Ultrastructure structure of antennal sensilla of carabid beetle

Carabus elysii Thomson, 1856 (Coleoptera: Carabidae)

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Abstract

The sensilla type, number and distribution in male and female adults of Carabus elysii Thomson, 1856 (Coleoptera: Carabidae) were studied using scanning electron microscopy. The results showed that there are seven categories and 12 types of sensilla in C. elysii adults: three types of S.Ch, three types of S.T, two types of S.B and one each of B.B, S.Co, S.Ca and S.Cam. There is no difference between male and female in the types of sensilla. Apart from the significant difference in the number and distribution of S.B.2, S.Co and S.Ca between male dorsal and ventral surfaces, there are no significant differences between male and female antennae. In general, the number of sensilla in females is larger than that in males. The results provide a basic reference for future ultrastructure, electrophysiological, and comparative behavioral studies of Carabus species.

Key words: antennal sensilla, Carabus elysii, scanning electron microscopy.

Introduction

Carabidae, which are predatory insects, are important natural enemies in forests and farmland, and play an important role in the maintenance of ecological balance (Park et al. 2013). Species of the genus Carabus, most of them without hind wings, are unable to fly, and move around by walking. Consequently, they have a larger dependence on the environment than the species of other genera, and are thus an ideal material for the study of relationships between habitat and distribution. Moreover, being large and brightly colored, Carabus species have important ornamental and collection value, and are referred to as “walking jewels” (Tian 2010). The carabid species Carabus elysii preys on the larvae of Lepidoptera, and in China it is mainly distributed in the provinces of Henan, Hunan, Jiangsu, and Anhui (Zhu et al. 1999).

The antennae are important organs of insect predation, mating, habitat searching, and environmental sensing (Hansson & Stensmyr 2011). Antennal sensilla play an important role in intraspecific and interspecific information exchange and host recognition. Although sensilla are found on every part of the insect body, they are most concentrated on the antennae, and they represent the main channel through which insects sense external stimuli (Schneider 1964). Scholars abroad have described the antennal sensilla in a number of beetle genera, including Platynus (Weis et al. 1999; Merivee et al. 2001; Ploomi et al. 2003), Bembidion (Merivee et al. 2000, 2002), Paussus (Giulio et al. 2012), and Typhlocharis (Perez-Gonzalez & Zaballos 2013), and have discussed the functions of the main sensilla based on electrophysiological studies (Weis et al. 1999; Giulio et al. 2012). However, to date, there have been only a few reports on the sensilla in Carabus (Kim & Yamasaki 1996, 1998; Liu & Tian 2008).

In this study, we compared the sensilla type, number, and distribution in male and female adult C. elysii using scanning electron microscopy, and present a preliminarily discussion on the functions of sensilla, in order to provide a basic reference for future ultrastructure, electrophysiological, and comparative behavioral studies of Carabus species (Figs 1 and 2).

Materials and methods

The source of sample insects

Carabus elysii adults were sampled from the village of Zhang, Qiaocheng District, Bozhou City, Anhui Province, China
Sample preparation and observation

Six male and female adults of *Carabus elysii* were selected, and from these the antennae were removed using a pair of forceps. The antennae were placed into distilled water and were cleaned by three to five 10-min ultrasonications. The antennae were maintained in 70% ethanol overnight, and the following day were dehydrated in an ethanolic series of 75%, 80%, 85%, 90%, and 100% (10 min at each concentration). The dehydrated samples were then placed in a clean place to dry, and glued onto sample grids according to the following categories: female, male, dorsal surface, and ventral surface. The mounted samples were subjected to gold spray treatment using a Hitachi E-1010 ion sputtering instrument, and were subsequently observed and photographed under a Hitachi S-4800 scanning electron microscope, with a working voltage of 5 kV.

The naming of the sensilla

Sensilla were named according to the classification method of Schneider (1964), and the type and location of antennal sensilla were also determined.

Data statistics and analyses

The length and diameter of sensilla were determined from scanning electron micrographs using ImageJ (Version 1.48) software, and the electron micrographs were processed using CS Photoshop (Version 6.0) software. All data were analyzed using SPSS (Version 15.0) statistical analysis software.
Results and analyses

Types of antennal sensilla

Using scanning electron microscopy, a large number of sensilla were visible on the surface of *C. elysii* antennae. According to the shape and distribution characteristics of the sensilla, they can be divided into seven categories and 12 types, namely, three sensilla chaetica, three sensilla trichodea, two sensilla basiconica, Böhm bristles, sensilla coeloconica, sensilla cavity, and sensilla campaniformia. The morphological characteristics of the various sensilla are described as follows.

