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Donato A. Grasso · Alessandra Mori
Francesco Le Moli · Johan Billen

Morpho-functional comparison of the Dufour gland in the female castes of the Amazon ant *Polyergus rufescens* (Hymenoptera, Formicidae)

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Abstract The Dufour gland is crucially involved in main aspects of the parasite habit of the slave-making ant *Polyergus rufescens*, i.e. slave-raids and host colony usurpation. Workers use chemicals from this gland as recruitment signals during raid organization, while newly-mated queens use its secretion to appease residents during host nest invasion. Here, we report a comparison of the general morphology and fine structure of the Dufour gland in the female castes of *P. rufescens*: queens, ergatogynes (intermediate forms), and workers. The analysis clearly shows the link between gland structure and its behavioural role in queens and workers. In particular, queens present a hypertrophied gland with an extended lumen and a thin epithelium no more active in secretory function. This is consistent with the fact that usurper queens use the Dufour gland contents only during the short phase of host nest penetration. Contrary to adult queens, the cytoplasmic organization of the Dufour gland epithelium of raiders is typical for a tissue with secretory activity (abundance of mitochondria, free ribosomes, strands of smooth endoplasmic reticulum and a Golgi apparatus). This is consistent with the continuous raiding activity performed by workers throughout their adult life. The biology of ergatogynes is still an enigmatic matter. Their Dufour gland is intermediate in shape and size between that of queens and workers. It presents a fairly thick epithelium with features that are typical of a quite active secretory tissue.

Keywords *Polyergus rufescens* · Dufour gland morphology · Worker · Queen · Ergatogyne

D. A. Grasso · A. Mori · F. L. Moli (✉)
Department of Evolutionary and Functional Biology,
University of Parma, Viale delle Scienze 11/A,
43100 Parma, Italy
E-mail: lemoli@biol.unipr.it
Fax: +39-521-905657

J. Billen
Zoological Institute, University of Leuven,
Naamsestraat 59, 3000 Leuven, Belgium

Introduction

The ecological success of ants is due to their highly developed social organization (Wilson 1990). Social mechanisms allowing colonies to be efficient superorganisms are largely based on the use of chemical substances. This is reflected in the amazing development of the exocrine system in these hymenopterans, in which several tens of different glands have been described (Hölldobler and Wilson 1990; Billen and Morgan 1998). Because of the crucial importance of the exocrine equipment for virtually all aspects of biology of these insects, investigations on the morpho-functional features of glands, their development, roles and diversity between and within the species may further clarify the astonishing puzzle of ant social life. In this context, slave-making ants offer interesting examples of behavioural and physical adaptations to the parasitic habit by eusocial insects. *Polyergus rufescens* is a specialized slave-maker that presents several chemical adaptations to this habit. For example, secretions from the mandibular gland are used by workers of *P. rufescens* as 'propaganda' substances during slave-raids (Visicchio et al. 2001). Morpho-functional peculiarities of the mandibular gland may also be found in queens. In fact, secretions from this gland are discharged in the air through a peculiar curved slit of the mandible cuticle and convey signals by which virgin females attract males for mating. Female calling by mandibular gland secretions is a rare phenomenon in ants, and in *P. rufescens* may be regarded as an adaptation to the parasitic life (Grasso et al. 2003, 2004).

Structural and functional adaptations of *P. rufescens* to the slave-maker habit involve also another important exocrine structure, the Dufour gland. Although this abdominal gland is universally present in ant workers and queens, remarkable differences in cellular organization have been found in species belonging to different subfamilies (Billen 1986). This is probably linked to the wide variety of functions mediated by its secretions,

ranging from alarm to sexual attraction and trail or territorial marking (Billen and Morgan 1998).

In *P. rufescens* the Dufour gland is crucially involved in the main aspects of the parasite habit, i.e. slave-raids and host colony usurpation. Workers use chemicals produced by this gland as recruitment signals during raiding swarm formation (Visicchio et al. 2001), while newly-mated females use its main secretion (decyl butyrate) during host nest invasion as an appeasement substance to cope with the fierce hostility offered by resident workers (Mori et al. 2000a, b, 2001).

Within the female caste of *P. rufescens* intercastes may occur, although in a small number per colony. These intermediate forms (ergatogynes) are permanently wingless females having an external appearance which combines typical features of both queens and workers, but their role in the economy of the slave-maker colony remains largely unknown (Buschinger and Heinze 1992; Visicchio et al. 2003). Nothing is known on the function of the Dufour gland in the biology of ergatogynes. Despite this lack of information structural differences are expected in the Dufour gland of female castes of *P. rufescens*, differences that can be related to their function. In order to test this we compared the general morphology and fine structure of the Dufour gland in the three female castes (queens, ergatogynes, workers) of *P. rufescens*.

