



## Occurrence of B chromosomes in *Tetragonisca* Latreille, 1811 (Hymenoptera, Apidae, Meliponini): A new contribution to the cytotaxonomy of the genus

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### Abstract

*Tetragonisca angustula* and *Tetragonisca fiebrigi* have recently been listed as valid species. This study aimed to cytogenetically investigate both species, emphasizing the new registry of B chromosomes in the tribe Meliponini. We analyzed colonies of *T. angustula* and *T. fiebrigi* collected at Tangará da Serra, Mato Grosso, Brazil, through conventional Giemsa staining, C-banding, and base-specific fluorochrome staining (CMA<sub>3</sub>/DAPI). *T. angustula* showed  $2n = 34$  chromosomes in females and  $n = 17$  in males, with karyotype formula  $2K = 34A^M$ . *T. fiebrigi* showed numeric variation, with chromosome number varying from  $2n = 34$  to  $2n = 36$  in females and from  $n = 17$  to  $n = 18$  in males, with karyotype formula  $2K = 32A^M + 2A^{Mc}$  and  $2K = 32A^M + 2A^{Mc} + 1$  or  $2$  B-chromosomes. The B chromosomes are heterochromatic. In *T. fiebrigi*, the CMA<sub>3</sub>/DAPI staining revealed four chromosomes with a CMA<sub>3</sub> positive band. All individuals from the same colony showed the same number of B chromosomes. *T. angustula* and *T. fiebrigi* showed karyotype divergence, principally due to the presence of B chromosomes, which are found only in *T. fiebrigi*. Our data corroborate the status of valid species for both *T. angustula* and *T. fiebrigi*, as recently proposed.

**Key words:** stingless bees, cytogenetics, interspecific differentiation, B chromosomes.

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Until recently, the genus *Tetragonisca* comprised two species, *Tetragonisca weyrauchi* and *Tetragonisca angustula*, the latter with two subspecies: *Tetragonisca angustula angustula* and *Tetragonisca angustula fiebrigi*. Moure *et al.* (2007) considered both subspecies as distinct species (*Tetragonisca angustula* and *Tetragonisca fiebrigi*), based on their morphological characteristics and sympatric distribution.

*Tetragonisca angustula* (Latreille, 1811) has a wide geographical distribution in the Americas, from Southern Mexico to the southernmost state of Rio Grande do Sul in Brazil (Schwarz, 1938; Moure *et al.*, 2007). This species, characterized by the black mesopleura, has been reported in all regions of Brazil (Nogueira-Neto, 1970; Moure *et al.*, 2007). *Tetragonisca fiebrigi* (Schwarz, 1938) differs morphologically from *T. angustula* in the ferruginous coloration of the mesopleura and lateral areas of the propodeum. This species ranges from the state of Rio Grande do Sul to the state of Mato Grosso, according to Schwarz (1938). But this distribution range has expanded to the northwestern border with Uruguay (Schwarz, 1938) and to the state of Goiás (Oliveira *et al.*, 2004).

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The validity of infraspecific taxa has been particularly debated over the last 50 years, with statements of some authors favorable to it (Wilson and Brown, 1953; Smith *et al.*, 1997; Isaac *et al.*, 2004) and others against it (Mayr 1982; Burbrink *et al.*, 2000; Ward, 2007). The need to use non-morphological characters for the study of subspecies was raised by Mayr (1982), referring to ecological and behavioral characteristics. On the other hand, the ambiguity of some morphological characters in species recognition, such as color pattern, has been demonstrated with molecular data (Burbrink *et al.*, 2000).

The occurrence of B chromosomes is regarded as one of the sources of numerical variation in the karyotype of some species. B chromosomes are rare in Meliponini bees, and have been reported to date in *Partamona helleri* (Costa *et al.*, 1992; Brito *et al.* 1997; Tosta *et al.*, 2004; Martins *et al.*, 2009) and *Melipona quinquefasciata* (Marla P. Rocha, personal communication).

This study was aimed at obtaining new cytogenetic information for *Tetragonisca angustula* and *Tetragonisca fiebrigi*, with emphasis on the new occurrence of B chromosomes in the tribe Meliponini.

We analyzed 20 individuals per colony, in 11 colonies of *Tetragonisca fiebrigi* and three colonies of

*Tetragonisca angustula*, collected in Tangará da Serra, in the state of Mato Grosso, Brazil (14°04' S, 57°03' W).

