Ultrastructures of the Sternal Glands in Two Thripine Thrips and One Phlaeothripine Thrips (Thysanoptera: Insecta)

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Abstract

We observed the ultrastructures of sternal glands of two thripine thrips (*Thrips hawaiiensis* and *Frankliniella intonsa*) and one phlaeothripine thrips (*Psalidothrips simplus*). The glands of the thripine thrips consist of many secretory cells, a cuticular ridge, some secretory ductules and a wide subcuticular space filled with secretion. On the other hand, the gland of the phlaeothripine thrips lacks a cuticular ridge and a secretory ductule. The secretory cells are characterized by an abundance of mitochondria and numerous apical microvilli. The size of a cuticular ridge was much larger in the genus Thrips [*T. hawaiiensis* and *T. validus* by Bode (1978)] than in *F. intonsa*. Some differences among genera belonging to the family Thripidae and an obvious difference in the gross structures between thripine thrips was speculated on the basis of morphological comparison with various secretory glands in other insects.

Introduction

Some adult male [and a few female, *e. g.*, *Chilothrips yamatensis* (Kudo, 1987)] thrips have glandular areas, which contain sternal glands, in their abdominal sternites. The area is present in each of the sternites (mostly in sternites III–VII) in thripine thrips but only in sternite VIII in phlaeothripine thrips. These glandular areas also vary in shape, size, and position according to taxa. For example, small oval, scattered numerous small circular, transverse elliptical and horseshoe-shaped glandular areas are observed in thripine thrips, and small circular, transverse band-like and broad glandular areas are observed in phlaeothripine thrips. However, the number of abdominal segments in which the glandular area is present and the shape of its area do not vary in the same species or genus. Thus, these are important characteristics for thysanopteran classification (cf. Tsutsumi, 1997).

Bode (1978) investigated the internal morphology of the sternal gland in *Thrips validus* by ultrastructural and histochemical examinations. He reported that: 1) the sternal gland consists of a large subcuticular space filled with secretion, 2) secretory ductules (120 Å in diameter) of the cuticle, a cuticular ridge and secretory cells in which numerous microvilli develop in the apical parts, and 3) remarkably elongated mitochondria and no secretory granules are observed in the cytoplasm of secretory cells. These results, however, were only for one species.

In the present study, we compared the structures of sternal glands of two thripine thrips and one phlaeothripine thrips using both light and electron microscopy in order to determine whether or not the sternal glands of *T. validus* and those of other thrips belonging to another genus or family have the same structural characteristics.

Materials and Methods

Two thripine thrips (family Thripidae), *Thrips hawaiiensis* and *Frankliniella intonsa*, were collected from flowers of *Trifolium pratense*, and one phlaeothripine thrips (family Phlaeothripidae), *Psalidothrips simplus*, was extracted using Tullgren funnels from leaf litter in a deciduous forest dominated by *Quercus serrata* and *Pinus densiflora* in the campus of Fukushima University, Fukushima Prefecture, Japan.

Adult males of these thrips were anesthetized by submerging them in 70% ethyl alcohol for a few minutes, and the abdomens were removed from their bodies in chilled fixative (Karnovsky's fixative: 2% paraformaldehyde +2.5%

glutaraldehyde) buffered at pH 7.2 with 0.1 M HCl–sodium cacodylate. The abdomens were refixed with the same fixatives followed by 1% osmium tetroxide diluted with the same buffer at room temperature. The fixed materials were then dehydrated in a graded acetone series and embedded in water-miscible epoxy resin, Quetol 651 (Nisshin EM, Tokyo). Silver-golden thick sections were prepared, double-stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope, JEM 1010 (JEOL, Tokyo), at 80 kV. Semi-thin sections of 1 μ m in thickness were stained with toluidine blue O (TBO) and observed under a light microscope.

