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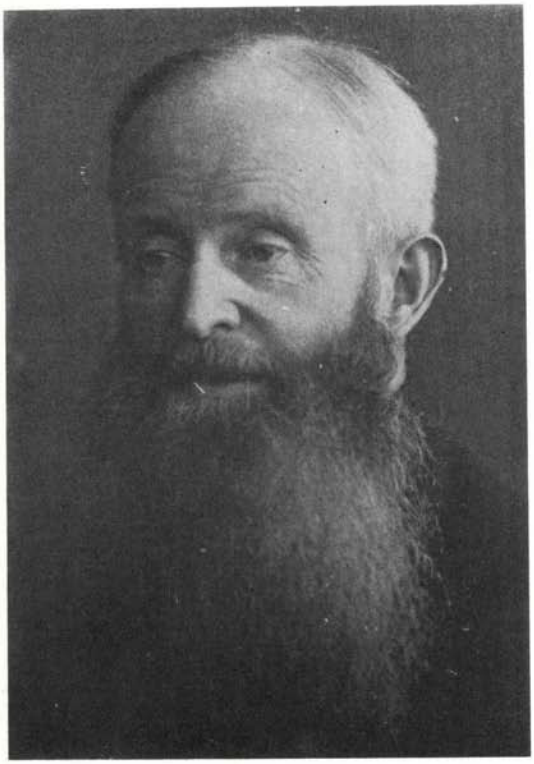

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COMPARATIVE ULTRASTRUCTURE OF THE POISON AND DUFOUR GLANDS IN OLD AND NEW WORLD ARMY ANTS (Hymenoptera, Formicidae)

by
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Summary: The ultrastructure of the poison and Dufour glands has been investigated in five African army ant species (*Anomma*, *Dorylus*) as well as in the New World species *Eciton burchelli*. The poison gland secretory cells in workers of both groups are characterized by a very well developed granular endoplasmic reticulum, according to the proteinaceous nature of the poison. The Dufour gland epithelium in the Dorylinae has a conspicuous crenellate appearance at its apical side and shows numerous basal invaginations. The Ecitoninae, on the other hand, have a very uniform epithelium without such invaginations, but are characterized by numerous foldings of the lateral cell membranes. These clear differences in the Dufour gland morphology between Old and New World army ants are in agreement with the assumed diphyletic origin of both groups.

Key-words: *poison gland*, *Dufour gland*, *morphology*, *ultrastructure*, *Dorylinae*, *Ecitoninae*, *Formicidae*.

Resumé: Ultrastructure comparative de la glande à poison et la glande de Dufour chez les Fourmis dorylines et ecitonines (Hymenoptera, Formicidae)

L'ultrastructure de la glande à poison et la glande de Dufour a été examinée chez cinq espèces dorylines africaines (*Anomma*, *Dorylus*) ainsi que chez *Eciton burchelli* provenant d'Amérique Centrale. Les cellules sécrétrices de la glande à poison chez les ouvrières des deux groupes sont pourvues d'un ergastoplasme très élaboré, ce qui est conforme à la nature proteineuse du venin. L'épithélium de la glande de Dufour chez les espèces dorylines montre un aspect très crénelé dans sa région apicale et contient de nombreuses invaginations basales. La glande des Ecitoninae, au contraire, montre un épithélium d'une hauteur très uniforme sans de telles invaginations, mais elle est

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caractérisée par des membranes intercellulaires latérales très sinueuses. Ces différences très nettes entre les glandes de Dufour des Dorylinae et des Ecitoninae sont en accord avec l'origine diphylétique supposée des deux groupes.

Mots-clés: *glande à poison, glande de Dufour, morphologie, ultrastructure, Dorylinae, Ecitoninae, Formicidae.*

INTRODUCTION

Due to their obvious behaviour of mass raiding and their extremely large colonies, army ants often form a spectacular and impressive appearance in tropical forests of both the Old and New World. The African members of the Old World Dorylinae undoubtedly beat the record size that insect communities can reach. Although their nests are monogynous, numbers of over 20 million workers were recorded for *Anomma wilverthi* (RAIGNIER and VAN BOVEN, 1955). The colonies of the New World Ecitoninae, in contrast, seem relatively small but still attain numbers of nearly a million individuals.