Metaphasic chromosomes were obtained from cerebral ganglia of prepupae following the method described by Imai *et al.* (1988). The slides were then submitted to conventional Giemsa staining, C-banding (Pompolo and Takahashi, 1990), and base-specific fluorochrome staining (CMA<sub>3</sub>/DAPI) (Schweizer, 1976). The slides were analyzed and the best metaphases were photographed using an Olympus photomicroscope. Karyotypes were arranged in decreasing order of length of euchromatic arms. Chromosome nomenclature followed Imai (1991), with pseudoacrocentric chromosomes showing an extraordinarily elongated heterochromatic short arm (A<sup>M</sup>), acrocentric with totally heterochromatic arms (A<sup>h</sup>), and pseudoacrocentric with pericentromeric heterochromatin (A<sup>Mc</sup>).

We found chromosome numbers  $2n = 34$  in females and  $n = 17$  in males of *Tetragonisca angustula* species. C-banding revealed heterochromatin in one arm of all chromosomes, and karyotypic formula  $2K = 34A^M$  (Figure 1 A, B). CMA<sub>3</sub>/DAPI showed no evident differential staining in chromosomes of this species (Figure 2 A, B).

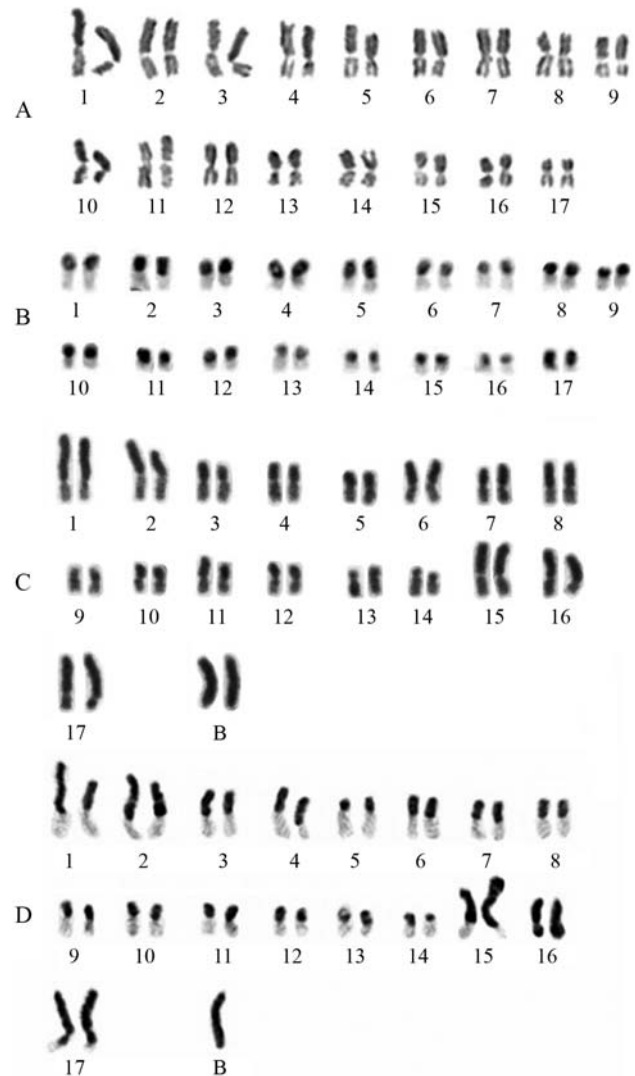
*Tetragonisca fiebrigi* showed variation in diploid chromosome number with  $2n = 34$  (one colony),  $2n = 35$  (eight colonies), and  $2n = 36$  (two colonies) (Figure 1 C, D). In three of the colonies in which we found females with  $2n = 35$ , we also found males with  $n = 18$ .

The differences in diploid number observed in these colonies ( $2n = 34$  to  $2n = 36$ ) were due to the presence of up to two B chromosomes per individual. The length of the B chromosome (about 5  $\mu\text{m}$ ) was similar to that of the regular complement, and was observed in all individuals of the same colony.

C-banding revealed that most chromosomes of *T. fiebrigi* were pseudoacrocentric with karyotype formulae  $2K = 32A^M + 2A^{Mc}$ ,  $2K = 32A^M + 2A^{Mc} + 1B$ , and  $2K = 32A^M + 2A^{Mc} + 2B$ . B chromosomes were entirely heterochromatic – A<sup>h</sup> (Figure 1D). Four CMA<sub>3</sub> positive bands were also observed in two chromosome pairs, one in the interstitial region, and one in the terminal region (Figure 2 C, D).

