Neotropical Entomology

ISSN: 1519-566X

journal homepage: www.scielo.br/ne

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Morphometry of the Midgut of *Melipona quadrifasciata anthidioides* (Lepeletier) (Hymenoptera: Apidae) during Metamorphosis

LC Cruz[1], VA Araújo[2], H Dolder[3], APA Araújo[4], JE Serrão[1], CA Neves[1]

[1]Depto de Biologia Geral, Univ Federal de Viçosa, Viçosa, MG, Brasil
[2]Instituto de Ciências Biológicas e da Saúde, Univ Federal de Viçosa, Rio Paranaíba, MG, Brasil
[3]Depto de Biologia Celular, Univ Estadual de Campinas, Campinas, SP, Brasil
[4]Depto de Biologia, Univ Federal de Sergipe, São Cristovão, SE, Brasil

Keywords
Cell volume, digestive cell, regenerative cell, stingless bee

Correspondence
Clovis A Neves, Lab de Biologia Estrutural, Depto de Biologia Geral, Univ Federal de Viçosa, 36570-000, Viçosa, MG, Brasil; caneves@ufv.br

Edited by Fernando L Cônsoli – ESALQ/USP

Received 28 October 2010 and accepted 30 March 2011

Abstract

In Hymenoptera, midgut changes begin in the last instar. At this stage, the larval epithelial digestive cells degenerate, leaving only the basal membrane and the regenerative cells which will develop into a new epithelium during the pupal stage and in the adult. Epithelium renewal is followed by changes in volume and shape of the midgut. Morphometric analysis of digestive cells and total midgut volume of *Melipona quadrifasciata anthidioides* (Lepeletier) were conducted to verify whether cell volume increase are sufficient to account for the total midgut volume increase that occurs during metamorphosis. An increase in midgut volume was verified in spite of the scarcity of cell proliferation found during metamorphosis. At the end of metamorphosis, the increase in cell volume was not sufficient to explain the increase in volume of the midgut, indicating that an increase in the number of digestive cells is apparently necessary. Nevertheless, the mechanism by which regenerative cells reconstitute the epithelium during metamorphosis remains unknown.

Introduction

The midgut epithelium of bees consists of three cell types: digestive, endocrine and regenerative cells (Cruz *et al* 2007). The regenerative cells may be found either isolated, in pairs, or forming small groups called cell nests, which are fundamental for digestive epithelium renewal. The more abundant digestive cells are responsible for the synthesis and release of enzymes and the absorption of digestive products (Lehane & Billingsley 1996). During metamorphosis, the larval digestive epithelial cells undergo apoptosis and are replaced by regenerative cells present in the larval midgut (Neves 2002, Martins *et al* 2005). During regenerative cell differentiation, cells elongate towards the midgut lumen and acquire microvilli, followed by an increase in nucleus and cytoplasm volume, to form the cells that will become the digestive cells of the adult insect (Werner *et al* 1991, Cruz-Landim *et al* 1996, Neves *et al* 2003, Martins *et al* 2005).

In Hymenoptera, changes in midgut begin in the last instar with the degeneration of the larval epithelium, so that only the basal membrane and the regenerative cells remain. Concurrently, regenerative cells proliferate and spread along the basal membrane to renew the epithelium (Gama *et al* 1984, Neves *et al* 2002, Martins *et al* 2005). During bee metamorphosis, proliferation of regenerative cells in the midgut epithelium is rarely observed (Neves *et al* 2002, Martins *et al* 2005).

Vertebrate and invertebrate digestive tracts are similar in development, cellular types and genetic control.

In *Drosophila*, midgut stem (regenerative) cells have been investigated as a new model to understand the fundamental biological mechanisms that control stem cell behavior (Ohlstein & Spradling 2006). Studies of other insect groups, such as social bees (Hymenoptera), may reveal new pathways for midgut epithelium renewal. This work aimed to study morphometrically the midgut of the stingless bee *Melipona quadrifasciata anthidioides* (Lepeletier), in an attempt to establish whether increased cell volume can explain the total volume changes described during metamorphosis.

Material and Methods

Insects

Brood combs were removed from colonies of *M. quadrifasciata anthidioides* and placed in Petri dishes in an environmental chamber at 29ºC. Thirty bees from each of the metamorphosis stages studied (prepupae, white eyed pupae and black eyed pupae) and adult forager workers were collected and further processed.

