Acta Zoologica (Stockholm) **87**: 101–111 (April 2006)

Functional morphology of the postpharyngeal gland of queens and workers of the ant *Monomorium pharaonis* (L.)

Dieter Eelen,[1] Lisbeth Børgesen[2] and Johan Billen[1]

[1]Laboratory of Entomology, K.U. Leuven, Naamsestraat 59, B-3000 Leuven, Belgium; [2]Institute of Zoology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark

Keywords:
Monomorium pharaonis, exocrine glands, postpharyngeal gland, histology, ultrastructure

Accepted for publication:
17 October 2005

Abstract

Eelen D., Børgesen L.W. and Billen J. 2006. Functional morphology of the postpharyngeal gland of queens and workers of the ant *Monomorium pharaonis* (L.). — *Acta Zoologica* (Stockholm) **87**: 101–111

The postpharyngeal gland (PPG) is unique to ants and is the largest exocrine gland in their head. In queens of the pharaoh's ant, *Monomorium pharaonis*, the gland contains approximately 15 finger-like epithelial extensions on each side and opens dorsolaterally in the posterior pharynx. In these ants the PPG morphology varies considerably according to age and mating status. The epithelial thickness increases with age and reaches a maximum at 3 weeks in both virgin and mated queens. A considerable expansion of the lumen diameter occurs in both groups between 4 and 7 days. Virgin queens release their secretion into the gland lumen from an age of 7 days, whereas mated queens accumulate large amounts of secretion in their epithelium. The increasing epithelial thickness, together with the increasing lumen diameter, the presence of numerous inclusions in the epithelium and the release of secretion, are indicative for increasing gland activity. The gland ultrastructure indicates involvement in lipid metabolism and *de novo* synthesis of lipids. The PPG of workers consists of 12 finger-like tubes at each side. There is a significant difference in epithelial thickness between nurses and repletes and between nurses and foragers. We suggest the PPG serves different purposes in pharaoh's ants: it is likely that the PPG of workers and virgin queens is used to feed larvae. In mated queens the gland probably plays a role in providing the queen with nutritious oils for egg production. The PPG may also function in signalling species nestmate and caste identity, as well as in the reproductive capacity of the queens.

Johan Billen, Laboratory of Entomology, K.U. Leuven, Naamsestraat 59, B-3000 Leuven, Belgium. E-mail: johan.billen@bio.kuleuven.be

Introduction

The postpharyngeal gland (PPG) is the largest gland in the head of ants and is associated with the pharynx. It is unique to the Formicidae and can therefore be considered as a neoformation of this family (Billen 1990). The postembryonic morphological development and anatomy of the PPG in ants was described by Emmert (1968) and by Peregrine *et al.* (1973). There are only a few descriptions of the ultrastructure of this gland (see Soroker *et al.* 1995; Schoeters and Billen 1996; Kaib *et al.* 2000), whereas the functions of the gland have been the target of numerous investigations they are still disputed and a spectrum of suggested functions has been put forward.

The gland occurs as a paired tubular structure consisting of a proximal sac-like part from which several finger-like tubes extend. Histologically, the PPG belongs to the epithelial type, which Noirot and Quennedey (1974) described as type-1 glands. The wall is made by monolayered epithelial cells. The basal side of this epithelium, next to the haemolymph, is lined by a basal lamina. The apical side is bordered by a well-developed layer of microvilli and is supported by a 1-µm thick cuticular intima surrounding the central lumen (Zylberberg *et al*. 1974).

All finger-like extensions open into a central sac-like structure on each side, which opens dorsolaterally into the posterior part of the pharynx (Pavan and Ronchetti 1955). Just behind this point, the pharynx continues into the oesophagus. The connection between the gland and the pharynx is associated with different muscle groups, which are probably responsible for the release or uptake of the contents (Peregrine *et al.* 1973; Phillips and Vinson 1980). These fingers are widely extended inside the head of ants and cover most of the brain.

Comparisons of this gland between subfamilies reveal a gradation of the number of extensions (Gama and Cruz Landim 1982). The PPG of the Ponerinae and Pseudomyrmecinae is characterized by only a few lobes, while the gland of the Dorylinae, Formicinae and Myrmicinae usually shows 20–30 finger-like protrusions (Gama and Cruz Landim 1982; Lukas *et al*. 2004). An exception to this is the fire ant (*Solenopsis*), where the PPG consists of about 85 flattened lobes (Phillips and Vinson 1980). Other shapes have also been reported, such as the peculiar appearance with basal protrusions of the epithelium described in the ponerine species *Dinoponera australis* and *D. quadriceps* (Schoeters and Billen 1996).

The gland is present in workers and queens as well as in males. In *Solenopsis invicta* there is a significant difference between the three castes. The gland is bigger in queens than in workers and males and has fewer lobes in workers and males (Phillips and Vinson 1980).

In *M. pharaonis*, the completely sterile workers can be divided into three temporary castes, based on their task in the colony. The nurses take care of the brood and stay in the nest; the repletes store liquid food in their crops, mainly from the larvae and stay in the nest (Børgesen 2000), and the foragers mainly go outside the nest looking for food sources.

Observations of the PPG in pharaoh's ant queens have shown conspicuous morphological and histological changes during their lifetime. The objectives of this investigation are to study the postimaginal development of the PPG and to contribute to the discussion of its functions by comparing histological sections of queens with respect to age and mating status and of the three different worker castes.

Materials and Methods

The *M. pharaonis* queens and workers studied in this project were obtained from stock colonies of the University of Copenhagen (Denmark).

The present study deals with 11 age stages of queens (0, 4, 7, 10 days and 2, 3, 6, 10, 16, 20 and 40 weeks) and two different mating conditions, virgin (Qv) and mated (Qm). To produce cohorts of queens of known ages, approximately 20 gyne pupae with the same degree of pigmentation were taken from the stock colony and isolated in small colonies together with 30–40 workers, and with the same number of worker larvae. In colonies with virgins, no male pupae were added, and the colonies were regularly checked for developing male pupae, which were removed; in colonies which were to contain mated queens, on the other hand, a high number of male pupae was present. As one male is able to transfer sperm to two to four of the monoandrous queens, all queens were assumed to be mated after a few days, as they are capable of mating at an age of 2–3 days (Petersen-Braun 1973). Some of the queens were controlled for mating status by dissection of the abdominal reproductive organs, and all of these queens were found to be consistent with the expected condition. When reaching the right age for our project, the queens prior to dissection were gently removed from their colony and killed by being placed on a frozen device, designed for keeping lunch boxes chilled.

Based on the task of the workers in the colony, their position (inside or outside the nest) and the morphometrical measurements of body length, abdomen length and abdomen width (Børgesen *et al.* in preparation), they were divided into three groups: nurses, repletes and foragers. Samples of each caste were killed in the same way as the queens, and like these, the anterior part of the head was fixed in 2% glutaraldehyde (4 °C, pH 7.3 and buffered with 0.05 M sodium cacodylate). Dehydration in a graded acetone series and embedding in Araldite followed postfixation in a buffered osmium tetroxide solution. Semi-thin sections (1 µm) for light microscopy were made with a Reichert OmU2 ultramicrotome and stained with methylene blue and thionin. The sections were observed with a Zeiss Axioskop and an Olympus BX-51 light microscope. Thin sections (70 nm), made with a Reichert Ultracut E, were stained with uranyl acetate and lead citrate in an LKB 2168 Ultrostainer and observed with a Zeiss EM900 transmission electron microscope. Glands for scanning electron microscopy (SEM) were first dehydrated in formaldehyde dimethyl acetate and then critical-point-dried in a Balzers CPD 030 critical-point-drying device. They were coated with gold and observed in a Philips XL 30 ESEM scanning microscope.

