Cytogenetic Characterization of the Lower-Attine *Mycocepurus goeldii* (Formicidae: Myrmicinae: Attini)

by

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ABSTRACT

The objective of this study was the cytogenetic characterization - number, morphology and banding pattern - of *Mycocepurus goeldii* Forel, 1893 as a contribution for a better comprehension of the evolution of the Attini tribe, a particular group of ants that obligately depend on the cultivation of fungus for food. The chromosome number observed was 2n=8 chromosomes (2K=8M). C-banding technique indicated positive markings in the centromeric regions of three pairs of chromosomes, and the second-largest pair had a pericentromeric band on its long arm. Also, C-banding markings were coincident with CMA_3 fluorochrome markings. The genus *Mycocepurus* is characterized by the lowest chromosome number in the Attini tribe, suggesting the karyotype to be relict, based on Minimum Interaction Theory. This plesiomorphy confirms this genus in a basal condition relative to the remaining Attini.

Key words: cytogenetics, ant, chromosome banding, evolution

INTRODUCTION

Cytogenetics has gained a deserved place in comparative biology as an important tool for the study of evolution, since chromosome alterations are important

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for species evolution and are able to influence their adaptation (Hoffmann & Rieseberg 2008), acting also as isolation factors that contribute to speciation (White 1970). Moreover, gene expression is regulated, at least partially, by the neighboring genes' location, and thus a chromosomal rearrangement can lead to phenotypic alterations (Ridley 2006). Thus, cytogenetic data are useful for phylogenetic, taxonomic and evolutionary studies (McGregor 1993).

Ants occupy a keystone position in most terrestrial environments and are useful as model organisms for many studies (Schultz 2000). There are almost 12,600 species described (Agosti & Johnson 2005) and chromosome numbers are known for more than 750 ant populations (Lorite & Palomeque 2010). Chromosome numbers range from 2n=2 chromosomes in *Myrmecia croslandi* Taylor (Crosland & Crozier 1986) to 120 chromosomes in *Dinoponera lucida* Emery (Mariano *et al*. 2008). Few cytogenetic data are available for the Attini tribe, a particular group of ants that obligately depend on the cultivation of fungus for food, where only 23 taxa have been studied to date (Goñi *et al*. 1983; Fadini & Pompolo 1996; Fadini *et al*. 1996; Murakami *et al*. 1998; Barros *et al*. 2008) which represents about 10% of described species. Within this tribe, chromosome numbers range from 2n=8 chromosomes in *Mycocepurus* sp. (Murakami *et al*. 1998) to 2n=54 chromosomes in *Mycetarotes paralellus* Emery (Fadini & Pompolo 1996).

The Neotropical genus of fungus-growing ants *Mycocepurus* (Myrmicinae, Attini) includes currently five valid species (Kempf 1963; Mackay 1998; Fernandez 2003) that have been until recently little studied probably because of the difficulties of excavations of ant nests (Rabeling *et al*. 2007, 2009). Morphologic and behavioral data suggesting that this genus is plesiomorphic within Attini (Schultz & Meyer 1995; Rabeling *et al*. 2007; Mehdiabadi & Schultz 2009), and therefore cytogenetic, are particularly relevant for the understanding of the evolution in the tribe. Most of the evolutionary studies are related to the genera of higher agriculturists (*sensu* Mehdiabadi & Schultz 2009), which are more derived and less informative for the elucidation of the events related to the first steps of the evolution of the group (Rabeling *et al*. 2007).

The morphology of *Mycocepurus* ants is easily characterized among the fungus-growing ants because of their numerous spines on most bodily surfaces; additionally, this is the only genus in the New World that have a crown of spines on the promesonotum (Mayhé-Nunes & Meneguete 2000; Mackay *et al*.

2004). *Mycocepurus goeldii* (Forel) is widely distributed, ranging from northern Argentina to the Guyana (Kempf 1963; Mackay 2004). An interaction between this species and the *Hymenaea courbaril* L. (Fabaceae: Caesalpinioideae) tree has been reported, where the removal of fruit matter by ant predation lowers fungal seed attack, enhancing seed germination (Oliveira *et al.* 1995). *Mycocepurus goeldii*, as well as *Mycocepurus smithii* (Forel), accumulates nutrients in low soil layers that can be utilized by higher plants after they leave the ant nest chambers (Rabeling *et al.* 2007, 2009).

Mycocepurus smithii is a rare case of thelytokous parthenogenesis in ants (Rabeling *et al.* 2009; Mehdiabadi & Schultz 2009), while *M. goeldii* reproduces sexually, being reported as mating with several males (polyandry) (Rabeling *et al.* 2009; Mehdiabadi & Schultz 2009). The only cytogenetic information available for this genus is for an unidentified *Mycocepurus* species from Panama (2n=8) (Murakami *et al.* 1998).

