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MORPHOLOGY AND ULTRASTRUCTURE OF THE METATIBIAL GLAND IN THE ARMY ANT *DORYLUS MOLESTUS* (HYMENOPTERA, FORMICIDAE)[1]

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Abstract: Beneath the cuticle of the ventral side of the distal fourth of the hindleg tibia of workers of the army ant *Dorylus molestus,* there is a conspicuous glandular epithelium with a thickness of approx. 35 µm. The columnar secretory cells are characterized by the presence of a well developed smooth endoplasmic reticulum and numerous secretory inclusions. Their basal cell membrane shows numerous invaginations, while an extensive but irregular microvillar differentiation occurs apically. The overlying cuticle is traversed by pores that guide the glandular secretion to the outside. The function of the gland, that is probably absent in queens and males, is unknown. In each of the three pairs of legs, there is an additional cluster of so far unknown glandular cells with accompanying duct cells in the most distal part of the tibia, as well as a glandular epithelium dorsally underneath the proximal part of the basitarsus.

Key words: ants, Dorylinae, exocrine glands, scanning microscopy.

INTRODUCTION

Social insects are well known for their elaborate exocrine system, in which 63 different glands have been distinguished so far (BILLEN, 1994). Although the majority of these are located in the head, thorax and abdomen, the appendages also contain exocrine glands. Recent research revealed that the legs of ants may show various glandular structures that can be located in the coxa (SCHOETERS and BILLEN, 1993), tibia (LEUTHOLD, 1968; PASTEELS *et al.*, 1970; BILLEN, 1984; HÖLLDOBLER *et al.*, 1996), basitarsus (HÖLLDOBLER and PALMER, 1989a; HÖLLDOBLER *et al.*, 1992; SCHÖNITZER *et al.*, 1996) and pretarsus (HÖLLDOBLER and PALMER, 1989b; BILLEN, 1993). Among these, various types of tibial and basitarsal glands can be distinguished, both in terms of their general occurrence and cellular organization.

Two kinds of tibial glands have been reported so far, both occurring in the hindlegs. An internalized epithelial gland in the metatibia is only known for species of the genus *Crematogaster,* where it is the source of the trail pheromone (LEUTHOLD, 1968). The exis-

[1] Dedicated to the memory of the late Professor J. VAN BOVEN.

tence of a metatibial gland underneath the tegumental cuticle was first mentioned by Bolton (1990) as a synapomorphic character of ants of the doryline section. Its distribution and general organization was recently described in a comparative study by Hölldobler *et al.* (1996). Since no ultrastructural data on this gland are available, we examined the well developed metatibial gland of the army ant *Dorylus molestus,* and report on our findings.

MATERIAL AND METHODS

Foraging workers of *Dorylus (Anomma) molestus* Gerstaecker, 1858 were collected from a natural raiding column in Nairobi, Kenya. Legs of minor, medium and major (soldier) workers were cut off near the proximal side of the tibia and fixed in 2% glutaraldehyde, buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose. Postfixation in a buffered osmium tetroxide solution was followed by dehydration in a graded acetone series and embedding in Araldite. Semithin sections (1 µm thickness) for light microscopy were stained with methylene blue and thionin. Thin sections for ultrastructural examination (70 nm thickness) were double stained with uranyl acetate and lead citrate in an LKB 2168 Ultrostainer and were examined in a Zeiss EM 900 microscope. Tissues for scanning microscopy were dehydrated in an ethanol series after postfixation and were critical point dried. They were coated with gold and viewed in a Philips SEM 515 microscope.

RESULTS

The ventral side of the distal fourth of the hindlegs of workers of *Dorylus molestus* shows an obvious elongated and flattened area which is devoid of the hairs and sculpture found elsewhere on the legs (Fig. 1). The same region on the fore- and midlegs, in contrast, clearly lacks this modification (Figs 2 and 3, respectively). This flattened area on the metatibia at first appears as a smooth zone, but at high magnification shows numerous very small pores with a diameter of approx. 50 nm that open through the cuticle at various angles (Fig. 4). Histological sections through the distal part of the metatibia reveal the presence of a conspicuous glandular epithelium with high columnar cells underneath the flattened area (Figs. 5, 6). This epithelium is a continuation of the squamous tegumental epidermis, and reaches a constant thickness of approx. 35 µm (range among individuals 28-44 µm). There is no correlation between epithelial thickness and worker size. When checking preserved queens and males, the hindleg tibia appears to lack the specialized distal area described for workers, which probably indicates the absence of the gland in the reproductive castes.

