Development of the labial gland of the ponerine ant *Pachycondyla obscuricornis* (Hymenoptera, Formicidae) during the pupal stage

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Abstract

The labial gland of adult workers of the ant *Pachycondyla obscuricornis* is made up of many acini, each consisting of one central cell surrounded by approximately 10 parietal cells. Both cell types are associated with a system of ramified canaliculi that remove the secretion towards a ductule outside the acinus. These ductules, each associated with one acinus, fuse together and form a ramified system of ducts, ending in two paired ducts. These paired ducts widen to form a reservoir and anteriorly join into a common unpaired duct, which ends at the base of the labium.

During development in the pupal stage, epithelial acini are formed first, consisting of a monolayered epithelium lining a central lumen. In these acini, one cell grows out to become the central cell, while the others will re-arrange around it to form the parietal cells. At the end of the pupal stage, the canaliculi are formed inside the acini by the central and parietal cells that secrete a lipidic substance and a cuticle.

This gland type, which also occurs in some other Hymenoptera, is structurally different from the epithelial glands and the glands consisting of bicellular units, that have been traditionally distinguished until now.

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Keywords: Morphology; Ultrastructure; Development; Labial gland; Formicidae; Hymenoptera

1. Introduction

Although functionally diverse, insect exocrine glands can be typically classified in two groups based on their structural organization. The first group consists of epithelial gland cells. These are described as type 1 by Noirot and Quennedey (1974). The other group is generally formed by bicellular units, in the Hymenoptera each containing a duct cell and a secretory cell, known as type 3 in the classification of Noirot and Quennedey (1974).

The labial or salivary gland of Hymenoptera contains a common duct that opens near the labium and splits into two paired ducts. Each paired duct continues in a proximal reservoir and a distal secretory part. In most Hymenoptera, the secretory part of the gland consists of one or more epithelial tubules. In some species, however, it shows a very peculiar structural organization. The secretory part of these labial glands consists of a ramified system of ductuli, each ending in an acinus. Each acinus comprises a central cell surrounded by parietal cells. Between both cell types, canaliculi carry the secretion to the ductuli. This structure is found in the social wasps (Cruz-Landim and Saenz, 1972; Landolt and Akre, 1979), in some Anthophorinae bees (Cruz-Landim, 1973; Cavasin-Olivera and Cruz-Landim, 1998) and in some ponerine ants (Gama and Cruz-Landim, 1982; Lommelen et al., 2002). Further on we will refer to this structure as the CP-structure, according to its structural organisation with a central cell (C) surrounded by parietal (P) cells.

Quennedey (2000) mentions the structure as belonging to type 1 gland cells, without providing further details. In the present paper, we study the development of the labial gland in *Pachycondyla obscuricornis*, a ponerine ant showing the CP-structure, during the pupal stage, in order to elucidate the precise structural characteristics of this peculiar gland type.

2. Materials and methods

2.1. Study species

Colonies of the ponerine ant *P. obscuricornis* were

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collected at La Selva (Costa Rica). They were kept in plaster nests in a climatized room at a temperature of 24 °C and 65% relative humidity. Every day, the new cocoons were colour marked with paint markers. Labial glands of workers and pupae of different stages (every 2 days) were dissected. Later on, additional dissections were done at stages where the development was in a crucial stage. In this article the stage is indicated as the proportionate age (PA), which indicates the proportion of the age of the cocoon with regard to the total duration of the cocoon stage. The cocoon stage starts after spinning the cocoon (PA 0%) and ends when the ant ecloses (PA 100%). The different stages are indicated in Table 1. During the first days the larva is still present inside the cocoon. This is called the prepupal stage. At a PA of 20%, the prepupa transforms into a pupa.

2.2. Light and transmission electron microscopy

Dissected labial glands were fixed in 2% cold glutaraldehyde and rinsed in a buffer of 0.05 M sodium cacodylate with 0.15 M saccharose. After postfixation in 2% osmium tetroxide, tissues were dehydrated in a graded acetone series and embedded in araldite. Semithin sections were made with a Reichert OmU2 microtome, coloured with methylene blue-thionine and observed in a Zeiss Axioskop light microscope. Ultrathin sections were made with a Reichert Ultracut E microtome, double stained with lead citrate and uranyl acetate and examined in a Zeiss EM900 transmission electron microscope.

2.3. Scanning electron microscopy

Dissected larval labial glands were fixed in glutaraldehyde, dehydrated in ethanol and critical point dried in a Balzers CPD 030 critical point drying device. They were coated with gold and observation took place in a Philips XL 30 ESEM scanning microscope.

