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Post-embryonic development of intramandibular glands of *Friesella schrottkyi* (Friese, 1900) (Hymenoptera: Apidae) workers

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ABSTRACT

Exocrine glands play important role in social organization of insects, such as caste and intercaste differentiation. Due their functional and structural plasticity, morphological studies on such glands contribute to better understanding the biology of social bees. Therefore, the aim of the study was to characterize the sequence of the post-embryonic development of intramandibular glands of Friesella schrottkyi *(Friese, 1900) (Hymenoptera: Apidae) workers using histological and histochemical analyses. The mandibles of pupae at different developmental stages and newly emerged adults were analyzed. The intramandibular glands of* F. schrottkyi *presented two types: class I glands, in the mandible epidermis and class III glands, inside the mandible cavity that open onto external surface. The intramandibular glands of* F. schrottkyi *developed during the transition from the prepupae to the white-eyed pupae, as shown by the morphological changes. Black-eyed pupae of* F. schrottkyi *presented fully developed intramandibular glands.*

Key-Words: Exocrine glands; Morphology; Development; Stingless bees.

INTRODUCTION

Stingless bee workers show plasticity of tasks they perform during their life, which can change according to the demands of the colony (Van Bethem *et al.,* 1995). The ability to perform different tasks in the colony is related to the worker bee age and morpho-physiology of the exocrine glands. The morphology of these glands and the chemical composition of their secretions are parameters to analyze the functional plasticity of these glands (Cruz-Landim & Abdalla, 2002).

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The main exocrine secretory cells of the insects are class I and III. In class I, the epidermal cells are in close association with the endocuticle and release their secretions to the tegument surface by diffusion through the cuticle, whereas in class III glands, the secretory cells are detached from the epidermis, releasing the secretion through a canaliculus connected to a pore in the body surface (Noirot & Quennedey, 1991).

Mandibular glands can be of two types: ectomandibular gland (mandibular gland) and mesomandibular gland (intramandibular gland). The ectomandibular glands are better studied than the mesomandibular glands. The term "mandibular glands" is used to distinguish them from the intramandibular glands (Cruz-Landim & Abdalla, 2002). According Nedel (1960), the mandibular glands of bees present secretory activity, spherical shape, and isolated cells with independent canaliculi. Moreover, epidermal hypertrophied cells inside the mandibles of some stingless bees were also described and classified as exocrine glands class I (Costa-Leonardo, 1978).

The function of the intramandibular gland is unknown, but its secretion may contribute to the lubrication of mandibles (Schoeters & Billen, 1994; Amaral & Caetano, 2006) or to release of scent trail pheromones (Martins *et al.,* 2013, 2015) in ants. In the stingless bee *Plebeia emerina* it is postulate that the intramandibular secretion helps in manipulation of propolis (Santos *et al.,* 2009). The post-embryonic development of the intramandibular glands has been reported in ants (Martins *et al.,* 2013), but is lacking in stingless bees.

Friesella is a monospecific genus of stingless bees with small workers (3-5 mm). *Friesella schrottkyi* (Friese, 1900) (Hymenoptera: Apidae) is a highly eusocial bee with labor division, overlapping generations, and food storage (Michener, 1974).

The aim of this study was to characterize the sequence of the post-embryonic development of intramandibular glands in the stingless bee *F. schrottkyi* using histological and histochemical analyses.

MATERIALS AND METHODS

Bee collection

Colonies of *F. schrottkyi* bees were maintained in Rio Paranaíba (19°11'37"S, 46°14'50"W), state of Minas Gerais, Brazil. Brood combs were removed from colonies and transferred to the Laboratory of Morphology of Arthropods at the Universidade Federal de Viçosa – Campus de Rio Paranaíba. Worker pupae at different developmental stages characterized by eye pigmentation (white-, pink-, brown-, and black-eyed pupae) were obtained from brood and newly emerged adults classified by tergite pigmentation collected in the nest (Bueno, 1981; Fagundes *et al.,* 2006).

