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Comparative morphology of cephalic exocrine glands among castes of the black ant *Lasius niger*

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Abstract

The glandular system is crucially involved in main aspects of ant social life. The function of glands has been primarily studied in the workers (the non-reproductive individuals in a colony). In contrast, little information is available on queens (the reproductive females in a colony) or males in spite of the obvious functional differences between these castes. Here we report a comparison of the general morphology of the mandibular, propharyngeal and postpharyngeal glands between the three castes of the black ant *Lasius niger*. The analysis clearly shows that all these cephalic glands differ in relative size between castes and suggests a link between gland structure and its behavioral role in queens, workers and males. In particular, males present a hypertrophied mandibular gland. This is consistent with the fact that these glands might be the source of the sex pheromone in this caste. By contrast, queens exhibited the most developed postpharyngeal glands. This is consistent with the production of particular cues by queens for workers to help them to distinguish between reproductive and non-reproductive females. Finally, the propharyngeal glands were most developed in the worker caste and of similar relative size in males and queens. Their function is still enigmatic.

Keywords: Mandibular gland; Propharyngeal gland; Postpharyngeal gland; Social insects; Formicidae

1. Introduction

The complex social system of ants and other social insects is based on a complex system of communication that occurs almost exclusively by pheromones (Hölldobler and Wilson, 1990). Accordingly, social insects possess a massive and very complex system of exocrine glands producing a great variety of pheromones. While several glands such as the mandibular glands occur in all insects, others are specific to particular families, subfamilies, genera or even species (Billen, 1990). For example, the postpharyngeal glands are found in no other family than the Formicidae. The function of many glands varies greatly between species and also between the queen, worker and male castes. For example, the mandibular glands of workers of some species are involved in defence systems and alarm communication (Billen et al., 1998; Buschinger and Maschwitz, 1984; Hölldobler and Wilson, 1990). In some other species, the mandibular glands of workers are involved in nestmate recognition (Hernandez et al., 2002) and fungal growth inhibition (Marsaro Junior et al., 2001). In males and queens these glands have a very different function, serving as a source of sex pheromones (Ayasse et al., 2001; Topoff and Greenberg, 1988).

The comparison of gland size between queens, workers and males can reveal caste specific gland functions and is thus useful to understand patterns of communication in ants. To date, only very few such studies have been performed for the three cephalic glands (the mandibular, postpharyngeal and propharyngeal gland) in males, queens and workers (*Formica rufa*, Bausenwein, 1960; *Solenopsis invicta*, Phillips and Vinson, 1980). The aim of the present work is to conduct, in the ant *Lasius niger*, a comparative study between virgin queens, males and workers of the mandibular, propharyngeal and postpharyngeal glands to see if there are differences in the

