Morphological changes in the cephalic salivary glands of females and males of *Apis mellifera* and *Scaptotrigona postica* (Hymenoptera, Apidae)

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The cephalic salivary glands of some species of bees are exclusive and well developed only in Apinae. These glands were studied with light and scanning electron microscopy in workers, queens and males from the honey bee *Apis mellifera*, and the stingless bee *Scaptotrigona postica* in different life phases. The results show that the cephalic salivary glands are present in females of both the species, and in males of *S. postica*. Nevertheless, they are poorly developed in young males of *A. mellifera.* In both species, gland growth is progressive from the time of emergence to the oldest age but, in *A. mellifera* males, the gland degenerates with age. Scanning electron microscopy shows that the secretory units of newly emerged workers are collapsed while in older workers they are turgid. Some pits on the surface of the secretory units correspond to open intercellular spaces. The possible functions of these glands in females and males of both species are discussed.

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1. Introduction

Insects have a salivary gland system associated with the mouth appendices. The labial or salivary glands consist of paired glands, whose secretory portion lies in the thorax. These are connected to the tongue by the excretory ducts. In adult bees, the salivary system consists of the labial or salivary glands, mandibular and hypopharyngeal glands. In spite of being a part of the salivary system, the functions of the various glands are not always related to food ingestion or digestion.

The labial glands of bees are known as the real salivary glands, because their secretion is delivered on the tongue. This condition facilitates the immediate mixing of secretion with the ingested food.

The labial glands are homologous with the so-called salivary glands of other insects and in holometabolous insects, as in bees they originate from the larval salivary glands during post-embryonic development (Snodgrass 1956; Cruz-Landim 1967; Cruz-Landim and Mello 1967; Silva-de-Moraes 2002).

Even though the secretions of the salivary glands are delivered on the tongue, the secretory units are located in the thorax constituting the thoracic salivary glands present in all species of bees. However, in some species of bees, an additional pair of salivary glands with a common excretory duct is present in the head. This cephalic salivary gland is present in some species of Megachilinae, but with plain development and function is found only in some species of Apinae (Cruz-Landim 1967).

The cephalic salivary gland grows into the head during pupation as an outgrowth from the remains of the common excretory duct of the larval salivary gland (Cruz-Landim and Mello 1967).

The thoracic and cephalic salivary glands produce different kinds of secretions and have different functions, even though they have a common embryonic origin and excretory duct. In the Meliponini and Bombini, there is a swelling called the salivary pouch, where the ducts of the thoracic and cephalic salivary glands converge.

Studies by Simpson (1960), Simpson *et al.* (1968) and Delage-Darchen *et al.* (1979) show that the secretion of the thoracic salivary gland is an aqueous solution of digestive enzymes whereas the secretion of the cephalic gland is oily.

Keywords. Anatomy; bee; labial gland; queen; scanning electron microscopy; worker

In general, the salivary glands of bees differ in females and males, being more developed in females. The workers perform various tasks in the colony in a sequence, according to their age and physiological state or the needs of the colony. Changes from one task to another are generally marked by changes in the development and function of the exocrine glands (Cruz-Landim 1994). Observations by Heselhaus (1922), Inglesent (1940) and Simpson (1960, 1961, 1963) suggest that the cephalic salivary glands in forager workers of *A. mellifera* are developed to a greater extent. Katzav-Gozansky *et al.* (2001) and Poiani (2007) have supported the observations by Heselhaus (1922) through statistical analysis.

Taking into account the relationship between the organ's morphology and its function, in the present study, the morphological and functional characteristics of the cephalic salivary glands were analysed in order to contribute to the knowledge of possible gland function. The study was performed in different life phases or colonial functions of workers, queens and males of two species of eusocial bees, *Apis mellifera* Linnaeus (1758) and *Scaptotrigona postica* Latreille (1807).

