

ORIGINAL ARTICLE

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Structural and functional changes of the Dufour gland in gynes of the amazon ant *Polyergus rufescens* (Hymenoptera, Formicidae)

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Abstract Colony foundation by the slave-making amazon ant *Polyergus rufescens* requires penetration of the young gyne soon after mating into a colony of the slave species. During this process, she uses decyl butyrate from her large Dufour gland as an effective appeasement allomone. The structural appearance and development of this gland shows a clear age-dependent evolution that is in line with its behavioural function. At the moment of eclosion, young females have a gland with an empty lumen but thick lining epithelium, of which the active secretory cells are characterised by a well-developed Golgi apparatus, abundant mitochondria and smooth endoplasmic reticulum. The basal plasmalemma shows deep invaginations that facilitate the uptake of precursors from the haemolymph, while intercellular contacts display conspicuous interdigitations in the apical cell part. During the first days of adult life, secretion starts to accumulate in the lumen. At the age of 3 weeks, the gland displays a large lumen filled with secretion and a thin epithelium that no longer displays secretory activity. By this time, the gynes are ready for penetration into a slave colony, being loaded with large quantities of the appeasement allomone that are necessary at this initial stage of the usurpation process.

A. Introduction

Dulosis (slavery) in ants represents a specialised form of social parasitism, in which the slave-making species organises raids against colonies of the slave species in order to pillage the latter's pupae. These are brought back to the colony of the slave-maker, where they eclose and be-

come well-integrated members of the slave force in the heterospecific mixed colony (Buschinger et al. 1980; Le Moli and Mori 1987; Hölldobler and Wilson 1990). The European amazon ant *Polyergus rufescens* (Latreille, 1798) is an obligate dulotic ant, that during summer conducts frequent raids against colonies belonging to the *Formica fusca* group (subgenus *Serviformica*), such as *Formica cunicularia* Latreille, 1798. During these spectacular events, hundreds of raiding *P. rufescens* workers enter the *F. cunicularia* nest and after a few minutes return to their own colony carrying *F. cunicularia* pupae as their booty (Mori et al. 1991; Le Moli et al. 1994). The penetration into the host colony evidently constitutes a very delicate confrontation with the resident workers. The *P. rufescens* workers can rely upon their powerful scissor-shaped mandibles in eventual physical encounters with defending slave workers, although it is possible, similar to other slave-raiding ants, that the attack goes along also with the use of 'propaganda substances' that facilitate the robbery of brood by creating panic among the slave workers (Hölldobler and Wilson 1990; Mori et al. 1991).

An even more delicate stage in the life history of slave-making ants occurs during colony foundation. As is the rule for obligatory social parasites, queens of *Polyergus* species are also not capable of founding new colonies independently. Therefore, before laying her eggs, a newly mated female generally must locate and penetrate a nest of her host, eliminate the resident queen/s, appropriate the resident brood and obtain the care of resident workers for herself and, subsequently, for her brood (Topoff et al. 1988; Mori et al. 1995). The very crucial phase of this usurpation process is the invasion of the host nest, where the dulotic queen faces the competition with the resident ants. During the initial period of this process, the penetrating dulotic queen undergoes fierce hostility from the resident workers. While her thick exoskeleton gives her a mechanical protection, she also uses chemical tools to allow an easier usurpation of the host colony. Mori et al. (2000a) first described the role of the Dufour gland secretion of newly mated females as ap-

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peasement substances that significantly lower the attacks by *F. cunicularia* workers against the intruding *P. rufescens* queen. Chemical analysis of this gland reveals the presence of decyl butyrate as the dominant compound (D'Ettorre et al. 2000; Visicchio et al. 2000). Although a different interpretation considers this compound as a repellent (D'Ettorre et al. 2000), behavioural assays demonstrated that decyl butyrate is responsible for the appeasement function (Mori et al. 2000b).

The Dufour gland is the largest exocrine gland in the abdomen of *P. rufescens* females, and has a characteristic bilobed shape. Earlier histological work (Beck 1972) described age-dependent changes in the epithelial thickness of the Dufour gland, that were seen as a transition from a secretory phase in the younger individuals to that of storing secretion in older ants. These differences were explained as a possibly ephemeral function of the Dufour gland secretion during copulation, with an eventual role as lubricant substance for the sagitta or as a kind of physiological agent for the sperm (Beck 1972).

