CELLULAR DEVELOPMENT AND HISTOCHEMICAL ASPECTS OF THE NASSANOFF GLANDS UNDER JUVENILE HORMONE ACTION.

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ABSTRACT

The Nassanoff glands are responsible for synthesis of species-specific pheromones, and secretory substances responsible for the colony scent. In the present work the Nassanoff glands of 12 and 25 day-old *Apis mellifera* workers were treated with juvenile hormone (JH) just after emerging, were studied through morphometric and histological analyses and compared to those of non-treated workers. The results showed that although JH administration caused an acceleration of worker maturation, it failed to affect the developmental pattern of the Nassanoff glands, showing that the period of administration of the JH is inappropriate to cause any influence to this pattern.

Keywords: *Apis mellifera*, histology, juvenile hormone, morphometry, Nassanoff glands.

INTRODUCTION

The Nassanoff glands, also known as scent glands, are unicellular glands, located in the intersegmentar membrane, between the sixth and seventh tergits of *Apis* workers, covered by the posterior border of the sixth tergit and are part of a group of free pheromone-producing cells (Snodgrass, 1956).

In *Apis*, these glands are present only in workers and absent in queens, being its secretion responsible to the mates nest recognition, demarcation of the entrance of colony and scent marking of water source (Free, 1980). The pheromone produced by the Nassanoff gland contains seven chemical volatiles compounds whose main function seems to be orientation (Boch and Shearer, 1964; Winston, 1987).

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The main compound of the gland secretion is the geraniol, but experimentally the most attractive element to antennal response is the (E)-citral and the geranic acid. In *A. mellifera,* these glands produce citral, geraniol, nerol, (E-E)-farnesol, geranic acid and nerolic acid (Bell and Carde, 1984).

The Nassanoff gland secretion is species-specific, but does not colony-specific, since is the bees that give a specific scent to the colony. The colony-specific scent is obtained with other members of the colony, faves, larva and flowers visited by the bees. By this way, different colonies have different scents. This specific scent facilitates the recognition of nest mates and the workers may also be attracted by the smell trail of nest mates, before other members of any other colony. The secretory compounds produced by the Nassanoff gland cells are absorbed by the wax that involves the cuticle of the insect (Chapman, 1972).

The juvenile hormone has an important function in the labor division and in the task changing during the worker life (Jaycox, 1976). The transition of nurse to forager workers is marked by physiological changes of exocrine glands and it is regulated by juvenile hormone titers variations (Robinson, 1987).

The aim of the present investigation is to test the action of the juvenile hormone as a modulador of the activity of Nassanoff glands cells, seeking to obtain information that may contribute to the understanding of the mechanisms involved in its secretory activativity.

MATERIAL AND METHODS

Workers of *Apis mellifera* L. were obtained in the apiary of Institute of Bioscience, UNESP - Rio Claro, Brazil. About 1000 newly-emerged workers were collected and divided in two groups, treated with JH and control, being the bees of each group marked in the thorax with two different colors with non-toxic Suvinil synthetic ink without dilution.

To the workers of treated group was applied, topically, on the abdominal cuticle, 1 ㎖ of JH-III (Sigma), diluted in acetone, in the concentration of 1 ㎎/㎖, according to Muller and Hepburn (1994). Workers of the control group just received application of 1 ㎖ of acetone.

As the workers treated with juvenile hormone (T) as the non-treated (NT), were marked with different colors, returned to the colony and were collected 12 and 25 days-old after the demarcation.

The Nassanoff glands were dissected in saline solution for insects (NaCl – 3.75 g, KH_2PO_4 – 1.76 g, Na_2HPO_4 – 1.98 g for 500 mL of distilled water) and fixed in alcoholic Bouin, during 24 hours. After fixation, the glands were dehydrated in an graded ethanol series and then embedded in JB–4 resin (Polysciences). Sections 4.5 ㎛ thick were obtained in a histomicrotome (BIO – RAD JB – 4) stained by hematoxylin and eosin and examined and photographed in a photomicroscope (ZEISS) and to accomplish the morphometric analyses.

Morphometry

Ten workers were studied for each age (12

and 25 days-old) in different situations (T and NT). As the Nassanoff glands are formed by a free cells group, 10 cells were measured by specimen (n = 100).

The cell area measurement was obtained through a Axioskop microscope (ZEISS), coupled to a microcomputer provided with Axiohome system. The averages and standard deviation of the cell areas were calculated by each gland measured, and applied the Kruskal- Wallis test, for determination of the rank sums and the Nemenyi test, with significance level at 5%, for comparison of the areas average among the bees studied (SAS Institute, 1985; Zar, 1996).

