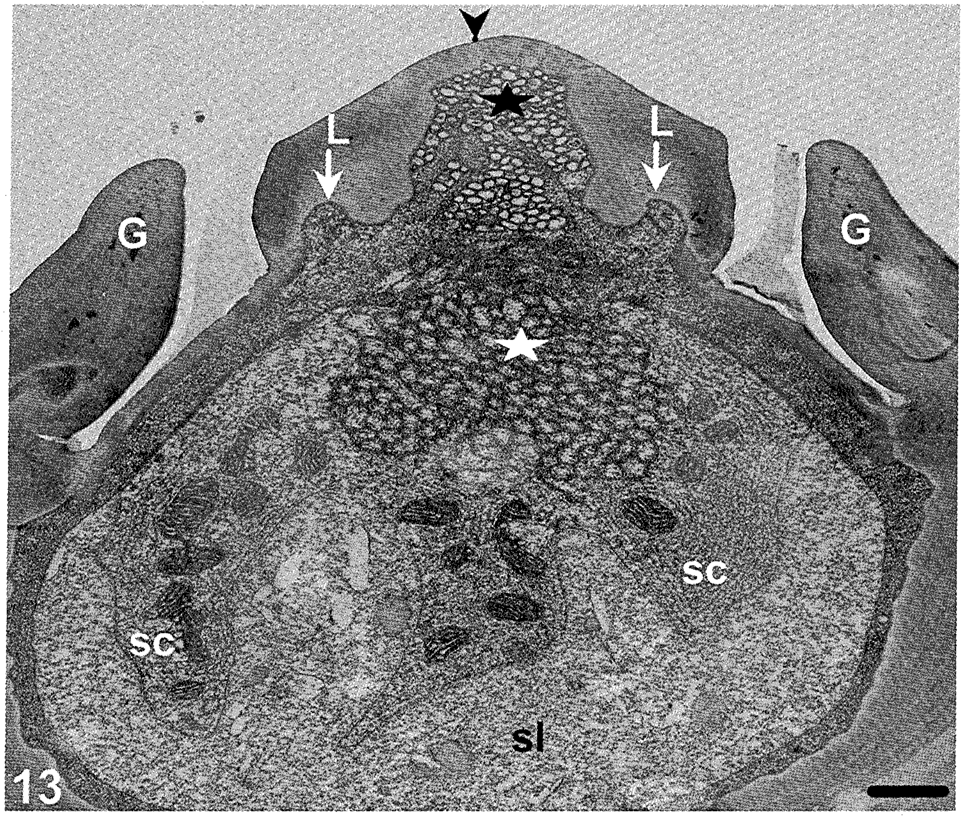
Table 1
Numbers of elongated placoid sensilla on flagellar subsegments of the antennae of male and female *M. croceipes* ($n = 3$)

	Flagellar subsegments (mean ± S.E.)					
	1st	2nd	8th	9th	15th	16th
Female	89 ± 8	123 ± 7	133 ± 2	126 ± 0	87 ± 2	101 ± 1
Male	225 ± 1	243 ± 3	240 ± 11	216 ± 7	153 ± 5	158 ± 6

The number of placoid sensillar per segment is higher on the medial and proximal segments than on distal segments. There are about twice as many placoid sensilla per flagellomere on male antennae compared with female antennae (Table 1).

3.2. Single-sensillum responses

We successfully established contacts with receptor neurons housed in s. placodea in 63 attempts to test various odorants at different concentrations. Of these contacts, 27



Figs. 12 and 13. Fig. 12 Longitudinal section from a placoid sensillum. Dendritic branches (arrows) run parallel to the sensillar axis and end with cuticular pores (arrowhead) at the sensillum tip. TEM, scale bar = 2 μm. Fig. 13 Transverse section in the terminal region of a placoid sensillum showing elevated grooves (*G*) that surround sensilla; lateral channels (*L*); and numerous inner- and outer-dendritic branches (open and filled asterisks, respectively), within the median channel. Within the sensillum lymph (*sl*) are supporting cells (*sc*) with numerous mitochondria. TEM, scale bar = 500 nm.

