Apidologie 36 (2005) 49–57

DOI: 10.1051/apido:2004068

Original article

Age-dependent morphology and ultrastructure of the hypopharyngeal gland of *Apis mellifera* workers (Hymenoptera, Apidae)

Jeroen DESEYN*, Johan BILLEN

Zoological Institute, University of Leuven, Naamsestraat 59, 3000 Leuven, Belgium

Received 19 February 2004 – Revised 20 May 2004 – Accepted 28 May 2004

Published online 16 March 2005

Abstract – The main secretory products of the hypopharyngeal gland are royal jelly compounds, as well as other substances such as α-glucosidase. Our study of the morphology and ultrastructure of this gland in relation to worker's age clearly shows a secretory cycle within the cells, although production of secretion is asynchroneous between different cells within an acinus. Secretory vesicles appear already in 3 day old bees, while peak production is around 6 days. Thereafter, the volume of the acini as well as the number of secretory vesicles decrease and no vesicles are visible after 3 weeks of age. Foragers display degenerative structures in their cells. The hypopharyngeal gland cells of winter bees contain large numbers of secretory vesicles, that are probably stored until spring.

exocrine gland / hypopharyngeal gland / morphology / ultrastructure

1. INTRODUCTION

Task regulation in worker honeybees is based mainly on age, with young individuals performing activities inside the nest such as brood care and wax production, while older bees become foragers with extranidal activities (Michener, 1974). Age-dependent changes in exocrine glands can be expected, because of changing functions. Hypopharyngeal glands are found in honeybees, as well as other bees and wasps (Snodgrass, 1956) and occur as 4 anatomical types, based on the number of secretory cells within the acini and the mutual position between the acini and ducts (Cruz-Landim and Costa, 1998). In honeybees, the function of the hypopharyngeal gland is mainly the production and secretion of components of the royal jelly, which is fed to the queen and brood (Michener, 1974). Royal jelly has a complex composition of water, sugars, proteins, cholesterol, amino acids and vitamins (Rembold, 1964). 90% of the proteins belongs to the same group and contains 5 kinds of proteins with a relatively high amount of essential amino acids (Schmitzova et al., 1998). The hypopharyngeal gland also produces α-glucosidase (Kratky, 1931), glucosidase oxidase (Ohashi et al., 1999) and other enzymes such as galactosidase, esterase, lipase and leucine arilamidase (Costa, 2002).

The secretions produced by the hypopharyngeal glands depend on the needs of the hive. Therefore, the gland has been reported to display a flexible secretory activity in relation to the needs for feeding brood (Free, 1961). Sometimes, older workers seem to retain secretory activity (Ohashi et al., 2000), but workers must always be stimulated by brood pheromone to elicit a maximum development of the gland. A diet of pollen is also necessary for full development of glands (Hrassnigg and Crailsheim,

* Corresponding author: jeroen.deseyn@student.kuleuven.ac.be

1998). A deficit of work within the colony is the signal for workers to become foragers with a corresponding degeneration of the hypopharyngeal glands (Cruz-Landim and Hadek, 1969). Different modes of cell death have been reported in the regressing hypopharyngeal glands of worker honey bees (Silva de Moraes and Bowen, 2000).

A previous study of Cruz-Landim and Hadek (1969) investigated differences of the hypopharyngeal glands between the three broad categories of newly emerged bees, nurse bees and foragers. A more detailed examination of the ultrastructural changes in the hypopharyngeal gland with age in the honeybee *Apis mellifera* L., however, has never been done. The aim of the present paper is to describe the morphological and ultrastructural changes of the hypopharyngeal gland of honeybee workers with respect to age.

2. MATERIALS AND METHODS

Workers of *Apis mellifera* L. were obtained from a large colony in an apiary in Roeselare, Belgium. The source hive contained 2 chambers with plenty of brood, 1 chamber with honey stores and a young, but fully active egg-laying queen. In the summer season, the queen was confined to 2 combs to get a concentration of brood. The queen was confined to a different comb each week for 3 weeks. We let workers emerge once a week in a breeding box, after which we marked the callow bees with different colours to know their age exactly. Winter bees were taken from the combs of the source colony in November. The age of the winter bees was not known precisely, but each was at least two months old. Three workers of each of the ages of 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33 days as well as winter bees were dissected in Ringer solution (Jolly). The hypopharyngeal glands were fixed in 2% glutaraldehyde buffered with 0.05M sodium cacodylate. 1% osmium tetroxide was used for postfixation and was followed by dehydration in acetone and embedding in Araldite.

Semi-thin sections (1 µm) for light microscopy were made with a Reichert OmU2 microtome and stained with methylene blue and thionine. Thin sections (70 nm) for electron microscopy were made with a Reichert Ultracut E microtome and double-stained with uranyl acetate and lead citrate. They were examined with a Zeiss EM900 electron microscope.