2. Materials and methods

2.1 *Materials*

The cephalic glands of workers, queens and males of *Apis mellifera* and *Scaptotrigona postica* were obtained. The workers were captured according to their state in the colony, i.e. when newly emerged (young), as nurses (middle-aged) and as foragers (old). Two stages of queens (virgin and egg-laying), and two stages of males (newly emerged and sexually mature) were captured.

2.2 *Methods*

2.2.1 *Light microscopy:* The cephalic glands were dissected in buffered saline solution for insects, spread onto histological slides and fixed with Bouin fixative. The images captured were obtained through a camera coupled to a light microscope and treated by Leica Qwin 550 Serves – Image with Peripheral Server Software.

2.2.2 *Scanning electron microscopy (SEM):* For the SEM studies, only workers and queens were used. The glands were dissected and fixed in Karnovsky fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4) for one hour. After washing in distilled water, the glands were dehydrated in a series of ethanol and through critical point drying. The glands were fixed on metallic supports with adhesive tape and covered with gold with a spray Sputtering Balzer SCD 050, and examined and photographed under a Philips scanning electron microscope.

3. Results

3.1 *Light microscopy*

The cephalic salivary glands of both species are located in the head and consist of a pair of lateral branches of the common excretory duct of the thoracic salivary glands. Each gland consists of alveolar secretory units and ducts. The alveoli are formed by an epithelium covered by a cuticle on the luminal surface. From the alveoli, ducts emerge and converge to form the excretory duct of the gland. The excretory duct of each gland joins the common excretory duct of the thoracic salivary gland to form the main excretory duct (figure 1A), which delivers the secretion on the tongue (i.e. glossa prementum).

The cephalic salivary glands of *S. postica* consist of spherical or elliptical alveoli and very slender ducts. The alveoli are few and form small clumps that are dispersed in the head. The junction of the cephalic and thoracic ducts is expanded and is called the salivary pouch (figure 1A).

In newly emerged workers of *S. postica*, the alveoli are shrivelled (figure 1B), whereas in foragers and egg-laying queens, they are turgid (figure 1A). In nurse workers and virgin queens, some alveoli are empty and shrivelled, or contain only a little secretion, while others are turgid and full of secretion (figure 1C).

In *A. mellifera*, each glandular pair possesses two branches, one located frontally and the other behind the brain. The alveoli are mainly piriform and the ducts wide and flattened (figure 1D, E and F).

The newly emerged workers of *A. mellifera* possess collapsed alveoli (figure 1D), which become increasingly turgid, attaining the maximum size and turgidity in forager workers and egg-laying queens (figure 1E and F).

In sexually mature males as well as females of *S. postica*, the cephalic salivary glands are developed (figure 1G). In contrast, in newly emerged males of *A. mellifera*, the glands are covered by fat body cells (figure 1H), and in sexually mature males, the glands are in a regressive state, so that practically only the excretory ducts are visible.

3.2 *Scanning electron microscopy (SEM)*

In both species, under SEM, the alveoli and ducts of newly emerged workers appear collapsed (figure 2A and D). In nurse workers and virgin queens, the majority of the alveoli are collapsed, but some are already turgid (figure 2B), while in forager workers and egg-laying queens, all the alveoli are turgid (figure 2C, E and F).

