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Ultrastructural aspects of two sting glands in social Hymenoptera

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ABSTRACT. Exocrine glands associated with the sting apparatus are widespread in social Hymenoptera. Well-known are the venom gland and the Dufour gland. However, some other glands have been reported in literature. One example is the 'sting gland' in the region of the triangular plate part of the sting apparatus. This gland belongs to the secretory unit type 3. The duct cells open through an intersegmental membrane near the base of the triangular plate. In the present study, this site of duct openings is discussed in the perspective of its association with the oviduct and not with the sting sensu stricto. The postulated involvement of the gland in reproduction gains in importance, because obvious differences concerning glandular size were found between queens and workers. This tendency is observed for some ants, for bumblebees and for social wasps.

In general, this triangular plate gland seems to metabolize lipids. The fact that some queens possess extremely developed glands most probably has little to do with lubricant production needed for facilitating the act of stinging, as is sometimes suggested in literature, since usually workers are more likely to sting throughout their lives, and queens will especially sting at the more critical stage of colony foundation.

A second gland, the quadrate plate gland was found near the quadrate plate of the sting in bumblebees. Ultrastructural characteristics of both glands are described.

KEY WORDS: exocrine glands, triangular plate gland, quadrate plate gland, social insects.

INTRODUCTION

Social insects are known to be well provided with exocrine glands (Billen, 1993; Billen & Morgan, 1998; Hölldobler & Wilson, 1990). Most of the glands play an essential role in the social life of these insects. Even today, new glands are being discovered. The objectives of the present study are 1) to provide more detailed data concerning a gland that was once mentioned briefly in literature, the triangular plate gland (or sting gland according to Robertson, 1968) and 2) to describe a novel gland in bumblebees, the quadrate plate gland.

Usually, concerning sting glands, two particular structures are of interest: the venom gland and the Dufour gland. The venom gland is involved in venom production in stinging species, but can have various other functions, such as the production of pheromones. The Dufour gland often produces hydrocarbon mixtures with various functions (Billen & Morgan, 1998).

However, the glands dealt with in the present study are rather intersegmental glands that are more indirectly associated with the sting. Jessen & Maschwitz (1983) have provided a detailed overview of abdominal glands in *Pachycondyla tridentata* (F. Smith, 1858), a ponerine ant. Most of the glands mentioned in their work are intersegmentally located. In that study, an account was also given of the glands associated with the sting apparatus itself (triangular plate gland, quadrate plate gland, spiracular plate gland, sting sheath gland). However, only light microscopical data are available.

MATERIAL AND METHODS

Foraging workers and queens of *Myrmecia pyriformis* F. Smith, 1858, *Diacamma* sp., *Odontomachus rixosus* F. Smith, 1857, *Pachycondyla* sp., *Bombus terrestris* (Linnaeus, 1758) and *Vespa crabro* Linnaeus, 1758 were dissected in insect Ringer solution (Jolly) and then fixed in glutaraldehyde. A first group of samples was dehy-

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drated in a graded ethanol series. After dehydration, samples were put into LRWhite resin (three times rinsed). Consequently embedding in LRWhite was performed (at 50 °C). LRWhite embedded material was then processed for histochemical staining (for protein) by means of Coomassie blue.

Sting glands were fixed during 2-20 hours in 2% glutaraldehyde (4 °C, pH 7.3 and buffered with 0.05 M sodium cacodylate). Postfixation in a buffered osmium tetroxide solution (1 hour) was followed by dehydration in an acetone series and embedding in Araldite.

Semi-thin sections (1 µm thickness) for light microscopy were made with a Reichert OmU2 ultramicrotome and stained with methylene blue and thionin.

Thin sections, made with a Reichert Ultracut E microtome, were stained with uranyl acetate and lead citrate in an LKB 2168 Ultrostainer, and examined in a Zeiss EM 900 electron microscope.

Samples for SEM analysis were critical point dried in a Balzers CPD 030 critical point drying device, after complete dehydration in formaldehyde dimethyl acetal (dimethoxymethane or methylal). They were coated with gold and viewed in a Philips SEM 515 microscope.

RESULTS

The triangular plate gland

A series of species belonging to different families of social insects were screened to check for the presence and development of their sting glands. Particular attention was paid to the occurrence and development of the triangular plate gland (or sting gland, following Robertson, 1968). Each triangular plate gland consists of a cluster of secretory units, belonging to type 3 according to Noirot & Quennedey (1974) and Quennedey (1998). This means that each secretory cell is provided with its own duct cell.

