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Karyotypic description and repetitive DNA chromosome mapping of *Melipona interrupta* Latreille, 1811 (Hymenoptera: Meliponini)

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Abstract. Heterochromatic patterns in the genus *Melipona* vary among subgenera species. *Melikerria* is the only subgenus that presents species with different content of heterochromatin. Thus, the cytogenetic knowledge of other species of this subgenus is important for the understanding of karyotype evolution in *Melipona*. Here, we describe the karyotype of *Melipona* (*Melikerria*) *interrupta* based on the chromosomal heterochromatic patterns, AT and GC richness, mapping sequences of rDNA, microsatellites and telomeric regions. We observed 2*n*=18 chromosomes, with a high heterochromatin content rich in AT and euchromatic regions rich in GC base pairs. The high GC content was observed at interstitial region near the junction of the stained euchromatin and heterochromatin of the first chromosomal pair, the same region marked for the rDNA 18S locus. Microsatellites hybridized only on euchromatin regions and the telomeric probe on terminal regions of all chromosomes. *Melipona* (*Melikerria*) *interrupta* belongs to previous described heterochromatic Group II, suggesting there has been an increase in heterochromatin content in *Melikerria*. The *M. quinquefasciata*, belonging to the same subgenus as *Melipona* (*Melikerria*) *interrupta*, has low content of heterochromatin and appears to be evolving independently. So, the differences in the content heterochromatin, in the marker regions of CMA_3 and the rDNA 18S locus in species of *Melikerria* is an important feature to be investigated further.

Keywords. Cytogenetics, Fluorescence *in situ* Hybridization (FISH), Heterochromatin, *Melikerria*, Stingless bee.

1. INTRODUCTION

Bees of the genus *Melipona* Illiger, 1806, are eusocial insects belonging to the Meliponini tribe and occur throughout the Neotropical region (Michener 2007; Camargo and Pedro 2013). This genus is represented by 73 species, 43

of which can be found in Brazil (Camargo and Pedro 2013; Pedro 2014). Morphologically, the genus *Melipona* is grouped into four subgenera: *Eomelipona*, *Melikerria*, *Melipona stricto sensu*, and *Michmelia* (Camargo and Pedro 2013). The *Melikerria*, *Melipona stricto sensu,* and *Michmelia* subgenera are considered monophyletic, whereas *Eomelipona* is polyphyletic according to molecular phylogenies (Rasmussen and Cameron 2010; Ramírez et al. 2010).

Cytogenetically, only 28 of all the *Melipona* species have had their karyotypes described. These species are characterized by a conserved diploid number of $2n = 18$ chromosomes in females and $n = 9$ in males, except for *M. seminigra merrilae* and *M. seminigra pernigra*, with $2n = 22$ (Francini et al. 2011). In addition, the pattern of heterochromatin distribution in some *Melipona* species differs from that observed in the majority of species in the Meliponini tribe (reviewed in Tavares et al. 2017; Cunha et al. 2018; Silva et al. 2018). The genus can be divided into two groups based on the pattern of distribution and content of heterochromatin: Group I is composed of species with low heterochromatin content present only in the pericentromeric regions, similar to that in the other Meliponini; and Group II comprised by species with a high heterochromatin content that covers large extensions of their chromosomes (Rocha and Pompolo 1998; Rocha et al. 2002).

From all the analyzed species, those belonging to the *Eomelipona* and *Melipona stricto sensu* subgenera present low levels of heterochromatin, whereas all those of the subgenus *Michmelia* have high levels of heterochromatin (Rocha and Pompolo 1998; Rocha et al. 2002; Rocha et al. 2003; Lopes et al. 2011; Cunha et al. 2018). However, *Melikerria* has only three species described cytogenetically that present the two patterns: *Melipona fasciculata* Smith, 1854 and *Melipona grandis* Guérin, 1844, with high content of heterochromatin and *Melipona quinquefasciata* Lepeletier, 1836 with a low content (Rocha et al. 2002; Rocha 2002; Lopes et al. 2011), making heterochromatic evolution in this group difficult to elucidate. Thus, cytogenetic studies with other species of the subgenus *Melikerria* are needed since they may help to elucidate the processes leading to the chromosomal alterations in the genus.

The aim of this study was to characterize the karyotype of *Melipona* (*Melikerria*) *interrupta* based on the heterochromatin distribution pattern and chromosomal regions rich in Guanine-Cytosine (GC) and Adenine-Thymine (AT) base pairs, as well as mapping the ribosomal 18S DNA sites, the microsatellites $GA_{(15)}$, $GAG_{(10)}$, $CAA_{(10)}$, and $CGG_{(10),}$ and the regions containing telomeric $TTAGG_{(6)}$ sequences.