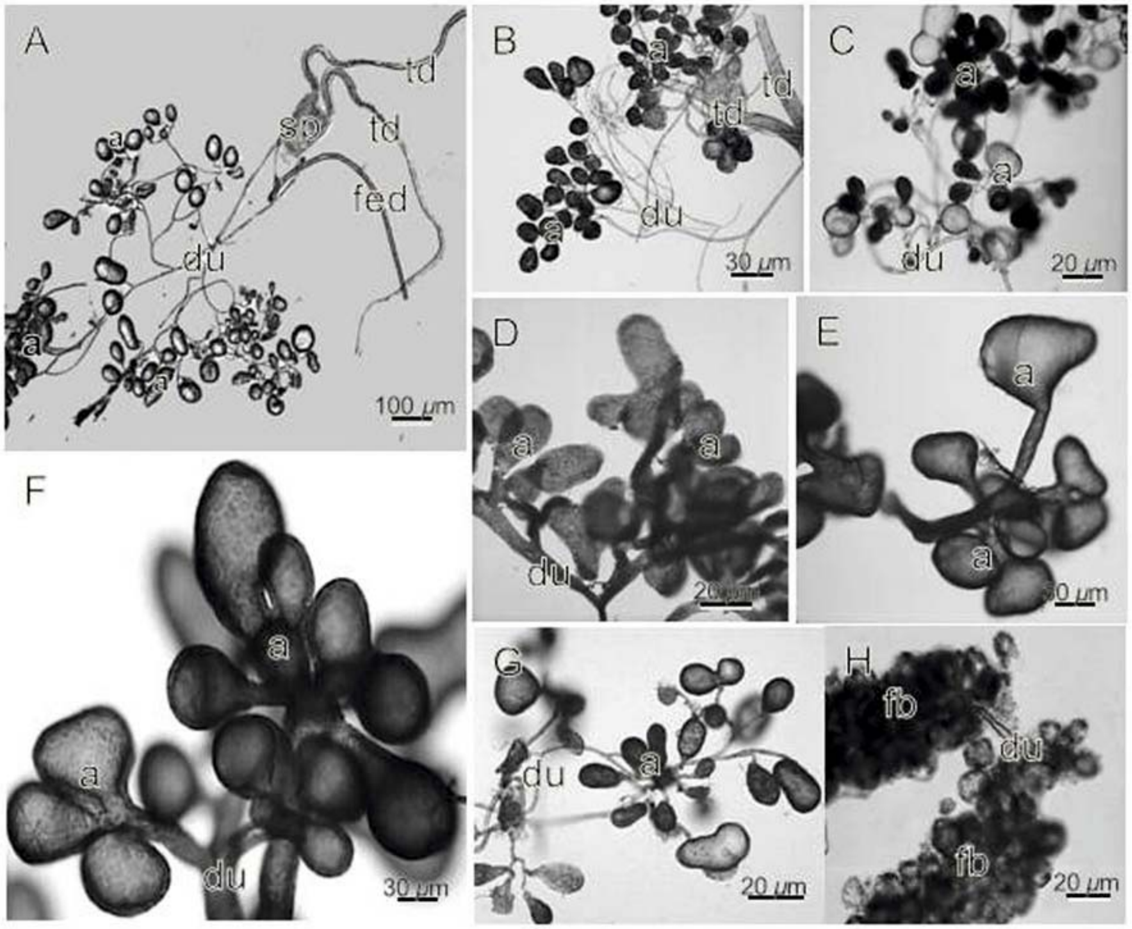

Figure 1. Light microscopy images showing the anatomical features of the cephalic salivary glands of *Scaptotrigona postica* and *Apis mellifera.* **(A)** General aspect of the cephalic salivary gland of an *S. postica* forager worker with slender ducts (du), turgid alveoli (a), a salivary pouch (sp), thoracic ducts (td), and a final excretory duct (fed). Newly emerged workers of *S. postica* **(B)** and *A. mellifera* **(D)** showing cephalic alveoli (a) and in *A. mellifera* robust flattened ducts (du). Nurse worker of *S. postica* **(C)** and *A. mellifera* **(E)**, showing some alveoli (a) with secretion. **(F)** Cephalic salivary gland of egg-laying queens of *A. mellifera* with maximum accumulation of secretion, showing turgid alveoli. **(G)** Gland of sexually mature *S. postica* male showing the same development as a worker. **(H)** Newly emerged males of *A. mellifera* showing gland alveoli covered by fat body (fb).

In newly emerged workers, the limits between the alveolar and duct cells are well seen (figure 2A), but in nurse and forager workers and in egg-laying queens, they are not visible (figure 2B and C). The external surface of the alveoli and ducts of the glands of *A. mellifera*, except in newly emerged workers, possess outer pits similar to pores (figure 2B and C).