Light microscopy

Bees were dissected in insect saline solution (0.1M NaCl, 0.1M KH_2PO_4, 0.1M $NaHPO_4$), and the midguts obtained were transferred to Carson's formalin fixative (Carson *et al* 1973) for 12h. Midgut samples were dehydrated in graded ethanol series and embedded in historesin (Historesin, Leica). Semi-thin (3 µm) sections were stained with buffered borax-1% toluidine blue (pH = 7.3) and photographed in an Olympus BX-60 microscope, with a Q color 3 – Olympus digital camera.

Morphometric analysis

Morphometric analysis was carried out using the Image-Pro Plus 4.0 (Media Cybernetics) program. The volume of the digestive cells and total midgut volume were calculated according to the cylinder volume formula: $V = \pi R^2 \times h$. To calculate its total volume, the midgut was measured using the length (h) and diameter/2 (R) for 30 individuals at each metamorphosis stage. The calculation of cell volume was obtained from the height averages (h) and diameter/2 (R) of 30 cells from each 30 midgut measured. Because of the presence of midgut folds in the black-eyed pupae and workers, the average distances were calculated between the basal membrane at the apex and that at the base of the fold, in longitudinal histological sections of the midgut (Fig 1a-b).

Dataset were submitted to statistical analyses to investigate the relationship between digestive cell volume and midgut volume (y-variables) in the different developmental stages (x-variable). Morphometric data were submitted to one-way analysis of variance (ANOVA) at 5% significance level. Volume measurements of the digestive tube were calculated as logarithms in order to adjust the normality predicts. All analyses were performed using the R software (R Development Core Team 2005), followed by residual analysis in order to verify their acceptability, error distribution and over-dispersion.

Results

The volume of digestive cells changes according to the developmental stages from prepupae to black eyed pupae ($F_{3.120}$ = 121.41 and P < 0.001) (Fig 2). However,

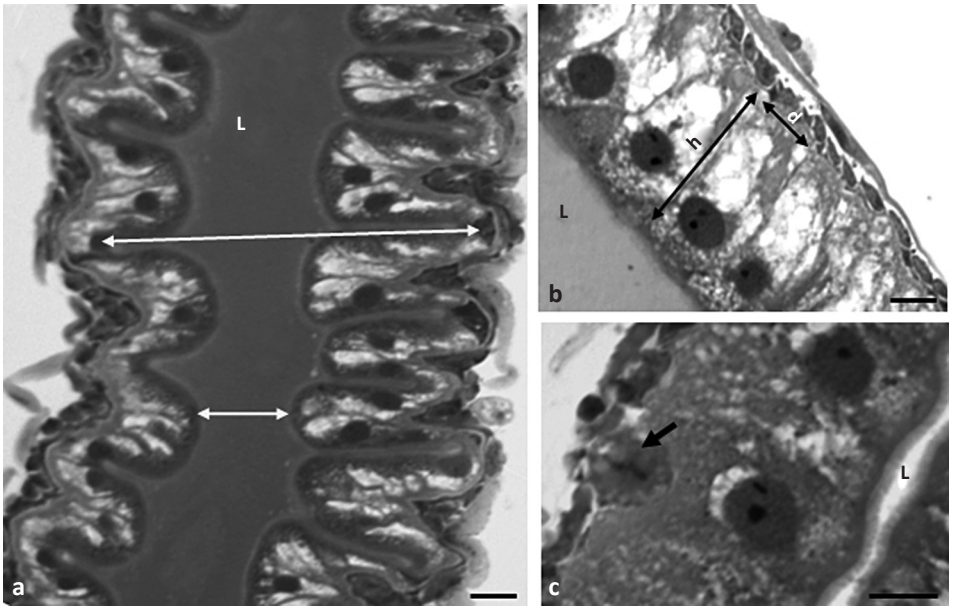

Fig 1 Longitudinal sections of the midgut of *Melipona quadrifasciata anthidioides*. a) Distances between the apex basal membrane and the fold base, used to calculate the total volume of the midgut; b) Height (h) and diameter measurements, used to calculate digestive cell volume; c) Black-eyed pupa, showing a cell in metaphase (arrow). Bar: a = 60 µm; b-c = 30 µm.