In the species studied, sting glands are usually well developed towards the region of the triangular plate (Fig. 1), where these glands are attached via their ducts to the intersegmental membrane. However, the position is not always the same, because of the glandular cluster size. In bumblebees, for example, and more particularly in their queens, a more ventral (with the sting as reference) location is found. Bumblebee queens are among the most interesting insects to demonstrate the position and development of the triangular plate gland, as is illustrated in Figs 2 and 3. From these figures, it is clear that, notwithstanding the fact that from a lateral view the gland seems to be located opposite to the triangular plate, it is also clearly associated with the posterior end of the oviduct, and hence masking its relevant position.

When the sting is moved backwards to allow egg-laying, however, the ducts most likely will discharge their secretion more ventrally into the posterior part of the central oviduct.

The ultrastructural characteristics of the gland include occurrence of smooth endoplasmic reticulum, the end apparatus (Fig. 5), lipid inclusions of varying diameter, electron-dense inclusions and multilamellar bodies. The last were conspicuously found in association with lipid droplets in the triangular plate gland of *Odontomachus rixosus* queens (Figs 5 and 6) and are known from other studies to be involved in the actual secretion process. Lamellar bodies, similar to those found in vertebrate surfactant producing cells, were also found near the end apparatus (Fig. 5). *O. rixosus* workers, however, do not seem to have this gland. In contrast, queens possess a glandular cluster of about 300 µm in diameter, with glandular cells having a diameter of approximately 40 µm.

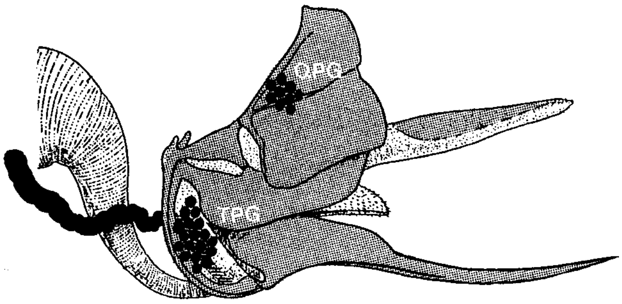


Fig. 1. – Schematical representation of two glands associated with the sting in bumblebees. Lateral view of sting apparatus; at the left are the Dufour gland (black) and the duct of the venom gland; QPG=Quadrate plate gland; TPG=Triangular plate gland.

Legends to the figures (see opposite page)

Fig. 2. – Scanning electron micrograph of the triangular plate gland in a *Bombus terrestris* queen. Lateral view of sting apparatus. The arrow indicates the position of the gland near a cluster of fat cells. DG=Dufour gland; St=sting; TPG=triangular plate gland. Scale bar 1 mm.

Fig. 3. – Scanning electron micrograph of the triangular plate gland in a *B. terrestris* queen. Ventral view of sting apparatus, showing both glands. OD= oviduct; St= sting. Scale bar 1 mm.

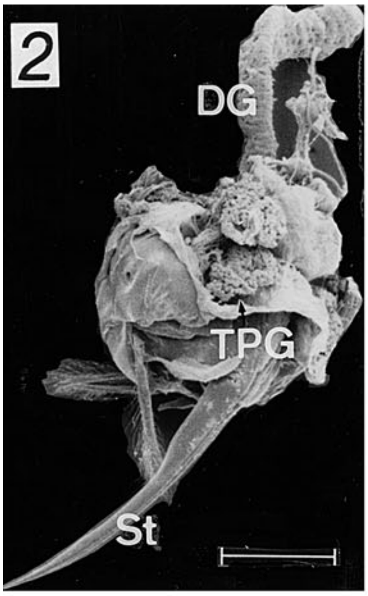
Fig. 4. – Semi-thin section of LRWhite-embedded triangular plate gland in a *Myrmecia pyriformis* worker. The dark staining end apparatus after application of Coomassie Blue for protein in each cell is clearly visible (arrows). DC=duct cells. Scale bar 10 µm.

Fig. 5. – Electron micrograph of the triangular plate gland in an *Odontomachus rixosus* queen, showing end apparatus (EA) with slight microvillar distortion. LB=lamellar bodies; mv=microvilli. Scale bar 1 µm.