The alveoli of *S. postica* workers are mainly spherical (figure 2D and E), but in egg-laying queens, the alveoli are predominantly elliptical (figure 2F). Pits were not observed on the alveolar surface, but in egg-laying queens, the cell contours were well delineated. The excretory ducts are cylindrical and slender. In both species, the alveoli are well supplied with tracheas, as seen in figure 2D, E and F.

4. Discussion

Well-developed cephalic salivary glands are seen in only eusocial species of Apinae. These, as well as the differences in secretory cycle and degree of development among the colony classes of individuals, point to the use of the secretion for activities linked to the special tasks of these individuals in society.

Bees from eusocial societies perform different tasks in the colony, which might be characterized as functional life phases. In queens there are two phases: virgin and fertilized egg-laying queens. Among workers of advanced eusocial species, the phases are characterized as a division of labour in which the different tasks are done according to worker age, characterizing a polypheny or an ethary polyethism.

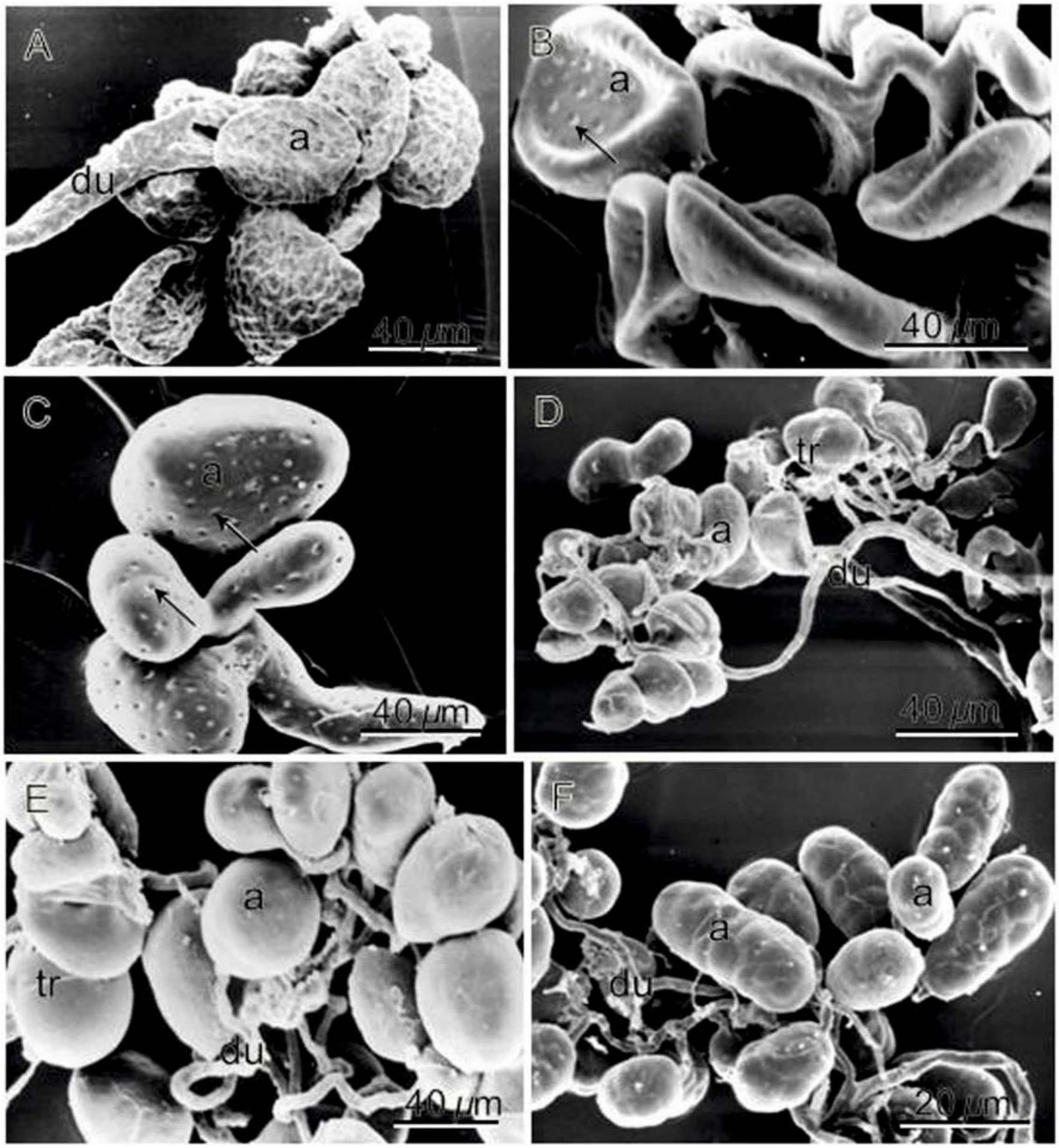

Figure 2. Scanning electron micrographs. **(A)** Newly emerged worker of *Apis mellifera* showing alveoli (a) and flattened ducts (du). The cellular limits are well seen in the alveoli and duct surface. **(B)** Virgin queen of *A. mellifera* with part of the alveoli turgid and part empty. **(C)** Turgid alveoli of several shapes in forager of *A. mellifera*. Surface of *A. mellifera* cephalic salivary gland alveoli (**B** and **C**) and ducts marked by punctuations (arrows) that correspond to the openings of intercellular spaces. Newly emerged workers **(D)** and foragers **(E)** of *Scaptotrigona postica*, showing round alveoli (a) turgid in the forager and flat in the newly emerged worker. **(F)** Egg-laying queen of *S. postica.* Elliptical alveoli with marked cellular contours on the surfaces. a, alveoli; du, duct; tr, tracheole.

The ability of workers to display functions as required by the division of labour is closely related to the functional cycle of the exocrine glands (Michener 1974; Cruz-Landim 1994).

