

OVARIAN GROWTH DURING LARVAL DEVELOPMENT OF QUEEN AND WORKER OF *Apis mellifera* (HYMENOPTERA: APIDAE): A MORPHOMETRIC AND HISTOLOGICAL STUDY

REGINATO, R. D. and CRUZ-LANDIM, C.

Departamento de Biologia, Instituto de Biociências de Rio Claro (UNESP), CEP 13506-900, Rio Claro, SP, Brazil

Correspondence to: Rejane Daniele Reginato, Departamento de Biologia, Instituto de Biociências de Rio Claro (UNESP), CEP 13506-900, Rio Claro, SP, Brazil, e-mail: cclandim@rc.unesp.br

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(With 15 figures)

ABSTRACT

The present work reports the differences between the ovarian growth in queen and worker larvae of *A. mellifera*, from the start of differential feeding. The observations made of the growth rates in larvae of both castes showed that the queen and worker larvae have the same rates of cephalic capsule growth from one instar to another but the weight gain is greater in queens. In the same way, the draw areas of ovaries of queens increase more and continuously, while from the 5th instar on the ovaries of workers decrease in size. The decrease is due to a loss of ovariole numbers that starts early in the worker larvae and increases in the 4th-5th instar. The ovarian shape in queens and workers became different in the last larval instars.

Key words: honeybee, castes, differentiation, ovary, ovariole loss.

RESUMO

Crescimento ovariano durante o desenvolvimento larval de operárias e rainhas de *Apis mellifera* (Hymenoptera: Apidae): estudos morfométricos e histológicos

O presente trabalho relata as diferenças verificadas no desenvolvimento das larvas de rainhas e operárias e de seus ovários, desde o início da alimentação diferencial, dadas as duas castas, ou seja, desde o 3^o dia de desenvolvimento larval. As taxas de crescimento em ambas as castas mostram que as larvas de rainhas e de operárias apresentam o mesmo índice de crescimento da cápsula cefálica de um instar para outro, mas que o ganho de peso é muito maior nas rainhas. Da mesma forma, os ovários das rainhas crescem mais e continuamente, enquanto os das operárias decrescem a partir do 5^o instar. O decréscimo se deve à perda de ovaríolos que começa cedo na larva de operárias e aumenta nos 4^o e 5^o instares. A forma dos ovários das rainhas e das operárias já é diferente no último instar larval.

Palavras-chave: abelha melífera, castas, diferenciação, ovário, perda de ovaríolos.

INTRODUCTION

In the eusocial Apinae bees a division of the reproductive work in colonies convey to two female castes, one fertile (the queen) and another sterile or partially sterile (the worker). This condition is reflected in a marked dimorphism in ovarian development between queens and workers. In *Apis mellifera*, the dimorphism is expressed as different numbers of ovarioles per ovary in the castes

(Snodgrass, 1956). Each queen ovary contains more than 100-180 ovarioles, while in workers there are generally less than ten (Oertel, 1930; Snodgrass, 1956; Chaud-Netto & Bueno, 1979; Morini & Bueno, 1995). The difference in ovariole numbers, between worker and queen, results from the worker ovary atrophy during the larval development, consequence of the differential feeding of the castes (Zander & Becker, 1925; Oertel, 1930; Lotmar, 1945; Kuwabara, 1947).

The differences in the food received by queen and worker larvae of *Apis mellifera* are both qualitative and quantitative (Haydak, 1943; Ribbands, 1953) and start in the 2nd or 3rd day after hatching. During all larval phases the queen larvae are fed with royal jelly, while the worker larvae, once they are 2-3 days old, are fed with a blend of jelly, honey and pollen.

The ovaries, present in larvae at the hatching time, undergo, according to Anglas (1900), during the larval phase a process of development without histolysis or reabsorption. Actually, Meier (1916) counted 62-72 ovarioles in 2 day old worker larva and 74-89 ovarioles in 3 day old larva, however, the numbers reached by workers were not ever equal to that achieved by the queen larvae at the same age (Löschel, cited by Meier, 1916). It seems therefore, that the ovariole number in ovaries is different during the larval phase in workers and queens. However, several other studies demonstrate that the ovariole numbers are the same in all early larvae and that the final differences between queen and worker are due to ovariole reabsorption in worker ovaries (Bueno, 1981; Hartfelder & Steinbrück, 1997).

Another aspect to consider is the time when the differences between worker and queen are established, or when worker ovarioles start to suffer reabsorption. According to Bueno (1981) and Hartfelder & Steinbrück (1997) reabsorption takes place by the end of the larval phase (4th, 5th instar), but previous results indicate an early initiation of the process (Lotmar, 1945; Beetsma, 1979).