Considering its involvement in the early stages of colony usurpation, we investigated the structural ontogeny of the Dufour gland in *P. rufescens* gynes with both light and electron microscopy, in order to check whether the gland structure showed a structural evolution in parallel to the behavioural differences in the various stages.

B. Materials and methods

Pupae of gynes and adult workers of the amazon ant *P. rufescens* (together with their slave workers *F. cunicularia*) were collected in the field in Casola, near Parma, in Northern Italy. The colony fragments with pupae were housed in plaster nests and kept at 25°C and 70% relative humidity. Callow gynes were colour marked immediately after eclosion, allowing a study of winged females of known age. Other winged and dealate newly mated gynes were collected in the field just before and after nuptial flights, respectively. The Dufour glands of such gynes, and of mated dealate queens at initial stages in the usurpation process of queenright colonies of *F. cunicularia* in the laboratory, were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose, and postfixed in 2% osmium tetroxide in the same buffer. Dehydration was carried out in a graded acetone series, and 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, and double-stained 70-nm-thin sections were examined in a Zeiss EM900 electron microscope. Glands for scanning microscopy were critical-point dried in a Balzers CPD 030 instrument and examined in a Philips XL30 ESEM scanning microscope.

C. Results

I. General structure and light microscopy of Dufour gland

The Dufour gland is the largest gland in the abdomen of *P. rufescens* gynes (Fig. 1A) and opens into the cloacal chamber ventrally to the venom gland and hindgut opening. It has a bilobed shape with each lateral lobe extend-

ing anteriorly into the abdomen for up to 1 mm, and then curving back in a posterior direction along the body's midline. Each lobe can reach a diameter of up to 500 µm.

Histologically, the Dufour gland is formed by a simple monolayered epithelium surrounding a central lumen. At its luminal side the epithelium is lined with a cuticle and at the haemolymph side it rests on a basement membrane. The gland is surrounded by a few tracheoles and muscle fibres, the latter becoming well developed in the duct region where they form a specialised control apparatus that regulates the discharge of secretion (Billen 1982).

The histological appearance shows a very clear evolution in relation to the gyne's age and condition (Fig. 2). Immediately after eclosion from the pupal cocoon, the gland's epithelial wall is formed by high cylindrical cells with a thickness of 43.2 ± 6.4 µm ($n=4$ callow gynes, with eight measurements per individual), while the lumen is empty (Fig. 1B). During the first few days of adult life, the epithelial thickness remains high (45.5 ± 8.4 µm, $n=6$), while the lumen becomes more prominent (Fig. 1C) due to accumulation of secretion. Between the ages of 1 and 3 weeks, the epithelium shows a drastic decrease in thickness, while the lumen is full of secretion. In alate females of 20 days old, in alate and dealate females just before and after mating, as well as in dealate females just after penetration into the host colony, the epithelium has a thickness of 15.0 ± 5.7 µm ($n=11$) and the lumen is turgid (Fig. 1D). One week after the onset of usurpation, the epithelial thickness shows a further slight decrease, and measures 12.2 ± 5.0 µm ($n=3$; Fig. 1E). At this stage, the volume of the lumen starts to become less turgid (Fig. 2).

II. Ultrastructure of Dufour gland in young gynes

At the moment of eclosion, the epithelium is considerably folded due to the empty lumen. At its apical side, it is lined by a cuticle with a thickness of approximately 0.5 µm, that shows the usual sequence of an electron-dense epicuticle, a fibrillar exocuticle and an electron-clear endocuticle (Fig. 3A). The cells have a cylindrical shape and their apical cell membrane displays an irregular topography. Conspicuous interdigitations of the lateral cell junctions occur in the apical region (Fig. 3A). Nuclei have an ovoid to slightly polymorphic shape and occur in the central or lower part of the cells. The cytoplasm is characterised by an abundant smooth endoplasmic reticulum, that also continues as parallel tubular formations along the interdigitating lateral cell borders (Fig. 3A). Mitochondria are fairly numerous, while the Golgi apparatus is also well developed (Fig. 3B). The basal cell membrane shows numerous deep invaginations (Fig. 3C), that may locally show narrow extracellular spaces (Fig. 3D). Small lipid droplets with a diameter of up to 1 µm can occur in the basal part of the cytoplasm (Fig. 3D). The basement membrane has a thickness around 0.2–0.3 µm, and separates the epithelium from underlying tracheoles and muscle fibres (Fig. 3C, D).

