DNA characterization and karyotypic evolution in the bee genus *Melipona* (Hymenoptera, Meliponini)

MARLA PIUMBINI ROCHA[1,2], SILVIA DAS GRAÇAS POMPOLO[1,*], JORGE ABDALA DERGAM[3], ANDERSON FERNANDES[1] and LUCIO ANTONIO DE OLIVEIRA CAMPOS[1]

[1] *Departamento de Biologia Geral, Universidade Federal de Viçosa, 36571-000 Viçosa, MG, Brazil*
[2] *Departamento de Biologia Celular CP 6109, Universidade Estadual de Campinas 13083-970, Campinas-SP, Brazil*
[3] *Departamento de Biologia Animal, Universidade Federal de Viçosa, 36571-000 Viçosa, MG, Brazil*

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We analyzed patterns of heterochromatic bands in the Neotropical stingless bee genus *Melipona* (Hymenoptera, Meliponini). Group I species (*Melipona bicolor bicolor*, *Melipona quadrifasciata*, *Melipona asilvae*, *Melipona marginata*, *Melipona subnitida*) were characterized by low heterochromatic content. Group II species (*Melipona capixaba*, *Melipona compressipes*, *Melipona crinita*, *Melipona seminigra fuscopilosa* e *Melipona scutellaris*) had high heterochromatic content. All species had $2n = 18$ and $n = 9$. In species of Group I heterochromatin was pericentromeric and located on the short arm of acrocentric chromosomes, while in Group II species heterochromatin was distributed along most of the chromosome length. The most effective sequential staining was quinacrine mustard (QM)/distamycin (DA)/chromomycin A_3(CMA$_3$)/4–6-diamidino-2-phenylindole (DAPI). Heterochromatic and euchromatic bands varied extensively within Group I. In Group II species euchromatin was restricted to the chromosome tips and it was uniformly GC$^+$. Patterns of restriction enzymes (*Eco*RI, *Dra*I, *Hind*III) showed that heterochromatin was heterogeneous. In all species the first pair of homologues was of unequal size and showed heteromorphism of a GC$^+$ pericentromeric heterochromatin. In *M. asilvae* (Group I) this pair bore NOR and in *M. compressipes* (Group II) it hybridized with a rDNA FISH probe. As for Group I species the second pair was AT$^+$ in *M. subnitida* and neutral for AT and GC in the remaining species of this group. Outgroup comparison indicates that high levels of heterochromatin represent a derived condition within *Melipona*. The pattern of karyotypic evolution sets *Melipona* in an isolated position within the Meliponini.

Silvia das Graças Pompolo – Departamento de Biologia Geral, Universidade Federal de Viçosa, 36571-000 Viçosa, MG, Brazil. E-mail:spompolo@mail.ufv.br

The genus *Melipona* Illiger, 1806, encompasses a group of stingless bees commonly known as 'indigenous bees'. They are widely distributed in the Neotropics. They are recognized as ecologically important pollinators of cultured (HEARD 1999) and wild plants (KERR et al. 1996).

So far, sixteen species have been analyzed cytogenetically. Fifteen of them are $2n = 18$, and one is $2n = 20$ (KERR 1948, 1952, 1972, KERR and SILVEIRA 1972; TAMBASCO et al. 1979; ALMEIDA 1981; HOSHIBA 1988; POMPOLO 1992, 1994; HOSHIBA and IMAI 1993; ROCHA and POMPOLO 1998).

ROCHA and POMPOLO (1998) applied C-banding on these bees. On the basis of the relative amount of heterochromatin of eight species of *Melipona*, they divided the species into two groups. Group I includes species characterized by low levels of heterochromatin such as *Melipona asilvae*, *Melipona bicolor bicolor*, *Melipona marginata*, and *Melipona quadrifasciata*. On the other hand, Group II includes species with high heterochromatin levels like *Melipona capixaba*, *Melipona captiosa*, *Melipona seminigra fuscopilosa*, and *Melipona scutellaris*. High heterochromatin contents have been reported for a few animal taxa such as Diplopoda (VITTURI et al. 1997), Coleoptera (JUAN and PETITPIERRE 1989; PLOHL et al. 1993; BRUVO et al. 1995), Hymenoptera (GOMES et al. 1998) and Amphibia (KING 1980; SCHMID et al. 1988).