The papers on honeybee castes differentiation (Rembold, 1987; Rachinsky *et al.*, 1990; Evans & Wheeler, 1999) do not consider the subject of ovary development. Therefore, the present work attempts to contribute in solving this pendent question by analysing the size and histology of worker and queen ovaries during larval development.

MATERIAL AND METHODS

Two day old larvae were collected from the colony and grafted to queen cups containing royal jelly for queen artificial production. The remaining larvae of the same age and egg-laying queen were left in the comb to develop into workers. Larvae from queen cups and from worker brood cells were collected daily from one to six

days after grafting. These larvae were weighed and the width of their cephalic capsules were measured at the level of the antennal imaginal discs in order to determine their developmental instar, by comparison with the previous data of Goitein (1989). Their ovaries were then excised, their contours outlined on paper with a camera lucida, and the relative areas of the drawings calculated. Some ovaries were fixed for light (LM) and scanning electron microscopy (SEM) studies, in 4% paraformaldehyde in 0,1M phosphate buffer, pH 7.4. For light microscopy, the ovaries were embedded in historesin and the sections stained with hematoxylin and eosin according to the usual procedures. The specimens used for SEM were dehydrated, dried at critical point and coated with gold.

RESULTS

The measurements of the larval cephalic capsule width (Fig. 1) show that until the 2nd day when grafting (transfer of larvae into queen cups) was done, queen and worker larvae were in the same instar, 3rd instar. One day after grafting the queen larvae was in the 4th instar, while the worker larvae remained in the 3rd instar. Five days after the grafting some of the queen larvae was still in the fifth instar but, most of the queen cups already contained pre-pupae and in the next day, pupae. In the brood comb, the worker larvae of the same age was in the 4th instar (5 days after grafting) and 5th instar (6 days after) according to the previous determination of Goitein (1989) for bees raised in the same conditions.

The results also show that the developmental rate of cephalic capsule (Fig. 1) from one instar to another is the same in queen and worker larvae. In spite of the same rate growth the gain in weight of worker and queen larvae is different. Queen larvae are already heavier than the workers one day after grafting, and the difference increases throughout the days (Fig. 2). Until three days after grafting there is little weight difference between worker and queen, but in the 5th instar it is great. Queen larvae four days after grafting weighs twice as much as the worker and five days after two folds and a half. The queen and worker larvae duplicate its weight from the 4th to the 5th instar.



Fig. 1 — Cephalic capsule width of queen and worker of *Apis mellifera* larvae, from same queen laying battery 1, 3, 5, 7 and 9, worker larvae respectively 1, 2, 3, 4 and 5 days after some larvae of the batch have been graft, into queen cups. 2, 4, 6 and 8, queen larvae respectively 1, 2, 3, 4 days after grafting.

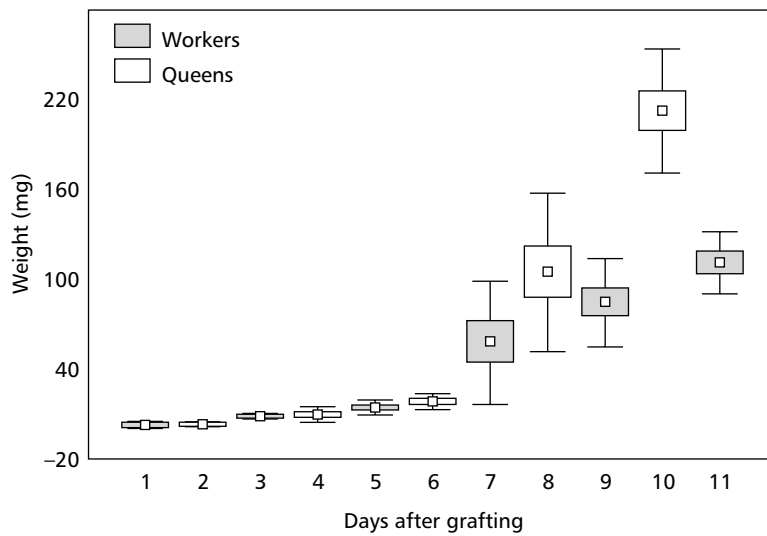


Fig. 2 — Average weight (mg) of queen and workers larvae of *Apis mellifera* during the development. 1, 3, 5, 7, 9 and 11, worker larvae respectively 1, 2, 3, 4, 5 and 6 days after some larvae of the same batch have been graft, into queen cups. 2, 4, 6, 8 and 10, queen larvae respectively 1, 2, 3, 4 and 5 days after grafting.