Histochemistry

To detect the polysaccharides and proteins presence were accomplished the following histochemistry tests:

Periodic acid-Schiff (PAS) and Alcian Blue: To detection of neutral polysaccharides with group's 1-2 glicol and acids polysaccharides, were used simultaneous coloration PAS/Alcian Blue. The slides contend the sections, were red-faced with Alcian blue for 30 minutes, washed in distilled water, oxides in periodic acid 1% for 5 minutes, and washed again in distilled water. After that, the samples were stained for 30 minutes with Schiff reactive, in the darkness, washed again in average water for 10 minutes, stained for 2 minutes with hematoxylin, washed in distilled water, dehydrated and mounted in Permount.

Bromophenol Blue (Pearse, 1960, modified): To detection of proteins with SH groups, the sections were red-faced with solution of Bromophenol Blue 0.5%, at surrounding temperature, for 1 hour, being washed in aqueous solution of acetic acid 0.5% for 5 minutes. The samples were differentiated in distilled water, dehydrated and mounted with Permount.

Results

The Nassanoff glands cells are spherical, with nuclei also spherical and central, quite stained by hematoxylin, into an eosinophilic cytoplasm (Fig. 1A).

In agreement with the morphometrics results, we can observe the occurrence of size significant differences of gland cells among 12 and 25 days-old NT, 12 and 25 days-old T, as well as 12 days-old NT and 25 days-old T. The treatment with juvenile hormone doesn't promote alteration of cellular area into the same age of the bees, does not being detected significant differences among treated individuals and non-treated with the same age (Table I).

In all treatments and ages (12 and 25 days-old T and NT) the Nassanoff glands present a weak positivity to the reaction with PAS (Fig. 1B, C, D), while the positivity to Alcian blue in the cytoplasm of the gland cells is just not weak in glands of 25 days-old NT workers (Fig. 1D). Positive reaction to Blue Bromophenol was observed in all the glands of the bees studied (Fig. 2A, B, C, D), indicating the presence of proteins with SH groups.

Discussion

A group of spherical cells individualized, with central nuclei and de-condensed chromatin forms the Nassanoff glands. These cells are provided by an excretory canalicule, which has intracellular origin, which connects them to the tegument, in the intersegmentar membrane between the sixth to the seventh tergit (Cruz-Landim, 1963). These glands have, therefore, unicellular secretory elements, belonging to the type III of Noirot and Quennedey (1974, 1991) exocrine gland insect cell classification.

The presence of acid polysaccharides detected in the cytoplasm of cell glands of workers T, as well as workers with 25 days-old NT, may represent intracellular component or compound of the secretion. In workers treated with juvenile hormone, further increasing the variability of the cell gland area along the age, as demonstrated in the morphometry, seems to do with 12 days-old T workers secrete the same type of material that 25 days-old NT workers secrets, inducing an acceleration of worker maturation, advancing, therefore, the secretory cycle. This aspect has support in the morphometric analyses, where the absence of significant differences was observed among gland cell areas of 12 days-old T workers and 25 days-old NT workers.

The Bromophenol blue test indicates the presence of great amount of structural proteins and/or enzymes that participates in several functions carried out by the active cells. As the secretion produced by these glands is composed of light and volatile substances (Boch and Shearer, 1964; Winston, 1987), the absence of proteins in glandular product was expected.

According Mota and Cruz-Landim (1988), the development of the Nassanoff glands, typical of *Apis* workers, have a gradative increase from the emergency of 15 days-old workers, when they reach the maximum size, being still well developed in workers with 30 days-old.

Our results confirm those obtained by the authors mentioned, already in the period of 12 and 25 days old, the gland cell areas suffer increases statistically significant, similaring increases, independently of the treatment. In this way, the topical application of juvenile hormone in workers doesn't promote significant increases of the gland cell areas, in comparison with the workers non treated of same age and also does not alter the pattern of development of these glands, however, it approached the areas of gland cell of 12 days-old T workers of those with 25 days-old NT, giving indications of an acceleration of maturation of workers treated. But the factor that more modified the development of the Nassanoff glands was the age of the workers. In such way this behavior was expected because these glands are typical of workers, and are completely formed in newly emerged workers and whose product is usually used by individuals that do tasks out of the colony.

