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THE MIDGUT OF THE PARASITOID *CAMPOLETIS FLAVICINCTA* (HYMENOPTERA: ICHNEUMONIDAE)

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Abstract

The midgut epithelium of insects is composed mainly of digestive cells, responsible for the digestion and absorption of food, and regenerative cells, which play a role in cell renewal. The morphological and histochemical analyses of the midgut of *Campoletis flavicincta* (Ashmead) (Hymenoptera: Ichneumonidae) showed that it is similar to those of other Hymenoptera. Morphometric analyses revealed differences in cell height, length of the striated border, and nuclear area of the digestive cells along the midgut. The nuclear area of regenerative cells was similar between the anterior and posterior midgut regions. These results demonstrate that the anterior and posterior midgut regions are morphologically distinct from each other.

Key Words: epithelium, morphometric, digestive cells and regenerative cells

Resumen

El epitelio del intestino medio de los insectos está compuesto principalmente por células digestivas, responsables de la digestión y absorción del alimento, y células regenerativas, responsables de la regeneración celular. El análisis de la morfología e histoquímica del intestino medio de *Campoletis flavicincta* (Ashmead) (Hymenoptera: Ichneumonidae) mostró que el intestino medio tiene una morfología similar a otros Hymenopteros. El análisis morfométrico mostró diferencias en la altura de las células, la longitud de las microvellosidades y el área nuclear de las células digestivas a lo largo del intestino medio. El área nuclear de las células regenerativas fue evaluado y no se encontraron diferencias significativas entre el intestino anterior y posterior. Los resultados mostraron que las regiones anterior y posterior del intestino medio son distintas.

Palabras Clave: epitelio, morfometría, células digestivas, células regenerativas

Campoletis flavicincta is an important parasitoid of Lepidoptera larvae found in subtropical and temperate regions of North and South America. This insect is a potential biocontrol agent of the caterpillar *Spodoptera frugiperda* (Smith) because it parasitizes the first instar of this pest, before it can cause significant damage to corn plantations (Dequech et al. 2005). Larvae of *C. flavicincta* feed on the internal contents of the host, and exit the caterpillar body to build a cocoon in the external environment at the onset of the pupal stage (Cruz et al. 1995; Cruz 1995). The life cycle of the parasitoid is 19 days, including 12 days from egg to pupa and 7 days of the pupal period. Adults feed upon nectar and have longevities of 23 days for females and 29 days for males (Ashley 1979; Patel & Habib 1987; Cruz et al. 1995; Cruz et al. 1997).

Storage of nutritional resources is important for holometabolous species that have developmental stages during metamorphosis when insects do not feed. The midgut is the main organ responsible for receiving food and selectively provides nutritional resources for development (Klowden 2007; Hakim et al. 2010).

The midgut of hymenopterans is a relatively long tube, lined with a single layered epithelium, 2 layers of circular (inner) and longitudinal (outer) muscles, and a peritrophic membrane enclosing the food (Snodgrass 1935; Serrão & Cruz-Landim 1996b). The midgut epithelium consists of 3 cell types: digestive or principal cells, regenerative cells and endocrine cells (Cruz-Landim & Mello 1970; Cruz-Landim et al. 1996; Neves et al. 2003; Teixeira et al. in press). Digestive cells have microvilli on their apical surfaces and invaginations in the basolateral plasma membrane. Digestive cells are responsible for the synthesis of digestive enzymes and absorption of digested products (Terra 1990; Hakim et al. 2010; Fialho et al. in

press). Regenerative cells differentiate into new digestive cells, constantly renewing the intestinal epithelium (Billingsley & Lehane 1996; Cruz et al. 2011). Endocrine cells are responsible for release of small peptides with hormonal characteristics. Although the biological role of these cells is not clearly established, they are involved in digestion, peristalsis, diuresis and development (Cruz-Landim 2009; Takashima et al. 2011).

Besides being the largest endocrine organ of insects, the midgut is responsible for digestion and absorption (Zitnan et al. 1993; Hakim et al. 2010). In order to contribute to a better understanding of digestive processes in parasitoids, this paper describes the morphology of different midgut regions of newly emerged *C. flavicincta* adults.