Although the stingless bees have been considered to lack suitable cytogenetic markers that could be used to construct a phylogeny (MICHENER 1990), the patterns of distribution of heterochromatin in *Melipona* can contribute to the knowledge of the phylogeny of this genus (ROCHA and POMPOLO 1998). We shall here describe the heterochromatin/euchromatin ratio of species from both groups, define the nature of the heterochromatin by means of a combination of fluorochrome staining with different banding techniques, and propose a phylogeny for the tribe Meliponini.

MATERIALS AND METHODS

Ten Brazilian *Melipona* species were cytogenetically studied (Table 1). We analyzed a mean of forty individuals per species, and five metaphases per specimen.

Mitotic metaphase spreads were obtained from cerebral ganglia of larvae in the final deffecation stage (IMAI et al. 1988). Conventional Giemsa staining and C-banding were carried out according to ROCHA and POMPOLO (1998), NOR banding followed HOWELL and BLACK (1980), with some modifications (MAFFEI et al. 2001).

Chromosome lengths were determined on four metaphase spreads of *M. bicolor bicolor* and *M. subnitida*, and values were compared to those of Group II species, *M. crinita*, *M. compressipes* and *M. seminigra fuscopilosa*. A mean value of linear length of euchromatic and heterochromatic regions was estimated with an ocular micrometer, and the euchromatin/heterochromatin ratio was calculated for each species.

Fluorochromes were applied sequentially as follows:

1. Distamycin/chromomycin A_3 (DA/CMA_3), according to SCHWEIZER (1980).
2. Distamycin/4–6-diamidino-2-phenylindole (DA/DAPI) according to SCHWEIZER (1980).
3. Distamycin/chromomycin A_3/4–6-diamidino-2-phenylindole (DA/CMA_3/DAPI) according to SCHWEIZER (1980). After DA/CMA_3 staining, slides were incubated in DAPI for 20 min, air dried and mounted on freshly filtered sucrose medium.
4. Quinacrine mustard/distamycin/chromomycin A_3/4–6-diamidino-2-phenylindole (QM/DA/CMA_3/DAPI). After staining with quinacrine mustard (SCHMID 1980), some slides were placed directly in McIlvaine's buffer (pH = 7.0) for 20 min. Good quality metaphases were photographed, and the material was treated for DA/CMA_3/DAPI. For all sequential staining protocols, some slides were subjected to C-banding protocol (BS) (ROCHA and POMPOLO 1998) prior and after fluorochrome treatments.

Table 1. *Sample localities of Melipona*

Species	Locals
Melipona asilvae	Pedra de Maria da Cruz – MG and Santana do Seridó – RN
Melipona bicolor bicolor	Caeté and Cunha – MG
Melipona capixaba	Venda Nova do Imigrante – ES
Melipona compressipes	São Luis – MA
Melipona crinita	Rio Branco – AC
Melipona marginata	Caeté – MG
Melipona quadrifasciata	Caeté and Viçosa – MG
Melipona seminigra fuscopilosa	Rio Branco – AC
Melipona scutellaris	Lençóis – BA
Melipona subnitida	Santana do Seridó – RN

Fluorescent in situ hybridization (FISH) followed VIEGAS-PÉQUIGNOT (1992) with a rDNA probe pDm 238. We also applied the following GIBCO-BRL restriction enzymes (RE): *Eco*RI (G↓AATTC), *Dra*I (TTT↓AAA), *Hind*III (A↓AGGTT). We applied 30 µl of a solution containing 10 U (1 µl) of each enzyme dissolved in 9 µl of appropriate buffer and 90 µl of ultra pure water. The slide was covered with a coverslip and placed in a humid chamber for 12 h at 37°C, it was afterwards rinsed with tap water and stained with 3,3 % Giemsa diluted in Sörensen buffer (0.06 M, pH 6.8).

Identification of homologues within species and putative homologues among species of Group I was based on banding patterns and chromosome morphology, and band size was used as complementary criterion. Finally, we attempted to unveil the nature of chromatin by applying sequential fluorochrome staining.

RESULTS AND DISCUSSION

Chromosome number and heterochromatin content

All species had a chromosome number typical of the genus *Melipona*, with 2n = 18 and n = 9 for females and males, respectively (Fig. 1, 2, 3, 4 and 5). The chromosome number was determined for *M. compressipes* and *M. crinita* (Fig. 2). Typically, the diploid number of *Melipona* is highly conservative, except for *M. quinquefasciata*, which was reported to be 2n = 20 (POMPOLO 1994).