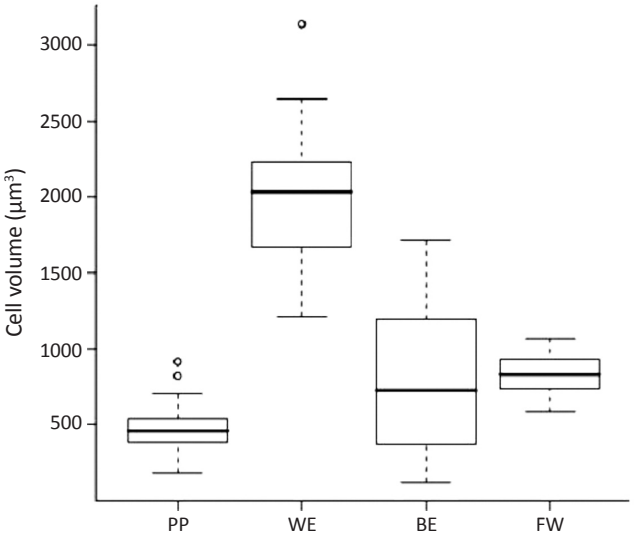

Fig 2 Variance analysis (ANOVA), with normal error distribution, relating developmental phases to cell volume. PP = prepupae; WE = white-eyed pupae; BE = black-eyed pupae; FW = forage workers.

no difference in cell volume was found between black-eyed pupae and workers ($F_{1.120}$ = 0.0838 and P = 0.7728). The largest cell volume was found in white-eyed pupae, followed by black-eyed pupae and workers, while the prepupae had the smallest digestive cell volume.

The midgut volume was different in all stages studied ($F_{3.120}$ = 517.89 and P < 0.001) (Fig 3), in which the largest volume was found in white-eyed pupae, followed by workers, whereas smaller midgut volumes were found in prepupae and black-eyed pupae. During metamorphosis,

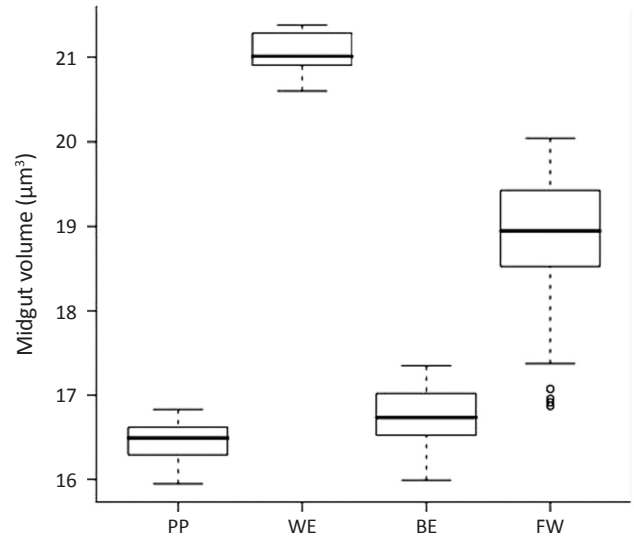

Fig 3 Variance analysis (ANOVA), with normal error distribution, relating the developmental stages of midgut volume. Measurements of digestive tract volume were shown as logarithms to adjust them to the distribution used. PP = prepupae; WE = white-eyed pupae; BE = black-eyed pupae; FW = forage workers.

an increase in cell number was not followed by an increase in cell volume and only one cell type was found proliferating in one of the specimens of black-eyed pupa (Fig 1c).

Discussion

In the present study, we investigated whether there are significant differences in cell volume and size during the metamorphosis of *M. quadrifasciata anthidioides*. This aspect of organ increase would be an interesting hypothesis to explain the growth and remodeling of the midgut considering the absence of an expressive amount of mitoses. Several investigations have focused on the reorganization of the bee midgut during metamorphosis (Neves *et al* 2002, 2003, Cruz-Landim & Cavalcante 2003, Martins *et al* 2005). Morphometric studies of insect midgut epithelial cells are rare, especially those concerning the distinct metamorphosis phases of the midgut. Most of the studies involve ultrastructural analysis in insect orders such as Diptera (Nopanitaya & Misch 1974, Wood & Lehane 1991, Andrade-Coelho *et al* 2001), Hemiptera (Billingsley 1988, Ranjini & Mohamed 2000) and Lepidoptera (Cioffi 1979, Lello *et al* 1984, Santos *et al* 1984).

The significant increase in the length of the midgut, as found for *M. quadrifasciata anthidioides,* has already been reported in Hemiptera (Billingsley 1988) and Lepidoptera (Pinheiro *et al* 2003, Pinheiro & Gregório 2006, Levy *et al* 2009). It is known that in stingless bees, the increase in midgut size during the larval stage is due to the growth of digestive cells (Cruz-Landim & Mello 1970), and to the increase in cell number due to proliferation and differentiation of regenerative cells (Serrão & Cruz-Landim 2000, Martins *et al* 2005).

