## SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

# Electrophoretic Protein Pattern and Acid Phosphatase Activity in the Midgut Extracts of *Apis mellifera* L. (Hymenoptera: Apidae) During Metamorphosis

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Padrão Eletroforético de Proteínas e Atividade de Fosfatase Ácida em Extratos do Intestino Médio de *Apis mellifera* L. Durante a Metamorfose

RESUMO - Foram pesquisadas variações no padrão eletroforético das proteínas e da atividade da fosfatase ácida contidas em extratos do intestino médio de *Apis mellifera* L. durante o último estágio larval e pupação com a finalidade de estabelecer um paralelo entre os resultados e os eventos da metamorfose. Verificou-se maior variedade de bandas protéicas durante o estágio de pré-pupa e menor na pupa de olho marrom. A atividade da fosfatase ácida foi maior durante o último estágio larval e menor na pupa de olho branco. A maior variedade de bandas protéicas na pré-pupa coincide com a histólise do epitélio larval e reconstituição do epitélio pupal, enquanto a menor variabilidade na pupa de olho marrom coincide com o fim da diferenciação do intestino médio. A maior atividade fosfatásica no último estágio larval pode ocorrer em razão da sua função na histólise do epitélio.

PALAVRAS-CHAVE: Desenvolvimento pós-embrionário, enzimas, histólise, larva, pré-pupa, pupa

ABSTRACT - The electrophoretic pattern of proteins and the activity of acid phosphatase were described in the midgut extracts during the post-embryonic stages of *Apis mellifera* L. in order to stablish a correlation with the metamorphosis events. The results show the greatest variation in proteic electrophoretical bands during prepupal stage and the smallest variation during the brown eyed pupae stage. The acid phosphatase activity was higher during the larval last instar and lower in white eyed pupae. The greatest variety of bands during the prepupal stage coincides with the histolysis of the larval midgut epithelium and the lowest variability in the brown eyed pupa coincides with the end of the midgut differentiation. In another hand, the greatest phosphatase activity in the last larval stage must reflect this enzyme actuation in the histolysis of the larval epithelium.

KEY WORDS: Differentiation, enzymes, histolysis, larva, prepupa, post-embryonic development, pupa

The larval digestive tract of *A.mellifera* L. is constituted by a foregut, whose single differentiation is the stomodeal valve; a midgut, totally undifferentiated; and a hindgut constituted by an ileum and a rectum (Snodgrass 1956).

The wall of these parts consists of two cellular layers, an inner of epithelial cells and an outer layer of muscular fibers. Each part of the digestive tract has specific functions in the food processing. The midgut function is to secret digestive enzymes, as well as to absorbe the resulting nutrients.

The changes that occur in the transformation of the larva into adult may be more or less extensive depending on the degree of differences between both phases, related to morphological and physiological traits. In the honey bee, although the midgut continues to be an anatomically undifferentiated tube, it is longer in adult than in larva. It has at least a Histologically differentiated segment around the cardiac valve and is physiologically differentiated along its lenght.

The food used by larvae and adults is the same, pollen and honey, but some differences must occur in its digestion since the passage from the midgut into the hindgut remains closed until the end of the larval phase, so blocking the food transit to the hindgut.

Before entering the pupation the midgut is emptied and during pupation it undergoes a series of modifications that result in the final adult form (Dobrovsky 1951, Cruz-Landim & Mello 1970). The cells of the midgut wall of the larva, mainly the digestive cells of the epithelial layer, die and are eliminated to the midgut lumen, where they are digested (Cruz-Landim & Mello 1970, Gama & Cruz-Landim 1984). During pupation new epithelial cells originated from proliferation of the larval regenerative cells and even these new cells, suffer several types of modifications that envolve elimination of cell parts to the lumen (Cavalcante 1998). Therefore during metamorphosis the midgut wall, the majority of the epithelial layer, undergoes extensive histolosis and histogenesis.