The ovaries have a continuous growth in both queen and worker during the larval development (Fig. 3), but the growth is greater in queen larvae mainly in the fifth instar. The queen larvae ovaries increase 15% from 2nd to 3rd instar, 78% from 3rd to 4th instar and 100% from 4th to 5th instar, while the worker larvae ovaries increase 10% from 2nd to 3rd instar, 63% from 3rd to 4th instar and from 4th to 5th instar it decreases. The queen ovaries continue to grow even after the fifth instar and during pupation (Fig. 3).

The examination of the ovary sections (Figs. 4-6) of queen and worker larvae on the 2nd instar with light microscopy shows a mass of cells, where large cells are surrounded by small flat ones and the cell group by a capsule of several layers of flat cells (Fig. 4).

From these large cells seem to originate the germ cells, while the small surrounding cells give rise to the ovariole somatic tissue. As the ovarioles individualization advance they become separated one from another by inter-ovariolar flat cells, the

stromatic cells, and their number becomes evident (Figs. 5 and 6).

At the 3rd larval instar the morphology of the ovaries and the number of ovarioles seem the same in queen and worker larvae, but in workers, cell deaths may be observed in them. In the next stages (4th and 5th instars) differences in the ovariole numbers between queen and worker, can be observed. Nevertheless only by the end of the 5th instar are they more evident and in pre pupae a great difference is established in the ovariole numbers between the two castes (Figs. 5 and 6).

The SEM examination (Figs. 7-15) show that after the 4th instar the shape of workers and queens ovaries becomes different. The workers maintain the elongated form (Fig. 11) while in queens the ovaries round up (Figs. 12-14). Freeze-fracture of a queen 5th instar ovary, shows a great number of ovarioles closely packed (Figs. 13 and 15). In pre-pupae the worker ovary has a cylindrical shape (Fig. 11) while the queen ovary has a round-flattened form (Fig. 14).

