

ANATOMY AND FINE STRUCTURE OF THE METAPLEURAL GLAND IN *ATTA* (Hymenoptera, Formicidae)

by

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SUMMARY

Representatives of the ant genus *Atta* Fabricius possess metapleural glands that are characterized by a complex morphological organization. In contrast to most other glands in these ants a muscular supply nearby the glandular orifice is lacking. An hypothesis is postulated concerning the implication of the morphology of the glandular opening for the release of the secretion, probably facilitated by the existence of a cuticular ridge between the glandular opening and the hindlegs. In three species (*Atta bisphaerica*, *Atta laevigata* and *Atta sexdens*) the ultrastructure of the gland was studied. The elongated secretory cells possess a fairly developed granular endoplasmic reticulum. Very often we observed the presence of inclusions of lysosomal origin.

Key-words : Morphology, ultrastructure, metapleural gland, *Atta*.

INTRODUCTION

The paired metapleural glands are complex structures located at the posterolateral edge of the metathorax, each consisting of a cluster of glandular cells, with each cell draining through a duct into a common chitinous collecting sac. The collecting sac leads into the storage chamber or reservoir, which is a simple sclerotized cavity. Because of the presence of a narrow ridge inside the reservoir and the lack of a muscular supply, transport of the secretion to the outside by capillarity seems the most probable mechanism. Although we previously assumed that it would be difficult to obtain an overview of the complete glandular morphology, our trials demonstrated that accurate dissections, followed by SEM, can give satisfactory results. MASCHWITZ *et al.* (1970) were able to isolate a strongly acid secretion out of the metapleural gland reservoirs of some myrmicine workers, including *Atta sexdens* (L.). According to these authors, the secretion smells like honey and would be an effective antibiotic against *Escherichia coli*, *Staphylococcus*

aureus and *Penicillium glaucum*. The main compound displaying an antibiotic activity is phenyl acetic acid. A more detailed analysis (MASCHWITZ *et al.*, 1970; MASCHWITZ, 1974) of the *Atta* metapleural gland secretion showed that beta-indolyl acetic acid and beta-hydroxy-hexanoic, — octanoic and — decanoic acid also occur in the secretion.