Table 1
Developmental stages of *Pachycondyla obscuricornis*, with proportionate age (PA) and absolute age of the pupa in days

Developmental stage	PA (%)	Age (days)
Larva in cocoon	0	0
Prepupa, beginning of differentiation of appendages		
Prepupa, intermediary phase of development of appendages	14	5
Prepupa, appendages fully developed, beginning of eye pigmentation	20	7
Pupa, white cuticle, pink eyes	28	10
Pupa, white cuticle, pale brown eyes	51	18
Pupa, white cuticle, brown eyes	63	22
Pupa, pale ochre cuticle, dark brown eyes	80	28
Pupa, ochre cuticle, black eyes	91	32
Pupa, yellow-brownish cuticle, black eyes	97	34
Imago, brown cuticle, black eyes	100	35
Imago, black cuticle, black eyes	103	36

2.4. 3D-Reconstruction

To understand the three-dimensional structure of some stages, serial semithin sections were photographed with a digital camera and parts of the glands were reconstructed using the Surfdriver 3.5 programme.

3. Results

3.1. Morphology of the adult labial gland

As described in Lommelen et al. (2002), the labial gland of an adult worker of *P. obscuricornis* starts with an unpaired duct (ud) that splits into two paired ducts (pd) at the posterior part of the head (Fig. 1). These paired ducts widen to form the reservoirs (r) of the gland. Distal to the reservoirs, the ducts ramify into many ductuli. Each ductulus ends in an acinus (a), consisting of a central cell (CC) surrounded by approximately ten parietal cells (CP, Fig. 2). This number was confirmed via three-dimensional reconstructions. Both cell types are associated with canaliculi, that remove the secretion from the cells into the ductulus. In the acinus near the ductulus, the (c) canaliculus splits into two branches. These are lying spirally in the central cell in order to reach all the parietal cells (Figs. 2 and 3F). Near every parietal cell, there is a ramification of the canaliculus (can) that invaginates into the parietal cell. The canaliculi are formed by a fenestrated cuticular canal surrounding a lumen. The canaliculi are surrounded by microvilli, that originate from the surrounding cells.

3.2. Development of the labial gland

Similar to the adult labial gland, the larval labial gland of *P. obscuricornis* contains an unpaired (ud) duct and two paired ducts (pd), the latter are widened to form the reservoir (r). Contrary to the adult gland, the secretory part consists of epithelial tubuli (gt) (Fig. 4).

In the prepupal stage (PA 0–20%), the posterior part of the larval gland degenerates. At the anterior part of the
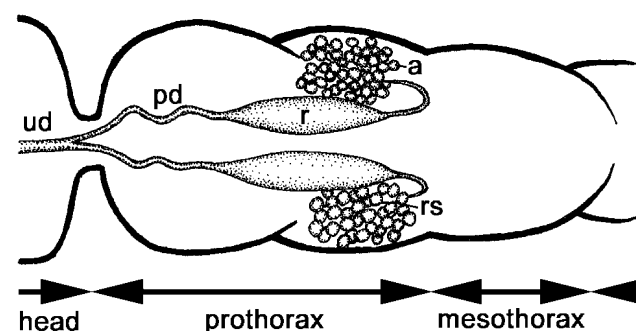

Fig. 1. Schematic top view of the labial gland in *Pachycondyla obscuricornis*. a = acini, pd = paired duct, r = reservoir, rs = ramified system of ducts (in between acini), ud = unpaired duct.
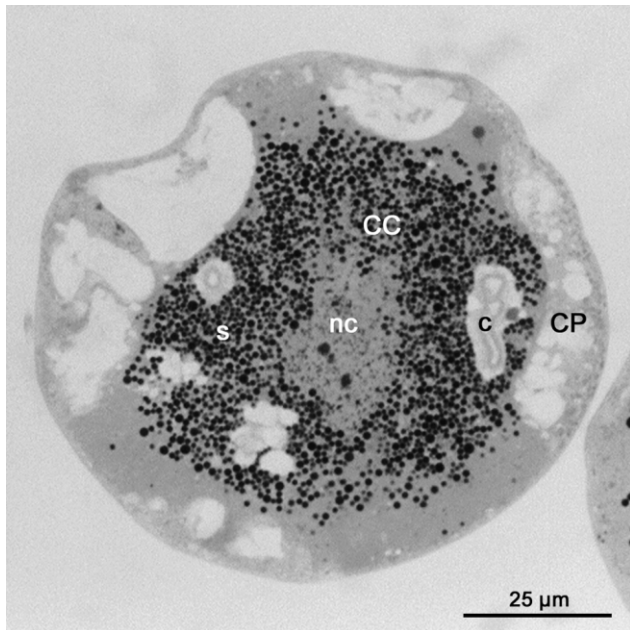
Fig. 2. Semithin section of acinus of adult worker, with central cell filled up with secretion. c = canaliculus, CC = central cell, CP = parietal cell, nc = nucleus of central cell, s = secretory vesicles.

paired duct, a thickened structure develops. Initially, there is no lumen inside this structure (PA 14%, Fig. 5), but it will be formed within two days (PA 20%, Fig. 7). At these stages the labial gland consists of epithelial cells with a large central nucleus and many hyaline droplets (hd) at the basal side (Figs. 5–7). Around these droplets no membrane is visible. In some individuals these droplets colour greenish with methylene blue-thionine. This colouration is similar to the staining results of triglyceride droplets when applying the same staining protocol, indicating the droplets are probably filled with a lipidic substance. Apically, the cytoplasm contains granular endoplasmic reticulum and numerous mitochondria (M, Fig. 6). The apical cell membrane shows conspicuous microvilli in the anterior region of the gland, whereas, in the posterior part only few or no microvilli are visible.