Measurements of the PPG were performed with the Olympus DP-Soft 3.2 software on digital photographs of histological sections, which were taken at a magnification of 400×. To work randomly, a grid is placed on the picture, so that the cross-points of these gridlines and the basal side of the epithelial cells can be used as measuring points. We measured the epithelial thickness by drawing a perpendicular line from the measuring point to the apical side of the cell. We only measured on straight parts of the epithelium. All data are presented as arithmetic means ± SD.

Results

Up to the age of 6 days, queens are always virgin in this investigation. For all the higher ages, a distinction has to be made between the virgin and mated status.

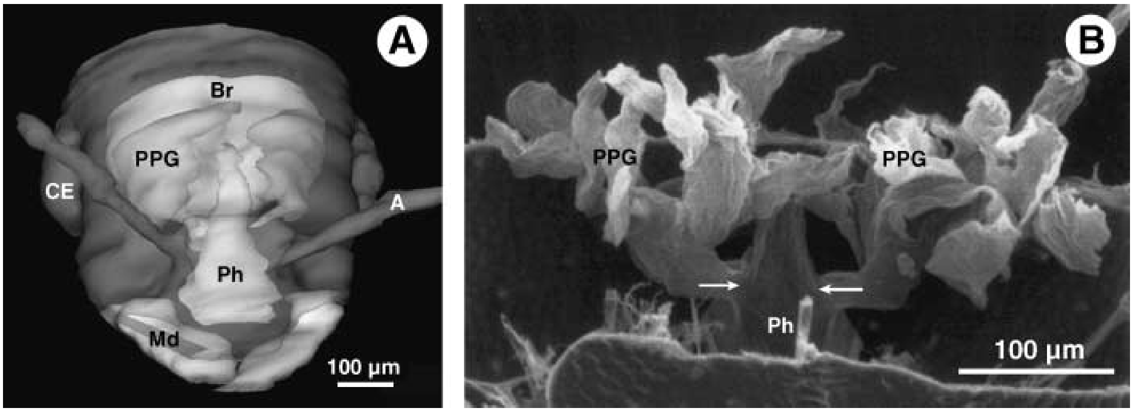

Fig. 1—**A**. Three-dimensional reconstruction of the head of a newly eclosed *Monomorium pharaonis* virgin queen showing the position of the PPG covering the brain and opening in the posterior pharynx; the tubes stick together; —**B**. Scanning electron micrograph of the PPG in a 4-day-old *M. pharaonis* queen (arrows indicate opening of PPG in pharynx); A, antenna; Br, brain; CE, compound eye; Md, mandible; Ph, pharynx; PPG, postpharyngeal gland.

Virgin queens

The PPG of a *M. pharaonis* queen is a glove-like structure (with approximately 15 finger-like tubes) which opens into the dorsolateral part of the posterior pharynx (Fig. 1A, B).

Eclosing queens The PPG is small, flaccid and colourless. At this stage the gland consists of flattened lobes (Fig. 2A), covering only part of the brain (Fig. 1). The epithelial thickness is 3.9 ± 0.51 µm (Fig. 3A). The epithelial walls more or less stick to each other, which makes the lumen of the gland hardly visible at this stage (Fig. 2B). In general the nucleus is situated in the centre of the cell and its approximate diameter varies between 5 and 6 µm during the lifetime of the queen. At this stage the nucleus has one big central nucleolus. The basal side of the epithelium shows invaginations and small vacuoles indicating transport in or out of the haemolymph. At this stage only a few oil droplets are present in some of the cells.

Four days The epithelium of these virgin queens has an irregular form, because some of the cells have started to develop while others next to them have not. The mean thickness is 4.7 ± 0.80 µm which is approximately 1 µm thicker in comparison to the eclosing queens (Fig. 3A). The lumen diameter remains inconspicuous as in the previous stage (Fig. 3B). The nucleus has very much the same rounded appearance as in eclosing queens (Fig. 2C). Basally we still find invaginations and small vacuoles. Numerous elongated mitochondria are mainly concentrated around the nucleus (Fig. 2C). Smooth endoplasmic reticulum (SER) is mainly concentrated at the apical side near the cuticle.

Seven days At this stage the epithelium is still irregular but it is much more even than in the 4-day-old queens. The mean thickness is 5.9 ± 0.62 µm, which is another increase with approximately 1 µm (Fig. 3A). The most remarkable change is the seven-fold increase of the lumen diameter between the age of 4 days (3.1 ± 1.35 µm) and 7 days (21.3 ± 3.19 µm) (Fig. 3B). Whereas the PPG consists of flattened lobes in the eclosing and in the 4-day-old queens, the PPG of the 7-day-old virgin queens is gradually taking the typical glove-like shape as described in most other ant species. Approximately 40% of the tubes at this age are filled with a greenish-grey secretion on methylene blue-stained sections. In dissected specimens the contents of the PPG resemble a clear yellowish oil.

Ten days The mean thickness of the epithelium is 6.0 ± 1.09 µm, which is almost the same as in the previous age stage (Fig. 3A). The epithelium is more regular than in the 7-day-old queens and at least 60% of the tubes are filled with secretion. The lumen diameter increases by approximately 5 µm and reaches a mean thickness of 26.1 ± 4.96 µm (Fig. 3B).

Two, 3 and 10 weeks The epithelial thickness increases and reaches approximately 6.7 ± 0.51 µm at an age of 2 weeks, 8.1 ± 1.18 µm at an age of 3 weeks and reaches a maximum of 8.3 ± 1.07 µm in the 10-week-old virgin queens (Fig. 3A). From this age, we find some greenish-grey spherical inclusions with a diameter of approx. 3 µm in the gland cells. Also the lumen diameter increases and reaches values of 29.6 ± 3.17 µm at an age of 2 weeks and 32.1 ± 3.42 µm at an age of 3 weeks, which each time represents an increase of 3 µm (Fig. 3B). Between the ages of 3 and 10 weeks there is a small decrease in the lumen diameter of about 1 µm. As in the sections taken from 10-day-old queens, some tubes of the 2- and 3-week-old virgin queens are filled with the greenish-grey secretion (Fig. 2D), but almost all of them were filled in the 10-week-old queens. This secretion stains positive (black) on semi-thin araldite sections which are treated with the histochemical Farrants' Gum method for lipid staining (Fig. 2E). Light-microscopical pictures show thread-like

