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FEULGEN STAINING IN MALPIGHIAN TUBULES OF MELIPONID BEES: A METHODOLOGICAL CONTRIBUTION*

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ABSTRACT

Whole-mounted Malpighian tubules of larvae from two meliponid bee species fixed in acetic acid-ethanol showed a positive cytoplasmic staining with Schiff reagent when submitted to the Feulgen reaction in which acid hydrolysis was done in 4 M HCl at room temperature. The ability of various treatments applied before the Feulgen acid hydrolysis to abolish this cytoplasmic staining was examined. The aldehyde groups of phospholipids present in the cytoplasm of the Malpighian tubules were blocked or removed by sequential treatment with 5% sodium borohydride and acetone-chloroform (1:1, v/v) for 15 min each prior to HCl hydrolysis. This treatment is recommended in order to abolish the cytoplasmic (plasmal) reaction and to allow the reliable quantification of DNA by the Feulgen reaction and the discrimination of nuclear phenotypes in the Malpighian tubules of meliponid bees.

Key words: Aldehydes, cytoplasmic phospholipids, Feulgen staining, Malpighian tubules, meliponid bees, plasmal reaction

INTRODUCTION

As in most insects with complete metamorphosis, the larval organs in bees grow by endopolyploidy rather than by cell division [4]. The degrees of ploidy in cells of the larval Malpighian tubules of some bee species has been estimated in cytological preparations submitted to the Feulgen reaction in which the acid hydrolysis step was done in 1 M HCl at 60°C [3]. Currently, the hydrolytic step of the Feulgen reaction is frequently done in 4 M HCl at room temperature, mainly because of operational facilities and more reliable results obtained [5]. Under these conditions, when analyzing the Feulgen response in Malpighian tubule cells from bees of the genus *Melipona* fixed in absolute ethanol-glacial acetic acid (3:1, v/v) for 1 min, we have also observed a deep, positive staining in the cytoplasm of these cells. This additional staining, which is typical of cytoplasmic phospholipid aldehydes (plasmalogens) that react with Schiff reagent [7], can hinder the reliable quantification of nuclear DNA and the discrimination of nuclear phenotypes by microspectrophotometry or video image analysis.

In this study, we examined the ability of various treatments used before the Feulgen acid hydrolysis step to block or destroy the cytoplasmic aldehydes responsible for the strong reaction, while retaining the positive Feulgen staining of nuclear DNA.

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MATERIAL AND METHODS

Fifth instar predefecating larvae from *Melipona quadrifasciata anthidioides* Lep. and *Melipona rufiventris* Lep. (Hymenoptera, Apoidea) reared and supplied by the Federal University of Vi osa were used. Whole-mounted preparations of Malpighian tubules removed from the larvae in Ringer solution were fixed in absolute ethanol-glacial acetic acid (3:1, v/v) for 1 min, followed by rinsing in 70% ethanol for 1-5 min. The tubules were subjected to the Feulgen reaction, with hydrolysis in 4 M HCl at 25°C for 90 min followed by treatment with Schiff reagent for 40 min. The preparations were then rinsed three times in sulfurous water and once in distilled water before being air dried. The organs were cleared in xylene and mounted in natural Canada balsam (Vetec, Rio de Janeiro).

To define the cytoplasmic staining as a plasmal reaction [2,7], some fixed preparations were simply treated with Schiff reagent without the acid hydrolysis step. The abolishment of the cytoplasmic reaction was examined by varying the duration of the sulfurous water rinses from 3 to 10 min each and by treating the preparations with (a) 5% sodium borohydride solution [2], pH 9.8, for 1 to 15 min, (b) acetone-chloroform (1:1, v/v) for 15 min, or 5% sodium borohydride solution followed by acetone-chloroform (1:1, v/v) for 15 min each, prior to acid hydrolysis.

The preparations were observed and photographed in a Zeiss Axiophot II microscope (Oberkochen, Germany).

RESULTS

The tubules treated with Schiff reagent without prior acid hydrolysis showed the cytoplasmic staining typical of plasmal reaction [2,7].

