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Functional morphology of the metapleural gland in workers of the ant *Crematogaster inflata* (Hymenoptera, Formicidae)

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Abstract. Workers of *Crematogaster inflata* possess the largest metapleural glands (relative to body size) known among ants, with reservoirs extending anteriorly up to the junction between the pro- and the mesothorax, and with over 1400 secretory cells on both sides together. This large secretory capacity is related to the gland's defensive function, which, in members of this species, is directed against larger arthropod and vertebrate enemies, and apparently not against microorganisms, in contrast to other ants, where the gland produces antibiotics. The gland is not equipped with any direct musculature. Secretion release is probably caused by contraction of the oblique longitudinal thorax muscles or by passive expulsion caused by external pressure.

Additional key words: exocrine glands, social insects, histology

Ants live in colonies that represent potentially very valuable resources for predators. The colony as a whole, however, is generally well protected by the combined defensive power of its individual members. The common defense mechanisms of ant workers include both mechanical and chemical elements. They can rely on their mandibles as powerful instruments for biting, spray formic acid if they are formicine ants, or use their sting, which acts as a syringe to inject the contents of the venom gland. Besides these defense strategies that involve the head and the abdomen, the thorax can also play an important role through the secretory products of the metapleural gland. This gland is known for the elaboration of antibiotics that suppress the development of microorganisms (Maschwitz et al. 1970; Schildknecht & Koob 1970, 1971; Beattie et al. 1986; Bot et al. 2001, 2002; Poulsen et al. 2002a,b).

In workers of the southeast Asian ant *Crematogaster inflata* Smith 1857, the posterior part of the thorax is bright yellow, in contrast to the otherwise black appearance of these ants. The yellow region corresponds with the position of an enlarged metapleural gland, which, in this species, functions in the elaboration of a sticky defensive secretion (Maschwitz 1974; Jones et al. 2005). The obvious black-and-yellow color pattern was also found to be a warning signal for predators, as exemplified by cases of Batesian mimicry by a similarly colored member of the genus *Camponotus* and of beetles in the genus *Drusilla* (Maruyama et al. 2003; Ito et al. 2004). We examined the morphology of this gland in workers of *C. inflata,* and linked its structural characteristics with the defensive function it performs. In his 1974 paper, Maschwitz also included the congeneric species *Crematogaster difformis* Smith 1857, workers of which also have enlarged metapleural glands, but with the production of antibiotics as their main function. *Crematogaster difformis* is probably a mis-identification of *Crematogaster sewardi* Forel 1901 (Hosoishi & Ogata 2009). As we had access to embedded material of *C. sewardi,* we also examined the metapleural gland of members of this species for comparison.

Methods

Foraging workers of *Crematogaster inflata* were collected at the Ulu Gombak Field Station, peninsular Malaysia. Posterior thorax halves were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with $50\,mol\,L^{-1}$ Na-cacodylate and $150\,mol\,L^{-1}$ saccharose. Postfixation was performed in 2% osmium tetroxide in the same buffer and was followed by dehydration in a graded acetone series. Tissues were embedded in Araldite and sectioned with a Reichert

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Ultracut E microtome (Reichert, Austria). Semithin sections (1 μm thick) were stained with methylene blue and thionin and viewed with an Olympus BX-51 microscope (Olympus, Hamburg, Germany). A thin layer of Pattex®-glue was applied onto the side of the tissue block first touching the knife, which allowed us to make aligned ribbons of serial sections that were used for 3D-reconstruction using the Amira software program (Visage Imaging, Berlin, Germany). Estimation of the number of secretory cells was carried out by counting the cumulative number of cells occurring in serial sections through the entire thorax at 30-μm intervals (which is the average cell diameter). Workers of *Crematogaster sewardi* (which, like *C. inflata*, belongs to the *Physocrema* group: Hosoishi & Ogata 2009), collected in the Bogor Botanical Gardens, Indonesia, were prepared the same way for comparison of the number of secretory cells in their metapleural glands.

Thin sections (70 nm) of the metapleural glands of members of *C. inflata* were double stained with lead citrate and uranyl acetate and examined in a Zeiss EM900 electron microscope (Zeiss, Oberkochen, Germany). For scanning microscopy, air-dried material was mounted on stubs, coated with gold, and examined using a JEOL JSM-6360 scanning microscope (JEOL, Tokyo, Japan).

