

CHANGES IN THE MORPHOLOGY AND ULTRASTRUCTURE OF THE DUFOUR'S GLAND DURING THE LIFE CYCLE OF THE BUMBLE BEE QUEEN, *BOMBUS TERRESTRIS* L. (HYMENOPTERA: BOMBINI)

by

FÁBIO CAMARGO ABDALLA^{1,*}, HAYO VELTHUIS², CARMINDA DA
CRUZ-LANDIM¹ and MARIE JOSÉ DUCHATEAU²

(¹ Departamento de Biologia, Instituto de Biociências de Rio Claro, UNESP, Caixa
Postal 199, 13506-900 Rio Claro, SP, Brazil; ² Vergelijkende Fysiologie, Projectgroep
Ethologie & Socio-Oecologie, Universiteit Utrecht, P.O. Box 80086, 3508 TB Utrecht,
The Netherlands)

ABSTRACT

The Dufour's gland is found closely associated with the sting apparatus of all female hymenopterans, playing multiple roles among bees. In some species of *Bombus* the gland may be involved in production of nestmate recognition pheromones, but in *B. terrestris* its function is not certain yet. The morphology of the Dufour's gland of *B. terrestris* queens and the ultrastructural features of its cells were studied in different ages and behavioural stages using routine transmission electron microscopy. Measurements of the length and the diameter of the gland in the same conditions were also made. The Dufour's gland of the queen increases significantly in size (both in length and in diameter) with age and reproductive activity. The ultrastructural features of the gland show electron-dense material that comes from the haemolymph. This material is also present in the intercellular spaces, and is conducted to the subcuticular space, to be released directly into the glandular lumen. Hence at least part of the secretion is probably taken up directly from the haemolymph. The ultrastructural features indicate a more active phase of the gland corresponding to the period of egg-laying of the queen, and a decrease in activity when the queen is in hibernation as well as after the competition point. In conclusion, the gland is probably involved in reproduction, more specifically, in the marking of eggs.

KEY WORDS: Dufour's gland, bumble bee, queen, Hymenoptera, morphology, ultrastructure.

INTRODUCTION

Exocrine glands play a fundamental role in social hymenopterans, producing a plethora of pheromones that are involved in most social interactions. The Dufour's gland (DUFOUR, 1841), one of two exocrine glands

* Corresponding author; E-mail: fabdalla@rc.unesp.br

associated with the sting apparatus, is found in all female hymenopterans (BORDAS, 1894; LELLO, 1968), located ventrally to the venom gland and opening, in bees, into the dorsal vaginal wall (BILLEN, 1987).

In solitary bees the Dufour's gland has many functions, including the production of a hydrophobic material used as nest cell lining (CANE, 1981; KRONENBERG & HEFETZ, 1984), pheromones for sex (SMITH *et al.*, 1985) or nestmate attraction (HEFETZ, 1990), nest entrance marking (SHIMRON *et al.*, 1985), trail pheromones (VINSON *et al.*, 1978) and also as larval food (NORDEN *et al.*, 1980).

The role of the Dufour's gland in social bees is not certain yet. Queens of *Apis* and *Bombus* have a larger Dufour's gland than workers (CRUZ-LANDIM, 1967) and, in *Apis*, the gland produces wax-like esters that are not found in the secretions of workers, except of egg-laying workers (KATZAV-GOZANSKY *et al.*, 1997). Because of the localization of its opening, its size, and its caste-specific pheromones in honey bees, it has been suggested that the gland may play a role in reproduction, producing egg-marking pheromones which may be involved in the marking of the queen eggs to prevent them from being devoured by inspecting workers (RATNIEKS, 1993, 1995; KATZAV-GOZANSKY *et al.*, 1997).

Although much progress has been made in the elucidation of the chemical composition of its secretion and its function, the morphology and the ultrastructure of the Dufour's gland, including its changes during the life of bees or during the colony cycle, as well as the differences between the castes of social bees, are largely unknown. The purpose of this study, therefore, was to describe the principal morphological and ultrastructural features of the Dufour's gland of the *Bombus terrestris* queen at different ages and behavioral stages in an attempt to elucidate the possible function of this gland.

