

Differential Morphology of the Hemocytes Present in Pupal Ovaries of the Queen and Worker Honeybee

Carminda da Cruz-Landim

Departamento de Biologia, Instituto de Biociências de Rio Claro,
Universidade Estadual Paulista (UNESP),
13506–900, Rio Claro, SP, Brasil

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Summary The location and morphological features of the blood cells found in the pupal ovary of workers and queens of *Apis mellifera* are described in relationship with their probable function in the ovary differentiation. The hemocytes from inside the ovarioles are different in both castes. In queens their morphology suggest an action in the tunica propria production, while in workers it suggest a phagocytic activity. The hemocytes present in the interstitial tissue are phagocytes in both castes, and may be responsible by the ovary shapping during metamorphosis.

Key words Ovary, Queen, Worker, Differentiation, Hemocytes, *Tunica propria*, Castes.

The functions reported to insect hemocytes are, among others, immunological defense, throughout encapsulation or phagocytosis, histolytic enzymes secretion during molt and metamorphosis, blood coagulation and tegument regeneration, nutrients transportation, synthesis of connective tissue components (Whitten 1964, Ashhurst 1979, Jones 1979, Wigglesworth 1979).

The diversity of morphologies described to these cells by different authors using light and transmission electron microscopy resulted in diferent systems of identification and classification of the insect hemocytes (Wigglesworth 1959, Jones 1979, Gupta 1979, 1985) and Brehélin and Zachary (1986) attempted a systematization that should universalise the authors interpretations of cell types, their origin, derivation and roles.

From the morphological standpoint the role of the hemocytes, in a given context, may be inferred by the cell features, its location and relationships with the neighbour cells or tissues (Anglas 1900, Lotmar 1945, Fyg 1975, Cruz-Landim and Giometti 1990, Cruz-Landim 1996).

Hemocytes are frequently found inside the gonads, mature and developing ones (King 1970, Büning 1994, Fyg 1975, Szollosi 1982, King and Büning 1985, Zacaro 1999), where several interpretations were given to their presence.

The present paper reports differences in the hemocytes presentation in ovaries of queen and worker honeybee pupae.

Material and methods

The bee under study is *Apis mellifera* Linné. Worker pupae were collected from queen right colonies in the red eyed stage of development. The queens were raised artificially and their pupae were also collected in the red eyed stage. All material come from the Department of Biology Apiary (Rio Claro, SP, Brazil).

The ovaries of both castes were prepared to light, scanning and transmission eletron microscopy.

For transmission electron microscopy (TEM) the ovaries were dissected and fixed in

Karnowsky in 0.1 M Na cacodylate buffer, pH 7.4 and after washing in the buffer, post-fixed in 1% osmium tetroxide in the same buffer. The embedding was done in epon-araldite and the sections stained with uranyl acetate and lead citrate.

For light microscopy (LM) and scanning electron microscopy (SEM) the ovaries were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. For LM the pieces were imbedded in historesin Leica and the sections stained with hematoxylin and eosin. For SEM the specimens were dehydrated, dried at the critical point and covered with gold, before observation in the microscope.

Results

Two locations were detected for the pupal ovarian hemocytes: 1) scattered in the interstitial tissue amongst the ovarioles, the stroma and 2) into the ovariole, between the peritoneal sheath and the tunica propria (Fig. 1A, B).

The hemocytes in the stroma appear as almost round cells (Figs. 1A, 4) while the ones inside