Materials and methods

The examined specimens (queens, ergatogynes and workers) were all adult and functionally active individuals. They were collected in the field near Parma (Northern Italy) while performing their typical above-ground tasks. In particular, dealate queens were captured on the ground just after nuptial flights and before attempting host-nest usurpation. Ergatogynes were collected on the ground while wandering around the parasite nest. Workers were collected from raiding columns during slave-raids.

Dufour glands were dissected and fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Postfixation was carried out in 2% osmium tetroxide in the same buffer. After dehydration in a graded acetone series, tissues were embedded in Araldite and sectioned with a Reichert Ultracut E microtome. Semithin 1 μm sections were stained with methylene blue and thionin and viewed in a Zeiss Axioskop microscope, 70 nm thin sections were double stained with uranyl acetate and lead citrate, and examined in a Zeiss EM900 electron microscope. Material for scanning microscopy was critical point dried in a Balzers CPD 030 instrument and examined in a Philips XL30 ESEM scanning microscope.

Statistical analyses of lumen diameter and epithelium thickness measurements were made by means of a one-way ANOVA. The Tukey test was used for post-hoc comparisons.

Results

As is usual for formicine ants (see Billen 1986), the Dufour gland in *P. rufescens* is a bilobed sac, that opens at the abdominal tip through a slit-like duct ventrally of the venom gland duct. In the workers, it is small and has a heart-like shape, whereas the two considerably enlarged lobes in queens give it an inward curled appearance. In ergatogynes, a somehow intermediate size and shape are found (Fig. 1).

Histologically, the Dufour gland shows a simple organization as its wall is formed by a monolayered epithelium which is apically lined by a cuticular intima and basally rests on a basal lamina. Some muscle fibres, nerves and tracheoles surround the gland on the hemolymph side. Differences and similarities among the three female groups can be seen in the thickness of the epithelium and the diameter of the lumen. Dealate queens show a gland with a very extended lumen (mean

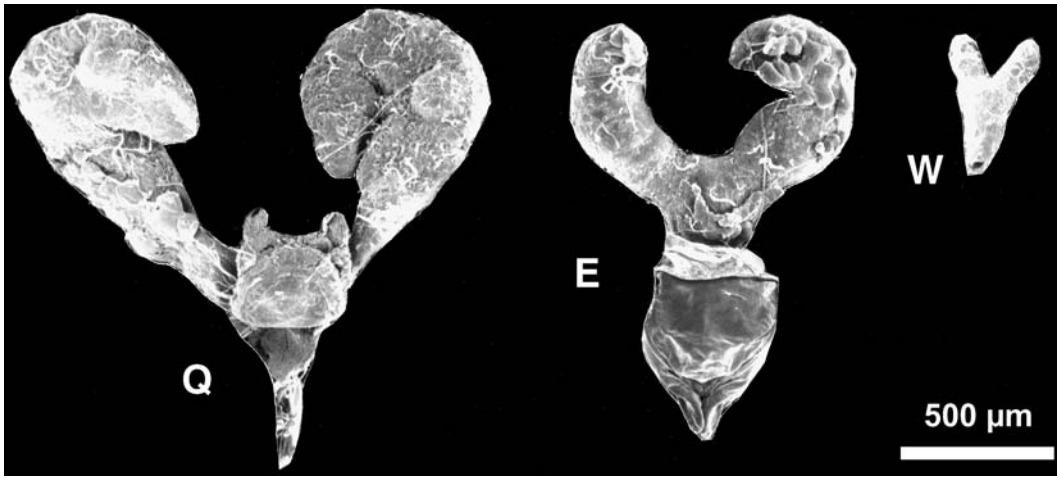

Fig. 1 Scanning micrographs at same magnification of Dufour gland of a dealate queen (*Q*), ergatogyne (*E*) and raiding worker (*w*), showing intermediate size and shape of the ergatogyne gland

value ± standard error of lumen diameter = 283.1 ± 23.1 μm, $n = 8$) and a thin epithelium (mean value ± standard error of epithelium thickness = 14.7 ± 1.2 μm, $n = 8$), whereas ergatogynes have a considerably smaller lumen (91.7 ± 13.5 μm, $n = 8$, $P < 0.001$) but thicker epithelium (25.6 ± 2.2 μm, $n = 8$, $P < 0.001$). Raiding workers are characterized by a gland with a lumen comparable to that of ergatogynes (131.3 ± 5.5 μm, $n = 8$, $P = 0.25$) and a fairly thin epithelium with a thickness comparable to that of the dealate queens (12.9 ± 0.9 μm, $n = 8$, $P = 0.7$) but significantly smaller than that of ergatogynes ($P < 0.001$).