Results

The paired metapleural gland of each worker of *Crematogaster inflata* occupies a large portion of the posterior part of the thorax. At each side, the gland opens to the outside through a large round opening with a diameter of ~80 μm just below the metathoracic spiracle (Fig. 1A–C). The opening is not associated with any muscular elements, and is surrounded by a smooth thick cuticle, which results in a permanently open contact with the exterior (Fig. 1C). At each side, this opening leads to a very large reservoir sac that stretches anteriorly to the border between the pro- and the mesothorax (Fig. 1D,E), which roughly corresponds with the part that has a yellow appearance externally. The reservoir wall is formed by a thin cuticle with a thickness of 1 μm and an underlying squamous epithelium. Its fragility often causes a partially collapsed appearance on histological sections. When fully extended, each reservoir reaches a length of ≤700 μm, a height of 400 μm, and a width of 350 μm.

The rounded glandular cells occur in the anterior of the reservoir, toward the body midline (Fig. 1F). The number of cells is estimated at 720±45 per side ($n=5$ ants) or >1400 for the entire gland. A cell count in two workers of the congeneric *Crematogaster sewardi* yielded a total number of 747 and 774 cells, approximately half the number found in workers of *C. inflata*. The cells have an average diameter of 30 μm, each cell being connected to the reservoir through an accompanying duct cell (according to the classification of class-3 exocrine glands of Noirot & Quennedey (1974)). The cells are often grouped in clusters, as can be seen by the occurrence of bundles of ten to 15 duct cells (Fig. 1G). Nuclei are spherical with a diameter of ~10 μm, and are centrally localized. At the ultrastructural level, the secretory cells are characterized by the presence of an end apparatus, comprising a central cuticular duct surrounded by microvilli, and numerous mitochondria (Fig. 1H). Free ribosomes are scattered over the cytoplasm, although no clear endoplasmatic reticulum could be found.

Muscle fibers that insert directly onto the metapleural gland were not found either surrounding the reservoir, or in the region of the lateral openings where secretion is discharged to the outside. Indirect muscular elements that attach to body parts other than the metapleural gland, however, are represented by the paired oblique longitudinal thoracic muscles. These run from the pronotum to the coxal region (Fig. 1D,E), and as such form contractile cord-like bundles touching the midventral side of the reservoirs (Fig. 1D–F).

Discussion

The metapleural gland of workers of *Crematogaster inflata* is much larger than that of other ants, both in the proportional extent of its reservoirs and in the absolute number of its secretory cells. With a total number of over 1400 secretory cells, metapleural glands of *C. inflata* have a secretory capacity that is considerably larger than that of the metapleural gland of any other ant species. Cell counts for the metapleural gland in members of the congeners *Crematogaster clariventris* Mayr 1895 and *Crematogaster depressa* Latreille 1802 yield a total of 350 and 400 cells, respectively, which is among the highest known number among myrmicine ants (Fanfani & Giovannotti 1994). Members of the congeneric *Crematogaster sewardi,* which also belongs to the *Physocrema* group, also have enlarged metapleural glands (Maschwitz 1974; Hosoishi & Ogata 2009), but with total cell numbers of 747 and 774 in the two workers checked, their size is only half that of the glands of *C. inflata*. Maschwitz (1974) reported that the metapleural glands of C. sewardi (misidentified as *Crematogaster difformis*) show the usual antibiotic function. Leaf-cutting ants are also noted for their large metapleural glands, with 400 cells per side in members of the genus *Atta* (Schoeters & Billen 1993) and 200–600 cells per side in workers of *Acromyrmex octospinosus* (Reichert

