

Karyotypes of two species of stingless bees, *Leurotrigona muelleri* and *Leurotrigona pusilla* (Hymenoptera, Meliponinae)

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ABSTRACT

In a cytogenetic study including heterochromatin distribution carried out on two *Leurotrigona* species, a difference was observed between the karyotypes of *Leurotrigona muelleri*: $n=8$, $2n=16$; 7(14) metacentric and 1(2) acrocentric chromosomes and low heterochromatin content and *Leurotrigona pusilla*: $2n=30$; 10 metacentric, 14 submetacentric and 6 acrocentric chromosomes and a high heterochromatin content. The cytological mechanisms that have led to karyotype differentiation in these two species are discussed.

INTRODUCTION

Several studies on the cytogenetics of meliponids are available in the literature (Kerr, 1948, 1952, 1969, 1972; Kerr and Araújo, 1957; Kerr and Silveira, 1972; Tarelho, 1973; Tambasco *et al.*, 1979; Hoshiba, 1988; Bravo and Arcos, 1991; Pompolo, 1992, 1994). On the basis of the chromosome numbers of 52 species, Kerr and Silveira (1972) and Tarelho (1973) have proposed polyploidization as the major mechanism for karyotype evolution in the Apoidea.

In ants, Imai *et al.* (1988) have proposed that the karyotype evolution was based on the mechanism of centric fission followed by heterochromatin addition. This addition would form non-specific association and the pericentric inversion accompanied by the excision of heterochromatin, would yield a relatively stable karyotype with new, higher chromosome number.

The geographic distribution of *Leurotrigona pusilla* includes the Amazon region, Colombia and the

Guyanas, whereas *L. muelleri* is distributed only in Brazil from the South to Tocantins (Camargo, J.M.F., personal communication).

Kerr and Silveira (1972) detected $n=8$ chromosomes in the gonadal cells of *L. muelleri* males.

In the present study we report the karyotype of two species of stingless bees, *Leurotrigona muelleri* and *L. pusilla*, including the distribution and content of heterochromatin visualized by C-banding (Sumner, 1972).

MATERIAL AND METHODS

Cytogenetic analyses were carried out on colonies of each species in 1991 and 1993. Three colonies of *L. muelleri* were from Senador Mourão (MG), Cajuru (SP), and the Kraho-Galheiros Indian reserve (45 km SE of Itacajá, TO). The two colonies of *L. pusilla* were collected in Manaus (AM). Approximately ten individuals per colony were studied and at least ten

metaphases per individual were examined. The technique for the preparation of metaphase chromosomes from the cerebral ganglion of prepupae was adapted from Imai *et al.* (1988). The time of ganglion permanence in colchicine hypotonic solution was increased to two hours in order to increase the number of metaphases. The C-banding technique used for both species was that of Sumner (1972), with some adaptations. For *L. muelleri*, three days after chromosome preparation the slides were immersed in sequence in the following solutions: 0.2 N HCl at room temperature for eight minutes followed by a rapid wash in distilled water; 5% Ba(OH)₂ at 60°C for seven minutes followed by a rapid wash in 0.2 N HCl and then in distilled water, and 2XSSC, pH 7.0, at 60°C for seven minutes. The same procedure was used for *L. pusilla*, except for the times of immersion in each solution, which were five minutes for HCl, six minutes for Ba(OH)₂, and six minutes for 2XSSC. The preparations of both species were stained with 6% Giemsa in Sorensen buffer for 15 minutes.

The metaphases of best quality were photographed with Agfa Copex Pan A.H.U. film and the karyotype was mounted in decreasing order of chromosome size.

RESULTS

In the present study, analysis of three colonies of *L. muelleri* showed $n=8$ chromosomes for males and $2n=16$ chromosomes for females. The morphological constitution of the chromosomes was 7(14) metacentrics and 1(2) acrocentrics (Figure 1A and B). Heterochromatin content visualized by C-banding in a

female was quite low and was limited to two pairs: the centromeric region of a metacentric pair and the short arm of an acrocentric pair; the remaining chromosomes did not show C-banding (Figure 1C).

In *L. pusilla* we obtained $2n=30$ chromosomes, in females, whose morphologic constitution was 10 metacentric, 14 submetacentric and six acrocentric (Figure 2A). The C-banding technique showed that the heterochromatin content was high and was present in all chromosomes. In two pairs (submetacentrics) heterochromatin was found only in the centromeric regions, and in the remaining ones on one of the arms (Figure 2B).

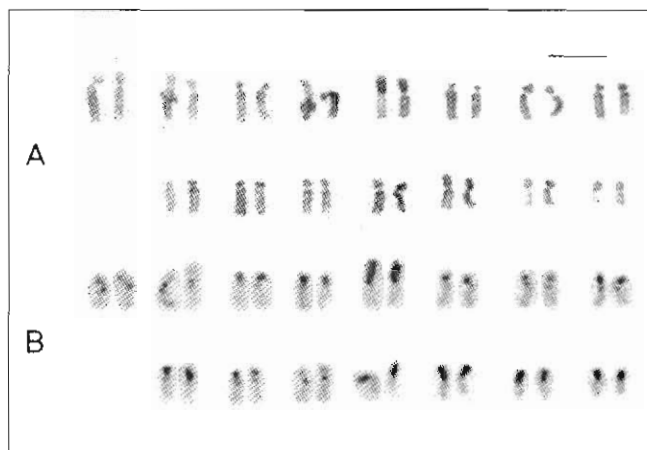


Figure 2 - Karyotype of *Leurotrigona pusilla* (from Manaus, AM). A, Female with $2n=30$ chromosomes. Standard staining. B, C-banding showing the distribution of heterochromatin in all chromosomes of a female. Bar represents μm .

