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Longitudinal differentiation in *Melipona mandacaia* (Hymenoptera, Meliponini) chromosomes

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Melipona mandacaia is a stingless bee endemic to northeast Brasil. We describe the *M. mandacaia* karyotype using C-banding technique, fluorochrome staining and treatment with restriction enzymes and discuss the position of this species in the context of the phylogeny of the genus. *Melipona mandacaia* has $2n = 18$ (14 SM + 2 M + 2 A). Heterochromatin was detected in the pericentromeric region of pairs 1, 2 and 8 and in the form of small blocks in the remaining pairs. Staining with base-specific fluorochromes showed that this heterochromatin was rich AT (QM and DAPI), except in the region corresponding to the NOR which was rich GC (CMA_3) and was cleaved by the *Hae*III enzyme. *Melipona mandacaia* is a member of Group I *Melipona*. Treatment with *Dra*I/Giemsa discloses a larger number of bands than treatment with *Dra*I/QM. Pre-cleavage with *Dra*I gave rise to a larger number of bands following QM staining; a circumstance evidently due to a removal of the DNA-protein complex that prevented the association of the fluorochrome with AT-rich DNA. The results highlight the complex nature of heterochromatin.

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INTRODUCTION

The major group of social bees in Brasil belongs to the tribe Meliponini. These "stingless" bees are highly diverse in the Neotropics, with approximately 43 genera and about 350 species (Michener 2000). They are very important pollinators in the ecosystem (Heard 1999). *Melipona mandacaia* is a stingless bee popularly known as 'mandaçaia', which is distributed in the Brasilian northeast, where it faces extinction due to habitat loss.

Studies on 27 genera of the tribe show that the haploid chromosome numbers range from 8 to 20 chromosomes (Kerr 1948, 1952, 1969, 1972; Kerr and Silveira 1972; Hoshiba 1988, Hoshiba and Imai 1993; Pompolo 1994; Brito-Ribon et al. 1999). Among these meliponine, *Melipona* shows especially interesting patterns of heterochromatin. Sixteen species of *Melipona* (out of 42 species described) have been studied cytogenetically. All have $n = 9$, $2n = 18$ for males and females respectively; *Melipona quinquefasciata* has, in addition B chromosomes (Rocha 2002). Rocha and Pompolo (1998) analyzed patterns of heterochromatin content and localization in 8 species of *Melipona*. The genus could be divided in two groups: Group I, formed by species with low content of heterochromatin that was either pericentromeric or present in the short arm of acrocentrics (*Melipona bicolor bicolor*, *M. quadrifasciata*, *M. marginata and M. asilvae*) and Group II with species with high heterochromatin content, which was distributed mainly along the chromosomes (*Melipona seminigra fuscopilosa*, *M. capixaba*, *M. scutellaris and M. captiosa*). Rocha et al. (2002) confirming Rocha and Pompolo (1998) findings on additional species. Other cytogenetic techniques (NOR banding, FISH, fluorochrome staining, digestion with restriction enzymes) were applied to study the molecular composition of chromatin, a fact that permitted proposing phylogenetic relations of the genus within the tribe Meliponini (Rocha et al. 2002).

In this study we characterize cytogenetically *Melipona mandacaia* and discuss the relevance of this species in the phylogeny of the genus.

MATERIAL AND METHODS

Samples of 40 bees of two *M. mandacaia* colonies from Irecê (BA), were studied. Metaphase chromosomes were obtained from cerebral ganglia of prepu-

