Genetics and Molecular Biology, 28, 3, 390-393 (2005)

www.sbg.org.br

Short Communication

Karyotype characterization of *Trigona fulviventris* Guérin, 1835 (Hymenoptera, Meliponini) by C banding and fluorochrome staining: Report of a new chromosome number in the genus

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Abstract

Although many species of the genus *Trigona* have been taxonomically described, cytogenetic studies of these species are still rare. The aim of the present study was to obtain cytogenetic data by conventional staining, C banding and fluorochrome staining for the karyotype characterization of the species *Trigona fulviventris*. Cytogenetic analysis revealed that this species possesses a diploid chromosome number of 2n = 32, different from most other species of this genus studied so far. This variation was probably due to the centric fusion in a higher numbered ancestral karyotype, this fusion producing the large metacentric chromosome pair and the lower chromosome number observed in *Trigona fulviventris*. Heterochromatin was detected in the pericentromeric region of the first chromosome pair and in one of the arms of the remaining pairs. Base-specific fluorochrome staining with 4'-6-diamidino-2-phenylindole (DAPI) showed that the heterochromatin was rich in AT base pairs ($DAPI^+$) except for pair 13, which was chromomycin A_3 (CMA_3) positive indicating an excess of GC base pairs. Our data also suggests that there was variation in heterochromatin base composition.

Key words: cytogenetics, heterochromatin, evolution, stingless bees.

Received: October 13, 2004; Accepted: May 4, 2005.

Despite the predominant role of bees in the pollination of native species in ecosystems such as the Atlantic Rain Forest, bee diversity has been poorly studied. According to Kerr *et al.* (2001), stingless bees, which until 1838 were the only species used for the honey production in Brazil and pollination of the native flora, have been endangered due to factors such as deforestation, clearance of ground by burning and extractive activities.

The tribe Meliponini is pantropically distributed but the highest concentration and diversity occurs in the American tropics, with 56 genera being found in Brazil (Roubik, Segura and Camargo, 1997; Costa *et al*., 2003).

The stingless bee *Trigona fulviventris* Guérin, 1835 is characterized by a black head and thorax and dark orange metasoma. The nest is subterranean and located between roots at the base of large trees or underground and this bee shows no aggressive behavior in the defense of the nest (Roubik, 1992).

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Cytogenetic analyses have contributed greatly to the studies of the phylogeny, speciation mechanisms and genetic variability of many plant and animal groups. Published data regarding the cytogenetics of Meliponini have indicated that the haploid number ranges from 8 to 18 chromosomes, although the predominant number is n = 17 (Kerr, 1952, 1969, 1972; Kerr and Silveira, 1972; Tarelho, 1973; Hoshiba, 1988; Alves, 1999; Rocha *et al*., 2002).

Kerr (1972), Kerr and Silveira (1972) and Tarelho (1973) have raised the hypothesis of polyploidy to explain the numerical changes observed in the karyotypes of these bees. However, subsequent studies on different meliponine species suggested that centric fission has been the main mechanism involved in the karyotype evolution within the group, since the polyploidy hypothesis does not explain the presence of heterochromatin or morphological chromosome variations observed in some species (Pompolo and Campos, 1995).

From a list of nineteen currently recognized *Trigona* species (Silveira *et al*. 2002), only seven have been studied cytogenetically. Some previous analyses was limited to a description of the chromosome number and of the pattern of heterochromatin distribution.

In the present study we developed a detailed analysis of the karyotype of *T. fulviventris* based on conventional staining, C banding and fluorochrome staining (4'-6-diamidino-2-phenylindole - DAPI and chromomycin A_3 - CMA_3).

Cytogenetic analysis was performed on a total of 25 samples from each of two colonies collected in the region of Pedra Branca (12°50'817" S, 39°30'766" W), near Salvador, the capital of the northeastern Brazilian state Bahia.

Slides were prepared using the cerebral ganglion of prepupal larvae, according to the technique proposed by Imai *et al*. (1988) and C banding was performed according to the method of Sumner (1972), modified by Pompolo and Takahashi (1990). Fluorochrome banding was carried out using the triple staining (DA/CMA_3/DAPI) method as modified by Schweizer (1980).

A minimum of 10 metaphases per individual was analyzed and those showing the best quality were photographed using Kodak Imagelink HQ film for conventional staining and C banding and Kodak T-MAX film for fluorochrome staining.

We determined that the chromosome number of *T. fulviventris* was 2n = 32. According to the nomenclature proposed by Imai (1991), the diploid karyotype consists of one metacentric chromosome pair which is very long compared to the remaining karyotype, two acrocentric pairs and 13 pseudoacrocentric pairs, differing little in size (Figure 1 A and B).