(male = 11, and female = 16) were used for dose-response curves. Generally, action potentials of varying heights occurred in each recording to the selected compounds (Fig. 14), suggestive of contacts with more than one receptor neuron. We were not able to resolve consistent differences in spike amplitudes and waveforms to identify activities of individual neurons.

For all compounds tested, the threshold value in males for the ensemble of neurons in each sensillum was at the 1-μg dose on filter paper, whereas in females the threshold value was between the 10- and 100-μg dose (Fig. 15). There were no significant differences in the responses of neurons to the different compounds tested in the same sex at a given dose. However, responses from the male neurons were of significantly higher frequency ($P < 0.05$) than those of females at most doses.

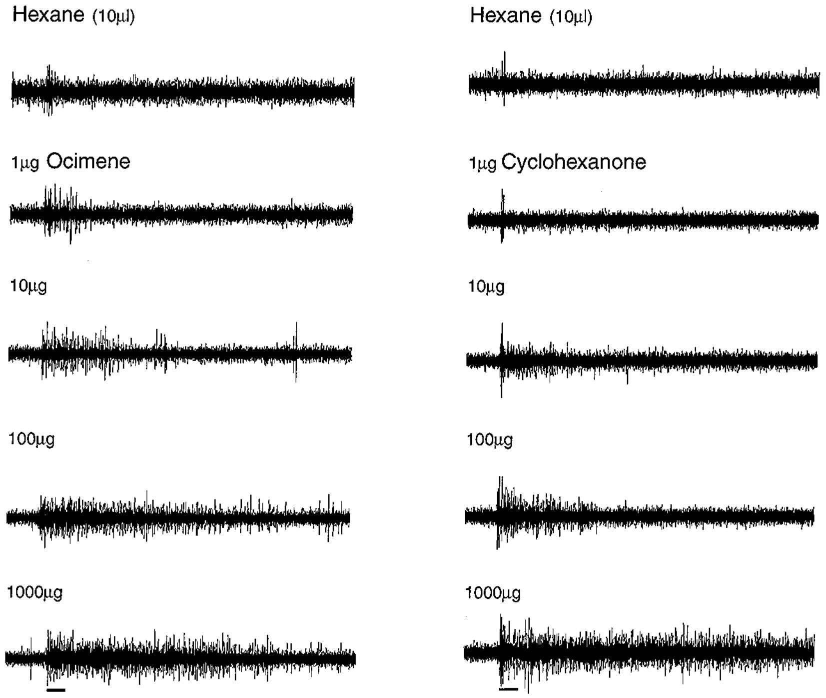


Fig. 14. Typical single-sensillum responses of male *M. croceipes* antennal neurons to emissions from 1- to 1,000-µg cartridges of ocimene and cyclohexanone. Scale bar represents stimulus duration (0.2 s).

4. Discussion

The antennae of *M. croceipes* play a vital role during host-finding and acceptance behaviors (Powell and King, 1984; Tumlinson et al., 1993; Alborn et al., 1995). Prior to this study, no ultrastructural investigation of antennal cuticular setae had been reported in *M. croceipes*. However, thin-walled and thick-walled chemoreceptors and tactile