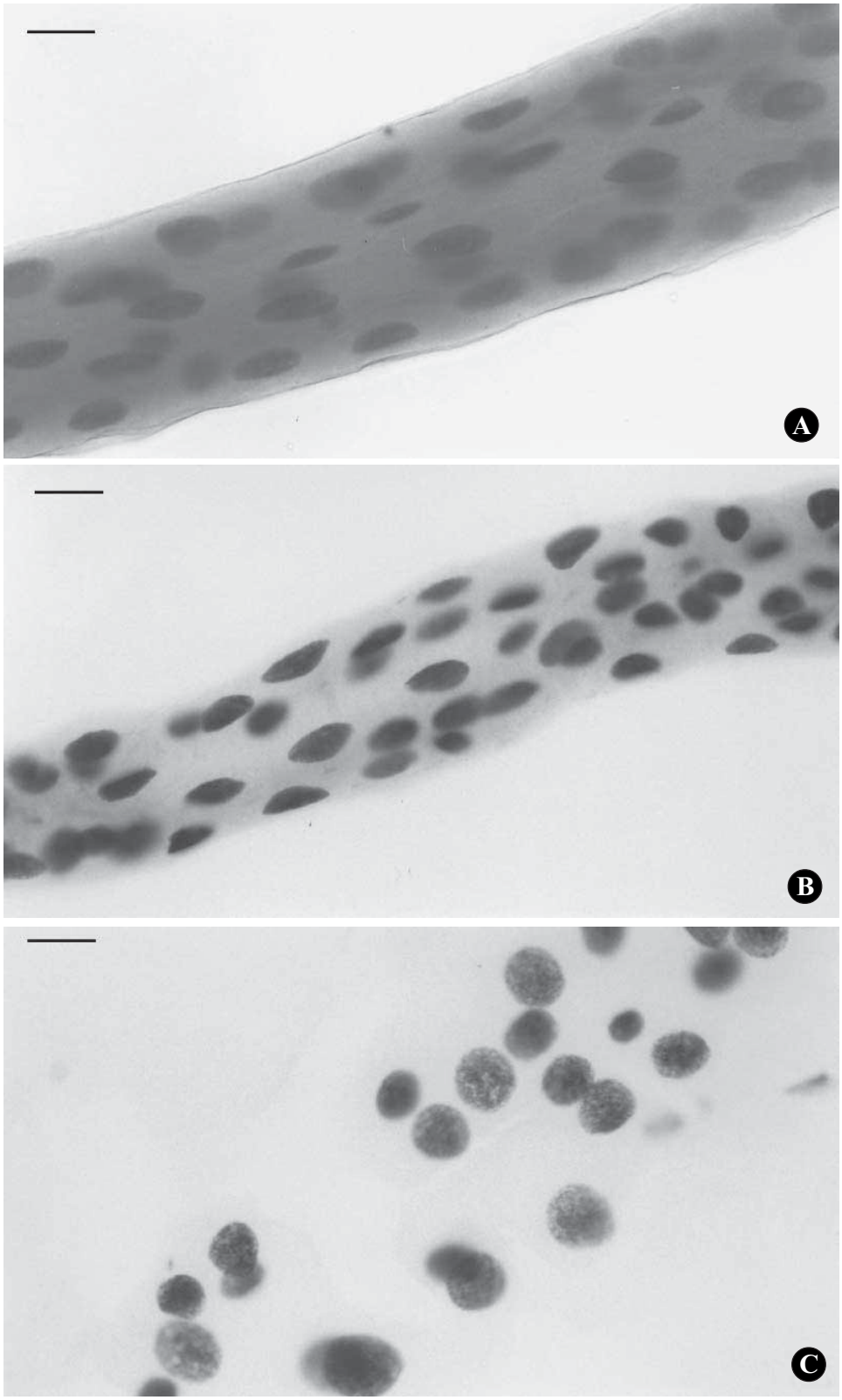

Figure 1. Feulgen-stained Malpighian tubules of *M. quadrifasciata*. **A** — Sulfurous water rinses lasting 10 min each. **B** — Reaction preceded by sodium borohydride treatment. **C** — Reaction preceded by treatment with sodium borohydride and acetone-chloroform. Bar = 30 μm.

Prolongation of the sulfurous water rinses up to 10 min each did not affect the positive cytoplasmic reaction in the Malpighian tubules of *M. quadrifasciata* submitted to the Feulgen reaction (Fig. 1A). Of the various treatments used prior to acid hydrolysis, that with borohydride solution decreased the intensity of the plasmal reaction after a 15 min exposure to this reducing agent (Fig. 1B), whereas the acetone-chloroform treatment did not affect the cytoplasmic reaction. In contrast, a combination of both treatments (sodium borohydride followed by acetone-chloroform for 15 min each) while using sulfurous water rinses lasting 3 min each after the Schiff reagent treatment totally abolished the plasmal reaction (Fig.1C). Similar results were obtained for *M. rufiventris.*

DISCUSSION

Our results indicate that aldehyde groups were responsible for the cytoplasmic staining seen in the Malpighian tubule cells of *M. quadrifasciata* and *M. rufiventris* after the Feulgen reaction. Alkaline solution of sodium borohydride reduces aldehydes to primary alcohols, [$4RCHO + NaBN_4 + 4H_2O \rightarrow 4RCH_2OH + B(OH)_3 + Na^+ + OH^-$] thus blocking the positive Schiff staining caused by aldehydes present in the tissue [2]. The complete removal of aldehydes present in the cytoplasm of Malpighian tubules required sodium borohydride treatment followed by acetone-chloroform (1:1, v/v). This observation indicated that the aldehydes involved were provided by phospholipids [2], a conclusion in agreement with the cytochemical demonstration of these components in the Malpighian tubules of *M. quadrifasciata* [6].

The use of whole-mounted preparations and of acid hydrolysis done at room temperature were probably responsible for preservation of the cytoplasmic aldehydes which reacted with the Schiff reagent. Similar reactions have been reported for frozen sections of other materials, but rarely in paraffin sections [7]. In a previous study of the DNA content of bee Malpighian tubules, no cytoplasmic staining was observed, probably because the Feulgen acid hydrolysis step had been done at 60°C [3]. In bee organs that do not contain phospholipid granules such as those present in Malpighian tubules, no cytoplasmic staining is seen after the Feulgen reaction, even when the acid hydrolysis step is done at room temperature [1].

In conclusion, our results show that the potential interference by cytoplasmic aldehydes in the Feulgen reaction with acid hydrolysis at room temperature used in whole-mounted Malpighian tubules of meliponid bees can be eliminated by the sequential treatment with 5% sodium borohydride and acetone-chloroform (1:1, v/v) for 15 min each prior to the HCl hydrolysis.

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