Variation of heterochromatin content was evident among these species, with heterochromatin/euchromatin percentage values ranging from 8 % (*M. bicolor bicolor*) to 17 % (*M. subnitida*) in the Group I species, a clear contrast with Group II species 54 % (*M. crinita*), 61 % (*M. compressipes*) and 73 % (*M. seminigra fuscopilosa*). Based on these data, we define Group I as composed by species with less than 50 % heterochromatin content, while Group II species have heterochromatin values typically higher than 50 %. These latter heterochromatin/euchromatin ratios are higher than the ones reported for heterochromatin-rich karyotypes (PLOHL et al. 1993; BRUVO et al. 1995; VITTURI et al. 1997).

Furthermore, we observed that chromatin distribution also characterizes both groups; in Group I spe-

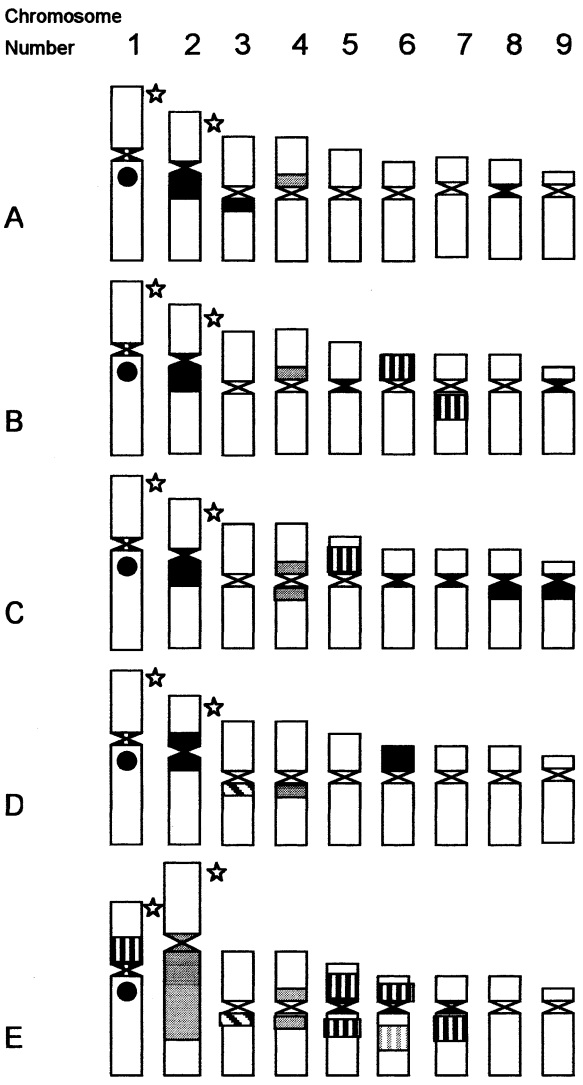

Fig. 1. Idiograms of the *M. bicolor bicolor* **A**, *M. quadrifasciata* **B**, *M. marginata* **C**, *M. asilvae* **D**, and *M. subnitida* **E** karyotypes, based on C-banding and sequential staining patterns. Dark blocks: B0. Checkered circles: B1. Gray blocks: B2. Vertical stripes: B3. Gray vertical stripes: B4. Black oblique stripes: B5. ☆ Band polymorphism.

cies the heterochromatin is pericentromeric and located on the short arm of acrocentric chromosomes (Fig. 1), while in Group II species the heterochromatin is distributed along most of the chromosome length, and the euchromatin is restricted to regions close to the telomeres (Fig. 2 and 5).

Chromatin composition

For all species, the most effective staining sequence for heterochromatin characterization was QM/DA/CMA_3/DAPI. In Group I species, we were able to differentiate six kinds of heterochromatic bands (JOHN et al. 1985) (Table 2); B1 and B2 bands were complementary to each other, while B0, B3, B4 e B5 bands were less common than the former and were not complementary among themselves (Fig. 1, Table 3). We observed fluorochrome-stained euchromatic bands of B3 type in the seventh chromosome pair of *M. quadrifasciata* and the fifth pair of *M. subnitida* (Fig. 1, Table 3). Because B3 bands were detected with AT-specific fluorochromes, and they were neutral for GC-specific fluorochromes, we concluded that they are moderately AT-rich. JOHN et al. (1985) did not find B3 and B4 bands in grasshoppers and they are characterized here for the first time in hymenopterans.

