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Histochemistry, immunohistochemistry and cytochemistry of the anterior midgut region of the stingless bee *Melipona quadrifasciata* and honey bee *Apis mellifera* (Hymenoptera: Apidae)

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ABSTRACT

The anterior midgut region of stingless bees is anatomically differentiated with tall and narrow cells, whereas in other social and solitary bees this anatomical gut region is lacking. The objective of the present study was to describe the histochemistry, immunohistochemistry and cytochemistry of the anterior midgut region of the stingless bee *Melipona quadrifasciata* in comparison with the honey bee *Apis mellifera*. The anterior midgut region of both species was evaluated for identification of the enzymes β- galactosidase, glucose-6-phosphatase, acid phosphatase, and alkaline phosphatase, the membrane transporter aquaporin, the hormone FMRF-amide, and lysosomes. Histology of the anterior midgut region showed that this region in *M. quadrifasciata* workers did not present external folds of the wall, whereas the following midgut wall presented many. In *A. mellifera*, folds in the midgut wall occur starting from the fore- midgut transition region. Despite these morphological differences, the tests evaluated were similar in both species. β-galactosidase was not found in the anterior midgut cells. Glucose-6-phosphatase and acid phosphatase occurred in the apical region of the gut epithelium. Alkaline phosphatase occurred in vesicles in apical cytoplasm and in the basal plasma membrane infoldings of the epithelial cells. Aquaporin was found in the basal region of the midgut epithelium and in the associated visceral muscles. FMRF-amide was found only in nerve endings in the anterior midgut region. All cells in the anterior midgut region were rich in lysosomes. These results suggest that in both bee species, although they have anatomically different anterior midgut regions, these regions present high metabolic activity and function in cellular homeostasis, lipid absorption and are under neurohormone control.

1. Introduction

Wild and managed bees have been used as a pollinators to increase agricultural yields. The honey bee *Apis mellifera* (Apidae: Apini) pollinates a variety of important crops (Aizen and Harder, 2009). However, ecosystem destruction (Grixti et al., 2009) and agricultural practices, especially pesticide use, have been decimating bee populations (Fairbrother et al., 2014; Goulson et al., 2015). Recently, there is a strong decline in global honeybee populations (van Engelsdorp et al., 2009; Lima et al., 2016), which has been associated with pathogens, pesticides and a lack of flowers (Goulson et al., 2015). This phenomenon has been characterized as colony collapse disorder (CCD), a disease in which adult workers disappear and die resulting in the collapse of the entire colony (Neumann and Carreck, 2010).

As a consequence of CCD in honeybees, native bees have emerged as potential candidates as a future alternative for commercial pollinating (Garibaldi et al., 2016). In Neotropical regions, stingless bees (Apidae: Meliponini) are the most abundant and common native bees, with social behavior, colonies with a high number of individuals and visiting a wide range of plants (Roubik, 1989). This scenario increases the need to understand fundamental aspects of these bees such as digestive tract morphology and physiology.

The digestive tract of insects is divided into foregut, midgut and hindgut (Snodgrass, 1935). In bees, the proventriculus is the most specialized region of the foregut responsible for food storage and transport to the midgut (Snodgrass, 1956; Serrão, 2001, 2005). The

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posterior region of the proventriculus protrudes into the midgut lumen, folds forward and connects with the midgut wall forming the stomodeal valve, which prevents food reflux (Snodgrass, 1956; Serrão and Cruz-Landim, 1995; Serrão et al., 2008).

A short region of flattened cells marks the transition from the stomodeal valve (foregut) to the midgut epithelium, which is characterized by many folds in the wall along the entire organ (Serrão et al., 2008). However, in stingless bees, the fore-midgut transition is quite different with the midgut wall presenting no folds until the end of the stomodeal valve, at which point the folds begin (Peixoto and Serrão, 2001). In this anatomically differentiated region in stingless bees, the epithelium has taller and narrower cells than those in the remaining midgut (Serrão and Cruz-Landim, 1996).

The function of the differentiated anterior midgut region in Meliponini is unknown.In Diptera, the anterior midgut region is termed cardia and plays a role in the production of the peritrophic matrix (King, 1988), but in bees, the peritrophic matrix is synthesized along the whole midgut (Serrão and Cruz-Landim, 1996; Marques-Silva et al., 2005; Teixeira et al., 2015). Serrão et al. (2008) suggested that this midgut region in stingless bees may have some role in transporting water from the gut lumen avoiding dilution of digestive enzymes.

The objective of this study was to compare the occurrence of enzymes, membrane transporters, hormone and lysosomes in the cells of the anterior midgut region of the stingless bee *Melipona quadrifasciata*, which has an anatomically differentiated anterior midgut in comparison with the honey bee *A. mellifera* without that anatomical region. Data were assessed by histochemistry, cytochemistry and immunohistochemistry, contributing to our understanding of the physiological function of this anatomically differentiated region in stingless bees.

2. Materials and methods

2.1. Bees

Melipona quadrifasciata and *A. mellifera* adult workers were collected from nests in the central apiary at the Federal University of Viçosa, Viçosa, Minas Gerais state, Brazil, when returning with their corbiculae loaded with pollen grains.

