Ultrastructural characteristics of the proboscis and the labial palp pit organ in the oriental fruit moth, *Grapholita molesta*

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Abstract

The ultrastructural characteristics of proboscis and the labial palp and its pit organ in the oriental fruit moth, *Grapholita molesta*, were studied using scanning electron microscopy. Four types of sensilla were identified on the proboscis of *G. molesta*: aportorous sensilla chaetica, uniporous sensilla basiconica (two subtypes), uniporous sensilla styloconica (two subtypes), and aportorous sensilla campaniformia. The sensilla campaniformia found in proboscises of Lepidoptera were only the second time. Three types of sensilla were found on the surface of the labial palp: sensilla chaetica, sensilla squamiformia, and sensilla campaniformia. In addition we found small holes sporadically located on the walls of the second segment of each labial palp. The pit organ, which is located on the terminal segment of each labial palp, is 86.61 µm deep with a 21.90 µm-wide opening. Each pit structure contains two types of sensilla: hair-shaped sensilla (containing some sensilla with forked tips) (located on the upper half of the pit) and club-shaped sensilla (located on the lower half of the pit).

Key words: *Grapholita molesta*, proboscis, labial palp pit organ, sensilla, morphology, scanning electron microscopy.

Introduction

Morphological studies of the proboscis in Lepidoptera have played a significant role in framing our understanding of the evolutionary history of this megadiverse taxon (Chauvin and Faucheux, 1981; Kristensen and Nielsen, 1981; Kristensen, 1984; Nielsen and Kristensen, 1996; Faucheux, 1999; 2013a). The form and function of the proboscis are closely related to the feeding behaviour of each species (Krenn, 2010). The first broad-scale studies that investigated the correlation between proboscis morphology and feeding habits were performed by Chauvin and Faucheux (1981), Faucheux (1999) and Krenn et al. (2001). The proboscis of higher Lepidoptera consists of the two extremely elongated galeae, which are connected and made fluid-proof by linking structures. The diversity of feeding habits within the Lepidoptera makes this an interesting family in which to study proboscis morphology.

Apart from feeding, the sensilla of the proboscis play a role in ovisposition by females. For example, in the common clothes moth, *Tineola bisselliella* (Hummel) and *Monopis crocicapitella* (Clemens), the bilateral ablation of the galeae added to the total palpectomy of maxillary and labial palps diminishes only marginally the volume of the ovispositor compared to that of exclusively palpectomized females and change in the egg distribution on the substrate (Faucheux, 1987). Previous investigations on proboscis sensilla in Lepidoptera mainly concentrated on families Noctuidae, Pyralidae, Nymphalidae, Erebididae, Neopseustidae, and Geometridae (Sellier, 1975; Faucheux, 1991; Büttiker et al., 1996; Krenn, 1998; Krenn and Penz, 1998; Pett and Stewart, 2004; Faucheux, 2008; Zaspel et al., 2011; Xue and Hua, 2014). To our knowledge, however, the proboscis sensilla of Tortricidae have little been investigated except the spruce budworm, *Choristoneura fumiferana* (Clemens) (Walters et al., 1998).

The oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera Tortricidae), is a prominent pest that attacks stone and pome fruit worldwide. Larvae bore to new growth shoots and fruits and cause economic damage (Rothschild and Vickers, 1991). Peach extrafloral nectar from leaf glands is an important natural food resource for *G. molesta* adult (Atanassov and Shearer, 2005). Previously, we have studied this insect’s adult antennal and larval mouthparts sensilla (Zhang et al., 2014; Song et al., 2014). In this study, we aimed to determine the ultrastructure and distribution of sensilla on the proboscis and within the labial palp pit organ of *G. molesta*. Gaining a better understanding of these sensory structures will aim electrophysiological and behavioural studies in the future.

Materials and methods

Insects

*G. molesta* were reared in a climate chamber at the College of Plant Protection, Northwest A & F University. Rearing condition was a photoperiod of 15:9 h (L: D) at 24 ± 0.5 °C and 70 ± 10% relative humidity. This colony was originally collected from infested fruits in the peach orchard in Yangling, Shanxi, China, in July 2010.