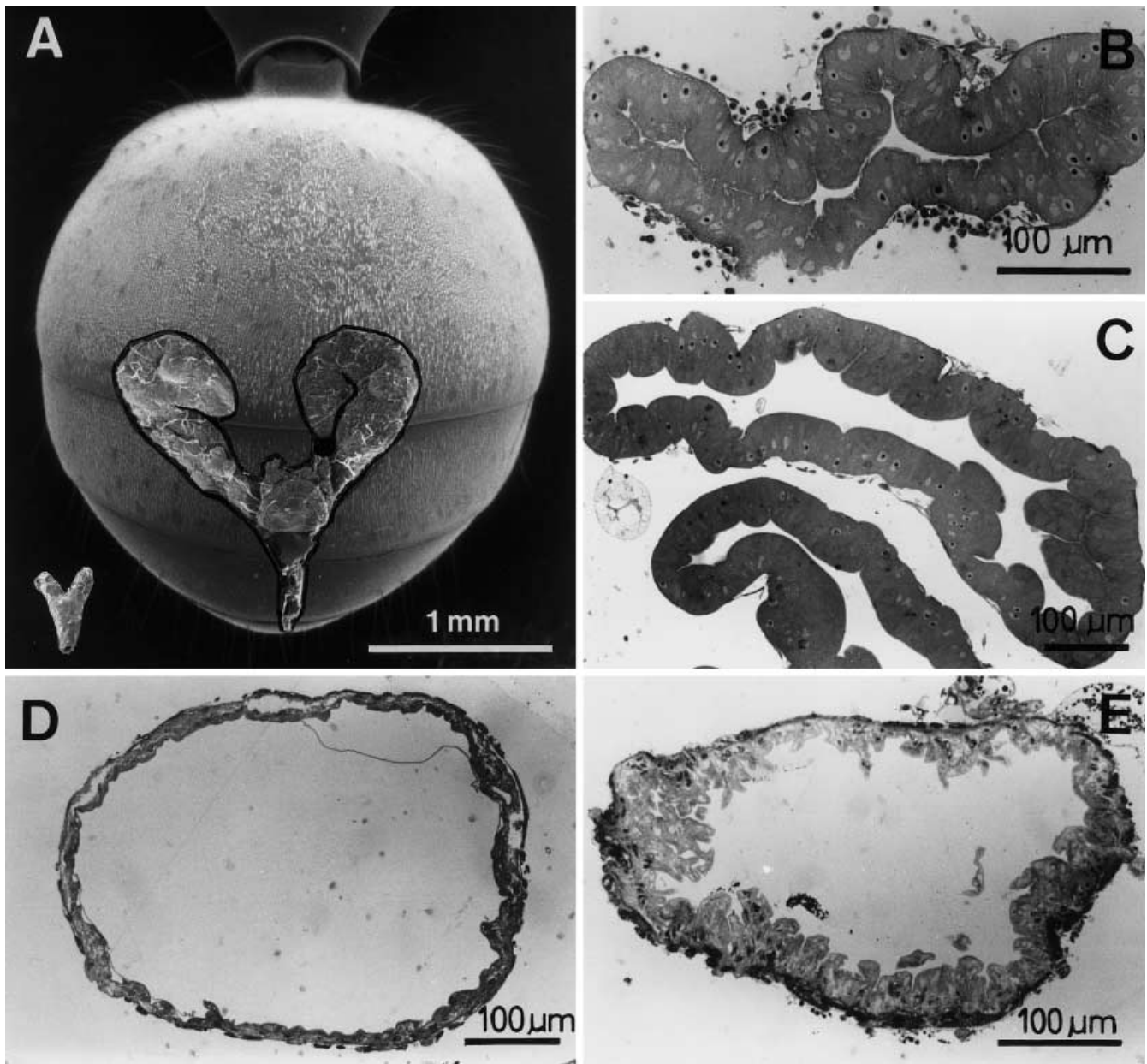


Fig. 1 A Scanning micrograph of the abdomen of a *Polyergus rufescens* queen with the large bilobed Dufour gland superimposed. The lower left corner shows the worker Dufour gland for comparison. B–E Semithin sections through outer lobe of Dufour gland at same section level in gynes of various age. B Few hours after eclosion. C Callow, 2 days old. D Callow, 20 days old. E One week after usurpation

III. Ultrastructure of Dufour gland in alate and dealate gynes at later stages

The epithelium of older alate as well as dealate gynes is formed by flattened cells. These continue to show very conspicuous intercellular digitations, while the subcuticular position of mitochondria remains an obvious characteristic (Fig. 4A, B). Due to the considerable decrease of

the epithelial thickness, the cytoplasm appears much reduced and contains increasing amounts of lamellar and vesicular inclusions as its main constituents (Fig. 4B–D). Other features such as irregular topography of the apical plasmalemma, presence of basal invaginations and a relatively thick basement membrane (Fig. 4C) remain as at earlier ages.

During the usurpation process, gynes still display a Dufour gland with flattened epithelial cells, in which the lamellar and vesicular bodies represent the most common cytoplasmic features, thus giving the cells a fairly disorderly appearance (Fig. 5).