Results

External morphology of the glandular area (pore plate)

Adult males of the thripine thrips *Thrips hawaiiensis* and *Frankliniella intonsa* have one transverse broad glandular area (*ca.* $42-67 \times 4-7 \mu m$ in *T. hawaiiensis* and *ca.* $42-65 \times 9-12 \mu m$ in *F. intonsa*) in each of the sternites III to VII (Fig. 1a). In these areas, the cuticle is a little thinner than the cuticle in the rest of the sternum, and many small pores are observed to aggregate densely.

The phlaeothripine thrips *Psalidothrips simplus* has a glandular area only in sternite VIII, and it is a slender transverse glandular area (*ca.* $120-130\times13-16 \ \mu$ m) (Fig. 1c). In this area, many small pores are observed to aggregate densely, as was observed in the two thripine thrips.



Fig. 1 a. Glandular areas of *Thrips hawaiiensis*. b. Sagittal section of abdomen of *T. hawaiiensis*, showing the sternal glands (arrows). Arrowheads show the region in contact with the cuticle of the glandular area. c. Glandular area of *Psalidothrips simplus*. d. Sagittal section of the median part of the sternal gland of *P simplus*. Arrowheads and an asterisk show the top of the sternal gland and the subcuticular space, respectively. Cu: cuticle of the glandular area, GA: glandular area, 3–9: 3rd to 9th abdominal segments. Scales=50 μm.

General structures and stainability of the sternal glands with TBO

Light microscopically, adult males of *T. hawaiiensis* and *F. intonsa* have sternal glands of the same type as those of *T. validus* reported by Bode (1978) and those of *Taeniothrips inconsequens* by Moritz (1997). Their sternal glands are semicircular in shape in sagittal section and composed of an outer part (*ca.* 5–10 μ m in width) that is not stained well by TBO and a well-stained inner part (*ca.* 20 μ m in width) (Fig. 1b). In *T. hawaiiensis*, the region that is in contact with the cuticle of the glandular area is also stained well by TBO (arrowheads in Fig. 1b).

In sagittal section, the median part of the sternal gland of *P* simplus is a distorted semicircular shape (Fig. 1d), but either side part of the sternal gland is anteroposteriorly slender in shape (cf. Fig. 2c). The cuticle of the glandular area is thickened (Cu in Fig. 1d), and there is a subcuticular space in contact with the cuticle of the glandular area, which is stained very weakly by TBO (asterisk in Fig. 1d). This space branches off toward the top of the sternal gland.

Ultrastructure of the sternal gland

In all of the thrips species examined in the present study, the sternal gland consists of many secretory cells connected by desmosomes and a cuticle of the glandular area (pore plate). The secretory cells are characterized by an abundance of mitochondria and the development of microvilli in their apical region facing a wide subcuticular space filled with secretion (asterisks in Fig. 2). The mitochondria in the secretory cells are divided into two types according to shape: a remarkably elongated type (Fig. 2b) and an oval one. Some secretory ductules (Fig. 3a, c) and a cuticular ridge (Fig. 3a, b) were observed in the cuticle of the pore plate of the two thripine thrips, but no secretory ductule was observed in the cuticle of the pore plate of the phaeothripine thrips, *P simplus* (Fig. 3d).

Mitochondria

In the secretory cells of *T. hawaiiensis* and *F. intonsa*, remarkably elongated mitochondria are situated at the base of the microvilli, and oval-shaped mitochondria exist throughout the cytoplasm (Fig. 2a, b). Some elongated mitochondria had almost penetrated completely the microvilli (Mt in Fig. 2b). In contrast, remarkably elongated mitochondria were not detected in *P. simplus* (cf. Fig. 2d).

The oval-shaped mitochondria (Mt in Fig. 2a) were observed in all of the thrips examined in the present study, and there were many variations in development of cristae.

Cuticular ridge

In the sternal glands of the thripine thrips, the cuticle on the edge of a pore plate protrudes into the subcuticular space, as a cuticular ridge (CR in Fig. 3a, b). This cuticular ridge, also described in *T. validus* by Bode (1978), was found in the two thripine thrips species but not in the phlaeothripine thrips, *P simplus* (cf. Fig. 2c). This ridge tapers off to a point and bends to the outside of the pore plate region. The size of this ridge is much larger in *T. hawaiiensis* (0.5 μ m in basal width, 1 μ m in height) (Fig. 3a) than in *F intonsa* (0.3 μ m in basal width, 0.3 μ m in height) (Fig. 3b).