These events, by affecting cell differentiation and function, must involve physiological modifications in the enzymes present in the differentiating epithelium during metamorphosis. As the enzymes are proteins, the aim of the present study was to compare the eletrophoretic protein pattern and the acid phosphatase, a hydrolase, activity in midgut extracts during metamorphosis.

## Material and Methods

The bees used in the experiment were captured from colonies of the Instituto de Biociências apiary, in Rio Claro, São Paulo State, Brazil. The characterization of the developmental stage of the specimens obeyed the following criteria:
Last instar larvae – larvae that have already the upright position in the brood cell.
Prepupae – pupae that have already everted the external appendages, but still remain in the larval cuticle.
Pupae – after the larval ecdysis.

The advance in the pupal development was evaluated taking in account the eye pigmentation progress and the cuticle darkning. The following phases were studied: white eyed, pink eyed, red eyed, brown eyed pupae, and pupae with darkened tegumentar cuticle.

Five midguts from the last larval instar, prepupae and mentioned stages of pupae, were dissected in ice cold 0.9% NaCl, and stored at -20°C for protein quantification and polyacrylamide gel eletrophoresis.

The midgut extracts were obtained by homogenizing the midguts, and taking to a centrifuge at l0.000 rpm during 5 min. The supernatant was dissolved in 100µl of distiled water. Aliquots of these extracts were used for total protein quantification by the method of Bradford (1976) using bovine serum albumin as standard. Separation of the proteins was routinely performed on a gradient polyacrylamide gel under denaturating conditions (SDS-PAGE). Samples from the midgut extracts containing 60 µg of protein diluted in the buffer (Tris-HCl, 0.15M, pH 6.8), were boiled and loaded in polyacrylamide gels according the protocol of Weber & Osborn (1969). The molecular weight markers were bovine albumin (66 kDa), pepsin (34.7 kDa), Trypsinogen (24 kDa), blactoglobulin (18.4 kDa) and lysosim (14.3 kDa). Only the midgut wall extracts were used, i.e., before the maceration the larval peritrophic membrane with the content was taken off. The runing of the proteins were repeated twice, for assurance of the results, and the gels stained with Coomassie Brilliant Blue G-250.

The acid phosphatase activity was detected by the Ryder & Bowen (1975) method, using paranitrophenilphosphate (pNPP) as substrate. The substrate was prepared by dissolving 209 mg of pNPP in 100 ml of Na acetate buffer 0.01M, pH 5.5. The midgut extrates were incubated in 0.1M sodium acetate buffer pH 5.0 containing 1.9 ml of substract during 1h at 37°C. The incubation was interrupted by addition of NaOH. The results were read in spectrophotometer at 405 nm wave lenght. The procedures were repeated twice and the mean values for enzyme activity expressed in nanomols of paranitrophenol liberated/min/protein µg.

The extracts of the total midgut, the peritrophic membrane, with its food content, and the midgut wall (midgut without the peritrophic membrane) also were assayed for acid phosphatase activity.

## Results

The eletrophoresis shows the mass range of different proteins varying from 19 to 142 kDa encreased greatly from larvae to prepupae and tend to decrease during pupation until the phase of brown eyed pupae, encreasing again in the pupae with pigmented body. The wide variety of proteins molecular mass was present in prepupae and the low variety in brown eyed pupae (Fig. 1)

Only prepupae presented proteins with molecular weight over 69 KDa. In larvae and other pupal phases all proteins present had molecular weights below to 51 KDa. All extracts presented the 51 KDa protein and all pupae presented the 43 KDa protein which is absent from the larval extracts. Below 69 KDa all protein present in pupae were also present in prepupae with exception of the 20 KDa protein that were present in all extracts, less in the prepupal extracts (Fig. 1).

The eletrophoretic pattern showed also quantitative variation in the different proteins in the extracts as may be observed by the staining intensity of the bands (Fig. 1).