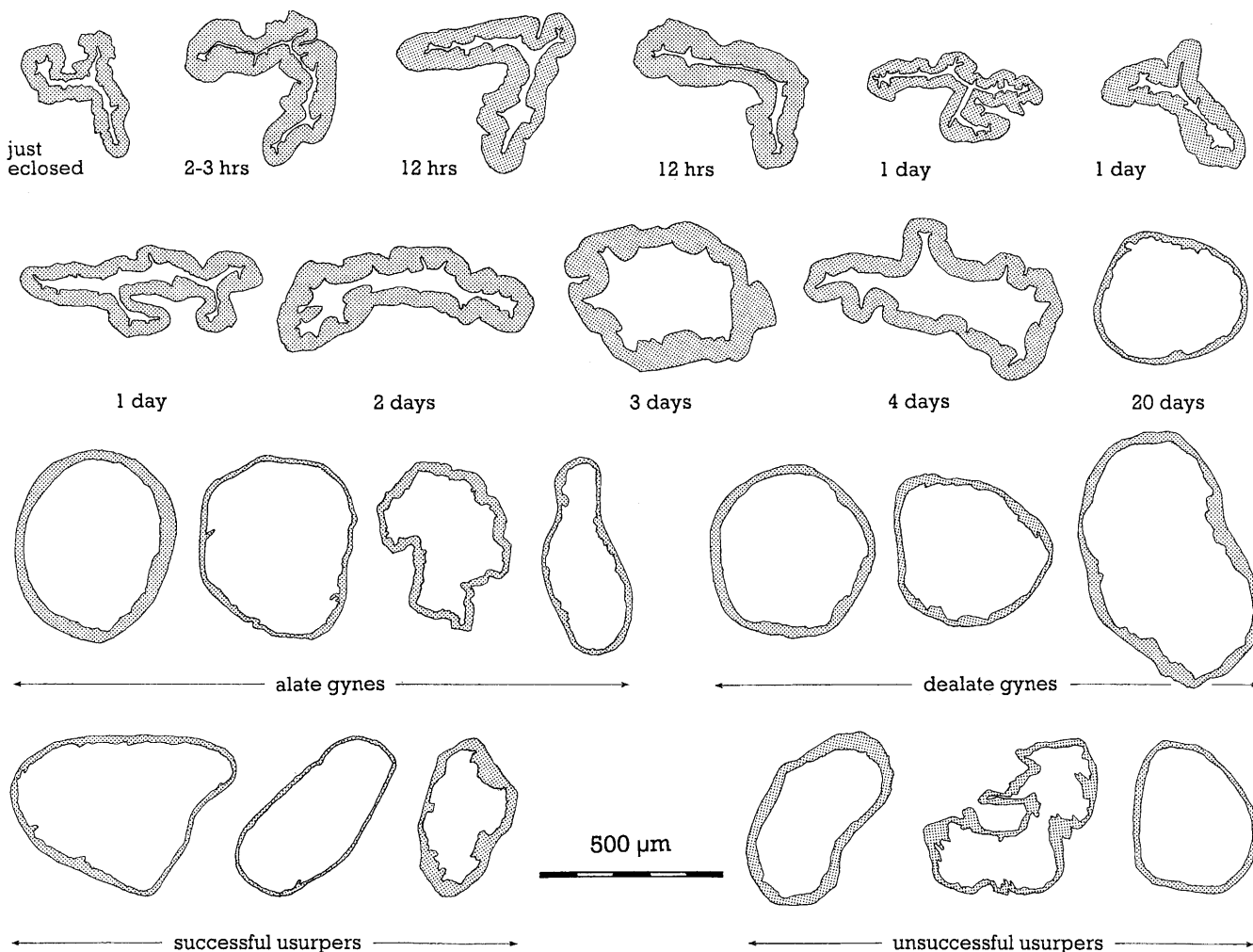


Fig. 2 Projection drawings of semithin cross-sections through outer lobe of Dufour gland at same section level in all 24 gynes investigated in this study, all at same magnification. Note obvious changes in extent of gland lumen and in epithelial thickness and appearance

D. Discussion

Communication, and the use of chemical messenger substances or pheromones in particular, is one of the keystones in the life of ants (Hölldobler and Wilson 1990). This is also reflected in the astonishing development of the exocrine system in these insects, in which over 60 different glands have been described so far (Billen and Morgan 1998).

In slave-making ants, the aspect of communication gets an additional dimension, as not only intraspecific communication with nestmates but also interspecific contacts with the slave species form part of the way of life. In species of the amazon ant genus *Polyergus*, this dual aspect is well exemplified in the first few weeks in the adult life of the young gyne: both in the North American *Polyergus breviceps* Emery, 1893 (see Topoff and Greenberg 1988) and in the European *P. rufescens* (see Mori et al. 1991, 1994), the young alate females join a

raid conducted by their nestmate workers towards a colony of the slave species, which already supposes communication between the young female and her sisters. During this phase of frenetic running, the female momentarily stops and attracts conspecific males with a sex pheromone. Immediately after mating (that in *P. rufescens* could also occur on the ground near the natal nest or after typical nuptial flights, see Mori et al. 1994), she sheds her wings and continues to follow the raid towards the target nest, where she will be faced with interspecific interactions with the slave species.

It is during this critical initial stage of usurpation that the Dufour gland secretion of the inseminated female plays a crucial role. Chemical analysis and behavioural observations in *P. rufescens* have revealed that its major component, decyl butyrate, is used as an appeasement allomone towards the resident slave workers in order to facilitate acceptance (Mori et al. 2000b; Visicchio et al. 2000). A similar appeasement function for the Dufour gland has also been described for usurping females of the American *P. breviceps*. In this species, a quite rapid decrease of the Dufour gland size after mating has been observed (Topoff et al. 1988).

The present work further illustrates the role of the Dufour gland in the usurpation process by gynes of the