Histology and histochemistry

The mandibles of *F. schrottkyi* pupae and adult workers were dissected and transferred to Zamboni fixative solution (Stefanini *et al.,* 1967), dehydrated in a graded ethanol series, embedded in historesin Leica, and sectioned at 3 µm thick. The sections were stained with toluidine blue-borax and analyzed using a light microscope.

Some mandible sections were submitted to Periodic acid-Schiff (PAS) staining (Bancroft & Gamble, 2008) for identification of neutral polysaccharides and glycoconjugates and to mercury-bromophenol (Bancroft & Gamble, 2008) for localization of total protein.

RESULTS

Histological analysis showed variations in the mandible according to the developmental phase of *F. schrottkyi* pupae. White- and pink-eyed pupae had only larval cuticle (Figs. 1A-B). The brown-eyed pupae showed both larval (external) and adult (onto the epidermis) cuticles (Figs. 1C-D). Black-eyed pupae had thicker adult cuticle (Fig. 1E).

The epidermis of mandibles in white- and pink-eyed pupae was formed by multiple cell layers with different morphology in the mandible regions. The mandible apex had irregular epidermal cells (Fig. 1A) and the basal region had columnar cells with basophil apex and central spherical nucleus with decondensed chromatin (Fig. 1B). Brown-eyed pupae showed columnar epidermal cells at the base of the mandible (Fig. 1C) and flattened ones in the middle and apical mandible regions (Fig. 1D). Black-eyed pupae and newly emerged workers showed flattened cells in the mandible (Figs. 1E-F).

Cells on the cavity of the mandible are involved in differentiation processes with granules and peripherical cytoplasm, and were found at higher quantity in the base mandible in white-, pink-, and brown-eyed pupae (Fig. 1C). Black-eyed pupae showed completely differentiated spherical cells with a large nucleus with decondensed chromatin and cytoplasm with many granules (Fig. 1E). These cells had a canaliculus that opens on to the mandible surface in black-eyed pupae and newly emerged workers (Fig. 1F).

From white to brown-eyed pupae, the epidermal and spherical cells showed a strong positive reaction for PAS (Fig. 2A) and proteins (Fig. 2B). Epidermal cells of black-eyed pupae and newly emerged workers were slightly positive for PAS (Fig. 2C) and the granules of spherical cells were positive (Fig. 2D) and both cell types were positive for protein in black-eyed pupae and newly emerged workers (Figs. 2E-F). Sensilla were present on the mandible (Fig. 1D).

DISCUSSION

The morphology of *F. schrottkyi* intramandibular glands is similar to other Hymenoptera species (Cruz-Landim & Abdalla, 2002; Cruz-Landim *et al.,* 2011; Martins *et al.,* 2013). Epidermal cells of exocrine glands are class I with secretory features in newly emerged *F. schrottkyi* workers. However, large nuclei with decondensed chromatin in the spherical class III secretory cells suggest high activity (Silva-Zacarin *et al.,* 2008).

The new cuticle of brown-eyed *F. schrottkyi* pupae is synthesized in the previous developmental phas-