At a PA of 40%, the gland looks a little messy, with different kind of cells (Fig. 8). In the centre there are numerous small cells with relatively large nuclei (SC). These cells did not occur in the previous stage. These are probably the result of a phase of strong cell dividing activity. In the periphery, some cells with a large amount of hyaline droplets (hd) still occur (Fig. 8). In between the central and pheripheral cells, there are some cells that are partly empty (DC, Fig. 9). They contain disorderly fragments of cytoplasmic material and probably are degenerating cells. After this stage the shape of the gland changes into a structure with epithelial acini. At a PA of 50% (Fig. 10), the acini and the lumina inside the acinar ducts already become clear. The gland will transform in such way that the acini become separated at a PA of 63%. Then the structure of an acinus consisting of epithelial gland

cells surrounding a lumen becomes very clear (Fig. 3A). Each acinus is associated with a ductulus (d), which is the smallest unit in the duct system of the labial gland. From reconstruction of serial sections, we know that this ductulus consists of a chain of single cells, each surrounding the lumen completely (Fig. 3A). Because of this invaginated appearance of the lumen, it is the two parts of the same cell that touch each other at one side of the ductulus (Fig. 11). In this contact region, the membranes are connected by septate junctions.

Until this stage, the development of the labial gland of *P. obscuricornis* is similar to that of the formicine ant *Formica pratensis* (Emmert, 1968), the result so far still being an epithelial gland. In contrast to the situation in *Formica*, however, *P. obscuricornis* shows during the second part of the development, that each epithelial acinus develops into a CP-structure. This process comprises a cellular reorganisation, followed by the formation of the canaliculi.

In order to form the central cell, the one cell situated opposite the ductulus will considerably enlarge (PA 75%). While growing, this cell pushes the lumen inside the acinus away until it reaches the ductulus (Fig. 3A–C). In the intermediate stage (Fig. 3B, PA 75%), the proximal part of the gland still has the lumen (la), while at the distal part the lumen already disappeared (Fig. 12). After the complete disappearance of the lumen, the other cells organise around this cell (CC) and thus form the parietal cells (CP, Fig. 3D). At this stage (PA 78%), the number of cells in an acinus is reduced approximately from 20 to 10. Around the acinus, some remnants of cells are visible, which indicates a degeneration of a number of cells.

Just after the CP-structure has originated (PA 78%), the ductulus (d) ends where it touches the central cell (CC, Fig. 3C and D). The canaliculi have not yet formed at this stage. Before the canaliculi are formed, there are many hyaline droplets visible, especially in the parietal cells. These droplets (hd) are filled with a lipidic substance, and are not lined with a membrane (Fig. 13). These droplets (hd) migrate in between the central and parietal cells (Figs. 3E and 14). By accumulation of many droplets in between the membranes, a tubular intercellular space will form (hd2, Fig. 3E). In order to obtain the ramified structure as in the adult gland (Fig. 3F), the intercellular space locally invaginates into the central and parietal cells (hd3, Fig. 3E). This results in the membrane of one cell surrounding the intercellular space completely at these particular spots (hd2, Fig. 15).