Samples for scanning microscopy were dehydrated in formaldehyde dimethyl acetal, after which the glands were critical-point dried. They were coated with gold and viewed with a Philips SEM 515 microscope.

3. RESULTS

3.1. General structure

The hypopharyngeal gland occurs as a paired structure, of which each part is formed by a long, slender main channel, into which alveolar clusters of glandular cells open (Fig. 1A). The number of alveolar units associated with each main channel is approx. 550 (Cruz-Landim and Hadek, 1969). Each acinus comprises approx. 8–12 glandular cells, that are each connected to the main channel through an accompanying duct cell (Fig. 1B), corresponding with class 3 exocrine glands following the classification of Noirot and Quennedey (1974). The junction between glandular cell and duct cell is formed by an end apparatus, which represents a specialized structure that allows secretions to leave the glandular cell to be carried off by the duct cell towards the main channel (Fig. 1C). The nucleus of the duct cells lies within the alveole (Fig. 1D). The ducts from the several glandular cells that make up an acinus form a bundle and open into the main channel through a sieve plate corresponding with this particular acinus (Fig. 1E). Secretory cells are linked by desmosome-like structures, which is an unusual structure between bicellular units (Fig. 1F). We found the same structures also between adjacent secretory cells in the mandibular glands of honeybee workers (Deseyn, unpublished data). Both main channels open into the suboral plate of the hypopharynx, so that secretions are ultimately released through the mouth. Many tracheoles penetrate between the secretory cells in the acinus. There are even tracheolar cells situated within the acinus.

3.2. Age-dependent ultrastructure

The size of the acini clearly changes with age (Fig. 2). The acini increase until 6 days of age and from then decrease. At 6 days, the diameter is doubled in comparison with newly emerged bees (Fig. 3).