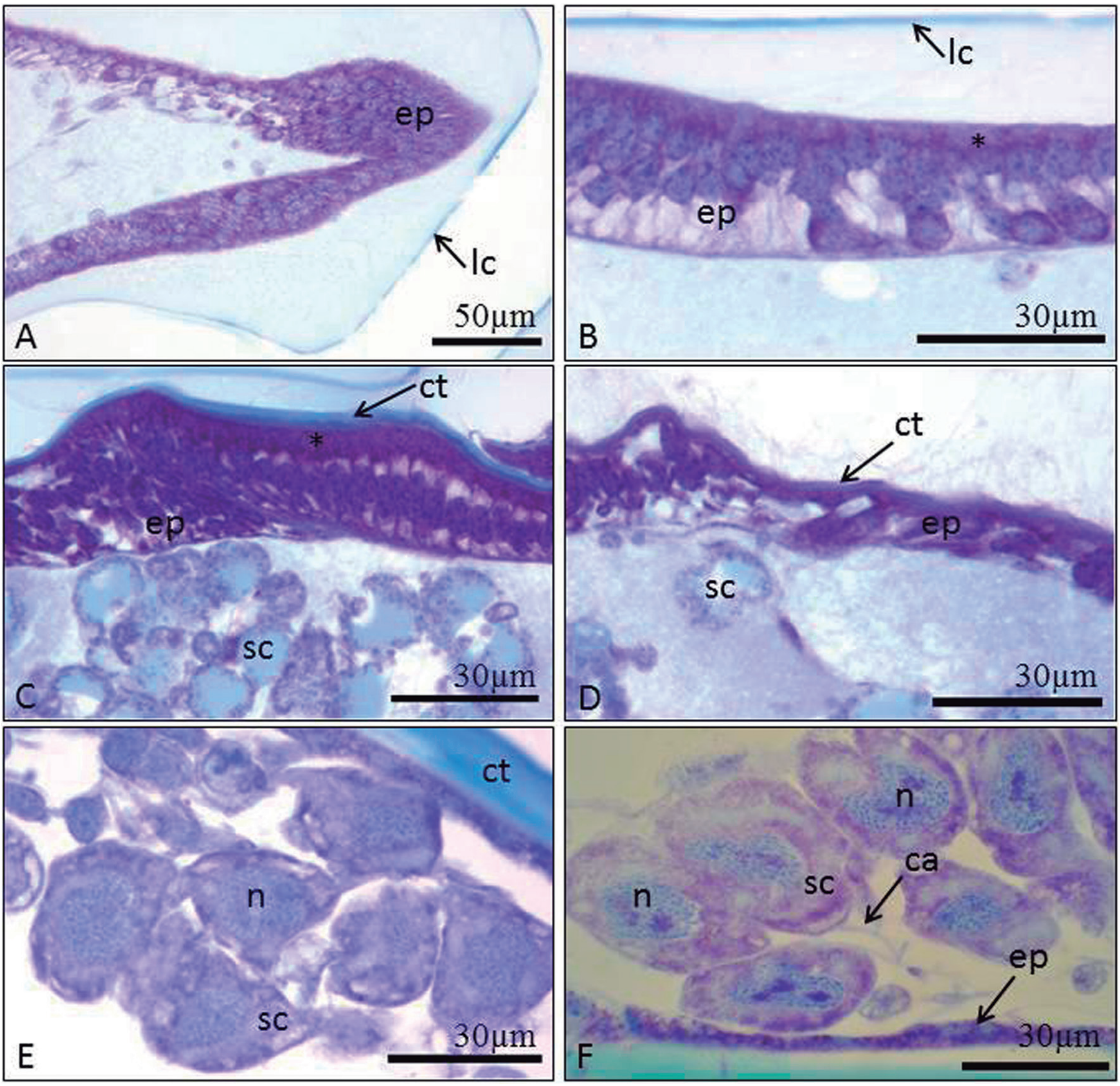

FIGURE 1: Mandible sections of *Friesella schrottkyi* (Hymenoptera, Apidae) worker pupae with toluidine blue. **(A):** Apex of the mandible in white-eyed pupae showing the larval cuticle (lc) and epidermal cells (ep). **(B):** Mandible of pink-eyed pupae showing epidermal cells (ep) with basophil apex (*). **(C-D):** Mandible in brown-eyed pupae showing the new cuticle (ct), epidermal cells (ep) and secretory cells (sc). **(E):** Mandible in black-eyed pupae showing secretory cells (sc) and epidermal cells (ep). ct: cuticle; n: nucleus. **(F):** Mandible in newly emerged worker showing epidermal cells (ep), secretory cells (sc) and canaliculus (ca). n: nucleus.

es because the apex of the epidermal cells was positive for proteins, which may be used in the production of the new cuticle. The epidermal cells of immature insects synthesize the cuticle partly constituted by proteins, and the cuticular layers are still deposited to a lesser extent in adult insects as reported for *Melipona quadrifasciata* (Cruz-Landim *et al.,* 2011).

