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Ultrastructure of female accessory glands in the scorpionfly *Panorpa sexspinosa* Cheng (Mecoptera: Panorpidae)

Na Ma [a,b], Meng Wang [b], Baozhen Hua [b,*]

[a] State Key Laboratory of Crop Stress Biology for Arid Areas, College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] Key Laboratory of Plant Protection Resources and Pest Management of the Education Ministry, Northwest A&F University, Yangling, Shaanxi 712100, China

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ABSTRACT

The histology and ultrastructure of the female accessory glands in the scorpionfly *Panorpa sexspinosa* Cheng was studied using light and transmission electron microscopy. The glands consist of a pair of distal elongate gland tubes and a basal common duct, which opens in the genital cavity at the dorsal side of the genital plate. The whole gland tubes and common duct are similar histologically and ultrastructurally. The epithelium of female accessory glands consists of two cell types: the outer secretory cells and the inner duct-forming cells. These two cells that join with a cuticular duct connecting to the inner intima constitute a functional glandular unit belonging to Class 3 glandular cells of epidermal glands. The secretory cells are rich in organelles, such as mitochondria, rough endoplasmic reticulum, and Golgi complex, indicating that they are active in secretion. The duct-forming cells are flattened with sparse-distributed organelles. These two kinds of cells are connected by septate junctions. The cuticular duct consists of a receiving and a conducting canal and is responsible for transferring the secretions of the secretory cell to the lumen. The receiving canal is formed of interrupted multilayered inner epicuticle and located in the irregular extracellular cavity of the secretory cell, bounded by microvilli. The conducting canal connects the inner intima and opens into the central lumen. The tentative functions of the secretions are briefly discussed.

1. Introduction

Epidermal glands are common in insects, occurring on various parts of the body, including the tergum, sternum, pygidium, legs, mouthparts, and genital apparatus, and take a tremendous diversity of morphology and function (Betz, 2010; Noirot and Quennedey, 1974; Quennedey, 1998).

Female accessory glands are often called colleterial or cement glands in insects. They are abdominal gland systems associated with the reproductive system and generally arise from the genital chamber or vagina (Büning, 1994; Chapman, 1998; Gullan and Cranston, 2005). As highly specialized secretory organs, the female accessory glands are derived independently in different orders and sometimes are even non-homologous within an order. These glands are ectodermal in most insects, but are mesodermal in origin and housed on the lateral oviduct in grasshoppers (Chapman, 1998). The synthesis and secretion functions of the glands are frequently in concert with ovarian and ovulation events, even the design of diverse mating systems (Degrugillier and Grosz, 1981; Hosken et al., 2001, 2002; Lococo and Huebner, 1980; Szopa, 1981).

According to previous studies, the female accessory glands exhibit various morphology and function among different species of insects (Gullan and Cranston, 2005). In most insect orders the female accessory glands are paired and produce a substance for protecting the eggs or attaching them to the substratum during oviposition (Chapman, 1998). In Dictyoptera the accessory glands are asymmetrical and involved in the elaboration of ootheca during oviposition (Courrent et al., 2008). The accessory gland of Thysanoptera, as a single structure with an apical bulb and a long duct opening into the vagina, produces secretion probably for lubrication during oviposition (Dallai et al., 1996). In Diptera the paired female accessory glands produce secretions that may contain factors affecting egg hatchability and sperm penetration (Degrugillier and Grosz, 1981; Degrugillier and Leopold, 1976). The secretion products of the accessory glands in the female Mediterranean fruit fly *Ceratitis capitata* are demonstrated to have antibacterial properties to protect eggs from the surroundings (Marchini et al., 1991, 1997). The parasitic ichneumonid *Pimpla turionellae* in Hymenoptera bears highly modified glands, among which the uterus glands produce hyaluronic acid and a lipoprotein coating the egg to prevent encapsulation by the host hemocytes (Blass and Ruthmann, 1989). The female accessory glands of the desert locust *Schistocerca gregaria* are tubular extensions of the paired genital ducts and play a major role in formation of the egg pod and transfer of behavioural gregarious phase characteristics from mother to offspring (Hägele et al., 2000; Szopa, 1981, 1982).

Panorpidae is the most species-rich family in Mecoptera, which is one of the most primitive taxa in Holometabola (Kaltenbach,

* Corresponding author. Tel.: +86 29 87091342; fax: +86 29 87091342.
E-mail address: huabzh@nwsuaf.edu.cn (B. Hua).

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1978; Penny, 2006; Willmann, 1987, 1989). They are commonly known as scorpionflies because the male genital bulb (the ninth abdominal segment and its appendages) are enlarged and recurved upward, superficially resembling the stinger of scorpions. The female accessory glands exhibit distinct morphological diversity in Panorpidae and other families, even different genera (Potter, 1938). However, the studies on the female accessory glands of Panorpidae are only concentrated on morphology and at most histology (Grell, 1942; Potter, 1938; Hou and Hua, 2008), no research has been conducted on their ultrastructure to date.

The objective of this study was to investigate the ultrastructure of the female accessory glands in *Panorpa sexspinosa* Cheng, 1949 using light and transmission electron microscopy to provide the first detailed description of the female accessory glands in *Panorpa*. These data can be used as a morphological and cytological basis for future studies on reproductive biology of scorpionflies, including oviposition.