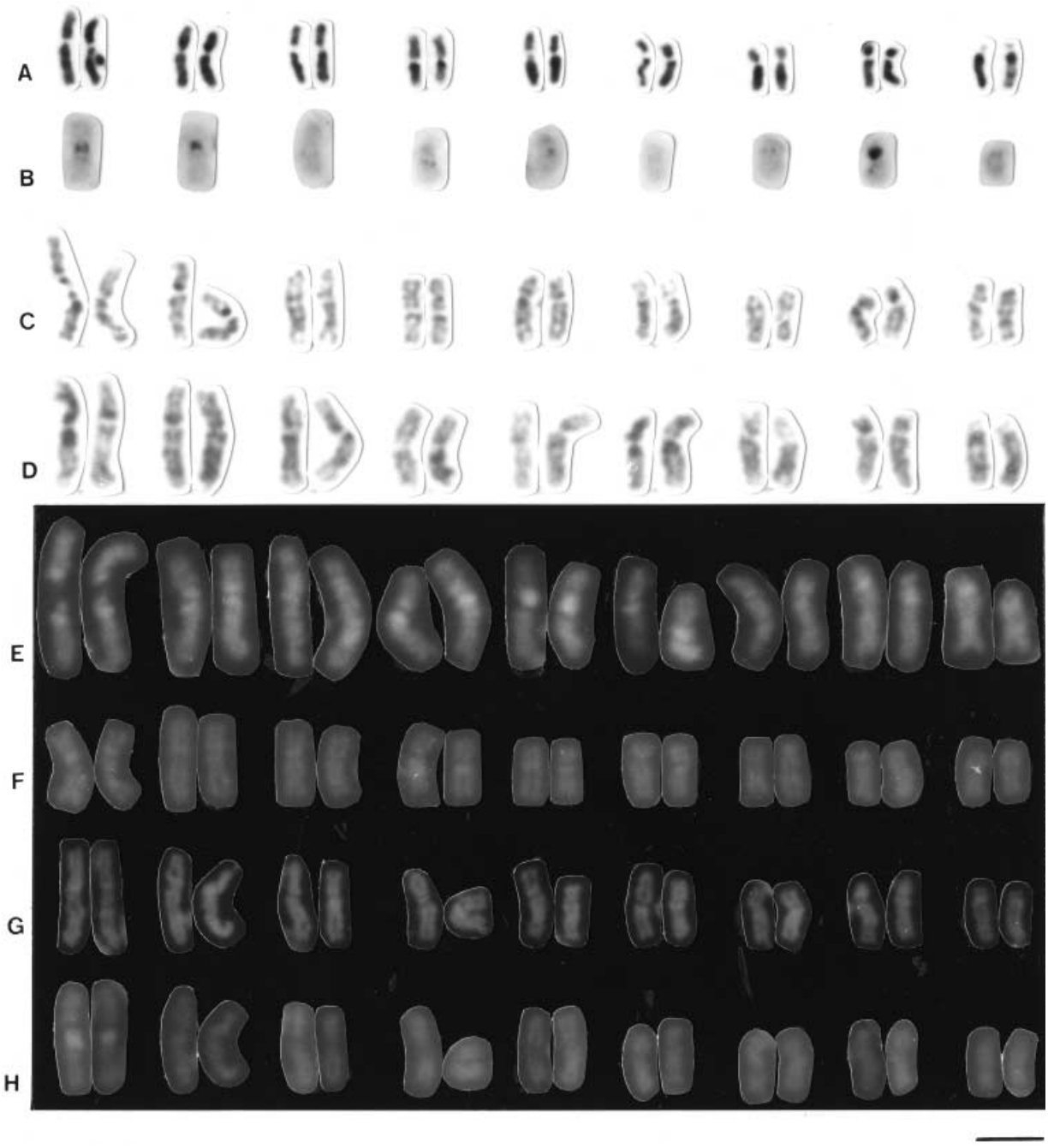

Fig. 1A-H. Karyogram of females (A, C, D, E, F, G) and male (B) of *Melipona mandacaia*. **A** Conventional staining with Giemsa; **B** C-band; **C** treatments with *Hae*III-Giemsa; **D** with *DraI*-Giemsa; **E** with *Dra*I-QM, **F** QM; **G** sequential staining with DA/DAPI and **H**-/CMA_3. Bar = 5 μm.

pae (Imai et al. 1988). C banding (BSG method) was carried out by the method of Sumner (1972), adapted by Rocha and Pompolo (1998). Sequential staining with the fluorochromes chromomycin A_3/DAPI followed Rocha et al. (2002).