Scanning electron microscopy

Scanning electron microscopy (SEM) was performed on 3-day-old male and female moths. Samples were fixed in Carnoy’s solution (95% ethanol: glacial acetic acid = 1:1, v/v) for 12 h, and dehydrated in graded ethanol series before CO₂ critical point dryer (Hitachi Koki Co., Tokyo, Japan). Dried samples were adhered to a sample block using double-sided adhesive tape and then sputter coated with gold (Hitachi E-1030). The gold-coated samples were observed in a Hitachi S-4800 SEM scanning electron microscope operated at an accelerating voltage of 10 kV.
Data analysis

We consulted a number of references (Altner, 1977; Zacharuk, 1980; Zhao et al., 2013; Chen and Hua, 2014) when identifying different types of sensilla. The images were marked using Adobe Photoshop CS4. The length and basal width of each sensillum type of at least ten adults were measured by ImageJ v2.1.4.7 software from scanning micrographs. Data (mean ± SEM) were analyzed using SPSS 17.0.

Results

G. molesta adult have siphoning mouthparts, consisting of labrum, proboscis, maxillary palpi, and labial palpi (figure 1a). The mandibles are reduced and nonfunctional. Because males and females within a species do not have differences in the anatomy of their mouthparts, results for males and females are combined.

Labrum

The labrum of G. molesta is a very small and triangular plate over the basal proboscis joint (142.66 ± 18.94 µm long; 29.76 ± 6.26 µm wide) (figure 1b). It bears numerous microtrichia, which are more or less symmetrically arranged on each side of the labrum. The bristles of pilifers located the basal galeal joint, are 23.36-83.44 µm long; these sensory bristles probably detect proboscis movements (figure 1b).

Maxillary palpi

The maxillary palpi located in both sides of galeae, are 93.55 ± 12.05 µm long and are divided into three segments. The terminal segment is equipped with a great scale and bristles (figure 1c).

Proboscis

The proboscis of G. molesta is about 914.07 ± 56.33 µm long (figure 2a), and comprises two extremely elongated maxillary galeae, which connected together to form a sucking tube (figure 2b). In the resting position, the proboscis is coiled in a tight spiral beneath the moth’s head between the labial palpi. The outer surface of the proboscis is densely covered with non-innervated microtrichia on the proximal two-thirds of its length, whereas on the distal one-third of proboscis has less microtrichia and becomes tuberculate (figure 2a, c). Both the female and male G. molesta possess four types of sensilla on their proboscis: aporous sensilla chaetica, two subtypes of uniporous sensilla basiconica, two subtypes of uniporous sensilla styloconica, and aporous sensilla campaniformia. Different types of sensilla are located on different regions of the proboscis and assume a specific functional distribution pattern along the proboscis.

Aporous sensilla chaetica (Sc) are 34.45 ± 9.73 µm in length and 1.5 ± 0.10 µm in diameter at the base, with a total number no more than ten sensilla per galea. Each sensillum is located on the outer surface of the galea, forming an angle of 45° with the surface of the proboscis, and inserted in a cone socket making possible the displacement of the sensillum. The outer surface of sensilla chaetica is adorned with 5-6 longitudinal ridges (figure 3a).

Uniporous sensilla basiconica (Sb1) existed on the external surface of the galea, are cone-shaped and 3.59 ± 0.94 µm in length, with smooth surface, and are surrounded by a 4.65 ± 0.52 µm wide flexible and shallow socket. These sensory pegs bear a terminal pore that is 0.19 ± 0.04 µm in diameter (figure 3c) and are interspersed in an irregular row between the sensilla styloconica of the two row (figure 2c), about 24 sensilla per galea.

Figure 1. Head and mouthparts of G. molesta. a: Ventral view showing the labial palpi (lp) and proboscis (pr); b: Labrum (la) with microtrichia and the pilifers (pi); c: Maxillary palpi (mp), segmented into three parts.
Uniporous sensilla basiconica (Sb2) existed on the internal surface of the galea are the only sensilla that exist on the internal wall of the food canal, where 13 pegs are irregularly arranged in a single row in the middle of food canal on each galea (figure 2b). These sensilla, 2.25 ± 0.09 μm long, have a basal diameter of 1.3 ± 0.02 μm and a terminal pore. Their broad socket, with 1.8 ± 0.03 μm in inner diameter and 5.34 ± 0.57 μm in external diameter probably allows ample movement of the pegs (figure 3f).