Although B chromosomes are frequently found in natural populations of animals and plants, they have been poorly studied in Hymenoptera. In the stingless bee *Partamona helleri*, B chromosomes have been reported to vary from none to four minute chromosomes per specimen (Costa *et al.*, 1992; Brito *et al.*, 1997; Tosta *et al.*, 2004). Recently, Martins *et al.* (2009) described the occurrence of a new B chromosome in this species, larger in size than those previously reported. In *Melipona quinquefasciata*, up to four B chromosomes have been observed (Marla P. Rocha, personal communication).

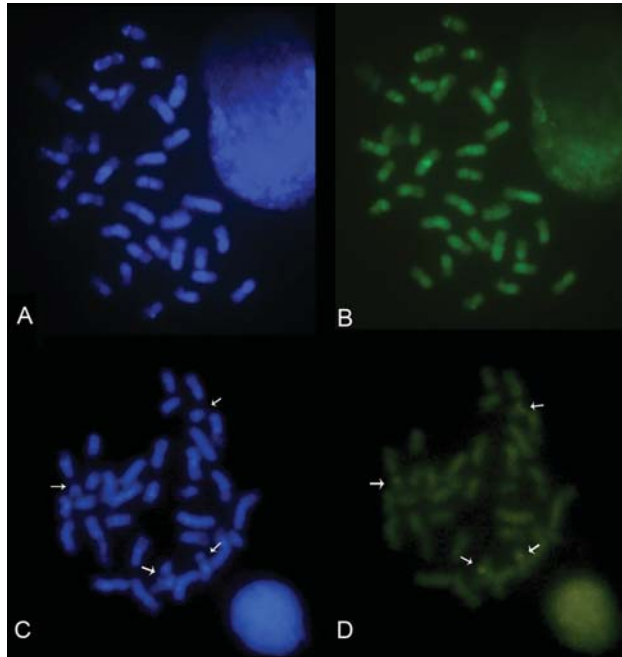
In the ant *Camponotus* sp, the occurrence of up to three small B chromosomes has been reported (Mariano *et al.*, 2001). In the wasp *Nasonia vitripennis*, a small submetacentric and heterochromatic B chromosome called



**Figure 1** - Female karyograms. A) *T. angustula* submitted to conventional Giemsa staining. B) *T. angustula* submitted to C-banding. C) *T. fiebrigi* submitted to Giemsa staining showing two B-chromosomes. D) *T. fiebrigi* submitted to C-band staining showing one B chromosome.

“psr chromosome” was reported to be involved in the elimination of the paternal chromosome set in fertilized eggs, converting them into haploid eggs (Nur *et al.*, 1988). The wasp *Trypoxylon albitarse* showed a constant distribution pattern of B chromosomes among individuals, with one and two B chromosomes in males and females, respectively (Rocha-Sanchez and Pompolo, 2004). In this case, the authors suggested that the B chromosome behaves as a chromosome of the regular complement during meiosis, undergoing balanced segregation at the end of cell division. A similar mechanism could explain the constant distribution of B chromosomes in *T. fiebrigi*.

In the colonies of *T. angustula* and *T. fiebrigi* we studied, karyotypes were distinct and conserved within each species, and no hybrid karyotypes were detected. This differentiation suggests reproductive isolation, which is reinforced by the presence of B chromosomes and specific



**Figure 2** - Female metaphases of A and B) *T. angustula* submitted to DAPI and CMA<sub>3</sub> staining, respectively, and C and D) *T. fiebrigi* submitted to DAPI and CMA<sub>3</sub> staining, respectively. Arrows in *T. fiebrigi* metaphases indicate positive CMA<sub>3</sub> bands.

CMA<sub>3</sub> bands only in *T. fiebrigi* species, allowing the use of these features as cytological markers to karyotypically distinguish the two species.

Our cytogenetic data corroborate the specific status of *Tetragonisca angustula* and *Tetragonisca fiebrigi* proposed by Moure *et al.* (2007). The numerical constancy of colonies with B chromosomes, however, demands more investigation to elucidate if the predominance of B chromosomes in the progeny of *T. fiebrigi* has been favored by events such as meiotic drive.

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