Most of our actual knowledge on army ants refers to behavioural and taxonomic studies (RAIGNIER and VAN BOVEN, 1955; RETTENMEYER, 1963; SCHNEIRLA, 1971; GOTWALD, 1982). Morphological research in these insects hitherto is very limited. A general anatomical description of the female castes in *Eciton burchelli* and *E. hamatum* was given by WHELDEN (1963), while in the latter species the poison apparatus sclerites (HERMANN and BLUM, 1967) was the subject of a morphological and histological study. Although the poison apparatus associated poison gland and Dufour's gland are known to play a major role in pheromonal communication in other ant subfamilies, neither their chemical secretion nor their morphological appearance have been investigated in army ants.

We here report on the morphology and ultrastructure of the poison and Dufour gland in *Eciton burchelli, Dorylus affinis* and four *Anomma* species.

MATERIAL AND METHODS

Workers of *Anomma kohli* Gerstaecker, *A. nigricans* Illiger and *Dorylus affinis* Shuckard were collected in Kigali (Ruanda), *A. molestum* (Mayr) in Nairobi (Kenya), *A. wilverthi* near Kizu (Zaire) and *Eciton burchelli* in Arima Valley (Trinidad).

Either abdominal halves or dissected Dufour glands were fixed in 2% cold glutaraldehyde buffered with 0.05 M sodium cacodylate and 0.15 M saccharose (pH 7.3). Postfixation was carried out in 2% osmiumtetroxide in the same buffer. Dehydration in an aceton series preceded embedding in Araldite. Semi-thin sections were stained with methylene blue and thionin and were used for light microscopy. Thin sections were cut with a Reichert Ultracut microtome, double stained with uranyl acetate and lead citrate and examined in a Philips EM 400 electron microscope.

RESULTS

The general anatomical appearance of both the poison gland and Dufour gland is very similar in doryline and ecitonine army ants and corresponds to that in most other ant subfamilies. The poison gland is formed by two long and slender secretory filaments that open in a relatively large reservoir sac. From this a narrow duct makes its way and enters the sting base dorsally to the duct from Dufour's gland. The Dufour gland is an elongate sac, the wall of which consists of a monolayered epithelium.

1. Poison gland ultrastructure

The poison gland secretory cells form the approximately 15 µm thick outer wall of the free filaments. The inner lining surrounding the central lumen is formed by a thin layer of duct cells with narrow cuticular ductules (internal diameter 0.3 - 0.4 µm), that form the link with the intracellular end apparatus of the secretory cells. In this region, the thick epicuticle becomes gradually narrower and fenestrated. It is accompanied by a fibrillar endocuticular layer surrounded by a sheath of microvilli (Fig. 1). The cytoplasm of the secretory cells is characterized by a very well developed granular endoplasmic reticulum, many free ribosomes and fairly abundant small mitochondria. Tracheoles and nerve fibres (Fig. 2) are often seen to penetrate between the secretory cells.

The poison gland reservoir consists of a very thin epithelial wall, covered with a cuticular layer that can reach a thickness of up to 7 µm in *Anomma*.

2. Dufour gland ultrastructure

Unlike the poison gland, the Dufour gland morphology in doryline and ecitonine ants is distinctly different.

The 5 Old World Dorylinae investigated all show a crenellate Dufour gland epithelium with a thickness of 10 tot 20 µm. Subsequent crenel tops are situated at 5 to 10 µm intervals and as a rule correspond to the apical region of the intercellular junctions (Figs. 3 and 6). Due to the much folded lateral cell membranes - particularly in the apical cell region - and the clear basal invaginations, the Dufour gland cells have a very capricious shape (Fig. 5). The slightly lobate nuclei are found in the lower half of the cells. The cytoplasm contains a well developed smooth endoplasmic reticulum, though less obvious compared with that in other ant subfamilies because of the numerous membrane foldings and the occurrence of multilamellar inclusions. Numerous slender and small mitochondria are randomly distributed in the cytoplasm. A relatively weak muscle layer as well as a few tracheoles and nerve fibres surround the gland.