2. MATERIAL AND METHODS

Larvae of *Melipona* (*Melikerria*) *interrupta* were collected from three colonies in Itacoatiara, Amazonas, Brazil and kept in the Meliponary of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazônia, Brazil. Mitotic chromosomes were obtained from the larval brain ganglia at the last larval instar as described by Imai et al. (1988), and stained with Giemsa. The heterochromatin regions were visualized by the C-band technique (Sumner 1972) and the DAPI and CMA_3 fluorochromes were used according to Schweizer (1980). Fifteen individuals were used, with 10 metaphases being analyzed on average for each slide. The images were obtained with an Olympus BX60 epifluorescence microscope, using Olympus Q-Color3™ software Olympus® images.

Fluorescent *in situ* Hybridization (FISH) was performed according to Pinkel et al. (1986), with modifications: (metaphase chromosomes were denatured in 70%/2xSSC formamide at 75 °C for 5 min; the probes were hybridized with chromosomes in 20 μL of hybridization mix and heated for 10 min at 85 ° C). The 18S ribosomal DNA probe was labeled with digoxigenin-11-dUTP (Roche Applied Science) and the signal was detected with anti-digoxigenin-rhodamine (Roche Applied Science). This probe was obtained by Polymerase Chain Reaction (PCR) amplification, using the primers F1(5′-GTCATATGTTGTCTCAAAGA-3′) and 18SR1.1 (3′-TCTAATTTTTTCAAAGTAAACGC-5′) designed for the species *Melipona quinquefasciata* (Pereira 2006). The microsatellites $GA_{(15)}$, $GAG_{(10)}$, $CAA_{(10)}$, $CGG_{(10)}$, and $TTAGG_{(6)}$ were labeled directly with Cy3 in the 5′ regions (Sigma, St. Louis, MO, USA). The metaphase images were obtained with an Olympus BX53 microscope fitted with an Olympus DP73F camera, using the CellSens Imaging software.

3. RESULTS AND DISCUSSION

The chromosome number observed in *M.* (*Melikerria*) *interrupta* was $2n = 18$ (Fig. 1a) similar the number finding by Barbosa (2018), which does not differ from that reported by Kerr (1969, 1972). The C-band revealed a karyotype with high heterochromatin content (Fig. 1b), making visualization of the centromere difficult. Thus, we could not identify chromosome morphology to determine the karyotype of this species. Based on the heterochromatic patterns, *M.* (*Melikerria*) *interrupta* can be classified as belonging to the group composed of species with more than 50% of heterochromatin in their chro-

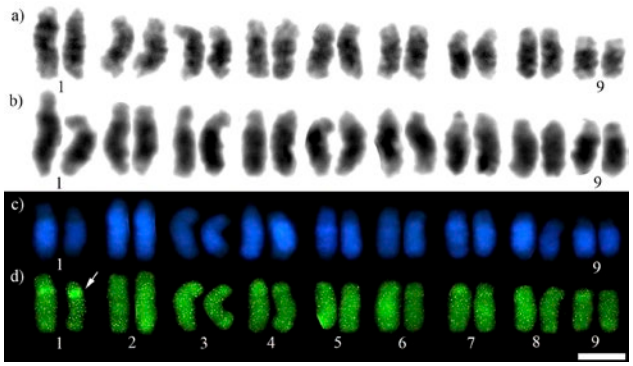

Fig. 1. Mitotic metaphase chromosomes of *Melipona* (*Melikerria*) *interrupta* stained with Giemsa (a), C- banding (b), DAPI (c), and CMA_3 (d). Scale bar = 5 µm.

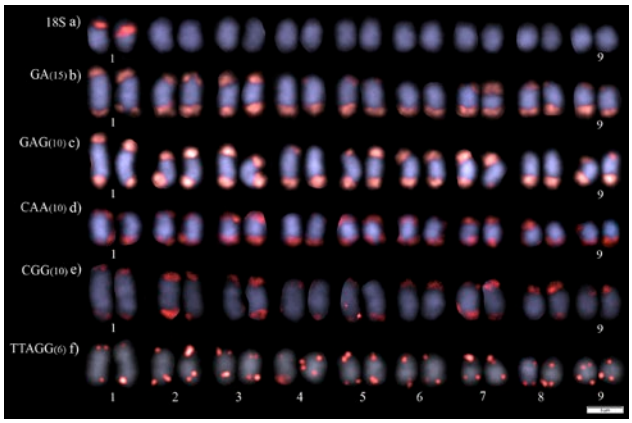

Fig. 2. Patterns obtained in metaphase chromosomes of *Melipona* (*Melikerria*) *interrupta* with FISH using the following repetitive DNA probes: 18S (a), $GA_{(15)}$ (b), $GAG_{(15)}$ (c), $CAA_{(10)}$ (d), $CGG_{(10)}$ (e), and $TTAGG_{(6)}$ (f). In blue: chromosomes stained with DAPI. In red: regions hybridized with probes. Scale bar = 5 µm.

mosomes, designated as Group II by Rocha and Pompolo et al. (1998). The species *M.* (*Melikerria*) *fasciculata* and *M.* (*Melikerria*) *grandis* were classified as belonging to this group (Lopes et al. 2011; Andrade-Souza et al. 2018) as well as *M.* (*Melikerria*) *interrupta*. Unlike, *M.* (*Melikerria*) *quinquefasciata* has a low heterochromatin content (Rocha et al. 2007), indicating that the karyotype of *M. quinquefasciata* may be evolving independently or have been the karyotype plesiomorphic within the *Melikerria*. So, in this subgenus may have been an increase in the levels of heterochromatin in the karyotype of the species. In fact, it has been, suggested that independent amplification of heterochromatin or differentiation in distinct *Melipona* subgenus (Piccoli et al. 2018).