Immediately after the intercellular space has formed, a cuticle (ct) will be formed around it by both central and parietal cells (Fig. 16). In these cells granular endoplasmic reticulum (RER) is abundant. Around the canaliculi, some fibrous secretion (s) is visible inside the cells (Fig. 16). During formation of the cuticle, the membranes around the canaliculi become folded, forming microvilli. In this stage (PA 80%), septate junctions are visible between the membranes that envelop the canaliculi.
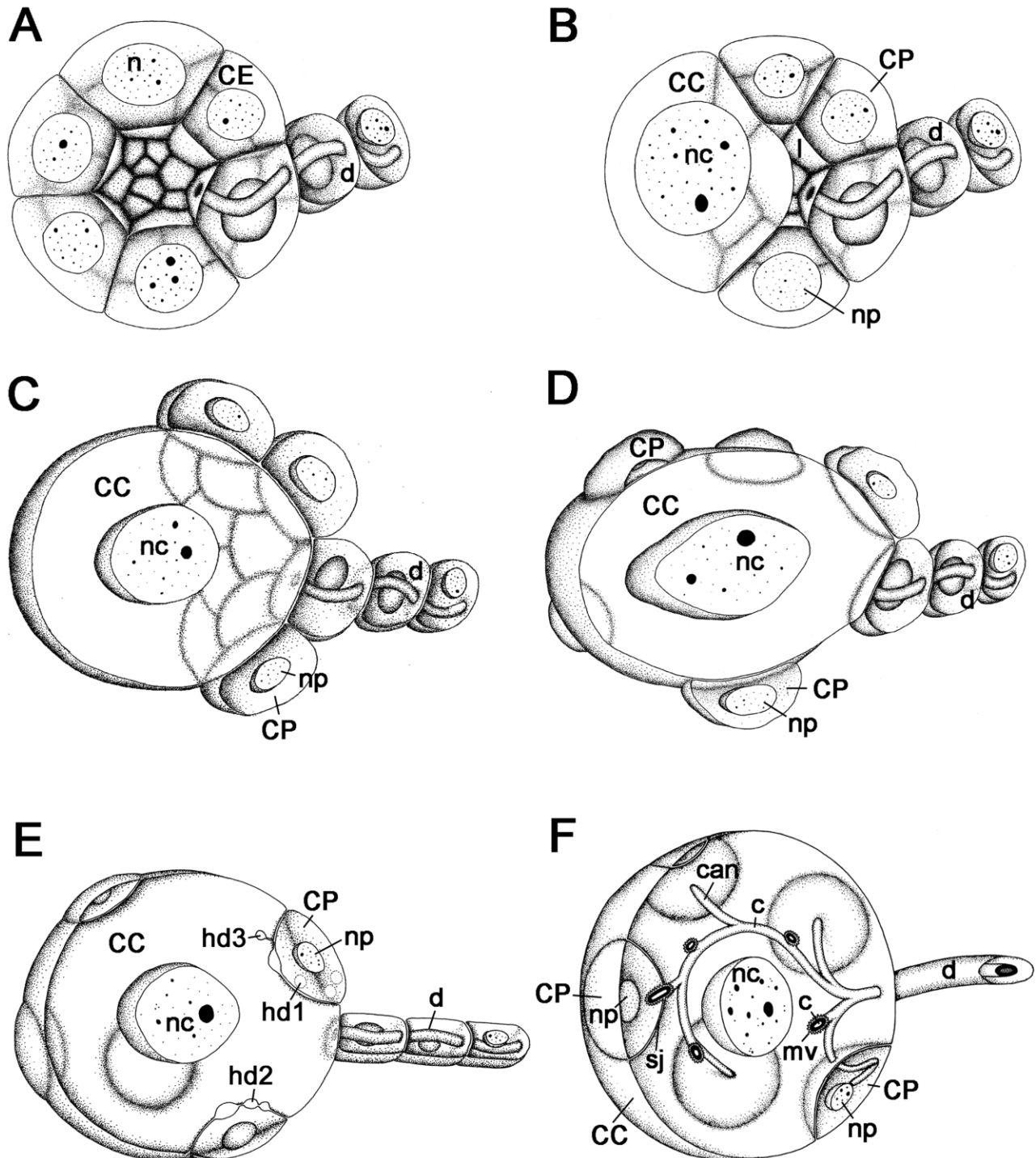
Fig. 3. Schematic representation of development of CP-structure starting from epithelial acinus. A. Epithelial acinus (PA 63%); B. Intermediate form (PA 75%); C. Intermediate form (parietal cells are not yet arranged around central cell); D. CP-structure without canaliculi (PA 78%); E. CP-structure with formation of canaliculi (PA 79%); F. CP-structure with canaliculi (adult). The invaginations of the membranes around the canaliculi are not shown for clarity. c = canaliculus (2 branches), can = ramification of canaliculus, CC = central cell, CE = epithelial cell, CP = parietal cell, d = ductulus, hd1 = hyaline droplet inside parietal cell, hd2 = hyaline droplet in between central and parietal cell, hd3 = hyaline droplet invaginated into central cell, l = lumen inside acinus, mv = microvilli, n = nucleus of epithelial cell, nc = nucleus of central cell, np = nucleus of parietal cell, sj = septate junctions.
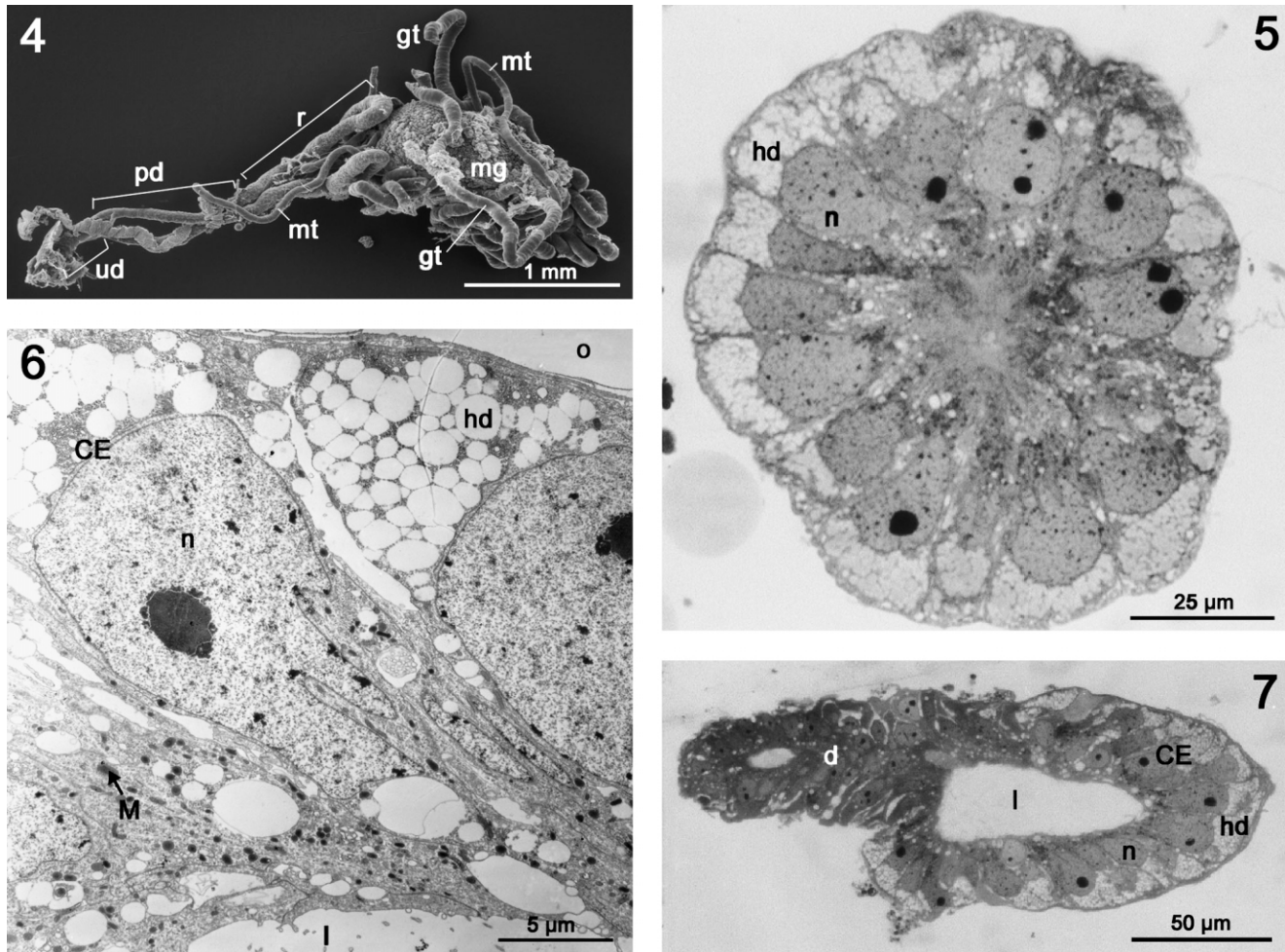
Figs. 4–7. Fig. 4: Scanning micrograph of larval labial gland. Fig. 5: Transversal semithin section of labial gland at PA 14%. Fig. 6: Ultrastructure of part of labial gland at PA 20%. Fig. 7: Longitudinal semithin section of labial gland at PA 20%. CE = epithelial cell, d = duct, gt = gland tubule (thicker tubes), hd = hyaline droplets, l = lumen inside gland, M = mitochondria, mg = midgut, mt = malpighian tubes (thinner tubes), n = nucleus, o = outside, pd = paired duct, r = reservoir, ud = unpaired duct.

