

## METHODOLOGY

# Extraction of genomic DNA from *Melipona quadrifasciata* (Hymenoptera: Apidae, Meliponinae)

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## ABSTRACT

The objective of the present study was to test three different procedures for DNA extraction of *Melipona quadrifasciata* based on existing methods for DNA extraction of *Apis*, plants and fungi. These methods differ in the concentrations of specific substances in the extraction buffer. The results demonstrate that the method used for *Apis* is not adequate for DNA extraction from *M. quadrifasciata*. On the other hand, with minor modifications this method and the methods for plants and fungi were adequate for DNA extraction of this stingless bee, both for adults and larvae.

## INTRODUCTION

Molecular markers (RAPD, RFLP, AFLP, etc.) have been widely used in plant and animal breeding as well as for the elucidation of molecular aspects of evolution, phylogeny and physiology.

These techniques require a fair amount of DNA of good quality. This is a particularly serious problem with small animals like insects. In these cases, specific methodologies to optimize the extraction of good quality DNA samples are needed.

Few methods are available for bee DNA extraction in the literature. To our knowledge, only Sheppard and McPheron (1991) and Hunt and Page (1992) have described DNA extraction procedures for the genus *Apis*. In this work three DNA extraction methodologies used in different systems were modified and tested with *Melipona quadrifasciata*, a stingless bee popularly known in Brazil as "mandacaiá".

## MATERIAL AND METHODS

Fifteen adult workers and 15 larvae from the same colony were used for DNA extraction of *Melipona quadrifasciata*. Three different methods were tested: A) method described by Doyle and Doyle (1990) for plants and by Schäfer and Wöstemeyer (1992) for fungi; B) method described by Hunt and Page (1992) for *Apis*, and C) modified method B.

Basically, the three methods tested differ in the concentrations of specific components of the extraction buffer. In method A, the extraction buffer was 100 mM Tris-HCl, pH 8.0, containing 2% CTAB, 1.4 M NaCl, 20 mM EDTA and 100 µg/ml proteinase K. In B, the extraction buffer was 50 mM Tris-HCl, pH 8.0, containing 1% CTAB (hexadecyl trimethyl ammonium bromide), 0.75 M NaCl, 10 mM EDTA and 100 µg/ml proteinase K. In C, the extraction buffer was 50 mM Tris-HCl, pH 8.0, containing 2% SDS (sodium dodecyl sulfate), 0.75 M NaCl, 10 mM EDTA and 100 µg/ml proteinase K.

Each adult bee was ground individually in a mortar and pestle containing liquid N<sub>2</sub> and homoge-

nized with extraction buffer. Each larva was ground in a microcentrifuge tube with a plastic pestle in the presence of extraction buffer. The samples were then incubated at 65°C for 30 min. Samples containing adult individuals were deproteinized twice with one volume each of chloroform. Larva homogenates were then deproteinized once with one volume phenol:chloroform (1:1) and once with one volume chloroform. After deproteinization the samples were centrifuged at 11,750 g for 5 min. Nucleic acid was precipitated by addition of one volume of cold isopropanol and incubation at -20°C for 2 to 24 h. After centrifugation at 16,000 g for 30 min the pellets were washed twice with 70% (v/v) ethanol and vacuum dried for approximately 15 min or dried in the air for 30 min. The pellet was resuspended in 200 µl TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and incubated with RNase A (100 µg/ml) for 30 min at 37°C. The integrity and purity of the DNA samples were checked on 0.8% (w/v) agarose mini-gels.

## RESULTS AND DISCUSSION

Quantitative and qualitative differences were observed in the DNA samples obtained by the three different methods (Figures 1 and 2). Figure 1 suggests that the method B was not as adequate for DNA extraction from *M. quadrifasciata* as methods A and C. Similar results were obtained for DNA extraction from larvae (Figure 2). Again methods A and C proved to be more adequate for DNA extraction from *M. quadrifasciata*.