Newly emerged workers have an end apparatus in the secretory cells with no clear

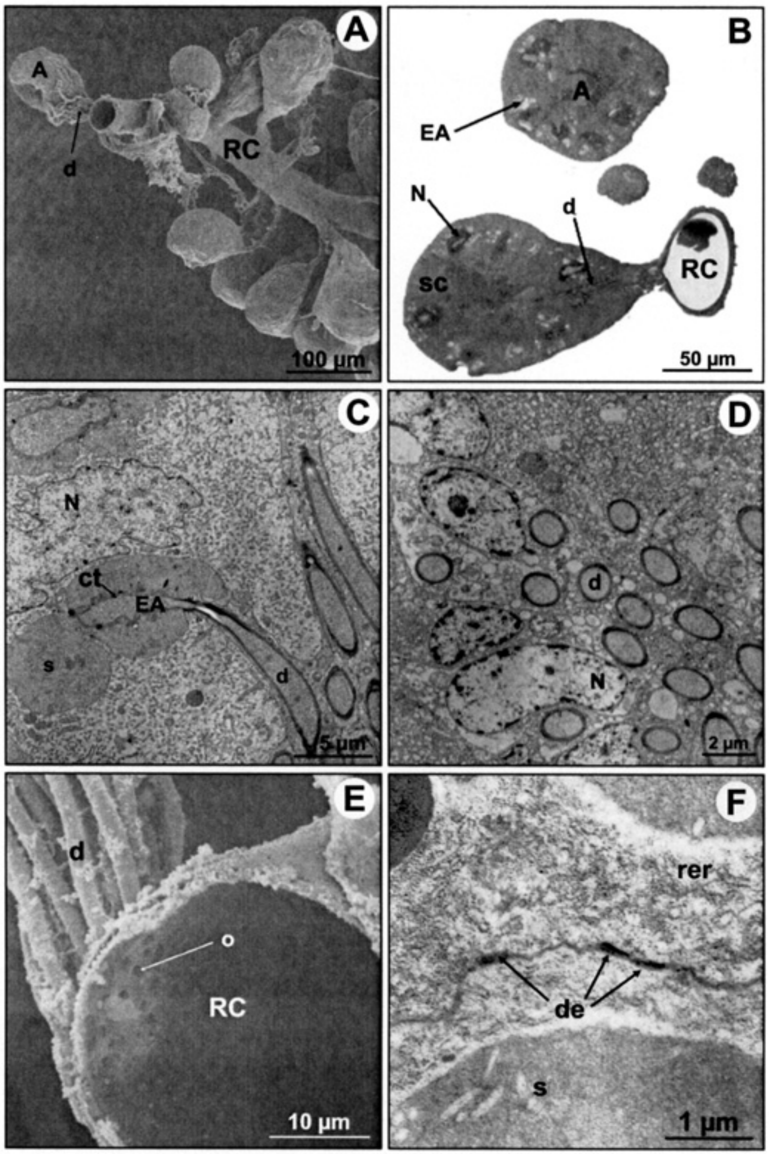

Figure 1. General organization of the hypopharyngeal gland. **A.** SEM-micrograph of part of the gland. **B.** Semithin section of 2 acini and the main channel. **C.** End apparatus between secretory cell and duct cell. **D.** Nuclei of duct cells within the acinus. **E.** Sieve plate of ducts openings into the main channel. **F.** Desmosomes forming junction of the basal membranes between adjacent secretory cells (A: acinus; ct: cuticle; d: duct; de: desmosome; EA: end apparatus; N: nucleus; o: opening; RC: main channel; rer: granular endoplasmic reticulum; s: secretion; sc: secretory cell).

microvilli (Fig. 4A). The cytoplasm contains a vesicular granular endoplasmic reticulum (RER) but secretory vesicles do not yet occur (Fig. 4B). No secretion is found around the end apparatus at this stage.

At the age of 3 days, secretory vesicles appear and the end apparatus displays well developed microvilli. The endoplasmic reticulum is now partially vesicular, partially reticular. Some lysosomes are clearly visible (Fig. 4C).

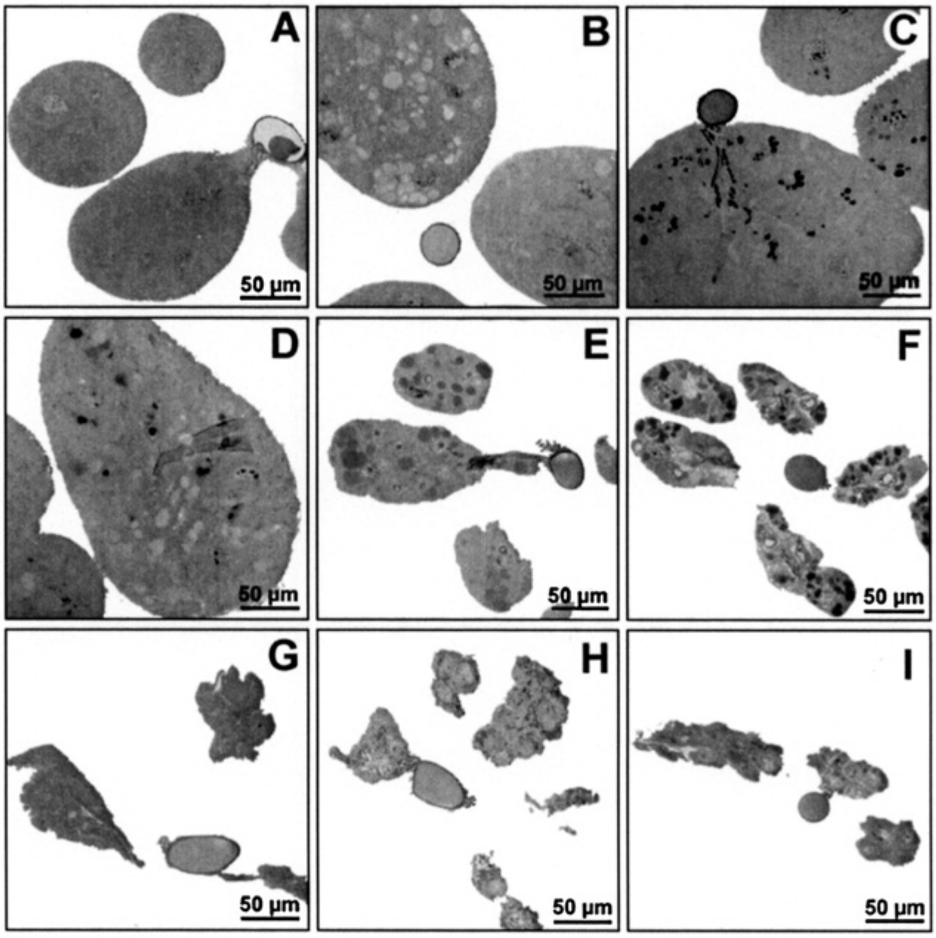

Figure 2. Light-microscopical micrographs of the acini (same magnification). **A.** 3 days. **B.** 6 days. **C.** 9 days. **D.** 12 days. **E.** 15 days. **F.** 18 days. **G.** 21 days. **H.** 24 days. **I.** 33 days.

At the age of 6 days, secretory vesicles concentrate around the end apparatus (Fig. 4D). The peak amount of accumulated secretion in the gland cells is found around this age, which corresponds with the previously mentioned metric results (Fig. 3).