This paper describes the morphological differences found in the salivary glands between the species studied, and correlates the changes along the life cycle of queens and workers of the two species studied for the possible use of secretion in colonial activities.

The main differences between the species were in gland size and presence of a salivary pouch in *S. postica*. This kind of structure is also present in *Euplusia violacens*, *Euglossa cordata*, *Eulaema nigrita* and in some species of *Bombini* (Cruz-Landim 1967), but its functional significance, if any, is unknown. Cruz-Landim (1967) suggested that it is a vestigial structure of the flat duct of the salivary glands of solitary bees, which do not have a cephalic salivary gland.

Another difference between the species was the gland size or the number of secretory units per gland, i.e. the alveoli. In *A. mellifera* the gland is larger in size than in *S. postica*, even taking bee size into account, which indicate a greater capacity for production of secretion by *A. mellifera.* In some workers of Meliponini, branches of the cephalic salivary glands may be found in the thorax, among the tubules of the thoracic gland (Cruz-Landim 1967; Graf 1968; Silva de Moraes 1978; Cavasin-Oliveira 1995), increasing the secretory capacity of the gland. This condition was not observed in *S. postica*, where the secretory units of the gland were limited to the head.

The morphology of the alveoli and ducts of the cephalic salivary glands varies between the species studied and between the sexes. In *A. mellifera*, the alveoli are predominantly piriform, arranged in two compact masses, and the ducts are robust and flattened. In *S. postica*, the alveoli are spherical or elliptical, distributed in small sparse groups, and the ducts are cylindrical and slender.

In spite of these anatomical differences between the two species, the glandular cycle of activity is similar among the workers and queens of both species. Secretion accumulates in the alveoli as the individuals get older.