MATERIAL AND METHODS

Measurements of the length and the diameter of the gland were made in *Bombus terrestris* L. queens. This concerned: newly emerged queens (NE), 6-day old queens that were ready to mate (6D), queens at the end of hibernation (EH), queens producing the first brood of the colony (1B), and queens from colonies after the competition point (ACP), with egg-laying workers (DUCHATEAU & VELTHUIS, 1988).

The queens were collected from colonies maintained at the Department of Comparative Physiology (Utrecht University, The Netherlands) and dissected directly in fixative solution. The glands were measured with an ocular micrometer adapted to a microscope. Variance analysis (Scheffé

F-test) with a 5% level of significance was used to verify differences among the queen groups.

Some glands of each group were fixed with Karnovski fixative (2% glutaraldehyde and 4% paraformaldehyde in 0.2 M phosphate buffer at pH 7.3) for transmission electron microscopy (TEM), washed twice in 0.2 M phosphate buffer, post-fixed in 1% osmium tetroxide in the same buffer, stained with 2% uranyl acetate in 10% alcohol, and dehydrated in a series of increasing concentrations of acetone, embedded in Epon resin, following usual procedures. Thin sections were cut with a diamond knife, contrasted with lead citrate, and examined in an electron microscope.

RESULTS

General morphology and ultrastructure

The length of the Dufour's gland varies among the queens. In NE, 6D, and EH queens, the length is statistically equal, while 1B and ACP queens have significantly longer glands. The diameter demonstrated a great variability among the queen groups. Like the length of the gland, the diameter increases with increasing age and reproductive activity of the queen (Table 1).

The glandular monolayered epithelium presents columnar cells lined on the luminal surface by a non differentiated cuticle. The cytoplasm of the cells has numerous polymorphic mitochondria that are randomly distributed in cells of young queens, but in older ones they can aggregate apically or basally, following the plasmic membrane invaginations often present in these cells. Smooth endoplasmic reticulum (SER), polyribosomes and some Golgi complexes as well as some secretion granules or vesicles were also observed (Fig. 1).

The intercellular space is very sinuous and, when opened, is often penetrated by the basal lamina and filled with electron dense material (Figs 3B, C). Very specialised structures are formed by invaginations of the basal and the lateral plasmic membrane into the cell, producing loop-like infoldings, often with just a single mitochondrion inside (Fig. 3C).

Ultrastructural distinction between the developmental phases of the queen

The cytoplasm of the secretory cells of NE queens contains abundant polyribosomes and mitochondria, but few other organelles, inclusions and vesicles. Some cytoplasmic structures presenting a double membrane with a ground matrix containing electron-dense granules inside were observed.

TABLE 1

The length and diameter of Dufour's glands of *Bombus terrestris* queens and variance analyses between the groups.

Queens	N+	Length*		Diameter*		
		Mean	F-Test***	Mean	F-Test	SD**
Newly Emerged	10	6.45	A	0.50	A	0.10
6-Day-Old	10	6.60	A	0.80	B	0.10
End of Hibernation	12	6.00	A	0.60	AB	0.20
First Brood	5	9.45	B	0.80	BC	0.20
After the Competition Point	10	9.30	B	0.90	C	0.20

* Length and diameter in millimeter (mm); ** Standard deviation; *** Variance analysis by Scheffé F-test with 5% significance, + number of individuals analyzed.

The loops of the plasmic membrane are not well structured. An extensive network of tracheoles is observed in NE queens (Figs 1-NE, 2A, B).

The cytoplasm of the secretory cells in 6D queens, ready to mate, have abundant randomly distributed mitochondria and some Golgi complexes. Basal invaginations of the plasmic membrane are observed, but often not deep into the cell. A non electron-dense basal lamina is also present. Apical invaginations are few, but deeper than in NE queens. There is also amorphous material in the subcuticular space. Granules containing a homogeneous ground matrix with dots of electron-dense material, are also frequently found in the cytoplasm (Figs 1-6D, 2C).