Secretory ductules

In sagittal sections, some pores were observed in the cuticle of the glandular areas. In the thripine thrips T. *hawaiiensis* and F *intonsa*, these pores had traversed the cuticle and penetrated the subcuticular space filled with secretory material, as ductules (SD in Fig. 3a, c). At the inner end, most of the ductules are covered by a mass of feltwork, which consists of radially arranged filaments (EF in Fig. 3a, c), as was reported in T validus by Bode (1978).

On the other hand, in the phlaeothripine thrips, very short tubular invaginations of the epicuticle were found (arrowheads in Fig. 3d), but a secretory ductule passing through the cuticle was not observed. Instead of the ductule, diffusion of filamentous material through the cuticle of the glandular area was observed (FM in Fig. 3d).

Discussion

Comparison of ultrastructures among species

Table 1 summarizes the ultrastructural features of sternal glands in the thrips examined (Bode, 1978; Sudo and Tsutsumi, herein). Many secretory cells, a cuticular ridge, secretory ductules passing through the cuticle, epicuticular filaments arranged radially at the inner end of the ductules and a wide subcuticular space separating the secretory cells from the cuticle were observed in the sternal gland in *Thrips hawaiiensis*. In their secretory cells, two types of





Fig. 3 TEMs of the cuticle of the glandular area. a. Cuticular ridge (CR) and secretory ductules (SD) in *Thrips hawaiiensis*. b. Cuticular ridge (CR) in *Frankliniella intonsa*. c. Secretory ductules (arrows) passing through the cuticle in *F intonsa*. d. Tubular invaginations (arrowheads) of the epicuticle and diffusion of filamentous material (FM) through the cuticle in *Psalidothrips simplus*. Cu: cuticle, EF: epicuticular filaments at the inner end of the secretory ductules. Scales =a, b, 200 nm; c, d, 500 nm.

mitochondria, a remarkably elongated type and an oval-shaped one, existed, and some elongated mitochondria had penetrated the microvilli. These ultrastructural features coincide with those in *Thrips validus* reported by Bode (1978). It appears that sternal glands in species belonging to the same genus show similar ultrastructural features.

In *Frankliniella intonsa*, a species of another genus belonging to the same family Thripidae as the genus *Thrips*, a cuticular ridge, secretory ductules and secretory cells with two types of mitochondria were observed, like in the genus *Thrips*. The cuticular ridge in this species, however, was much smaller than those in *T. hawaiiensis* and *T.*

Fig. 2 TEMs of the sternal glands. a. Gross structure of the sternal gland (sagittal section) of *Thrips hawaiiensis* (anterior to the right). b. Remarkably elongated mitochondria penetrating microvilli in *Frankliniella intonsa*. c. Gross structure of the sternal gland (sagittal section of a little side part) of *Psalidothrips simplus* (anterior to the right). d. Enlargement of the subcuticular space and its vicinity in *P simplus*. Note that there are no remarkably elongated mitochondria in the secretory cells. Asterisks show the subcuticular space filled with secretion. CR: cuticular ridge, Cu: cuticel, De: desmosome, LD: lipid droplet-like secretory granule, Mt: mitochondria, Mv: microvilli, N: nucleus of secretory cell. Scales=a, c, 5 μ m; b, d, 500 nm.

Family	Species	Secretory cells			Cuticle of glandular area		
		Microvilli in apical region	Remarkably elongated mitochondria	Oval-shaped mitochondria	Secretory ductules	Filamentous material through cuticle	Cuticular ridge
Thripidae	Frankliniella intonsa	developed	present	present	present	absent	small
	Thrips hawaiiensis	developed	present	present	present	absent	large
	Thrips validus*	developed	present	present	present	absent	large
Phlaeothripidae	Psalidothrips simplus	developed	absent	present	absent	developed	absent

Table 1 Ultrastructures of the sternal glands in the thrips species examined in the present study.