2.2. Histology

Ten *M. quadrifasciata* and 10 *A. mellifera* workers were dissected in 125 mM NaCl and the anterior midgut region was separated and transferred to Zamboni fixative solution (Stefanini et al., 1967) for 2 h. The samples were dehydrated in a graded ethanol series (70%, 80%, 90%, and 95%) and embedded in historesin Leica. Two μm thick sections were stained with hematoxylin (15 min) and eosin (30 s) and analyzed with an Olympus BX 60 light microscope and photographed using the Q color 3 Olympus camera, Q capture software, 1024 × 768 pixels in size, with an objective lens of 4 x (numerical aperture 0.10) and 10 × 14 (numerical aperture 0.30).

2.3. Histochemistry

Fragments of the anterior midgut region of worker bees were obtained by dissection in 125 mM NaCl solution as described. All samples were analyzed with an Olympus BX 60 light microscope and photographed using the Q color 3 Olympus camera, Q capture software at 682 × 512 pixels, 8 bit with objective lens of 10 x (numerical aperture 0.30), 20 x (numerical aperture 0.50) and 40 x (numerical aperture 0.75). They were submitted to the following histochemical tests.

2.3.1. β – Galactosidase

Samples of the anterior midgut regions of five *M. quadrifasciata* and five *A. mellifera* were incubated in 1 mL of polyvinylalcohol (18 g of polyvinyl alcohol in 100 mL of 0.1 M sodium acetate buffer, pH 4.0), 10 μl of NaCl (580 mg/mL) and 20 μL of 5-bromo-4-chloro-3-indolyl- B-galactopyranosidase (150 mg dissolved in 20 μL of 2- ethoxyethanol) at 37 °C for 2 h. After incubation, the samples were washed in distilled water at 60 °C and dehydrated at room temperature in a graded ethanol series and embedded in historesin Leica. Negative control was performed by omission of the substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranosidase from the incubating medium. 4 μm thick sections were counterstained with 1% hematoxylin for 10 min and analyzed with a light microscope.

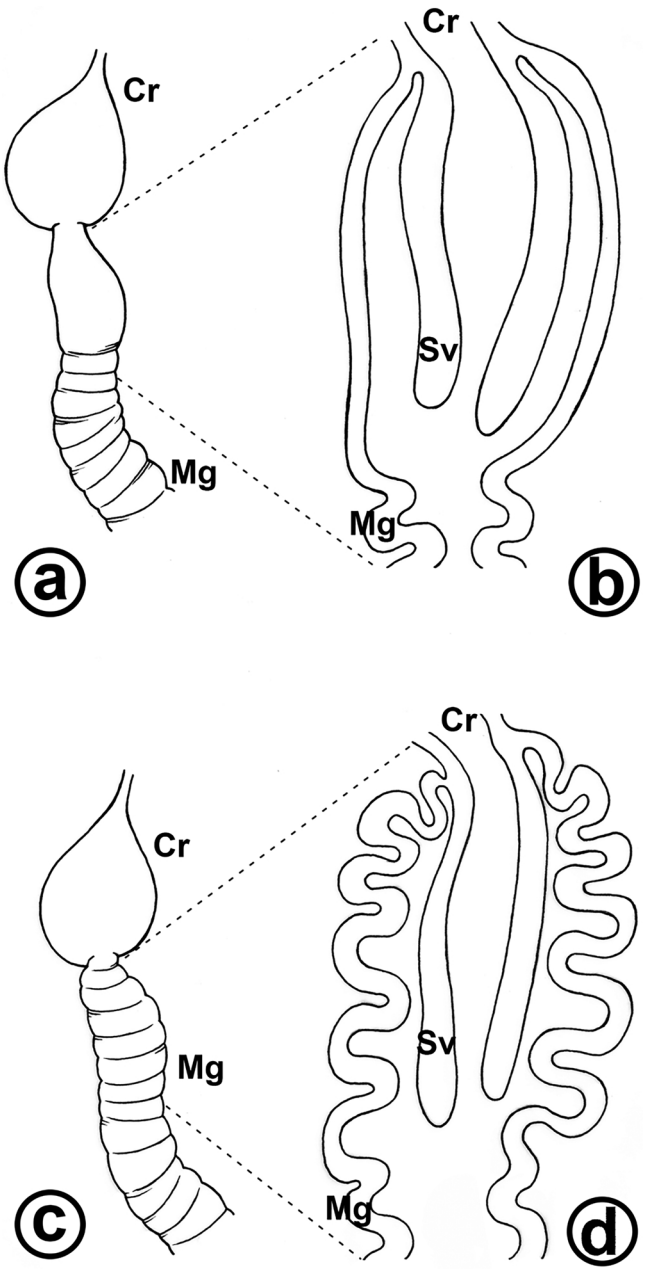

Fig. 1. [a] Schematic representation of the midgut in *Melipona quadrifasciata* showing crop (Cr) and midgut (Mg) without folds in the anterior region. [b] Schematic representation of the anterior midgut in *M. quadrifasciata* showing crop (Cr), stomodeal valve (Sv) and midgut wall without epithelial folds (Mg). [c] Schematic representation of the midgut in *Apis mellifera* showing crop (Cr) and midgut (Mg) with folds in the anterior reigon. [d] Schematic representation of the anterior midgut in *A. mellifera* showing crop (Cr), stomodeal valve (Sv) and midgut wall with epithelial folds (Mg). Not drawn with scale.