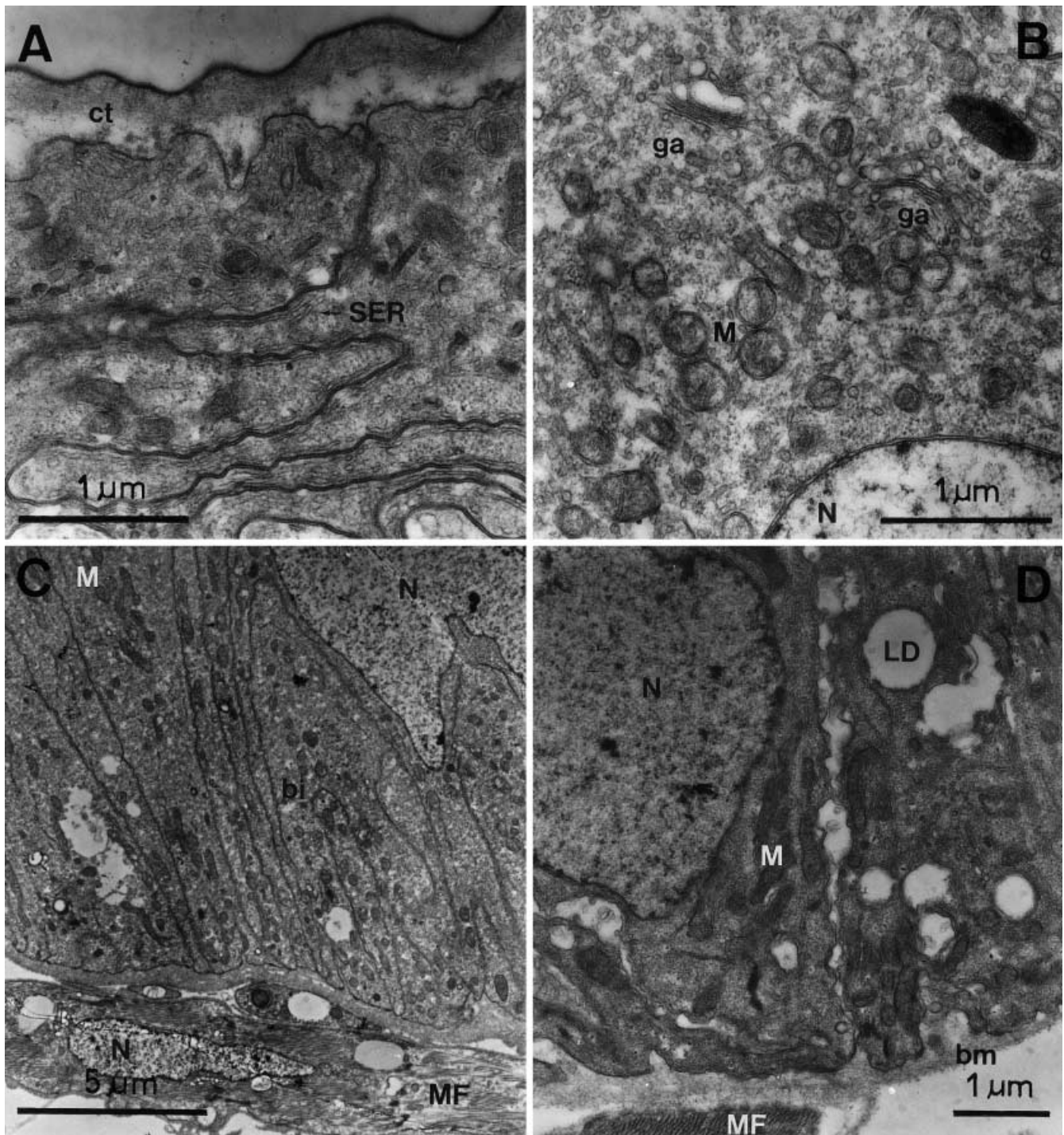


Fig. 3 **A** Apical region of epithelium in 1-day-old gyne, showing interdigitating lateral cell contacts. **B** Cytoplasm in 1-day-old gyne. **C** Basal region of epithelium in callosa gyne just after eclosion, showing basal invaginations. **D** Detail of basal region of epithelium in 1-day-old gyne. *bi* Basal invaginations, *bm* basement membrane, *ct* cuticle, *ga* Golgi apparatus, *LD* lipid droplets, *M* mitochondria, *MF* muscle fibres, *N* nucleus, *SER* smooth endoplasmic reticulum

European *P. rufescens*. At the moment of eclosion, the young callosa gyne possesses a gland with very reduced lumen without secretion, but with a thick epithelial wall, of which the cylindrical cells display a cytoplasm with active secretory capacity. The presence of a well-developed smooth endoplasmic reticulum and Golgi apparatus are in agreement with the production of fatty acid esters, such as decyl butyrate, that will indeed be needed soon when the young gyne, after having mated, will enter a host colony for usurpation. The presence of numerous invaginations of the basal plasmalemma in this context

