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Ultrastructure of antennal sensilla of an autoparasitoid *Encarsia sophia* (Hymenoptera: Aphelinidae)

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ABSTRACT

Encarsia sophia (Hymenoptera: Aphelinidae) is a parasitoid utilized for biological control of *Bemisia tabaci*, with selection of prey aided by chemoreceptor organs. The morphology and distribution of the antennal sensilla (chemoreceptors) of *E. sophia* were examined using Transmission electron micrographs. The total antennal length for *E. sophia* was 429.28 ± 0.95 μm for females and 437.19 ± 8.21 for males, and each antennae was found to consist of seven sensilla of different types. Both sexes possessed sensilla chaetica, sensilla trichodea, basiconic capitate peg sensilla, multiporous grooved-surface placoid sensilla (MG-PS), uniporous rod-like sensilla, nonporous finger-like sensilla, and sensilla coeloconica. Transmission electron micrographs of longitudinal sections of female antennae showed that they were composed of fat body, cuticle, mesoscutello-metanotal muscles, neurons, and glandular tissue, and cross-sections of the basal MG-PS showed sensillar lymph cavities and dendrites. The MG-PSs were imbedded in an electron-dense mass with cuticular invaginations which acted as pores that connected to a central lumen. The possible function of each type of sensilla is discussed.

1. Introduction

Chemoreceptors are found on the antennae and on a series of functional ultrastructures on the surface of insects. The ability of insects to detect environmental signals with these receptors has been well studied (Schneider, 1964). Chemoreceptors are involved in a variety of processes, such as searching for prey, courtship, and communication. Chemoreceptors detect volatile-chemical trails that are emitted by animals and plants, and aid in the selection of a suitable host via direct contact (Hays and Vinson, 1971; Vinson, 1984). The morphological differences among the sensilla of various species of insects demonstrate significant variation in function and the types of environmental signals detected (Schneider, 1964).

Many studies of the ultrastructure of sensilla have focused on deducing function, and have revealed that the type and structure of sensilla located on the antenna greatly influence the type of information that insects are aware of (Schmidt and Smith, 1985; Zhou et al., 2013). Many hymenopteran species are parasitoids, including members of the genus *Encarsia*. Host specificity is common in these parasitoids; they preferentially parasitize specific hemipterans and lepidopterans, with host selection behaviors during oviposition of the male and female eggs (Hunter and Woolley, 2001). As a key environmental sensor, the sensillas of parasitoids are prime candidates for determining behavioral relationships between the parasitoid and the host.

Certain members of the genus *Encarsia*, which are quite small in size, are important obligate parasitic enemies of the globally distributed homopteran pest *Bemisia tabaci*, and are effective in controlling the spread of *B. tabaci* if introduced early during an outbreak. A member of the family Aphelinidae, *Encarsia sophia* is a parasitic wasp that is native to India, and has been shown to be an effective biological control species against *B. tabaci* (Antony et al., 2003). *E. sophia* exhibits arrhenotokous, heteronomous, and autoparasitoid reproduction. Female eggs are laid internally in whitefly nymphs and develop as primary parasitoids, whereas males develop as hyperparasitoids either on females of their own species or on other primary aphelinid parasitoids. Further, females

Abbreviations: SEM, scanning electron microscopy; TEM, transmission electron microscopy; SEMGs, scanning electron micrographs; TEMG, stransmission electron micrographs; RA, radicula; SC, scape; PE, pedicel; F1–F6, the six flagellar segments; CH, sensilla chaetica; ST, sensilla trichodea; BCPS, basiconic capitates peg sensilla; MG-PS, grooved-surface placoid sensilla; CS, sensilla coeloconica; PO-UP, uniporous rod-like sensilla; FL-NP, nonporous finger-like sensilla; FG, flagellum; GL, glandular tissue; TB, tubular body; epd, epidermal cells; cut, cuticle; FB, fat body; MMM, mesoscutello-metanotal muscle; MC, molting channel; DE, dendrites; NE, neurons; mit, mitochondria; SL, sensillum lymph cavities; edm, electron-dense mass; OSL, outer sensillum lymph; SW, thick sensillar wall; ISL, inner sensillar lymph.

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develop as primary endoparasitoids in whitefly nymphs, and males develop as secondary ectoparasitoids on female *E. sophia* or other primary aphelinid parasitoids (Antony et al., 2003). Thus, host selection is very important for *E. sophia*, both for reproduction and for controlling the sex ratio of their offspring (Kochetova, 1977). Parasitoid species display behavioral adaptations for the following four aspects of oviposition selection: habitat selection, host tracking, host acceptance, and host suitability (Viggiani, 1984). It is likely that the antennal sensillar play an important role in these behaviors.

E. sophia was recently imported to the northern, central, and southeastern regions of mainland China for biological control applications. Despite its widespread use, basic research on the host-selection behavior of *E. sophia* remains in its infancy. Studies of the microstructure of *Encarsia* sensilla are scarce, and are limited primarily to those of *E. amicula* (Wang and Huang, 2007) and *E. guadeloupae* (Zhou et al., 2013). *Encarsia* have abundant mechanosensory and olfactory antennal sensilla, including sensilla chaetica, sensilla trichodea, multiporous sensilla placoid, uniporous rod-like sensilla, and nonporous finger-like sensilla, which they use to locate whitefly nymphs. Studies of the microstructure of the sensilla of *E. sophia* might identify structural variations that influence detection efficiency of *B. tabaci* through variable uptake of chemical messengers. We examined the distribution and structure of the various antennal sensilla and the internal structure of the antennae of *E. sophia* using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to gain insight into functions, and to further our understanding of the chemical basis of their host-selection behavior.