* From the description by Bode (1978).

validus. Thus, in the sternal gland of thripine thrips, there seem to be some differences in ultrastructural features among genera.

In the secretory cells of *Psalidothrips simplus*, a species belonging to another family Phlaeothripidae, remarkably elongated mitochondria were not found. Moreover, in the cuticle of the glandular area, a cuticular ridge and secretory ductules passing through the cuticle were not observed (cf. Figs. 2c, 3d). Instead of the ductule, diffusion of secretory material including filamentous structures through the cuticle of the glandular area was observed. Thus, an obvious morphological difference between the sternal glands in thripine thrips and phlaeothripine thrips was found. This finding suggests that the sternal glands in thripine thrips and phlaeothripine thrips have originated independently.

Comparison with secretory glands in other insects

The absence of sternal glands in most females and in larvae of both sexes, sudden appeasement of an excited female of *Taeniothrips dianthi* after the mounting of the male, and gathering of all other females of *T. dianthi* around the copulating couple had led researchers to the hypothesis that the secretion of the sternal gland of thrips is a pheromone secretion that acts as an attractant and as an approximate (Perikán, 1951). Ultrastructural and histochemical studies on the sternal gland in *T. validus* also supported this hypothesis regarding the function of this organ (Bode, 1978).

Generally, insect gland cells are characterized by numerous microvilli, abundant mitochondria, microbodies and an endoplasmic reticulum. The insect gland cells are classified into three categories (class 1, class 2 and class 3); class 1 cells adjoin the glandular cuticle, and the secretion is released by microvilli and then by a secretory ductule; class 2 cells are not in contact with the cuticle, and the secretion must pass through surrounding class 1 cells; and class 3 cells are composed of several cells connected to the cuticle by a secretory ductule draining the secretion outside (Quennedey, 1998). The cells in all of the sternal glands in thrips examined in the present study are class 1 cells as described by Quennedey (1998), as was also reported in the sex-pheromone glands in the females of the eastern spruce budworm Choristoneura fumiferana (Percy, 1974), the cabbage looper Trichoplusia ni (Percy, 1979) and the corn earworm Helicoverpa zea (Raina et al., 2000). The secretory cells of these sex-pheromone glands are characterized by numerous microvilli, desmosome by which the secretory cells are connected, rough-surfaced endoplasmic reticulum, Golgi complex, abundant mitochondria and lipid droplets. The ultrastructural features of these sex-pheromone glands are very similar to those of the sternal glands of thrips observed in the present study, though no obvious Golgi complex was observed in the secretory cells in the sternal glands of either thripine thrips and phlaeothripine thrips and no lipid droplets were observed in the sternal glands of thripine thrips. Epicuticular filaments at the inner end of the secretory ductules in thripine thrips (EF in Fig. 3a, c) have been also detected in the pheromone-secreting glands in males of the hangingfly Harpobittacus australis (Crossly and Waterhouse, 1969), in females of C. fumiferana (Percy, 1974), T. ni (Percy, 1979) and H. zea (Raina et al., 2000) and in the scent glands in the larvae of the hemipteran Apateticus bractratus (Percy et al., 1980). Moreover, a structure similar to the subcuticular space of the sternal gland of thrips appears in the sex-pheromone gland cell region during scotophase in H. zea as pockets of granular material (Raina et al., 2000). Thus, the sternal glands in all of the thrips species examined in the present study showed ultrastructural features common to other insect secretory glands. It is likely that the sternal glands of thrips are pheromone-secreting glands, as was pointed out by Perikán (1951) and Bode (1978). As mentioned above, however, we detected neither secretory granules

nor Golgi complexes in the secretory cells of sternal glands of thrips, though some lipid droplet-like secretory granules were found in the sternal glands of phlaeothripine thrips, *P. simplus*. The sternal glands of thrips, therefore, might have specific systems for synthesis and secretion of secretory material in contrast to those of other insects. Further detailed ultrastructural and histochemical studies are needed to test the validity of our hypothesis.

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