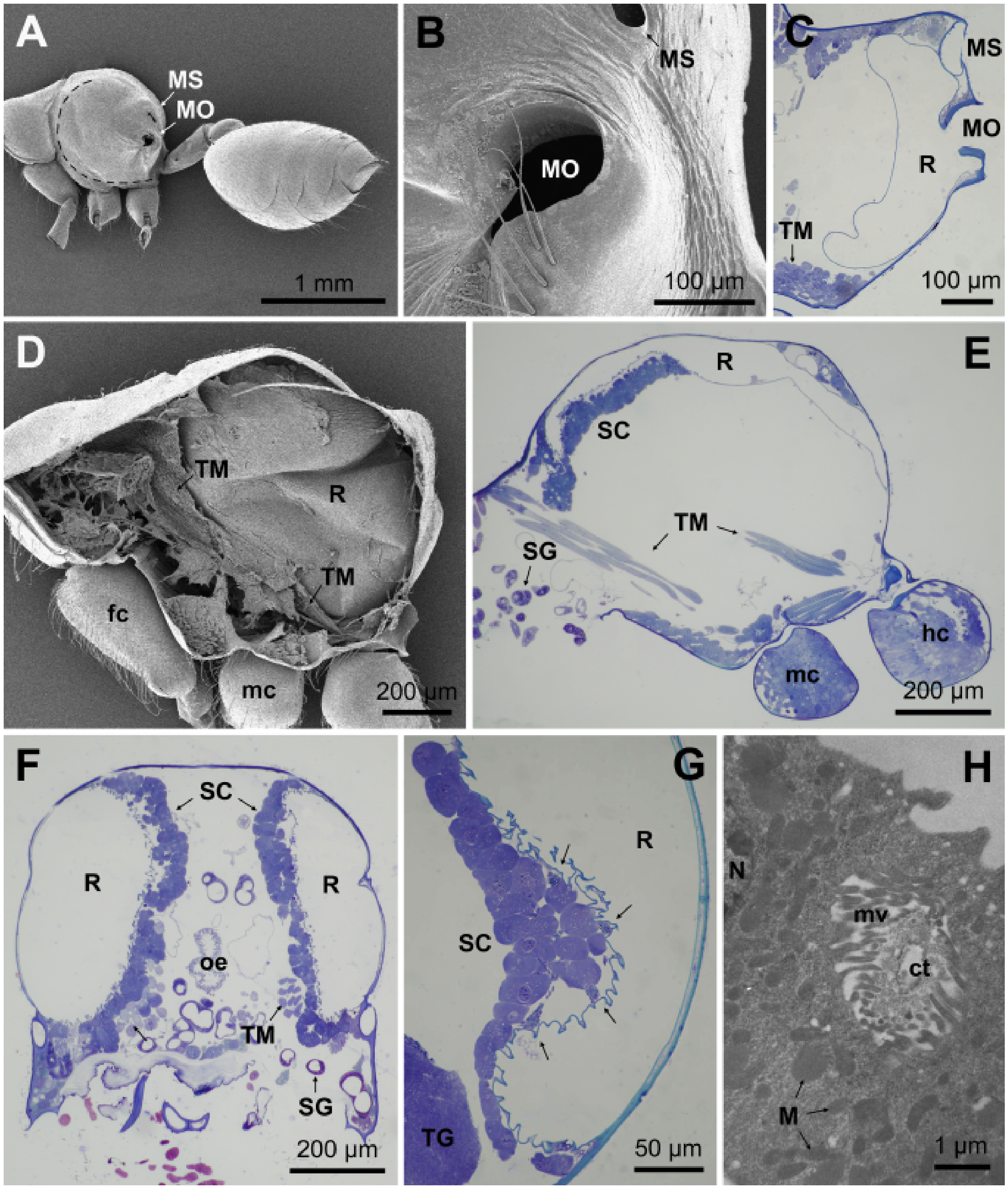

Fig. 1. Metapleural glands of workers of *Crematogaster inflata*. **A.** Scanning electron micrograph of the thorax and abdomen of a worker of *C. inflata,* showing the position of the large opening of the metapleural gland (MO). The dashed line indicates the internal extent of the metapleural gland. **B.** Detail of a metapleural gland opening. **C.** Semithin cross section through the posterior part of a metapleural gland showing the large lateral opening surrounded by a thickened cuticle. **D.** Scanning electron micrograph of KOH-treated half thorax (for removal of soft tissues), showing a large metapleural gland reservoir (R) and thoracic muscles (TM). **E.** Semithin longitudinal section near the body midline, showing clustering of secretory cells at the anterior side of the reservoir. **F.** Semithin cross section through the mesothorax in the anterior region of the metapleural gland, with massive clusters of secretory cells near the side of reservoirs closest to the body midline. **G.** Detail of secretory cells and reservoir; arrows indicate clustered duct cells. **H.** Transmission electron micrograph showing detail of the secretory cell cytoplasm, with numerous mitochondria (M) and an end apparatus formed by a cuticular ductule (ct) surrounded by microvilli (mv). fc, foreleg coxa; hc, hindleg coxa; mc, midleg coxa; MO, metapleural gland opening; MS, metathoracic spiracle; N, nucleus; oe, oesophagus; R, reservoir; SC, secretory cells; SG, salivary gland; TG, thoracic ganglion; TM, thoracic muscles.