MATERIALS AND METHODS

Twelve males of *C. flavicincta* were obtained from the Entomology Laboratory, Embrapa Maize and Sorghum, Sete Lagoas, Minas Gerais state, Brazil. These parasitoids were reared on an artificially controlled environment (25 ± 2 °C, $70 \pm 10\%$ RH and 12:12 h L:D and fed a 10% honey solution. Twenty-four h old adults were anesthetized at 4 °C and their midguts dissected in saline solution (0.1 M NaCl, 20 mM KH_2PO_4 and 20 mM Na_2H-PO_4). The midguts were transferred to Carson's formalin fixative solution (Carson et al. 1973) for 24 h at room temperature. After fixation, 6 midguts were washed in distilled water and stained with diamidino-2-phenylindole (DAPI) for 30 min, mounted in 50% sucrose solution and observed under an epifluorescence microscope (Olympus BX-60) with a UV excitation filter. Images were obtained with a digital camera (Q-Color, 3 Olympus) coupled to the microscope. Six more midguts were dehydrated in a graded ethanol series, embedded in histoResin (HistoResin Leica), sectioned into 3-μm thick slices and stained with hematoxylin and eosin. Some sections were subjected to Alcian Blue (AB) histochemical tests at pH 2.5 conjugated with the Periodic Acid Schiff (PAS) (Gajger et al. 2011) to determine the presence of glycogen, glycoproteins, glycolipids, acid mucins, acid glycoconjugates and neutral glycoconjugates. The images obtained from DAPI stained whole midguts were used to count the number of digestive and regenerative cells. Twenty digestive and 10 regenerative cell nuclei were used to measure nuclear area. The histological sections were used to measure the height of digestive cells, the length of the striated border, the nuclear area and the area of the regenerative cell nests. Morphometric data were obtained from the computer program Image-Pro Plus 4.5 (Media Cybernetics) and analyzed through Student's *t* test or Mann-Whitney statistical tests for data with normal and non-normal distribution, respectively(5% significance level).

RESULTS

The midgut epithelium of *C. flavicincta* was formed by a single layer of columnar digestive cells with a well-developed striated border and oval nuclei possessing 1 or 2 evident nucleoli. Scattered regenerative cells nests were present at the base of the digestive cells, close to the basal membrane. External to the midgut epithelium of *C. flavicincta*, an inner circular and an outer longitudinal muscular layers were present (Figs. 1 and 2). The median-apical region of digestive cells was strongly basophilic while the basal portion was predominantly acidophilic (Fig. 2).

Alcian blue/PAS (AB/PAS) histochemical test showed a strong reaction to PAS in the striated border and cytoplasmic granules in the apical portion of digestive cells. The basal and peritrophic membranes exhibited a moderate reaction to PAS, allowing their identification; whereas the regenerative cell nests and muscle layers were negative for this histochemical test (Fig. 1). The negative reaction for AB indicated the absence of acid glycoconjugates such as gastrointestinal acid mucins. However, some regions had neutral glycoconjugates that showed PAS positive reactions.

Morphometric data of the histological sections showed significant differences among the anterior and posterior midgut regions of *C. flavicincta*. The anterior midgut region showed longer digestive cells (34 ± 4.6 μm) and a longer striated border (3.6 ± 0.6 μm) than the posterior midgut region [($P < 0.05$) (Figs. 5 and 6)].

The morphometry of the entire midgut under DAPI staining showed that nuclei of digestive cells were larger in the anterior midgut region (45 ± 3.36 μm^2) compared with the posterior midgut region (25 ± 2.04 μm^2) [($P < 0.05$) (Fig. 7)]. The number of digestive cells was greater in the posterior midgut region, with 107 ± 3.8 cells, whereas the anterior midgut region had 90 ± 5.3 cells [($P < 0.05$) (Fig. 8)].