The ultrastructural organization of the Dufour gland epithelium in the dealate queens reveals a disorderly appearance. Most of the cytoplasm is filled up with spherical clear vacuoles with a diameter of up to 2 μm, that often contain vesicular and lamellar material (Fig. 2A, B). Other cell organelles are limited to some mitochondria that are mainly found in the apical part. Polymorphic nuclei are situated in the basal cell region, while the lateral cell membranes are characterized by very sinuous turns in their apical area (Fig. 2A, B).

In ergatogynes, similar foldings of the intercellular borders are found (Fig. 2C), but the cytoplasmic organization corresponds to that of an active tissue: abundant tubules of smooth endoplasmic reticulum and several mitochondria are found, with the peculiar occurrence of smooth endoplasmic reticulum tubules parallel to the lateral cell membranes (Fig. 2C), as has also been reported for young queens (Billen et al. 2001). Also a well developed Golgi apparatus and free ribosomes occur (Fig. 2D). The basal cell membrane displays numerous invaginations, while the basal region of the cytoplasm contains many small spherical vesicles with a diameter between 0.1 and 0.3 μm (Fig. 2E). Also in raiding workers, the sinuous appearance of the lateral cell membranes in the apical region of the epithelium occurs (Fig. 2F). The cytoplasm contains mitochondria in the subcuticular region of the cells, as well as numerous free ribosomes, isolated strands of smooth endoplasmic reticulum and a Golgi apparatus (Fig. 2F).

Discussion

The present investigation shows the strict link between histological features of the Dufour gland and its functional role in the different female castes of *P. rufescens*. Newly-mated females of this social parasite are adult individuals ready to start a new colony by usurpation. This is the very crucial phase in the life cycle of a parasite queen since its reproductive success will largely depend on the success in invading a host colony and being accepted by resident workers. In this context the Dufour gland provides an extremely efficient tool for usurper queens. Morphological investigation of newly-mated queens showed a very large gland with a big lumen full of secretion. However, ultrastructural analysis of gland features of these individuals revealed that the epithelium is no more active in producing secretions. The epithelium is thin and formed by flattened cells with a reduced cytoplasm containing large amounts of lamellar and vesicular inclusions that are indicative for autolytic processes during cell degeneration. This is in clear contrast with the cytoplasmatic organization we previously found in callow queens, where the thick epithelium is made of cylindrical cells showing a cytoplasm with a clear secretory capacity (see Billen et al. 2001). Therefore, the Dufour gland of adult usurper queens is a fully loaded and trigged chemical weapon, ready to be used at the onset of the critical stage of host nest invasion. In this limited phase, the queen needs a sufficient amount of appeasement secretion (the ester decyl butyrate) and thereafter no longer needs to keep her gland active. Once coped with the first hostility of resident workers and after killing the host queen, the *Polyergus* queen progressively acquires the colony chemical labels and becomes accepted by host workers (Johnson et al. 2001; Lenoir et al. 2001; Mori et al. 2001). From that moment, size reduction of the Dufour gland may facilitate the increase of ovary size (Topoff et al. 1988).

On the contrary, raiding workers of *P. rufescens* possess a well-developed and active gland epithelium. Not only is the gland turgid and full of secretion but, although the epithelium thickness is small and comparable to that of queens, the cytoplasmic organization of the gland is typical of a secretory active tissue (abundance of mitochondria in the subcuticular region of the cells, numerous free ribosomes, strands of smooth endoplasmic reticulum and a Golgi apparatus). This is consistent with raid organization which in *Polyergus* is generally performed by adult workers that may repeatedly engage in this task throughout the entire summer or even in consecutive seasons (Cool-Kwait and Topoff 1984; Mori et al. 1991; Le Moli et al. 1994). This means that raiders need to keep their glandular equipment active for a long time.

The biology of ergatogynes of *Polyergus* and their role in the economy of the colony remain enigmatic questions. These permanently wingless intermediate forms present an external morphology more similar to workers than to queens (Buschinger and Heinze 1992; Visicchio et al. 2003), but some aspects of their internal morphology and behaviour (large and well developed ovaries; egg laying) make them closer to queens, although we never observed them mating (see also Terayama and Yamaguchi 1993). In the field they do not engage in typical worker tasks and generally move cautiously around the nest in a way more similar to queens than to workers. Dissection revealed the presence of large and well developed ovaries and egg laying was recorded in the laboratory (Grasso et al. unpublished). Our data show that their Dufour gland is intermediate (in shape and size) between that of queens and workers. The gland epithelium is thick and the ultrastructural investigation clearly revealed a cytoplasmic organization which corresponds to that of an active tissue. This sug-