At the age of 9 days, we assume an asynchronic secretory phase (Fig. 4E). Some cells still have vesicular RER, while others already have reticular RER. Probably these different cells do not have the same function at this time. More electron dense secretion is found around the end apparatus. Probably this is the enzyme-rich secretion, which follows the production of the royal jelly (Sasagawa et al., 1989).

Secretion still gathers around the end apparatus at the age of 15 days, but the amount is considerably diminished (Fig. 4F).

At the age of 18 days, the RER is almost completely reticular (Fig. 5A). A large amount of electron dense secretion accumulates around and in the end apparatus.

From 21 days on, granular structures appear. Cruz-Landim and Hadek (1969) suggest these are segrossomes, which are a kind of lysosomes that are important in degenerative processes.

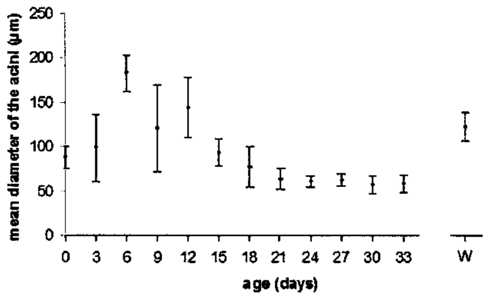

Figure 3. Mean diameter of the acini in function of the age of workers (days). W: winter bees (N = 3 for each category).

They accumulate and are most abundant at an age of 24 days (Fig. 5B). Other organelles disintegrate and the surrounding cytoplasm is less dense. Probably, these structures break down by active substances of the lysosomes.

From an age of 27 days, the RER is electron dense and completely reticular (Figs. 5C, D). Distinction between different structures and organelles becomes more difficult.

In winter bees, 2 types of vesicles with different electron densities are visible (Figs. 5E, F). The more electron dense vesicles are smaller in size, randomly distributed within the cytoplasm and have a diameter between 1–3 µm. The clear masses around the end apparatus correspond with the secretion in summer bees and probably are royal jelly. There is still production of secretory vesicles, but no active discharge. We assume that both types of secretion are stored until spring. As a consequence, this gland stays hypertrophied as described by Cruz-Landim and Hadek (1969). The diameter of the acini in winter bees is not as big as in summer bees.

4. DISCUSSION

The hypopharyngeal gland in the honeybee *Apis mellifera* L. is well investigated and the chemical composition of its secretions have been characterized. A preliminary morphological study was performed by Cruz-Landim and Hadek (1969). The hypopharyngeal gland has also been studied in other species of bees, such as the stingless bee *Melipona quadrifasciata anthidioides* Lep. (Cruz-Landim et al., 1987). This species exhibits a similar secretory cycle to the one we describe here for the honeybee.

The structure of the hypopharyngeal gland we report is in agreement with earlier descriptions (Cruz-Landim and Hadek, 1969), however, we also noticed pronounced age-dependent differences, which were revealed through anatomical measurements and light- and electron-microscopy.

In summer bees, the size of the acini changes with age. The peak size is found around 6 days of age, when workers are known to feed the larvae with royal jelly (Hrassnigg and Crailsheim, 1998). From the age of 15 days, we observed a decrease in size of the acini. The size of the glands in foragers corresponds with that of the still undeveloped gland of newly emerged bees. This was also observed by Simpson et al. (1968). Therefore, size of the gland is positively correlated with gland activity.

The amount of secretion in the secretory cells is also positively correlated with size of the acini. When the worker becomes a forager, the gland no longer has a food-producing function. This partially explains the decrease of size as illustrated by the metric results, but also the cytoplasmic structures start to disintegrate.

Organelles modify with age, such as the RER. The endoplasmic reticulum is almost vesicular and becomes more reticular. This situation is also seen in a *Melipona* species (Cruz-Landim et al., 1987).

The amount of secretion clearly changes. We observed a secretory cycle, reflected by the occurrence and amount of secretion around the end apparatus, which reaches a peak value around the age of 6 days. These secretory products probably correspond with the royal jelly, which young bees at this age produce in vast amounts for nursing the larvae (Snodgrass, 1956). Halberstadt (1980) described 2 phases in the secretory cycle: production of royal jelly, followed by production of enzymes. Production of α-glucosidase increases with the age of the worker bee. Even at an age of 18 days we observe secretory masses, but these are more electron dense. We assume this is another type of secretion than in earlier stages. Based on chemical research (Halberstadt, 1980), we assume that this different type of secretion is enzyme secretion. Probably production goes on, also after partial degeneration of the gland.