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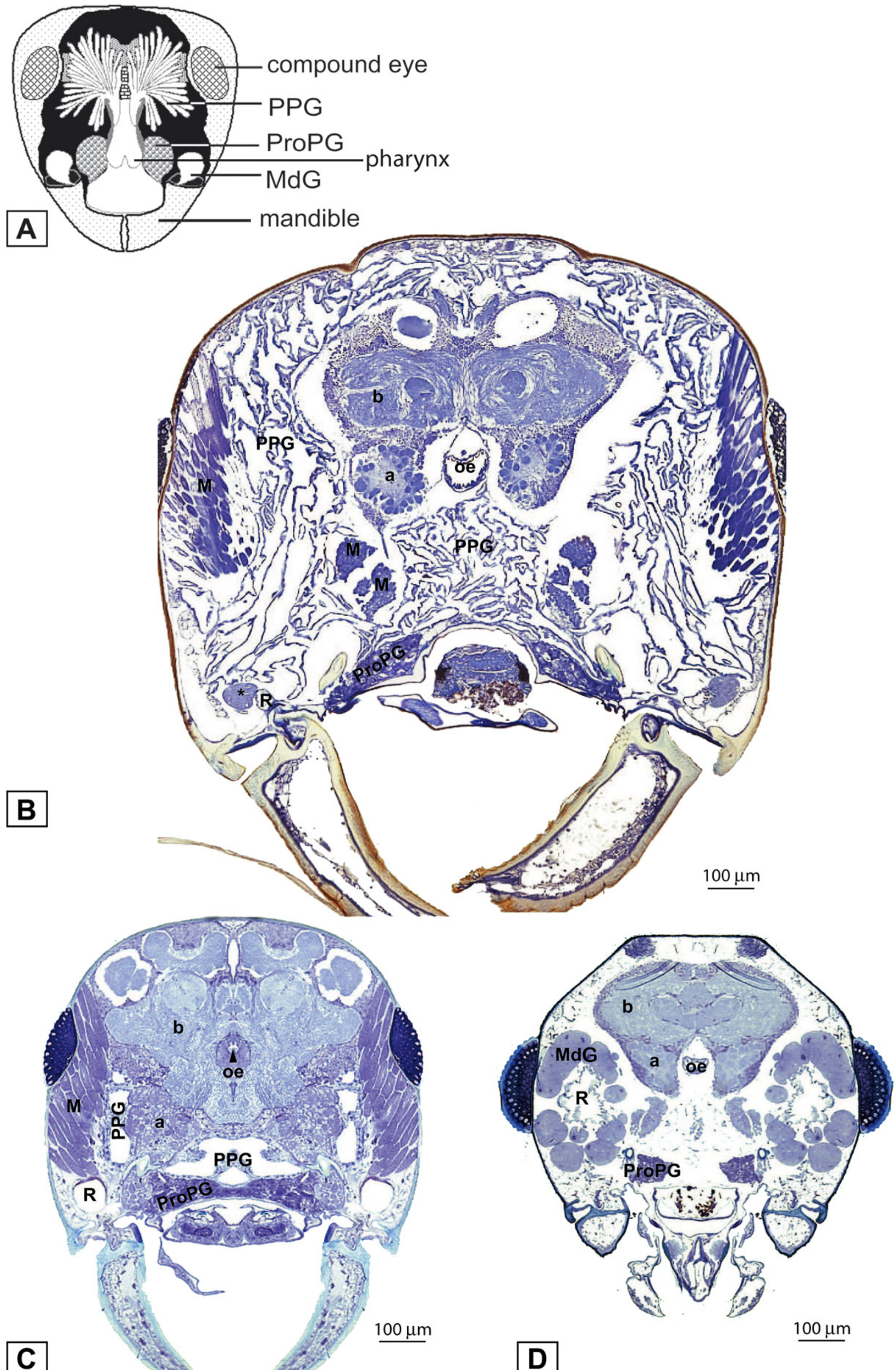
compound eye
PPG
ProPG
pharynx
MdG
mandible
A
b
PPG
M
a
oe
M
M
PPG
ProPG
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R
B
100 µm
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oe
M
PPG
a
PPG
R
ProPG
C
100 µm
b
MdG
a
oe
R
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100 µm

development of these glands between the castes. This species is very interesting for such a comparative study because it is the ant with the longest-lived individuals, the queens reaching the extreme age of 28 years (Kutter and Stumper, 1969; Keller and Genoud, 1997). By contrast, workers and males have a much shorter lifespan (workers: 1−3 years; males: a few weeks) as typical for other ants. Mature colonies of this species contain a single queen and up to 50,000 workers (Fjerdingstad et al., 2002). These life-history characteristics make this species an ideal system to test for the impact of glandular secretions in the complex interactions between individuals in a long-lived ant with a well marked queen worker dimorphism.

2. Materials and methods

2.1. Specimen preparation

Virgin winged queens, males and black workers of *L. niger* Linnaeus were collected just before nuptial flights from field colonies located in a meadow on the University campus in Lausanne, Switzerland. The specimens were narcotized in ice prior to dissection or fixation.

For size measurements, the glands were dissected under PBS buffer and the surrounding tissues removed.

