Brief Report

Occurrence of pre-nucleolar bodies and 45S rDNA location on the chromosomes of the ant *Mycocepurus goeldii* (Forel) (Formicidae, Myrmicinae, Attini)

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The ant *Mycocepurus goeldii* (Forel) is known for having a relict karyotype with low chromosome number and the present study help the understanding of this ant cytogenetics by describing the occurrence of pre-nucleolar bodies in their chromosomes using impregnation with silver nitrate (Ag-NOR) and the location of 45S rDNA sites by means of the FISH (fluorescent *in situ* hybridization) technique. Several spots were observed surrounding all chromosomes when submitted to the Ag-NOR technique. These unusual markings were observed in both chromatids of metaphase and early anaphase chromosomes, and are associated to the presence of pre-nucleolar bodies, allowing the observation of the phenomenon of nucleologenesis. Although recent studies have shown that all chromosomes of *M. goeldii* exhibit centromeric or pericentromeric markings for the CMA_3 fluorochrome, the FISH technique indicated the presence of 45S rDNA in only one pair of chromosomes that differed in the number of CMA_3 markings observed for this species, pointing that the other markings observed with this fluorochrome do not match the sequences in ribosomal genes.

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The *Mycocepurus* ant genus is widely distributed along the Neotropical region, however only five species are known (Mayhé-Nunes and Meneguete 2000; Fernandez 2003). Similarly to other Attini, these gardening ants depend on a symbiotic fungus for food (Mehdiabadi and Schultz 2009). These species are relatively common with a propensity to cryptic life (Rabeling et al. 2007, 2009). It was shown recently that the common *Mycocepurus smithii* (Forel) performs an unusual case of thelytokous parthenogenisis first hypothesized by Fernández-Marín et al. (2005) and verified by Rabeling et al. (2009). A combination of data suggests the plesiomorphic condition of the genus *Mycocepurus* inside the Attini tribe such as: molecular phylogeny studies (Schultz and Brady 2008), nest structure/architecture (Rabeling et al. 2007), the information on the evolution of fungus-growing ants (Mehdiabadi and Schultz 2009), and finally the low chromosome number observed in the two populations of the genus studied so far (Murakami et al. 1998; Barros et al. 2010).

Only two *Mycocepurus* populations have been studied by means of cytogenetics: an unidentified species from Barro Colorado Island, Panama, which had $2n = 8$ chromosomes (Murakami et al. 1998) and *M. goeldii* (Forel) from Viçosa, Minas Gerais, Brazil with the same chromosome number (Barros et al. 2010). When submitted to the C-banding technique and stained with CMA_3 fluorochrome, all *M. goeldii* chromosomes revealed centromeric or pericentromeric marks for both techniques indicating the presence of GC-rich heterochromatin blocks. The second pair of chromosomes showed stronger markings for both techniques and also coincided with a region of secondary constriction (Barros et al. 2010).

The CMA_3 fluorochrome has already been applied to chromosomes of the family Formicidae (Lorite et al.

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1997, Mariano et al. 2008, Santos et al. 2010), which has marks on one single pair of chromosomes. This GC-rich chromatin coincided with the location of nucleolus organizing regions (NOR), as occurs in other organisms (Schmidt and Guttenbach 1988; Reed and Phillips 1995). Therefore, it is expected that in species with one single pair of chromosomes carrying the CMA_3 marks these regions probably will contain the NOR.

The nucleus is a complex region within which the various functions and components are spatially and temporally organized and regulated (reviewed by Matera et al. 2009). One such component is the nucleolus, representing the most prominent cellular structure of the interphase nucleus whose primary function is the biogenesis of ribosomal subunits (Dundr et al. 2000; Boisvert et al. 2007) since rRNAs are synthesized, processed, cleaved and assembled with ribosomal proteins in the nucleolus before being exported to the cytoplasm. Other functions, however, have been attributed to the nucleolus such as mitosis regulation, stress responses, and it is related to some human diseases (Boisvert et al. 2007). The nucleolus is considered a dynamic structure because it becomes disorganized when the cell enters mitosis and reorganizes itself gradually in the late anaphase or early telophase when rDNA transcription is reactivated (Ochs et al. 1985; Dundr et al. 2000; Boisvert et al. 2007).

During mitosis, part of the nucleolar proteins may remain associated with NORs, may be bounded at the periphery of mitotic chromosomes and, may also be dissolved in the cytoplasm (Ochs et al. 1985; Dundr et al. 1997, 2000). At the end of anaphase the rDNA transcription reactivation leads to the gradual reorganization of the nucleolus. This reorganization begins when the nucleolar material appears in the vicinity and on the surface of chromosomes, where it gathers in discrete structures called pre-nucleolar bodies (PNB), which fuse at chromosomal NORs in telophase or early interphase, thus completing the formation of the mature interphase nucleolus (Ochs et al. 1985; Dundr et al. 2000; Savino et al. 2001). The molecular interactions between the PNBs components that occur during the assembly of the nucleolus are not well known (Bell and Scheer 1996).