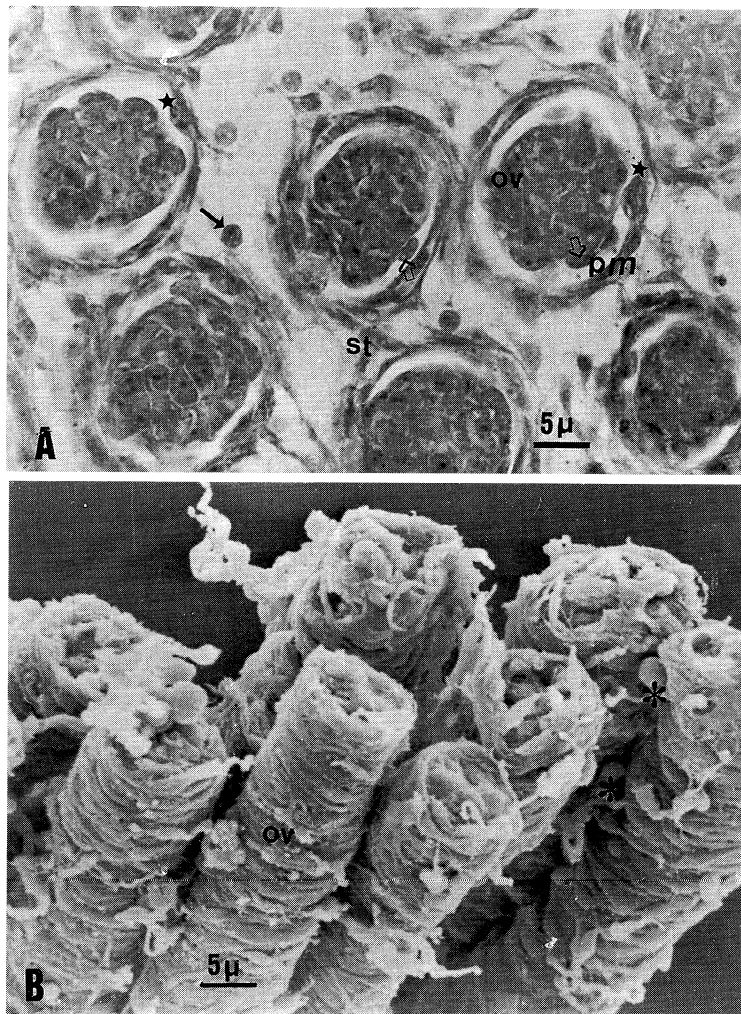


Fig. 1. A) Light micrograph of cross sections of an ovary showing the localization of hemocytes in the stroma (st-arrow) and inside the ovariole (ov) (arrows and star). B) SEM micrograph showing stromatic hemocytes (asterisks). pm=peritoneal membrane; ov=ovariole.

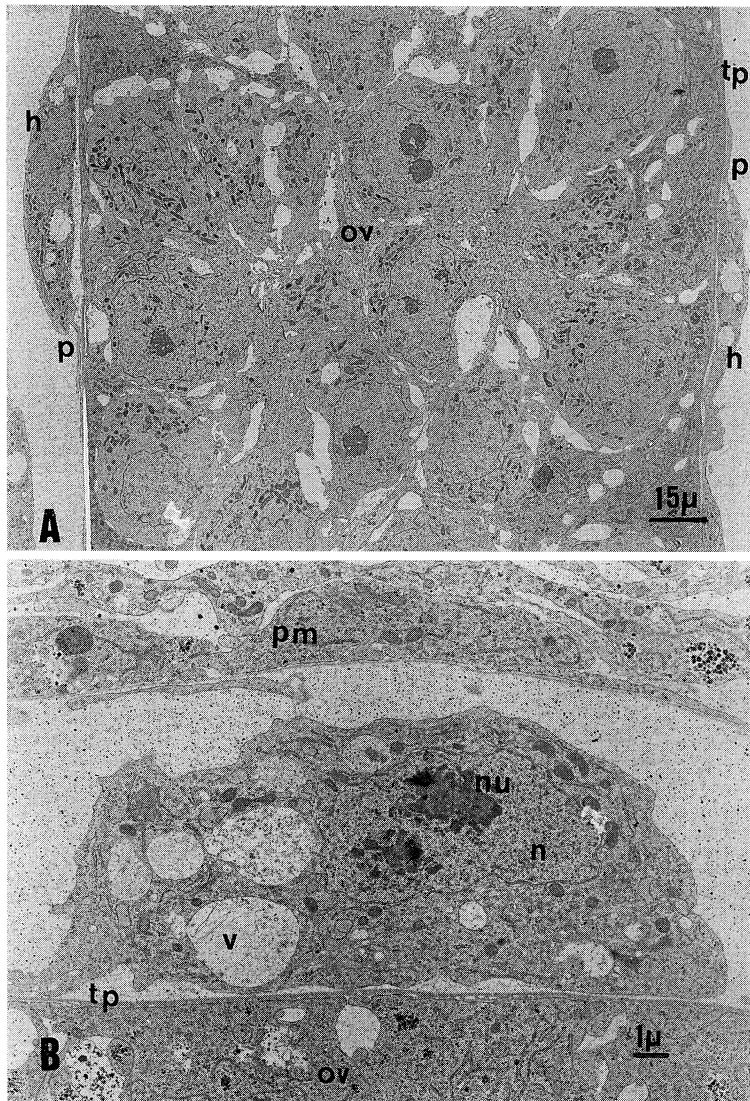


Fig. 2. TEM micrographs of plasmotocyte hemocytes (h) into the ovariole (ov) of a queen. A) Hemocytes (h) applied to the ovariole (ov) tunica propria (tp). B) Magnification of a hemocyte showing the cell organelles. pm=peritoneal membrane, n=nucleus, nu=nucleolus, v=vacuole, p=pseudopodia.

the ovarioles are flattened against the tunica propria or the peritoneal membrane that envelopes each ovariole (Figs. 1A, 2). At TEM the hemocytes features are different in queen and worker red eyed pupae.

