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Anatomical organization of antennal-lobe glomeruli in males and females of the scarab beetle *Holotrichia diomphalia* (Coleoptera: Melolonthidae)

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ABSTRACT

The glomerular organization of the primary olfactory brain center, the antennal lobe, was studied in males and females of *Holotrichia diomphalia* adults using serial histological sections labeled by the reduced silver-stain technique. The results revealed an apparent sexual dimorphism. Whereas an enlarged cap-shaped glomerulus was found at the antennal nerve entrance into the antennal lobe in males, no such unit was present in females. Also the size of the antennal lobe differed between the sexes, the antennal lobe of males being larger than that of females. We estimated the total number of glomeruli at approximately 60 units in the female antennal lobe. In males, we could discriminate only those glomeruli that were located in the anterior area of the antennal lobe.

1. Introduction

The olfactory sense is essential for insects, as it plays a paramount role in locating food sources, oviposition sites, and conspecific individuals, mates included (Hartlieb and Anderson, 1999). The primary olfactory brain center of insects, the antennal lobe (AL), constitutes the first synaptic relay station of the antennal afferent pathways, as it receives input from antennal olfactory sensory neurons and sends the output to higher brain centers (Rospars, 1988; Hildebrand and Shepherd, 1997; Anton and Homberg, 1999). The building blocks of the AL found in most insect orders are the olfactory glomeruli, in which the interactions between antennal and deutocerebral neurons take place (Schachtner et al., 2005). Thus, the glomerular array is thought to constitute a chemotopic map, which ultimately leads to olfactory coding (Christensen and White, 2000; Galizia and Menzel, 2000, 2001; Ignell and Hansson, 2005).

Due to their functional significance glomeruli have been anatomically mapped in several insect species. The size, number, and organization of glomeruli are shown to be conserved within individuals of one species, meaning that insects seem to have a species-specific glomerular arrangement. Their relatively small number of glomeruli, as compared to those of mammals for example, allows for the identification of individual glomeruli within a species (Rospars, 1988; Anton and Homberg, 1999; Schachtner et al., 2005). The numbers of glomeruli were ranging from 43 in fruit fly (Stocker, 1979, 1994; Stocker et al., 1990) to 442 in ants (workers) (Kuebler et al., 2010). In some species, as *Drosophila melanogaster* (Stocker, 1979, 1994; Stocker et al., 1990) and *Pieris brassicae* (Rospars, 1983), the glomeruli of males and females are of approximately equal size and shape. However, sexually dimorphic AL glomerular organization has also been reported in a number of Hymenoptera, Lepidoptera, and Blattaria species (Rospars, 1988; Anton and Homberg, 1999; Schachtner et al., 2005). In particular, an arrangement of enlarged glomeruli situated at the antennal nerve entrance, a so-called macroglomerulus (MG) or macroglomerular complex (MGC), has been described in males of several species. This male-specific structure is responsible for processing sex pheromone information underlying reproductive behavior (Rospars, 1983; Boeckh and Selsam, 1984; Arnold et al., 1985; Rospars and Hildebrand, 1992; Hansson, 1997).

Thus far, no study has been conducted on elucidating the complete antennal-lobe glomerular organization of Coleoptera species (beetles). A few publications have reported about individually recognizable glomeruli in beetles (reviewed by Schachtner et al., 2005). Recently, Dreyer et al. (2010) described the structure and arrangement of a part of the antennal-lobe glomeruli of the red flour beetle, *Tribolium castaneum*. Regarding the peripheral system, the fact that semiochemicals were identified in some economically important plant-feeding scarabs (Leal, 1998) promoted studies on

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the function of olfactory receptor neurons of beetles. Thus, antennal sensilla housing receptor neurons specifically tuned to female-produced pheromones and plant volatiles have been identified in several scarab beetles (Renou et al., 1998; Hansson et al., 1999; Larsson et al., 2001). This information represents a foundation for investigating the structure and function of scarab beetles' central nerve system. As severe pests during the larval stage, these insects cause substantial damage to agricultural crops and forestry productions worldwide (Lee, 2003). The significance of beetles is difficult to ignore from several points of view, the economical one not being the least.

Some species of scarab beetles show an obvious sexual dimorphism of peripheral olfactory organs. This is expressed by enlarged antennal lamellae and thereby a considerably higher number of olfactory sensilla in males as compared to females (Meinecke, 1975; Ågren, 1985). For other species, the size of lamellae and number of sensilla may be similar between sexes, but the type of olfactory sensilla and/or the sensitivity of olfactory receptor cells (ORC) are significantly different (Kim and Leal, 2000; Larsson et al., 2001; Nikonov et al., 2001; Stensmyr et al., 2001). Generally, sexual dimorphism of antennal sensilla often correlates with dimorphism of the AL (Rospars, 1988). In a previous study, using scanning and transmission electron microscopes, the types and ultrastructure of antennal olfactory sensilla of *Holotrichia diomphalia* Bates, an important soil pest in the Northeastern region of China, was characterized. The results revealed that *sensilla basciconica* and *sensilla placodea* were two predominant types of olfactory sensilla in both sexes and that the number of sensilla was significantly higher in males than that in females (Sun et al., 2007).

