Protein content and electrophoretic profile of fat body and ovary extracts from workers of *Melipona quadrifasciata anthidioides* (Hymenoptera, Meliponini)

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ABSTRACT. Workers of *Melipona quadrifasciata anthidioides* (Lepeletier, 1836) develop their ovaries and lay eggs, therefore the production of vitellogenin is expected. In electrophoretic profiles only fat body extracts from nurse workers and ovary extracts from newly-emerged workers show protein with molecular mass similar to vitellogenin. However, an increase in the protein content was detected in forager fat body. This increase was attributed to storage of vitellogenin or other proteins in the previous phase and not discharged into the hemolymph or to an effect of the increased titre of juvenile hormone in this phase of worker life over the fat body functioning.

KEYWORDS. Stingless bees, fat body, ovary, vitellogenin, oogenesis.

INTRODUCTION

The insect fat body has basically two functions. One is related to the intermediate metabolism, which is responsible for the metabolic processes of synthesis of hemolymph proteins, storage of substances, such as lipids, proteins and carbohydrates (Wigglesworth, 1942; Wyatt, 1980; Keeley, 1985; Rosell & Wheeler, 1995) and accumulation of toxic materials such as urate (Babthan & Gilbert, 1972; Locke, 1984; Cruz-Landim, 1985; Dean *et al*., 1985). The other is the active participation in vitellogenesis, supplying the soluble precursor for the yolk, i.e., vitellogenin (Engelmann, 1971; Behan & Hagedorn, 1978; Tadbowski & Jones, 1979). In bees the first function of these cells is carried out in the immature phases, mainly in the larval phase, and the second mainly in the adults (Paes-de-Oliveira & Cruz-Landim, 2003b).

Fat body is the main source for the proteins found in the hemolymph (Palli & Locke, 1988). There is a constant exchange between the fat body and the hemolymph with alternate phases of release and absorption of proteins (Cruz-Landim, 1983; Marx, 1987). Ultrastructural studies have shown that some proteins of the fat body are not synthesized in its cells, but just stored there after intake from the hemolymph (Locke & Collins, 1966; Price, 1973; Thomsen & Thomsen, 1978; Wyatt, 1980).

In adult female insects, vitellogenin, a lipoglycoprotein with molecular mass of 180 kDa, is synthesized in the fat body cells and later secreted into the hemolymph from where it is absorbed by the oocytes in the ovary through the follicular epithelium (Engels, 1973; Engels *et al.*, 1990). The production of this protein, therefore, is the main function of the fat body cells of adult females (Brookes, 1969; Pan *et al*., 1969).

The aim of the present study was to quantify the total proteins in the fat body and ovaries of adult workers of *Melipona quadrifasciata anthidioides* (Lepeletier, 1836), and to observe eventual differences and similarities in the molecular masses of the proteins present in each phase, in comparison with the stages of ovarian development.

MATERIAL AND METHODS

Adult workers of *Melipona quadrifasciata anthidioides* were obtained from the apiary of the Instituto de Biociências, Universidade Estadual Paulista, Rio Claro. Workers were classified as newly-emerged when captured just emerging from the brood cells, as nurses when captured in the brood area of the colony, while building and provisioning the brood cells, and as forager workers when returning from the field to the colony.

Portions of the parietal fat body and entire ovaries of 10 newly-emerged, nurse and forager workers, with one replicate, were used, for a total of 60 individuals. The material was dissected and stored at -20°C for protein quantification and polyacrylamide gel electrophoresis. The tissue was homogenized in 40 µl distilled water and centrifuged at 8,160 g for five minutes. Aliquots of the supernatant extracts were used for total protein quantification by Coomassie Blue staining according to the method of Sedmak & Grossberg (1977), modified.

Electrophoretic separation of the proteins present in the fat body and ovary extracts was routinely performed in a 5-20% gradient of polyacrylamide slab gels run under denaturing conditions – Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Samples from fat body and ovary extracts containing 60 µg protein diluted in buffer were boiled for 5 min, centrifuged at 8,160 g at 5°C and loaded onto the polyacrylamide gels. After electrophoresis, the gels were stained with Coomassie Brillant Blue R-250. Molecular weight markers (MW standard mixture, P7708S-BioLabs) were used as standards to determine the relative molecular mass of protein in the fat body and ovary.

RESULTS

The concentration of proteins was higher in the ovary extracts than in the fat body extracts in newly-emerged and nurse workers, but this situation was inverted in forager workers (fig. 1).

The fat body of newly-emerged workers (fig. 2) lacks the proteins of high molecular mass present in the ovaries, whereas in fat body extracts from nurse workers there is a protein of high molecular mass. There is a similarity between the molecular masses of the bands present in the fat body and ovaries in each stage and little variation among the same tissues in the different stages.

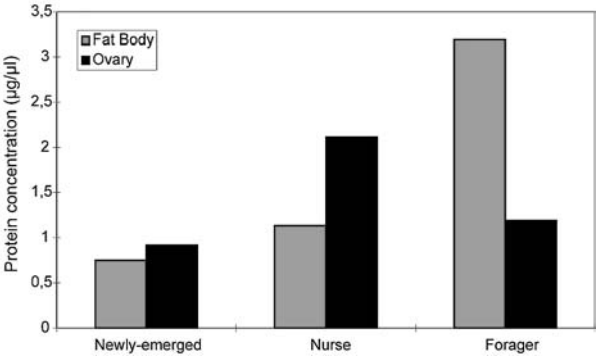

Fig. 1. Average protein concentration (µg/µl) in the fat body and ovaries of *Melipona quadrifasciata anthidioides* workers in different life phases.