Staining with the base-specific fluorophores, DAPI and CMA_3, indicated that the heterochromatic regions were $DAPI^+$ (Fig. 1c) and the euchromatic regions were CMA_3^+. Stronger labeling with CMA_3 was seen in the interstitial region near the euchromatin and heterochromatin junction of the first chromosomal pair (Fig. 1d), coincident with the hybridization site of the 18S rDNA probe (Fig. 2a). Staining with DAPI has shown that heterochromatin in eusocial bees is generally AT-rich (Brito et al. 2003; Rocha et al. 2003; Lopes et al. 2011; Godoy et al. 2013). This characteristic seems to be shared by *Melipona* species that contain either high or low levels of heterochromatin.

Although the numbers of CMA_3^+ and rDNA markers on a single pair of chromosomes are conserved traits in the genus (Cunha et al. 2018; Andrade-Souza et al. 2018), the chromosomal positions differ among species, even in those with a high heterochromatin content. According to the results of CMA_3^+ and rDNA probe, species with a low heterochromatin content exhibited pericentromeric markings in the first chromosome pair, while Group II species showed terminal markings (reviewed in Cunha et al. 2018). Although *M.* (*Melikerria*) *interrupta* is classified as belonging to Group II, CMA_3^+ and rDNA markings were observed in the interstitial region of the first chromosomal pair. *Melipona* (*Melikerria*) *fasciculata* and *M.* (*Melikerria*) *grandis*, which belongs to the same subgenus, also showed CMA_3^+ markings in this region (Lopes et al. 2011; Andrade-Souza et al. 2018), different to that observed in the other species with high heterochromatin content (reviewed in Cunha et al. 2018). These results suggest that variation in the positions of GC rich regions and 18S rDNA sites may be results of divergent heterochromatin evolutionary pathways in the *Melipona* as suggested by Cunha et al. (2018) and Picolli et al (2018). As the markings in other species of Group II is at the chromosome end, the occurrence of rearrangements, such as inversion, would result in a portion of heterochromatin at the ends of the chromosome. However, we did not find heterochromatin in these regions suggesting that the position of CMA_3 and rDNA no result of chromosome inversion events.

The microsatellite probes $GA_{(15)}$, $GAG_{(10)}$, $CAA_{(10)}$, and $CGG_{(10)}$ labeled only euchromatin regions (Fig. 2b-e), while the telomeric probe $TTAGG_{(6)}$ showed staining in the terminal regions of the chromosomes of *M.* (*Melikerria*) *interrupta* (Fig. 2f). This staining pattern in regions of euchromatin was also observed in the chromosomal mapping of *Melipona scutellaris* Latreille, 1811 using different repetitive DNA sequences ($CA_{(15)}$, $GAC_{(10)}$, and $TAA_{(10)}$) (Piccoli et al. 2018). The $TTAGG_{(6)}$ labeling on the terminal regions of the chromosomes indicated the presence of the TTAGG sequence in the

telomeric sites in the karyotype of *M.* (*Melikerria*) *interrupta*. Studies have shown that TTAGG (Sahara et al. 1999) and TCAGG (Mravinac et al. 2011) were observed in telomeres of several Hymenoptera species, including Apidae (*Apis mellifera*) (Meyne et al. 1995; Sahara et al. 1999) and Formicidae (*Tapinoma nigerrimum*, *Myrmecia* spp. and *Acromyrmex striatus*) (Meyne et al. 1995; Lorite et al. 2002; Frydrychová et al. 2004; Pereira et al. 2018). However, in many other Hymenoptera the TTAGG sequence was not observed in the telomeres of the chromosomes, suggesting that it has been lost and recovered in Apidae and Formicidae or that multiple losses of this region have occurred throughout the evolutionary history of the groups (Menezes et al. 2017). In this study, we report for the first time the telomeric sequence based on the FISH technique on a bee species of Meliponini tribe. This information added to the cytogenetic characteristics of *Melipona* already described in the literature may contribute to the understanding of karyotype evolution in these bees.

We conclude that *M.* (*Melikerria*) *interrupta* is classified as belonging to Group II based on heterochromatic patterns, which suggests an increase in the amount of heterochromatin in the subgenus *Melikerria*. It further suggests that karyotype the *M. quinquefasciata* be plesiomorphic or this may be evolving independently in the group, and that the differences in the CMA_3 and 18S marker regions interstitial, reported in the two species of *Melikerria* subgenus with high heterochromatin content, are an important feature to be further investigated.

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