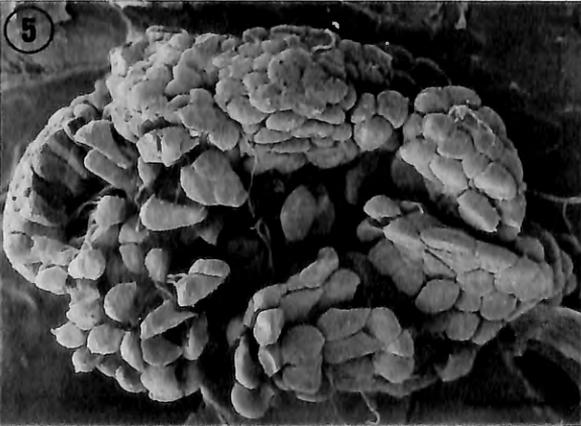
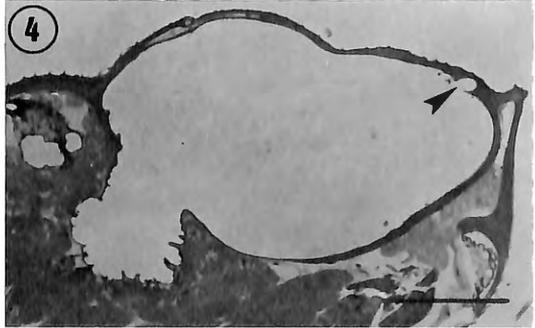
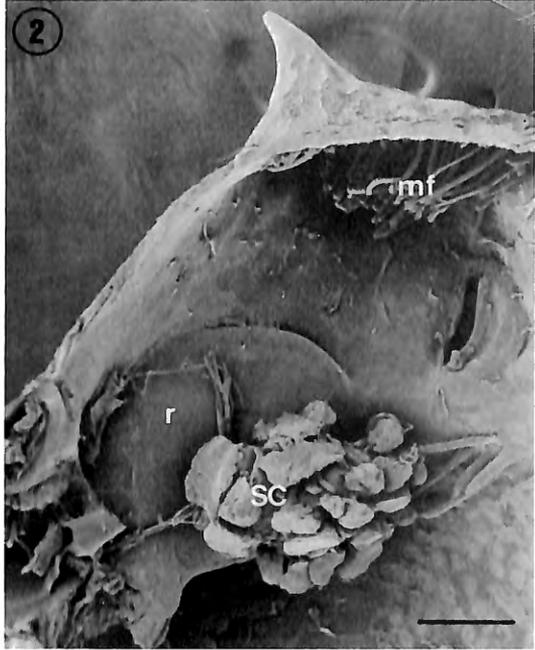
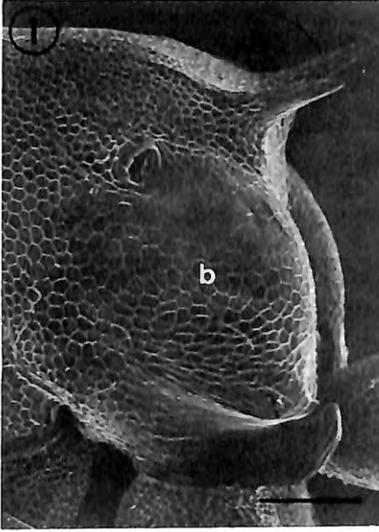
The morphology and ultrastructure of the metapleural gland in *Atta* are the subject of the current study. Until now, morphological studies on the metapleural gland are limited to those of HÖLLDOBLER and ENGEL-SIEGEL (1984), covering many ant species belonging to several subfamilies, and FANFANI and DAZZINI VALCURONE (1991), dealing with some dolichoderine species. Ultrastructural details have been reported by TULLOCH *et al.* (1962), Billen and VAN BOVEN (1987) for Old World army ants, and by SCHOETERS and BILLEN (1992) for *Diacamma* MAYR.

MATERIAL AND METHODS

The ants examined were collected from their nest in Viçosa, MG, Brasil (*Atta bisphaerica* FOREL soldiers, *Atta laevigata* (FR. SMITH) queen and soldiers), and from two laboratory colonies. An incipient colony of *Atta sexdens sexdens* (L.) was collected after the nuptial flight in December 1973 in Cayenne, French Guyana. A colony of *Atta sexdens rubropilosa* (FOREL) was likewise collected in Viçosa, MG, Brasil (nuptial flight in November 1987). Tissues were fixed in 2 % glutaraldehyde, buffered at pH 7.3 with 0.05M Na-cacodylate and 0.15M saccharose. After postfixation in 2 % osmium tetroxide, samples were dehydrated in acetone and embedded in Araldite. Thin sections for transmission EM were double stained with a LKB 2168 Ultrastainer, and examined with a Zeiss EM 900 electron microscope. Material for scanning EM was coated with gold and viewed with a Philips SEM 515 microscope.

Double-fixed glands for scanning EM of their internal anatomy were examined after critical point drying. Removal of tissue to see cuticular parts of the gland happened in a 5 % potassium hydroxide solution.

Figs. 1-6. — 1. Scanning electron micrograph of the bulla of the metapleural gland in a *A. sexdens sexdens* minor worker. b = bulla. (Scale 100 μ m). - 2. Survey of the metapleural gland in a *A. bisphaerica* soldier, showing the internal anatomy. mf = muscle fibres, r = reservoir, SC = secretory cells. (Scale 200 μ m). - 3. KOH-treated collecting chamber in a *A. laevigata* queen. Note the irregular surface. r = reservoir. (Scale 200 μ m). - 4. Longitudinal semi-thin section through a *A. sexdens sexdens* metathorax (minima worker). The arrow points to the specialized cuticular ridge. (Scale 100 μ m). - 5. Secretory cell clusters in a *A. sexdens rubropilosa* worker. (Scale 50 μ m). - 6. Morphological adaptation towards an optimization of secretion transport once it is out of the reservoir (arrow) (*A. sexdens rubropilosa* minor worker). (Scale 50 μ m).