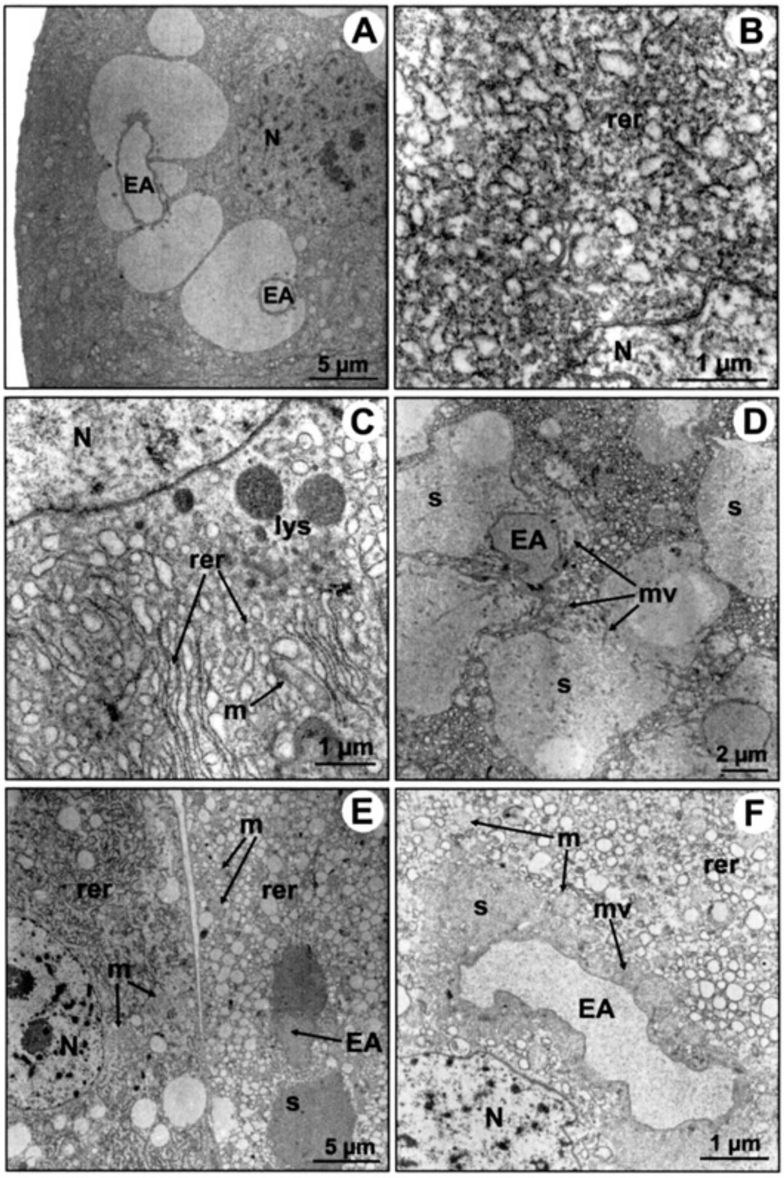

Figure 4. Age-dependent ultrastructure of the hypopharyngeal gland (0–15 days). **A.** End apparatus in newly emerged worker. **B.** Cytoplasm in newly emerged worker. **C.** Cytoplasm in 3 days old worker. **D.** End apparatus and secretion in 6 days old worker. **E.** Cells with different cytoplasm in 9 days old worker. **F.** End apparatus in 15 days old worker (EA: end apparatus; lys: lysosomes; m: mitochondria; mv: microvilli; N: nucleus; rer: granular endoplasmic reticulum; s: secretion).

Only from 21 days on does secretion disappear completely.

The development of the secretory cells is not synchronous within an individual bee and even within an acinus, as we noticed the occurrence of cells with a different cytoplasmic organization and different amounts of secretion at the age of 9 days. We suppose these cells have an asynchronical secretory phase. Probable function can be a prolongation of secretion.