**Sensilla chaetica (S.Ch):** This type of sensilla grows perpendicular to the antennal surface, and generally has varying lengths. It has either an acute or obtuse apex, and either a wide or narrow basal fossa. It can be further divided into three subtypes: S.Ch.1, S.Ch.2, and S.Ch.3. The length of S.Ch.1 is $28.53 \pm 209 \mu m$, the base diameter is $4.41 \pm 0.23 \mu m$, and it has an acute apex. There are longitudinal ridges on the lower half of the surface, but the upper half of the surface is smooth without any holes. The basal fossa is narrow (Plate I: A). The length of S.Ch.2 is $56.49 \pm 9.36 \mu m$, the base diameter is $6.12 \pm 0.82 \mu m$, and it has an acute apex. Some of these sensilla are bent toward the base. There are longitudinal ridges surrounding the surface, and the basal fossa is narrow (Plate I: B). The length of S.Ch.3 is $172.49 \pm 50.17 \mu m$ and the base diameter is $10.96 \pm 2.5 \mu m$. This is one of the longest sensilla and has a thorn shape. The apex is obtuse, and the sensilla are generally slightly curved. There are broadly impressed fossae in the base, and there are a small number of fossae mainly present on the top of each section (Plate I: C).
Sensilla trichodea (S.T): This type has a hairy appearance and varies in length. It has either an acute or obtuse apex, either a wide or narrow basal fossa, and is generally either upright or slightly curved. It can be further divided into three subtypes: S.T.1, S.T.2, and S.T.3. The length of S.T.1 is 168.42 ± 25.09 μm, the base diameter is 10.73 ± 200 μm, and it generally has a large degree of bending, like a sickle. The apex is obtuse, and there are convex longitudinal ridges on the surface. The basal fossa is narrow (Plate I: D). The length of S.T.2 is 125.46 ± 35.53 μm, and the base diameter is 7.68 ± 1.14 μm. It is generally upright, but curves slightly at two-thirds along its length. The apex is acute, the surface has convex longitudinal ridges, and the basal fossa is wide (Plate I: E). The length of S.T.3 is 143.43 ± 37.40 μm, and the base diameter is 7.07 ± 1.19 μm. The slender terminal has a significantly curved shape, the apex is acute, the surface has convex longitudinal ridges, and the basal fossa is narrow (Plate I: F).

Sensilla basiconica (S.B): This type of sensilla has a shape similar to an awl, and grows on or inside a circular cavity. It is short compared to the other sensilla, the apex is obtuse, and the basal fossa is either wide or narrow. It can be further divided into two subtypes: S.B.1 and S.B.2. The length of S.B.1 is 5.52 ± 1.72 μm, and the base diameter is 4.77 ± 0.34 μm. There is a depression on the surface and a cone inside. The apex is obtuse, the surface is smooth without longitudinal ridges, and the basal fossa is wide (Plate II: A). The length of S.B.2 is 8.36 ± 1.86 μm, and the base diameter is 2.69 ± 0.27 μm. There is a foundation support in the base. It has an overall conical shape with a low number. The apex is obtusely rounded, the surface is smooth without longitudinal ridges, and the basal fossa is narrow with a shell-shaped pattern (Plate II: B).

Böhm bristles (B.B): The length is 32.76 ± 10.05 μm, and the base diameter is 5.69 ± 1.02 μm. Most are perpendicular to the antennal surface, and distributed around the internode of the scape and pedicel in clusters of small thorns. They are shorter and more acute than thorn-shaped sensilla, smooth without any holes, and the basal fossa is wide (Plate II: C).

Sensilla coeloconica (S.Co): The sensilla diameter is 1.45 ± 0.97 μm, without edge hair. It has an epidermal depression, and one or no sensory cones in the center (Plate II: D).

Sensilla cavity (S.Ca): The diameter of the sensilla cavity is 8.09 ± 2.02 μm. Epidermal invaginations form a cavity. There are a large number of this type of sensilla (Plate II: E).

Sensilla campaniformia (S.Cam): The length is 0.65 ± 0.11 μm, and the base diameter is 8.09 ± 0.26 μm, with semi-elliptical or hemispherical shape. It grows from a disk cavity, which is surrounded by a smooth circular thick wall edge. The shape is similar to that of a button. There are papillary projections in the middle, and the end is bluntly rounded (Plate II: F).

