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Research article

Electrophoretical studies of proteins of the hypopharyngeal glands and of the larval food of *Melipona quadrifasciata anthidioides* Lep. (Hymenoptera, Meliponinae)

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Summary

The electrophoretical protein patterns of hypopharyngeal glands, larval food of *Melipona*, and royal jelly of *Apis* were compared.

Since protein patterns of hypopharyngeal glands from newly emerged workers, brood cell provisioners and foragers are similar to freshly deposited larval food, the identical protein bands probably represent actual gland secretion. This suggests that, as in *Apis*, the glands secrete proteins to the larval food, and maintain this ability throughout life, although at slightly different intensities, according to the activity of the bees.

The similarity on the electrophoretic profiles of the major larval food protein in *Apis* and *Melipona* is an interesting finding because of its probable evolutionary significance.

Introduction

The larvae of most meliponines develop in brood cells, provisioned with pollen and with a fluid that has been described by Michener (1961, 1974) as a gland secretion. The growth and regression cycle of the hypopharyngeal glands is quite similar to that of *Apis* in workers of *Scaptotrigona postica* (Dias and Simões, 1972) and of *Melipona* (Martinho, 1975). Furthermore, the food for meliponine larvae is similar in appearance to the royal jelly of *Apis*, and in the same manner as *Apis* queens eat royal jelly directly offered by nurse workers, the queens of some meliponines also eat the liquid food soon after it is deposited into the brood cells by the workers (Michener, 1974).

The findings of Hartfelder and Engels (1989) suggest that in some meliponines the secretion produced by the hypopharyngeal glands of workers serves as food for larvae and queens as occurs for *Apis* (Jung-Hoffmann, 1966; Halberstadt, 1980; Takenaka and Kaatz, 1987; Knecht and Kaatz, 1990; Klaudiny et al., 1994).

To contribute to the better understanding of this subject we studied the protein patterns of hypopharyngeal glands of *Melipona* in relation to workers' activity and their band correspondence with those of the larval food. *Apis* royal jelly was also analyzed.

Materials and methods

Workers of *Melipona quadrifasciata anthidioides* were collected from the apiary of the Department of Biology, Bioscience Institute of Rio Claro, during three different phases of adult life: newly emerged (NE), brood cell provisioners (P), and foragers (F). The brood cell provisioner workers were collected inside the colony when provisioning of the brood cells was being carried out. The foragers were the darkest bees collected at the entrance of the hives coming back from the field with pollen on their legs. As the hair and the chitin pigmentation of the workers' body increases with the age (Kerr and Santos, 1953), the darkest foragers were the oldest among the studied bees.

We have worked with three replications each containing 37 workers for NE phase, 10 for the P phase and 40 for the F phase. The hypopharyngeal glands of these individuals were dissected out, and those from the same phase, in each replication, were pooled, resuspended in 0.4 ml insect saline and centrifuged at 1400 × g for 3 minutes for three consecutive times for washing.

For total protein extraction the glands were submitted to osmotic shock by suspension in distilled water, followed by three cycles of freezing in liquid N_2 and thawing, in order to induce cell membrane rupture and consequent extravasation of intracellular content.

The material was centrifuged at 14000 × g 15 min and the supernatant (glandular extract) was reserved for analysis of the total protein by the Comassie blue binding method (Sedmak and Grossberg, 1977) and for electrophoresis by 5–20% SDS – PAGE (14,5 × 16,5 × 1,5 cm) (Hames and Rickwood, 1981). The Dalton Mark VI (Sigma) or bovine albumin (66KDa), ovalbumin (45KDa) and ribonuclease (14,5 KDa) were used as molecular weight (MW) markers.

The *Apis* royal jelly was removed from provisioned queen-rearing cells which had been produced according to Doolittle (1899). The larvae introduced were one day old and the royal jelly was collected 72 h after grafting. The larval food of *Melipona* was collected from cells right after provisioning. As larval food of *Melipona* usually contains some pollen (Sakagami and Zucchi, 1966), this pollen was separated by resuspending the larval food in 100 µl of twice distilled and deionized water and centrifuging for 15 min at 14000 × g. The supernatant (larval food without pollen) as well as the royal jelly were resuspended separately in twice distilled and deionized water and submitted to the same analysis as for the glandular extracts.

The gel in Figure 1 is a representative one of several.