Fig. 6. – Electron micrograph of the triangular plate gland in an *O. rixosus* queen, showing secretory vesicles of mixed composition. li=lipid material; mt=mitochondria. Scale bar 1 µm.

Fig. 7. – Electron micrograph of the quadrate plate gland in a *Bombus terrestris* worker, showing end apparatus without microvillar distortion. lu=lumen of the end apparatus. Scale bar 1 µm.

Fig. 8. – Electron micrograph of the quadrate plate gland in a *B. terrestris* worker, showing inclusions of mixed composition. my=myeloid secretion. Scale bar 1 µm.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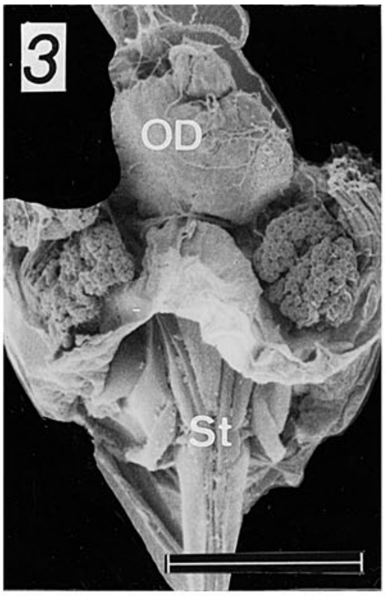
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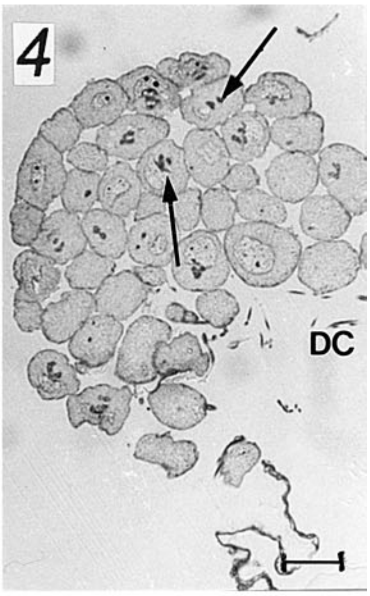
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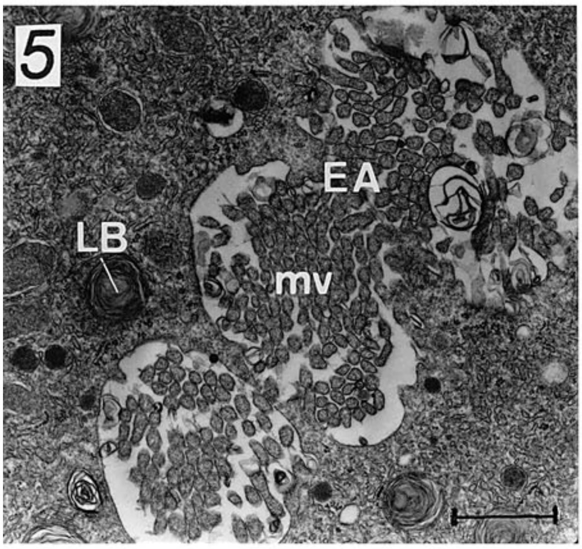
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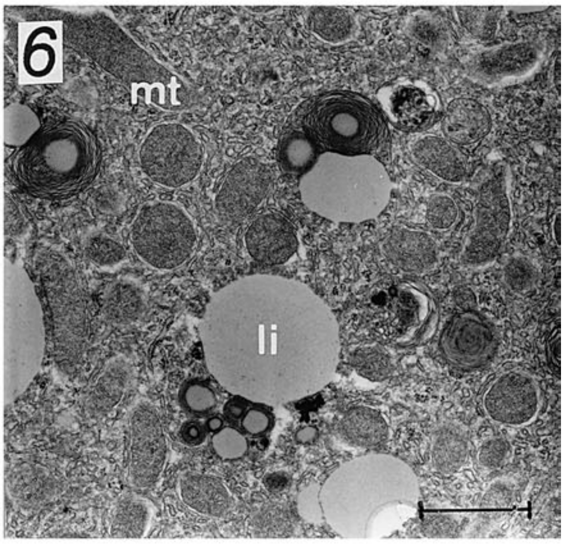
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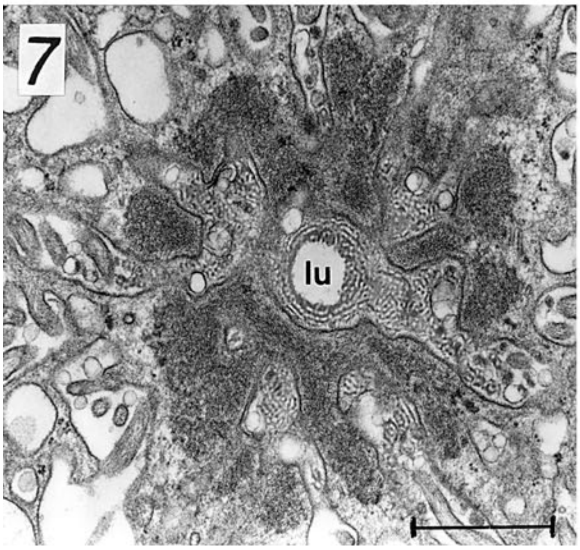
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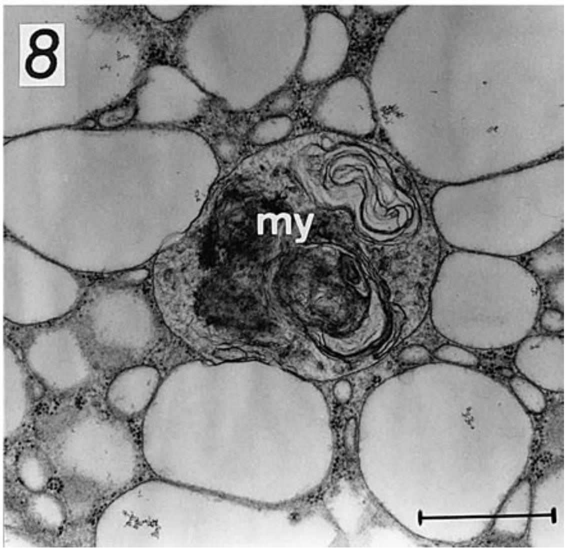
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my