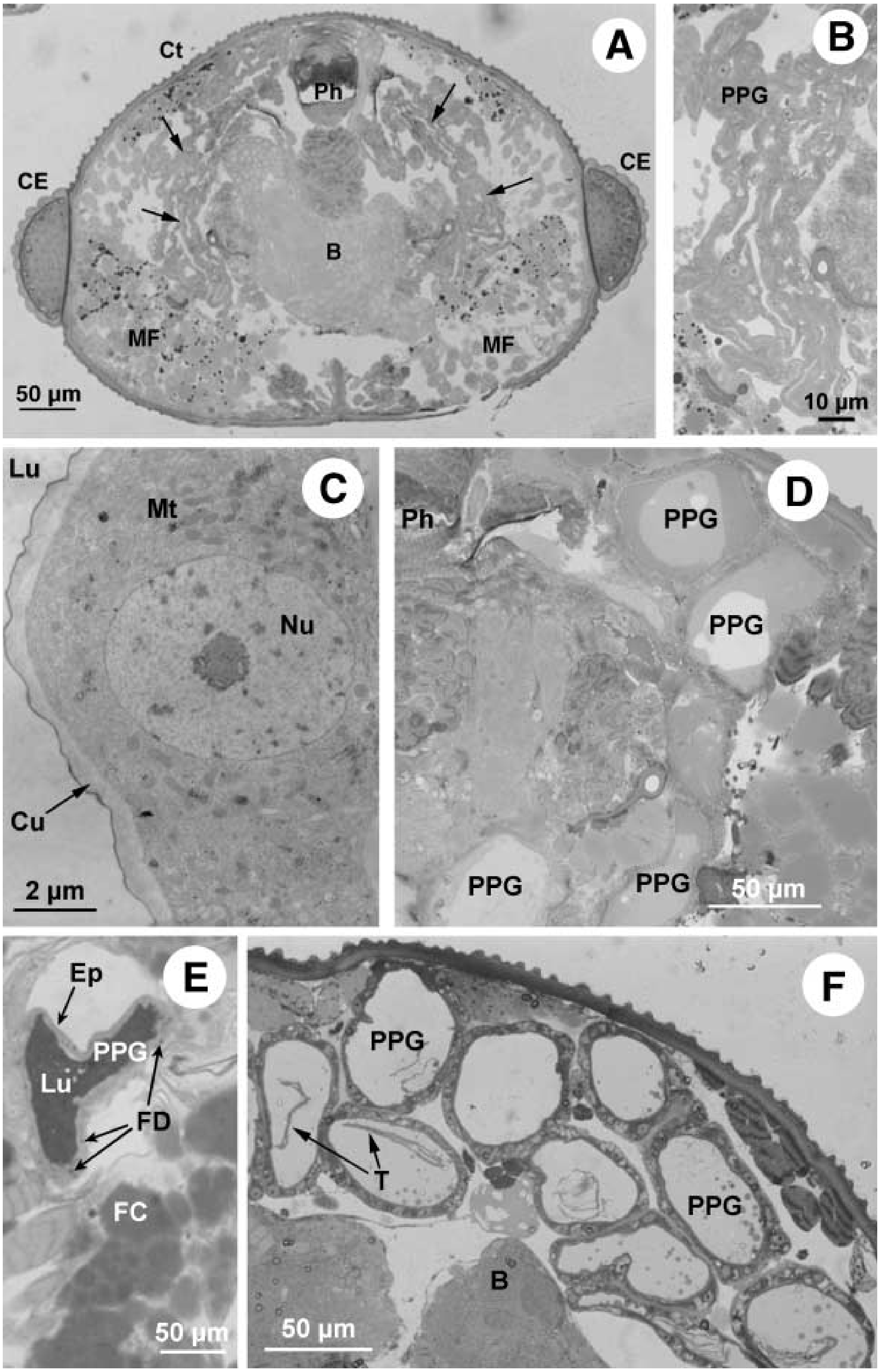

Fig. 2—**A**. Light micrograph (LM) of eclosing queen (arrows indicate postpharyngeal gland); —**B**. LM detail of PPG of eclosing queen; **C**. Electron micrograph (EM) detail of epithelium of 4-day-old queen showing nucleus and mitochondria; —**D**. LM 2-week-old virgin queen showing typical finger-like tubes filled with secretion; —**E**. LM with histochemical staining (Farrants' Gum with 2% ethyl gallate) for lipids on a 3-week-old virgin queen; —**F**. LM of 10-week-old virgin queen with thread-like structures in lumen; B, brain; CE, compound eye; Cu, cuticle; Ep, epithelium; FC, fat cells; FD, fat droplets; Lu, lumen; MF, muscle fibres; Nu, nucleus; Ph, pharynx; PPG, postpharyngeal gland.

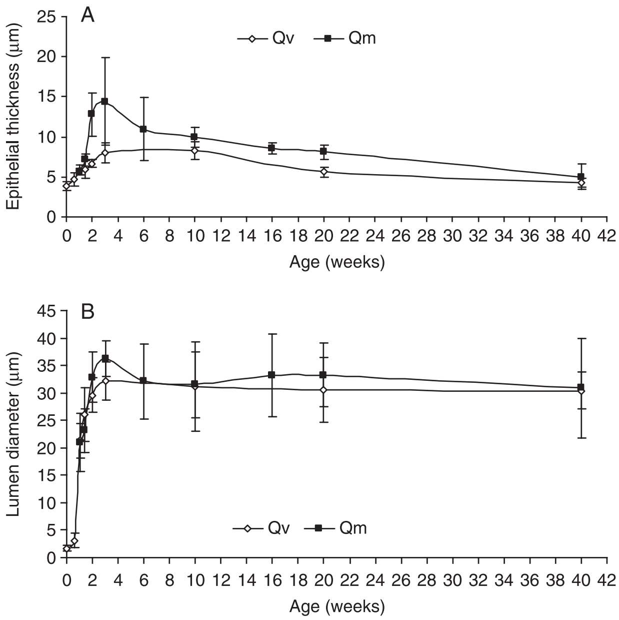

Fig. 3—**A**. Epithelial thickness and —**B**. lumen diameter of the PPG of queens with respect to age and mating status. Queens of 0 and 4 days have not yet mated. A lack of sexual pupae resulted in the absence of virgin individuals of 6 and 16 weeks.

structures in the tubes of the PPG at an age of 10 weeks (Fig. 2F). The number of invaginations at the basal part tends to increase until 10 weeks. The spherical and centred nucleolus observed in young queens is still present but becomes smaller in the older ants with an appearance of more and smaller nucleoli throughout the nucleus indicating increased activity (Fig. 4A). The mitochondria are more evenly dispersed. From an age of 2 weeks we observe that glycogen is present throughout the cytoplasm of the epithelium (Fig. 4A).

From the age of 2 weeks the PPG is formed by the characteristic finger-like tubes, which now also extend into the anterior part of the head.

Twenty and 40 weeks In the older virgin queens the epithelial thickness has decreased to 5.6 ± 0.61 µm and 4.3 ± 0.51 µm, respectively, in the 20-week and 40-week-old queens (Fig. 3A). The lumen diameter decreases with another 1 µm compared with the 10-week-old queens, but stays about the same for the 20-week-old (30.6 ± 5.93 µm) and 40-week-old (30,4 ± 3.43 µm) queens (Fig. 3B).