After fixation, the chromosomes were treated with the restriction enzyme (RE) *Hae*III (GC↓GC) and *Dra*I (AAA↓TTT) diluted in buffers indicated by the manufacturer (1 μl enzyme containing 10 units in 9 μl buffer) and then diluted in 80 μl milli-Q water. Each slide received 30 μl of the final solution and was incubated for 12 hours in a moist chamber at 37°C. After washing in running water, the slides treated with *Hae*III were stained with Giemsa and the slides treated with *Dra*I were stained with Giemsa or the fluorochrome quinacrine mustard (QM). Staining with quinacrine mustard was performed by the method of Schmid (1980).

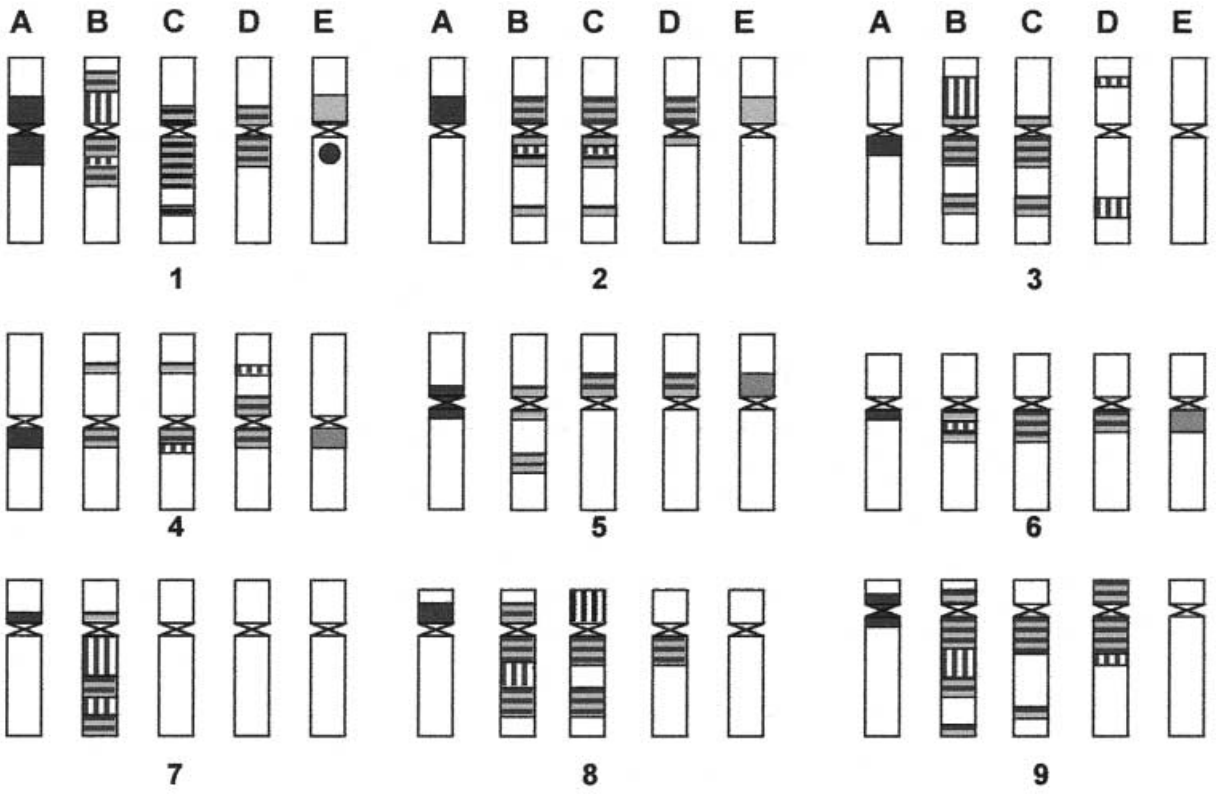

Fig. 2A-E. Ideogram of *Melipona mandacaia*. **A** C-band; **B** treatament with *Hae*III-Giemsa; **C** with *Dra*I-Giemsa; **D** with *Dra*I-QM, **E** staining with QM, DA/DAPI/CMA_3. ■ C^+ band; ● GC^+ band; ■ AT^+ band; □ Negative band; ■ Positive band.

