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Morphological features and occurrence of degenerative characteristics in the hypopharyngeal glands of the paper wasp *Polistes versicolor* (Olivier) (Hymenoptera: Vespidae)

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Abstract

Different histochemical techniques were applied to examine the morphological features of the secretory cells of hypopharyngeal glands in the wasp *Polistes versicolor*. The results showed that most analyzed individuals present active glands with secretion stored in the cytoplasm. In some glands, morphological analyses revealed the presence of degenerative characteristics. Analyses of cellular integrity, however, did not detect dead cells. The results showed that, in *P. versicolor*, the development and regression of the hypopharyngeal glands were not age related, unlike glands of social bees.

Keywords: Hypopharyngeal glands; Salivary system; *Polistes versicolor*; Wasps; Hymenoptera; Cell death; Histochemistry; Histology

1. Introduction

In Hymenoptera, several glands are associated with the mouthparts and the alimentary canal. In general, these glands are called salivary glands and constitute the salivary system. The main glands that compose this system are the mandibular glands, the hypopharyngeal glands and the thoracic salivary glands (Bordas, 1895).

Hypopharyngeal glands are exclusively found in Hymenoptera and their function is only known in social bees. In *Apis mellifera*, the secretion of hypopharyngeal glands is the main constituent of royal jelly. This substance is produced by workers and is used to feed the brood (Dixon and Shuel, 1963; Cruz-Landim and Saenz, 1972). Thus, in this species, glands are developed only in workers, and are atrophied in queens and males (Crailsheim and Stolberg, 1989). Nonetheless, even in workers these glands degenerate when they stop feeding the brood and start foraging (18 days after emergence) (Costa, 2002).

In other social Hymenoptera, such as ants (Gama, 1985; Caetano et al., 2002; Amaral and Caetano, 2005) and wasps (Cruz-Landim and Saenz, 1972; Britto et al., 2004; Britto and Caetano, 2005), only few studies have examined the hypopharyngeal glands. Their function and importance in a social context remain unclear in these insects, especially in most primitive groups. Among these groups the genus *Polistes* is one of the most studied (Reeve, 1991; Gobbi, 1977; Giannotti and Machado, 1999; Zara and Balestieri, 2000). It is considered the "key genus" for understanding social insects' evolution, due to slight morphological differences between casts and, also, the wide distribution of the genus, which is found in different climatic areas. It allows effective comparisons between species of temperate and tropical climates (Evans, 1958).

In the life of social insects, there are important physiological changes that lead to differentiation of behaviors and, even, morphological changes (i.e. casts differentiation). Considering that these events are followed by morphological and functional changes in exocrine glands, the study of these structures provides important contributions to understanding the biology of social insects. Thus, the present work aims to analyze the morphological features of the secretory cells of hypopharyngeal glands in the paper wasp *Polistes versicolor*, as well as correlate their developmental level to the age of specimens.

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2. Materials and methods

2.1. Monitoring the specimens' age

Newly emerged wasps from three nests were captured daily and anaesthetized under low temperature (4 °C). After this procedure the wasps were marked with Testors modeling paint on the central area of the thorax. Each wasp was marked with different colors for monitoring their ages. Soon after, wasps were reintroduced in their respective colonies.

2.2. Processing the hypopharyngeal glands

After reaching appropriate ages, wasps were recaptured and, once more, anaesthetized under low temperature. With the aid of stereomicroscope, tweezers and microscissors, hypopharyngeal glands were removed on dissection plate with physiologic solution. Glands were then submitted to different procedures.

2.2.1. Morphological characterization and histochemical techniques

The hypopharyngeal glands of 55 wasps between ages 0 and 92 days (Table 1) were fixed in paraformaldehyde 4% in phosphate buffer (0.1 M, pH 7.4) dehydrated in ethanol, and embedded in resin (Leica®), during 24 h, at 4 °C. Resin blocks obtained after polymerization were sectioned at a thickness of 5 μm with glass knives using a Leica RM 2145 microtome. Slices were placed on histological slides and submitted to the following techniques: (1) Methyl Green-Pyronin (Moffitt, 1994) that stains DNA and RNA differently; (2) Mercuric Bromophenol Blue (Pearse, 1985) for proteins, and (3) PAS for demonstrating neutral glycoconjugates (McManus, 1946).

Table 1
Age and number of wasps utilized in the morphological and histochemical analyses of hypopharyngeal glands ($n = 55$ wasps)

Age (days)	Amount of wasps captured
0	6
2	2
6	1
7	2
8	3
9	3
10	2
15	5
17	4
18	3
20	1
25	1
26	3
27	1
28	2
29	1
30	3
31	1
33	1
34	1
35	2
38	1
40	1
50	2
70	2
92	1

2.2.2. Techniques of cellular integrity evaluation

Sixteen additional glands were obtained from eight 10-day-old wasps and eight 35-day-old wasps. The "35-day-old" group was chosen since 40-day-old wasps were uncommon. In addition, this age was considered above the average lifespan of *P. versicolor*, as observed by Gobbi (1977). According to this author the average lifespan of workers is 17 days.

After dissection, glands were directly processed according to the following techniques: (1) Nile Blue (Saunders et al., 1962) for detection of cells in necrosis and/or apoptosis; (2) demonstration of acid phosphatase activity (Hussein et al., 1990). Each technique was carried out with four glands of 10-day-old wasps and four glands of 35-day-old wasps.

3. Results

3.1. Morphological characterization of secretory cells

Cells of hypopharyngeal glands presented secretion dispersed throughout the cytoplasm in small vesicles stained by Mercuric Bromophenol Blue (proteins) and PAS (glycoconjugates) (Fig. 1).

Pyronin in the Methyl Green-Pyronin technique strongly stained the peripheral portion of the cytoplasm of most cells, indicating the presence of RNA (Fig. 2A). Some glands presented cells with cytoplasm exhibiting small vacuoles (Fig. 2B). Both glandular characteristics were common and present in approximately 80% of the glands analyzed. Also, secretion vesicles were always present in the cytoplasm of cells of these glands, as revealed by the Mercuric Bromophenol Blue and PAS techniques. Nuclei were weakly stained with Methyl Green, indicating that most chromatin was slightly condensed (Fig. 2).

Some glands, found in newly emerged wasps, exhibited cells with a homogeneous cytoplasm and absence of secretion, as well as a regular nucleus and numerous nucleoli (Fig. 2C).

Another morphological aspect found was glands with secretory cells exhibiting degenerative characteristics, indicated by numerous large vacuoles in the cytoplasm (Fig. 2D). Some of these cells presented large amounts of secretion, while in others concentration was low. Vacuoles were not stained by Methyl Green-Pyronin and Mercuric Bromophenol Blue, however, when submitted to PAS technique, weakly stained vacuoles were observed in all wasps. The presence of glands with these characteristics was only observed in 5 of the 55 glands of specimens submitted to the morphological analysis (10% of the wasps). These individuals were 9, 15, 26, 30 and 38 days old. The nuclei of the cells of these glands did not exhibit different characteristics from those of cells considered healthy (Fig. 2A, B and D). In some cases, they presented irregularities similar to those found in cells with large amounts of secretion.

An anomalous characteristic was observed in a gland that presented asynchronism in the development of secretory cells.