Images obtained from DAPI demonstrated that digestive cells are surrounded by regenerative cells or form nests with up to six cells (Figs. 3 and 4). The nucleus size of regenerative cells (4.13 ± 1.2 μm^2) and the number of cells (250 ± 24 cells) were similar in the anterior and posterior midgut regions [($P = 0.879$) (Figs. 9 and 10)]. No digestive or regenerative cells in the process of cell division were found.

DISCUSSION

The midgut of *C. flavicincta* showed morphological differences between the anterior and posterior regions. The midgut is constituted by a simple epithelium formed by columnar digestive cells with a well-developed striated border surrounded by 2 muscle layers similar to other Hymenoptera (Serrão & Cruz-Landim 1995; Bution et al. 2006;
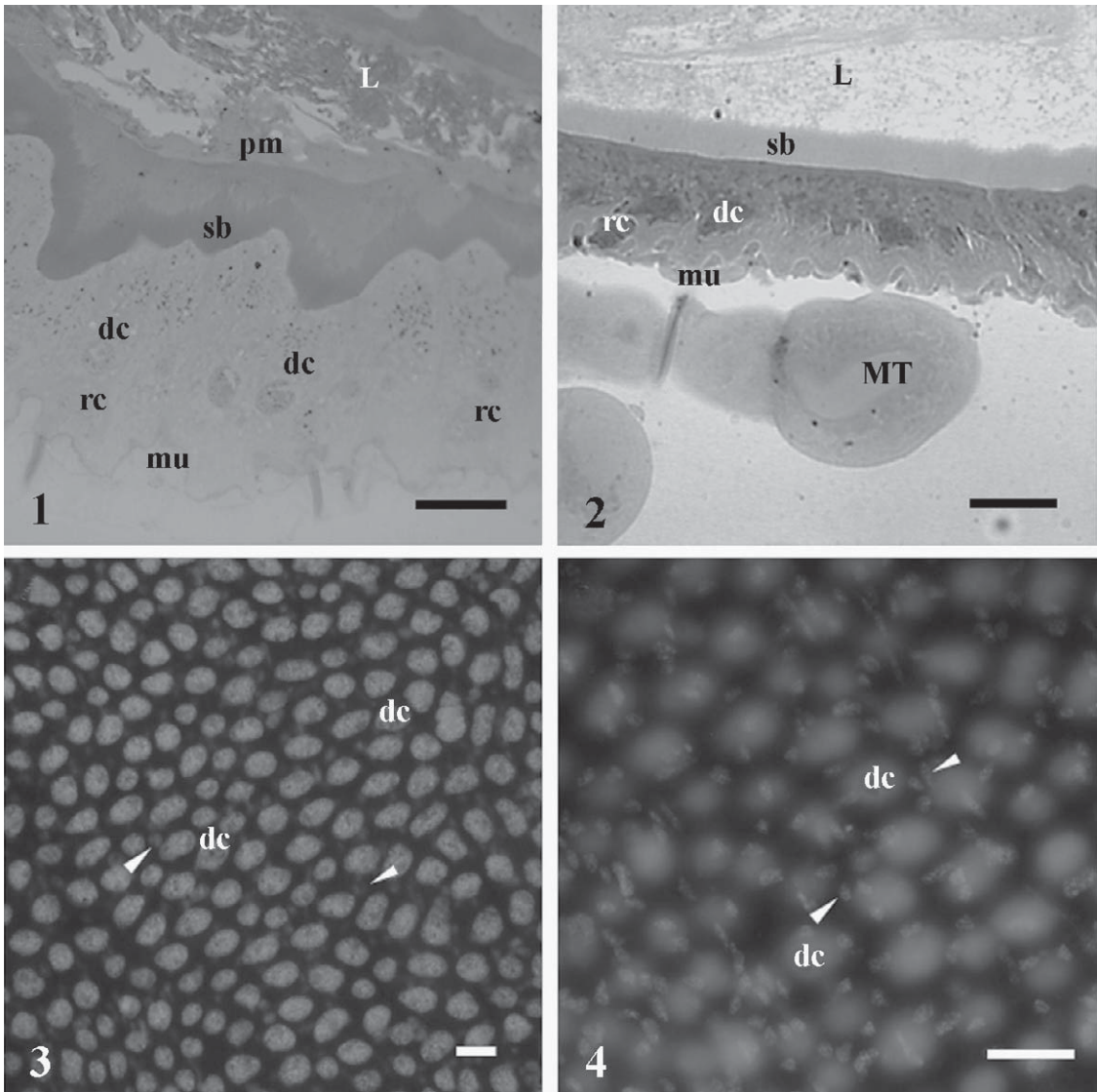
Figs. 1-4. Midgut of *Campoletis flavicincta* male. (1) Longitudinal section of the anterior region, Staining AB/PAS showing the digestive cells (dc), regenerative cells (rc), muscle (mu), striated border (sb), peritrophic membrane (pm) and lumen (L). (2) Longitudinal section of the posterior region, H.E. staining, showing the digestive cells (dc), regenerative cells (rc), muscle (mu), striated border (sb) and lumen (L). (3) MT - Malpighian tubules and (4) Posterior region of *Campoletis flavicincta* in total assembly showing nuclei of regenerative cells (arrowheads) and nuclei of digestive cells (dc). Bars = 20 µm.