Except for one of the acrocentric pairs which was completely euchromatic (second pair - Figure 1B), we observed C^+ band regions on most chromosomes. Heterochromatin was detected in the pericentromeric region of the large metacentric pair, on the short arm of the second acrocentric pair, and throughout the extension of one of the arms of all pseudoacrocentric chromosomes. One of the pseudoacrocentric pairs (pair 13) presented heterochromatin that was more strongly labeled than in the others and showed a conservative pattern in all metaphases and individuals analyzed, suggesting a difference in molecular composition.

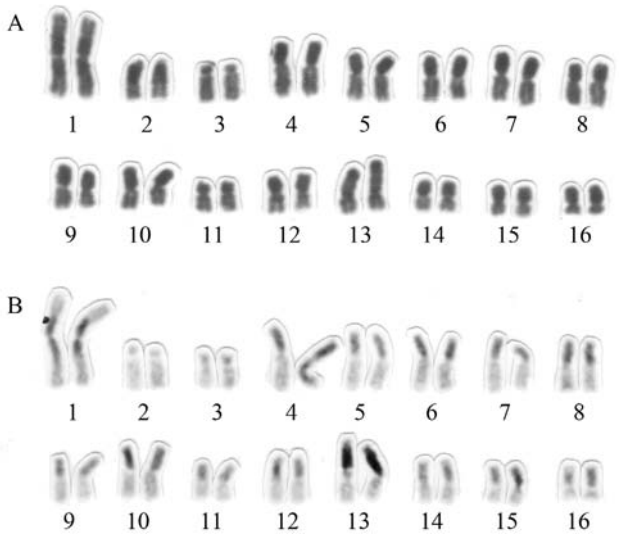

Figure 1 - A) Karyotype of a *Trigona fulviventris* worker with 2n = 32 chromosomes. B) C banding pattern. The chromosomes are arranged in decreasing order of size of the euchromatic arm.

Triple staining revealed CMA_3^+ labeling only in pair 13 heterochromatin, while in the remaining chromosome pairs heterochromatin regions were $DAPI^+$ (Figure 2). The euchromatic acrocentric chromosome (pair 2) was not labeled by any of the fluorochromes used.

In this paper we present the first report chromosome number variation within *Trigona*, 2n = 34 having been the only karyotype reported for all previously studied species, *e.g. T. spinipes* (Kerr, 1969; Tarelho, 1973; Brito and Pompolo, 1997), *T. cilipes* (Kerr, 1969), *T. recursa* (Tarelho, 1973; Costa *et al*., 2004), *T. fuscipennis* (Tarelho, 1973), *T. branneri*, *T. hyalinata* and *T. chanchamayoensis* (Costa *et al*., 2004). We also found another karyotypic difference, a large metacentric chromosome pair in the karyotype of *T. fulviventris* which was absent, or has not been reported, in the above cited species.

Previous studies have pointed to some possible mechanisms generating karyotype changes within Hymenoptera. Kerr and Silveira (1972) proposed the occurrence of polyploidy in a bee ancestral karyotype with n = 8 chromosomes. Subsequent changes in the chromosome number would have been the result of Robertsonian rearrangements. In contrast, data published by Pompolo (1992), Rocha *et al*. (2003) and Gomes (1995) suggested that the karyotype evolution of bees and wasps follows the same cycle of changes proposed by Imai *et al*. (1988), Imai and Taylor (1989), Imai (1991) and Hoshiba and Imai (1993) for different groups. Hoshiba and Imai (1993) suggested karyotype evolution according to the minimum interaction theory, which assumes that the main mechanisms involved in this process favor an increase in the chromosome number by centric fission, resulting in a reduction of size and, consequently, of a physical interaction between chromosomes during meiosis. This process would reduce the occurrence of deleterious chromosome translocations (for details, see Imai, 1991, 2001).

We observed a large number of chromosomes with pseudoacrocentric morphology in the karyotype of *T. fulviventris*, suggesting the occurrence of centric fission followed by the tandem growth of terminal heterochromatin. However, the first chromosome pair of the *T. fulviventris* karyotype, showing a metacentric morphology and heterochromatin distributed in the pericentromeric region, was considerably longer than the remaining chromosomes of the karyotype, a fact providing strong evidence for centric fusion between two pseudoacrocentric chromosomes. Centric fusion can be an important mechanism for eliminate excess of heterochromatin and thus reduce the risk of deleterious chromosome interactions (Imai, 1991).