During the prepupal stage, the midgut volume decreases due to the elimination of the larval digestive cells together with the feces, defining the end of the larval stage. Newly differentiated digestive cells are tall and narrow resulting in a small cell volume. At the pupal stage, there is no evidence of cell proliferation, likely reported by Martins *et al* (2005) who detected the proliferation of regenerative cells only in the prepupae of *M. quadrifasciata anthidioides* by the incorporation of BrdU, which could possibly mark the origin of regenerative cell nests in adults.

An increase of the cell and midgut volume was observed in white-eyed pupae. The increased cell size results in progressive development of a well organized cell monolayer, without the increase in cell number. Cell volume increase can explain by itself the increase in the total midgut volume herein observed. On the other hand, the increase in the midgut volume in black-eyed pupae was not accompanied by an increase in cell volume. Instead, there is a decrease in cell size, and cells

already display the dimensions they will have in adult bees. Furthermore, during this period, of the black-eyed pupae as in adults, the onset of regenerative cell nests was identified.

These data indicate the need for new digestive cells in order to increase the midgut volume. However, in this study, only a single cell in mitosis was observed, despite the expressive number of images analyzed. It is noteworthy that Moreira *et al* (2008) found many proliferating cells in histological sections of termite midgut using the same procedures employed in this study. Rost (2006) suggested that the regenerative cells divide synchronically, and due to the short chromosome condensation period during mitosis, cell proliferation may have been underestimated in the midgut sections analyzed. Another possible hypothesis would be that undifferentiated cells from the hemolymph could migrate across the basal membrane to participate in the epithelium (Baldwin & Hahim 1991). However, this has not been demonstrated in bees yet.

In conclusion, the cellular proliferation level observed in the midgut of *M. quadrifasciata anthidioides* pupae is insufficient to promote the increase in number of digestive and regenerative cells. Perhaps, the midgut epithelium of adult *M. quadrifasciata anthidioides* might develop a mechanism of stem-like cells migration or mitoses that occur in undetected synchronous phases.

Acknowledgments

This research was supported by the financial agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, and Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG.

References

Andrade-Coelho CA, Santos-Mallet J, Souza NA, Lins U, Meirelles MNL, Rangel EF (2001) Ultrastructural features of the midgut epithelium of females *Lutzomia intermedia* (Lutz and Neiva, 1912) (Diptera: Psychodidae: Phlebotominae). Mem Inst Oswaldo Cruz 96: 1141-1151.

Baldwin KM, Hakim RS (1991) Growth and differentiation of the larval midgut epithelium during molt in the moth, *Manduca sexta*. Tissue Cell 23: 411-422.

Billingsley PF (1988) Morphometric analysis of *Rhodnius prolixus* Stal (Hemiptera:Reduviidae) midgut cells during blood digestion. Tissue Cell 20: 291-301.

Carson FL, Martin JH, Lynn JA (1973) Formalin fixation for electron microscopy: a re-evaluation. Am J Clin Pathol 59: 365-373.

Cioff M (1979) The morphology, fine structure of the larval midgut of a moth (*Manduca sexta*) in relation to active ion transport. Tissue Cell 11: 467-479.

Cruz LC, Araújo VA, Dolder H, Neves CA (2007) Midgut ultrastructure of queens and foraging workers of *Melipona quadrifasciata anthidioides* (Hymenoptera, Apidae, Meliponini). Sociobiology 50: 1117-1125.

Cruz-Landim C, Cavalcante VM (2003) Ultrastructural and cytochemical aspects of metamorphosis in the midgut of *Apis mellifera* L. (Hymenoptera: Apidae: Apinae). Zool Sci 20: 1099-1107.

Cruz-Landim C, Mello MLS (1970) Post-embryonic changes in *Melipona quadrifasciata anthidioide*s Lep. IV. Development of the digestive tract. (1). Bol Zool Biol Marinha 27: 229-263.

Gama V, Cruz-Landim C (1984) Morfologia do tubo digestivo de *Camponotus* (*Myrmothix*) *rufipes* (Fabricius, 1975) (Hymenoptera, Formicidae) durante a metamorfose. Naturalia 9: 43-55.

Kerr WE, Carvalho GA, Nascimento VA (1996) Abelha uruçu – biologia, manejo e conservação. Belo Horizonte, Fundação Acangaú, 144p.

Lehane MJ, Billingsley PF (1996) Biology of the insect midgut. London, Chapman & Hall, 235p.