Moreira et al. 2008; Bution & Caetano 2008; Gajger et al. 2011).

The midgut digestive cells showed polarity with the basophilic apex and PAS-positive granules protruding into the lumen, suggesting the secretion of neutral glycoconjugates along the entire midgut, similar to other hymenopterans (Neves et al. 2002; Bution et al. 2006; Bution & Caetano 2008). Insects release compounds to the midgut lumen such as digestive enzymes (Serrão & Cruz-Landim 2000; Fialho et al. 2013) and peritrophic membrane compounds (Marques-Silva et al. 2005) via apocrine secretion, which may be carbohydrates or glycoconjugates as shown by PAS-positive granules along the entire midgut of *C. flavicincta*.

No acidic glycoconjugates were detected by the Alcian blue test to acidic mucins; however, presence of neutral glycoconjugates was shown by the PAS test to the peritrophic membrane. The presence of abundant glycosylated molecules on the peritrophic membrane may be associated with lu-
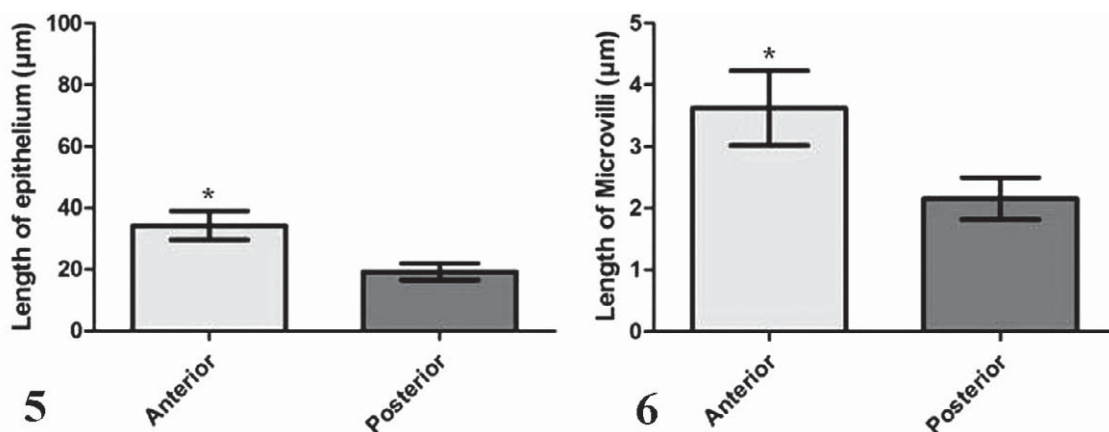
Figs 5-6. Morphometric analysis of midgut regions *Campoletis flavicincta* in histological section. (5) Epithelial height. (6) length of the striated border (microvilli). Vertical bars represent the standard deviation. * Indicates significant difference (t Student test at $P < 0.05$).

brication and epithelial protection against proteolysis and microorganism invasion (Vuoculo et al. 2001). The origin of the peritrophic membrane is unclear. However, the PAS-positive reaction of the peritrophic membrane, and the cytoplasm granules in the apical portion of the digestive cells, corroborate the hypothesis that the peritrophic membrane is synthesized along the whole midgut surface, and not only in the anterior region, as previously reported (Bowen 1968; Serrão & Cruz-Landim 1996a; Marques-Silva et al. 2005).