EH queens show a cytoplasm full of myelinic figures and glycogen deposits. Lysosomes were also present (Figs 1-EH, 2D, E, F).

The most complex ultrastructural organisation of the glandular cells was observed in queens of colonies in the first brood phase (1B). The loop-like infoldings of the plasmic membrane are present and very well organized, being lined with SER. Very electron-dense basal lamina and amorphous material penetrate into the dilated intercellular space. This material probably accumulates in the haemolymph (the amorphous matrix outside the gland, containing electron-dense dots) that is held between the gland and its surrounding muscular sheet (Figs 1-1B, 3A, B, C, D).

The main aspect of the glandular cells of AFC queens is the presence of high electron-dense granules in the cytoplasm. They are round to

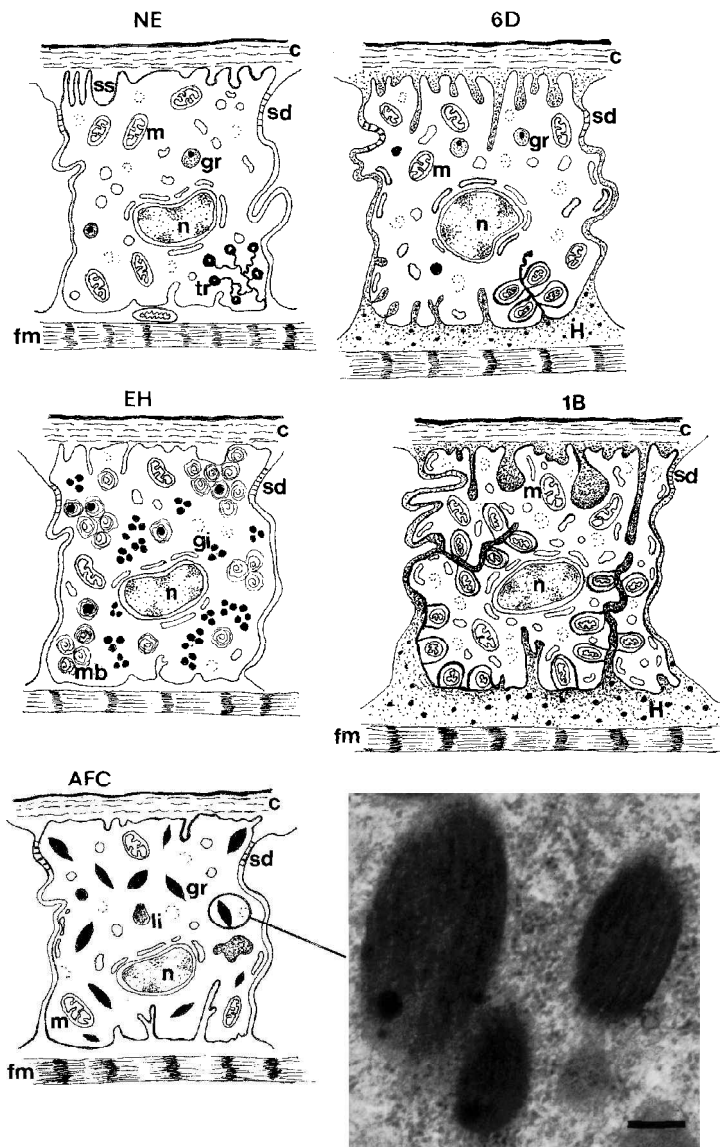


Fig. 1. Schematic representation of the main cell ultrastructural changes of the Dufour's gland of *B. terrestris* during the queen's life cycle. Legend: NE = newly-emerged queen, 6D = six day old queen, ready to mate, EH = end hibernation queen, 1B = first brood queen, AFC = after the competition point queen, with detail of cytoplasmic electron-dense granules, scale bar $0.1 \mu\text{m}$. c = cuticle, gi = glycogen deposits, gr = intracellular granules, H = amorphous material accumulated outside gland, m = mitochondria, mb = myelinic body, fm = muscle fiber, n = nucleus, sd = septated desmosome, ss = subcuticular space.