The number and distribution of antennal sensilla

The base of the Carabus antenna has fewer types and numbers of sensilla than elsewhere. There are four sensilla of three types (S.Ch, S.B, and B.B). The 3rd to 9th knots of the flagellum have more types and a large quantity of sensilla. There are six types of sensilla in this section: S.Ch, S.T, S.B, S.Ca, S.Co, and S.Cam. No significant differences have been noticed between the sexes, with the exception of the significant difference in the number and distribution of S.B.2, S.Co, and S.Ca between the dorsal and ventral surface of male antennae.

S.Ch: There is a large number of this type of sensilla, although it is not present in the pedicel. Among these, S.Ch.1 is distributed in clusters in the 5th to 8th knots of the flagellum. There are 134 and 131 S.Ch.2 on the dorsal surface of the female and male antennae, respectively, and 114 and 100 on the ventral surface, respectively. S.Ch.2 is distributed in the 3rd to 8th knots of the flagellum. There are 255 and 315 S.Ch.3 on the dorsal surface of the female and male antennae, and 202 and 248 on the ventral surface, respectively. S.Ch.3 is only distributed at the end of each knot. There are 49 and 55 S.Ch.3 on the dorsal surface of the female and male antennae, and 43 and 47 on the ventral surface, respectively.

S.T: There is a large number of this type of sensilla, which are distributed on both the dorsal and ventral surface of the flagellum. Among these, S.T.1 are denser in the 3rd to 9th knots of the flagellum. There are 275 and 452 on the dorsal surface of the female and male antennae, and 244 and 278 on the ventral surface, respectively. S.T.2 is evenly distributed in the 3rd to 9th knots of the flagellum. There are 613 and 832 on the dorsal surface of the female and male antennae and 492 and 764 on the ventral surface, respectively. S.T.3 are less numerous and are distributed in the 3rd to 9th knots of the flagellum. There are 613 and 832 in the pedicel. These are distributed in the 3rd to 8th knots of the flagellum, and 244 and 278 in the ventral surface, respectively. S.T.3 are less numerous and are distributed in the 3rd to 9th knots of the flagellum in a scattered pattern. There are 66 and 45 on the dorsal surface of the female and male antennae, and 72 and 46 on the ventral surface, respectively.

S.B: There is a large number of this type of sensilla and they are distributed in each node of the antenna. Among these, S.B.1 is distributed in 1st to 2nd knots of the scape, pedicel, and flagellum. There are 241 and 200 on the dorsal surface of the female and male antennae, and 244 and 278 on the ventral surface, respectively. There are fewer S.B.2 sensilla, which are distributed in the 3rd to 8th knots of the flagellum. There are 61 and 98 on the dorsal and ventral surfaces of the female antennae, respectively, and 432 on the ventral surface of the male antennae, but none on the dorsal surface.

B.B: These are clustered in the scape base, and are absent from the remainder of the sections. There are 33 and 29 on the dorsal surface of the female and male antennae, and 55 and 52 on the ventral surface, respectively.

S.Co: These are distributed in the 3rd to 9th knots of the flagellum section, but are absent from other sections. There
are 26 and 47 on the dorsal surface and ventral surfaces of the female antennae, and 0 and 44 on the dorsal and ventral surfaces of male antennae, respectively.

S.Ca: There is a large number of this type of sensilla, distributed in the 3rd to 9th knots of the flagellum section. The other sections have no S.Ca. There are 183 and 616 sensilla of this type on the dorsal and ventral surfaces of the female antennae, and 0 and 5 on the dorsal and ventral surfaces of the male antennae, respectively.

S.Cam: These are distributed in the 3rd to 6th and 8th to 9th knots of the flagellum section; however, there are no sensilla of this type in other sections of the antenna. There are 5 and 2 on the dorsal surface of the female and male antennae, and 11 and 13 on the ventral surface, respectively.

Discussions

The results of this study showed that there are seven categories and 12 types of sensilla in *C. elysii* adults: three types of S.Ch, three types of S.T, two types of S.B, and one each of B.B, S.Co, S.Ca, and S.Cam. Apart from the significant difference in the number and distribution of S.B.2, S.Co, and S.Ca between male dorsal and ventral surfaces, there were no significant differences between male and female antennae. In general, the number of sensilla in females is larger than that in males, which may be related to the specific circumstances of the living environment (Schneider 1964; Merivee et al. 2000, 2002, Ploomi et al. 2003).