At the end of the pupal stage (PA 97%), the parietal cells become partly embedded into the central cell forming a globular acinus (Figs. 3F and 17). At the moment the ant ecloses, secretory vesicles are not yet visible, production of secretion therefore apparently starts in the adult ant stage (Fig. 2).

4. Discussion

Structural studies dealing with the labial (salivary) gland in social insects are fairly common for adult individuals (among others Kürschner, 1971; Gama and Cruz-Landim, 1982; Gama, 1985; Lommelen et al., 2002 for ants; Landolt and Akre, 1979 for wasps; Cavasin-Olivera and Cruz-Landim, 1998; Cruz-Landim, 1968; Cruz-Landim, 1973 for bees; Anstee, 1975; Billen et al., 1989; Bland and House, 1971; Costa-Leonardo, 1997; Kessel and Beams, 1963 for termites), while also a few reports are available on the larval labial gland (Grosch, 1952; Schmidt and Gürsch, 1970;

Petralia et al., 1980; Petralia and Haut, 1986). The development of the gland, however, has only been described for the termite *Prorhinotermes simplex* by Šobotník and Weyda (2003) and for the formicine ant *F. pratensis* in the pioneer work by Emmert (1968). In this species, the adult labial gland belongs to the structurally simple epithelial type, which therefore involves relatively simple developmental changes. In wasps and many ponerine ants, on the other hand, the adult labial gland corresponds with the acinar type with one central cell surrounded by several parietal cells (and hence referred to as the CP-structure). Its development therefore is of a more complex nature, which we studied in the ponerine species *P. obscuricornis*.