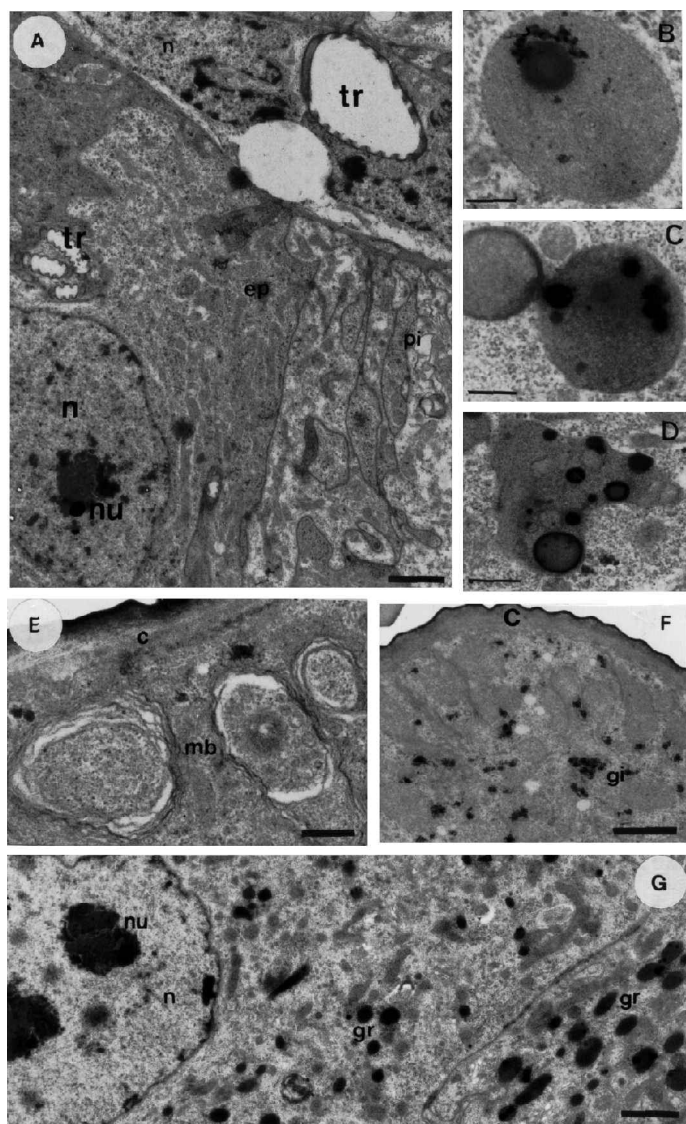


Fig. 2. Main Dufour's gland ultrastructural aspects of the different life phases of the queen. A. Detail of the glandular epithelium (ep) of the NE queen, scale bar $0.2\ \mu\text{m}$. B. Detail of a cytoplasmic structure with a discrete double membrane and internal lamellae, scale bar $0.3\ \mu\text{m}$. C. Detail of a cytoplasmic ground granule with electron-dense dots inside, scale bar $0.3\ \mu\text{m}$. D. Detail of a lysosome, scale bar $0.2\ \mu\text{m}$. E. Myelinic bodies (mb) in the EH queen cell luminal region, scale bar $0.2\ \mu\text{m}$. F. Glycogen granules (gi) in EH queen cell, scale bar $0.6\ \mu\text{m}$. G. Portion of cytoplasm of AFC queen, showing abundant electron-dense granules (gr), scale bar $0.6\ \mu\text{m}$. c = cuticle, n = nucleus, nu = nucleoli, tr = tracheole.