Fluorochromes are substances that can emit fluorescence under specific wavelengths. They are very useful in cytogenetics by their binding to the DNA with different affinities regarding the base pair composition. Usually fluorochromes are classified in two categories: AT-and GC-specific (Sumner 2003). Heterochromatin study and characterization is also important in cytogenetic studies of the family Formicidae, due to its central role in chromosome evolution of the group (Imai *et al.* 1988; Imai 1991). The fluorochrome Chromomicin A_3 (CMA_3) marks regions which are rich in GC pairs and has already been used for ant chromosomes such as in *D. lucida* (Mariano *et al.* 2008), *Wasmannia auropunctata* (Roger) (Souza 2007), the social parasite *Acromyrmex ameliae* Souza, Soares & Della Lucia and its hosts A*cromyrmex subterraneus subterraneus* Forel and *Acromyrmex subterraneus brunneus* Forel (Barros *et al.*, 2008), *Tapinoma nigerrimum* (Nylander) (Lorite *et al.* 1997), and *Gnamptogenys striatula* Mayr (Barros *et al.*, unpublished data). Typically, all studied ants have CMA_3 markings on only one pair of chromosomes. In *D. lucida* and *T. nigerrimum*, the pair of chromosomes marked by CMA_3 overlaps with nucleolus organizing regions (NOR) evidenced with FISH through rDNA hybridization.

This study focused the cytogenetic characterization of *M. goeldii* (chromosome number, morphology and banding pattern), in a putative plesiomorphic position of this genus within Attini (Mehdiabadi & Schultz 2009).

MATERIALS AND METHODS

A colony of *M. goeldii* with a single queen was collected in the Forest Reserve Mata do Paraíso in Viçosa, State of Minas Gerais, Brazil (20°41'20"S - 20°49'35"S; 42°49'36"W - 42°54'27"W) and reared at the Insect Cytogenetics Laboratory, Federal University of Viçosa, to warrant brood production used for cytogenetic studies. The metaphases were obtained from brain ganglia of pharate pupae of 20 workers, according to Imai *et al*. (1988). Eight to 10 metaphases were observed per individual. No male brood was found in the colony.

A portion of the obtained slides was stained with Giemsa to define chromosome morphology and the remaining part was submitted to chromosome banding techniques. The chromosomes were classified according to the nomenclature proposed by Imai (1991).

The distribution pattern of heterocromatin was obtained by C banding technique (Sumner 1972). The characterization of GC and AT base pairs richness along their chromosomes was obtained with Chromomycin A_3 (CMA_3) and by 4'6- diamidin-2-phenylindole (DAPI) fluorochromes according to Schweizer (1980).

The metaphases were analyzed with an Olympus BX 60 microscope coupled to an image digitalizing system (Q color 3 Olympus®) . The Q Capture® program was used to obtain the images. All slides submitted to the CMA_3 and DAPI fluorochrome techniques were analyzed with an epifluoerescence microscope with a WB filter (450 to 480 nm) for the CMA_3 and the WU filter (330 to 385 nm) was used for the DAPI.

Imago vouchers were deposited in the collection Myrmecology Laboratory at the Cocoa Research Center (CEPEC), Ilheus, State of Bahia, Brazil.

RESULTS AND DISCUSSION

The chromosome number of *M. goeldii* was 2n=8 chromosomes (Fig. 1) in all individuals (workers). All chromosomes were metacentric according to Imai (1991) and karyotypic formula 2K=8M. However, according to the chromosome classification proposed by Levan *et al*. (1964), both the biggest and smallest chromosome pairs are assorted as metacentric, and the other two pairs are submetacentric. The *Mycocepurus* sp. karyotype studied in Panama by Murakami *et al*. (1998) had the same chromosome number and type. In

some metaphases observed in *M. goeldii*, the second pair of chromosomes presented secondary constriction on the long arm, close to the centromere region, which can be seen at least in one of the homologous chromosomes (Fig. 2).

The C-banding technique indicated positive markings at centromeric and pericentromeric regions. Three pairs of chromosomes presented centromeric markings and the second-largest pair presented pericentromeric markings on its long arm.

Fig. 1. a) Female metaphase of *Mycocepurus goeldii* stained with Giemsa; and b) its karyotype. Bar=5 μm.

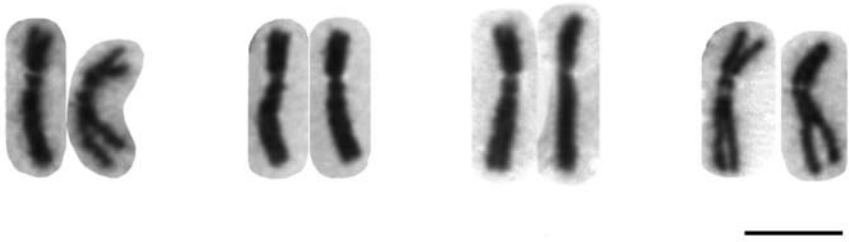

Fig. 2. Second-largest pair of chromosomes indicating the presence of the secondary constriction at least in one of the homologous pairs. Bar=5 μm.

