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Short Communication

Cytogenetic characterization of two species of *Frieseomelitta* Ihering, 1912 (Hymenoptera, Apidae, Meliponini)

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Abstract

The cytogenetic analysis of *Frieseomelitta dispar* and *F. francoi* revealed the chromosome numbers 2n = 30 and n = 15 and a karyotypic formula $2K = 4M+2M^{t}+4A+20A^{M}$. The number of chromosomes observed was consistent with those reported for other *Frieseomelitta* species. The occurrence of the M^{t} chromosome and other features of the karyotype formulae suggest a close relationship between *F. dispar*, *F. francoi* and *F. varia*. Nevertheless, it was possible to differentiate the karyotypes of the species by DAPI/CMA_3 staining, which revealed GC-rich regions on two chromosome pairs of *F. dispar*: one acrocentric and one pseudoacrocentric. In *F. francoi,* the same kinds of regions were observed on a pair of metacentrics and on a pair of acrocentrics. Our analysis also confirmed the chromosome number conservation in *Frieseomelitta* and suggests that infrequent pericentric inversion could constitute a synapomorphy for the group including *F. dispar*, *F. francoi,* and *F. varia.*

Key words: Hymenoptera, *Frieseomelitta,* interspecific differentiation, C-banding, heterochromatin.

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Frieseomelitta is comprised of 16 species (Moure *et al.*, 2007) of which only five have been cytogenetically analyzed (Rocha *et al.*, 2003; Célia M.L.C., Moreira and Kleber F. Costa, personal communication). Previous analyses were restricted to the description of chromosome numbers and heterochromatin distribution. Although northeastern Brazil is a center of endemism for several meliponine species (Silveira *et al.*, 2002; Moure *et al.*, 2007), the local fauna is poorly represented in earlier cytogenetic surveys. The objective of the present study was to analyze the karyotypes of *Frieseomelitta dispar* and *F. francoi*, two closely related species from the northeastern Brazilian fauna in order to understand the types of chromosome changes that might have occurred during the differentiation of these species. To this aim, we used different cytogenetic techniques to characterize the molecular nature and patterns of distribution of constitutive heterochromatin. The placement of these two species in the available phylogeny (Camargo and Pedro, 2003) is also discussed at the light of the present results.

We analyzed specimens of *F. dispar* from four nests collected in the region of Ilhéus (14°47' S, 39°12' W) and of *F. francoi* from one nest collected in Boipeba Island, municipality of Cairu (13°30' S, 39°02' W), in the state of Bahia, Brazil.

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Metaphases of 20 individuals from each nest were obtained from cerebral ganglion cells at the prepupal stage. Chromosome preparations were done according to Imai *et al.* (1988). A minimum of ten metaphases was analyzed per specimen. C-banding followed the protocol of Sumner (1972), with the modifications described by Pompolo and Takahashi (1990). DAPI and CMA_3 staining was done according to Schweizer (1980). The best quality metaphases were photographed using a CX41 Olympus photomicroscope.

Chromosome classification and karyograms followed the nomenclature of Imai (1991), which includes metacentric chromosomes with centromeric C-bands (M) and acrocentric chromosomes with small (A) or large heterochromatic arms (A^{M} or pseudoacrocentrics). A type of metacentric chromosome with centromeric and telomeric C-bands was also observed and it is herein called M^{t}. Voucher specimens were deposited in the Coleção Entomológica do Departamento de Zoologia, Universidade Federal do Paraná (DZUP), in Curitiba, Paraná State, and in the entomological collection of the Universidade Estadual de Santa Cruz (UESC).

Both species showed the chromosome numbers 2n = 30 in females and n = 15 in males. These numbers were also found in all *Frieseomelitta* species previously studied (Rocha *et al.*, 2003), confirming the chromosome number conservation within the genus. These numbers are uncommon in the tribe Meliponini and have only been observed in

the Neotropical genera *Duckeola*, *Geotrigona,* and *Leurotrigona* (Kerr, 1972; Pompolo and Campos, 1995; Rocha *et al.*, 2003). Except for *Duckeola,* these Neotropical genera are not closely related to *Frieseomelitta* (Rasmussen and Cameron 2010).