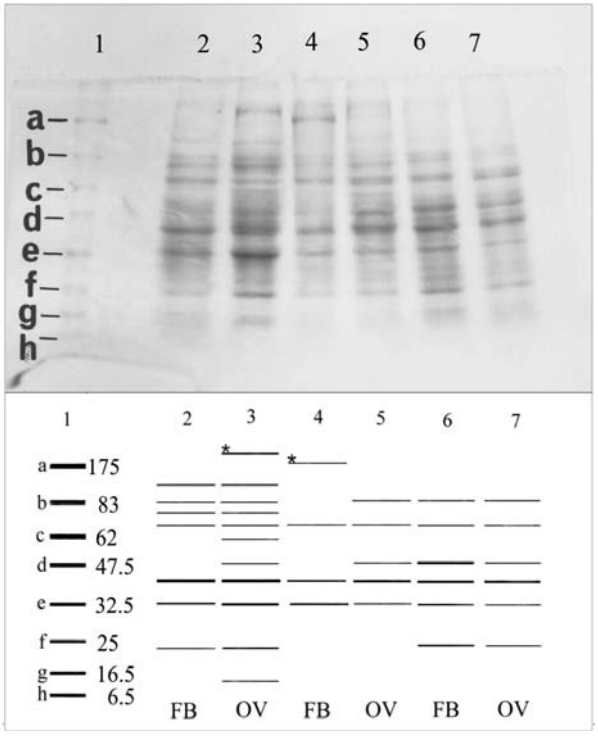

Fig. 2. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (5-20% gradient) protein separation of respectively fat body and ovaries in newly-emerged (2 and 3), nurse (4 and 5) and forager (6 and 7), proteins marked (1) (FB, fat body; OV, ovary; *, bands with molecular mass similar to vitellogenin).

DISCUSSION

The protein content in worker fat body extracts increased with aging, while in the ovaries, as expected, the protein content presented a peak in the nurse phase, during which the workers have vitellogenic ovaries and may lay eggs (SAKAGAMI *et al*., 1963; BEIG & SAKAGAMI, 1964; BEGO, 1983; PAES-DE-OLIVEIRA & CRUZ-LANDIM, 2003a).

The largest protein content in the ovary extracts coincides with the higher developmental rate of the ovaries in nurse workers. Even for the points considered here as the peak of protein concentration, the values were low as a consequence of the poor ovarian development of workers compared to physogastric queens.

The most intriguing data related to protein content in the fat body extracts are those for forager workers, which show 3-fold higher levels than newly-emerged workers and 2-fold higher levels than nurse workers. Protein content would be expected to be high at the beginning of development (newly-emerged) and to decline with time since morphometric analysis of the fat body cells demonstrates a decrease in cell size related to workers' aging, attributed to the use of cell reserves in order to supply vitellogenin to the ovaries during the vitellogenic period (PAES-DE-OLIVEIRA & CRUZ-LANDIM, 2003a). Nevertheless, the higher protein content observed during this phase agree with the great development of the granular endoplasmic reticulum observed in the fat body cells of foragers and with the hypothesis that not all produced vitellogenin is used during the nurse phase or that not every produced protein is vitellogenin, since proteins of high molecular weight are not observed in the fat body or ovary extracts of forager workers (fig. 2). HARTFELDER & ENGELS (1998) demonstrated that vitellogenin is present in the hemolymph of queens and workers of *Apis mellifera* Linnaeus, 1758 at almost the same rates; therefore it is probable that, due to the short duration of the vitellogenic phase in workers, vitellogenin or even other proteins are stored in the fat body. In this respect, the divergence between the cell size and the protein content may be explained by the consumption of lipids, which occupy a lot of space and are present in smaller amounts in nurse workers compared to newly-emerged workers (PAES-DE-OLIVEIRA & CRUZ-LANDIM, 2003a)

Since the change from intranidal to extranidal work is mediated by an increase in juvenile hormone concentration in the hemolymph (JAYCOX *et al*., 1974; FLURI *et al*., 1982; HUANG *et al*., 1991), the foragers have higher titres of this hormone than nurse workers. It is known that juvenile hormone controls vitellogenin synthesis by the fat body cells in adult females (ADAMCZYK *et al*., 1996) and also has a somatotrophic function, activating the general metabolism of the insect (JAYCOX *et al*., 1974; HUANG *et al*., 1991). This condition would explain the development of the granular endoplasmic reticulum in the fat body cells of workers during this phase and eventually the occurrence of synthesis of vitellogenin or other proteins in these cells, targeted by the increased hormonal levels. The electrophoretic pattern here observed (fig. 2) does not show proteins of high molecular

mass in the extracts of the fat body in this phase. Thus, other proteins may be produced, but not vitellogenin. The high energy consumption by foragers during flight may be responsible for the decrease in lipid storage in the fat body and therefore for the increase in the relative protein rates in the cells. In this case, the decrease in cell size may be due to the lipid loss. The relatively low protein rate in forager workers' ovaries is due to the regressive phase in this organ (Paes-de-Oliveira & Cruz-Landim, 2003a).

In conclusion, there is an unexpected discrepancy between the protein content and electrophoretic pattern of the fat body and ovarian extracts. Nevertheless these discordances may be explained by taking into account the chronological differences in the development of these organs and the general physiological conditions that can interfere with this development.

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