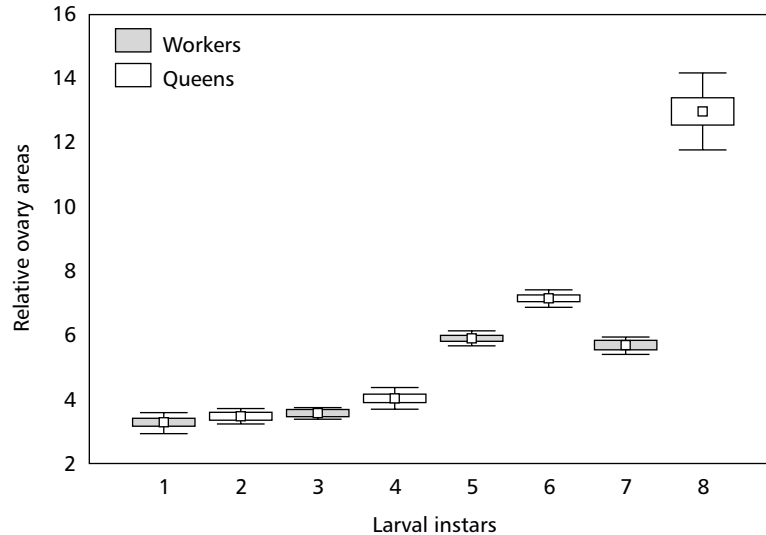
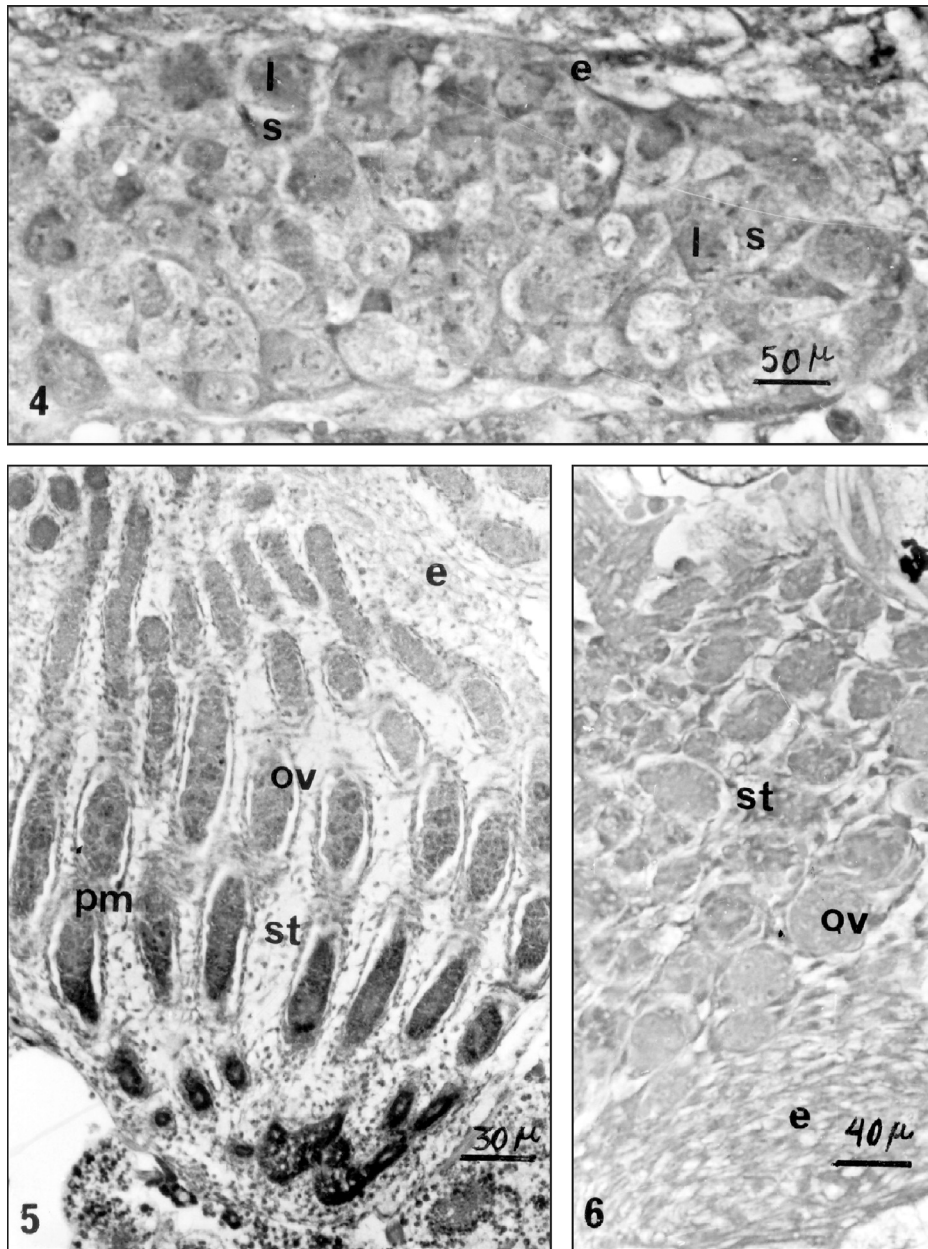
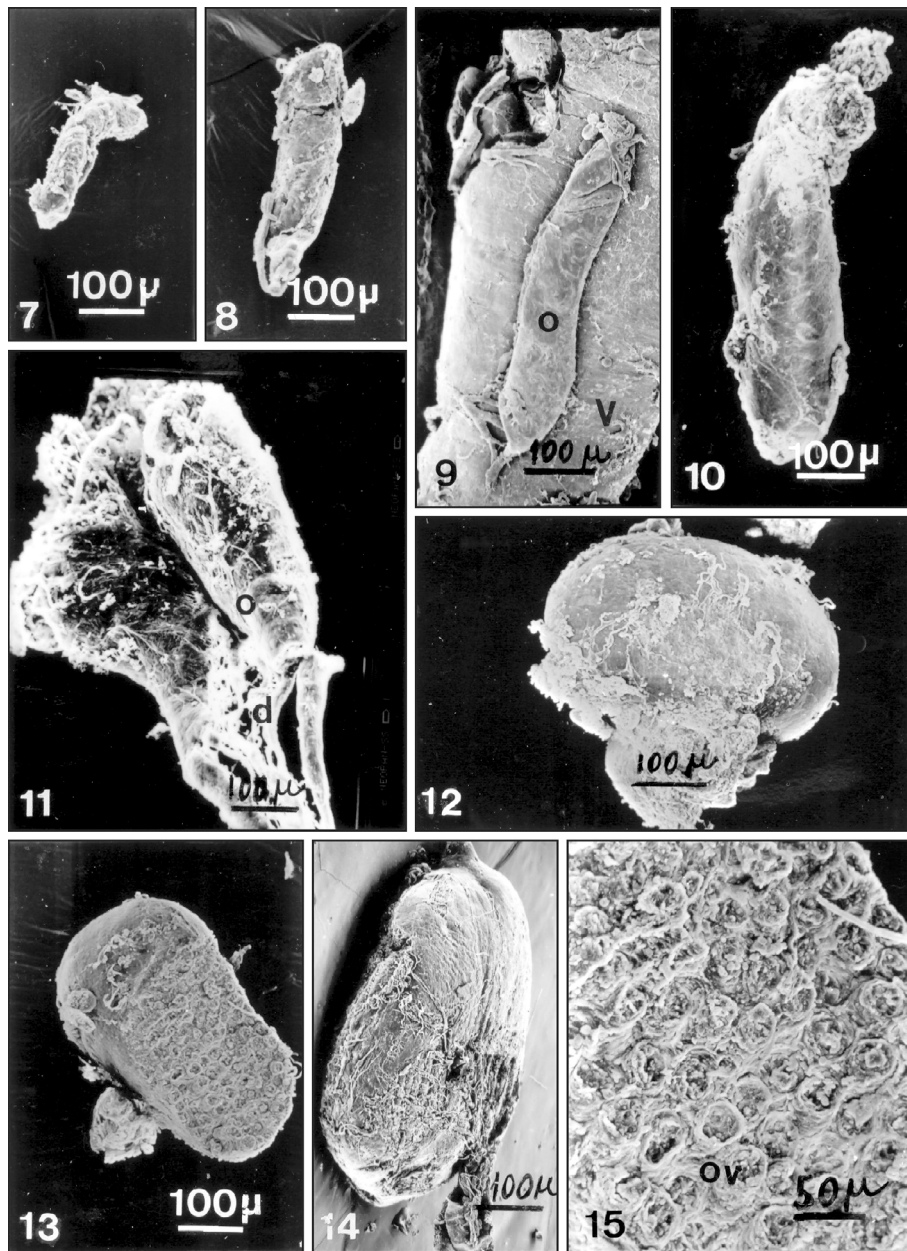