We also observed the karyotypic formula 2K = $4M+2M^t+4A+20A^M$ in both species. The high number of pseudoacrocentric chromosomes in these karyotypes is consistent with previous results reported for *Frieseomelitta* species (Rocha *et al.*, 2003), except for *F. trichoceratta* that had the karyotypic formula 2K = $4M+16A+10A^M$ (K.F. Costa, MSc Dissertation, Universidade Federal de Mato Grosso, Cuiabá, 2003).

C-banding revealed the presence of constitutive heterochromatin in the centromeric region of metacentric chromosomes and on the short and long arms of acrocentrics and pseudoacrocetrics of both species. An M^t chromosome, which is uncommon in the Meliponini, was observed in the karyotypes analyzed herein (Figure 1). This type of chromosome was previously found in *F. varia.* However, it was defined as an A^{ct} chromosome by C.M.L.C. Moreira (MSc Dissertation, Universidade Federal de Viçosa, Viçosa, 1997) and as the 10th pair by Rocha *et al.* (2003). According to Imai (1991), M^t chromosomes would have likely originated from an ancestral acrocentric through a pericentric inversion.

The CMA_3/DAPI staining in male metaphases of *F. dispar* revealed CMA_3^+/$DAPI^-$ heterochromatic regions on the small arms of the 4th chromosome pair and on the large arms of the 7th pair (Figure 2A and B). *F. francoi* had CMA_3^+/$DAPI^-$ bands in the centromeric regions of the 1st chromosome pair and in the telomeric regions of the 4th pair (Figure 2C and D). The molecular characterization of the heterochromatin revealed that putative homeologous heterochromatic regions are different in base composition and probably had different origins. Inferences drawn exclusively from the comparison of the C-banding results, as it is usually done, would thus be misleading. This technique was much more informative when combined with the fluorochromes staining.