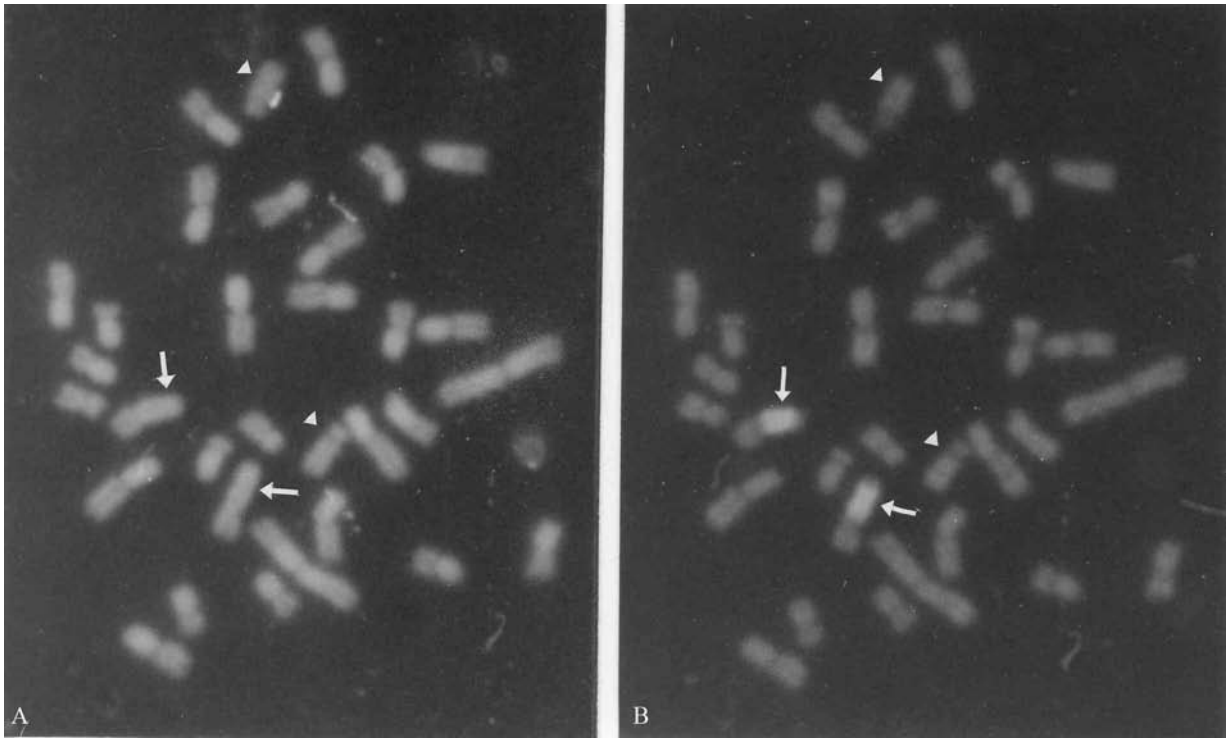

Figure 2 - Metaphases of a *Trigona fulviventris* worker with 2n = 32 chromosomes treated with A) DAPI fluorochrome and B) CMA_3 fluorochrome. Arrows indicate the chromosome pair 13 and the arrowheads the chromosome pair 2.

Triple staining provided an analysis of complementarity of bands produced by DAPI and CMA_3. These results showed divergent base composition in the heterochromatin of this species. Positive CMA_3 labeling observed in the chromosome pair 13 heterochromatin (Figure 2B), suggests that this particular heterochromatin is rich in GC base pairs, since the CMA_3 fluorochrome is specific for these bases. In contrast, $DAPI^+$ labeling was observed on chromosomes whose heterochromatin was weakly labeled by C banding, indicating a widespread distribution of the AT-rich heterochromatin. Costa *et al*. (2004) found a predominance of GC-rich heterochromatin in *T. branneri* chromosomes but not in *T. recursa*. These authors did not perform DAPI staining on these two species. Present and published data indicate that, besides being quantitatively variable, heterochromatin content may have different base composition and hence independent origins. Comparative studies based only on C-banding information may be misleading and molecular characterizations such as the fluorochrome staining described in our present study is an essential tool for future comparative analyses.

More conclusive evidence regarding the origin of the large metacentric pair and the reduced chromosome number observed for *T. fulviventris* should be obtained with the use of more accurate techniques, such as *in situ* hybridization or banding methods that permit a more detailed characterization of the chromosome arms. Such studies performed on *T. fulviventris* and compared to the patterns observed for other closely related species will certainly be enlightening and will contribute to a better understanding of the mechanisms involved in the karyotype evolution of the genus *Trigona*.

Acknowledgments

This study was developed at UESB, Jequié Campus, with the financial support of PRODOC/CADCT. We thank Dr[a] Silvia das Graças Pompolo (UFV, Viçosa, MG) for technical support and indispensable contributions to the work and Dr. Gabriel A.R. Melo (UFPR, Curitiba, PR) for the Identification of *Trigona fulviventris*.

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Associate Editor: Catarina S. Takahashi