RESULTS AND DISCUSSION

The chromosome number of *M. mandacaia* was 2n = 18 for females and n = 9 for males. There are: 7 submetacentric pairs, 1 metacentric pair and 1 acrocentric pair (Fig. 1). Heterochromatin, visualized by C banding, was found to be limited to the pericentromeric region of pairs 1, 2, 8, and in the remaining pairs it appeared in the form of small blocks (Fig. 1B, 2A). Heterochromatin was mostly rich AT (Fig. 1F, G, H and 2E). These data are similar to those obtained for Group I species and described by Rocha and Pompolo (1998) and Rocha et al. (2002), with a greater karyotypic similarity to the species *Melipona subnitida*. Rocha et al. (2002) hypothesized that the Group I was more generalized than Group II; the latter are characterized by high levels of heterochromatin.

RE treatment showed regions with different staining intensities in all chromosomes, with some areas being completely cleaved (gaps) and other resistant (Fig. 1C–E and 2B–D). According to Lorite et al. (1999), the resistant regions correspond to heterochromatin. Indeed, some of them corresponded to the regions visualized by C banding whereas the remaining regions, although probably being heterochromatic, were not visualized by C banding. According to Camacho et al. (1984), C banding may not detect all heterochromatin. The use of RE permitted the differentiation of some types of heterochromatin, not visualized by standard C banding.

*Hae*III/Giemsa pretreatment allowed an accurate chromosome comparison. The banding pattern (Fig. 1C and 2B) was similar to the G banding pattern of the species *M. quadrifasciata* described by Tambasco et al. (1979). A correspondence between the *Hae*III and G banding patterns has been detected in many animals (Miller et al. 1983; Babu and Verma 1986; Lorite et al. 1999).

After staining with base-specific fluorochromes, only the pericentromeric region of the first pair showed a predominance of GC pairs (Fig. 1F–H and 2E). This region exhibited band heteromorphism and it was sensitive to *Hae*III (Fig. 1C and 2B). *Hae*III has been found to cleave the NOR in other organisms (Gosálvez et al. 1987; Juan et al. 1991; Padilla et al. 1993; Lorite et al. 1999). The presence of this heteromorphic block was observed in five *Melipona* species and it was found to be NOR-positive in *M. asilvae* (Rocha et al. 2002) and *M. marginata* (Maffei et al. 2001). In *M. bicolor* and *M. quadrifasciata* it was

labeled by FISH (Rocha 2002). Accordingly, the pericentromeric block of the first pair of *M. mandacaia* contains the genes of the NOR. We observed that *Hae*III + to CMA_3 + correlate to each other in *M. mandacaia*.

*Dra*I/Giemsa treatment (Fig. 1D and 2C) showed a larger number of bands in different chromosomes than *Dra*I/QM treatment (Fig. 1E and 2D). This is attributable to a possible camouflaging artifact of small negative bands caused by strong fluorescence of QM.

Comparison of the banding patterns after staining with QM and after the *Dra*I/QM treatment showed that QM labeled a larger number of bands when the chromosomes were pretreated with the *Dra*I enzyme. This treatment revealed an extra block in the first pair, which was not observed when QM staining alone was used. In *M. subnitida* and *M. quinquefasciata* (Rocha et al. 2002), only one block was detected in the first pair. These species may also possess the other block, which, however, was only identified in *M. mandacaia* following treatment with *Dra*I. The observation of a larger number of bands after treatment with *Dra*I may have been due to the removal of the DNA-protein complex, which prevented the association of fluorochromes with AT-rich DNA.

The AT rich pericentromeric regions are expected to be digested by *Dra*I (Fig. 1D–E and 2C–D). The non-digestion of these regions may have been due to the size of the cleaved fragments, since fragments larger than 1 kb pairs remain in the chromatin after cleavage and only fragments smaller than 200 pb are extracted (Babu and Verma 1986; Verma and Babu 1995). Alternatively, in spite of the abundance of AT, AAATTT sites were not frequent in these regions. The results highlight the complex nature of heterochromatin in these stingless bees.

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