The first steps in the development of the acinar CP-structure are very similar to those described by Emmert (1968) for the epithelial gland. In its development, the acinar labial gland also goes through a clear initial epithelial stage, but the second part of the development is characterized by very different processes. A first step in these development towards the adult CP-structure is the cellular
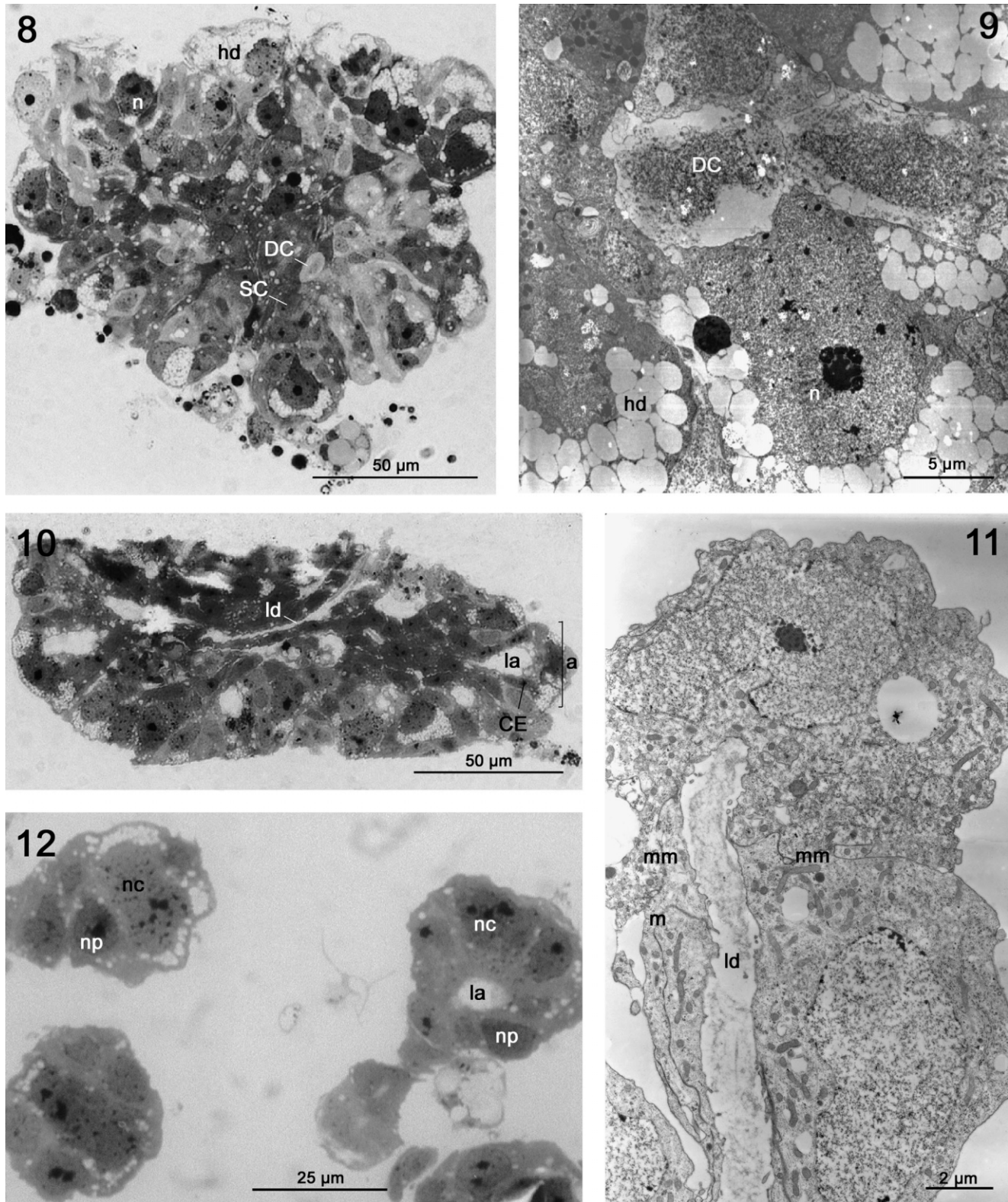
Figs. 8–12. Fig. 8: Semithin section of labial gland at PA 40%. Fig. 9: Ultrastructure of part of labial gland at PA 40%. Fig. 10: Semithin section of labial gland at PA 50%. Fig. 11: Ultrastructure of ductulus at PA 63%. Fig. 12: Semithin section of some acini at PA 75%. Left acinus: section at anterior side of acinus; right acinus: section at posterior side of acinus. a = epithelial acinus, CE = epithelial cell, DC = degenerating cell, SC = small cell with large nucleus, hd = hyaline droplet, la = lumen inside acinus, ld = lumen inside ductulus, m = two parts of membrane of the same cell touch each other, mm = membranes of two different cells touch each other, n = nucleus, nc = nucleus of central cell, np = nucleus of parietal cell.