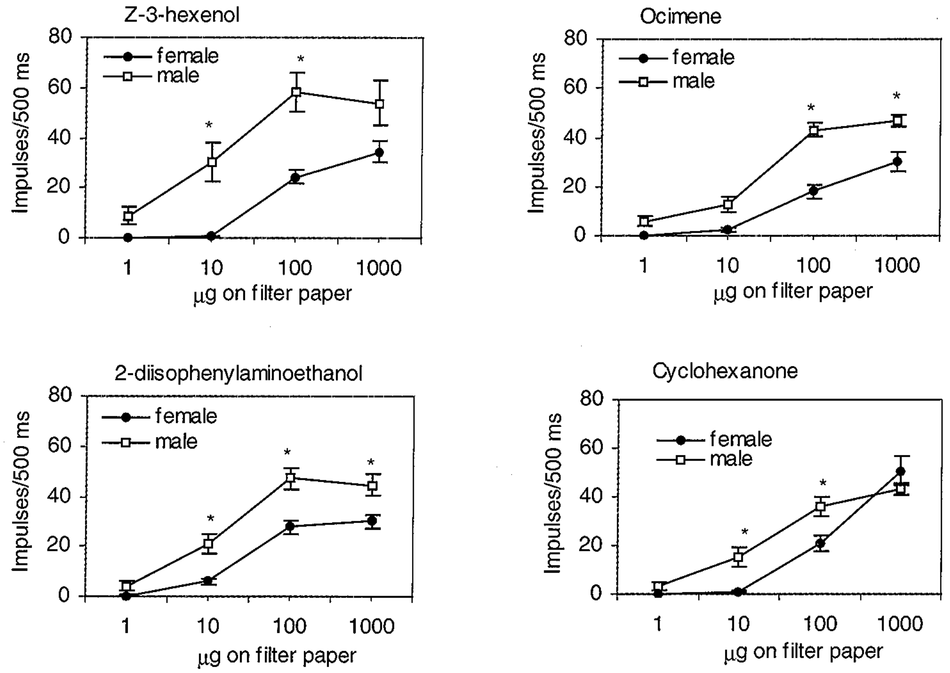


Fig. 15. Dose-response curves from receptor neurons of male ($n = 11$) and female ($n = 16$) *M. croceipes* to serial stimulus loads of cis-3-hexenol, ocimene, 2-diisophenylaminoethanol and cyclohexanone. The response of a stimulus was quantified as the net number of spikes during initial 500 ms minus the net blank (hexane) response. Action potentials of different sexes at the same dose with asterisk are significantly different ($P < 0.05$; Duncan's multiple-range test). Error bars are ±S.E.M.

receptors have been found on the antennae of various parasitic Hymenoptera (Norton and Vinson, 1974b; Barlin and Vinson, 1981; Navasero and Elzen 1991). These studies, in agreement with our TEM examination, did not find any pore systems on the s. trichodea and therefore no olfactory function could be attributed to this sensillar type. But due to their socket-like insertion into the antennal cuticle and their spatial arrangement, s. trichodea may serve mechanoreceptive functions in *M. croceipes*.

Sensilla chaetica may also serve a mechanofunction in *M. croceipes*, and possibly as proprioreceptors perceiving antennal movement and position based on their location in the proximal region of the pedicel as well as the intersegmental membrane between the scape and head.

TEM investigations in our study revealed multiple pores in the cuticle of the smooth, thin-walled s. basiconica type 2, which suggests an olfactory function (Steinbrecht, 1984). The s. basiconica type 2 with multiple cuticular pores were not observed in previous studies of *M. croceipes* (Norton and Vinson, 1974a; Navasero and Elzen, 1991). Furthermore, our study extends the findings of Navasero and Elzen (1991) that both s. basiconica types 1 and 2 are present on the antennae of both sexes.

Similar to previous studies, we found s. coeloconica in both sexes numbering between one and two per flagellomere. The shape and cuticular insertion of this sensillar type are similar to that of other insects (Altner et al., 1983; Steinbrecht, 1984). Coeloconic sensilla are found in many Hymenoptera belonging to several families and have been described by different terminologies, such as 'small subterminal sensilla' (Weselow, 1972), 'multiporous grooved sensilla' (Barlin et al., 1981), 'bulb sensilla' (Cave and Gaylor, 1987), and 'smooth basiconic sensilla' (Norton and Vinson, 1974a). Olson and Andow (1993) suggested an olfactory function of this sensillar type in *Trichogramma nubilale* (Ertle and Davis), but as revealed in several insect species (Altner et al., 1983), this sensillar type may be involved in thermo- or hygroreception.