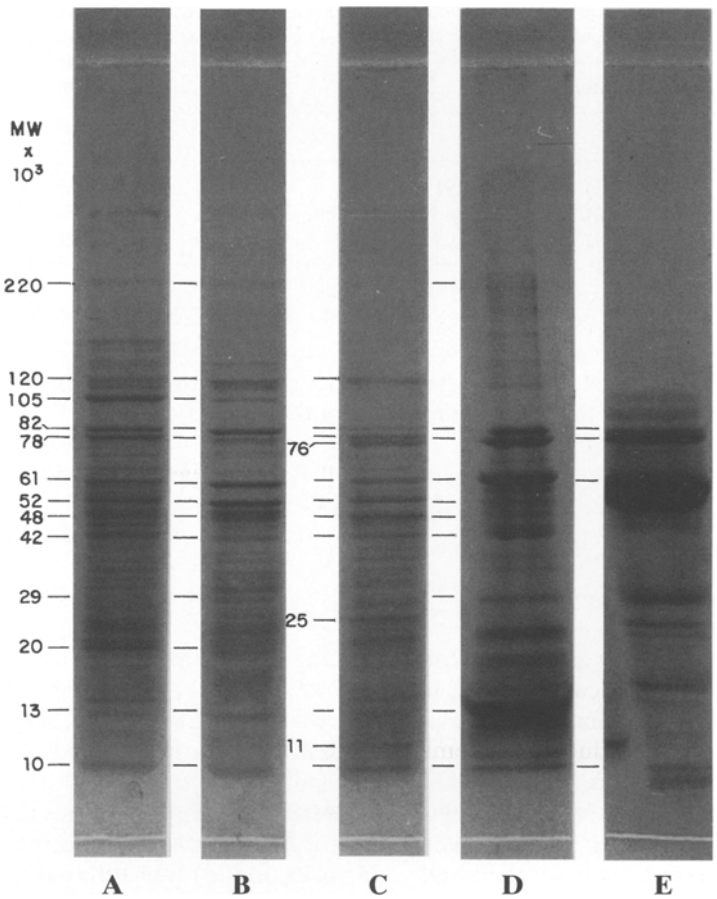

Figure 1. Polypeptide patterns from *Melipona quadrifasciata anthidioides* and *Apis mellifera*
A Hypopharyngeal glands from *Melipona newly* emerged workers (n = 37 workers)
B Hypopharyngeal glands from *Melipona* brood cell provisioners workers (n = 10 workers)
C Hypopharyngeal glands from *Melipona* forager workers (n = 40 workers)
D Larval food of *Melipona*, removed from provisioned cells
E Royal jelly of *Apis* removed from provisioned queen-rearing cells

Results

Total protein content of the hypopharyngeal glands of *Melipona* presented higher values in brood cell provisioners workers (Table 1).

The electrophoretical protein patterns (Fig. 1) also varied in relation to worker activity. The spectrum obtained with glands from foragers (Fig. 1C) is different from those of the other two groups (Fig. 1A, 1B) by the lower band intensities (82 and 61 KDa), the absence of some of them (105, 29 and 20 KDa) and the presence of new bands (76, 25 and 11 KDa).

Table 1. Values of the protein content (μg/μl) of the hypopharyngeal gland of *Melipona quadrifasciata anthidioides*. Values in parentheses, indicating number of glands, and the average ± standard deviation ($\bar{x} \pm sd$) are shown

Phase	Replications			$\bar{x} \pm sd$
	1	2	3	
NE	4.10 (37)	3.91 (37)	4.35 (37)	4.12 ± 0.20 (111)
P	36.60 (10)	28.40 (10)	32.60 (10)	32.53 ± 3.35 (30)
F	1.28 (40)	1.03 (40)	1.49 (40)	1.26 ± 0.17 (120)

Comparison of the protein pattern of the hypopharyngeal glands (Fig. 1A, B, C) with the pattern of the *Melipona* larval food (Fig. 1D) indicated some identical bands (220, 82, 78, 61, 52, 48, 42, 29, 13 and less than 10 KDa). Between all these protein patterns and those of *Apis* royal jelly (Fig. 1E) correspondence of at least four major bands (82, 78, 61 and 10 KDa) was also demonstrated.

Discussion

Our results, showing glandular protein content changes in relation to worker activity of *Melipona*, indicate that the higher protein content for the brood cell provisioners can correspond to a more intense activity of the hypopharyngeal glands for the production of secretory protein as suggested by Hartfelder and Engels (1989). Besides this, the electrophoretic protein patterns also showed slight changes throughout the life of adult bees. It can be seen, for example, changes in band intensities such as 82 or 61 KDa (probably secretory proteins) that are prominent in cell provisioners, but not in foragers and young bees. In the forager glands, the most different of them, some protein bands are lacking (105, 29 and 20 KDa) and new ones appear (76, 25, 11 KDa). These results support, respectively, two hypothesis: 1. the occurrence of a differential stage specific gene expression, presumably for a higher production of secretory proteins in brood cell provisioners; 2. the occurrence of a new gene expression during the last phase of adult life.

By finding that glandular protein patterns from workers of the three studied phases share many bands with larval food, we conclude that hypopharyngeal glands contribute to larval food, as in *Apis* (Haydak, 1970; Halberstadt, 1980; Knecht and Kaatz, 1990; Hanes and Sïmúth, 1992; Klaudiny et al., 1994). Furthermore, since foragers, which are the oldest among them, still contain some correspondent bands with those found in larval food, these workers probably retain the ability of larval food production. This is similar to the finding for honey bees by Takenaka and Kaatz (1987) and Knecht and Kaatz (1990), but it disagrees with the results of Halberstadt (1980). In order to prove that a major part of the larval food proteins of *Melipona* actually originates from these glands further studies will be developed for the determination of their molecular identity.

The most important difference between the larval food of the honey bee and of the stingless bee resides in the protein quantity (Takenaka and Takahashi, 1980).

However, we have observed apparent similarities between their electrophoretical patterns. Other similarities are the findings that the proportions of sugar and free amino acids in stingless bee larval food lie exactly within the range for *Apis mellifera* royal jelly (Shuel and Dixon, 1968).

Since it is suggested that *Melipona* and *Apis* evolved their sociality separately (Winston and Michener, 1977), the similarities on the electrophoretic protein patterns could be explained assuming either that these proteins arose from convergent evolution or that they were present in the pre-social, common ancestor to the two lines. Then, due to the action of the same selective pressure (first hypothesis) or by the fact they have been maintained in evolution (second hypothesis), those similarities would occur because presumably, the hypopharyngeal glands play an important role in social organization as they produce larval food, a vital part of brood care.

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