The triangular plate gland thus has ultrastructural characteristics of a gland producing lipoid substances. However, histochemical investigation for protein in *Myrmecia pyriformis* has revealed the presence of proteinaceous material in the lumen of the end apparatus of each secretory cell (Fig. 4). Additional histochemical analyses have shown that a minor fraction of the secretion contains polysaccharides.

The quadrate plate gland in *Bombus terrestris*

In bumblebees we found a glandular cluster (see Fig. 1), near the quadrate plates associated with the sting.

Each cluster consists of secretory units. The ducts of these units discharge their secretion through the intersegmental membrane between the quadrate plate and the spiracular plate. The quadrate plate gland of the bumblebee *Bombus terrestris* is fairly similar in ultrastructure to the triangular plate gland. Sections through the end apparatus can be encountered (Fig. 7), as well as inclusions consisting of variable contents (Fig. 8), which can be designated as myeloid secretion.

DISCUSSION

The present study is the first to provide more accurate data concerning the morphology, the ultrastructure and the histochemistry of the triangular plate gland in social Hymenoptera. The only available literature data dealing with this gland are simple descriptions for some species on the occurrence or absence of the gland, without further specification of cellular morphology and/or ultrastructure (ROBERTSON, 1968). We have tried to fill this gap and have shown that, at least in bumblebees, even another ‘sting gland’ occurs near the quadrate plate. In earlier literature on honeybee glands by SNODGRASS (1956), it was already suggested that this glandular mass of unicellular glands producing their secretion to the outside of the quadrate plates of the sting in *Apis mellifera*, might lubricate the shaft of the sting when venom is ejected. However, it has been shown by GHENT & GARY (1962) that an attractant is produced, which stimulates other bees to continue their attacks at the initial stinging site. This assumption remains controversial, because the gland as we found it in bumblebees is not very likely to serve a similar function, knowing that bumblebees usually are far less aggressive.

In general, the triangular plate gland seems to metabolize lipids, but apparently lipids are not the only constituents of the secretion, since histochemical staining with Coomassie blue has revealed the presence of protein in the end apparatus of each secretory unit (in bulldog ants of the genus *Myrmecia*). Even sugars are present.

