Note

Cytogenetic analysis of the Amazon stingless bee *Melipona seminigra merrillae* reveals different chromosome number for the genus

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ABSTRACT: Cytogenetic analysis of the Amazon stingless bee *Melipona seminigra merrillae*, by conventional Giemsa staining and C-banding, revealed a different chromosome number for *Melipona*: 2n = 22 for females and diploid drones while the haploid drones present n = 11. There is no evidence of B chromosomes. This result contrasts with previous studies, in which the chromosome number of 19 *Melipona* species was determined as 2n = 18 for females and n = 9 for haploid males. Based on cytogenetic information available for other *Melipona* species, we propose that *M. s. merrillae* has a more derived diploid number. This indicates that chromosome number is not a conservative characteristic within the genus as previously thought. Cytogenetic data for stingless bees are scarce, especially in Amazon region. Additional studies will be very important in order to promote *Melipona* karyoevolution discussion and consequently a taxonomy review.
Keywords: Meliponini, Neotropics, cytogenetics, diploid male chromosomes

Introduction

The majority of the stingless bee species from the Meliponini tribe are represented by *Melipona Illiger*, 1806 genus (Camargo and Pedro, 2008). Although widely distribute throughout the Neotropics (Michener, 2007) and found in all Brazilian States, *Melipona* species richness is higher in the Amazon basin region, where possibly many of them still undescribed (Silveira et al., 2002). The stingless bees, as a group, are relating to forest environments (Brosi, 2009) being one of the main native pollinators (Michener, 2007), besides some species are important in crop and greenhouse pollination as well (Heard, 1999; Nicodemo et al., 2009; Slaa et al., 2006). Cytogenetically, *Melipona* is the best-studied genus of the Meliponini, with 19 species studied so far, all presenting 2n = 18 chromosomes for females and n = 9 for males (Rocha et al., 2007).

Melipona seminigra merrillae Cockerell, 1919, is an abundant bee in Central Amazon (Camargo and Pedro, 2008). It is a honey-producing species and one of the most reared by Amazonian people in Meliponiculture, which is a significant activity for biodiversity conservation and local economic development (Cortopassi-Laurino et al., 2006). In this study, a different chromosomes number for *M. s. merrillae* in an urban population was described, being an important contribution for cytogenetic of *Melipona* also Hymenoptera.

Materials and Methods

Cytogenetic analysis was carried out on 81 individuals (40 females-workers, 36 haploid males and 5 diploid males), from ten hives of *Melipona seminigra merrillae*, in an urban population at Manaus, state of Amazonas, Brazil (3º 05.838' S, 59º 59.103' W) from August to October 2008. Mitotic chromosomal preparations were obtained based on the technique described in Imai et al. (1988), by using cerebral ganglion of pink-eyed pupae. The bees' pupae dissected under a stereomicroscope (40x) and the cerebral ganglions isolated and immersed in hypotonic solution of colchicine-citrate 0.005 % for one hour and twenty minutes. All chromosomal preparations were stained with Giemsa solution 5 % for 20 min. The constitutive heterochromatin was detected according to Sumner (1972). The metaphases was observed with a microscope (100x) and photographed with a digital camera (Sony DSC–W150). An average of 30 metaphases per individual was analyzed.

Results and Discussion

All analyzed females and diploid drones of *Melipona seminigra merrillae* showed a diploid number of 2n = 22 (Figure 1a and c) while all haploid drones were n = 11 (Figure 1b), without evidence of B chromosomes. Although deserving more detailed analysis, the karyotype of *Melipona seminigra merrillae* seems to consist of four pairs of metacentric, six pairs of submetacentric and one pair of acrocentric chromosomes. *Melipona* species present two patterns in heterochromatin distribution, the Type I showing C-bands around the pericentromeric region and the Type II with heterochromatin distributed along all chromosomes and/or terminally (Rocha et al., 2007). *Melipona*

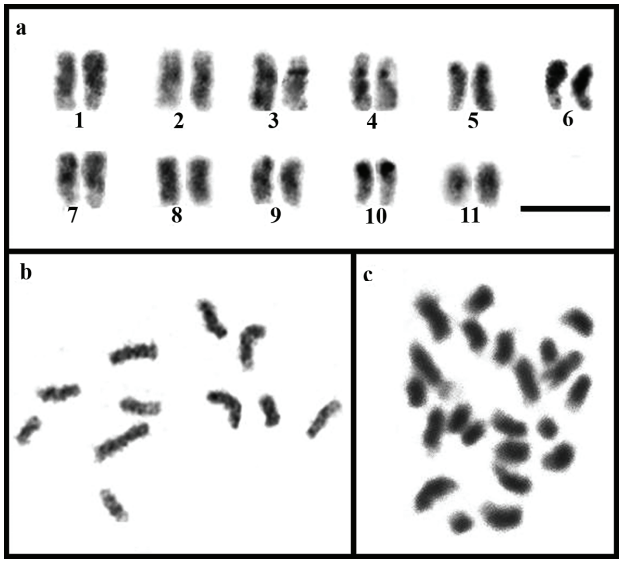

Figure 1 – *Melipona seminigra merrillae:* a. Female karyotype in C-banding (showing 2n = 22) b. Metaphase in Giemsa of male (showing n = 11); c. Metaphase in Giemsa of diploid male (showing 2n = 22) (Bar: 5 µm).

seminigra merrillae seems to present heterochromatin distributed according to Type I as evidenced by conspicuous blocks in the pericentromeric region and short arms of the 6th and 10th chromosome pairs (Figure 1a).

Since the first karyotype described to *Melipona* (Kerr, 1948) to the present, the chromosome number available for all *Melipona* species was conservative, all of them presenting 2n = 18 (females) and n = 9 (males) (Rocha et al., 2007). Variations in the number of chromosomes were registered for *Melipona quinquefasciata* Lepeletier, 1836 (Rocha et al., 2007) and *Melipona rufiventris* Lepeletier, 1836 (Lopes, 2008), but these variations were attributed to the presence of B chromosomes. Thereby the results observed for *M. s. merrillae* contrast with the current view that chromosome number is conservative in *Melipona* (Rocha et al., 2007).

Cytogenetic data for stingless bees are scarce, especially in the Amazon region. Only *Melipona interrupta manaosensis* Schwarz, 1932 (as *Melipona compressipes manaosensis*) was cytogenetically studied so far (Kerr, 1952). Other two species had also been cytogenetically characterized but from sample collected in Acre state (Rocha and Pompolo, 1998; Rocha et al., 2002).

M. s. merrillae is considered to be one of the seven geographic subspecies of *Melipona seminigra* Friese, 1903, and an endemic bee of Amazonas state, Brazil (Camargo and Pedro, 2008). The higher chromosomes number of *M. s. merrillae* reflects its more derived status in relation to other *Melipona* species for which cytogenetic information is available (Kerr and Silveira, 1972; Hoshiba and Imai, 1993; Rocha et al., 2002).

These are very important and intriguing results, especially due to the diploid males'methaphase presented. Therefore, additional cytogenetic analyses on *Melipona* species will certainly clarify the evolutionary relationship among species and lead to a better understanding of the modifications in the Meliponini karyotype. Furthermore, it will contribute to the identification of species, which will certainly be useful for information regarding the biodiversity conservation and ecological pollination dynamics in the Central Amazon.

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