The acid phosphatase activity, in the extracts of the examined specimens, was also variable. The higher activity of the enzyme was observed in the last-instar larvae extracts, followed by a drastic fall in the prepupae extracts. In larval midgut the higher activity was observed in the peritrophic membrane extracts. The total midgut show an activity that is the sum of the peritrophic membrane and midgut wall activities (Fig. 2).

The midgut acid phosphatase activity during pupation was very low when compared to the levels found in this larval organ. If the total midgut is considered, the enzyme activity in larva is 13 times higher than the highest level that occurs during pupation, which is present in prepupae and brown eyed pupae (Fig. 3). However if only the larval midgut wall is considered, the differences are lower. The enzyme actitivy in the larva midgut wall is only 6 times higher than in prepupae and brown eyed pupae (Fig. 3).

## Discussion

During the last larval instar, the midgut presents the food content enveloped by the peritrophic membrane. Although the digestive enzymes are produced by the epithelial cells of the midgut wall, they are delivered to the peritrophic membrane lumen where food digestion takes place. The higher levels of acid phosphatase activity in the content of the larval peritrophic membrane extracts may be due to the enzyme presence in the digestive juice, in the food itself, mostly constituted by pollen grains, or in the midgut bacterial flora.

By the end of the last larval instar, after the midgut is emptied, the transformations that will turn the larval midgut into the adult organ begin. The functional larval cells are replaced by others that will function in adult. This replacement depends upon death of the larval digestive cells and proliferation of the regenerative cells in order to constitute a

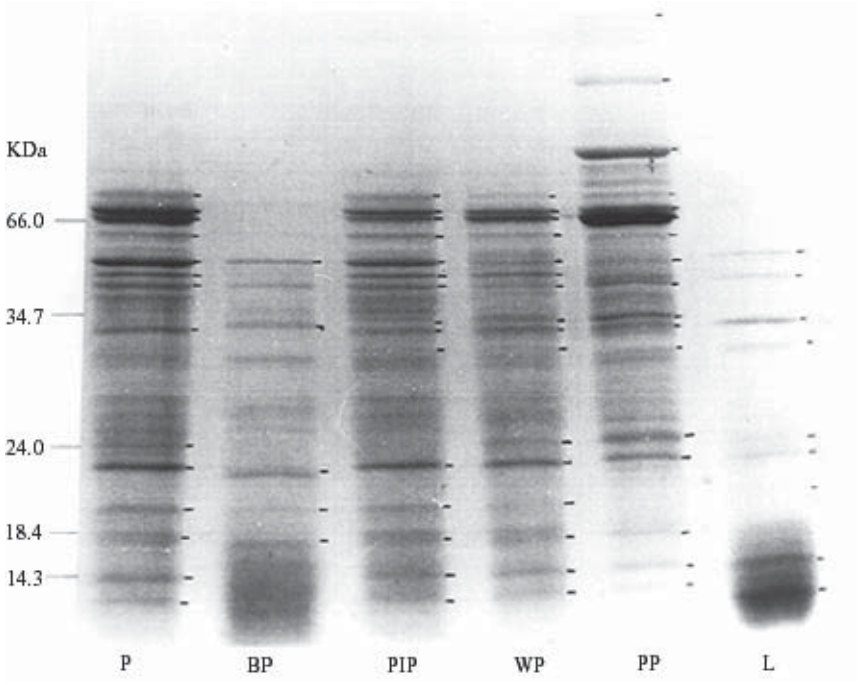


Figure 1. Polyacrilamide gel eletrophoresis from midgut of *A.mellifera* extracts of last instar larvae (L); prepupae (PP), white eyed pupae (WP), pink eyed pupae (PIP), brown eyed pupae (BP) and pupae with darkened body (P) stained with Coomassie Brilliant Blue G-250.