There are several situations in which the foragers workers expose the seventh tergit area, where is the Nassanoff gland, for example, when a queen is losted of the colony, in the entrance of the colony (Sladem, 1901), close to the food source (Kaltoven, 1951) and during the collection of water (Free and Williams, 1970). To those exhibitions the liberation of the characteristic glandular scent is preceded.

Mota and Cruz-Landim (1988) observed that the nuclei of these glands cells present activity signs from the emergency of worker, when the chromatin is de- condensed, and the cytoplasm gradatively grows, reaching the maximum size with 15 days-old. Through the morphometric analyses we can concluded that the secretion stayed stored in the cytoplasm still the 25 days. This indicates that there wasn't need the use of the secretion still this age and it suggests the existence of just one secretory cycle for these glands, with liberation of its products in more advanced ages.

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REFERENCES

1. BELL, W.J., CARDÉ, R.T. *Chemical ecology of insects*. Sounderland, Mass.: British Library Cataloguing in Publication Data, 1984. p. 387-428.
2. BOCH, R., SHEARER, D.A. Identification of nerolic and geranic acids in the Nassanoff pheromone of the honey bee. *Nature*, London, v. 202, p. 320-21, 1964.
3. CHAPMAN, R.F. *The insects: structure and function.* London: The English Universities Press, 1972. p. 692-716.
4. CRUZ-LANDIM, C. Evolution of the wax and scent glands in the Apidae (Hymenoptera, Apidae). *J. New York Entomol. Soc.*, v. 71, p. 2-13, 1963.
5. FREE, J.B. *A organização social das abelhas (Apis).* São Paulo: EDUSP. 1980. III + 79p. (Temas de Biologia, v.13).
6. FREE, J.B., WILLIANS, H. Exposure of Nassanov gland by honeybee (*Apis mellifera*) collecting water. *Behavior,* v. 37, p. 286-90, 1970.
7. JAYCOX, E.R. Behavioral changes in worker honey bees (*Apis mellifera* L.) after injection with synthetic juvenile hormone (Hymenoptera; Apidae). *J. Kans. Entomol. Soc.*, v. 49, p. 165-70, 1976.
8. KALTOVEN, R.S. Das problem des volkeduftes bei der Honigbiene. *Z. vergl. Physiol.*, v. 33, p. 462-75, 1951.
9. MOTA, M.H.V.B., CRUZ-LANDIM, C. Ocorrência e morfometria de glândulas tegumentares abdominais em *Apis mellifera* L. (Hymenoptera: Apidae) *Rev. Bras. Zool.*, v. 5, n. 1, p. 119-54, 1988.
10. MULLER, W.J., HEPBURN, H.R. Juvenile hormone III and wax secretion in honeybees (*Apis mellifera capensis***)** *J. Insect Physiol.*, v. 40, n. 10, p. 873-81, 1994.
11. NOIROT, C., QUENNEDEY, A. Fine structure of insect epidermal glands. *Annu. Rev. Entomol.***,** v. 19, p. 61-80, 1974.
12. NOIROT, C., QUENNEDEY, A. Glands, gland cells, glandular units: some comments on terminology and classification. *Na. Soc. Ent. Fr. (N.S.)*, v. 27, n. 2, p. 123-8, 1991.
13. PEARSE, A.G.E. *Histochemistry:* theoretical and applied. London: J. & A. Churchill, 1960. 998p.

14. ROBINSON, G. E. Regulation of honey bee age polyethism by juvenile hormone. *Behav. Ecol. Sociobiol*. ,v. 20, p. 329-38, 1987.

15. SAS INSTITUTE INC. *SAS user's guide:* statistics. Carry, North Carolina, 1985 (CD-Rom).

16. SLADEM, F.W.L. A scent organ in the bees. *Amer. Bee J.*, v. 29, p. 142-3, 1901.

17. SNODGRASS, R.E. *Anatomy of the honey bee*. London: Constable and Co., 1956. XVI + 334p.

18. WINSTON, L.W. *The biology of the honey bee*. Cambridge: Mass., Harvard Univ. Press, 1987. p. 129-49.

19. ZAR, J.H. *Biostatistical analysis*. 3°ed. New Jersey: Prentice-Hall, 1996. p. 226-9.

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TABLE I. Nemenyi test for verification of significant contrasts among the treatments (T) and age (A) of workers, for the values of Nassanoff gland cells.