Uniporous sensilla styloconica (St1) appear in the distal of the galea and arrange in two rows on each galea, an inner and an outer one, with about 30 sensilla for each galea (figure 2a, c). Sensillum styloconicum each consists of a short sensory cone with a terminal pore, 5.52 ± 0.82 μm long and 1.68 ± 0.15 μm in basal diameter, which is located at the top of a larger stylus, 16.07 ± 2.88 μm long and 8.08 ± 1.94 μm in basal diameter, which has 6-7 smooth cuticular ridges that extend lengthwise to the top of the stylus (figure 3d).

Another type of uniporous sensilla styloconica (St2) bears at the apex of per galea (figure 2e, f). These sensory pegs are composed of two segments: upper portion containing a short sensory cone with a terminal pore, 3.50 ± 0.03 μm long and 1.32 ± 0.02 μm in basal diameter; bottom portion not have 6-7 smooth cuticular ridges, 3.44 ± 0.13 μm long and 2.57 ± 0.09 μm in basal diameter.

Aporous sensilla campaniformia, 6.68 ± 1.77 μm in diameter and 2.05 ± 0.18 μm in height, arrange along the sides of the proboscis. The number of sensilla is at most seven (figure 3e).
Labial palpi

Labial palpi are about 625.03 ± 26.38 μm long and divided into three segments (figure 4a, b), extending from the prementum sclerite and forming prominent structures in front of the head, and are entirely covered with scales (figure 1a). The terminal segment is the shortest one (122.07 ± 9.41 μm long, figure 4c) and bears sensilla chaetica (33.85 ± 7.36 μm long and 1.81 ± 0.02 μm in basal diameter). Each sensillum is inserted into a small socket and have ring-shaped grooves on their surface (figure 4d). The second segment is the longest (338.14 ± 20.41 μm long), and presents sensilla squamiformia which are inserted into a distinct bulgy socket (figure 4e). The walls of the second segment present a few very small randomly located holes (diameter 0.44 ± 0.08 μm) that penetrated through the surface of the labial palp (figure 4f). Five aporous sensilla campaniformia (8.18 ± 1.13 μm in outer diameter and 3.89 ± 0.43 μm in internal diameter) arrange in an arch along the medial wall of the basal segment (201.51 ± 17.47 μm long) (figure 4g, h).

The pit organ, located at the tip of the terminal segment in each labial palp, is about 86.61 ± 14.83 μm deep with an opening of 21.90 ± 3.12 μm in diameter (figure 5a, b). There are three types of sensilla in the pit: hair-shaped sensilla and club-shaped sensilla. Hair-shaped sensilla are distributed in the most distal part of the pit, from the opening to the middle of the upper half, while the club-shaped sensilla, which have ridges like leaf veins, are located in the lower half of the pit (figure 5b, c).
Hair-shaped sensilla are either unbranched (6.69 ± 1.05 µm long and 0.59 ± 0.01 µm in diameter) or furcate (6.94 ± 1.15 µm long and 0.46 ± 0.01 µm in diameter), and both sensilla have smooth surfaces (figure 5d). Club-shaped sensilla, 10.48 ± 3.41 µm long and 2.07 ± 0.02 µm in diameter, show a grooved cuticle (figure 5e). Finally, small mastoid structures, 0.32 ± 0.02 µm long and 0.27 ± 0.01 µm in diameter, are scattered among hair-shaped sensilla (figure 5f).

**Discussion**

The change of adult and larval mouthparts in same species among holometabolous insects is both tremendous and puzzling. In the study, the adult mouthparts of *G. molesta* were subjected to a detailed microscopic investigation. Compared to the *G. molesta* larvae (Song et al., 2014), the labrum of *G. molesta* have changed into a very small plate, and the mandibles of *G. molesta* have
reduced and nonfunction. The pilifers on the labrum that touch the basal galeal joint bear many bristle-shaped sensilla probably detecting proboscis movement (Zacharak, 1980; Faucheux, 1999). The maxillary palpi consist of three segments and bear a great scale and bristles, but their function is unknown. Like C. fumiferana, the proboscis of G. molesta comprise the paired galea modified into a tubelike structure adapted for imbibing liquid foods (Walters et al., 1998).