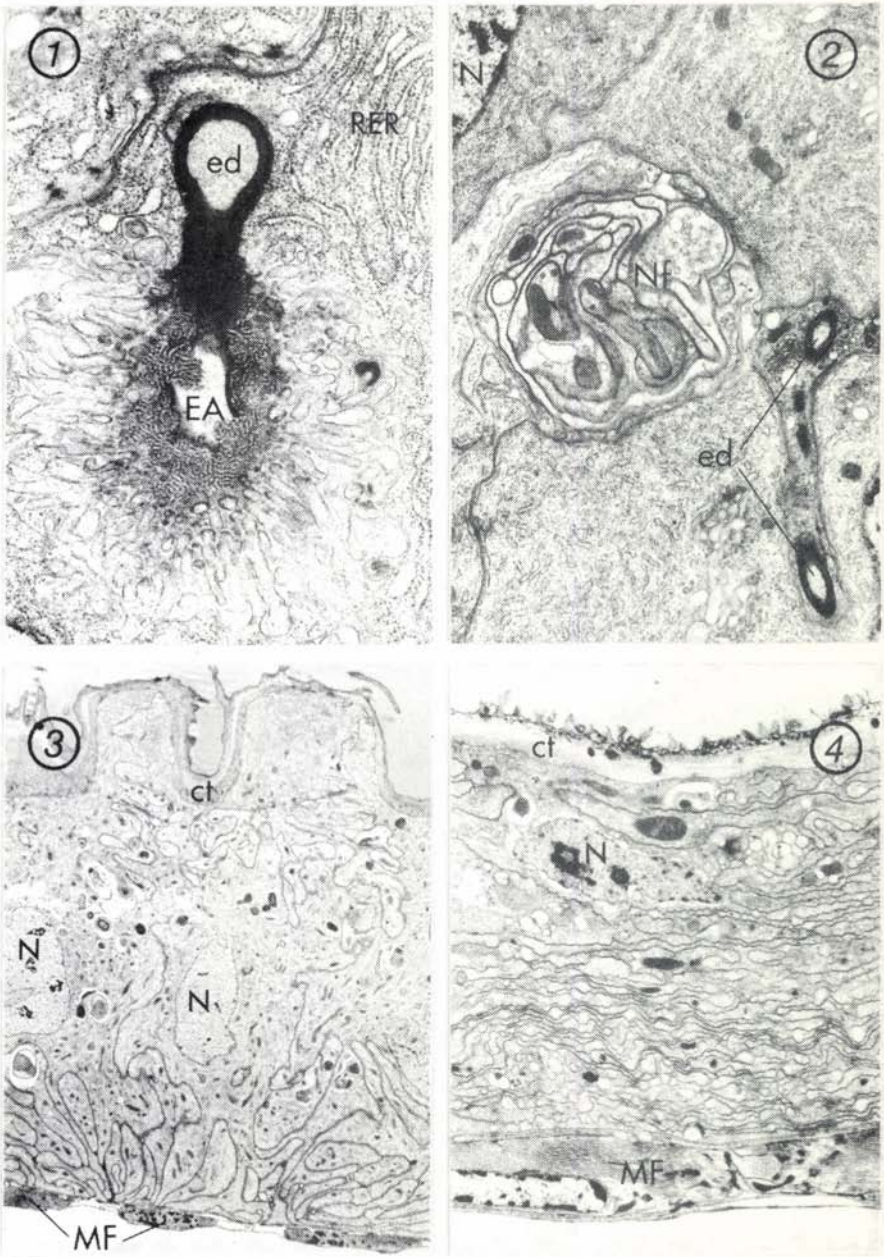
Fig. 1. Poison gland secretory cell in Eciton burchelli *showing the junction between the intracellular end apparatus and the duct cell.* *(x 24,750)*

Fig. 2. Poison gland in Anomma molestum *with extracellular duct and nerve fibre penetrating between the secretory cells.* *(x 13,500)*

Fig. 3. Dufour gland epithelium in Anomma nigricans. *(x 3,600)*

Fig. 4. Dufour gland epithelium in Eciton burchelli. *(x 8,000)*

ct = *cuticle*, EA = *end apparatus*, ed = *extracellular duct*, MF = *muscle fibres*, N = *nucleus*, Nf = *nerve fibre*, RER = *granular endoplasmic reticulum*.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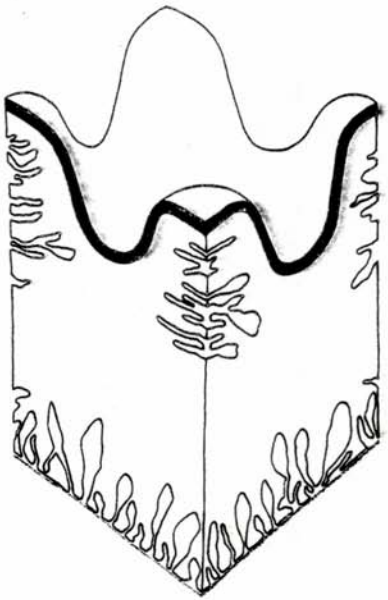


