

**CYTOCHEMICAL STUDY OF SILK PRODUCTION PHASE IN *Pachycondyla villosa* ANT LARVAE.** Fernando J. Zara (1) and Flávio H. Caetano (2). (1) Universidade Estadual Paulista (UNESP), Campus do Litoral Paulista, 11330-205, São Vicente, SP, Brasil; (2) Universidade Estadual Paulista (UNESP), Depto. Biologia – P.O.box 199, 13506-900, Rio Claro, SP, Brasil.

The salivary gland of ant larvae reflects possible functions in digestion and/or silk production (Petralia et al., 1980). Protease activity has been observed in *Solenopsis invicta* [1], however, the digestive function was not clearly confirmed. There is only one ultrastructural study of the silk glands in ants [2], but the silk production was not performed. Here we demonstrate silk production in *Pachycondyla villosa* using cytochemical methods. Identification of the last larval instar was performed through the rule of Dyar according to Zara & Caetano [3]. For both TEM routine and cytochemical techniques, the salivary glands were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). For the osmium tetroxide technique to Golgi bodies visualizing, the glands were dissected and transferred to unbuffered osmium solution. The other tests performed were: ethanolic phosphotungstic acid (EPTA) and ammoniacal silver for basic proteins; Imidazole-buffered osmium tetroxide for unsaturated lipids, p-Nitrophenyl phosphate for acid phosphatase; and the Afzelius, for glycogen. The basal lamina appears thin (Fig. 1) and comprised by three layers (Fig. 2). The inner and middle ones are positive to EPTA (Fig. 3), and middle is positive to ammoniacal silver (Fig. 4). This result can identify the collagen presence in the basal lamina, such as proposed in *Periplaneta* and *Locusta* [4]. The basal infoldings are shallow (Figs. 1, 3 and 4) and with many mitochondria, glycogen granules, few lipid droplets positives to unsaturated fatty acids (Fig. 5) as may be an energy source for absorption of products from the haemolymph. The absorption has been demonstrated in *Rhynchosciara americana* [5]. It was observed in *P. villosa* through the p-Nitrophenyl phosphate technique for acid phosphatase, with positive reaction on the basal lamina and basal infoldings (Fig. 6). These results are similar to *Calliphora erythrocephala* [6, 7]. The large nuclei possess great amount of heterochromatin and many nucleoli (Fig. 7), showing positive reaction to the EPTA (Fig. 8) and ammoniacal silver (Fig. 9) techniques. The rough endoplasmic reticulum is developed and disposed in parallel lamellae (Fig. 10). It was positive to the osmium tetroxide technique for the regions next to the Golgi bodies (GB), as similar by to observe in *Drosophila auraria* [8] and can be a transition zone to GB. The GB is a set of vesicles filled with fibrous material (Fig. 10). The osmium tetroxide technique was performed in order to visualize the GB and the set of vesicles showed positive reaction, indicating that they are part of the GB (Fig. 11), like occurred in *D. auraria* [8]. The GB also presented positive reaction for acid phosphatase in the membrane of the vesicle (Fig. 12), being rarely observed in the fibrous materials. Nevertheless, lysosomes were not observed, and their absence might be explained by at this stage they are not produced or the degradation process will only occur during the pre-pupa stage. The secretion vesicles have fibrous materials on their inside and are eliminated into the lumen through exocytosis (Fig. 13 and 14). These vesicles showed positive reaction to the osmium tetroxide technique, indicating their Golgian origin, being this positive reaction disappeared when the vesicle passes through exocytosis (Fig. 14). Close to these vesicles, numerous mitochondria and glycogen granules occur (Fig. 13). The glycogen appears the main energy supply to vesicle exocytosis. This secretion mechanism was similar to that observed for *Bombyx mori* [9]. In the lumen, masses of secretion tend to fuse forming tactoids. The tactoids showed positive reaction to EPTA at the cortex (Fig. 15) and to ammoniacal silver in the medulla (Fig. 16). This result agrees with the silk protein constitution proposed for Hymenoptera, which appear to have a  $\alpha$ -helix arrangement and large amounts of lysine, arginine, and histidine [10]. Supported by FAPESP 98/00576-8

#### References

- [1] R. S, Petralia et al., Cell Tiss. Res (1980) 206, 145.
- [2] R. S, Petralia and C. F, Haut. Iowa Acad. Sci (1986) 91, 16.
- [3] F.J, Zara and F.H. Caetano. Sociobiology (2001) 38, 679
- [4] D.E, Ashhurst. Connective tissue. In: KING, R.C. and AKAI, H. Insect ultrastructure (1985) 2, 249.
- [5] W. R, Terra and A. G, Bianchi. Insect Biochem. (1974) 4, 173.
- [6] M, Levy, M and A. M, Bautz. Int. J. Insect Morphol. Embryol. (1985) 14, 281
- [7] L, Ambruster et al., Comp. Biochem. Physiol. (1986) 84B, 349.
- [8] G. N, Thomopoulos et al., J. Cell Sci. (1992) 102, 169.
- [9] H. Akai. Experientia (1983) 39, 433.
- [10] F, Lucas and K. M, Rudall. Extracellular fibrous proteins: the silks In: M, FLORKIN and E. H, STOTZ, Comprehensive biochemistry (1968) 26B, 475