For histology and ultrastructure analyses, the ant heads were fixed at 4 °C in a solution containing 4% paraformaldehyde/PBS pH 7.4 and 0.1% (w/v) Brij 35 (Sigma). They were then rinsed in 0.1 M sodium cacodylate pH 7.2 and placed in 10% sucrose cacodylate buffer at room temperature and, after dehydration in a graded ethanol series and embedding in unicryl resin (British Biocell International) at 56 °C, sectioning was done in the frontal plane of heads with a Diatome diamond knife on a Reichert Ultracut S microtome. Semi-serial and semithin sections were performed to estimate the number of secretory cells per glandular cluster. After double-staining with 1% azur blue and 1% methylene blue (11:1) in 1% sodium tetraborate for 1 min and washing with distilled water, the sections were examined under a Leica Diaplan microscope and photographed with a Leica DC300 digital camera. For transmission electron microscopy, 70 nm fine sections were placed on coated parallel bar copper grids and contrasted with a 2% aqueous solution of uranyl acetate followed by lead citrate. The grids were then examined with a Philips CM12 transmission electron microscope.

Five randomly sampled specimens per caste were taken for each experiment.

2.2. Image analysis

The head width (HW), the diameter of the mandibular and propharyngeal glandular cluster, and the number of postpharyngeal gland lobes were measured using a Leica microscope and photographed with a Leica DC300 digital camera. The number of secretory units was determined by the compilation of all serial sections. All measures are made with NIH Scion Image. For comparisons across castes, we determine the gland diameter divided by head width and conducted all analyses with these relative sizes. We also calculated the relative reservoir size for the mandibular glands by dividing the reservoir diameter by the gland diameter. All values were normalized using an arcsine(sqrt(x)) transformation. We used one-way ANOVA to compare the relative sizes of glands and of mandibular gland reservoirs between castes. Post hoc pairwise comparisons were performed with the Tukey's method when the ANOVA revealed significant differences between castes. *P*-values smaller than 0.05 were considered as significant. All statistical analyses were performed with S-Plus 6.2.

3. Results

The morphology of heads and the relative size of most tissues varied between queens, males and workers (Fig. 1).

3.1. Mandibular glands

The mandibular glands are paired spherical structures located between the base of the mandible and the inner surface of the compound eye at each side of the head (Fig. 1A). In females, they are small structures containing only five secretory cells per gland in workers and 12 per gland in queens (Fig. 1B and C; Table 1). These cells are located near the apical region of the mandibular gland reservoir. In males, the mandibular glands are hypertrophied and occupy approximately half of the entire head (Fig. 1D). Ten clusters of approximately 10 cells completely surround an impressive mandibular gland reservoir (around 267 μm in diameter). Even the relative size of the mandibular gland (mandibular gland diameter to head width ratio) is significantly greater in males than in females ($F[2,12] = 2413$, $p < 0.001$; Table 1). In contrast, in spite of the strong difference between worker and queen head width, the gland size of these two castes did not differ significantly. Surprisingly, the relative size of mandibular gland reservoir varied significantly between males, queens and workers ($F[2,12] = 8923$, $p < 0.001$; all pairwise comparisons $p < 0.001$) with males having the largest reservoir and workers having larger ones than queens.

The structural examination of the mandibular glands reveals the occurrence of a bicellular system consisting of a secretory cell connected to the glandular reservoir through an accompanying duct cell (Fig. 2). The junction between glandular cell and duct cell is formed by an end apparatus. This

Fig. 1. Histological aspects of the head glands of *Lasius niger*. (A) Schematic drawing of an ant head showing the position of the mandibular gland (MdG), the propharyngeal gland (ProPG) and the postpharyngeal gland (PPG) inside the head capsule. (B−D) Frontal sections of the head showing consistent differences in gland size between castes. The postpharyngeal glands (PPG) occupy half of the entire head in queens (B) and the mandibular glands (MdG) are particularly hypertrophied in males (D). The propharyngeal glands (ProPG) touch each other on the axial region of the head only in workers (C). Abbreviations: a = antennal lobe, b = brain, M = muscle, oe = oesophagus, R = reservoir of the mandibular gland, * = mandibular gland in queens, MdG = mandibular gland.