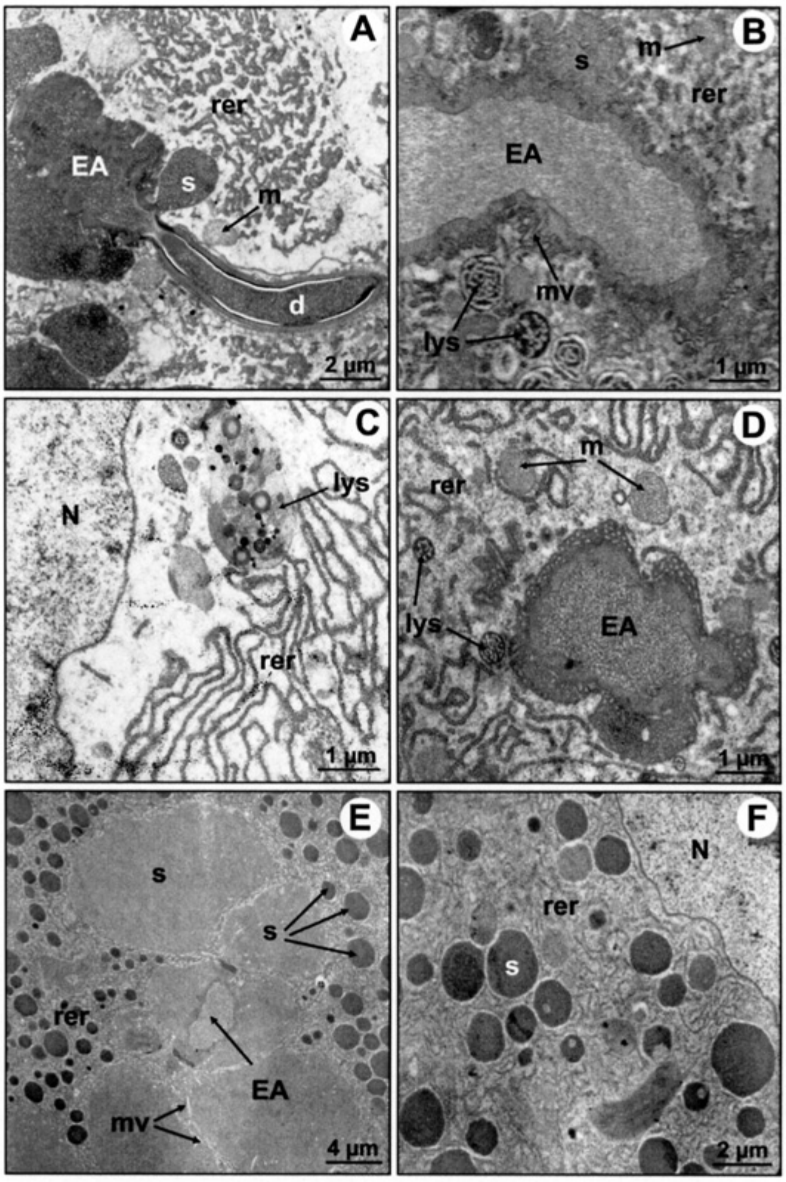

Figure 5. Age-dependent ultrastructure of the hypopharyngeal gland (18–33 days and winter bees). **A.** End apparatus in 18 days old worker. **B.** Granular secretion in 24 days old worker. **C.** RER in 27 days old worker. **D.** Granular secretion and less RER in cytoplasm of 33 days old worker. **E.** Big masses of secretion around the end apparatus and abundant electron dense secretory vesicles in the cytoplasm of winter bees. **F.** Reticular RER between electron dense vesicles in winter bees (d: duct; EA: end apparatus; lys: lysosomes; m: mitochondria; mv: microvilli; N: nucleus; rer: granular endoplasmic reticulum; s: secretion).

Hypopharyngeal glands in winter bees have hypertrophied acini as previously described (Cruz-Landim and Hadek, 1969). Huang et al. (1989) reported that the size of the acini is not directly correlated with the activity of gland cells, in contrast with the situation in summer bees. Undeveloped or hypertrophied cells have less activity than glands with medium size.

Two types of secretion are observed in winter bees: (1) the secretion around the end apparatus, which is probably the same as that observed in summer bees, and (2) electron dense secretory vesicles. These are not observed in summer bees and probably contain enzymes, which have been observed in winter bees (Cruz-Landim and Hadek, 1969).

There is no secretory cycle visible during winter, when the gland shows a reduced activity (Brouwers, 1982). Because of the large quantities of secretory vesicles, we assume the secretion in winter bees is produced but not discharged and stored until the bees reactivate in spring.

In conclusion, the development and physiological activity of the hypopharyngeal gland varies with the task of the worker bee. The gland is fully developed when the worker performs tasks inside the hive for feeding larvae. Activity and developmental stage are age-dependent and can be linked with the behaviour of the worker (Wilson, 1971; Michener, 1974). The observed ultrastructure is in agreement with the secretory phase of royal jelly with a mean activity between an age of 6 and 12 days. This happens asynchronously between different secretory cells. Thereafter, secretion still occurs, but the production of royal jelly probably stops. When the bee becomes a forager, the gland begins to degenerate. In winter bees, secretions are probably stored until spring, when the reactivated workers use them to feed the new generation of larvae.

ACKNOWLEDGMENTS

We greatly acknowledge the help of K. Collart and J. Cillis in tissue preparation for microscopy, and of two anonymous referees for valuable comments on an earlier version of the manuscript.