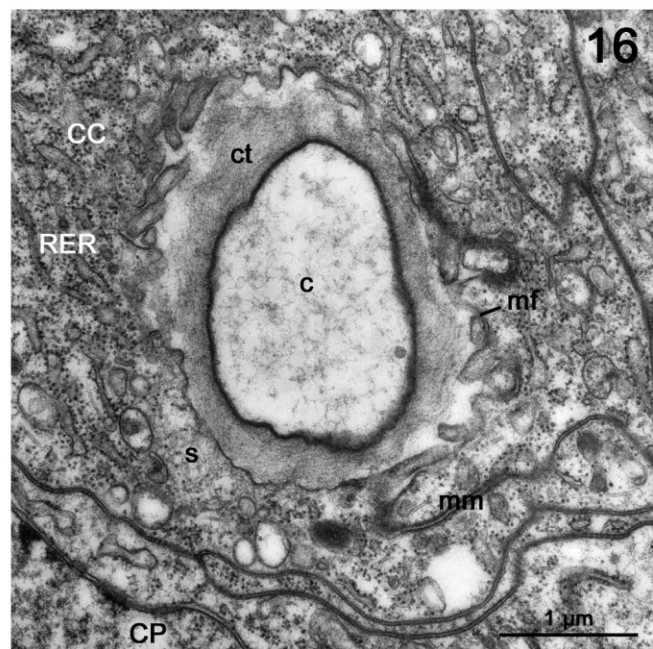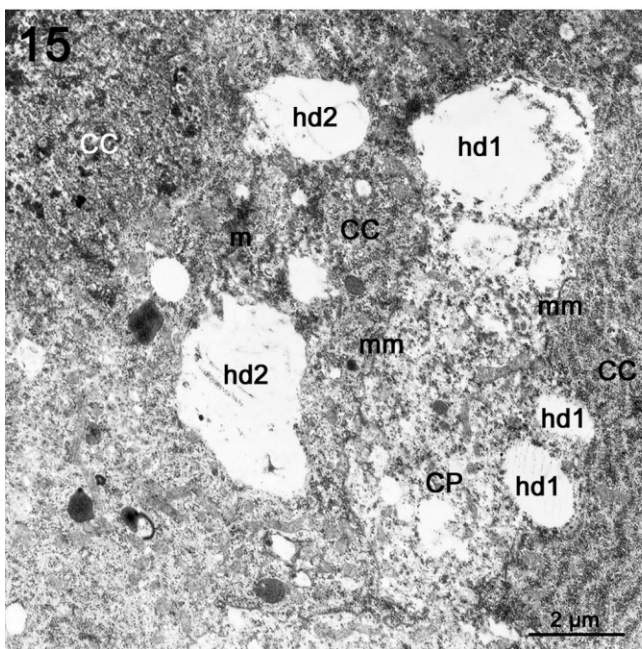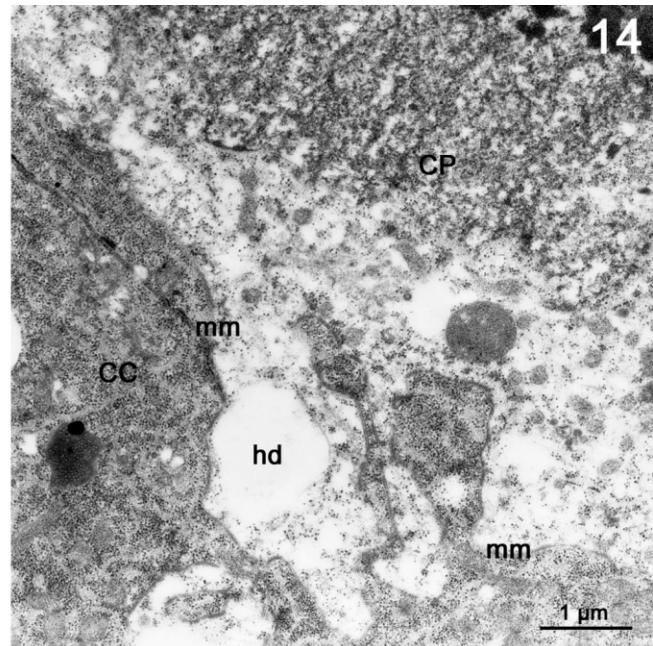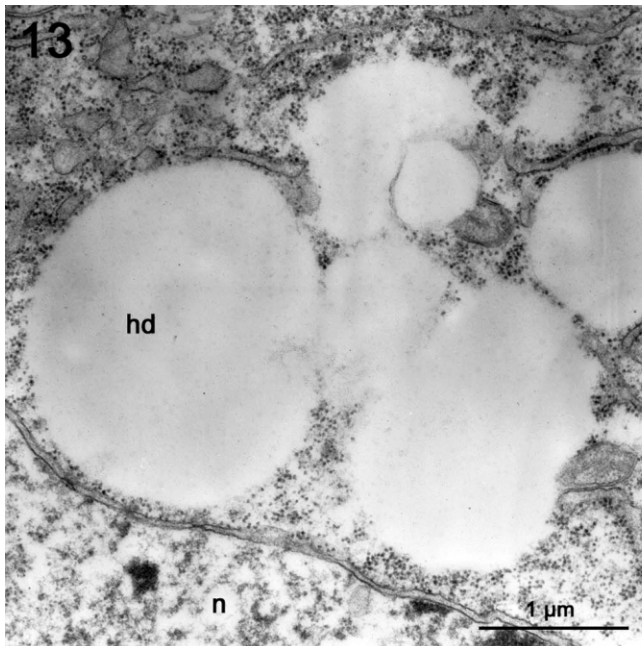
Figs. 13–16. Formation of canaliculi. Fig. 13: Ultrastructure of hyaline droplets (PA 63%). Fig. 14: Ultrastructure of hyaline droplet in parietal cell that migrates towards the membrane in between the central and parietal cell (PA 78%). Fig. 15: Ultrastructure of hyaline droplets in between the central and the parietal cell (hv1) and invaginated into central cell (hv2) (PA 78%). Fig. 16: Ultrastructure of canaliculus during formation of the cuticle (PA 79%). c = canaliculus, CC = central cell, CP = parietal cell, ct = cuticle, hd = hyaline droplet, m = different parts of membrane of central cell, mf = folded membrane, mm = membranes of central and parietal cell, n = nucleus, RER = granular endoplasmic reticulum, s = secretion.