In this study, the anatomical organization of antennal-lobe glomeruli is described in males and females of *H. diomphalia*. The results, obtained by investigating serial antennal-lobe sections labeled via the reduced silver-stain technique, demonstrate a clear sexual dimorphism expressed by an enlarged male-specific macroglomerulus situated at the entennal nerve entrance. The data provide a sound foundation for functional investigations of the insect's central nervous system.

2. Material and methods

2.1. Insects

H. diomphalia eclose in the soil at the end of July and overwinter in the original place. Adults come out of overwintering in early June the following year and the adult stage lasts about 30 days. Wild adults of *H. diomphalia* were collected in Sun Island Park, Harbin, Heilongjiang province on June 15th, 2008, between 18:00 and 20:00.

2.2. Preparations of brain tissues for histological observation

Heads of males and females were fixed in Bouin's fixation solution (Kermel Chemical Reagent Company, Tianjin, China) for 2 days. The brains were dissected under a stereo microscope and pre-stained by placing them in 1% silver nitrate solution (Beijing Chemical Plant, Beijing, China) in darkness (24 h). The pre-stained brains were then embedded in paraffin and sectioned at 9 μm thickness from anterior (A) to posterior (P) or from ventral (V) to dorsal (D) with a Leica RM 2015 microtome. The serial sections were dried on histological slides before being rehydrated and stained according to Rowell's method (Rowell, 1963). The stained sections were then dehydrated in ascending concentrations of ethanol (Restoration Technology Development Co., Ltd. Tianjin, China) (30%, 50%, 70%, 90%, 95%, 100% × 3, 10 min each time), diaphanized in xylene (Kermel Chemical Reagent Company, Tianjin, China), and finally mounted using Canada balsam (Shanghai molding plant specimens. Shanghai, China). Observations and photos were made by using a light microscope equipped with a 20×/0.50 Ph2 dry objective lens (Olympus BX51X) and a digital camera (Olympus DP71).

2.3. Image processing and statistical analysis

The stained sections of at least 20 scarab beetle were compared. Although the AL of both hemispheres were stained in some preparations, we only chose a single AL from each specimen for further analysis, disregarding possible differences between left and right hemispheres. Four brains of each sex stained with sliver-labeling technique were chosen for statistical analysis. The images were processed using Photoshop 10.0. Sections containing glomeruli were photographed in the same manner (direction and magnification) in both males and females. Adjacent sections that contain same glomeruli were matched by overlapping images from consecutive cuts, comparing shapes and locations on each image, etc. The 3-dimensional measurements of the ALs were measured (two perpendicular lengths in μm) on the section of a given glomerulus where it appears with the largest area. In the direction perpendicular to the sectioning plane, we multiplied the number of sections on which the glomerulus was visible by the section thickness (9 μm), giving a third number (depth in μm). Methods of measurements of the MG size were the same as AL. Here, the maximum length of AL and MG was measured using the DP controller 3.1.1.267 Software. All the data were analyzed with one-way ANOVA (SPSS 13.0).

2.4. Glomerular nomenclature

The glomerular nomenclature used in the present study is based on the general position (Boyan et al., 1993; Nishino et al., 2005; Ghaninia et al., 2007) of single glomeruli in the AL, where two of the following capital letters denote the position; A (anterior), P (posterior), V (ventral), D (dorsal), L (lateral), M (medial), and C (central) (Figs. 1, 3–5). Positions are given according to the neuroaxis and not to the coordinates of the body. According to the sectional series from anterior to posterior, the first 9 sections of female ALs were designated as region A and the rest were

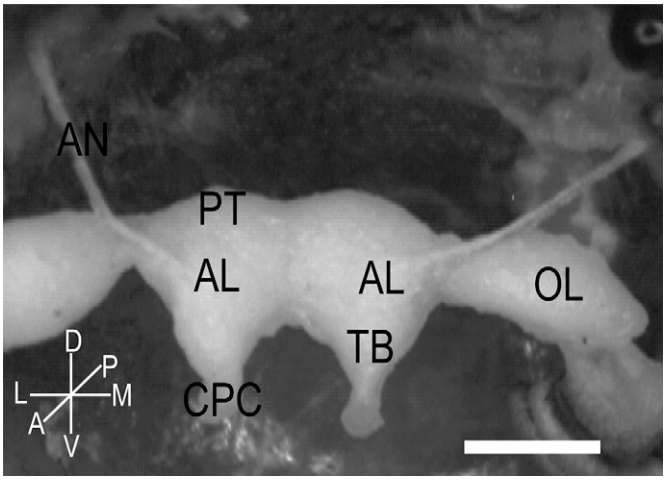

Fig. 1. Photomicrograph of the dissected brain of a female *H. diomphalia* Bates. AL: antennal lobe; AN: antennal nerve; CPC: circumoesophageal connective; OL: optic lobe; PT: protocerebrum; TB: tritocerebrum; A: anterior; D: dorsal; L: lateral; M: medial; P: posterior; V: ventral. Scale bar = 0.5 mm.