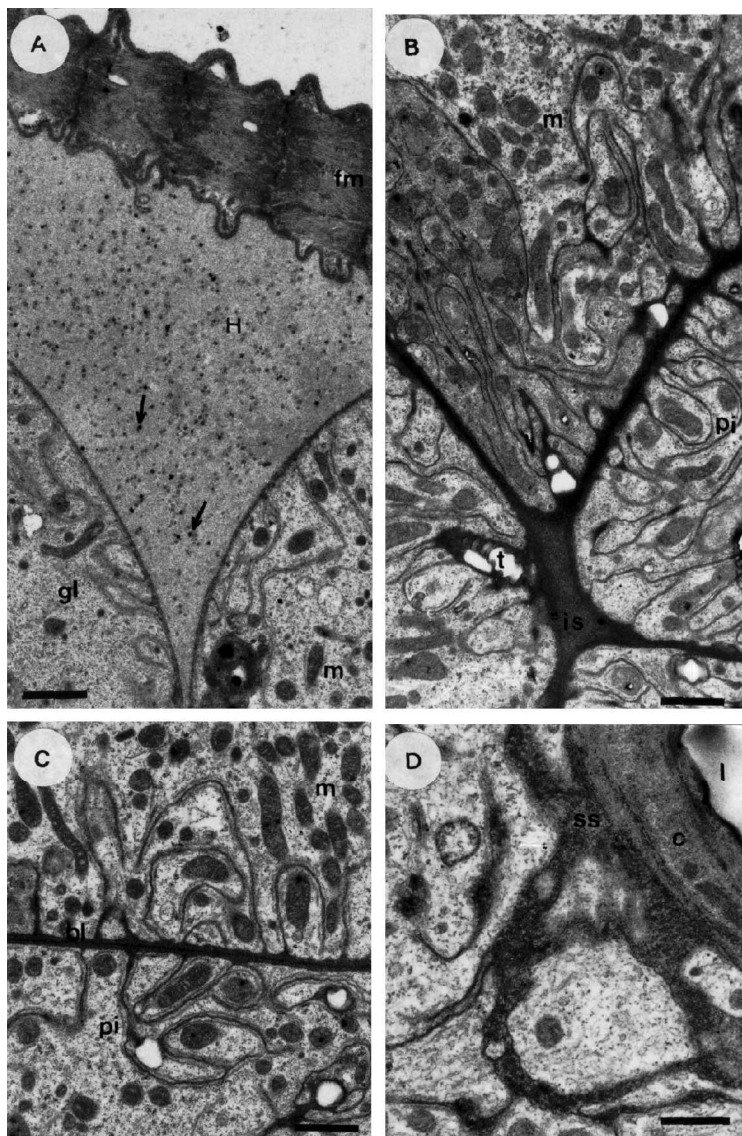


Fig. 3. Dufour's gland cells of the 6D queens. A. Amorphous material from the haemolymph (H) containing little dots (arrows) accumulated between the gland (gl) and sheet of muscle fibers (mf), scale bar $0.6 \mu\text{m}$. B. Very electron-dense material in the intercellular space (is), scale bar $0.8 \mu\text{m}$. C. Invaginations of the plasmic membrane (pi) form loop-like infoldings with a single mitochondrion (m) inside, scale bar $0.8 \mu\text{m}$. D. Material accumulated in the subcuticular space (ss) comes from the intercellular space and, probably from that outside the gland (A, B), does not pass into the gland, scale bar $0.3 \mu\text{m}$. bl = basal lamina, c = cuticle, l = lumen, m = mitochondria.

elliptical in shape and present a very electron-dense matrix with parallel lamellar organization, resembling cristae, suggesting that these granules may be modified mitochondria (Fig. 1-ACP, detail). Apical and basal invaginations are present, but the former appear to be undergoing a disorganisation process, having an irregular shape and a dilated lumen. The loops are poorly organised, but the intercellular space is very electron-dense and sinuous. (Figs 1-ACP, 2G).