In both castes the intra-ovariolar hemocytes are closely apposed to the tunica propria and emitting slender pseudopodia along the ovariole outer surface (Figs. 2, 3). Nevertheless, while in queens they have few vacuoles in the cytoplasm, containing homogeneous, low electron density substances (Fig. 2A, B), in worker they present vacuoles with heterogeneous content (Fig. 3A, B). Besides the vacuoles, some hemocytes present also electron dense granules (Fig. 3B). Cellular debris are frequently found around these hemocytes (Fig. 3A). The ovarioles to which the hemocytes are apposed are also different in worker and queen. While in queen they have regular contours (Fig. 2A, B), in worker the contours are very irregular (Fig. 3A, B).

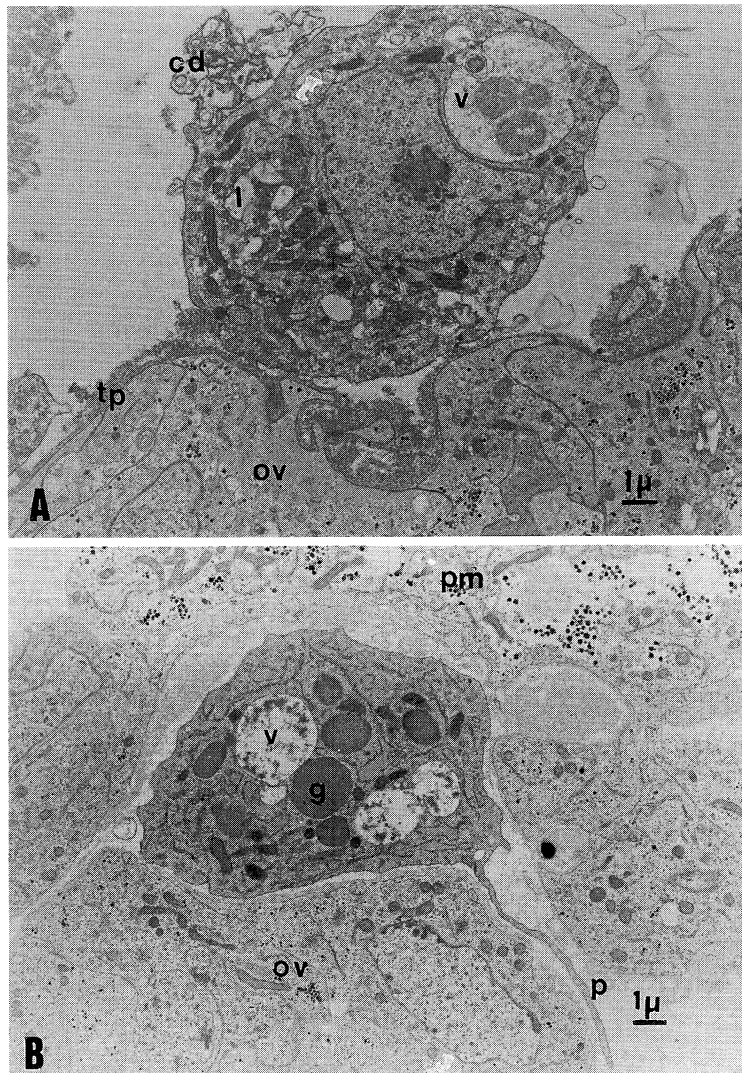


Fig. 3. A, B) TEM micrographs of granulocyte hemocytes into the ovariolar of a worker. Notice the irregular outlines of the ovariolar (ov) and cellular debris (cd) around the hemocytes, in A. Notice in B, the presence of electron-dense granules (g) in the hemocyte cytoplasm. v=heterophagic vacuoles, l=lipid, pm=peritoneal membrane, p=pseudopode, tp=tunica propria.

The hemocytes scattered in the ovarian interstitial tissue (stroma) have in both castes, characteristics of fagocytes (Fig. 4). Although all them present cytoplasmic vacuoles, in worker they have certainly heterophagic origin and in some cases, as in Fig. 4D, remains of foreign cells and glycogen may be recognised in it.

The morphology of the hemocytes permit their identification as plasmatocytes or granulocytes. These types of hemocytes were found inside and outside of the ovariolar and in the interstitial tissue (Fig. 4A, C) in worker and queens. It seems that in workers, occurs preferentially granulocytes in both locations.