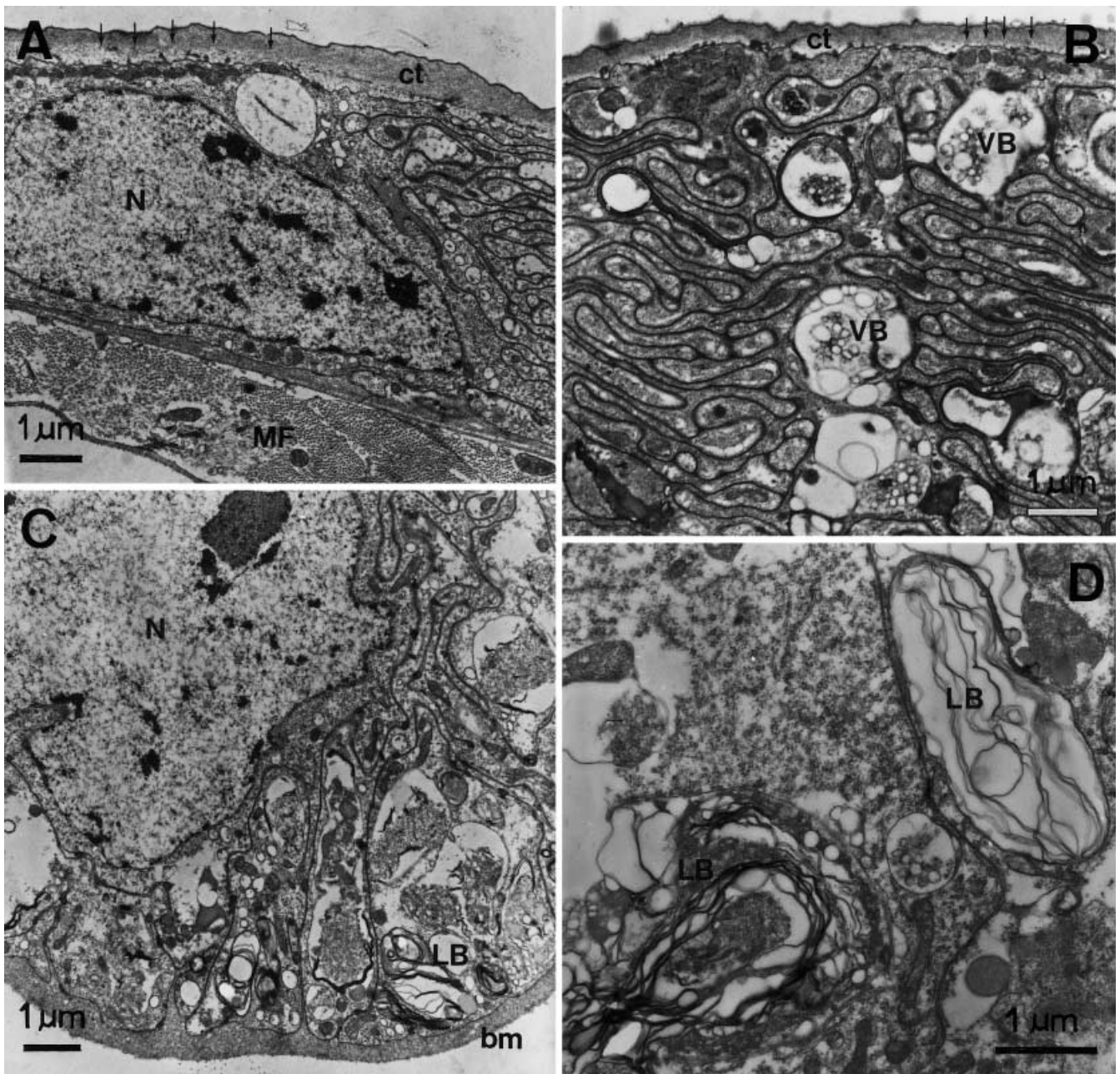


Fig. 4 **A** Dufour gland epithelium of alate female (*arrows* indicate subcuticular position of mitochondria). **B** Apical region of epithelium in dealate gyne (*arrows* indicate subcuticular mitochondria). **C** Basal region of epithelium in alate gyne, showing basal invaginations and lamellar inclusions. **D** Detail of cytoplasm with lamellar bodies in 20-day-old gyne. *bm* Basement membrane, *ct* cuticle, *LB* lamellar bodies, *MF* muscle fibres, *N* nucleus, *VB* vesicular bodies