DISCUSSION

The colony development of *B. terrestris* can be characterized by three successive phases: first, the colony is initiated by a solitary queen who produces her first workers by laying diploid eggs; second, once the workers of the first brood emerge, the social phase begins, during which the queen switches from laying diploid eggs to laying haploid eggs, from which males will arise; and third, workers start to lay eggs. In this last phase, mutual overt aggression starts between workers and between egg-laying workers and the queen. The transition from the second to the third phase is rapid, and has been termed the competition point (DUCHATEAU & VELTHUIS, 1988).

During the life cycle of the queen, it is possible to identify four important physiological phases: 1. from emergence until the queen becomes ready to mate (6 days old); 2. the hibernation period; 3. from the start of egg-laying (first brood) until the competition point; and 4. After the competition point. The development of the queen's Dufour's gland was studied under these different physiological conditions to elucidate its possible function.

The Dufour's gland of *B. terrestris* increases in size during the queen's life cycle and thus with age. Both length and diameter of the gland are maintained until the end of the hibernation phase. When the queen lays the first eggs, the size of the gland has increased significantly (Table 1). The gland also undergoes a major increase of its membrane surface during the queen's egg-laying period (1B queens), produced by several plasmic membrane infoldings, and basal or apical invaginations, resulting in an increase in exchange-surface with both the haemolymph and the gland lumen (Figs 3A, B, C, D).

The subcuticular space and the space between the muscle sheet and the gland becomes filled with amorphous material in 6D and 1B queens. This fact may be responsible for the evident increase in the diameter of the gland in both phases (Table 1, Figs 1-6D, 1B). Also the major increase in total glandular area (length times diameter) is reached in 1B queens,

indicating that the gland may be involved in reproduction, perhaps in the egg-marking activity as in *Apis* (KATZAV-GOZANSKY *et al.*, 1997).

The Dufour's gland appears to undergo restructuring twice: first when the queen is in the hibernation period and second after the competition point. In both phases the apical and basal invaginations and the loops of the lateral and basal plasmic membranes of the glandular cells are low or do not exist. The occurrence of reabsorption in the cell apices is evident from membranous debris, forming myelin figures in this region (Fig. 2E). These results support the probable participation of the Dufour's gland of *B. terrestris* in reproduction.

In *B. terrestris* the Dufour's gland cells are morphologically similar to those of class I of NOIROT & QUENNEDEY (1974, 1991), but they seem not to have a prominent secretory function, because the cells are very poor in secretion vesicles or granules. Furthermore, morphological evidence shows that at least part of the products sent to the lumen and constituting the secretion, are taken up directly from the haemolymph. This exogenous material may be taken to the lumen from the space between the gland and the muscle sheet through the intercellular space, and is not passing the epithelial cells. The secretion may also be stored in the subcuticular space (Figs 3A, B, C, D).

Since the gland does not have associated oenocytes (a characteristic of the class II cells of NOIROT & QUENNEDEY (1974, 1991), and its epithelial cells do not show strong evidences of secretory activity (characteristic of class I cells), the gland is composed of a new type of glandular cells, in which most of the constituents of its secretion may come directly from the haemolymph.

The chemical studies about the secretion of *Bombus* support this theory: the same hydrocarbons present on the exoskeleton are found in the Dufour's gland secretion (TENGÖ *et al.*, 1991; OLDHAM *et al.*, 1994). According to these authors, the composition of the secretion of the gland varies among the individuals of the same species from different colonies and probably the secretion serves for nestmate recognition. One explanation for the differences in the composition of the Dufour's gland secretion among the colonies is the difference in the quality of the food stored in them (HEFETZ *et al.*, 1993). The products of digestion, absorbed by the midgut and delivered to the haemolymph, may be taken up by the Dufour's gland of *Bombus*; food differences among the colonies are thus reflected in the composition of the secretion.

In conclusion, the Dufour's gland presents changes in its morphology during the life cycle of the queen, reflecting a possible complex function of the gland in *B. terrestris*. Its development during the life cycle, reaching a higher developmental level in the critical reproduction period, suggests

that the gland may play some role in reproduction. Perhaps, and more appropriate to social bees, it may supply the queen's eggs with specific pheromones.

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