Table 1
Mean ± SD size of the mandibular gland in workers, queens and males ($n = 5$ per caste)

Caste	Gland diameter (μm)	Relative gland size	Reservoir diameter (μm)	Relative reservoir size	Number of secretory cells per gland
Worker	59 ± 4.2	0.054 ± 0.004	69.6 ± 3.6	0.063 ± 0.003	5
Queen	135 ± 7.9	0.068 ± 0.004	31.8 ± 2.4	0.016 ± 0.001	12
Male	505 ± 21.8	0.505 ± 0.020	267.6 ± 2.5	0.267 ± 0.002	>100

The relative gland size was calculated by dividing the gland diameter by the head width and the relative reservoir size by dividing the reservoir diameter by the gland diameter.

specialized structure allows secretions leaving the glandular cell to be carried off by the duct cell towards the reservoir (not shown, similar to Fig. 3D). This bicellular system is a common characteristic for the type-3 secretory cells described by Noirot and Quennedey (1974, 1991). In queens, males and workers, the secretory cells are large (diameter approximately 35 μm) and possess an eccentrically located nucleus of similar diameter (12 μm).

3.2. Propharyngeal glands

The propharyngeal glands (ProPG) are a pair of spherical structures located close to the lateral pouches of the pharynx in males and queens, but besides and above the pharynx in workers (Fig. 1). This particularity is translated by a significant difference in the relative size of the propharyngeal gland between castes ($F[2,12] = 247$, $p < 0.001$), with workers having a relatively larger gland than queens and males (both $p < 0.001$, Table 2).

The difference in gland size is explained by a difference in the number of secretory cells between castes: each gland contains 14 secretory cells in males, 15 in queens and 20 in workers. In all castes, the secretory cells have an irregular shape and contain a centrally located nucleus (diameter 20 μm) (Fig. 2). The histological examination of the propharyngeal glands reveals also the occurrence of end apparatuses (Fig. 3), which is characteristic of the type-3 secretory cells (Noirot and Quennedey, 1974; Noirot and Quennedey, 1991). As in all type-3 secretory glands, the secretory unit of propharyngeal glands consists of a secretory cell connected to the glandular reservoir through an accompanying duct cell.

3.3. Postpharyngeal glands

The postpharyngeal glands have a glove-shaped appearance (Fig. 1), their general morphology corresponding to the glandular organisation of type-1 secretory cells as described by Noirot and Quennedey (1974, 1991): the glandular cells are arranged in a simple monolayered epithelial wall that surrounds the lumen (Figs. 1 and 3B). These secretory cells vary in height from 7.5 μm to 12 μm and contain a centrally located rounded nucleus (diameter 6.5 μm). In queens, the glands have 80–100 finger-like lobes and are enormous, occupying a large proportion of the head capsule where they completely cover the brain, extending forward to the propharyngeal and mandibular glands. By contrast, in workers and males the postpharyngeal glands are relatively small and are confined to the front of the head (one finger-shaped lobe per gland in males and two in workers, Table 3). Lipid inclusions were observed in the lumen and in the cells surrounding the lumen, and are equally noticeable in all castes (data not shown). Their diameter varied significantly between worker, male and queens ($F[2,78] = 24.6$ $p < 0.001$), with significantly bigger inclusions in queen glands than in workers and males (both $p < 0.001$).

4. Discussion

4.1. Mandibular glands

L. niger males possess mandibular glands that are much more developed than in queens and workers. A similar pattern has been observed in some other species in the genus *Lasius* (Billen and Schoeters, 1994), where the mandibular glands are thought to be the source of the sex pheromone (Law et al., 1965). It is thus well possible that the hypertrophy of the mandibular glands in *L. niger* males is associated with the production of the sex pheromone. 1-Octanol and 1-nonanol were identified as the main components of the mandibular gland secretion of *L. niger* (Bergström and Löfqvist, 1970). It remains to be investigated whether these two compounds play a role in attracting females in *L. niger*.

The relative size of the mandibular gland reservoir is higher in workers than queens, suggesting that this gland might also have a worker specific function. In several ants and other Hymenoptera, the mandibular glands are involved in the alarm-defence system (Billen and Morgan, 1998; Hölldobler and Wilson, 1990). A larger relative size of the glands in workers than queens has also been observed in other species such as *Leptanilla* sp. (Billen et al., 1998) and in species of the *Camponotus cylindricus* complex (Maschwitz and Maschwitz, 1974; Buschinger and Maschwitz, 1984). These data are consistent with the idea that the mandibular glands might also be involved in the alarm-defence system of *L. niger*.