Fig. 3. a) Metaphase submitted to the C-banding technique for heterochromatin detection; and b) its karyotype. Bar=5 μm.

Centromeric regions of three chromosome pairs were rich in GC base pairs (CMA_3^+), and one pair presented a marking on the pericentromeric region of the long arm (CMA_3^+) (Fig. 4a). The centromeric and pericentromeric regions that were rich in GC base pairs evidenced by the CMA_3 fluorochrome were the same regions highlighted by heterochromatin using C-banding technique. The fluorochrome DAPI did not present a specific marking pattern but the CMA_3^+ regions showed $DAPI^-$, indicating that these fluorochromes were complementary (Fig. 4b). Unlike the pattern observed in different ant species studied with only one pair of markings for CMA_3 fluorochrome, *M. goeldii* showed four marks for this fluorochrome.

Cytogenetic studies in ants indicate conservative pattern of NORs restricted to a single pair of chromosomes, in a GC-rich base pairs region. In *M. goeldii*, the CMA_3 fluorochrome marked all chromosomes and therefore did not show a NOR specific location. It is unlikely that all markings are NOR bearers. However, considering that the second pair of chromosomes showed pericentromeric heterochromatin, secondary constriction and large CMA_3 fluorescent marking without DAPI marking, we concluded that this pair likely bears NOR regions in this species.

Based on the Minimum Interaction Theory (Imai *et al*. 2002), lower chromosome numbers are expected to be a plesiomorphic trait. The *Mycocepurus* genus presents to date the smallest chromosome number for the Attini tribe,

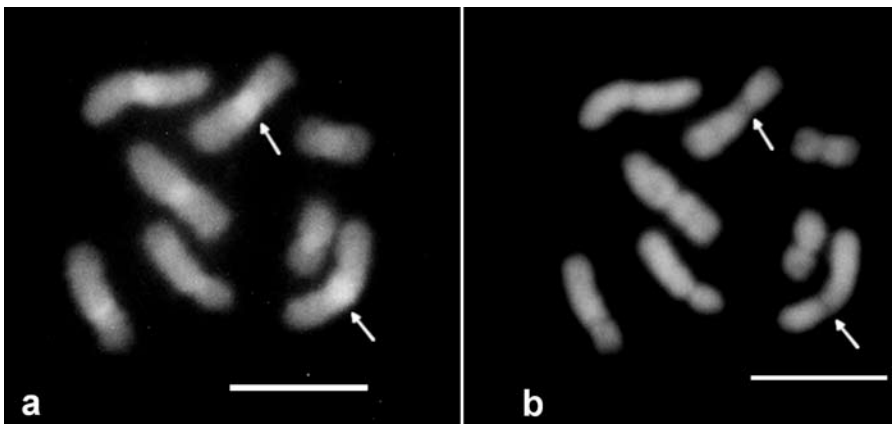

Fig. 4. a) Metaphase stained with the fluorochrome CMA_3 and b) DAPI. Arrows point to the positive and negative marks, for the fluorochromes CMA_3 and DAPI, respectively, in the second pair of chromosomes, the likely NOR bearer pair. Bar=5 μm.

a potential plesiomorphic characteristic and so, a relict karyotype (Imai *et al* 2002), that suggests an older origin for this genus that confirms the phylogenetic molecular study by Schultz and Brady (2008) and other information about fungus-growing ant evolution (Mehdiabadi & Schultz 2009).

Cytogenetic data for the Attini tribe indicate patterns of variation in chromosome numbers that range from 2n=8 chromosomes in *Mycocepurus* sp. (Murakami *et al*. 1998), *M. goeldii* (present work) to 2n=54 chromosomes in *Myce. paralellus* (Fadini & Pompolo 1996). Most genera of this tribe for which cytogenetic information is available present variation species-level variation in their chromosome numbers. In the genus *Mycetarotes*, for example, *Mycetarotes carenatus* Mahye-Nunes presents 2n=14 and *Myce. paralellus* 2n=54 (Fadini *et al*. 1996). However, species of *Atta* and *Acromyrmex* have uniform chromosome numbers: 2n=22 and 2n=38 respectively, with the exception of *Ac. ameliae* which has 2n=36 chromosomes (Barros *et al*. 2008). The karyotypic conservatism of these two genera denotes evolutionary stability or recent differentiation from a common ancestor. Then, the low chromosome number observed in *M. goeldii* suggests retention of the low chromosome number which is certainly an ancestral character of the tribe.

The applying of CMA_3 fluorochrome to different ant subfamilies studied to date presented a marking on only a single pair of chromosomes including the *A. ameliae* species and its hosts (Barros *et al.* 2008), that belong to the same tribe as *M. goeldii*. Thus the study of the location and composition of the heterochromatin in other species of this tribe might be informative on the retention or loss of heterochromatin regions that are rich in GC pairs.

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