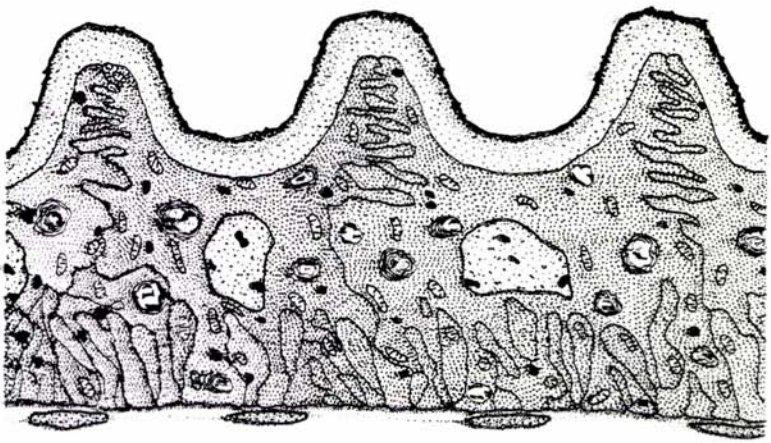
Fig. 5. Theoretical model of a Dufour gland cell in doryline army ants, conveniently drawn for a four-corner-cell. The intercellular junctions correspond with crenel tops, resulting in a multicellular origin for each crenel.

The New World *Eciton burchelli*, on the other hand, displays a Dufour gland epithelium with a very constant thickness between 7 and 10 µm. The elongate nuclei are located in the upper half of the epithelium. Some scattered mitochondria and numerous small vacuoles are found along with smooth endoplasmic reticulum. The basal epithelium half is characterized by the overwhelming number of lateral cell membrane foldings (Figs. 4 and 6). The basal cell membrane is tightly appressed to the thin basement membrane. The gland is surrounded by a muscle layer, some nerve fibres and tracheoles.

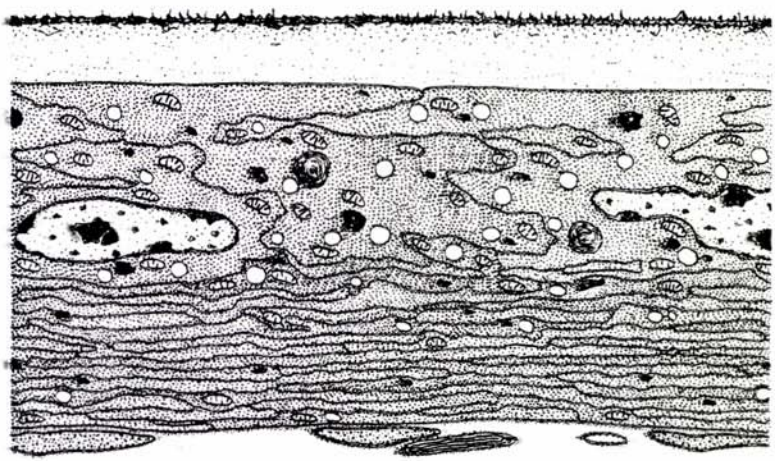
DISCUSSION

The poison and Dufour glands in ants generally are involved in the alarm-defence system or in the production of trail pheromones. Although their function in the army ants has been hardly investigated, it seems most likely they serve a similar role in this group.

As in other ant subfamilies, the poison gland in Ecitoninae is reported to contain a proteinaceous venom (BLUM & HERMANN, 1978). In accordance with this is the very well developed granular endoplasmic reticulum we find in the secretory



DORYLINAE



ECITONINAE

Fig. 6. Schematical comparison between the Dufour gland epithelium in the Dorylinae and Ecitoninae.

cells of *Eciton* as well as these of the doryline species. A similar ultrastructural organization, including the appearance of the end apparatus, has been described for the poison gland in wasps (KANWAR & KANWAR, 1975) and honeybees (OWEN & BRIDGES, 1976). Moreover, the very thick cuticular lining of the reservoir may be considered as an individual protection of the ant against its own powerful poison contents.