4.2. Propharyngeal glands

We found that the propharyngeal glands are more developed in workers than in the other two castes suggesting that these glands might have a workerspecific function. Currently, very little is known about the function of propharyngeal

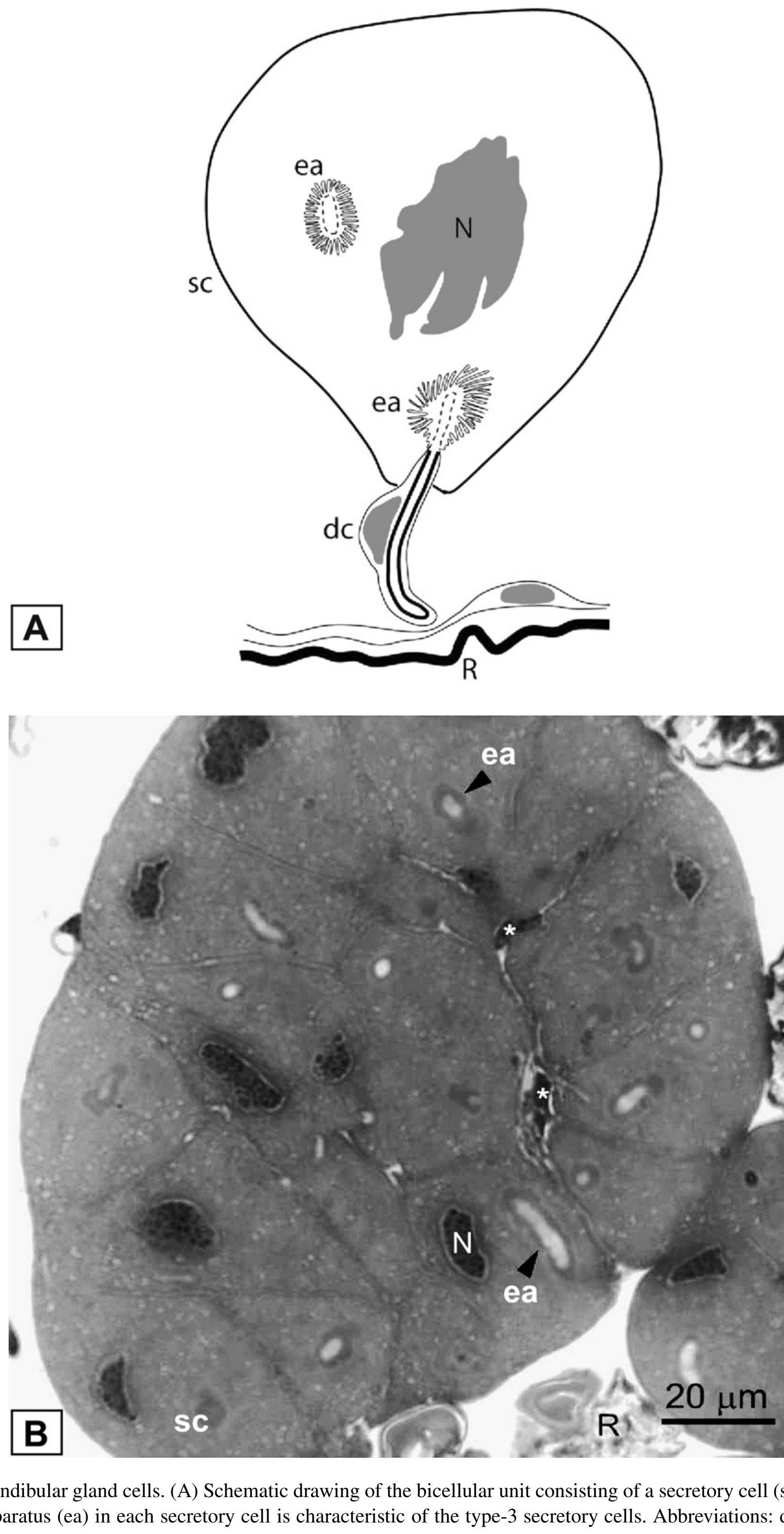

Fig. 2. Histological aspect of mandibular gland cells. (A) Schematic drawing of the bicellular unit consisting of a secretory cell (sc) associated with a duct cell (dc). (B) The presence of the end apparatus (ea) in each secretory cell is characteristic of the type-3 secretory cells. Abbreviations: dc = duct cell, ea = end apparatus, N = secretory cell nucleus, R = reservoir of mandibular gland, sc = secretory cell, * = duct cell nucleus.