RESULTS

In the case of *Atta*, the metapleural gland can externally be recognized by a bulla, and a curved slit-shaped opening to the outside (Fig. 1 and 6). Observations under a dissecting microscope make it possible to determine whether the reservoir is empty or not. The collecting chamber and the reservoir are clearly distinguishable, as can be seen on scanning micrographs of the sclerotized internal parts (Fig. 2). Scanning microscopy of the collecting chamber shows that its surface is very irregular (Fig. 3).

Scanning microscopy of the internal appearance of the metapleural gland in different *Atta* species invariably shows a large bean-shaped reservoir and several secretory cell clusters surrounding the collecting chamber (Fig. 2 and 5). Similar observations were done under the light-microscope (Fig. 4). The secretory cells are usually grouped into relatively large clusters of about 20 cells. This clustered organization can easily be recognized on scanning micrographs (Fig. 2 and 5). The well-defined clustered organization (sometimes up to 18 clusters) of the secretory cells surrounding this collecting chamber (Fig. 4 and 5) is different from the one found in other ant species. In other species we can readily distinguish fewer cell clusters, which are usually situated more closely to each other.

The externally visible part of the metapleural gland is a narrow, horizontally located slit-like opening near to the hindleg coxae (Fig. 7) on both sides. At its posterior end, each slit widens into a narrow semicircular slit. A non-sculptured chitinous area of the exoskeleton surrounds the opening of the gland (Fig. 6), which is even more conspicuous when comparing this smooth appearance with the characteristic reticulate body sculpture elsewhere on the body of the ant. The typical hexagonal pattern found there is lacking near the opening (Fig. 6).

The secretory cells

The number of remarkably white coloured glandular cells seems to vary considerably when comparing workers of different size. Giving precise cell numbers for all worker sizes is difficult, but it seems that about 400 cells occur in medium workers, soldiers and queens. Minor and minima workers possess fewer secretory cells, but in these castes the highest relative development of the gland was recorded (when referring to size of thorax). The secretory cells possess a large distal portion and a rather slender proximal part, the latter in direct connection with a duct cell. Each secretory cell is consistently equipped with a more straight zone in the proximal part of the cell, which becomes more curved in the distal part, corresponding with the so-called end apparatus. In semi-thin sections this end apparatus appears darker than the cytoplasm and is surrounded by a narrow microvillar lining which is usually only faintly stained (Fig. 4).

The rounded secretory cell nucleus is usually located in the broader distal part of the cell. It is characterized by several dark chromatin condensations. Several vesicle types were observed in the cytoplasm (Fig. 8). Also inclusions were found