Non-innervated microtrichia are widespread on the proboscis and probably have function as mechanoreceptors (Krenn and Penz, 1998; Krenn, 1998; 2010; Faucheux, 1999; Krenn et al., 2005; Molleman et al., 2005). They also aid in gathering and fixing pollen, which plays a role in zoochore (Gilbert, 1972). Aporous sensilla chaetica on the galeae are different in shape from sensilla chaetica on the labial palpi, but they are similar to sensilla chaetica on the antenna of G. molesta (Zhang et al., 2014). Sensilla chaetica are the most frequent type in the proximal region of the proboscis. They are innervated by a single sensory cell and have mechanical functions (Städler et al., 1974; Gnatzy and Tautz, 1980; French and Sanders, 1981).

The mouthparts of G. molesta have two types of sensilla basiconica that are present in different locations and both have a terminal pore. This result is same with Faucheux, 1999; 2013a. These sensilla in C. fumiferana were innervated by four or five sensory cells and comprised a mechanoreceptor and 3-4 chemoreceptors, and they are bimodal taste/tactile sensilla (Walters et al., 1998). These sensilla in Vanessa cardui (L.) and Papilio xuthus L. house only two sensory cells whose dendrites reach the terminal pore and regarded as unimodal contact chemosensilla (Krenn, 1998; Inoue et al., 2009).

Figure 5. Sensilla inside the labial palp pit organ. a: Opening of the labial pit organ as viewed from the distal tip of the palp, scales have been mechanically removed; b: Longitudinal section of the pit organ; c: An inserting picture from b; d: Hair-shaped sensilla with smooth surface. The arrow shows a hair-shaped sensillum with forked tip; e: Club-shaped sensory with grooved on the cuticle; f: Small mastoid structures between hair-shaped sensilla.
Uniporous sensilla styloconica are commonly found on the proboscises of Lepidoptera, according to many different studies (reviews in Faucheux, 1999; 2013b). Gould and Vrba (1982) and Altner and Alnet (1986) suggested that sensilla styloconica evolved from sensilla basiconica. The sensilla styloconica may respond to various chemical substances, especially saccharides (Fring and Frings, 1956; Blaney and Simmonds, 1988). During feeding, sensilla styloconica may provide the insect with important information, such as the location of nectar and the floral tube depth; they may also have the function of pollen collection (Krenn, 1998; Krenn and Penz, 1998). Uniporous sensilla styloconica are therefore bimodal taste/tactile sensilla.

The observation of aporous sensilla campaniformia along the proboscis of *G. molar* is interesting because it is only the second time that they are described in Lepidoptera (Faucheux, 2013b). They are proprioceptors which are stimulated during coiling and folding of the proboscis. Aporous sensilla campaniformia in the proximity of the basal area of the labial palpi of *G. molar* have so far never been mentioned in Lepidoptera except *Synempora andesae* Davis et Nielsen, with proprioceptive functions (Faucheux, 2008), but the number of aporous sensilla campaniformia and their locations are different depending on the insect species. However, little is known of the biology, ecological tolerance, and requirements of the Tortricidae family, of which *G. molar* is a member. The small holes we found on the second segments of the labial palpi in *G. molar* have not been identified before, and their functions must be studied further; probably they are only glandular pores. The sensilla chaetica with tubular bodies located in the distal region of the labial palpi possess a flexible socket, and in other species are known to be mechanosensilla (Lee, 1987). The sensilla squamiformia on the second segment of the labial palpi in *G. molar* are only found in antennae of moths, and their possible function is unknown (Liu et al., 2013; Faucheux, 2014).

The club-shaped sensilla in the pit organ of each labial antennae of moths, and their possible function is unknown. *B. armigera* (Lepidoptera: Noctuidae) has uniporous sensilla styloconica that function to detect carbon dioxide. However, the structures and types of sensilla in the labial palp organ still need to be confirmed by both behavioral and electrophysiological studies.

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