Mated queens

Seven days The mated queens generally have a thicker epithelium than the unmated ones (Fig. 3A). As in the virgin queens, there is a conspicuous expansion of the lumen diameter between the ages of 4 and 7 days (Fig. 3B). Almost none of the tubes contain secretion.

Three weeks Approximately half of the tubes of the mated queens are filled with the greenish-grey secretion, similar to the virgin queens. The other tubes are empty and look the same as in the previous age stages. The mean epithelial thickness in the mated queens has its maximum value (14.4 ± 5.47 µm) at an age of 3 weeks (Fig. 3A), because of the accumulation of lipids in the epithelium (Fig. 4B, C).

Older than 3 weeks Between 3 and 6 weeks the epithelial thickness decreases considerably, to approx. 11.0 ± 3.97 µm, and after that to approximately 8 µm in the 20-week-old queens (Fig. 3A). We still find the greenish-grey substance in the epithelium and the lumen, even in the 40-week-old queens (Fig. 4D), where the epithelial thickness decreases to approximately 5 µm (Fig. 3A).

In general the shape of the nucleus in the epithelial cells of mated queens seems to be more elongated than described for the virgins, but the pattern of the nucleoli is consistent with our observations of the unmated queens older than 4 days. The number of invaginations is comparable with that of the virgins. There is a tendency for the mitochondria to have moved from the area of the nucleus to the apical part of the cell (Fig. 4E) where the majority of the SER is situated. In our material there seem to be more mitochondria in the mated queens than in the old virgins. The oil droplets in the epithelium are larger (8 µm) and much more abundant than in virgin queens (Fig. 4B, C). Also, the accumulation of oil in the epithelium develops more quickly in young mated queens than in virgins of comparable age. Ultrastructural analysis shows the fusion of many small lipid vesicles into much bigger drops (Fig. 4C). The presence of these oily inclusions in the epithelium was confirmed by a positive staining for lipids using Farrants' Gum on araldite sections.

Workers

Instead of the approximately 15 finger-like tubes in queens, the PPG of the workers consists of 12 tubes at each side of the pharynx. For each caste of the workers, nurses, repletes and foragers, the epithelial thickness of the PPG of eight individuals was measured. A one way analysis of variance (ANOVA) test on the mean values of the eight individuals of the three different groups showed that there is a difference between the nurses, repletes and foragers, with a mean value of 6.3 ± 0.84 µm, 7.5 ± 0.68 µm and 8.3 ± 0.44 µm, respectively. A Tukey-test on these data showed a significant difference between the nurses and the repletes ($P = 0.0053$), and between the nurses and the foragers ($P = 0.00015$). The differences between epithelial thickness of the PPG of repletes and of foragers were too small to be statistically significant ($P = 0.063$). Histological sections showed that there were no big differences for the lumen diameter between the three categories. In 20% of the investigated foragers, we found the greenish-grey oil in the lumen. In repletes and nurses (Fig. 4F) this proportion was 60% and 80%, respectively.

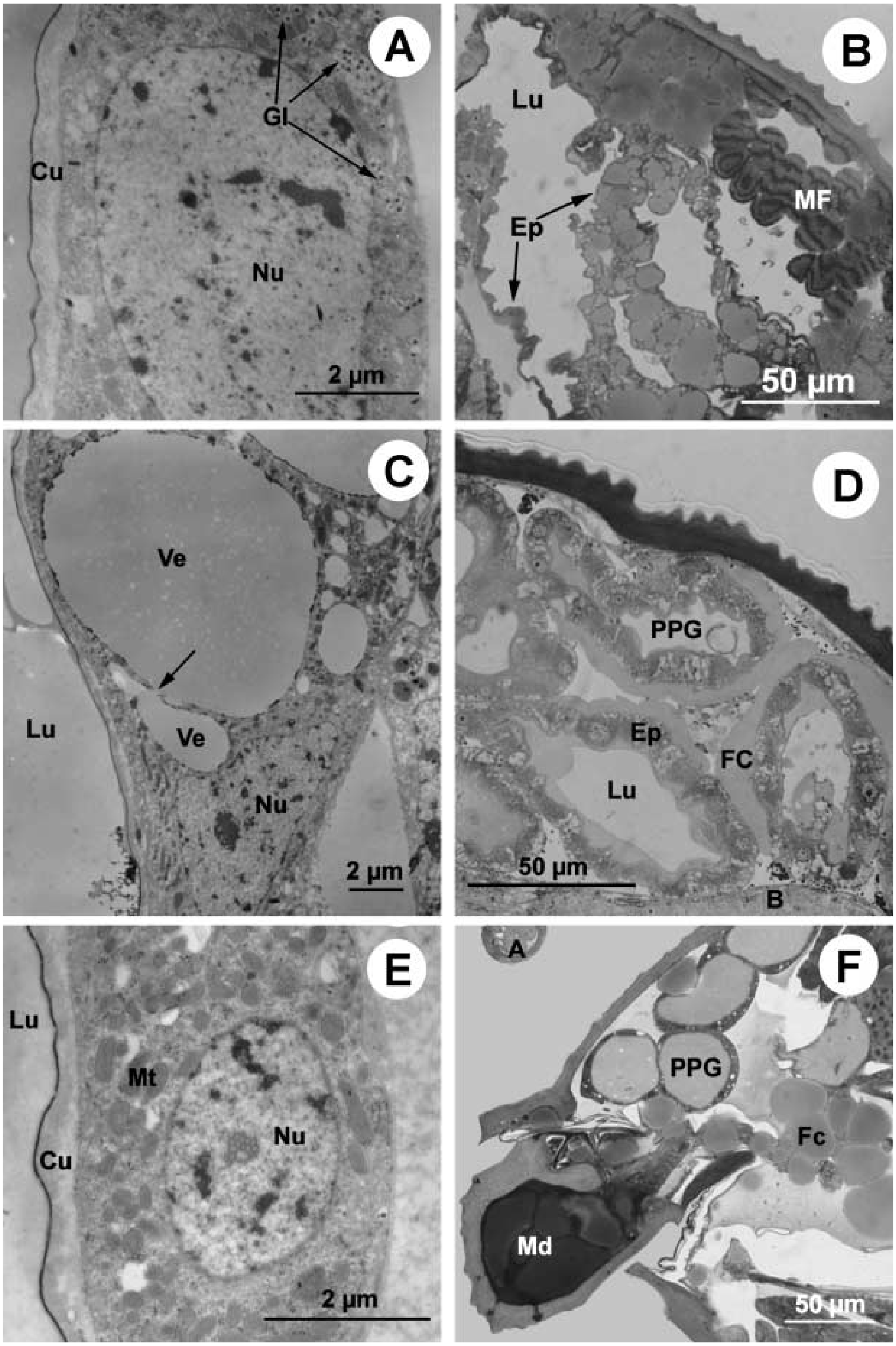

Fig. 4—**A**. EM detail of epithelium of 2-week-old virgin queen showing nucleus and glycogen; —**B**. LM of accumulation of vesicles in 2-week-old mated queen; —**C**. EM detail of lipid drops in epithelium, arrow indicates fusion of vesicles; —**D**. LM of 40-week-old mated queen still showing vesicles in their epithelium; —**E**. EM of 20-week-old mated queen showing mitochondria concentrated at apical part of cell; —**F**. LM longitudinal section of nurse worker, showing the PPG filled with secretion; A, antenna; B, brain; Cu, cuticle; Ep, epithelium; FC, fat cells; Gl, glycogen; Lu, lumen; Md, mandible; MF, muscle fibres; Mt, mitochondria; Nu, nucleus; PPG, postpharyngeal gland; Ve, vesicles.