glands. The fact that they are widespread in the Formicidae suggests that they play an essential role in some aspect of ant biology. The well developed granular endoplasmic reticulum and the presence of numerous secretory vesicles is suggestive that propharyngeal glands might be involved in the production of proteinaceous secretions (Billen and Peusens, 1984). It has also been suggested that they may be involved in the production of digestive enzymes such as

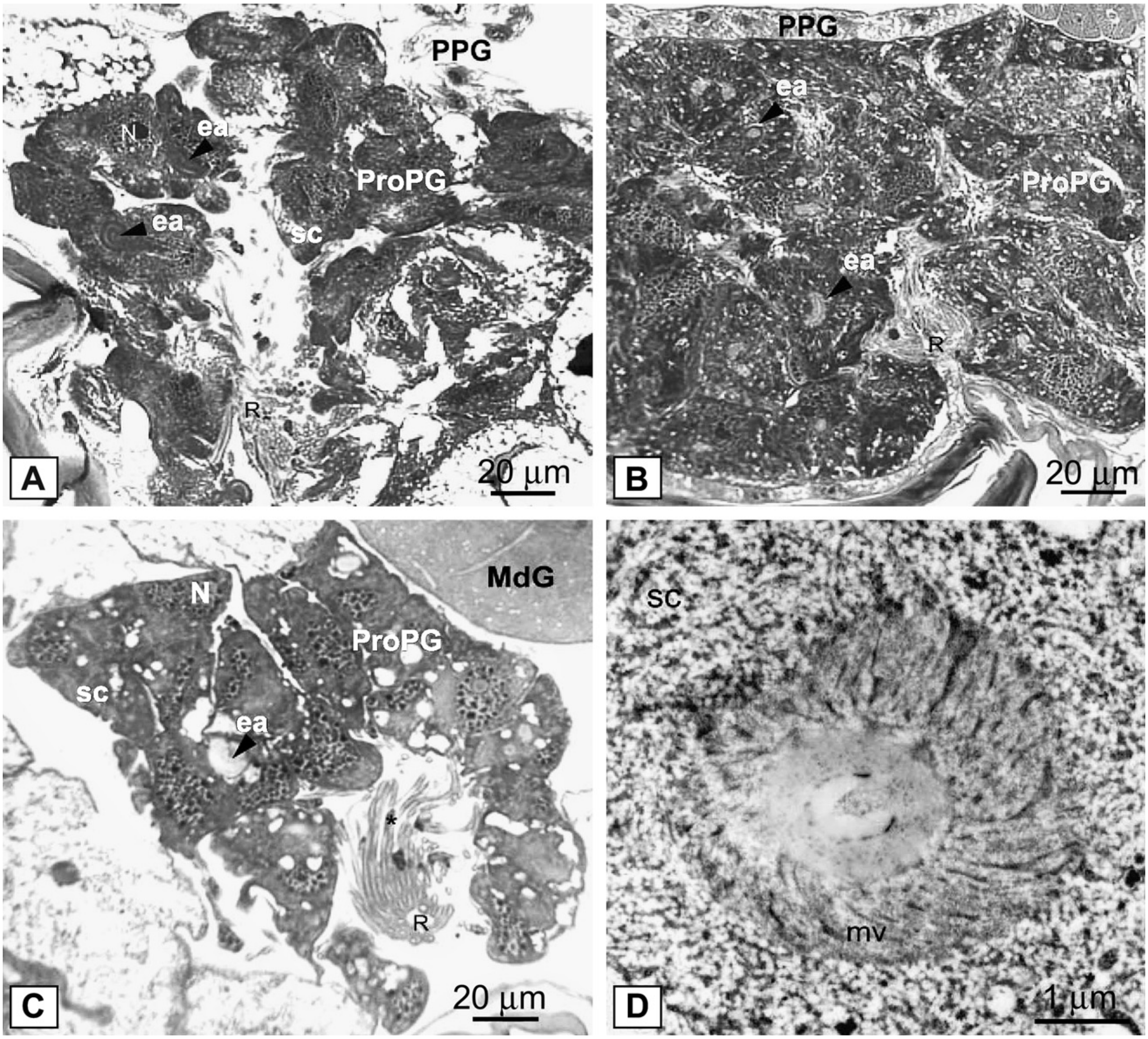

Fig. 3. Histological aspect of the propharyngeal gland in queen (A), worker (B) and male (C) and postpharyngeal gland in queen (A) and worker (B). The postpharyngeal gland (PPG) is close to the propharyngeal gland in females (queens and workers). In males, the mandibular gland (MdG) and not the PPG, is observed at the proximity of the propharyngeal gland. The presence of the end apparatus (ea) in each secretory cell (sc) is characteristic of the type-3 secretory cells. The microvillar pattern of the end apparatus is confirmed by an electron micrograph in (D). Abbreviations: ea = end apparatus, MdG = mandibular gland, mv = microvilli, N = secretory cell nucleus, ProPG = propharyngeal, PPG = postpharyngeal gland, R = reservoir of propharyngeal gland, sc = secretory cell, * = excretory duct of a duct cell.

invertase and amylase in some ant species (Ayre, 1967). There are only few comparative studies of the size and morphology of these glands. In the fire ant *S. invicta* the glands are relatively more developed in large (major) workers than small (minor) workers (Phillips and Vinson, 1980), but the reasons underlying this difference are not known. The dimorphism for the propharyngeal glands between castes might encourage further investigations on these enigmatic glands.

Table 2
Mean ± SD size of the propharyngeal gland in workers, queens and males ($n = 5$ per caste)

Caste	Gland diameter (μm)	Relative gland size	Number of secretory cells per gland
Worker	256 ± 15.2	0.233 ± 0.001	20
Queen	248 ± 8.4	0.124 ± 0.004	15
Male	132 ± 5.7	0.132 ± 0.006	14