2. Materials and methods

2.1. Insects

The female adults of *P. sexspinosa* Cheng, 1949 were collected from the Taibai Mountain, Shaanxi Province, China in August 2010, and were reared in screen-wire cages in the laboratory and fed freshly killed flies, grasshoppers, katydids or caterpillars. Since the morphology and function of the female accessory glands may change in different developmental stages (Rosay, 1968; Sturm, 2002; Sturm and Pohlhammer, 2000; Szopa, 1982; Tirone and Avancini, 1997), we choose gravid female adults to dissect the female accessory glands, which were in the actively functional condition.

2.2. Sample preparation and light microscopy

The specimens were anesthetized in ethyl ether and dissected rapidly in the Ringer's solution (Xie and Hua, 2010) under Nikon SMZ 1500 microscope. The habitus of the accessory glands was observed and recorded. To compare the structures of different regions, we divided the right and left gland tubes of the accessory glands into three parts (the apical, middle, and basal parts) equally. The dissected accessory glands were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in the phosphate buffered saline (PBS, 0.1 mol/L, pH 7.2) for 12 h at 4 °C and then rinsed with the same buffer. Then the specimens were dehydrated through a graded acetone series, infiltrated with the graded mixture of acetone and Epon 812 resin, and finally embedded in the pure Epon 812 resin. After the samples completely polymerized, they were cut into semi-thin sections on LKB2088 microtome. After stained with 0.5% toluidine blue, they were observed under an Olympus BX-51 light microscope and photographed with a digital camera mounted on the microscope.

2.3. Transmission electron microscopy (TEM)

For the TEM, the procedure of sample preparation is similar to the above, except for the post-fixation performed with 1% osmium tetroxide for 2 h at 4 °C and rinsing with the PBS before dehydration. After completely polymerized, the samples were cut into ultrathin sections with a diamond knife on an ultramicrotome (Reichert Ultracut E) and routinely stained with uranyl acetate and lead citrate, and finally examined in a JEOL JEM-1230 transmission electron microscope (JEOL, Tokyo, Japan) at 80 kV.

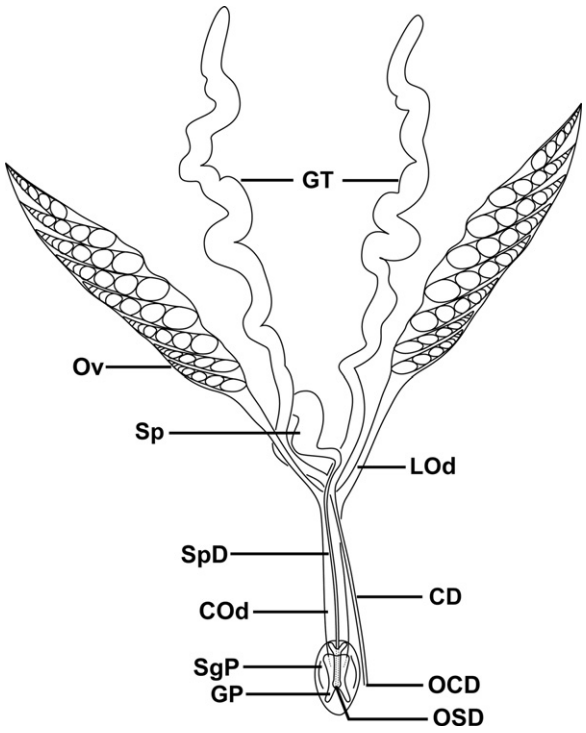

Fig. 1. Schematic diagram of accessory glands associated with other female reproductive system of *Panorpa sexspinosa*. The common duct of the accessory glands is originally situated dorsally to the spermathecal duct, and here pulled laterally to show the latter. CD, common duct of accessory glands; COd, common oviduct; SgP, subgenital plate; GP, genital plate; GT, gland tubes of accessory glands; LOd, lateral oviduct; OCD, opening of the common duct of accessory glands; OSD, opening of spermathecal duct; Ov, ovary; Sp, spermatheca; SpD, spermathecal duct.

3. Result

3.1. Gross morphology of the female accessory glands

The female accessory glands of *P. sexspinosa* are composed of a pair of gland tubes and a thin common duct (Fig. 1). The two gland tubes closely adhere to the ventral side of the alimentary canal and tortuously extend forward to the second abdominal segment between the two lateral oviducts (Fig. 1). The tubes are approximately uniform in length (mean 7.20 mm, $n = 4$) and diameter (mean 0.28 mm, $n = 4$). The two gland tubes are united to form a common duct in the seventh abdominal segment. The common duct is less than half the length of the tube (2.07 mm long) and considerably thinner (0.16 mm in diameter) (Fig. 1). It extends backward dorsally along the common oviduct and spermathecal duct, and opens in the genital cavity between the orifice of the spermathecal duct and anus. The newly dissected accessory glands from the gravid female adults are bulgy and full of reddish orange liquid secretions. Accompanying with female ovulation, the secretions are expelled in milky white colour. The secretions on the egg surface turn dark brown in several hours.

3.2. Histology and ultrastructure of the female accessory glands

The gland tubes and common duct have no significant differences histologically and ultrastructurally at various regions, except for the size. Therefore, the different region sections we chose here are used to represent the whole accessory glands. The female accessory glands consist of a muscle layer and an epitheical layer, enclosed by tunica externa outside (Figs. 2 and 3A and B). In the muscular layer, a few tracheoles can be seen to penetrate between muscular cells (Fig. 3A). Mitochondria are abundantly distributed in