DISCUSSION

The cytogenetic study of these two species of *Leurotrigona* showed a considerable difference between them. On the basis of data obtained by Kerr and Silveira (1972) and Pompolo (1994) for 48 species of Meliponinae, the haploid number for *Melipona* is uniformly nine; in the *Trigona*, *Scaptotrigona*, *Nannotrigona*, *Plebeia*, *Oxytrigona*, *Partamona*, all species have 17 chromosomes; and in *Frieseomelitta* and *Duckeola* all species studied have 15 chromosomes. Therefore it was a surprise to find two very similar species with such a different number of chromosomes. Costa *et al.* (1992) recently detected variation in chromosome number among colonies and among individuals of *Partamona cupira* ($2n=34$ to 37), but this difference was due to the presence of supernumerary chromosomes.

In other Hymenoptera such as ants, differences in chromosome number within a genus are common,

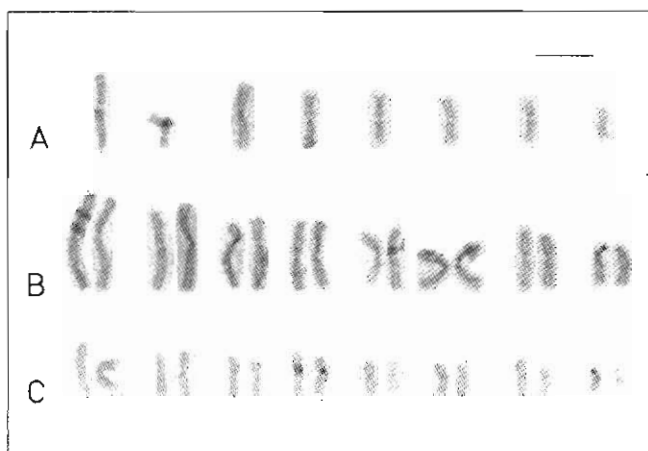


Figure 1 - Karyotype of *Leurotrigona muelleri* (from Cajuru, SP). A, male with $n=8$ chromosomes. B, Female with $2n=16$ chromosomes. Standard staining. C, C-banding showing the distribution of heterochromatin among chromosome pairs in a female. Bar represents $5 \mu\text{m}$.

and are generally due to centric fission (Imai *et al.*, 1988).

Kerr and Silveira (1972) suggested that polyploidy was the major event in the evolution of the karyotype of bees. This was based on data obtained for 52 species, in which a group with a chromosome number ranging from six to nine and another with a chromosome number corresponding to approximately double this value (14-20) were found (Tarelho, 1973). However, after 1988 many species were found with an intermediate number (see Pompolo, 1992, 1994) which made the poliploidy hypothesis less acceptable.

The karyotypes of the two *Leurotrigona* species studied here are quite different, a fact thus far unreported for any other genus of Meliponinae. Their morphological constitution and heterochromatin distribution were quite different (Figures 1 and 2). These data suggest that karyotypic differentiation from $n=8$ to $n=15$ between the two species occurred due to seven fissions and to the addition of heterochromatin to one of the chromosome arms, as observed in *L. pusilla* (Figure 2B). One of the arms in all metacentric and in 10 of the submetacentric chromosomes of *L. pusilla* was completely heterochromatic, in contrast with those of *L. muelleri* which have centromeric distribution in only one pair (Figures 1C and 2B). The addition of heterochromatin to the chromosome arms may play a role in the stabilization of the telomeric condition after fission (Imai *et al.*, 1988). Centric fusion and inversion may play an important role in eliminating heterochromatin by a mechanism which Imai (1991) denoted "splicing at the cytologic level", in order to free the chromosomes from excess centromere and interstitial telomere integrated into its structure and to reduce the risk of nonspecific association in the interphase nucleus, as proposed by Imai *et al.* (1986). In the two chromosome pairs of *L. pusilla* which present heterochromatin in the centromeric region, fission, heterochromatin addition and then inversion may have occurred. A C-banding positive region was observed at the end of the arm. The polyploidy hypothesis (Kerr and Silveira, 1972) could not explain the heterochromatic pattern or the chromosome morphology in the polyploid species. Imai's hypothesis seems adequate because it does not require large chromosome rearrangements and explains the accumulation of heterochromatin observed in *L. pusilla*.

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RESUMO

Em um estudo citogenético, incluindo a distribuição da heterocromatina, realizado em duas espécies *Leurotrigona*, observou-se diferença nos cariótipos *Leurotrigona muelleri*: $n=8$, $2n=16$; 7(14) cromossomos metacêntricos e 1(2) acrocêntricos e baixo conteúdo de heterocromatina e *Leurotrigona pusilla*: $2n=30$, 10 cromossomos metacêntricos, 14 cromossomos submetacêntricos e seis cromossomos acrocêntricos e alto conteúdo de heterocromatina. São discutidos os principais mecanismos citológicos que levaram a diferenciação dos cariótipos nas duas espécies como uma contribuição à citogenética dos Meliponídeos.

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