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The antennal sensilla of the praying mantis *Tenodera aridifolia*: A new flagellar partition based on the antennal macro-, micro- and ultrastructures

Thomas Carle [a,*], Yoshihiro Toh [b], Yoshifumi Yamawaki [b], Hidehiro Watanabe [a], Fumio Yokohari [a]

[a] Division of Biology, Department of Earth System Science, Faculty of Science, Fukuoka University, Fukuoka 814-0180, Japan
[b] Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan

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ABSTRACT

In insects, the antenna consists of a *scapus*, a *pedicellus*, and a *flagellum* comprising many segments (flagellomeres). These segments possess many morphological types of sensory organs (sensilla) to process multimodal sensory information. We observed the sensilla on flagellomeres in praying mantis (*Tenodera aridifolia*) with both scanning and transmission electron microscopes. We classified the sensilla into six types: chaetic, campaniform, coelocapitular, basiconic, trichoid and grooved peg sensilla, and inferred their presumptive functions on the basis of their external and internal structures. In addition, based on their distribution, we newly divided the *flagellum* into 6 distinct parts. This new division leads to a better understanding about the sexual dimorphism and the antennal development in the mantises. The sexual difference in distribution of the grooved peg sensilla suggests that this type of sensilla may play a role in sex-pheromone detection in mantis, which is a rare case of double-walled sensilla mediating this function.

1. Introduction

For animals, peripheral sensory systems are pivotal structures enabling them to receive information about their environment to seek food, habitats and sex partners, and also to avoid danger. The praying mantis is basically an ambush predator mostly waiting for prey. To detect and attack prey, it is equipped with binocular vision using a pair of compound eyes (Rossel, 1979, 1986). Because of these characteristics, their visual system has been well studied (Prete, 2004; Yamawaki, 2006), but other species have been preferred for investigation of the insect olfactory system (Hansson, 1999). However, although mantises rely highly on their vision in their courtship and feeding behaviors (Lelito and Brown, 2008; Rilling et al., 1959), their olfaction also plays an important role in these behaviors (Holwell et al., 2007; Prete et al., 1992). For example, *Sphodromantis lineola* can make ingestion decisions based on the odor of banana (Prete et al., 1992). In the courtship behaviors of some genera (*Pseudomantis albofimbriata* (Holwell et al., 2007), *Hierodula majuscula* (Allen et al., 2012), and *Tenodera aridifolia* (Bartley, 1982; Lelito and Brown, 2008)), males are able to use olfaction to find females. In these species, males may detect the sex-pheromones released by females and fly toward a sexual partner (Maxwell et al., 2010; Roeder, 1935).

In insects, the sense of smell is mediated by antennae that are important macrostructures providing chemosensory (olfactory or gustatory), mechanosensory, hygrosensory, and thermosensory information (Iwasaki et al., 1999; Ozaki and Tominaga, 1999; Steinbrecht, 1999; Yokohari, 1999). Each antenna consists of antennomeres, and every insect species shows a similar antennomere organization with a *scapus*, *pedicellus*, and *flagellum* (Hansson, 1999). The *flagellum* comprises segments, called flagellomeres, whose number and shape vary among species. The sensory organs of antennae (sensilla) are microstructures distributed on their surface, and their shapes differ depending on their functions. Each sensillum contains sensory neurons whose cilia spread along its cuticular apparatus (Altner et al., 1980; McIver, 1974, 1978; Steinbrecht and Gnatzy, 1984). Usually, the sensilla are classified based on their ultrastructures, such as the presence or

* Corresponding author.
E-mail address: th.carle@gmail.com (T. Carle).

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absence of wall pores, the structure of the side wall (single-walled or double-walled) and the socket (joint membrane) (Hansson, 1999). The study of these ultrastructures is essential for a better understanding of the functional aspects of sensilla (Hansson, 1999).

Although to date mantis antennae have only been investigated using light and scanning electron microscopes (Allen et al., 2012; Faucheux, 2008; Holwell et al., 2007; Slifer, 1968), observations of their internal structures are essential to improve their classification and to identify their functions and transduction mechanisms. The aim of the present paper is to investigate the macro-, micro-, and ultrastructures of antennae in *T. aridifolia* using scanning and transmission electron microscopes. Our results produced clear information about the ultrastructure of the sensilla, leading to a better understanding of their functions in mantises. In addition, we investigated the variations and sexual difference in sensillar distribution along the antennae, and devised a new division of the *flagellum*. This division enabled us to better understand sexual dimorphism in mantises and, in a general manner, their antennal development.

2. Methods

2.1. Insects

Male and female mantises (*T. aridifolia*) were used in this experiment. Oothecae were collected in the wild around Fukuoka City (Japan), and the nymphs were bred by methods previously described (Yamawaki, 1998; Yamawaki and Toh, 2003). From the 1st to the 3rd/4th instars, the nymphs were bred in groups with a food diet consisting only of fruit flies. After reaching the 3rd/4th instars, they were individually housed and fed with crickets until adulthood.