Discussion

Pharaoh's ant queens receive their food directly from large larvae or indirectly from replete workers, which collect the surplus of liquid produced by the larvae (Børgesen 1989, 2000). Besides the mating process, which usually takes place within 4 days after eclosure, the quality and quantity of food are important for the fecundity of the queen. Virgin queens cease to produce (haploid) eggs when they reach an age of about 6–8 weeks. At this age these virgins have been observed having difficulties achieving labial secretions from larvae (Børgesen, unpublished results). In contrast, mated queens can produce more or less distinct batches of eggs until they reach an age of about 1 year if kept under good conditions and with access to larvae developed from the previous batch of eggs. Under these conditions the queens remain highly fertile from an age of 10 weeks to 40 weeks. The development of the thick epithelium of the PPG containing large stores of lipids, which reaches its peak development a few weeks after eclosure, is probably part of the cascade of processes which changes the young mated queen into an egg-laying machine. The following decrease in thickness from a maximum of 14 µm to about 10 µm at 10 weeks, gradually decreasing to 8 µm and the stabilization of the lumen diameter at a high level is congruent with the period of high fecundity in mated queens. The number and distribution of mitochondria near the lumen of the PPG, together with the thickness of the epithelium and the diameter size, which are all higher in egg-laying, mated queens compared to virgins, are all signs of a high metabolic rate in the PPG.

Workers of *M. pharaonis* display temporal castes, where the youngest workers stay inside the nest and nurse the brood, while the oldest tend to become foragers. The repletes are a temporal caste based on their function inside the nest. This is supported by morphometric data on body length, abdomen length and abdomen width (Børgesen *et al.* in preparation). The thickness of the PPG increases with age. If the youngest workers, which are considered to be the nurses, are producing glandular secretions in the PPG to feed to the larvae, one would expect the epithelium in this caste to be very productive and thus enlarged, as compared to replete workers and foragers. This is not the case in our samples. To explain this we may reject the hypothesis of the PPG being the source of the yellowish larval food in pharaoh's ants. There is good evidence, however, that they really are the main source for nourishment for larvae in several other ant species, so it is not possible to rule this theory out completely. Another possibility is that its physiological activity is not necessarily correlated with the PPG epithelium thickness. This possibility is suggested by our data in the mated queens, where the highly fecund queens have an epithelium of reduced thickness as compared to the young 3-week-old queens.

In the following discussion we incorporate this knowledge of the biology of the pharaoh's ant with morphological data into the results of suggested functions of the PPG in other ants. In relation to these functions, we discuss the origin of the PPG contents.

Previous investigations have suggested a spectrum of functions for this unique gland, such as involvement in digestion or caste determination, provisioning of food to queens and larvae and being a source of recognition pheromones.

Digestive gland

Forbes and McFarlane (1961) proposed, based on studies on *Camponotus*, that the contents of the PPG are used by the individual ant for digestion. Ayre (1963) later came to the conclusion that this was not likely because of his findings of only trace amounts of amylase and lipase in the PPG. In the pharaoh's ant we find it unlikely that the PPG serves as a digestive gland, because the presence of SER in the cells strongly indicates a production of lipid substances rather than digestive enzymes, for which we would expect a very well-developed granular, or rough, endoplasmic reticulum (RER) (Billen 1991). The fact that there is a difference in epithelial thickness with respect to age and mating status does not support the hypothesis of the production of digestive enzymes.

Caste determination

Some early work by Brian (1968) and Brian and Blum (1969) indicated that primer pheromones originating from the queens of *Myrmica* accumulate in the lumen of the PPG of workers as a result of worker feeding activity. Based on experiments where dispersal of fatty acids from extracts of queen heads on the bigger larvae seemed to have a negative effect on their growth and reduced the number of developing queens, they suggested that the PPG is involved in caste determination. In later experiments, Brian (1973) and Vinson *et al.* (1980) showed that there is no support in these experiments for the theory that the PPG is directly involved in caste determination. In the pharaoh's ant there is no indication that PPG contents have an effect on caste determination. In this species caste determination is regulated by the specific behaviour of workers towards the brood based on whether the nurses as callows have undergone a process of imprinting on queen-specific cues or not (Børgesen, in preparation). According to the experiments previously mentioned, mature mated queens of *M. pharaonis* would suppress the development of new sexuals by releasing their PPG contents to the larvae. On the contrary, our histological sections, both light- and electron-microscopical, show that the mated queens of the pharaoh's ant accumulate lipids in their PPG epithelium instead of releasing them into the lumen of the gland. Egg-laying virgin queens would benefit from the development of larvae into new sexuals and especially into males to mate with; so they are not expected to release their PPG contents on the larvae according to the caste determination hypothesis. In contrast to the mated queens of similar age, the lumen of the PPG of most of these virgins is filled with secretion ready to secrete, which indicates different functions of the PPG in the two categories of queens. Our results in the pharaoh's ants do not support the hypothesis suggested by Brian and Blum (1969).

Provision of food to queens

Observations by Vinson *et al.* (1980) of depletion of the fat body in *Solenopsis* queens that have had their PPG removed, indicate that the gland is involved in providing nutrition to the egg-laying queens – especially lipids. Queens need plenty of lipids and other nourishment to produce eggs. The authors experimentally showed that only the lipids in the PPG of mated queens are absorbed into the haemocoele, whereas neither lipids in the thoracic and gastric crop nor those in the oesophagus are absorbed. For further information about the origin of the PPG contents, please see below. Also in pharaoh's ants, the mated queens need a large quantity of nutrition to produce eggs. As in the fire ants, there is a positive correlation between the number of larvae available for the queen and the fecundity in pharaoh's ants (Børgesen 1989; Tschinkel 1995). In the present investigation, we observed that the lumen of almost all of the finger-like tubes in virgins are filled with lipids (histochemical staining). Mated queens on the contrary accumulate lipid drops in the epithelium of the PPG which could give an indication of involvement in egg production by transporting lipids into the haemolymph, as is the case in fire ant queens. The pharaoh's ant workers do not lay eggs because they are completely sterile, therefore they do not need the abundance of lipids. This finding is in agreement with the observations of histological sections on the three castes of workers. They do not show accumulation of lipid drops in the PPG epithelium. The epithelial thickness in workers measures 6–8 μm, whereas the epithelium in mated queens can be twice as thick at the same age.

Provision of food to the larvae

This function in workers was suggested by several investigators including Ayre (1963, 1967), Barbier and Delage (1967), Markin (1970), Naarmann (1963), Paulsen (1969) and Vinson *et al.* (1980). Of these Markin, Naarmann, and Vinson *et al.* produced experimental evidence to support their hypothesis in the Argentine ant *Iridomyrmex humilis* (now *Linepithema humile*), in two species of *Formica* (*F. polyctena* and *F. nigricans*), and in the red imported fire ant (*Solenopsis invicta*), respectively.