The relative gland size was calculated by dividing the gland diameter by the head width.

Table 3
Comparative development of the postpharyngeal gland in workers, queens and males ($n = 5$ per caste)

Caste	Lobes number per gland	Relative gland index
Worker	2	0.002
Queen	80–100	0.05
Male	1	0.001

Relative gland development index is calculated here as number of lobes divided by head width.

4.3. Postpharyngeal glands

These exocrine glands are hypertrophied in the queen caste where they occupy approximately half of the entire head. By contrast, they are much smaller in males and workers. A similar pattern has been observed in *F. rufa* (Bausenwein, 1960), *Camponotus pennsylvanicus* (Forbes and McFarlane, 1961), *S. invicta* (Phillips and Vinson, 1980) and *Atta sexdens* (Schoeters and Billen, 1991). It has been shown that the content of the postpharyngeal gland closely reflects the cuticular hydrocarbons profile in several ants (Bagnères and Morgan, 1991; Jackson and Morgan, 1993). In *Myrmecia gulosa*, the cuticular hydrocarbon profiles differ between reproductive and non-reproductive females and serve as cues for workers to discriminate between reproductive and non-reproductive females (Dietemann et al., 2003). In queens of *Monomorium pharaonis*, the postpharyngeal gland shows structural differences that can be linked to age and mating status (Eelen et al., 2006). It remains to be investigated whether the hypertrophy of the postpharyngeal glands of queens might be associated with a specific role of this gland in indicating the reproductive and caste status of females in *L. niger* and other ants.

In conclusion, our comparison in the black ant *L. niger* of the cephalic glands between queens, workers and males clearly shows that all these cephalic glands differ in relative size among castes. This morphological difference suggests a link between the gland structure and its behavioral role in queens, workers and males. Further investigations on the chemical content of these glands in each caste will help to characterise these behaviours.

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