2.2. Scanning electron microscopy (SEM)

Adult mantis antennae were sampled and kept in a solution of acetone (50%). Isolated antennae were ultrasonically cleaned in 50% acetone for 3 min (Bransonic B1510, Yamato). Thereafter, they were dehydrated in an ascending series of acetone solutions (from 50 to 100%), air dried, and then coated with platinum-palladium by an ion sputter (E-1045; Hitachi, Tokyo, Japan). Digital images were acquired using a field emission scanning electron microscope (S-4800; Hitachi), and were analyzed with GIMP (GNU software).

2.3. Transmission electron microscopy (TEM)

Adult mantis flagellomeres were doubly fixed in 3% glutaraldehyde and 2% OsO_4, dehydrated through a graded series of ethanol solutions, and embedded in Epon 812 using the standard methods described previously (Toh, 1977). Ultrathin sections were cut on a Porter-Blum MT2 ultramicrotome and double-stained with uranyl acetate and lead tartrate. The collected stained sections were then examined with a transmission electron microscope (H-7000, Hitachi).

2.4. Electrophysiology

Adult mantises were placed in a supine position on an acrylic plate, and the head, thorax, abdomen, and legs were fixed using utility wax. The antennae were stuck on a small layer of bees wax by making fixation points along the antennae. To measure the activity of sensilla, tungsten electrodes (0.5 mm in diameter) were used. We electrolytically sharpened the tip end of electrodes in a potassium nitrite solution. The mass electrode was inserted at the base of antennae and the active electrode was inserted into the basiconic sensillum just deep enough to make an electrical contact. The potentials were recorded using a microelectrode amplifier (MEZ-8301, Nihon Kohden). The signals were amplifier at X500 and filtered at 2 kHz and 300 Hz through a high/low pass filter (Dagan corporation), and were usually digitized and recorded at a sampling rate of 10 kHz/s using a power lab 4/30 (ADInstruments) and the Labchart software (ADInstruments). The data were converted and further analyzed using Clampfit 10.3 (Axon Instruments).

Three different odors were used: 1-Hexanol (Wako pure chemical industries, Ltd.), α-Terpineol (TCI), and banana flavor (Mikoya). The odors were diluted at 1/10 in ethanol (100%) and 50 μl was put on an absorbent paper placed inside a capillary pipette. The olfactory stimuli were applied through an apparatus as previously described (e.g. Yokohari and Tateda, 1976). The duration of the stimuli was 2 s followed by a 30-s interval; the stimuli were applied using a stimulator (Nihon Kodhen) synchronized with the signal recording.

3. Results

3.1. Sensilla identification of the flagellum

Based on the morphology of external features of sensilla observed with the SEM, we classified the antennal sensilla into 6 types: chaetic (*s. chaetica*), campaniform (*s. campaniformia*), coelocapitular (*s. coelocapitula*), grooved peg, basiconic (*s. basiconica*), and trichoid (*s. trichodea*) (Fig. 1 and Table 1). In order to standardize the nomenclature with that of other insect species, our classification was correlated to that established by Hansson (1999). Except for the grooved peg sensilla, each type of sensillum had a specific and restricted distribution pattern along each flagellomere. The trichoid sensilla were further classified into 3 types, labeled 1–3, based on distinct localization patterns and their length (Fig. 1B and Table 1). The type 1, which was the longest of the three types (50 μm at the distal part of the antennae), was observed in the distal part of each flagellomere, whilst the type 2 (40 μm) occupied the proximal part. The type 3 was the shortest (20 μm) and was found around the chaetic sensilla. Although the length of the cuticular apparatus differed between these subtypes, there were no differences in internal structures.

3.2. Structure of sensilla

The chaetic sensilla are very long (~110 μm) with a grooved surface, a terminal pore at the tip and a flexible socket at the base (Fig. 2A–C). Transmission electron micrographs revealed that a thick single-walled cuticle lacks pores and surrounds the sensillar outer lymph cavity (Fig. 2D). In the inner lymph cavity, four sensory cilia spread along each sensillum and are isolated from the outer lymph by a dendritic sheath (Fig. 2E). Near the base of the cuticular apparatus, another sensory cilium appears whose profile might be a tip of the tubular body (arrow in Fig. 2G). At this level, each sensory cilium is partially isolated due to compartmentalization of the inner lymph cavity by thin walls. None of the sensory cilia ramifies along the sensillum. Inside the cilia, the number of microtubules depends on the diameter of the cilium.

The grooved peg sensilla are short (~5–15 μm) and stand in a small shallow depression (Fig. 3). The outer surface of the cuticle is sculptured with many longitudinal grooves along most of its length and has a smooth surface at its basal part. Although every grooved peg sensillum shares these features, there are some differences in length, number of grooves (12–22 grooves), and terminal structure (clogged or open with an irregular pore) (Fig. 3A–D). Internally, the grooved peg sensilla possess a thick double-walled cuticular apparatus (Fig. 3E) equipped with spoke canals (Fig. 3F) around an