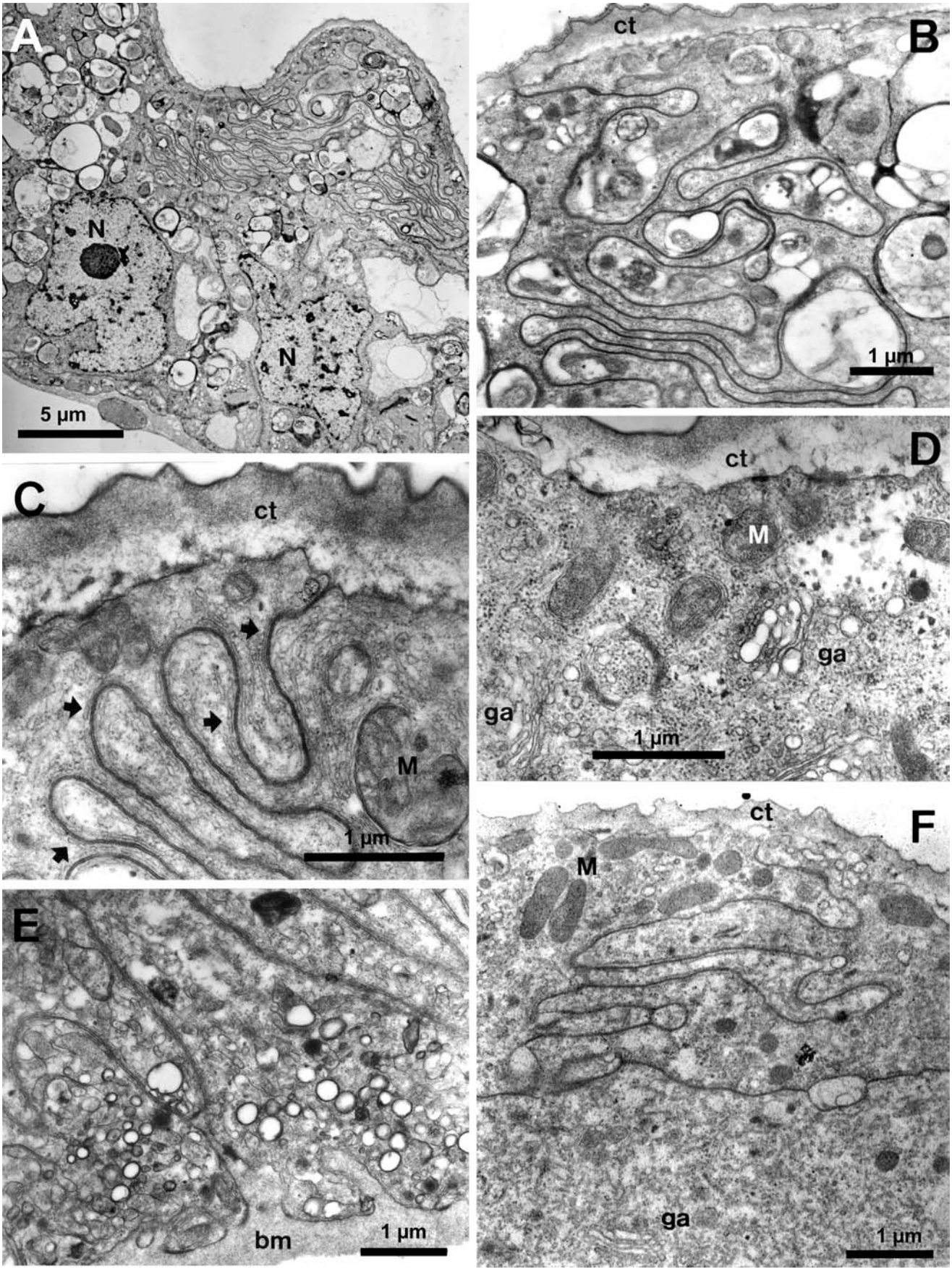
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Fig. 2 Electron micrographs of the Dufour gland in the three female groups. **A** Epithelium in a dealate queen, showing the disorderly arrangement of the cytoplasm, **B** apical region in a dealate queen with sinuous lateral cell membranes, **C** apical region in an ergatogyne showing tubules of smooth endoplasmic reticulum running parallel to the sinuous lateral cell membranes (*arrows*), **D** cytoplasm with Golgi apparatus and free ribosomes in an ergatogyne, **E** basal region in an ergatogyne with spherical vesicles, **F** apical region of epithelium in a raiding worker with subcuticular mitochondria. *bm* basal lamina, *ct* cuticle, *ga* Golgi apparatus, *M* mitochondria, *N* nucleus

gests that the gland may be involved in tasks that, contrary to queens, require a continuous production of secretion. Future field and laboratory studies on the life cycle of these intercastes as well as on the chemical nature of gland secretions will hopefully clarify this intriguing matter.

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