In *M. quadrifasciata*, BS-DA/CMA_3 and BS-DA/DAPI staining yielded a lower number of bands than slides not previously treated with BS-banding protocol, which may result from removal of euchromatin. The staining sequence BS-DA/CMA_3/DAPI provided similar results, e.g., a given heterochromatic region stained positive for CMA_3 and DAPI. This staining behavior may be explained also by the removal of DNA that stains positively for fluorochromes, or by an alteration of suitable conditions for fluorochrome staining. However, when applying the same sequential technique in Orthoptera, BELLA et al. (1993) obtained a complementary staining pattern. We concluded that our results may be more adequately explained by peculiar genomic characteristics of *Melipona*. Except for *M. asilvae*, we were unable to resolve the patterns of heterochromatic bands when BS-banding protocol was applied after fluorochrome staining.

The heterochromatin in chromosomes of Group I showed different staining intensity after C banding treatment (Fig. 1, Table 3). This behavior has also been reported for grasshoppers and may be explained by variations on the amount of heterochromatin among different regions (CAMACHO et al. 1984).

In Group I species, homologue pairing and homeologue identification among species were based on the presence of heterochromatic blocks in chromosomes. Thus, the GC-positive heterochromatic block in one pair of chromosomes of *M. subnitida* allowed us to identify as the homeologue of the first pair of chromosomes of the other species, despite the fact that it was not the largest of *M. subnitida*. This species was also karyotypically the most divergent, with interstitial heterochromatin and B3, B4 and B5 band types (Fig. 1, Table 3).

BS-banding patterns were similar for all Group I species. The application of different sequential staining protocols allowed to determine the existence of a highly heterogeneous heterochromatin in *Melipona* (Fig. 1 and 2, Table 3). Heterochromatin heterogeneity was first described in mouse (PARDUE and GALL