Prior to our study, physiological recordings from individual s. placodea have only been reported from honey bee, *Apis mellifera* L. (Lacher and Schneider, 1963 as cited by Bin et al., 1989), and more recently from the scarab beetles (Hansson et al., 1999; Larsson et al., 1999). We have therefore shown that the elongated s. placodea in parasitic wasps are indeed olfactory receptors. Richerson et al. (1972) suggested that placoid sensilla in *Coloides brunneri* Viereck could be involved in infrared radiation detection. In subsequent behavioral investigations, however, Mills et al. (1991) failed to demonstrate infrared detection ability in the braconids *C. bostrychorum* Gir. and *Dendrosoter middendorffii* (Ratz.), as well as in the pteromalid, *Rhopalicus tutela* Walk.

Sensilla placodea have been found in abundance on the antennae of nearly all parasitic Hymenoptera studied to date (Barlin and Vinson, 1981). In most of previous reports, a putative function for s. placodea has been assigned based purely on morphology, and their possession of a multiple cuticular pore system typical of olfactory chemoreceptors (Steinbrecht, 1984).

Our study also has shown that neurons in the placoid sensilla responded in a dose-dependent manner to plant volatiles as well as to the anthropogenic compounds cyclohexanone and 2-diisopropylaminoethanol. The importance of plant volatiles in host finding has been proven for several parasitic wasps, including *M. croceipes* (Eller at al., 1990; Whitman and Eller, 1990; Tumlinson et al., 1993; Röse et al., 1998). Female parasitic wasps use plant volatiles in the initial stages of orientation to and location of host insects for oviposition. Males, in contrast, may use plant-related chemical cues, possibly in conjunction with sex pheromones, to locate conspecific females (Powell and King, 1984; Whitman and Eller, 1992; Alborn et al., 1995).

In parasitic wasps, EAG responses to host-plant volatiles have been recorded from *M. croceipes* (Li et al., 1992; Park et al., 2001) and *M. demolitor* Wilkinson (Ramachandran and Norris, 1991). In these EAG studies, no marked differences in dose-response curves to plant volatiles were discerned between males and females. However, in the present single-sensillum recordings, dose-response curves revealed relatively higher action potential frequencies from males than from females to all compounds tested. The higher sensitivity of male receptor neurons compared with those of females to volatile odorants would be understandable if it were related to sex pheromone reception, but it is not. This finding is surprising and thus far unexplainable. The ability of cis-3-hexenol and ocimene to excite antennal receptor neurons is consistent with these compounds as being part of the plant-produced blend that evokes upwind flight in females (Whitman and Eller, 1990, 1992; Lewis et al., 1988; Turlings et al., 1990; McCall et al., 1993; Tumlinson et al., 1993).

The significant neuronal responses to the anthropogenic compounds used in our study demonstrates the olfactory flexibility of *M. croceipes* antennal receptors, even responding to compounds that the wasp would not have heretofore encountered in nature. Female *M. croceipes* are able to associatively learn host odors, but they also are able to be conditioned to fly to a novel plant-related odor such as vanilla extract (Lewis et al., 1991). The receptor system of *M. croceipes* seems to be broadly tuned and able, therefore, to respond to a wide array of odorants and odor blends with which they can associate their host larvae. *M. croceipes* females have also demonstrated an ability to distinguish host-related odors from non-host odors in choice tests (Eller et al., 1988; Zanen and Cardé, 1991), as well as to differentiate odors emitted from different caterpillars feeding on the same plant variety (Turlings et al., 1995).

Studies of female parasitic Hymenoptera are common, mainly because they attack the herbivore immature stages for oviposition and are thus important for biological control of herbivore pests. However, in morphological studies of several parasitic wasp species, contact chemoreceptors have been reported in both sexes (Bin et. al. 1989). We found abundant contact chemoreceptors on male *M. croceipes* antennae as well as on female antennae, but we do not know what role contact chemoreceptors would play in influencing male behavior.

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