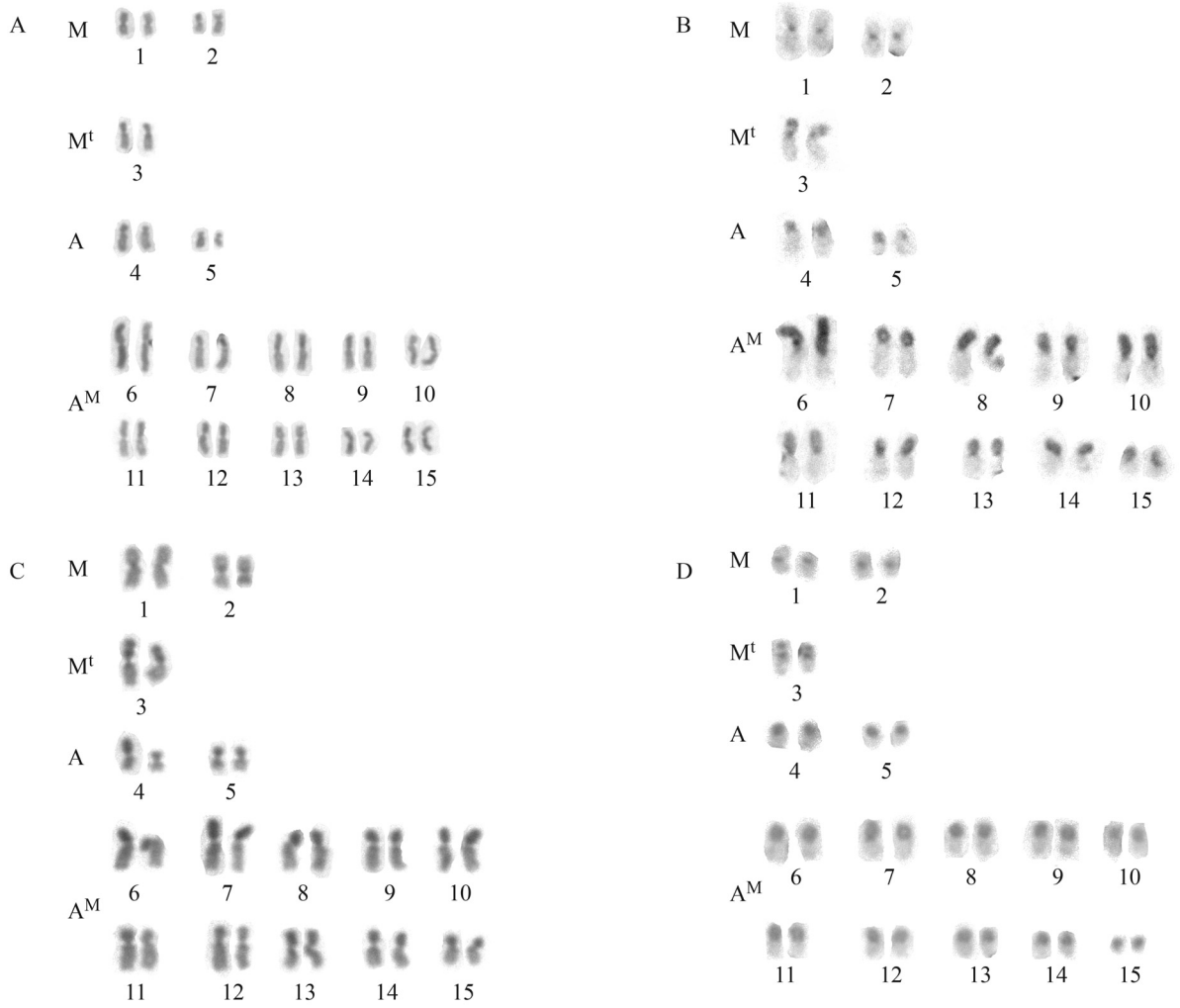

Figure 1 - Karyotypes of *Frieseomelitta dispar* (A and B) and of *Frieseomelitta francoi* (C and D) after: conventional coloration (A and C) and C-banding (B and D).

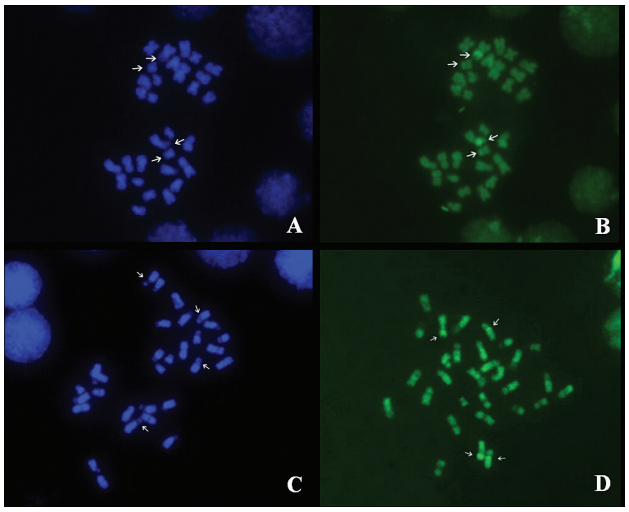

Figure 2 - Metaphases of a *Frieseomelitta dispar* male, n = 15 chromosomes (A and B) and of a *Frieseomelitta francoi* female, 2n = 30 (C and D) after: DAPI (A and C) and CMA_3 (B and D) staining. The arrows indicate CMA_3^+/$DAPI^-$ bands.

The occurrence of the M^t chromosome and other features of the karyotype formulae suggest a closer relationship between *F. dispar* and *F. francoi*. However, the available phylogeny separates these species in different groups (Camargo and Pedro, 2003) and our results with fluorochromes staining also point in that direction. In case that the phylogenetic hypotheses based on morphology are correct, the M^t chromosomes had an independent origin, which, however, is inconsistent with the karyotypic conservation observed in this genus.

Our results confirmed the chromosome number conservation in the karyotypes of *Frieseomelitta* species and suggest that a rare pericentric inversion could constitute a synapomorphy for a group including *F. dispar*, *F. francoi,* and *F. varia.* Further cytogenetic analyses including other *Frieseomelitta* species using more refined cytogenetic techniques may help to explain the origin of this inversion. The inclusion of species of related genera, such as *Trichotrigona* and *Duckeola*, may also help to clarify the cytological mechanisms involved in the origin of the divergent chromosome number n = 15 amongst the Meliponini.

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