Observations of pharaoh's ant larvae by Peacock and Baxter (1950) seem to confirm that young larvae are fed only with special nourishment as all first instar larvae irrespective of sex and caste display gold-coloured gut contents. In the second and third instar, worker larvae are also fed with solid food particles, which give their midgut a dark appearance. Besides their larger size and general lack of body hairs, the sexual larvae in pharaoh's ants, both males and females (gynes), are easily recognized with the human eye because of their bright golden to light green midgut having a large content of a clear oil-like substance. Qualities such as colour and texture of the oil may vary in the larval gut but it always has a remarkable similarity to the oil in the PPG of workers and queens in the same colony. We find plenty of secretions in the lumen of the PPG of the young workers. Most of the nurses (80%) have the secretion in their lumen, whereas only 20% of the foragers have it. This corresponds with the tasks of the workers; nurses take care of the brood and stay most of the time inside the nest, whereas foragers go searching for food and spend more time outside the nest. Sixty per cent of the investigated specimens of the repletes contain the secretion in the lumen. The repletes, which stay inside the nest to collect and store larval secretions, also spend much time nursing the young brood and probably feeding them, so our observations generally support the hypothesis that the PPG serves as a source of food for the larvae. Though no chemical analysis has yet been made on these oils in pharaoh's ants, we find it likely that one function of PPG in workers of pharaoh's ants is to feed this substance to young larvae in general and to the sexual larvae in particular.

In colonies with virgin queens, these are not able to suppress the development of a new sexual brood, which is also not in their interest because they need males to mate with to continue the colony. Virgins usually tend the brood, something which mated queens rarely do. Histological sections of virgin queens show some tubes filled with secretion while others are empty. This could indicate that the secretion of the lumen is frequently released to feed the larvae.

Buschinger and Kloft (1973) showed that virgin queens of pharaoh's ants obtain most of their nourishment directly from food brought into the colony by foragers. In contrast, the mated pharaoh's ant queens get most of their food from various larval secretions as observed by Børgesen (1989), they do not normally tend brood or stay with the larvae except when they want food from them. It has also been observed by Børgesen that virgin queens have difficulties in getting labial secretions directly from the larvae.

Recognition pheromones

Recent investigations in several ant species show that the hydrocarbon contents of the PPG, which essentially contain the same hydrocarbon mixture as in the cuticular lipid layer, are involved in recognition on the social level from individual to species. It is not in the scope of this paper to give a view of this large and fast-expanding field (Bagnères and Morgan 1991; Lahav *et al.* 1998; Morel *et al.* 1988; Soroker *et al.* 1995; Vander Meer and Morel 1998; Lahav *et al.* 1999; Kaib *et al.* 2000; Heinze *et al.* 2002; Boulay *et al.* 2003). We only want to draw the reader's attention to the levels of social organization in ants, where hydrocarbons have been found to play important roles in olfactory communication. Hydrocarbons, and probably also other chemical substances, are important recognition cues at all levels of ant societies. The recognition of species, nestmate, gender, caste and task is dependent on qualities such as specific chemical components, their mutual proportions and their quantity.

Unfortunately there are no investigations on the cuticular hydrocarbon and PPG profiles in *M. pharaonis*. Many years of work with this species leave us in no doubt that the pharaoh's ant can also discriminate all the social levels mentioned. The fact that the epithelial thickness of the PPG is more pronounced in the mated queens than in the virgin queens, and that the mated queens accumulate vesicles in their epithelium whereas the secretion in the virgin queens is found in the lumen of the PPG, may indicate mating status of the queen. Our morphological measurements of PPG in workers show a significant increase in epithelial thickness with task and age between the PPG in nurses and repletes and between nurses and foragers, as well as significant differences with regard to the amount of oils in the PPG. On histological sections we find most of the individuals of the group of nurses containing secretion in the lumen, whereas this is less in repletes and much less in foragers. Beside the assumed main function of the PPG in providing food for larvae, the differences in the PPG between the worker castes may indicate the different tasks these workers accomplish for their nestmates, when the content is applied onto the cuticle during trophallaxis or grooming. Two recently discovered possible sources of cuticular hydrocarbons may, however, also be involved in social recognition in ants. They are the subepithelial gland found by Gobin *et al*. (2003) and the infrabuccal epithelium gland described by Eelen *et al*. (2004). Both glands have a conspicuous development of SER indicating a main function in lipid metabolism and production of substances other than proteins. They are both exocrine glands secreting their products onto the surface of the cuticle – directly or indirectly.

There is evidence in all species investigated so far, that the PPG plays a role in the sociochemical information of the cuticular hydrocarbons but the subject needs further investigation.

Origin of the PPG contents

There is much controversy with regard to the source of the contents of the PPG. The literature points in three main directions with regards to the origin of the hydrocarbons.

(1) Synthesis de novo of hydrocarbons in the PPG epithelial cells This was shown in *Formica polyctena*, *Cataglyphis* workers and queens, and in *Dinoponera australis* by Naarmann (1963), Lahav *et al*. (1999) and Caetano *et al*. (2002), respectively. Vander Meer *et al*. (1982) found that the composition of the PPG in the fire ants changes with age.

The basic conditions for synthesis of hydrocarbons in the PPG are evident in *M. pharaonis* from the presence of well-developed SER mainly concentrated at the apical side having numerous mitochondria near them. The lamellar inclusions, a very active nucleus as well as the microvilli border are also indications of high activity in the metabolic pathway of lipids, such as the hydrocarbons. The increasing epithelial thickness of the PPG in both virgin and mated queens with age supports the production of hydrocarbons within the cell.

(2) Synthesis of hydrocarbons in other cells Some investigations show that the contents of the PPG, especially hydrocarbons, are synthesized in tissue associated with the integument (i.e. fat body, oenocytes) from where they are transported to the cuticular lipid layer and stored in the PPG by means of self-grooming, trophallaxis, body contact (Soroker *et al*. 1995; Vienne *et al*. 1995) or by allogrooming (Soroker *et al*. 1998). As mentioned, the subepithelial gland may also be involved in hydrocarbon synthesis in ants as indicated by the ultrastructure of the cells (Gobin *et al*. 2003). Preliminary investigations show that the pharaoh's ants still have undescribed glands near the cuticle, which may correspond to these subepithelial glands described by Gobin *et al*. (2003).

Soroker and Hefetz (2000) suggest, in a model of hydrocarbon synthesis and circulation, that the hydrocarbons are produced in the fat bodies, most probably in the oenocytes, from where they are transported to the PPG via the haemolymph.

In pharaoh's ant queens these oenocytes only occur in the subepidermal fat bodies of the gaster (Jensen and Børgesen 2000). The long distance between oenocytes in the abdomen and the PPG in the head makes it less likely that oenocytes are the main source of the hydrocarbons in the PPG of pharaoh's ants. Light-microscopical observations of the head of queens show large fat cells situated near the PPG. These fat cells in the head and in the nearby thorax are more likely to be a source of precursor molecules for the hydrocarbons, but this is pure speculation. The large amount of invaginations and lipid vacuoles at the basal part of the epithelial cell is a clear indication of extensive transport of lipids, which seems to favour the hypothesis of producing precursor molecules for hydrocarbons in other cells in the neighbourhood of the PPG and transporting them to the PPG.