Worker groups treatments (A x T)	**Ra – Rt**	**SE**	**Q**	**q(0,05)**
1x2	186	36,968	5,031*	3,633
1x3	114	36,968	3,084	3,633
1x4	72	36,968	1,948	3,633
2x3	300	36,968	8,115*	3,633
2x4	114	36,968	3,084	3,633
3x4	186	36,968	5,031*	3,633

* Significant at 5%

1 = 12 days-old workers treated

2 = 25 days-old workers treated

3 = 12 days-old workers non-treated

4 = 25 days-old workers non-treated

Ra – Rt= difference of rank sums

SE= standard error

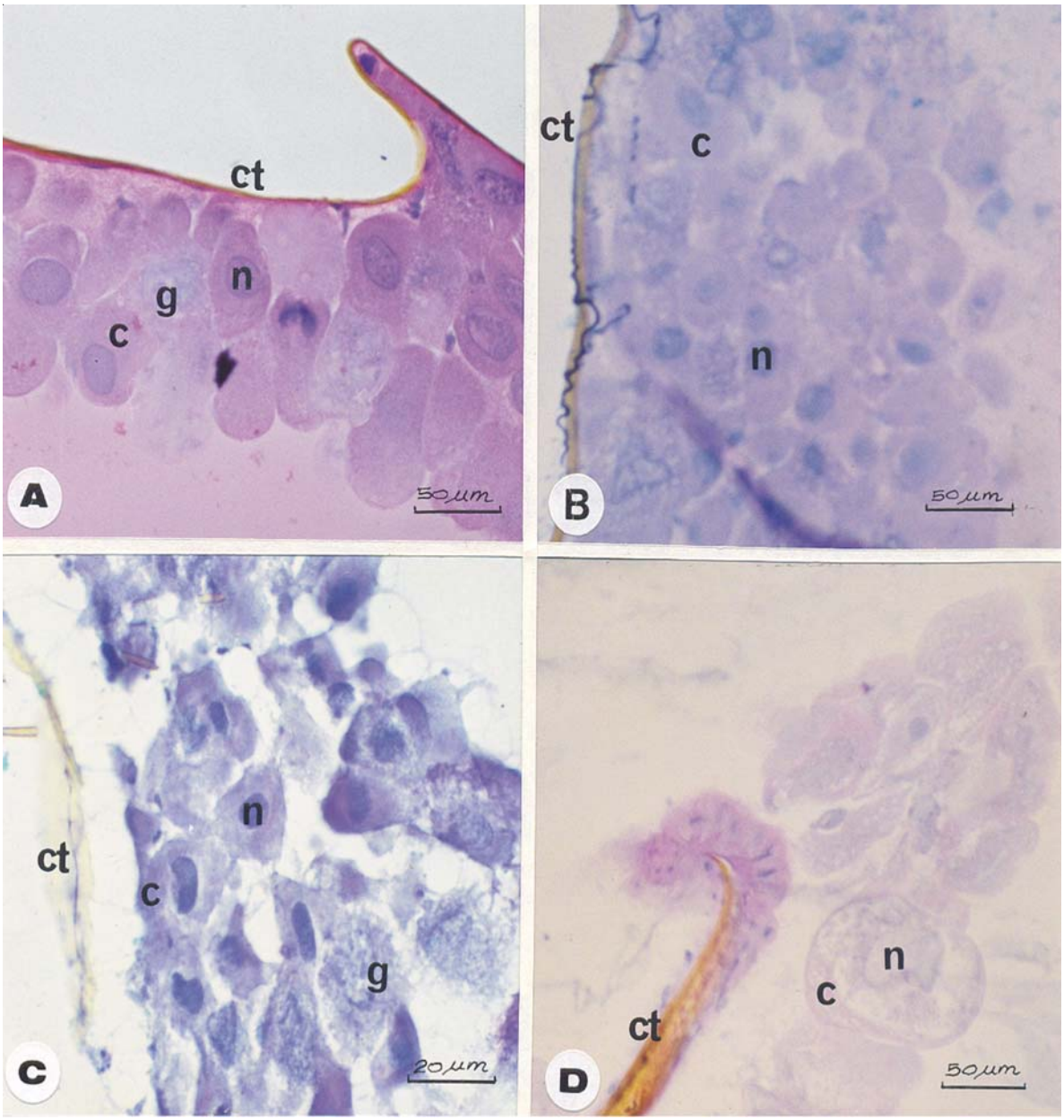

FIGURE 1. Secretory cells of Nassanoff evidencing the cuticle of the tergite (ct), nuclei (n), cytoplasm (c) and fatty body cells (g).