arrangement: in the epithelial acinus the cell opposite to the duct will enlarge, forming the central cell of the CP-structure. The other cells will gather around this cell to form the parietal cells. The cell opposite the ductulus enlarges to become the central cell in *P. obscuricornis*, is in *F. pratensis* also different from the other gland cells. Emmert (1968) describes the different functions of this cell: at the end of the larval stage, this cell produces silk for spinning the cocoon, during the pupal stage it is involved in the building of the

gland tube and in the adult labial gland it is larger than the other cells and produces a secretion different from the other cells. In *P. obscuricornis*, it is the cell in the same position that will develop into the central cell and produce the greater part of the secretion.

The second step of the development, which is the formation of canaliculi, starts with the emission of hyaline droplets. These droplets without membrane are present in most of the earlier pupal stages. The precise composition of
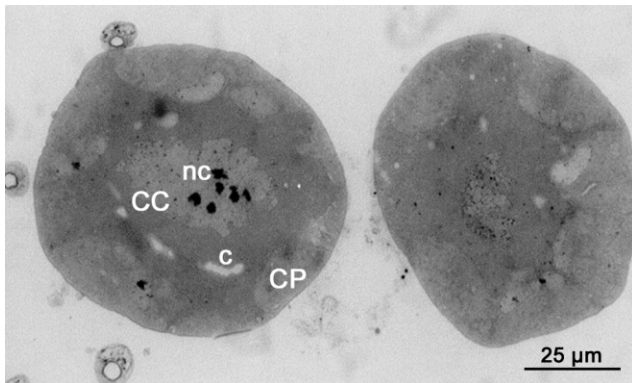
Fig. 17. Semithin section of some acini at PA 97%. c = canaliculus, CC = central cell, CP = parietal cell, nc = nucleus of central cell.

their lipidic substances inside is still unknown, as well as their precise function. Similar lipid-like droplets are described for water sac cells in the labial gland in the termite *P. simplex* (Šobotník and Weyda, 2003). The lipidic substance inside the droplets creates a space in between the membranes. Directly after, this space will be surrounded with a cuticle. This proteinaceous substance is produced by both central and parietal cells, which is indicated by the presence of granular endoplasmic reticulum and proteins in these cells.

The CP-structure of the labial gland, as observed in the social wasps and many ponerine ant species, thus is a complex type of gland in the adults, although its development shows a clearly epithelial origin. In their review work, Noirot and Quennedey (1974) classify the exocrine glands in two main categories, with epithelial class-1 glandular cells and class-3 glandular cells that together with their accompanying duct cells form bicellular units. The adult labial gland CP-structure neither corresponds with the bicellular unit category with class-3 cells, nor reflects its origin from the epithelial class-1 cells.

Structures that are similar in the epithelium and its derived CP-structure also occur in the bicellular unit (Billen, 1991). These are linked with the general morphology of glandular cells, such as the surface increasing apical microvilli and basal invaginations. Microvilli occur when the cell produces secretion and the abundance of basal invaginations is dependent on the amount of exchanges between the cell and the hemolymph (Noirot and Quennedey, 1974). Another structure that occurs in all three gland types are septate junctions, that assure a strong intercellular contact.

Since the CP-structure is described several times in different social insect species (Landolt and Akre, 1979; Cavasin-Olivera and Cruz-Landim, 1998; Lommelen et al., 2002), the morphology of the structure is well known. However, the consequences and potential advantages of this peculiar structure for the production of secretion is still unknown and require further research.

Acknowledgements

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