Sociobiology 60(1): 125-127 (2013)

Sociobiology
An international journal on social insects

SHORT NOTE

Karyotype Description of *Cephalotrigona femorata* Smith (Hymenoptera: Apidae) and the C-banding Pattern as a Specific Marker for *Cephalotrigona*

RV Miranda[1], A Fernandes[2], DM Lopes[1]

1 - Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil

2 - Universidade do Estado de Mato Grosso, Tangará da Serra, Mato Grosso, Brazil.

Article History
Edited by:
Cândida M. L. Aguiar, UEFS - Brazil
Received 31 October 2012
Initial acceptance 07 December 2012
Final acceptance 14 December 2012

Keywords
Hymenoptera, stingless bees, cytogenetics, heterochromatin.

Corresponding author
Denilce Meneses Lopes
Universidade Federal de Viçosa
Departamento de Biologia Geral
Av. Peter Henry Rolfs, s/n. Campus
UFV, 36570-000, Viçosa, MG, Brazil.
E-Mail: denilce.lopes@ufv.br

Abstract

Cephalotrigona femorata (Smith, 1854) was submitted to cytogenetic techniques to study and describe its karyotype. Conventional staining allowed the counting (2n=34) and observation of chromosome morphology. The amount and distribution of heterochromatin in this species was different from *Cephalotrigona capitata* (Smith, 1854), another species of the genus already analyzed. Our results indicate that heterochromatin is a potential marker for the genus, at least for the species found in Brazil. This region was marked by DAPI, revealing a high content of A:T. The CMA_3 marked two pairs, and it seems to be polymorphic in one pair.

Cytogenetic analyses allow for morphological and quantitative characterization of the chromosomes of a determined species, which may contribute to the understanding of phylogeny and improvements in the taxonomy of some groups of Hymenoptera (Rocha et al., 2003). A very peculiar taxon within Hymenoptera is the tribe Meliponini, the stingless bees. These bees play an important role in economic and ecological processes of tropical regions because they are important pollinators of native plants (Heard, 1999; Michener, 2000). In Brazil two species of the genus *Cephalotrigona* Schwarz, 1940 are found among the five species described taxonomically, *Cephalotrigona capitata* and *C. femorata* (Moure, 2011). *Cephalotrigona capitata* is the only species of the genus for which the karyotype has already been described, and 2n = 34 chromosomes were observed (Rocha et al., 2003). The present study evaluates the species *C. femorata* in order to characterize it cytogenetically and thus to obtain more knowledge on stingless bees.

Post-defecant larvae of four colonies of *C. femorata*, were obtained in the municipality of Urbano Santos, Maranhão, Brazil (3°12'29"S; 43°24'18"W), as part of a project to rescue wildlife in the area of deforestation. Larvae (n= 40) were collected and processed according to the method described by Imai et al. (1988) to acquire the mitotic metaphase chromosomes. Conventional staining was performed using diluted Giemsa in Sörensen buffer (0.006 M, pH 6.8) at the concentration of 4% for 20 minutes. For C-banding the BSG (Barium hydroxide/Saline/Giemsa) method was used according to Sumner (1972). Fluorochrome staining (CMA_3/DA/DAPI) was performed according to the protocol proposed by Schweizer (1980). Ten slides from each hive were analyzed along with an average of 10 metaphases per slide. The material was observed under an Olympus BX60 microscope and images were used for assembly of the karyotypes utilizing the image analysis program Image-Pro PlusTM (version 6.3, Media Cybernetics®, 2009). Karyo-

types were organized as reported by Imai (1991), taking into consideration heterochromatin distribution.

The conventional staining technique showed that *C. femorata* presents a diploid number of 2n = 34 in females (Fig. 1A and B). This data is similar to that of the other species of the genus, *C. capitata*. The C-band technique permitted observation of heterochromatic arms on all chromosomes (Fig. 1B). Often these portions represent regions larger than the euchromatic arms. Two pairs also showed heterochromatin in the pericentromeric region (Fig. 1B). The observed heterochromatin pattern differed significantly from *C. capitata* where only 8 of the 17 pairs had a heterochromatin arm. According to the classification proposed by Imai (1991), *C. femorata* had a karyotypic formula of $2k=30A^{M}+4A^{Mc}$ while for *C. capitata* this was $2K=18A+16\ A^{M}$, this variation can therefore be used to distinguish the two species since they are morphologically similar.

The heterochromatic regions were positively stained by DAPI revealing a high content of A:T in heterochromatin similar to other Meliponini (Fig. 1C). The fluorochrome CMA_3 showed four markings. In one of the pairs this staining showed a large polymorphism of size (Fig. 1C) in one homolog. These polymorphisms are common in bees and have been well characterized for other stingless bee species such as *Oxytrigona flaveola* (Krinski et al., 2010). As previously observed in other stingless bees, these GC-rich regions may be related to the nucleolus organizer regions (Brito et al., 1997, Maffei et al., 2001; Rocha, 2002; Brito-Ribon et al., 2005; Lopes et al., 2011).

Thus, although the number of chromosomes in general is constant in the genus, other characteristics such as the amount and distribution of heterochromatin vary among species and may be used as an important tool that may aid in the taxonomy and conservation of this species.