A. Coloration with HE of 12 days-old workers treated.
B. Reaction of PAS/ Alcian blue of 12 days-old workers non-treated.
C. Reaction of PAS/ Alcian blue of 12 days-old workers treated.
D. Reaction of PAS/ Alcian blue of 25 days-old workers non-treated.

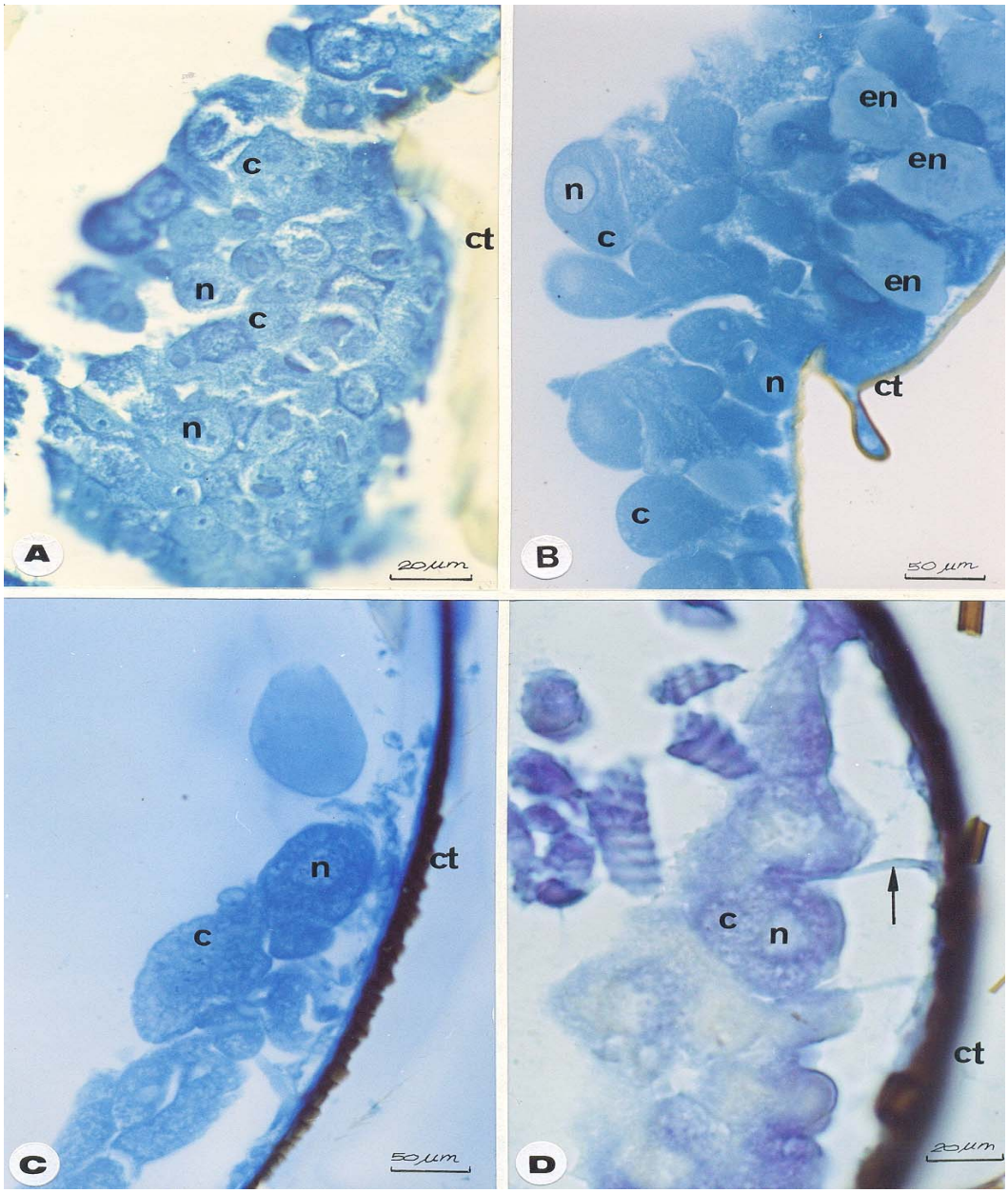

FIGURE 2. Reaction with Bromophenol blue of secretory cells of Nassanoff glands evidencing the cuticle of the tergite (ct), nuclei (n) and cytoplasm (c), enocytes (e) and canaliculle (arrow).

A. 12 days-old workers non-treated.
B. 12 days-old workers treated.
C. 25 days-old workers non-treated.
D. 25 days-old workers treated.