A variation in the number of digestive cells between the anterior and posterior midgut regions was also reported in the midgut of the stingless bee *Melipona quadrifasciata anthidioides* (Fernandes et al. 2010), which has fewer digestive cells in the anterior midgut region. In *C. flavicincta* the digestive cells were larger in the anterior region, resulting in a low number of digestive cells in this region. A difference in size of the digestive cells also occurs in *Diatraea saccharalis* (Lepidoptera), which exhibits larger cells in the posterior midgut region (Pinheiro et al. 2003; Pinheiro et al. 2006).

In *C. flavicincta* the striated border is longer in the anterior midgut region, similar to that found in the stingless bees *Trigona hypogea* and *Trigona spinipes,* which have longer microvilli in their midgut region (Serrão & Cruz-Landim 1996b). Longer microvilli result in increased surface area of the digestive cells, suggesting high nutrient absorption in the anterior midgut region as reported in some species of bees (Serrão & Cruz-Landim 2000), *Brontocoris tabidus* (Hemiptera: Pentatomidae) (Fialho et al. 2009) and the mosquito *Aedes aegypti* (Diptera: Culicidae) (Valotto et al. 2011).

The histological sections showed no significant differences in the nuclear area of digestive cells along the midgut; however, the DAPI stained digestive cells had larger nuclei in the midgut anterior region, suggesting a possible relationship with DNA amplification, necessary for increased cell activity (Billingsley 1989; Zudaire et al. 2004; Fernandes et al. 2010).

Our observations support the endo-ectoperitrophic digestion hypothesis proposed for bees, where the posterior midgut region is responsible for water release into the lumen, whereas the anterior midgut region plays a role in nutrient absorption. Nutrients are transported through water flux (Terra 1990; Shumaker et al. 1993). We suggest that the anterior midgut of *C. flavicincta* plays a role in absorption due to long striated border, whereas the posterior midgut may be responsible of water release to the midgut lumen.

Regenerative cells in the midgut of hymenopterans are located in the basal region of the epithelium, never reaching the lumen of the organ, and are found either isolated or forming nests (Raes et al. 1994; Martins et al. 2006; Fernandes et al. 2010). Cytoplasm projections form points of contact between the nests which could be responsible for cell differentiation synchronization of regenerative cells (Martins et al. 2006). However, the arrangement of regenerative cells is quite different in *C. flavicincta*, which are also found surrounding the digestive cells. Such organization suggests that digestive cells play a role in synchronization of cell differentiation of the regenerative cells.