As described in Material and Methods, the basic difference between the three methods is the concentration of specific components of the extraction buffer. In method A the concentrations are doubled in relation to B and C, while the main difference between methods B and C is the use of CTAB in B and SDS in C. Under the buffer and salt concentrations used in methods B and C, SDS but not CTAB was effective for DNA extraction. The only difference observed was that during larval DNA extraction using SDS (method C), contaminating material (proteins?) seems to retain part of the DNA in the wells, indicating that one more deproteinization step is necessary. On the other hand, when methods A and B are compared the concentrations of the other components of the extraction buffer seem to be crucial for obtaining reasonable DNA yields (Figures 1 and 2).

Although the same concentration of RNase was used for both adults and larvae, RNA was still present in the material extracted from larvae (Figure 2, arrow). This is explained by the larger amounts of RNA

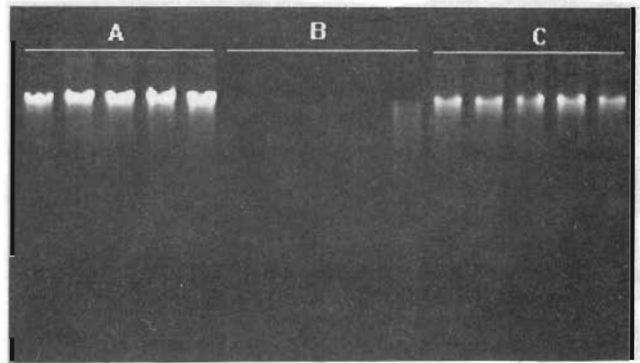


Figure 1 - Electrophoretic analysis of genomic DNA extracted from *Melipona quadrifasciata* (adults). A, Method described by Doyle and Doyle (1990) for plants and by Schäfer and Wöstemeyer (1992) for fungi; B, method described by Hunt and Page (1992) for *Apis*; C, method B modified.

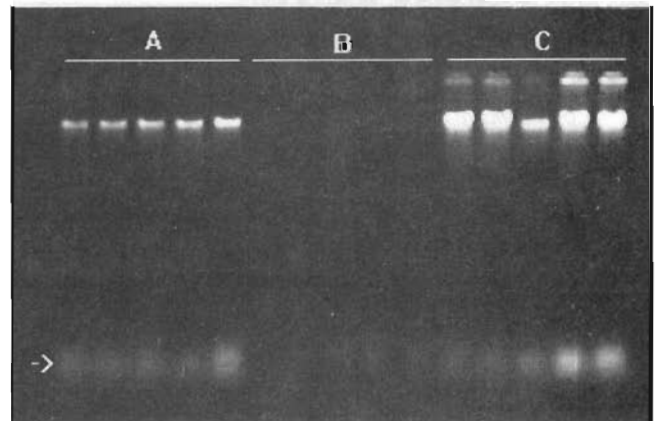


Figure 2 - Electrophoretic analysis of genomic DNA extracted from *Melipona quadrifasciata* (larvae). A, Method described by Doyle and Doyle (1990) for plants and by Schäfer and Wöstemeyer (1992) for fungi; B, method described by Hunt and Page (1992) for *Apis*; C, method B modified. Arrow indicates RNA.

normally present during larval development as compared to the adult phase. Consequently, it is recommended that a higher concentration of RNase be used for larvae.

The electrophoresis data (Figures 1 and 2) suggest that roughly the same amounts of DNA were obtained for adults and larvae in methods A and C. For this reason, the choice of the extraction method will depend on the objective of the work. If discrimination between the sexes is an important concern, adult individuals should be used, and in this case, workers (diploids) should be preferred as a starting material for DNA extraction as they have twice as much DNA as drones (haploids).

## ACKNOWLEDGMENTS

This work was supported by FAPEMIG. A.M.W. was the recipient of a CNPq fellowship.

## RESUMO

O objetivo deste trabalho foi o de testar três protocolos de extração de DNA utilizados para *Apis*, plantas e fungos, visando determinar um que seja eficiente para extração de DNA de *Melipona quadrifasciata*. Esses métodos diferem nas concentrações de componentes específicos do tampão de extração. Os resultados obtidos mostraram que a metodologia recomendada para extração de DNA de *Apis* não é adequada para a extração de DNA de *M. quadrifasciata*. Entretanto, com pequenas modificações, este método, bem como aquele utilizado para a extração de DNA de plantas e fungos, mostrou-se eficiente para a extração de DNA desta abelha sem ferrão, tanto de indivíduos adultos como de larvas.

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(Received April 1, 1997)