2. Materials and methods

2.1. Insects

The jiyou-768 transgenic strain of *Gossypium hirsutum* (cabbage), which expresses a toxin derived from *Bacillus thuringiensis* (BT-cotton), was used as the host plant for *E. sophia*, and was kindly provided by the Texas AgriLife Research, Texas A&M University System at Weslaco. *E. sophia* were reared on *B. tabaci* nymphs in a greenhouse at 25–28 °C at the Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, China. Cotton leaves with parasitized nymphs were kept in 9-cm petri dishes. The newly enclosed male and female adult parasitoids were maintained in separate petri dishes, and were fed 10% aqueous solution of honey.

2.2. Scanning and TEMGs methods

For SEM, 30 adult *E. sophia* were placed in a 1.5-mL centrifuge tube, and rinsed three times with phosphate-buffered saline (PBS) at pH 7.0. The insects were fixed in 2.5% glutaraldehyde for 24 h at 4 °C, and washed three times for 15 min with 0.1 M phosphate buffer, pH 7.0. The specimens were dehydrated in a graded ethanol series by incubation in 30%, 50%, 70%, 80%, 90%, and 95% ethanol for 10 min each. The dehydrated specimens were incubated in isoamyl acetate for 15 min, and critical point dried for 1.5 h in an HPC-2 oven (Hitachi, Tokyo, Japan), generally following Zhou et al. (2013). The antennae were removed using an insect needle, and examined using an MZ205 stereomicroscope (Leica, Wetzlar, Germany). The antennae were coated with gold using an E-1010 ion sputter coater (Hitachi Ltd., Tokyo, Japan), and observed using an S-3000N scanning electron microscope (Hitachi Ltd., Tokyo, Japan).

We used TEM to examine the antennae of female insects only. For TEM, female insects were washed in PBS and fixed as described above for SEM. The fixed specimens were dehydrated in 100% ethanol for 20 min, and postfixed in 2% osmium tetroxide for 1.5 h. The postfixed specimens were incubated in acetone for 20 min, and embedded in Epon-812 at 70 °C for 24 h. Ultrathin sections were prepared using an EM UC7 microtome (Leica, Germany). The sections were stained with lead citrate and uranyl acetate, and observed using an H-7650 transmission electron microscope (Hitachi Ltd., Tokyo, Japan).

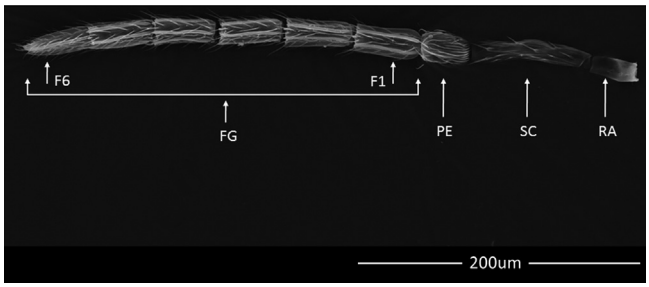

Fig. 1. Scanning electron micrographs (SEMGS) of the antennae of a male *E. sophia*. Radicula (RA), scape (SC), pedicel (PE), flagellum (FG), six flagellar segments (F1–F6).

2.3. Statistical analysis

The number and sizes of 30 pairs of antennal sensillas of the antennal segments were measured using the Photoshop CS3 software (Adobe System, Mountain View, CA, USA), and lengths were measured by CAMSONAR Images MP 1.0 software, based on the SEM photomicrographs of the dorsal and ventral surfaces of the antennae.

3. Results

3.1. Gross morphology of the antenna of E. sophia

The SEM analysis showed that the antennae of *E. sophia* consisted of nine segments, which included a radicula, an elongated scape, a thick pedicel, and a six-segmented flagellum (Fig. 1). Many sensilla were found on the antennae of *E. sophia* in which host choice was sex-dependent. The types of sensilla identified were sensilla chaetica, sensilla trichodea, basiconic capitates peg sensilla, multiporous, grooved-surface placoid sensilla (MG-PS), uniporous rod-like sensilla, nonporous finger-like sensilla, and sensilla coeloconica. The mean total length of the antennae of females and males was 429.28 ± 0.95 μm and 437.19 ± 8.21 μm, respectively. The radicula had a smooth surface (Fig. 2A). The mean length of the radicula in females (41.25 ± 1.23 μm) was longer than that of the males (32.44 ± 0.73 μm), comprising 9% and 7.4% of the total length of each antenna in females and males, respectively. The SC was approximately two times longer than the radicula, measuring 91.14 ± 1.92 μm in females and 86.98 ± 4.43 μm in males, and comprising 21% and 19.9% of the total length of the antenna in females and male, respectively. The PE was relatively short and was conical in shape. The mean length of the PE was 39.94 ± 0.73 μm in females and 38.15 ± 2.17 μm in males, comprising 9.3% and 8.7% of the total length of the antenna in females and males, respectively. The mean total length of the flagellum was 256.81 ± 1.09 μm in females and 279.59 ± 3.01 μm in males, comprising 59.8% and 64% of the total length of the antenna in females and males, respectively.

The TEM analysis showed that the antennae of females contained fat body (FB) (Fig. 3D), cuticle, mesoscutello-metanotal muscle (MMM), and neurons (NE). A transverse cross-section of the region containing multiporous grooved-surface placoid sensilla (MG-PS) showed outer sensilla lymph cavities (OSL), inner sensillum lymph (ISL) and dendrites (DE) (Fig. 4.1F). In a TEM image of a longitudinal section of an MG-PS, the pore, which contained