The S.Ch type of sensilla, which is present in large numbers, is considered most likely to have the dual functions of sensing mechanical stimulation and in chemosensory contact (Daly & Ryan 1979). This type is extensively distributed over the antenna, with the exception of the pedicel. S.Ch.2 and S.Ch.1 are, however, absent from the 1st and 2nd knots of the scape, pedicel, and flagellum. This is similar to the results of Daly and Ryan (1979) and Kim and Yamasaki (1996), but, Merivee et al. (2000) who showed that the S.Ch of *Bembidion lampros* are distributed in the knots of the antenna, and some are even distributed in the radical of the scape and pedicel.

S.T are large in number, of various lengths, hairy, with an acute or blunt apex, and found in many Carabidae insects (Kim & Yamasaki 1996, 1998; Weis et al. 1999; Merivee et al. 2000, 2001, 2002; Liu & Tian 2008; Giulio et al. 2012). The distribution pattern of S.T is similar to that reported by Kim and Yamasaki (1996), that is, they are mostly distributed in the 3rd to 9th knots of the flagellum in large numbers. Among these, ST.1 and ST.3 are thought to have a mechanoreceptive function (Daly & Ryan 1979), ST.2 is likely to be a receiver of the aggregation pheromone (Merivee et al. 2000, 2002; Ploomi et al. 2003).

The S.B has a shape similar to that of an awl, and grows on or inside a circular cavity. It is short compared with other sensilla, the apex is blunt, and the basal fossa is either wide or narrow. S.B.1 is only distributed in the 1st and 2nd knots of the scape, pedicel, and flagellum, and is believed to have the function of sensing mechanical stimulation (Kim & Yamasaki 1998; Merivee et al. 2000, 2001, 2002; Liu & Tian 2008). There is a foundation support in the S.B.2 base, it has an overall conical shape, the apex is bluntly rounded, the surface is smooth without longitudinal ridges, there is a narrow or no basal fossa, and it has a shell-shaped pattern. It is speculated to have the function of a chemosensory sensilla (Kim & Yamasaki 1996).

The B.B grow mostly perpendicular to the antennal surface, and are distributed around the internode of the scape and pedicel, like small thorns in a clustered pattern (Schneider 1964; Kim & Yamasaki 1996). It has been confirmed to have a mechanical receptor function based on electrophysiological studies (Merivee et al. 2001, 2002; Ploomi et al. 2003; Giulio et al. 2012).

The S.Co has no edge hair or thorns, and there is a depression on the surface. There is one or no sense cone in the center, which is very common in Carabidae (Merivee et al. 2001, 2002; Ploomi et al. 2003; Giulio et al. 2012), and it is accordingly defined as an altitude-shaped sensilla (Kim & Yamasaki 1996). Conical cavity sensilla and the altitude-shaped sensilla are both tiny depression structures, but the altitude-shaped sensilla has a deeper epidermal depression, and a narrower orifice than the cone cavity sensilla, which has a bolt-shaped structure and also grows in a deeper position. These two sensilla have often been reported to have the function of chemical, thermal, or moisture sensation, but not all of these functions have been confirmed (Schneider 1964).

The S.Co is a cavity formed by an epidermal depression. This type of sensilla is numerous, and has been reported in other types of Carabidae. It is generally considered to have the function of sensing chemical stimulation, temperature, and humidity (Schneider 1964). Kim and Yamasaki (1996) has defined it as a bottle-shaped sensilla.

The S.Cam is also described in the antennae of other carabid species (Kim & Yamasaki 1996) (Merivee et al. 2001, 2002; Ploomi et al. 2003; Giulio et al. 2012). It is distributed in the flagellum in a scattered pattern, however, the bell sensilla of *C. fiduciaries saishutoicus* are only distributed at the end portion of the antenna, and the difference in the size and surface structure among different insects is very significant (Kim & Yamasaki 1996). Sensory cells in bell sensillae at the end of the antennae of *Pterostichus aethiops* have been confirmed to be extremely sensitive to low temperature (Ploomi et al. 2003). In some other insects, this type of sensilla often grows in a position close to a certain joint of the halteres, wing, and foot for the sensation of epidermal pressure caused by mechanical deformation (Schneider 1964).
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