The PNBs contain processing-proteins, pre-snoRNAs and partially processed rRNAs (Ochs et al. 1985; Dundr et al. 2000; Boisvert et al. 2007). Some of these proteins can be stained with silver nitrate solution (Ochs et al. 1985). The PNBs were described in plants and animals which indicate their conserved character and important function in the cell (Boisvert et al. 2007) occurring both naturally and through drug induction (Ochs et al. 1985).

In this work we describe the location of 45S rDNA sites and the presence of pre-nucleolar bodies in *M. goeldii.*

MATERIAL AND METHODS

A colony of *Mycocepurus goeldii* was collected at the Estação de Pesquisa, Treinamento e Educação Ambiental 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). It was kept in a chamber incubation (B.O.D) at 25°C at the Insect cytogenetics laboratory, Univ. Federal de Viçosa, to obtain larvae. The metaphases were obtained from cerebral ganglia of 15 pre-pupa stage workers (after *meconium* elimination) following the protocol of Imai et al. (1988). Eight to ten metaphases were observed per individual.

Some slides were submitted to the silver staining method Ag-NOR (Howell and Black 1980) and others to the FISH technique using a 45S rDNA probe isolated from *Arabidopsis thaliana* following the procedures by Moscone et al. (1996) with modifications. Metaphases and interphase nuclei submitted to the silver staining methods were observed and photographed with a BX 60 microscope coupled to an Olympus Q Color 3 digital image system. An epifluorescence Leica DMRA2 microscope and IM50 software (ver. 5 Release 190) were used to analyze and capture images of the FISH technique. The images were organized using the Adobe Photoshop C3 and Corel Photopaint X4 Corel Corporation software.

Adult workers were placed as vouchers in the collection of the Laboratório de Mirmecologia at the Centro de Pesquisas do Cacau (CEPEC) Ilhéus, Bahia, Brazil.

RESULTS AND DISCUSSION

Two markings on interphase nuclei (Fig. 1a), corresponding to nucleoli, were identified by silver staining but there were no markings on the corresponding metaphase chromosomes. Several structures, however, were observed at the periphery of the chromatids of all eight chromosomes of this species, both in the metaphase (Fig. 1b) and in the early anaphase (Fig. 1c) which suggested the presence of pre-nucleolar bodies (PNBs). The PNBs could be visualized because some nucleolar proteins of these corpuscles have silver affinity (Ochs et al. 1985; Zatsepina et al. 1997) allowing observation of the nucleologenesis phenomenon.

It has been proposed that rDNA transcription activation facilitates the PNBs fusion (Benavente et al. 1987; Zatsepina et al. 1997), a useful feature in nucleologenesis studies since the drug actinomycin D is known as a transcription inhibitor (Ochs et al. 1985; Benavente et al. 1988) allowing the observation of the PNBs. Moreover, the use of antibodies against RNA polymerase I prevents the activation of gene transcription in rDNA at telophase transition - G1, which still allows the formation