(3) Sequestered oils from ingested food Markin (1970) found that the contents of the PPG in workers comprises a mixture of secretory products from the glandular cells and lipids from ingested food. Also, experiments by Barbier and DeLage (1967) and Delage Darchen (1968), as well as by Phillips and Vinson (1980), support the idea that the contents of the PPG are derived from the ants' diet. Phillips and Vinson (1980) suggested that the PPG is not a gland but functions as a cephalic caecum. The extraction of lipids from food, the further transport into the haemocoele, as described by Vinson *et al*. (1980), and the transportation and incorporation into the developing eggs, is an interesting idea, because

it partly explains how highly productive queens may achieve important energy resources for the large amount of eggs they produce. Mature mated queens of *M. pharaonis* can lay up to about 100 eggs a day. Our observations of the accumulation and possibly transport of extensive amounts of lipids in the epithelium of the PPG of mated queens of *M. pharaonis,* does not contradict the hypothesis that lipids are transported into the haemocoele and finally end up in the developing oocytes. The virgin queens, who are producing only a few eggs and who feed larvae, have the lipids in the lumen and have only a few lipid vesicles in the epithelium. Their PPG clearly do not have the same main function as in the mated queens.

The pharaoh's ants workers collect proteinaceous food (egg yolk, boiled liver) and carbohydrates for their diet. They prefer eating insects like crickets, mealworms, flies, etc. They also like almonds and nuts, which contain oils. The nourishment given to the ant colony influences the colour and texture of oils. Even if the crop contents are very similar to those of the PPG in both workers and queens, and to the contents of the midgut of the first larval instars as well as the sexual larvae, we can only conclude this after chemical analysis.

Conclusion

The PPG seems to be a very simple structure with regard to morphology and ultrastructure. When it comes to understanding the functions of this curious appendix to the anterior alimentary canal, however, we are puzzled by the many, and apparently opposing, conclusions which a large number of investigators have come up with, based on their experimental data. It is confusing having all these qualified suggestions and evidences of specific functions for the same gland, which is found only in ants. Some of the functions have proven not to be valid when investigated further, but many studies in different ant species show that there are indeed several functions which are not easily ruled out from the list of possibilities. A logical explanation is that the PPG has several functions depending on age, sex, caste and mating status, and not only one.

In the pharaoh's ant the morphology and ultrastructure of the PPG shows conspicuous differences with respect to size and epithelial development according to sex and caste, the queens having the largest pair of PPG, the workers the medium size, and the males having the smallest. The postimaginal development varies considerably with respect to age and mating status in queens, and with respect to temporal caste development in workers. The ultrastructure of the epithelial cells of the PPG in all groups investigated shows that the cells are mainly involved in lipid metabolism and synthesis of non-proteinaceous substances. There are indications of transport of lipids either way across the epithelium.

Based on our data and on our knowledge of the literature we suggest different functions of the PPG according to age, caste and mating status in *M. pharaonis*. The PPG of workers and virgin queens is mainly used to feed the youngest instars of worker larvae and the sexual larvae. In the mated queens the PPG is most likely involved in supplying the developing oocytes with lipids. In addition to these main functions, there is strong evidence that the PPG, among other possibilities, are involved in signalling the identity of species, nestmates and castes and the reproductive capacity of the reproductives.

Acknowledgements

We thank Koen Collart and Julien Cillis for technical assistance in preparing the tissues for microscopy. D.E. acknowledges the support of a specialization grant of the 'Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen)'. This research was supported in part by grant OT/2001/24 from the K.U. Leuven Research Fund and by the European Community's Improving Human Potential Programme under contract HPRN-CT-2000–00052 'INSECTS'.

References

Ayre, G. L. 1963. Feeding behaviour and digestion in *Camponotus herculeanus* (L.) (Hymenoptera: Formicidae). – *Entomologia Experimentalis et Applicata* **6**: 165–170.

Ayre, G. L. 1967. The relationships between food and digestive enzymes in five species of ants (Hymenoptera: Formicidae). – *Canadian Entomology* **99**: 408–411.

Bagnères, A. G. and Morgan, E. D. 1991. The postpharyngeal glands and the cuticle of the Formicidae contain the same characteristic hydrocarbons. – *Experientia* **47**: 106–111.

Barbier, M. and Delage, B. 1967. Le contenu des glandes pharyngiennes de la Fourmi *Messor capitatus* Latr. (Insecte, Hymenoptère, Formicide). – *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* **264**: 1520–1522.

Billen, J. 1990. Phylogenetic aspects of exocrine gland development in the Formicidae. In Veeresh, G.K., Mallik, B. and Viraktamath, C.A. (Eds): *Social Insects and the Environment*, pp. 317–318. Oxford and IBH Publishing Co, New Delhi, Bombay, Calcutta.

Billen, J. 1991. Ultrastructural organization of the exocrine glands in ants. – *Ethology Ecology and Evolution* **1**: 67–73.

Børgesen, L. W. 1989. A new aspect of the role of larvae in the Pharaoh's ant's society *Monomorium pharaonis* (L.) – Formicidae, Myrmicinae: producer of fecundity–increasing substances to the queen. – *Insectes Sociaux* **36**: 313–327.

Børgesen, L. W. 2000. Nutritional function of replete workers in the pharaoh's ant, *Monomorium pharaonis* (L.). – *Insectes Sociaux* **47**: 141–146.

Boulay, R., Katsav-Gozansky, T., Vander Meer, R. K. and Hefetz, A. 2003. Colony insularity through queen control on worker social motivation in ants. – *Proceedings of the Royal Society: Biological Sciences* **270**: 971–977.

Brian, M. V. 1968. Regulation of sexual production in an ant society. – *Colloques Internationaux du Centre National de la Recherche Scientifique, Paris* **173**.

Brian, M. V. 1973. Queen recognition by brood-rearing of the ant *Myrmica rubra* (L.). – *Animal Behaviour* **21**: 691–698.

Brian, M. V. and Blum, M. S. 1969. The influence of *Myrmica* queen head extracts on larval growth. – *Journal of Insect Physiology* **15**: 2213–2223.

Buschinger, A. and Kloft, W. 1973. Zur Funktion der Königin im sozialen Nahrungshaushalt in der Pharaoameise *Monomorium pharaonis* (L.) (Hym., Formicidae). – *Forschungsberichte des Landes Nordrhein-Westfalen* **2306**.

Caetano, F. H., Zara, F. J. and Gregorio, E. A. 2002. The origin of droplets in the post-pharyngeal gland of *Dinoponera australis* (Formicidae: Ponerinae). – *Cytologia-Tokyo* **67**: 301–308.

Delage Darchen, B. 1968. Recherches sur les fourmis moissonneuses du bassin Aquitain: éthologie et physiologie de l'alimentation. – *Annales Des Sciences Naturelles* **10**: 197–265.

Eelen, D., Børgesen, L. W. and Billen, J. 2004. Morphology of a novel glandular epithelium lining the infrabuccal cavity in the ant *Monomorium pharaonis* (Hymenoptera, Formicidae). – *Arthropod Structure and Development*.