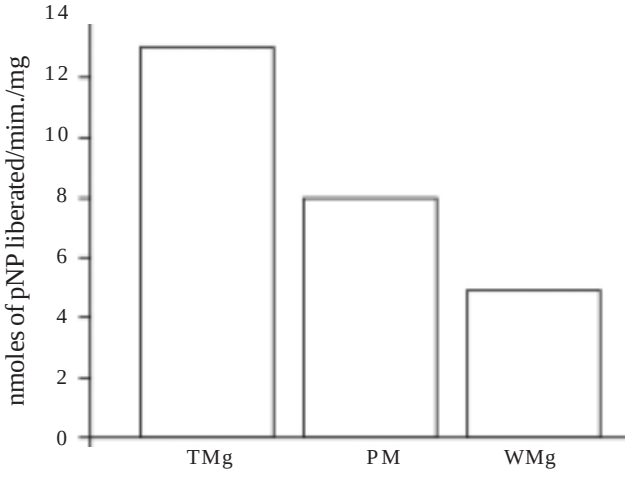


Figure 2. Acid phosphatase activity in different compartiments of the midgut of last larval instar of *A. mellifera*. TMg total midgut; PM peritrophic membrane; WMg midgut wall

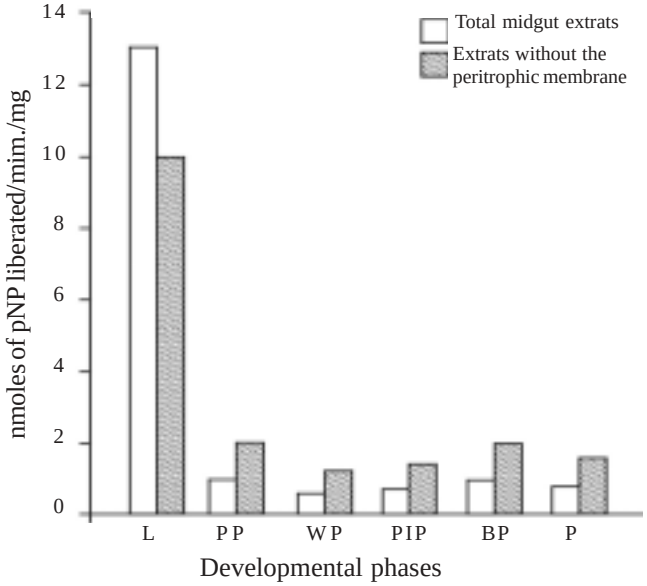


Figure 3. Comparison among acid phosphatase activity in extrats of the total midgut of *A. mellifera* larvae and pupae, and without the peritrophic membrane. (L) last larval instar; (PP) prepupae; (WP) white eyed pupae; (P/P) pink eyed pupae; (BP) brown pupae; (P) darkened pupae.

new epithelium of digestive cells to the adult (Cruz-Landim & Mello 1970, Grecorc & Bowen 1997).

During the epithelial renewing the dead cells are released to the midgut lumen, and there digested (Cruz-Landim & Silva de Moraes 2000). During this transition the midgut lose their ability of produce digestive enzymes and a peritrophic membrane is not present. Nevertheless, at the beginning of the pupation, in prepupae, eletrophoresis shows a great variety of protein types, some of which could be enzymes. Some have molecular weights similar to the chemotrypsin and trypsin found in the adult midgut of *A. mellifera* by Giebel *et al.* (1971) and Dahlman *et al.* (1978). In prepupae if present, these enzymes may be engaged in the dead cell digestion. The bands present in all extrats examined probably correspond to constitutive proteins of the midgut tissues.

The low variety of protein types observed during the white, pink and brown eyed pupa stages may reflet the absence the digestive enzymes, and represent only the structural proteins of the cells that constitute the organ. The higher levels of acid phosphatase activity and the lower number of different proteins in brown eyed pupae may be linked to the end of the adult midgut differentiation. The recovery of the protein variety at the end of the pupation (pupae with darkened body) represents a preparation to enter adult life.

As the midgut epithelium is remodelated during pupation, with discharge of cell parts to the lumen, the presence of acid phosphatase during the metamorphosis may be related to digestion of this material.

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