ATTYGALLE et al. (1996) screened a set of small dermal complex glands in the large ponerine ant *Pachycondyla tridentata* (F. Smith, 1858), a glandular system which consists of groups of gland cells located dorsally, ventrally, dorsoventrally, dorsolaterally and ventrolaterally in the intersegmental membranes between all gaster segments, and also near the sting apparatus. For the single species mentioned by these authors, oily substances were found and hence a lack of pheromone function and antibiotic effects was shown together with more evidence for a lubricative function. Significant amounts of linoleic acid and palmitic acid, together with trace amounts of other fatty acids and corresponding methyl esters have been demonstrated. According to the same authors, similar glands can be expected to be found in association with the sting apparatus as the sclerites of the sting apparatus derive from abdominal sclerites. However, our observations on *Odontomachus* ants, also belonging to the same subfamily as *P. tridentata* investigated by ATTYGALLE et al. (1996), suggest a shift in glandular function, since the lubricative gland seems absent in workers but is very obviously present in queens. It is indeed important to bear in mind that functional specialization within one particular category of glandular structures can occur, as is known for other sting glands as there are venom glands (with production of protein, alkaloids, pyrazines, formic acid, etc.) and Dufour glands.

In bumblebees and ants, cautious interpretation is recommended, because bumblebee and ant queens might need a powerful sting especially during the critical time of colony foundation, but this argument does not explain why e.g. in *Odontomachus rixosus*, the queen has such a large gland whereas the workers apparently lack it. *Odontomachus* workers frequently need their sting for predator-prey interactions and they do not have the gland, so the argument of a lubricative function is not valuable in this case. The fact that in *Odontomachus* ants, queens possess highly developed glands most probably has little to do with lubricant production needed for facilitating the act of stinging, as is sometimes suggested in literature, since usually workers are also very likely to sting. It would then be very unlikely to find an extremely large gland in the queen. In workers of *O. rixosus* we failed to trace the gland, so the difference between the castes is even more pronounced. In various social insect groups, workers are more likely to sting throughout their life, when facing prey and/or possible predators, whereas queens will sting especially during the critical time of colony foundation.

The observation that protein is present in the secretion is important in the light of the findings of CASSIER & LENSKY (1994) for the Nasanov gland of the honeybee. These authors pointed out that the protein present in the secreted material would enhance the effect of the biological activity of the released chemicals. Usually, the problem in analysing secretions by means of one particular technique, e.g. during the search for pheromones by gas chromatography, is that often other constituents of the secretion are being overlooked. An example from literature is known for tergal glands of cockroaches (QUENNEDEY & BROSSUT, 1975). These authors found that protein is indeed present in the gland cells, but the direct function of these molecules has not been elucidated. Because of its very obvious presence, the protein is not likely to be a structural element within the endocuticula of the gland end apparatus, at least for *Myrmecia*. The histochemical results in this study clearly point in the same direction, i.e. that a more cautious interpretation should be recommended. In general, proteins, often in combination with lipids or glucids, are common in secretions, but are generally underestimated because of the analytical techniques used in one particular research field.

The precise site where the ducts of the secretory units open can be situated in the intersegmental membrane near the triangular plate, but if the insect extrudes its sting, the ducts will open more ventrally into the cavity of the central oviduct. This is the controversial point we would like to emphasize in the present study. From our semi-thin sections and dissected stings, we therefore propose not to exclude a role in reproduction for the triangular plate gland. When the female starts egg-laying, the egg will pass directly underneath the sting base, and hence contact of the egg with glandular secretion is a possibility. One could imagine some kind of egg-marking. The myeloid secretion found in the glands studied, is also known from mandibular glands of the cockroach *Blaberus craniifer* (Quennedey & Brossut, 1975).

We have checked the occurrence of the triangular plate gland in several species of *Myrmecia* (Myrmeciinae), in several representatives of the Ponerinae, such as *Pachycondyla*, *Odontomachus* and *Diacamma* species, in social wasps and in bumblebees. Its widespread occurrence suggests a fundamental function in the biology of these insects.

Summarizing our findings, we can conclude that: 1) the triangular plate is present in more species than previously reported, 2) its secretion is lipoidal, but not uniquely. Part of the secretion is protein, 3) the gland shows, at least in some species, a clear queen-worker dimorphism, 4) the triangular plate gland is also present in other groups of social insects, such as bumblebees, 5) the triangular plate in bumblebees is also closely associated with the end of the oviduct, which probably implies a reproductive function and 6) other intersegmental glands such as the quadrate plate gland, are present in bumblebees.

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