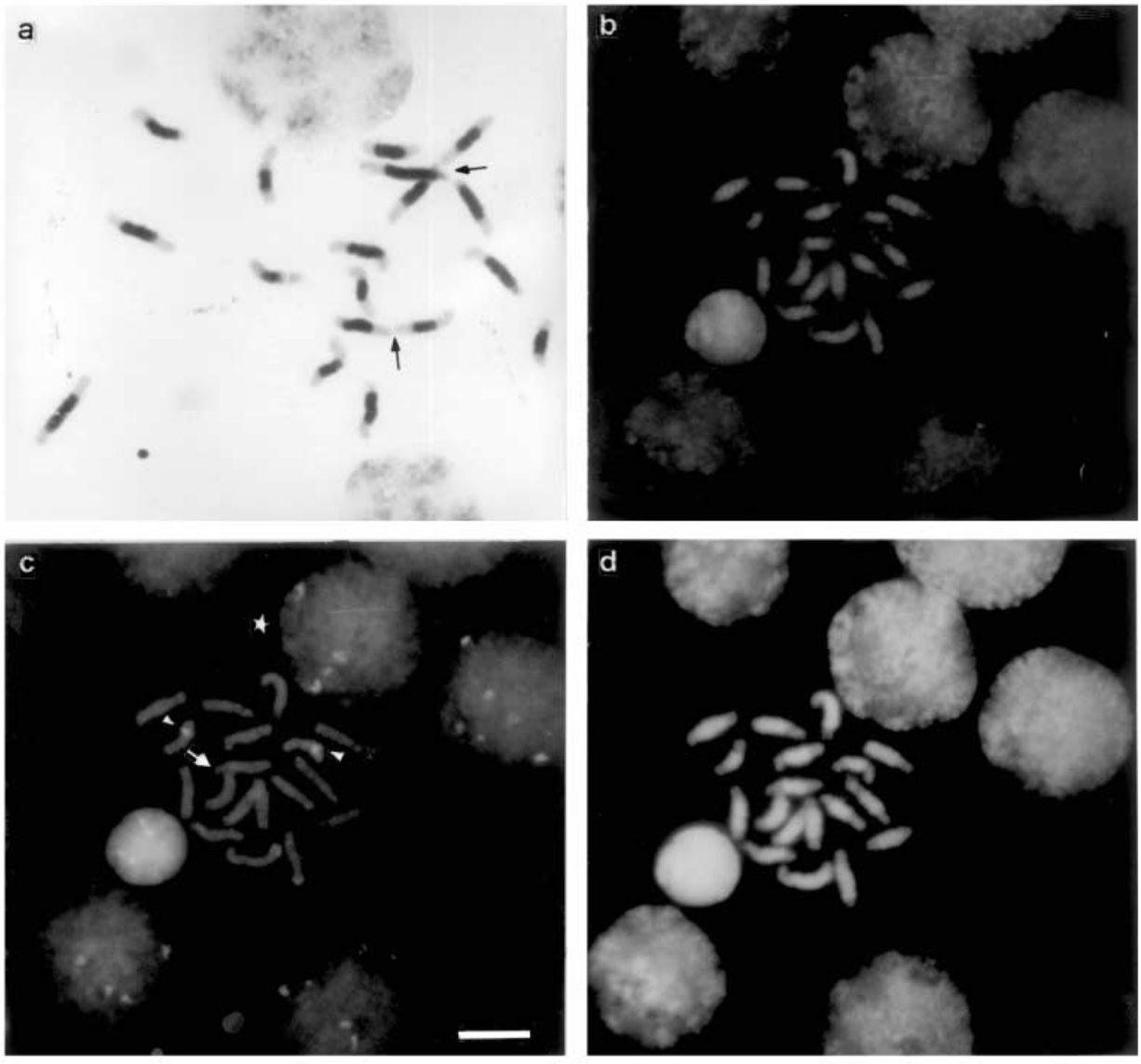

Fig. 2. Metaphases of females of *M. crinita* subject to C-banding **a** and sequential staining with QM **b**/DA/CMA_3 **c**/DAPI **d** fluorochromes. Arrowheads indicate CMA_3^+ regions; * indicates interphasic nucleus and arrows indicate euchromatic associations. Scale bar = 5 μm.

1970), and other organisms (ROCCHI 1982; JOHN et al. 1985; BELLA et al. 1993; MARTÍNEZ-LAGE et al. 1994).

In species of Group II sequential staining QM/DA/CMA_3/DAPI evidenced $CMA_3(GC^+)$ euchromatic chromosome ends (Fig. 2c and 5d); QM and DAPI (both with AT affinity) stained heterochromatin uniformly (Fig. 2 and 5). Staining patterns remained unaltered with either DA/CMA_3 or DA/CMA_3/DAPI sequential staining. Based on these data, we inferred that both euchromatin and heterochromatin alike are moderately rich in GC and AT base pairs respectively.

Fluorochrome staining data were complemented with RE protocols. In Group II species, RE banding patterns allowed for accurate chromosome pairing and to characterize high levels of heterogeneity of heterochromatin in these species (data not shown).

The heterochromatic blocks of the first and second chromosome pairs of both species groups were heteromorphic. Because we did not observe all possible character state combinations, we did not consider it as an instance of band polymorphism as reported for other insects (SENTIS et al. 1986; PANZERA et al. 1992; WARCHALOWSKA-SLIWA and BUGROV 1997). In these bees, constant size differences suggest the existence of gene regulation between the homologues, possibly involving NORs, as reported in aphids (MANDRIOLI et al. 1999). For species of Group I, the

Table 2. *Kinds of heterochromatic bands, based on fluorochrome affinities*

Kind of band	GC specific (CMA_3)	AT specific (DAPI and QM)
BO	Neutral	Neutral
B1	Positive	Negative
B2	Negative	Positive
B3	Neutral	Positive
B4	Neutral	Negative
B5	Positive	Positive

heterochromatic block of the first chromosome pair was GC positive (Fig. 1). In *M. asilvae* this pair bore Ag-NOR (Fig. 3), as it was also the case for *M. marginata* (MAFFEI et al. 2001). A direct relationship between GC positive bands and NORs was reported in grasshoppers *Eyprepocnemis plorans* and *Locusta migratoria* (CAMACHO et al. 1991), the ant *Tapinoma nigerrimum* (LORITE et al. 1997), the bee *Tetragonisca angustula* (MENEZES 1997), and *Partamona helleri* (BRITO-RIBON et al. 1998). This region was also seen in the first chromosome pair of *M. compressipes* (a Group II species). Hybridization of this region with the rDNA probe provides further support its NOR^+ nature (Fig. 4) and its homology with the NOR of the first pair of chromosomes of Group I species. In these species, the second pair of homologues bore a block that was neither GC^+ or $Ag\text{-}NOR^+$; this block was AT^+ in *M. subnitida* and neutral in the other species (Fig. 1, Table 3).