The Dufour gland ultrastructure, on the other hand, shows a well developed smooth endoplasmic reticulum in addition to numerous multilamellar inclusions. The latter perhaps may be considered as secretion bodies for a hydrocarbon-like fluid (HEFETZ & ORION, 1982). The presence of the smooth endoplasmic reticulum can be related to an eventual hydrocarbon metabolism. Although as yet, no chemical analyses have been made on the army ant Dufour gland, we may tentatively propose from our preliminary chemical investigations on *Anomma nigricans* they are involved in hydrocarbon production. In contrast with species from other subfamilies, however, we find here a complex mixture of high molecular weight compounds in the C_{18} to C_{25} range, with a very small total quantity of less than 100 ng per worker.

The morphological variation between the doryline and ecitonine Dufour gland as reported here, clearly illustrates the taxonomic difference of both groups. It moreover supplies additional evidence for their diphyletic origin in addition to obvious characters such as the blind and more or less stingless condition of Dorylinae in comparison with the powerful stinging and sighted Ecitoninae.

According to GOTWALD (1979), army ants even have to be considered as a triphyletic group, with the Ecitoninae occurring in the New World, and the Old World species being separated in the mainly African Dorylini and the mainly Indo-Australian Aenictini. Data on the *Aenictus* Dufour gland morphology so far are restricted to a brief report on the West African *A. asantei* that shows epithelial cells of varying height, "causing the surface of the lumen to appear irregular" (CAMPIONE *et al.*, 1983). Whether this particular arrangement is to be compared with the crenellate condition of the doryline Dufour gland or not, shall need more careful and perhaps ultrastructural research on *Aenictus*. Research in that direction therefore is planned and hopefully will shed more light on the relationships between the fascinating army ant species.

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REFERENCES

BLUM M.S., HERMANN H.R., 1978. - Venoms and Venom Apparatuses of the Formicidae : Myrmeciinae, Ponerinae, Dorylinae, Pseudomyrmecinae, Myrmicinae, and Formicinae. In : *Arthropod Venoms*, S. Bettini Ed., Springer-Verlag, Berlin - Heidelberg - New York, p. 801-869.

CAMPIONE B.M., NOVAK J.A., GOTWALD W.H., Jr., 1983. - Taxonomy and Morphology of the West African Army Ant, *Aenictus asantei* n.sp. (Hymenoptera : Formicidae). *Ann. Entomol. Soc. Am.*, **76**, 873-883.

GOTWALD W.H., Jr., 1979. - Phylogenetic Implications of Army Ant Zoogeography (Hymenoptera : Formicidae). *Ann . Entomol. Soc. Am.*, **72**, 462-467.

GOTWALD W.H., Jr., 1982. - Army Ants. In : *Social Insects*, vol. IV, H.R. Hermann Ed., Academic Press, New York, p. 157-254.

HEFETZ A., ORION, T., 1982. - Pheromones of Ants of Israel : I. The Alarm-Defense System of some Larger Formicinae. *Isr. J. Entomol.*, **16**, 87-97.

HERMANN H.R., BLUM M.S., 1967. - The Morphology and Histology of the Hymenopterous Poison Apparatus. III. *Eciton hamatum* (Formicidae). *Ann. Entomol. Soc. Am.* , **60**, 1282-1291.

KANWAR K.C., KANWAR, U., 1975. - Fine Structure of the Venom Gland of *Vespa orientalis*. *Toxicon*, **13**, 102-103.

OWEN M.D., BRIDGES A.R., 1975. - Aging in the Venom Glands of Queen and Worker Honey Bees (*Apis mellifera* L.) : Some Morphological and Chemical Observations. *Toxicon*, **14**, 1-5.

RAIGNIER A., VAN BOVEN J., 1955. - Etude taxonomique, biologique et biométrique des *Dorylus* du sous-genre *Anomma* (Hymenoptera Formicidae). *Ann. Mus. R. Congo Belg.*, 2 1-359.

RETTENMEYER C.W., 1963. - Behavioral Studies of Army Ants. *Univ. Kans. Sci. Bull.*, **44**, 281-465.

SCHNEIRLA T.C., 1971. - *Army Ants. A Study in Social Organization*. H.R. Topoff Ed., Freeman, San Francisco, 349 p.

WHELDEN R.M., 1963. - The Anatomy of the Adult Queen and Workers of the Army Ants *Eciton burchelli* Westwood and *Eciton hamatum* Fabricus. *J. N.Y. Entomol. Soc.*, **71**, 90-115.