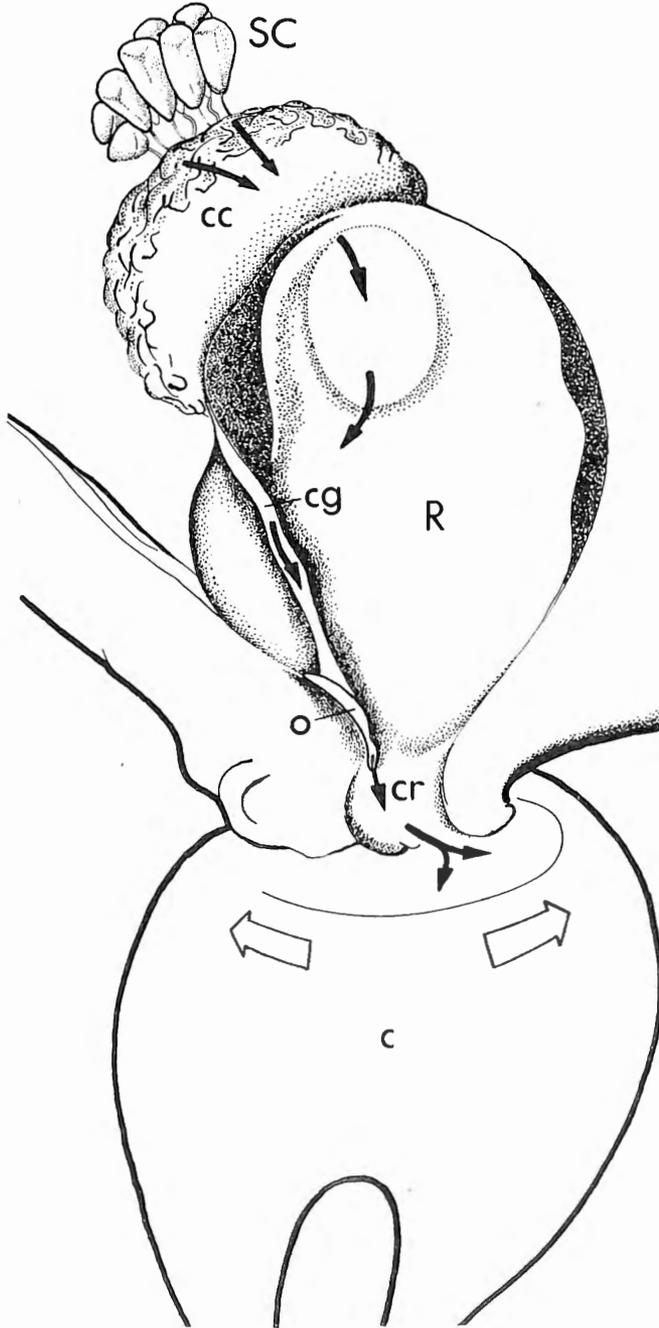
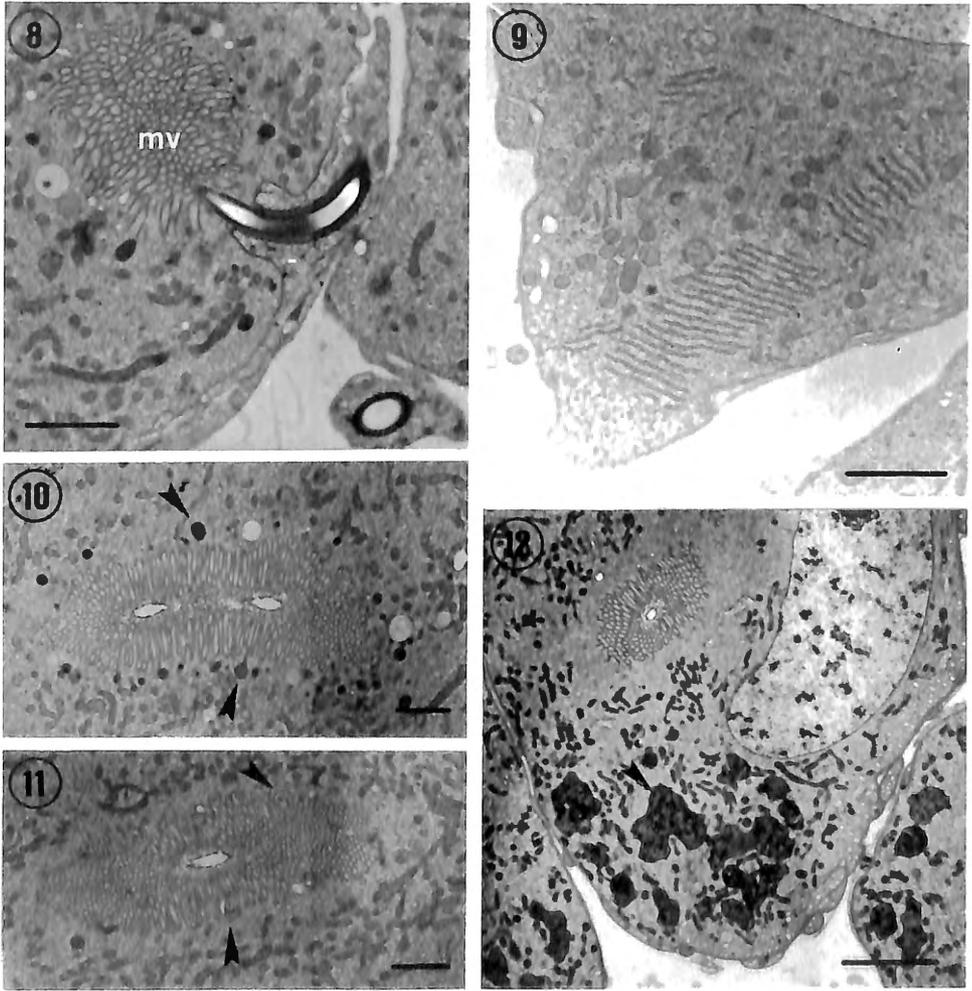


