The Post-embryonic Changes in *Melipona quadrifasciata anthidioides* Lep. (Hym., Apoidea)

V. Polyploidy in the larval Malpighian tubules¹

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Introduction

In bees, polyploidy has been demonstrated mainly for glandular cells (Painter 1945, Risler 1954, Painter and Biesele 1966, Cruz-Landim and Mello 1969a). Risler (1954), in his exhaustive research, investigating the occurrence of somatic polyploidy in *Apis mellifera*, pointed out that mitoses are not observed in Malpighian tubules during larval stages; nevertheless, there is an apparent enlargement of nuclei and an increase in number of nucleoli.

The larval Malpighian tubules of *Melipona quadrifasciata*, eight in number (Mello 1968), open into the anterior blind end of the hind intestine. The larval tubules appear unquestionably to be ectodermal in their origin; the imaginal or pupal tubules also belong to the ectodermal proctodaeum (Snodgrass 1956). According to onthogenetic ecdysis, *Melipona* have five larval instars (Oliveira 1960). During the larval stages, Malpighian tubules apparently increase in size and in early prepupa they are autolysed; the adult tubules begin their development in 5th instar larva (Cruz-Landim and Mello 1969b).

The occurrence of polyploidy and the number of DNA replications in cells of larval Malpighian tubules are investigated in this study.

Material and methods

The present work was performed on whole mounted Malpighian tubules of *Melipona quadrifasciata anthidioides* Lep.

Malpighian tubules of alive bees of the five larval instars and of prepupa stage were dissected in saline solution. They were fixed in Carnoy's fixative for one hour, washed in 95% ethanol, hydrated and then submitted to Feulgen reaction. After staining, the tubules were washed in 45% acetic acid and dehydrated and then mounted in euparal.

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For nuclei measurements we have choosed the distal portion of the tubules, because there is some evidence for different patterns of nuclear activity in insects, regarding proximal and distal regions of these organs (Risler 1954, Pavan 1965).

The quantitative determination of DNA contents was made with the aid of a cytophotometer. For each instar 100 nuclei were measured.

DNA contents were determined by the formula $Q = D^2(2 - \log I_n)$. D^2 is the square of the mean diameter (nuclear optical surface) and I_n is the light transmittance.

The nuclear volume determination was performed measuring camera lucida drawings of the nuclei and considering them as ellipsoids, with three axis, being the 3rd one equal to the 2nd. Nuclear volume was calculated through the formula $V = 4/3\pi ab^2$. For 1st axis (a) we have considered the higher value.

According to Bucher and Horisberger (1950) and Hintzche (1954), nuclear volumes have a log-normal distribution. Therefore all calculations have to be done on a logarithmic basis.

To avoid the search for logarithms, a scale of values in geometric progression was elaborated in such a way that between the values 1 and 2, ten intermediary classes were placed and original data were distributed in these classes (Ferreira *et al.* 1967).

Fig. 1. Malpighian tubules throughout larval and prepupal development. Only two tubules are represented for each instar. Camera-lucida drawings. hg: hindgut, L₁ to L₅: larval instars, mg: midgut, P: pupa, PP: prepupa.

Results and discussion

An increase in length of larval Malpighian tubules is anatomically evidenciated (Fig. 1) and the size of the nuclei follows this enlargement (Fig 2).

Values for DNA contents and nuclear volumes were plotted as frequency histograms (Figs. 3 and 4) and confirm the occurrence of polyploidy.

Geometric means of the values plus the standard deviation can be taken as representatives of only few stages, as the imaginal one and also the 4th and 5th larval instars. For the 1st, 2nd and 3rd instars, the mean is not the best representative (Table 1).

If we consider the imaginal

Malpighian tubules in a diploid stage, as was suggested for *Apis* (Risler 1954), the larval tubules go through four DNA replications.

In the 1st larval instar there are two DNA replications and in the 2nd

Fig. 2. Photomicrographs of whole mounted Malpighian tubules.

Fig. 3. Frequency histograms of nuclear volumes.

Fig. 4. Frequency histograms of DNA content.

instar there is another replication. In our 1st instar sample the nuclei are, in their majority, $8n$; the $4n$ stage probably occurs in very young larvae of the 1st instar.

The last DNA replication begins in the nuclei of late 3rd instar and it is more improved in the early 4th instar (Figs. 3 and 4).

In the 4th and 5th larval stages the nuclei are at least $32n$.

In all instars the nuclear volumes follow the increase in DNA contents, except in the 4th larval instar.

In the prepupa, the values for nuclear volume are slightly lower than in the 5th larval instar, on account of beginning the autolysis of larval Malpighian tubules, when the

nuclei become pycnotic (Cruz Landim and Mello 1969b).

As a specialized and differentiated unit of the larval organism, the Malpighian tubules have probably acquired this process of endopolyploidy to perform better their necessities of excretory purposes.

Table 1. Nuclear volume and DNA content in Malpighian tubules during larval and prepupal stages ($V \pm Sv$ =geometric mean of the nuclear volume in arbitrary units and its corresponding standard-deviation; $Q \pm Sq$ =geometric mean of DNA content in arbitrary units and its corresponding standard-deviation; n =number of measurements for each instar=100)

Stages		$V \pm Sv$	$Q \pm Sq$	Probable degree of ploidy
Larval	1st instar	14.90 ± 2.00	0.49 ± 0.16	8n
	2nd instar	29.75 ± 1.45	1.10 ± 0.14	16n
	3rd instar	26.10 ± 1.38	1.29 ± 0.13	16n
	4th instar	39.87 ± 1.55	1.32 ± 0.13	32n
	5th instar	36.37 ± 1.55	1.29 ± 0.16	32n
Prepupal		27.34 ± 1.48	1.14 ± 0.14	32n
Pupal		2.15 ± 0.17	0.09 ± 0.01	2n

Summary

In the course of the larval stages, the Malpighian tubules of *Melipona quadrifasciata* go through a process of endopolyploidy.

There are two DNA replications in the 1st larval instar, one in the second and another in the early 4th instar. After this stage the cells are at least 32n.

During all this process, the enlargement of nuclear volume always follows the increase in DNA contents, except in the 4th larval instar.

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