Lello EH, Bishoff ST, Misch DW (1984) Histopathological effects of tobacco hornworn larvae (*Manduca sexta*): low doses compared with fasting. J Invert Pathol 43: 169-181.

Levy SM, Moscardi F, Falleiros AMF, Silva RJ, Gregório E A (2009) A morphometric study of the midgut in resistant and non-resistant *Anticarsia gemmatalis* (Hubner) (Lepidoptera: Noctuidae) larvae to its nucleopolyhedrovirus (AgMNPV). J Invert Pathol 101: 17-22.

Martins GF, Neves CA, Campos LO, Serrão JE (2005) The regenerative cells during the metamorphosis in the midgut of bees. Micron 37: 161-168.

Moreira J, Neves CA, Araújo VA, Araújo APA, Cruz LC, Moreira PA, Rocha SL (2008) Digestive system morphology of *Nasutitermes rotundatus* (Isoptera: Termitidae, Nasutitermitinae). Sociobiology 51: 563-578.

Neves CA (2002) Estudo ontogenético, comparativo e ultraestrutural das células enteroendócrinas FMRFamida-"like" imunorreativas e descrição ultraestrutural do intestino médio de *Melipona quadrifasciata anthidioides* (Hymenoptera, Apidae, Meliponini) durante a metamorfose. Rio de Janeiro, RJ. Tese de doutorado, Instituto de Ciências Biomédicas, UFRJ, 113p.

Neves CA, Serrão JE, Gitirana LB (2003) Ultrastructural study of the metamorphosis in the midgut of *Melipona quadrifasciata anthidioides* (Apidae, Meliponini) worker. Sociobiology 41: 443-459.

Nishiura JT (2002) Coordinated morphological changes in midgut, imaginal discs, and respiratory trumpets during metamorphosis of Aedes aegypti (Diptera: Culicidae). Ann Entomol Soc Am 95: 498-504.

Nopanitaya W, Misch DW (1974) Development cytology of the midgut in the Flesh-fly, *Sarcophaga bullata* (Parker). Tissue Cell 6: 487-502.

Ohlstein B, Spradling A (2006) The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. Nature 439: 470-474.

Pinheiro DO, Gregório EA (2006) Morphometric study of the midgut epithelium in larvae of *Diatrea saccharalis* Fabricius, 1794 (Lepidoptera) parasitized by *Cotesia flavipes* Cameron, 1891 (Hymenoptera). J Invert Pathol 93: 60-62.

Pinheiro DO, Silva RJ, Quagio-Grassioto I, Gregório EA (2003) Morphometric study of the midgut epithelium in larvae of *Diatrea saccharalis* Fabricius (Lepidoptera: Pyralidae). Neotrop Entomol 32: 453-459.

Rost MM (2006) Ultrastructural changes in the midgut epithelium in *Podura aquatica* L. (Insecta, Collembola, Arthropleona) during regeneration. Arthr Struc Devel 35: 69-76.

Santos CD, Ribeiro AF, Ferreira C, Terra W (1984) The larval midgut of the cassava hornworm *(Erinnyis ello*): ultrastructure, fluid fluxes, the secretory activity in relation to the organization of digestion. Cell Tissue Res 237: 565-574.

Serrão JE, Cruz-Landim C (1996) Ultrastructure of digestive cells in stingless bees of various ages (Hymenoptera, Apidae, Meliponinae). Cytobios 88: 161-171.

Serrão JE, Cruz-Landim C (2000) Ultrastructure of the midgut epithelium of Meliponinae larvae with different developmental stages and diets. J Apic Res 39: 9-17.

Silveira FA, Melo GAR, Almeida EAB (2002) Abelhas brasileiras – sistemática e identificação. Belo Horizonte, Editora Composição e Arte, 253p.

Tettamanti G, Crimaldi A, Casartelli M, Ambrosetti E, Ponti B, Congiu T, Ferrrese R, Rivas-Pena ML, Pennacchio F, Eguileor M (2007) Programmed cell death and stem cell differentiation are responsible for midgut replacement in *Heliothis virescens* during prepupal instar. Cell Tissue Res 330: 441-449.

Werner K, Moutairou K, Werner G (1991) Formation and structure of the surface coat in the midgut of a waterstrider *Gerris najas* Deg. (Heteroptera: Gerridae). Int J Insect Morphol Embryol 20: 69-77.

Wood A R, Lehane M J (1991) Relative contributions of apocrine ad eccrine secretion to digestive enzyme release from midgut cells of *Stomoxys calcitrans* (Insecta: Diptera). J Insect Physiol 37: 161-166.