Fig. 7. — Reservoir morphology and presumed pathway the secretion follows (black arrows) before release into the outside world. White arrows indicate spreading of secretion through coxal movements. c = coxa of the hindleg, cc = collecting chamber, cg = cuticular gutter inside the reservoir, cr = cuticular ridge nearby hindleg coxa, o = glandular orifice, R = reservoir, SC = secretory cell.



Figs. 8-12. — Junction of the collecting canal and the conducting canal in a *A. sexdens rubropilosa* worker. mv = microvilli. Scale bar 2 μ m. - RER in the vicinity of the nucleus of a secretory cell (*A. sexdens rubropilosa* worker). Scale bar 2 μ m. - Electron-dense inclusions (arrows) surrounding the microvillar region of the collecting canal (*A. sexdens rubropilosa* worker). Scale bar 2 μ m. - Lamellar inclusions (arrows) in the vicinity of the end-apparatus (*Atta sexdens rubropilosa* worker). Scale bar 2 μ m. - Large inclusions of lysosomal origin (arrow), probably corresponding with autophagosomes, observed in a *A. laevigata* soldier. Scale bar 5 μ m.

consisting of two separate subunits, apparently fusing. One of both subunits contains parallel lamellar structures (length 0.5 μm), resembling membranes. Microvilli with a length of about 1.7 μm form the lining of the end apparatus (Fig. 10 and 11). These microvilli project into an extracellular space, the width of which is rather small in the gland of a recently emerged worker (0.2 to 0.5 μm broad). In the vicinity of the microvilli electron-dense (diameter about 0.6 μm) and electron-lucent vesicles (diameter from 0.5 to 0.8 μm) were observed.

The amount of RER (Fig. 9) and vesicles, when considered over the whole cytoplasm, varies considerably. Most of the electron-dense vesicles were found surrounding the end apparatus. In the young workers, the RER stacks showed a broad lumen. The Golgi-apparatus was not very common. If present, the dictyosomes had a length of 0.3 to 0.9 μm . In general mitochondria are about 1.5 μm long.

The stacks of RER show a typical parallel organization (Fig. 9) and were found on various locations in the cell. These stacks sometimes show an orientation parallel to the nuclear membrane, whereas in other cases it is perpendicular to it. We also observed inclusions of lysosomal origin, probably corresponding to autophagosomes filled with remnants of membranes and undigested material. Such inclusions, which in the case of *Atta laevigata* do not seem to be spherical, were fairly abundant (Fig. 12). Their contents are clearly electron-dense. This is also the case for the numerous mitochondria.