1793), depending on worker size (Bot et al. 2001). From the cell counts, probably expressed per side, in a review article on ant metapleural glands (Hölldobler & Engel-Siegel 1984), we can calculate an average cell number of 120 for workers representing 44 different species (ranging between 14 in *Aneuretus simoni* Emery 1893 and 473 in *Atta sexdens* (Linnaeus 1758)). The ultrastructural organization of the cytoplasm in the metapleural gland of workers of *C. inflata*, with no noticeable granular endoplasmic reticulum, is indicative of the elaboration of a non-proteinaceous secretion. This is in agreement with the chemical composition of the glands of *Crematogaster difformis* and *C. inflata*, in which 6-alkylsalicylic acid and 6-alkylresorcylic acid were identified as the major compounds (Jones et al. 2005).

The main function of the metapleural gland secretions of ants is that of producing antibiotics to protect them against microorganisms, as first reported by Maschwitz et al. (1970). This also explains the large size of the gland in leaf-cutting ants that have to protect themselves and their precious subterranean fungus gardens from infection (do Nascimento et al. 1996; Bot et al. 2001, 2002; Poulsen et al. 2002a,b). The gland secretion can freely flow out via the large lateral openings through which the reservoirs connect to the exterior (Hölldobler & Engel-Siegel 1984), allowing a continuous release of secretion. This could be associated with the constant need for protection of the ants against microorganisms, although Fernández-Marín et al. (2006) question whether or not secretion is continually released by the metapleural glands, and show that secretory activity is correlated with the degree of exposure to fungal infection.

Members of *C. inflata* always nest in tree cavities. Most arboreal ants, however, have atrophied metapleural glands, as they are much less exposed to microorganisms than terrestrial ants (Hölldobler & Engel-Siegel 1984). The very large metapleural glands of members of *C. inflata,* therefore, may be an exception among the ants, as they are apparently not active against microorganisms. This was experimentally shown by Maschwitz (1974), who found that their secretions did not inhibit the growth of the bacterium *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919, although data on the effect against other potential pathogens, including fungi, are not available. The possible inactivity of the metapleural gland secretion against microorganisms in *C. inflata* can thus probably be linked with their arboreal way of life. Instead, the enlargement of the glands may be a consequence of their use as defenses against considerably larger arthropod and vertebrate enemies by the discharge of sticky substances; in addition, these substances have a communicative function, serving as an attractant for nestmates (Maschwitz 1974). Workers of *C. inflata* are able to release large amounts of secretion instantly through the large and permanently open dorsolateral metathoracic openings (due to the rigidity of the thick cuticular lining) when confronted with predators or disturbed by enemies (Maschwitz 1974). This is probably the result of active contraction of the paired strands of the oblique longitudinal thoracic muscles (as also suggested for the metapleural glands of leaf-cutting ants [Bot et al. 2001]), as this exerts pressure onto the thin-walled reservoir sacs and hence pushes secretion out through the large lateral openings. Also, increased external pressure onto the fairly soft thorax integument when a worker is bitten by a predator can probably cause expulsion of secretion. Vertebrate predators avoid attacking *C. inflata* as they are deterred by the bright yellow thorax (Ito et al. 2004). Preliminary unpublished observations of contacts between individuals of other ant species (*Oecophylla smaragdina* (Fabricius 1775)) and workers of *C. inflata* showed how *O. smaragdina* specifically targets the yellow thorax of *C. inflata* (whereas in confrontations with members of other species of *Crematogaster*, *O. smaragdina* targets the head). This thoracic attack leaves members of *O. smaragdina* covered with metapleural gland secretion of *C. inflata,* which may therefore escape the attack.

In addition to the release of secretion, Maschwitz (1974) noted that the large external drops of secretion can be resorbed after exposure. This is possibly linked to the elasticity of the reservoir wall, which resumes its normal shape after external pressure comes to an end. The secretion's acidic composition (as described by Jones et al. (2005)) should yield a very viscous fluid (E.D. Morgan and T.H. Jones, pers. comm.), which is also in agreement with the possibility of secretion resorption after exposure. The functional morphology of the unusual metapleural glands of *C. inflata* is thus well in line with the behavioral characteristics of workers of the species.

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