Résumé – Modifications de la morphologie et de l'ultrastructure des glandes hypopharyngiennes des ouvrières d'*Apis mellifera* en fonction de l'âge. La glande hypopharyngienne, située dans la tête des ouvrières d'abeilles domestiques, est constituée de groupes de cellules sécrétrices qui déversent leur sécrétion dans un conduit principal débouchant dans la partie proximale du pharynx (Fig. 1). Les glandes hypopharyngiennes ont été étudiées du point de vue des modifications liées à l'âge. Les glandes ont été extirpées de la tête, disséquées, fixées, déshydratées et incluses dans de l'araldite. Des coupes ont été faites pour l'étude en microscopie photonique et électronique.
Nos mesures sur les abeilles d'été montrent une taille maximum des glandes chez les ouvrières âgées de 6 j, ce qui correspond à la période de production de gelée royale pour nourrir les larves (Figs. 2 et 3). Notre étude montre clairement un cycle de sécrétion à l'intérieur des cellules, bien que la production de la sécrétion soit asynchrone entre les diverses cellules au sein d'un même acine ou lobe. Les vésicules sécrétrices apparaissent dès l'âge de 3 j (Fig. 4C), leur nombre augmente pour atteindre un pic à l'âge de 6 j (Figs. 4D, E), ce qui est en accord avec nos mesures de la taille des glandes. Ensuite le volume des acines, de même que le nombre de vésicules sécrétrices, diminue (Fig. 4F) et au bout de 3 semaines plus aucune vésicule n'est visible.
Les organites aussi se modifient : le reticulum endoplasmique granulaire (RER), passe d'un aspect vésiculaire à un aspect réticulaire. Lorsque la production cesse, le RER semble dégénérer. Les butineuses présentent dans leurs cellules des structures dégénératives (Figs. 5A–D), mais pas de sécrétion. Nous pensons que les lysosomes, présents en grand nombre dans ces cellules, sont responsables de la désintégration des organites et de l'arrêt de la production de sécrétion.
Chez les abeilles d'hiver nous n'avons pas trouvé de cycle semblable de sécrétion. Les cellules des glandes hypopharyngiennes renfermaient de grandes quantités de vésicules sécrétrices (Figs. 5E–F). Elles stockent vraisemblablement les produits de sécrétion jusqu'à la réactivation de l'activité de couvain au printemps.

***Apis mellifera* / glande exocrine / glande hypopharyngienne / morphologie / ultrastructure**

Zusammenfassung – Altersabhängige Veränderungen in der Morphologie und Ultrastruktur der Hypopharynxdrüse von *Apis mellifera* Arbeiterinnen (Hymenoptera, Apidae). Die im Kopf von Arbeiterinnen der Honigbiene befindliche Hypopharynxdrüse besteht aus Gruppen sekretorischer Zellen, die ihre Sekretionsprodukte in einen Hauptkanal abgeben, der in den proximalen Abschnitt des Pharynx mündet (Abb. 1). Die Hypopharynxdrüsen von Arbeiterinnen wurden hinsichtlich altersabhängiger Veränderungen untersucht. Die aus dem Kopf freigelegten Drüsen wurden fixiert, entwässert und für die Erstellung licht- und elektronenmikroskopischer Schnitte eingebettet. Metrische Analysen an Sommerbienen ergaben ein Maximum der Zellgrössen bei 6-Tage alten Arbeiterinnen, was dem Zeitraum entspricht, in dem diese Gelée Royal an Larven verfüttern (Abb. 2 und 3). Unsere Untersuchung gibt auch klare Hinweise auf einen sekretorischen Aktivitätszyklus, obwohl die Sekretproduktion der Zellen innerhalb eines Acinus durchaus eine gewisse Asynchronie aufweisen kann. Sekretorische Vesikel sind bereits bei 3-Tage alten Bienen zu

beobachten (Abb. 4C), und ihre zunehmende Zahl erreicht ein Maximum bei 6-Tage alten Bienen (Abb. 4 D, E), was somit den metrischen Analysen der Drüsengrösse entspricht. Danach nimmt sowohl das Volumen der Acini als auch die Zahl der sekretorischen Vesikel ab (Abb. 4 F), und letztere sind bei 3-Wochen alten Bienen völlig verschwunden.

Auch die Organellstruktur, z.B. die des rauhen endoplasmatischen Retikulums (RER), ändert sich von einem vesikulären zu einem retikulären Erscheinungsbild. Mit dem Ende der Sekretproduktion scheint das RER abgebaut zu werden, und bei Sammlerinnen zeigen diese sekretionslosen Zellen degenerative Strukturen (Abb. 5 A–D). Wir nehmen an, dass die grosse Zahl der nun in diesen Zellen gefunden Lysosomen für den Abbau dieser Organelle und damit den Stop der Sekretproduktion verantwortlich sind.