DISCUSSION

Our observations concerning the variation of cell numbers and the gland development in relation to the size of the ant correspond with the results of WILSON (1980). The occurrence of a large number of secretory cells in each metapleural gland of *Atta* makes it an important source of both volatile and non-volatile chemicals (own observations), in all the (sub)species investigated. The cellular organization of the metapleural gland is consistent with the bicellular secretory unit, with a secretory cell and a duct cell (NOIROT and QUENNEDEY, 1974; BILLEN, 1987; BILLEN, 1991). The microvillar lining in the end apparatus showed little variation in its appearance, and certainly between secretory cells within the same individual. The presumable absence of enlarged extracellular spaces and the corresponding lack of creation of additional volume for the storage of secretion, could be explained by the presence of a large collecting chamber and an even larger atrium. The production of secretion is not likely to happen under neuroendocrine control, since no nerve fibres were seen in association with the glandular cell clusters.

The obvious occurrence of inclusions of lysosomal origin, such as autophagosomes, could lead to the assumption that the cells in which they were found are degenerating. As a consequence, these cells could be in an advanced stage of their secretory cycle (BAZIRE-BÉNAZET and ZYLBERBERG, 1979). Our observations on the presence of end-apparatuses without signs of microvillar distortion in cells with plenty of the inclusions mentioned above, however, probably indicate that signs of

cellular degeneration and end-apparatuses with dilated extracellular spaces do not necessarily have to occur together.

The presence of the glandular orifice so close to the hindleg coxa suggests, in combination with movements of the latter, a role in making the secretion accessible for spreading over the body. The pathway the secretion follows is obviously compartmentalized. A first barrier the secretory molecules have to pass is the end apparatus. After passage through the duct the secretion is accumulated in the collecting chamber that is morphologically separated from the glandular reservoir. Although this has not been elucidated yet, there must be a well defined reason for the compartmentalization of the glandular reservoir. Compartmentalization of structures involved in secretion is also found in the venom gland (with its convoluted gland part). After temporary storage in the collecting chamber, the secretion passes through a more or less circular opening in order to get into the reservoir and reach the glandular orifice to the outside. The reservoir wall is provided with a narrow channel, coming from the reservoir region close to the collecting chamber and going to the slitlike opening. This well defined morphological adaptation strongly suggests a secretion transport towards the gland opening, possibly by capillarity. Once the secretion is out of the reservoir, its spreading could be enhanced by coxal movements. Our observations concerning the appearance of the glandular bulla correspond to those reported by HÖLLDOBLER and ENGEL-SIEGEL (1984).

In the secretory cells of a callow *A. sexdens rubropilosa* (FOREL) worker we observed the presence of a fairly well developed RER. Until now, the few ultrastructural observations on this gland in other species revealed the developed smooth endoplasmic reticulum, probably in agreement with the production of more or less volatile substances. This leads us to the assumption that proteinaceous substances could be present in the secretion. An observation we made outside the nest (under a binocular microscope) is that the secretion quickly loses its volatile constituents and that a sticky residual mass is left on the substrate (for weeks, or even months). So, the most remarkable aspects recorded in our investigation are the presence of a well developed RER and the sticky residu found in the glandular reservoirs. These two aspects do not seem to be contradictory.

This investigation forms part of a comparative research on the metapleural gland in ants. When we compare our morphological data with those obtained for the metapleural gland in other representatives of the Formicidae, we are able to emphasize the following characteristics of this gland in *Atta*. The gland is very well developed in all workers, ranging from minima to soldier, as well as in females. The glandular opening is located relatively close to the hindleg coxae, whereas more primitive species (belonging to Nothomyrmecinae, Myrmeciinae and Ponerinae) usually display glandular openings that are located further away from the coxae and lack the adaptation for further transport of secretion towards the coxae. Cuticular hairs associated with the gland opening are totally absent, whereas in some ant species these are obviously present outside (*Cephalotes* LATREILLE, *Lasius* FABRICIUS) or inside the reservoir (*Amblyopone* Erichson). The cuticular collecting chamber of the metapleural gland in *Atta* reaches one of the highest degrees of development in ants. In some Ponerinae and Formicinae the elaboration of the

collecting chamber seems to be less important. Apparently the presence, size, and activity of the metapleural glands in *Atta* are to be considered as important characters contributing to the dominance of these ants in the neotropical region.

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