Emmert, W. 1968. Die Postembryonalentwicklung Sekretorischer Kopfdrüsen von Formica pratensis Retz. und Apis mellifer L. (Ins., Hym). – *Zeitschrift für Morphology der Tiere* **63**: 1–62.

Forbes, J. and McFarlane, A. M. 1961. The comparative anatomy of digestive glands in the female castes and the male of *Camponotus pennsylvanicus* DeGeer (Formicidae, Hymenoptera). – *Journal of the New York Entomological Society* **69**: 92–103.

Gama, V. and Cruz Landim, C. 1982. Estudo comparativo das glândulas do sistema salivar de formigas (Hymenoptera Formicidae). – *Naturalia* **7**: 145–165.

Gobin, B., Ito, F. and Billen, J. 2003. The subepithelial gland in ants: a novel exocrine gland closely associated with the cuticle surface. – *Acta Zoolocica* **84**: 285–291.

Heinze, J., Stengl, B. and Sledge, M. F. 2002. Worker rank, reproductive status and cuticular hydrocarbon signature in the ant, *Pachycondyla inversa*. – *Behavioural Ecology and Sociobiology* **52**: 59–65.

Jensen, P. V. and Børgesen, L. W. 2000. Regional and functional differentiation in the fat body of the pharaoh's ant queens, *Monomorium pharaonis* (L.). – *Arthropod Structure and Development* **29**: 171–184.

Kaib, M., Eisermann, B., Schoeters, E., Billen, J., Franke, S. and Franke, W. 2000. Task-related variation of postpharyngeal and cuticular hydrocarbon composition in the ant, *Myrmicaria eumenoides*. – *Journal of Comparative Physiology* **186**: 939–948.

Lahav, S., Soroker, V., Vander Meer, R. K. and Hefetz, A. 1998. Nestmate recognition in the ant *Cataglyphis niger*: do queens matter? *Behavioural Ecology and Sociobiology* **43**: 203–212.

Lahav, S., Soroker, V., Hefetz, A. and Vander Meer, R. K. 1999. Direct evidence for hydrocarbons as ant recognition discriminators. – *Naturwissenschaften* **86**: 246–249.

Lukas, C., Pho, D. B., Fresneau, D. and Jallon, T. 2004. Hydrocarbon circulation and colonial signature in Pachycondyla villosa. – *Journal of Insect Physiology* **50**: 595–607.

Markin, G. P. 1970. Food distribution within laboratory colonies of the Argentine ant, *Iridomyrmex humilis* (Mayr). – *Insectes Sociaux* **17**: 127–158.

Morel, L., Vander Meer, R. K. and Lavine, B. K. 1988. Ontogeny of nest-mate recognition cues in the red carpenter ant (*Camponotus floridanus*); behavioural and chemical evidence for the role of age and social experience. – *Behavioural Ecology and Sociobiology* **22**: 175–183.

Naarmann, H. 1963. Untersuchungen über Bildung und Weitergabe von Drüsensekreten bei *Formica* (Hymenoptera: Formicidae) mit Hilfe der Radioisotopenmethode. – *Experientia* **19**: 412–413.

Noirot, C. and Quennedey, A. 1974. Fine structure of insect epidermal glands. – *Annual Review of Entomology* **19**: 61–80.

Paulsen, R. 1969. Zur Funktion der Propharynx-, Postpharynx- und Labialdrüsen von *Formica polyctena* Foerst. (Hymenoptera, Formicidae). – Doctoral Dissertation, Natur Fakultät, Würzburg.

Pavan, M. and Ronchetti, G. 1955. Studi sulla morfologia esterna e anatomia interna dell′ operaia di *Iridomyrmex humilis* Mayr, e richerche chimiche e biologische sulla iridomircina. – *Atti Della Societa Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano* **94**: 379–477.

Peacock, A. D. and Baxter, T. 1950. Studies in Pharaoh's ant, Monomorium Pharaonis (L.) 3. Life history. – *Entomologists Monthly Magzine* **86**: 171–178.

Peregrine, D. J., Mudd, A. and Cherrett, J. M. 1973. Anatomy and preliminary chemical analysis of the postpharyngeal glands of the leaf-cutting ant, *Acromyrmex octospinosus* (Reich.) (Hymenoptera Formicidae). – *Insectes Sociaux* **20**: 355–363.

Petersen-Braun, M. D. 1973. Untersuchungen zur sozialen Organization der Pharaoameise *Monomorium Pharaonis* (L.). – Dissertation, p. 204. Rheinische Friedrich-Wilhelms-Universität, Bonn.

Phillips, S. A. and Vinson, S. B. 1980. Comparative morphology of glands associated with the head among castes of the red imported fire ant, *Solenopsis invicta* Buren. – *Journal of the Georgia Entomological Society* **15**: 215–226.

Schoeters, E. and Billen, J. 1996. The postpharyngeal gland in *Dinoponera* ants (Hymenoptera: Formicidae): unusual morphology and changes during the secretory process. – *International Journal of Insect Morphology and Embryology* **25**: 443–447.

Soroker, V. and Hefetz, A. 2000. Hydrocarbon site and synthesis and circulation in the desert ant *Cataglyphis niger*. – *Journal of Insect Physiology* **46**: 1097–1102.

Soroker, V., Hefetz, A., Cojocura, M., Billen, J., Franke, S. and Franke, W. 1995. Structural and chemical ontogeny of the postpharyngeal gland of the desert ant *Cataglyphis niger*. – *Physiological Entomology* **20**: 323–329.

Soroker, V., Fresneau, D. and Hefetz, A. 1998. Formation of colony odor in ponerine ant *Pachycondyla apicalis*. – *Journal of Chemical Ecology* **24**: 1077–1090.

Tschinkel, W. R. 1995. Stimulation of fire ant queen fecundity by a highly specific brood stage. – *Annals of the Entomological Society of America* **88**: 876–882.

Vander Meer, R. K., Glancey, B. M. and Lofgren, C. S. 1982. Biochemical changes in the crop oesophagus and postpharyngeal gland of colony founding red imported fire ant queens *Solenopsis invicta*. – *Insect Biochemistry* **12**: 123–128.

Vander-Meer, R. K. and Morel, L. 1998. Nestmate recognition in ants. In Vander-Meer, R. K., Breed, M. D., Espelle, K. E. and Winston, M. L. (Eds): *Pheromone Communication in Social Insects. Ants, Wasps, Bees and Termites*, pp. 79–103. Westview Press, Boulder.

Vienne, C., Soroker, C. and Hefetz, A. 1995. Congruency of hydrocarbon patterns in heterospecific groups of ants: transfer and/or biosynthesis? *Insectes Sociaux* **42**: 267–277.

Vinson, S. B., Phillips, S. Jr and Williams, H. J. 1980. The function of the postpharyngeal gland of the red imported fire ant, *Solenopsis invicta*. – *Journal of Insect Physiology* **26**: 645–650.

Zylberberg, L., Jeantet, A.-Y. and Delage-Darchen, B. 1974. Particularités structurales de l'intima cuticulaire des glandes post-pharyngiennes des fourmis. – *Journal of Microscopy* **21**: 331–342.