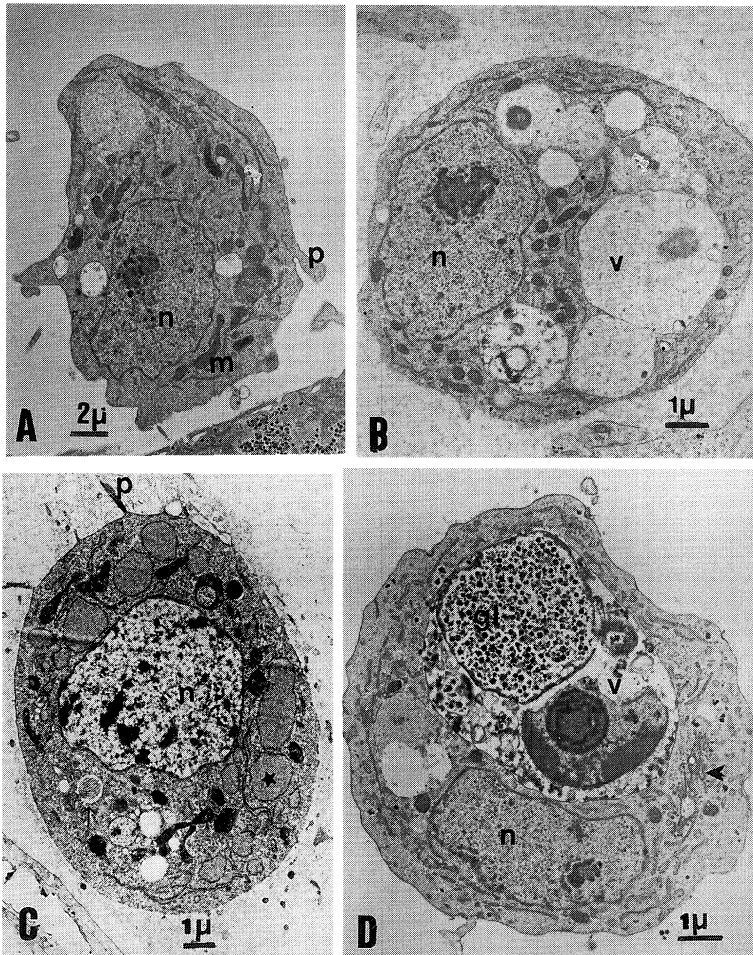


Fig. 4. TEM micrographs of stromatic hemocytes. A, B) Hemocytes from queen ovarian stroma. C, D) Hemocytes from worker ovarian interstitial tissue. A and C are plasmatocytes and B and D granulocytes. p=pseudopodia, n=nucleus, v=vacuole, arrow head=Golgi. Notice in C the enlarged lumen of the endoplasmic reticulum (star) and in D the great heterophagic vacuole (v) containing glycogen (g) and other foreign cellular debris.

Discussion

Anglas (1900) and Lotmar (1945) stated that in the honeybee the number of hemocytes increases during metamorphosis, and are characterized by the unusual cell size and cytoplasmic vacuoles. Whereas, the diameter of larval hemocytes vary from 6–10 μm during prepupal and pupal stage have a diameter of 15–25 μm . According to Anglas these larger hemocytes should play a part in the larval organs destruction that takes place during pupation. This special type of histolytic hemocyte was then denominated lyocyte. In the other hand Anglas described different function for other, equally large and vacuolated hemocytes, not involved in organ histolysis, but in transportation of nutrients from the fat body cells to the imaginal developing tissues. These were, therefore, named “formative leucocytes”.

There are a significative difference between the pupal ovary of worker and queen. In queen the ovary during pupation is being modelated by the progressive disappearance of the stroma among the ovarioles, but the ovarioles are already differentiated and with their final number established. In

worker where the ovariole number per ovary is very small in relation to the queen number, the pupal ovary are also being modeled but two events are in course: 1) ovariole destruction and 2) stromatic tissue elimination (Reginato 1998, Reginato and Cruz-Landim 2001).

The ovarioles destruction that begins in the worker 3rd instar larvae proceeds along all post-embryonic development (Reginato, 1998, Reginato and Cruz-Landim 2001) and is characterized by ovariole collapsing and desintegration as seen in Fig. 3 (compare the outlines of the ovariole in Figs. 2, 3). So the intra-ovariolar hemocytes that in queen have a formative role, participating in the production of the tunica propria, in worker have an additional role in destruction of the impaired ovarioles, and become filled up with engulfed material, that forms numerous and huge heterophagic vacuoles. Therefore the ovarian hemocytes may be included in the Anglas classification, as the morphological features of them permit identification as destructive or formative hemocytes.

The stromatic hemocytes have similar roles in queens and workers, i.e., the destruction of the interstitial tissue, that gives the ovary the adult shape. However, as seen in Fig. 4D their fagocytic activity seem to be greater in the workers, where they seem to participate also, in the ovariole reabsorption.

In conclusion, although the ovarian hemocytes should be seen as having a general part in the ovary structuration, this part is exerted through the activities proper of the hemocytes, forming the basal lamina known as part of the connective tissue (Ashhurst 1979) or fagocytosing unnecessary cells (Anglas 1900).

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