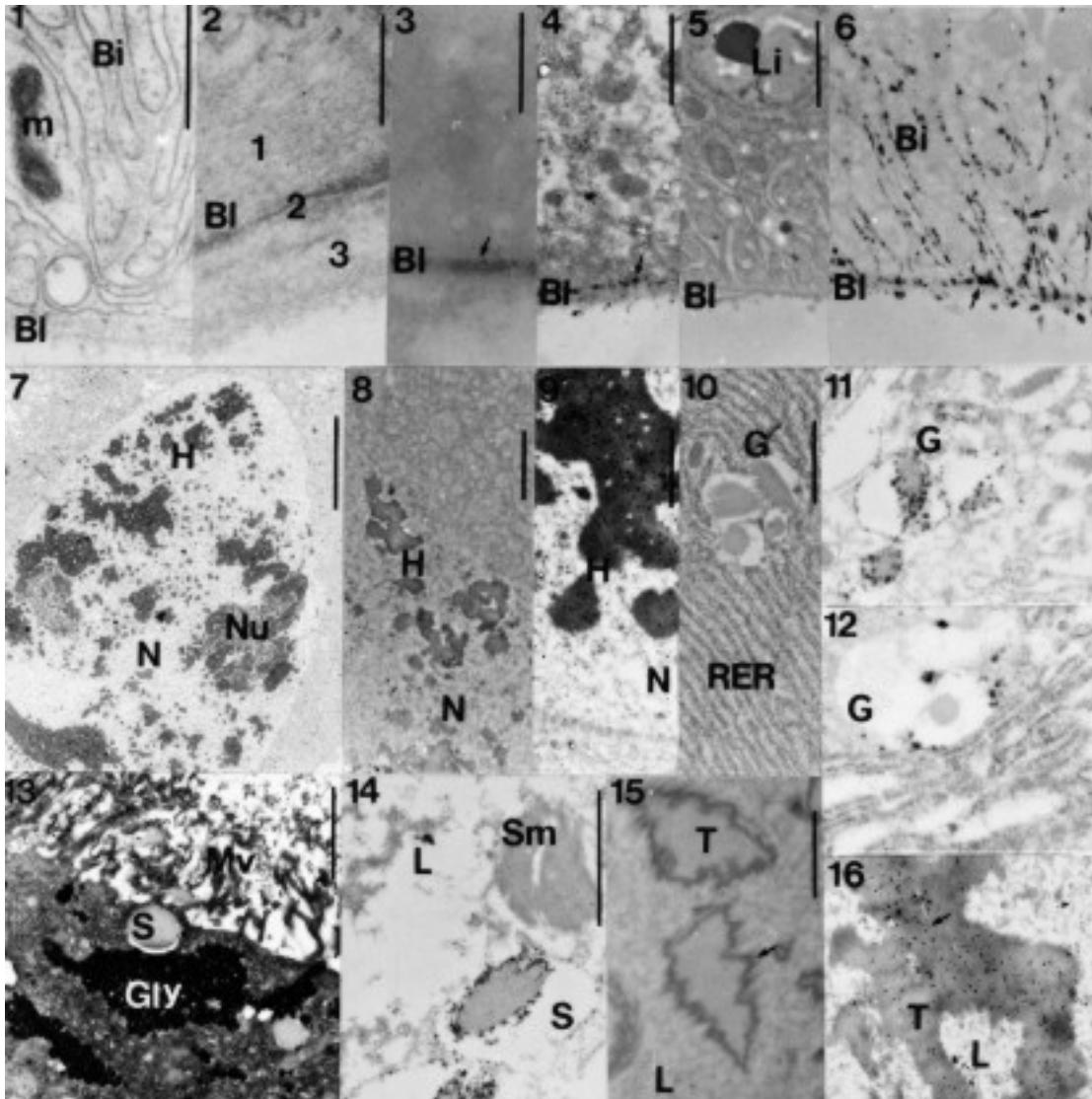


Fig 1 – A view of basal infolding (Bi), thin basal lamina (Bl) and mitochondria (m) of the salivary glands. Bar=0,5 $\mu$ m. Fig 2 – Detail of the Bl displaying three layers (1, 2 and 3). Bar = 0,25 $\mu$ m. Fig 3 – Unstained EPTA test showing positive reaction at inner and middle layers of Bl (arrow). Bar = 0,5 $\mu$ m. Fig 4 – Positive ammoniacal silver reaction in middle layer of Bl (arrow). Bar = 1 $\mu$ m. Fig 5 – Unstained Imidazole-buffered osmium tetroxide technique showing unsaturated fatty acids in lipid droplet (Li). Bar = 1 $\mu$ m. Fig 6 – Unstained p-Nitrophenyl phosphate technique with acid phosphatase activity at Bl and Bi (arrow). Bar = 1 $\mu$ m. Fig 7 – General view of the nucleus (N). Bar = 6 $\mu$ m, H = heterochromatin blocks, Nu = Nucleoli. Figs. 8 and 9 – The nucleus show positive reaction for EPTA and ammoniacal silver. Bar = 2 $\mu$ m and 1 $\mu$ m (RER). Bar = 1 $\mu$ m. Fig 11 and 12 – Positive reaction at Golgi bodies for osmium tetroxide and acid respectively. Fig. 10 – General aspect of the Golgi body (G) and lamellar rough endoplasmic reticulum phosphatase techniques. Bars = 1 $\mu$ m. Fig 13 – General view of the cell apex showing glycogen (Gly) near the secretory vesicles (S). Bar = 1 $\mu$ m, Mv = microvilli. Fig 14 – Exocytosis of the secretory vesicle showing a negative reaction for the osmium tetroxide technique, in the secretory mass (Sm) at the lumen (L). Bar = 1 $\mu$ m. Fig 15 and 16 – Positive reaction (arrow) for EPTA and ammoniacal silver technique in the tactoids (T) at the gland's lumen (L). Bars 1 $\mu$ m.