Ultrastructural examination of the gland reveals the modified appearance of the cuticle overlying the glandular epithelium, with conspicuous and irregular pores that traverse the cuticle from the apical side of the secretory cells towards the surface, where they open as holes with a diameter of approx. 50 nm (Figs 6,7). This porous appearance is only found in the cuticle overlying the glandular epithelium; elsewhere the usual horizontally layered

cuticle occurs (Fig. 7). The cuticle has a constant thickness of 13 μm. The apical region of the glandular cells is characterized by an extensive though irregular microvillar differentiation (Figs 6, 8). The cytoplasm is occupied by a well developed tubular smooth endoplasmic reticulum, and, especially in the apical part of the cell, numerous electron-dense secretory vesicles with a diameter of approx. 0.15 μm (Figs 8, 9). Granular endoplasmic reticulum does not occur, while free ribosomes are scattered through the cytoplasm (Fig. 9). The basal cell membrane displays obvious invaginations. Elongated mitochondria also occur in this region (Fig. 10). The cells are characterized by rounded to ovoid nuclei with a diameter around 5 μm (Figs 6, 10).

Examination of the leg sections revealed, in addition to the metatibial gland, the existence of another gland in the very distal part of the tibia, as well as the presence of a glandular epithelium in the proximal part of the basitarsus. The additional tibial gland is formed by a cluster of 5-10 rounded secretory cells with a diameter around 25 μm with accompanying duct cells that open through the tibio/basitarsal articulation membrane (Fig. 11). The glandular epithelium in the basitarsus is a differentiation of the tegumental epidermis. It occurs at the dorsal side, and reaches a thickness of approx. 15 μm (Fig. 11). Both glands appear in the three pairs of legs, and represent hitherto unknown exocrine structures.

Figs 1-4. – Scanning micrographs showing the ventral side of the tibiae near the articulation with the basitarsus in major workers of *Dorylus molestus*. Fig. 1. – hindleg with flattened area overlaying the metatibial gland (bar 100 μm). Fig. 2. – foreleg (with antennal cleaning apparatus, bar 100 μm). Fig. 3. – midleg (bar 100 μm). Fig. 4. – detail of the cuticle of the flattened area in the hindleg with numerous small pores (bar 1 μm).

Fig. 5. – Semi-thin cross section through the hindleg tibia of a minor worker showing the metatibial gland (MG). ct: cuticle, M: muscles, N: nerves, T: tibial tendon, Tr: trachea (bar 20 μm).

Figs 6-10. – Electron micrographs of the metatibial gland. Fig. 6. – survey of the glandular epithelium and overlying cuticle (major worker, bar 5 μm). Fig. 7. – porous cuticle overlying the glandular epithelium, normal cuticle at right (medium worker, bar 5 μm). Fig. 8. – apical cytoplasm with microvillar differentiation and secretory vesicles (major worker, bar 1 μm). Fig. 9. – detail of cytoplasm showing abundant tubules of smooth endoplasmic reticulum (medium worker, bar 1 μm). Fig. 10. – basal cytoplasm showing conspicuous invaginations of cell membrane (medium worker, bar 5 μm).

bi: basal invaginations, ct: cuticle, mv: microvilli, N: nucleus, SER: smooth endoplasmic reticulum, sv: secretory vesicles.

[See figures pages 162 and 163]