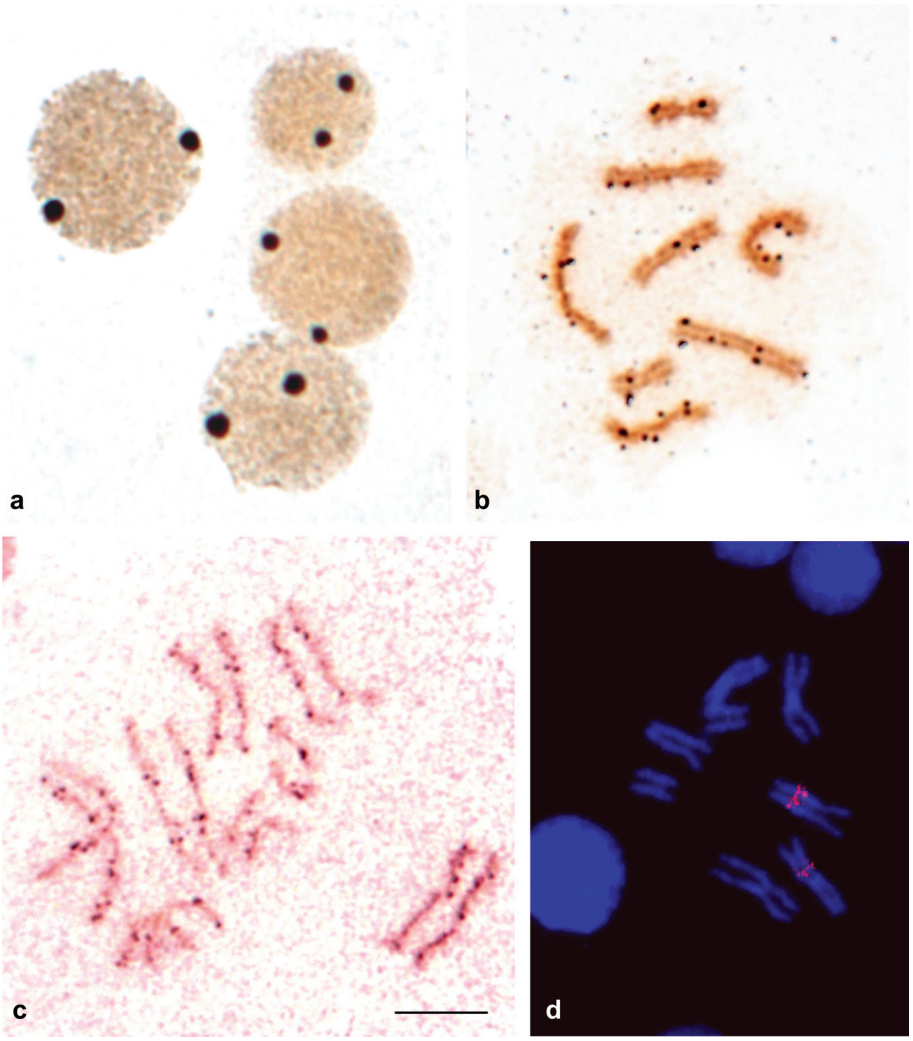

Fig. 1a–d. Dynamics of *Mycocepurus goeldii* NOR sites during the cell cycle. (**a**) interphase nucleus with two markings corresponding to NOR, **(b)** metaphase and **(c)** early anaphase with pre-nucleolar bodies (PNBs) along the chromosomes, **(d)** metaphase submitted to the FISH technique showing 45S rDNA sequences in the second largest chromosome pair. Bar = 5 µm.

of daughter cells that are capable of gathering PNBs but fail in merging to the nucleolus (Benavente et al. 1987). A similar effect can be observed in cells after prolonged exposure to antimitotic drugs such as colchicine, because the process is blocked in the PNBs nucleologenesis stage (Benavente et al. 1988). This drug is an alkaloid commonly used to block the cell cycle at the metaphase stage allowing the chromosome study and it is believed that by preventing the polymerization of spindle fibers, colchicine keeps the chromosomes in metaphase while nucleologenesis proceeds to the formation of PNBs. Thus, the PNBs maintain their formation and growth so the stages of this phenomenon can be observed around the chromosomes by silver impregnation, as observed in the metaphase and anaphase chromosomes of *M. goeldii* in this study.

PNBs reports are more common in mammals than in other *taxa* (Ochs et al. 1985; Zatsepina et al. 1997; Dundr et al. 2000; Savino et al. 2001) and for Formicidae there is no information about such structures probably because of the lack of cytogenetic data for this large group of insects. Moreover, it is possible that a few ant karyotypes may have the PNBs which were clouded by the ant cytogenetic technique limitations on the fixation of the ganglia. However, when an ant karyotype with only eight large chromosomes was analyzed some limitations were surpassed and the PNBs were seen. It must be pointed out that the improvement of cytogenetic protocol surely will unveil several unknown nuclear structures allowing a best comprehension of nuclear cycle.

The lack of Ag-NOR markings on the chromosomes corresponding to the 45S rDNA was due to the low

accessibility of the ribosomal gene chromatin by the argentophilic proteins (Imai et al. 1992).

Only one pair of chromosomes hybridized with the 45S rDNA probe (Fig. 1d), and two markings were observed in the interphase nuclei. The pair of chromosomes labeled by the FISH technique coincided with the same pair bearing the secondary constriction and also the highest GC-rich heterochromatic block at the pericentromeric region of the long arm, by means of C-banding and CMA_3 techniques (Barros et al. 2010). Together, all this evidence implies that this GC-rich heterochromatic region flanks the 45S rDNA genes. The FISH technique results indicated the absence of 45S rDNA genes on other chromosomes, showing the absence of any chromosomal rearrangements involving these genes.

The labeling pattern observed with the CMA_3 fluorochrome for *M. goeldii* indicated markings for the four pairs of chromosomes of this species and differed from that observed for other ants in the Attini tribe: *Acromyrmex ameliae* and its hosts *Acromyrmex subteraneus subterraneus* and *Acromyrmex subterraneus brunneus* (Barros et al. unpubl.), because these three ants presented markings on only a single pair of chromosomes using CMA_3 which probably corresponded to the NOR. On the other hand, only a single chromosome pair presented 45S rDNA sites in *M. goeldii* by means of the FISH technique. Since there are GC-rich regions in the species which do not correspond to ribosomal genes, the study of the heterochromatin location and composition in other Attini species may be very informative since it will contribute to the chromosomal mapping for retention or loss of GC-rich regions. These data, coupled with studies of 45S rDNA location will allow a better understanding of the chromosomal evolution of this tribe.

Although the results of the staining patterns obtained in this study were not complementary, the occurrence of PNBs is registered in ants. This observation raised the need to study these structures and the phenomenon of nucleologenesis in other ants, as a contribution to better understand some instigative gaps in the evolution of ant karyotypes. The number of markings observed for *M. goeldii* with the FISH technique differed from that observed with the CMA_3 (Barros et al. 2010) showing the importance of the combination of conventional cytogenetic techniques with molecular cytogenetic techniques.

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