Fig. 3 — Relative average areas of worker and queen ovaries of *Apis mellifera* during larval development. 1, 3 and 5, 7 worker larvae respectively from 2nd, 3rd, 4th and 5th instar. 2, 4, 6 and 8 queen larvae respectively from 2nd, 3rd, 4th and 5th instar.



Figs. 4-6 — Light micrographies of *Apis mellifera* ovaries. **Fig. 4** — Ovary of a 2nd instar larva showing a mass of large round cells (l) probably germinative cells, surrounded by flat small cells (s). **Fig. 5** — Queen ovary of a 5th instar larva showing the differentiated ovarioles (ov) separated by stromatic (st) cells. **Fig. 6** — Worker ovary of a 5th instar larva, showing few ovarioles (ov). o = ovary capsule; pm = peritoneal membrane surrounding the ovariole.



Figs. 7-15 — Scanning electron micrographs of the ovarian in different larval developmental phases. **Figs. 7-11** — Worker ovaries: 7 = 2nd instar; 8 = 3rd instar; 9 = 4th instar, 10 = 5th instar, 11 = prepupa. **Figs. 12-15** — Queen ovaries of larvae ending the 5th instar. 12 = outer view; 13 = inner view showing the ovarioles, 14 = ovary of a late 5th instar larvae 15 = cross section of the ovarioles. o = ovary; v = midgut; ov = ovarioles.

DISCUSSION

Some authors (Lotmar, 1945; Beetsma, 1979) observed a difference in ovariole number in the ovaries of worker and queen larvae, starting with the differential feeding of the larvae, but others show that the ovaries from worker and queen larvae grow continuously during the larval phase (Meier, 1916; Bueno, 1981), without a great difference between them, suggesting that only by the end of the larval phase are the differences between the ovaries of queen and worker established (Bueno, 1981; Hartfelder & Steinbrück, 1997).

These results seem conflicting but the present work shows that the differences in ovary size clearly starts once the feeding of the larvae becomes different. Reabsorption of ovarioles were seen by Reginato & Cruz-Landim (2002) since the 3rd instar in worker larvae and confirmed in this work. The ovariole reabsorption, however, does not totally reflect in the ovary size because the worker ovary continues to growth and stromatic cells fill up most of the space which before were occupied by the reabsorbed ovarioles, contributing to maintain the ovary total volume almost the same. Nevertheless, from the 4th instar on, the number of ovarioles become much lower in workers and the size and shape of the ovaries in both castes started to change.

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