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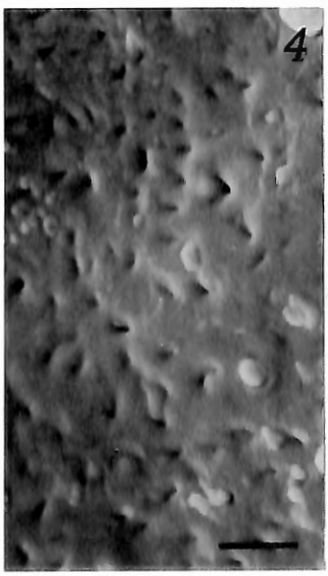
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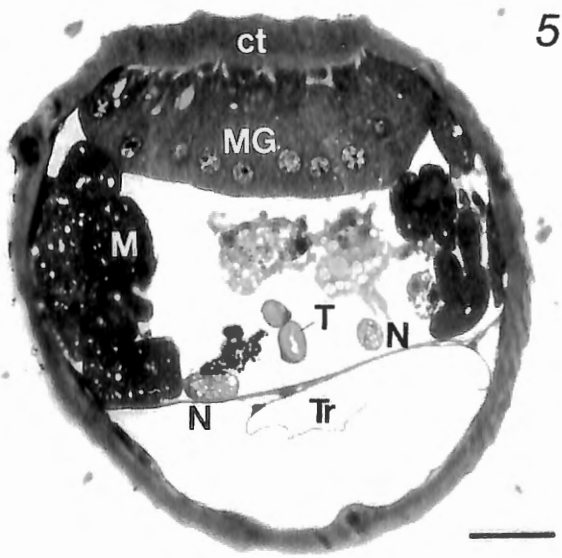
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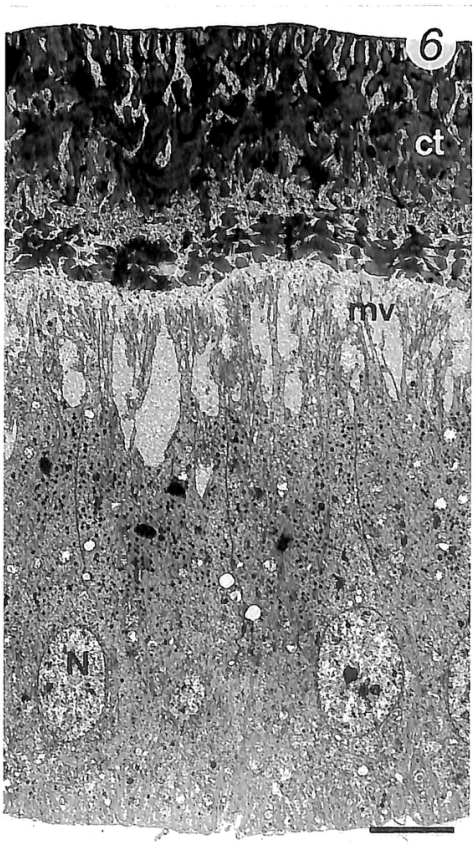
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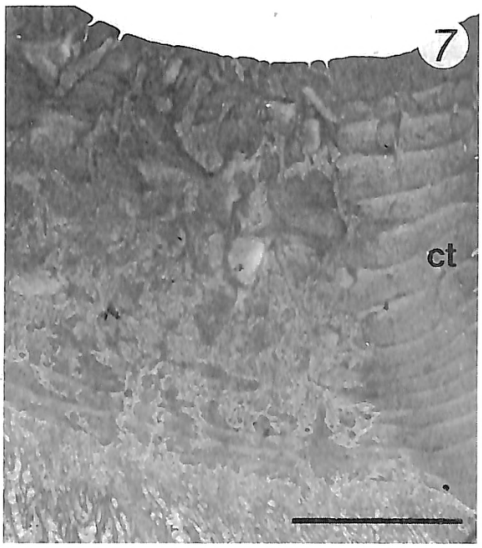
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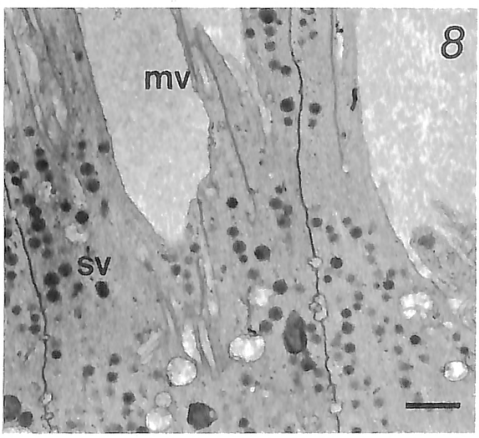
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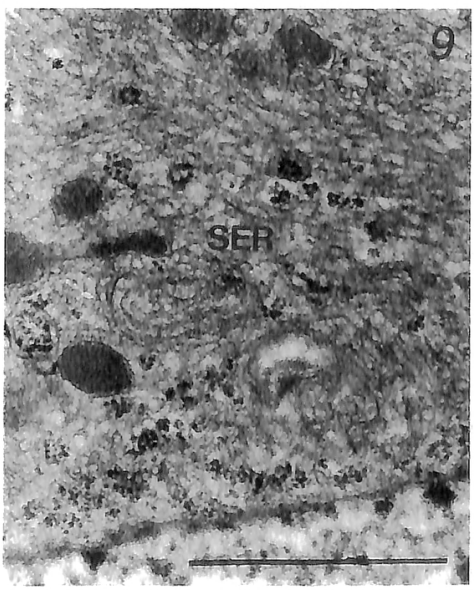
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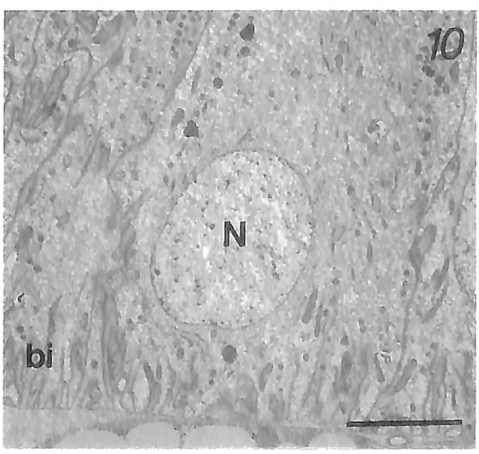
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Fig. 11. – Semi-thin longitudinal section through the articulation between the foreleg tibia (Tb) and basitarsus (bt) of a major worker, showing cluster of metatibial glandular cells of which corresponding ducts open through the articulation membrane (arrow), as well as glandular epithelium (GE) in proximal part of basitarsus (bar 50 μm).