The multiple layers of epidermal cells in the mandible of white-eyed pupae changes from a single layer of squamous cells to the cubic epidermal cells in black-eyed pupae, as found for *Plebeia emerina* (Santos *et al.,* 2009), *Melipona rufiventris* and *M. quadrifasciata anthioides* (Costa-Leonardo, 1978). The presence such epithelial cells, only in workers, could suggest some exclusive function for workers. Some authors attribute to the intramandibular secretion role in handling adhesive resins and propolis in *P. emerina* (Santos *et al.,* 2009; Cruz-Landim *et al.,* 2011).

Precursor cells of the class III glands found in the mandible of white-eyed pupae of *F. schrottkyi* are

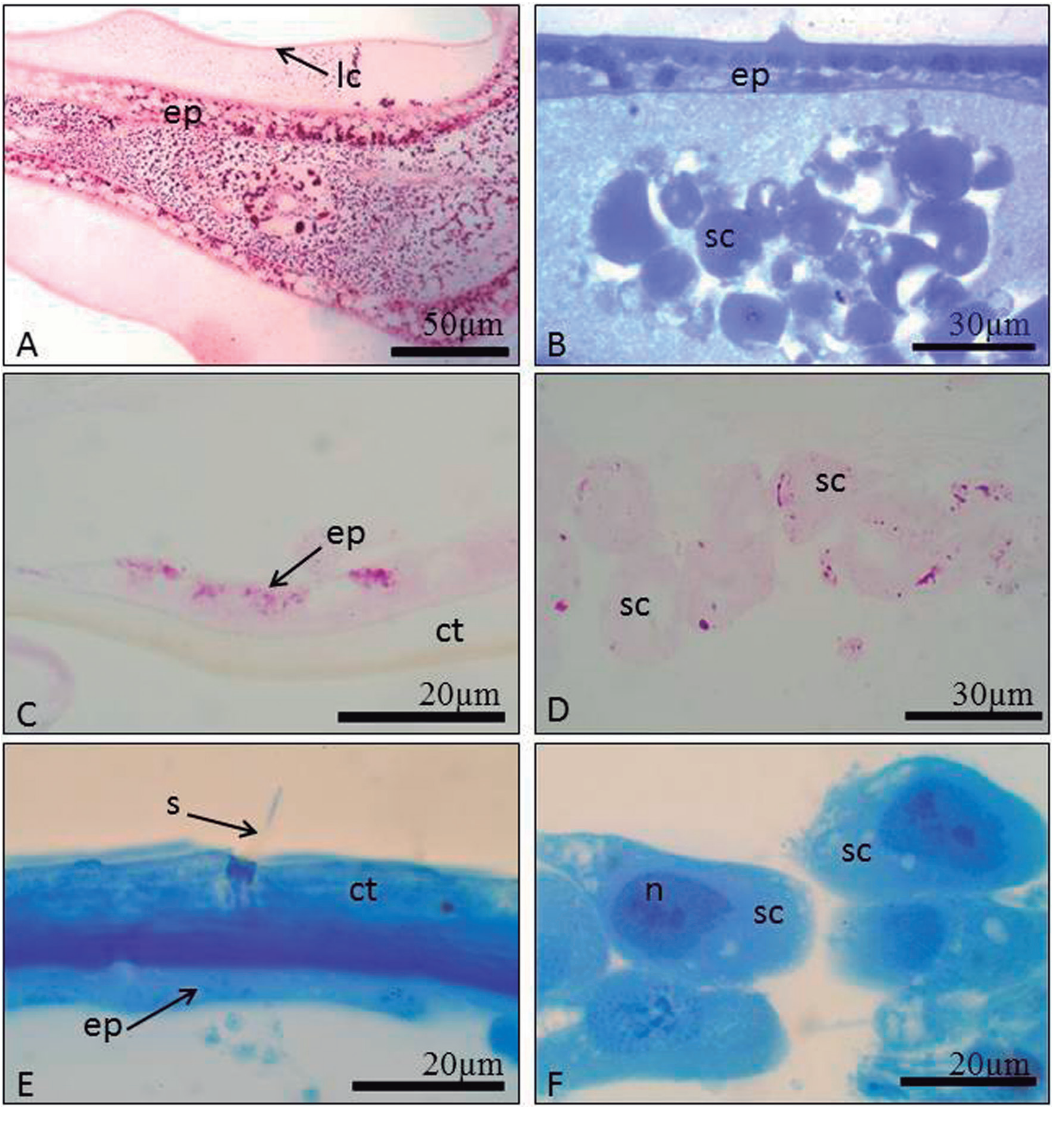

FIGURE 2: Mandible sections of *Friesella schrottkyi* (Hymenoptera: Apidae) worker pupae submitted to histochemical tests. **(A):** White-eyed pupae showing the mandible cavity and epidermal cells (ep) Periodic acid-Schiff (PAS) positive. lc; larval cuticle. **(B):** Brown-eyed pupae showing the secretory cells (sc) and epidermal cells (ep) positive to protein. **(C-D):** Black-eyed pupae showing the epidermal cells (ep) and secretory cells (sc) Periodic acid-Schiff positive. ct: cuticle. **(E-F):** Black-eyed pupae showing the epidermal cells (ep) and secretory cells (sc) positive to protein. n: nucleus; ct: cuticle; s: sensilla; ca: canaliculus.

characterized by cytoplasm granules, but with complete differentiation in black-eyed pupae. According to Martins *et al.* (2013), the cells of the class III of the ant *Pachycondyla verenae* are also completely differentiated in black-eyed pupae.

The large amounts of glycoconjugates and proteins in the mandibular cells from white to brown-eyed pupae of *F. schrottkyi* suggest a high metabolic rate, perhaps due to the cellular reorganization. The positive staining for polysaccharides and glycoconjugates in the mandibles of the pupae is probably due to glycogen, which is an energetic fuel for high metabolic activity as shown in the pupae of the ant, *Solenopsis invicta,* which use about 75% of their carbohydrates during the pupal stage (Wheeler & Buck, 1992). Mandibles of white-eyed pupae of *Pachycondyla verenae* showed intense cell reorganization with positive reactions for glycoconjugates and proteins (Martins *et al.,* 2013).