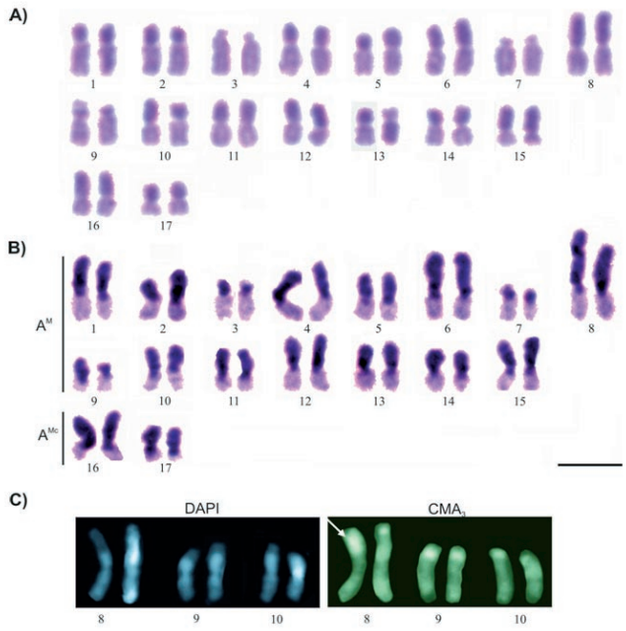

Figure 1. Karyotype of a *Cephalotrigona femorata* female. A. Conventional staining. B: C-banding. C: Fluorochromes DAPI and CMA3. Polymorphic pair indicated by arrow. Scale bar: 5 µm.

Acknowledgments

The authors thank the following research funding agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundações de Amparo à Pesquisa de Minas Gerais (FAPEMIG) and Mato Grosso (FAPEMAT), and Fundação Arthur Bernardes (FUNARBE) for their financial support to the research.

References

Brito, R.M., Costa, M.A. & Pompolo, S.G. (1997). Characterization and distribution of supernumerary chromosomes in 23 colonies of *Partamona* helleri (Hymenoptera, Apidae, Meliponinae). Braz. J. Genet. 20: 185-188.

Brito-Ribon, R.M., Pompolo, S.G., Martins, M.F., Magalhães, M.F.M., Barros, E.G. & Sakamoto-Hojo, E.T. (2005). Cytogenetic Characterization of Two *Partamona* Species (Hymenoptera, Apinae, Meliponini) by Fluorochrome Staining and Localization of 18S rDNA Clusters by FISH. Cytologia 70: 373-380.

Heard, T.A. (1999). The role of stingless bees in crop pollination. Annu. Rev. Entomol. 44,183-206.

Imai, H.T. (1991). Mutability of constitutive heterocromatin (C-band) during eukaryotic chromosomal evolution and their cytological meaning. Jap. J. Genet., 66: 635-661.

Imai, H.T., Taylor, R.W., Crosland, M.W.J. & Crozier, R.H. (1988). Modes of spontaneous evolution in ants with reference to the minimum interaction hypothesis. Jap. J. Genet. 63:159-185.

Krinski, D., Fernandes, A., Rocha, M.P. & Pompolo, S.G. (2010). Karyotypic description of the stingless bee *Oxytrigona cf. flaveola* (Hymenoptera, Apidae, Meliponina) of a colony from Tangará da Serra, Mato Grosso State, Brazil. Genet. and Mol. Biol. 33: 494-498. http://dx.doi.org/10.1590/S1415-47572010000300020

Lopes, D.M., Fernandes, A., Praça-Pontes, M.M., Werneck, H.A., Resende, H.R. & Campos, L.A.O. (2011). Cytogenetics of three *Melipona* species (Hymenoptera, Apidae, Meliponini). Sociobiology 58: 185-194.

Maffei, E.M., Pompolo, S.G., Silva-Junior, J.C. & Caixeiro, A.P. (2001). Silver staining of nucleolar organizer regions

(NOR) in some species of Hymenoptera (bees and parasitic wasp) and Coleoptera (lady-beetle). Cytobios 104: 119-125.

Michener, C.D. (2000).The Bees of the World. Johns Hopkins University Press, Baltimore, 913 pp.

Moure, J.S., Urban, D. & Melo G.A.R. (2011). Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region - online version. Available from http://www.moure.cria.org.br/catalogue.

Rocha, M.P. (2002). Análises citogenéticas em abelhas do gênero *Melipona* (Hymenoptera, Meliponinae) Ds. Universidade Estadual de Campinas, Campinas, São Paulo, 86pp.

Rocha, M.P., Pompolo, S.G. & Campos, L.A.O. (2003). Citogenética da tribo Meliponini (Hymenoptera, Apidae), pp 311-320. In G.A.R. Melo & I. Alves dos Santos (Eds.) Apoidea Neotropica:Homenagem aos 90 Anos de Jesus Santiago Moure., UNESC, Criciúma.

Schweizer, D. (1980). Simultaneous fluorescent staining of R bands and specific heterochromatic regions (DA-DAPI bands) in human chromosomes. Cytogen. Cell Genet. 27: 190-193.

Sumner, A.T. (1972). A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. 75: 304-306.