One characteristic found only in *A. mellifera* and, more specifically, in nurse and forager workers, and in virgin and egg-laying queens, is the pitted surface of the alveoli and ducts as revealed by SEM examination. The pits correspond to the openings of the intercellular spaces in the basal, outer region of the alveoli as shown by Poiani and Cruz-Landim (2009). This feature is perceived only when the alveoli are turgid, which is a condition found in older individuals. The intercellular spaces that open to the outer environment suggest that substances from the haemolymph are allowed to enter through these spaces and eventually reach the gland lumen to be eliminated with the secretion, thus revealing that the gland has an additional excretory function. The presence of pits from the nurse phase onwards and the increase in size of the alveoli in the forager phase suggest an intensification of glandular activity with the age of the bee. In some insects such as the Colembola, which do not have Malpighian tubules, the salivary glands have similar characteristics and also function as excretory organs (Chapman 1998). Pits are absent in *S. postica.*

The continuous increase in alveolar size and turgidity from the moment of emergence to the forager phase indicates that the secretion is not used, or used only little, in intra-colonial tasks. Taking into account the similarities between the cephalic salivary glands of the bee species analysed in this study and those of some species of *Bombus,* in which the glandular secretion is related to individual recognition (Bertsch *et al.* 2005; Terzo *et al.* 2007a, b), it is possible to suggest that the glandular secretion is used by older workers of *A. mellifera* and *S. postica* to identify individuals as belonging to a particular colony. Furthermore, the glandular secretion is used for communication regarding the location of food sources in the meliponines *Trigona recursa* and *T. spinipes* (Jarau *et al.* 2004; Schorkopf *et al.* 2007), to which *S. postica* is closely related. Yet, a study by Santos *et al.* (2009) in *Plebeia emerina* shows that the glandular secretion from middle-aged nurse workers is used to soften the propolis balls dispersed in the colony as a defence against invaders. In this species, the peak of glandular production occurs in middle-aged workers in contrast to the species analysed in this study. Since the gland is more developed in older workers of *S. postica* and *A. mellifera*, it is probable that the secretion could aid in the collection of resins in the field. The same age-dependent morphology and activity of the cephalic salivary glands were found in bumblebee males (Ågren *et al.* 1979; Šobotník *et al.* 2008) and was related to the use of secretion.

In *A. mellifera* and *S. postica*, the cephalic salivary glands are similar in workers and queens. In contrast, in males, these glands are different in the two species. In *S. postica,* the glands are developed in sexually mature males as well as in females. Males of *S. postica* are independent of the workers' care and collaborate in colony tasks (Nogueira-Neto 1997). This fact may be related to the presence of developed cephalic salivary glands. Males of *A. mellifera* do not accumulate secretion since they regress with age. This condition may be related to the fact that the males of *A. mellifera* have a single function, namely, to fertilize the queen and, because they are fed by the workers, they do not need structures that are related to feeding (Winston 1987).

The hypothesis that the size of the cephalic salivary glands is related to their role in the life history of bees is supported by the observation that males of *Bombus pomorum* (Ribbands 1953), *B. atratus* and *B. morio* (Lauer 1992) have well developed cephalic salivary glands, which are sometimes larger than those of females of the same species. In *Bombus*, the secretion of the male cephalic salivary glands is species-specific and produces a sexual marking pheromone (Šobotník *et al.* 2008). At least the males of *B. pratorum* and *B. lapidaries* demarcate their territories and attract females with the secretion of these glands (Bergman and Bergström 1997). Nevertheless, reduced cephalic salivary glands were found in the males of bumblebees of the subgenus *Rhodobombus*. In *Rhodobombus*, secretion of the reduced glands is atypical in composition and results in atypical mating behaviour (Terzo *et al.* 2007a, b).

As the queens are confined to a colony and only lay eggs, it has been hypothesized that the secretion of the cephalic salivary glands must have a pheromonal function such as perhaps to identify individuals as in *Bombus*, which is different from the function in workers.

In conclusion, the data presented here show that glandular secretion of the same gland may have different functions in different species as well as within species, according to the sex and age. This supports once more the theory of functional plasticity of bee glands (Katzav-Gozansky *et al.* 1997, 2000).

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