Bei Winterbienen fanden wir keinen derartigen Sekretionszyklus. Vielmehr enthielten deren Hypopharynxdrüsenzellen grosse Mengen an sekretorischen Vesikeln (Abb. 5 E, F) und speichern darin vermutlich die Produkte bis zur Reaktivierung der Brutaktivität im Frühling.

***Apis mellifera* / Drüse mit äußerer Sekretion / Hypopharynxdrüse / Morphologie / Ultrastruktur**

REFERENCES

Brouwers E.V.M. (1982) Measurement of hypopharyngeal gland activity in the honeybee, J. Apic. Res. 21, 193–198.

Costa R.A.C. da (2002) Glândulas Hipofaringeas, in: Cruz-Landim C. da, Abdalla F.C. (Eds.), Glândulas Exocrinas das Abelhas, Funpec, Brasil, pp. 91–110.

Cruz-Landim C. da, Costa R.A.C. (1998) Structure and function of the hypopharyngeal glands of Hymenoptera: a comparative approach, J. Comp. Biol. 3, 151–163.

Cruz Landim C. da, Hadek R. (1969) Ultrastructure of *Apis mellifera* hypopharyngeal gland, Proc. 6th Int. Congr. IUSSI, Bern, pp. 121–130.

Cruz-Landim C. da, Silva de Moraes R.L.M., Costa-Leonardo A.M. (1987) Ultra-estrutura das glândulas hipofaringeas de *Melipona quadrifasciata anthidioides* Lep. (Hymenoptera, Apidae), Naturalia 11/12, 89–96.

Free J.B. (1961) Hypopharyngeal gland development and division of labour in honey bee (*Apis mellifera* L.) colonies, Proc. R. Entomol. Soc. Lond. B 36, 5–8.

Halberstadt K. (1980) Elektrophoretische Untersuchungen zur Sekretionstätigkeit der Hypopharynxdrüse der Honigbiene *Apis mellifera* L., Insectes Soc. 27, 61–77.

Hrassnigg N., Crailsheim K. (1998) Adaptation of hypopharyngeal gland development to the brood status of honeybee (*Apis mellifera* L.) colonies, J. Insect Physiol. 44, 929–939.

Huang Z.-Y., Otis G.W., Teal P.E.A. (1989) Nature of brood signal activating the protein synthesis of hypopharyngeal gland in honey bees *Apis mellifera* (Apidae: Hymenoptera), Apidologie 20, 455–464.

Kratky E. (1931) Morphologie und Physiologie der Drüsen in Kopf und Thorax der Honigbiene (*Apis mellifica* L.), Z. Wiss. Biol. 139, 120–200.

Michener C.D. (1974) The Social Behavior of the Bees, Harvard University Press, Cambridge, Massachusetts.

Noirot C., Quennedey A. (1974) Fine structure of insect epidermal glands, Annu. Rev. Entomol. 19, 61–80.

Ohashi K., Natori S., Kubo T. (1999) Expression of amylase and glucose oxidase in the hypopharyngeal gland with an age-dependent role change of the worker honeybee (*Apis mellifera* L.), Eur. J. Biochem. 265, 127–133.

Ohashi K., Sasaki M., Sasagawa H., Nakamura J., Natori S., Kubo T. (2000) Functional flexibility of the honey bee hypopharyngeal gland in a dequeened colony, Zool. Sci. 17, 1089–1094.

Rembold H. (1964) Die Kastenentstehung bei der Honigbiene *Apis mellifica* L., Naturwissenschaften 51, 49–54.

Sasagawa H., Sasaki M., Okada I. (1989) Hormonal control of the division of labor in adult honeybees (*Apis mellifera* L.). I. Effect of methoprene on corpora allata and hypopharyngeal gland, and its α-glucosidase activity, Entomol. Zool. 24, 66–77.

Schmitzova J., Klaudiny J., Albert S., Schröder W., Schreckengost W., Hanes J., Judova J., Simuth J. (1998) A family of major royal jelly proteins of the honeybee *Apis mellifera* L., Cell. Mol. Life Sci. 54, 1020–1030.

Silva de Moraes R.L., Bowen I.D. (2000) Modes of cell death in the hypopharyngeal gland of the honey bee (*Apis mellifera* L.), Cell Biol. Int. 24, 737–743.

Simpson J., Riedel I., Wilding N. (1968) Invertase in the hypopharyngeal glands of the honeybee, J. Apic. Res. 7, 29–36.

Snodgrass R.E. (1956) Anatomy of the Honeybee, Cornell University Press, Ithaca, New York.

Wilson E.O. (1971) The Insect Societies, Belknap Press of Harvard University, Cambridge.