The decrease of polysaccharides and glycoconjugates in the mandibles of *F. schrottkyi* during development of class III cells along the pupae suggest that this gland may be associated with secretion, likely found in the ants *Atta sexdens rubropilosa* (Amaral & Caetano, 2006) and *Pachycondyla verenae* (Martins *et al.,* 2013)

The development of intramandibular glands during the transition from prepupae to white-eyed pupae of *F. schrottkyi* differs from the lack of differentiation of the intramandibular glands in *P. verenae* prepupae (Martins *et al.,* 2013). The process of differentiation of intramandibular glands beginning in white-eyed pupae and completed in black-eyed pupae is still little known in stingless bees, but epidermal and non epidermal organs have complete differentiation in black-eyed pupae (Neves *et al.,* 2002, 2003; Azevedo *et al.,* 2008).

The post-embryonic development of intramandibular glands in *F. schrottkyi* occurs during the pupal period and completes the development during the black-eyed pupal stage.

RESUMO

As glândulas exócrinas desempenham importantes funções na organização social dos insetos, como a diferenciação de castas e inter-castas. Devido à sua plasticidade estrutural e funcional, estudos morfológicos destas glândulas contribuem para o melhor entendimento da biologia das abelhas sociais. Por isso, o objetivo deste estudo foi acompanhar o desenvolvimento pós-embrionário das glândulas intramandibulares de operárias da abelha sem ferrão Friesella schrottkyi *(Friese, 1900) (Hymenoptera: Apidae) com análises histológicas e histoquímicas. As mandíbulas das pupas em diferentes estágios do desenvolvimento e operárias recém-emergidas foram analisadas. As glândulas intramandibulares de* F. schrottkyi *são divididas em dois tipos: glândulas da classe I na epiderme da mandíbula e da classe III, inseridas na cavidade da mandíbula que se abrem na superfície externa. As glândulas intramandibulares de* F. schrottkyi *se desenvolvem durante a transição de pré-pupa para pupa de olho branco como observado pelas alterações morfológicas nas células. As pupas de olhos pretos de* F. schrottkyi *apresentaram glândulas intramandibulares completamente desenvolvidas.*

Palavras-Chave: Glândulas exócrinas; Morfologia; Desenvolvimento; Abelhas sem ferrão.

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