DISCUSSION

The metatibial gland was recently described in a comparative survey study by Hölldobler *et al.* (1996) for ants of the poneroid group. Its presence was thus also reported for the Old World army ant *Dorylus nigricans,* for which it was briefly described as similar to that of the New World army ant *Eciton hamatum,* where it occurs as a «relatively thin glandular epithelium extending more than three-quarters of the length of the tibia». In all specimens of *Dorylus molestus* we examined, regardless of worker size, the gland is less extended in length (it occurs in the distal quarter of the hindleg tibia only) and is considerably more pronounced in thickness.

The ultrastructural characteristics of the gland as reported here for *Dorylus molestus* are indicative for an active transport of substances through the epithelium and overlaying cuticle. Precursor molecules from the haemolymph are probably taken up through the numerous basal invaginations, and undergo further metabolic processes in the well developed smooth endoplasmic reticulum. The secretory products appear as small rounded electron-dense vesicles. The extensive apical microvilli and the conspicuous cuticular pores form an easy pathway for secretion to the outside. The cytoplasmic organization with an extensive smooth endoplasmic reticulum is in agreement with that of pheromonal glands (Noirot and Quennedey, 1974; Billen, 1991). The function of the metatibial gland, however, is still unknown (Hölldobler *et al.*, 1996). For several queenless *Diacamma* species, the gland appears to be involved in sexual calling by the dominant worker

(HÖLLDOBLER *et al.*, 1996), but this is not applicable for army ants workers. The apparent absence of the metatibial gland in the army ant queen and male moreover excludes a role in reproductive regulation. An eventual function in trail laying, as has been demonstrated for leg glands of other ants (LEUTHOLD [1968] for the tibial gland in *Crematogaster,* HÖLLDOBLER and PALMER [1989a] and HÖLLDOBLER *et al.* [1992] for the basitarsal glands in *Onychomyrmex* and *Prionopelta,* respectively, and the pretarsal gland in *Amblyopone* (HÖLLDOBLER and PALMER [1989b]), does not seem to be the case for *Dorylus,* as these army ants rely on venom gland secretions as the source of their trail pheromone (BILLEN and GOBIN, 1996). The intense and obvious inter-individual contacts in the extremely large army ant colonies (GOTWALD, 1995) may indicate the distribution of chemical signals, although this remains purely speculative.

Our discovery of additional glandular structures in the legs is a clear illustration of the overwhelming extent of the exocrine apparatus of social insects in general and ants in particular. Their functions remain to be discovered, but they confirm the description of ants as walking glandular batteries (HÖLLDOBLER and WILSON, 1990).

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