can be regarded as a surface increase that facilitates the uptake of precursor molecules from the haemolymph. The absence of an apical microvillar border is surprising, as this often occurs in exocrine glands of the epithelial type (Soroker et al. 1995; Billen and Morgan 1998). Also the presence of numerous mitochondria is a common feature in exocrine glands. Their obvious subcuticu-

lar position may be related to the energetic costs of transporting the secretory products towards the lumen, and has been found to be a common characteristic for the Dufour of formicine ants (Billen 1986). The metabolic machinery for the elaboration of the appeasement substance is thus already fully active during the first days of adult life, and results in the rapid accumulation of secretion in the lumen. After 3 weeks, the gland has a turgid appearance with a full lumen, and is lined with a considerably reduced epithelium, that no longer displays the cytoplasmic features of an actively secreting tissue. It now gives a rather disorderly appearance with numerous lamellar and vesicular inclusions. These form a common element in insect pheromonal glands (Noirot and Quenney 1974; Billen and Morgan 1998), where they have

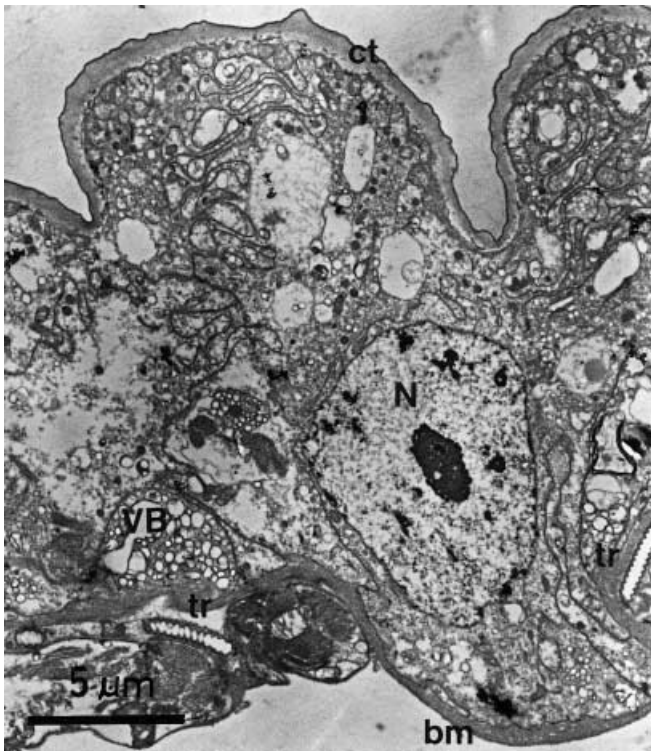


Fig. 5 Electron micrograph showing epithelium of Dufour gland in queen 1 week after invasion of the host colony, showing disorderly appearance of cytoplasm. *bm* Basement membrane, *ct* cuticle, *N* nucleus, *tr* tracheoles, *VB* vesicular bodies

been reported to represent secretory products (Hefetz and Orion 1982). During this stage of cell degeneration, they may also represent signs of massive autolytic processes. The Dufour gland thus is fully loaded at the onset of the critical usurpation stage, during which it will discharge the appeasement substance. Once the queen of the dulotic *Polyergus* species has been adopted by the resident slave workers, she no longer needs to keep her Dufour gland active, which also becomes apparent in its size reduction (Topoff et al. 1988). At this stage, the usurper's ovaries start to develop as from now on she will have the major task of producing offspring of her own. The size reduction of the Dufour gland will also facilitate the obvious increase of ovary size (Topoff et al. 1988).

These structural changes in the Dufour gland of *P. rufescens* gynes allow to shed more light on the functionality and adaptive value of this gland, and also illustrate the high level of adaptation that has been reached by *P. rufescens* in its parasitic habits.

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