The literature reports occurrence of mitosis in the midgut of *M. quadrifasciata anthidioides* pupae, but not in the adult stage (Cruz et al. 2013). Our study demonstrates that mitosis does not occur in digestive or regenerative cells of *C. flavicincta* adults, corroborating the hypothesis that no cell division occurs in the midgut cells of adult Hymenoptera (Cruz-Landim et al. 1996;
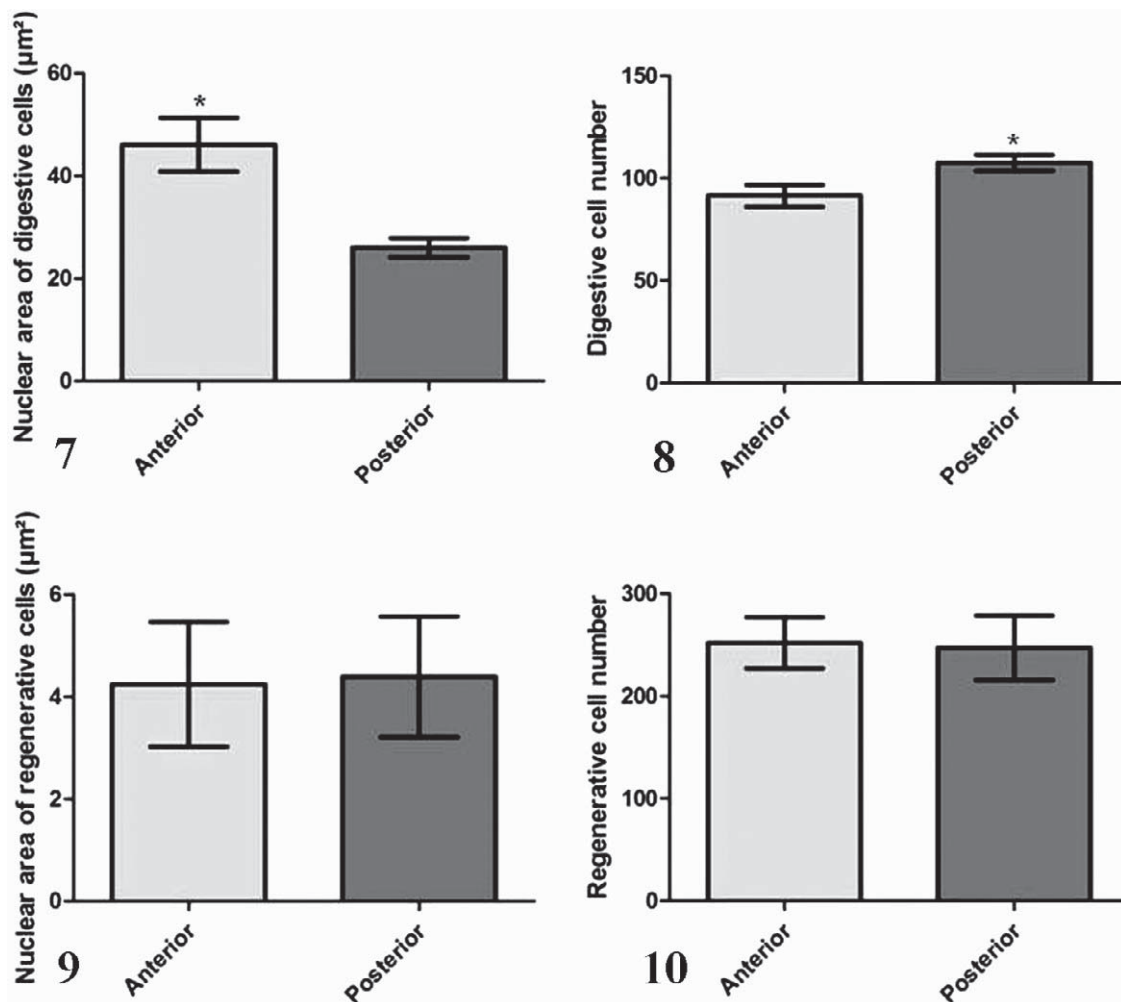
Figs. 7-10. Morphometric analysis of midgut regions of *Campoletis flavicincta* in total assembly (DAPI). (7) Area of nuclei of digestive cells. (8) Number of cells digestive. (9) Area of nuclei of regenerative cells. (10) Number of regenerative cells. Vertical bars are standard deviation. * Indicates significant difference (t Student test at $P < 0.05$).

Cruz-Landim & Moraes 2000). However, some adult Hymenoptera with long lifespan, such as bee queens, have replacement of digestive cells by mitosis (França et al. 2006).

This study showed functional and morphological differences between the anterior and posterior midgut regions of *C. flavicincta*. The high metabolic activity of the anterior midgut region did not result in differences in the number of regenerative cells between the regions.

The results of this study suggest the midgut of adult *C. flavicincta,* despite having carnivorous larval habits, is similar to necrophage and pollen feeding bees (Serrão & Cruz-Landim 1995). Although the gut of *C. flavicincta* does not have not specialized structures, it seems adapted to food digestion and absorption for insect development.

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