Chromatin behavior

In species of Group II, we observed an unusual chromatin behavior. In most nuclei, the heterochromatic regions were located in the peripheral region of the nuclei, while euchromatin occupied the central region. Likewise, HSU (1975) reported that heterochromatin lines the interphasic nuclei in some spe-

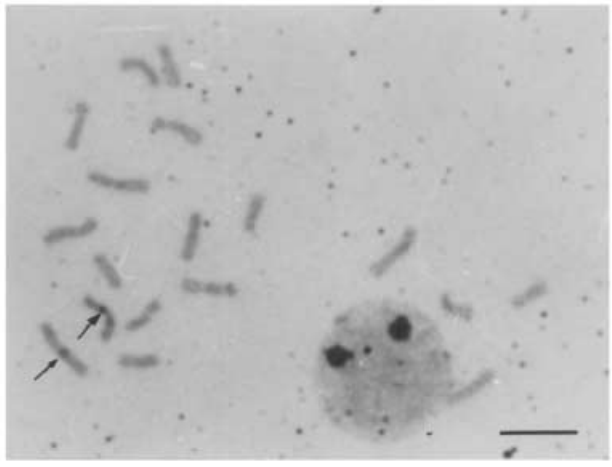

Fig. 3. Ag-NOR metaphase of *M. asilvae*. Arrows indicate NOR sites. Scale bar = 5 μm.

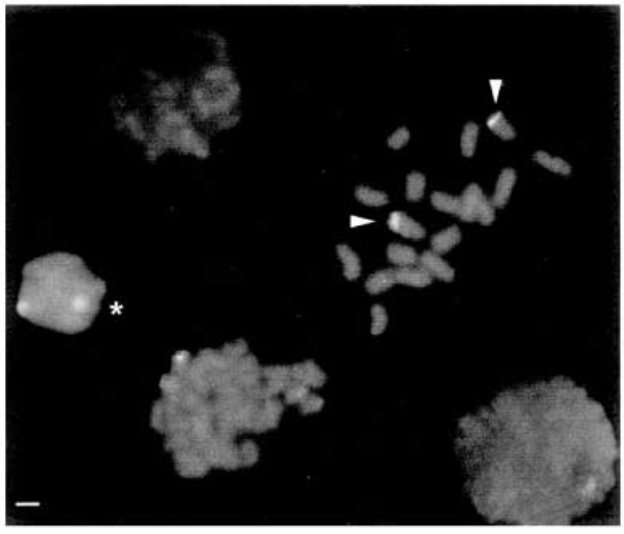

Fig. 4. Metaphase of *M. compressipes* after treatment with FISH rDNA probe. Arrowheads indicate hybridized regions, * indicates interphasic nucleus. Scale bar = 5 μm.

Table 3. *Position and nature of bands of five species of Melipona bees*

Chromosome number	*M. bicolor*	*M. quadrifasciata*	*M. marginata*	*M. asilvae*	*M. subnitida*
1	P*(B1)	P*(B1)	P*(B1)	P*(B1)	P*(B3, B1)
2	PW*(B0)	P*(B0)	P*(B0)	P*(B0)	PI*(B2)
3	PW(B0)	A	A	PW(B5)	P(B5)
4	PW(B2)	P(B2)	P(B2)	P(B2)	PW(B2)
5	A	PW(B0)	PW(B3)	A	P(B3)-E(B3)
6	A	C(B0)	PW(B0)	C(B0)	P(P-B3,I-B4)
7	A	A-E(B3)	PW(B0)	A	PW(B3)
8	PW(B0)	A	PW(B0)	A	A
9	A	PW(B0)	P(B0)	A	A

A – absence of C-banding; E-euchromatic band; P – pericentromeric C-banding; C – C-banding restricted to short arm; I – interstitial C-banding; W – weakly stained C-banding. Kinds of bands: (B0), (B1), (B2), (B3), (B4) e (B5); * – occurrence of band heteromorphism.

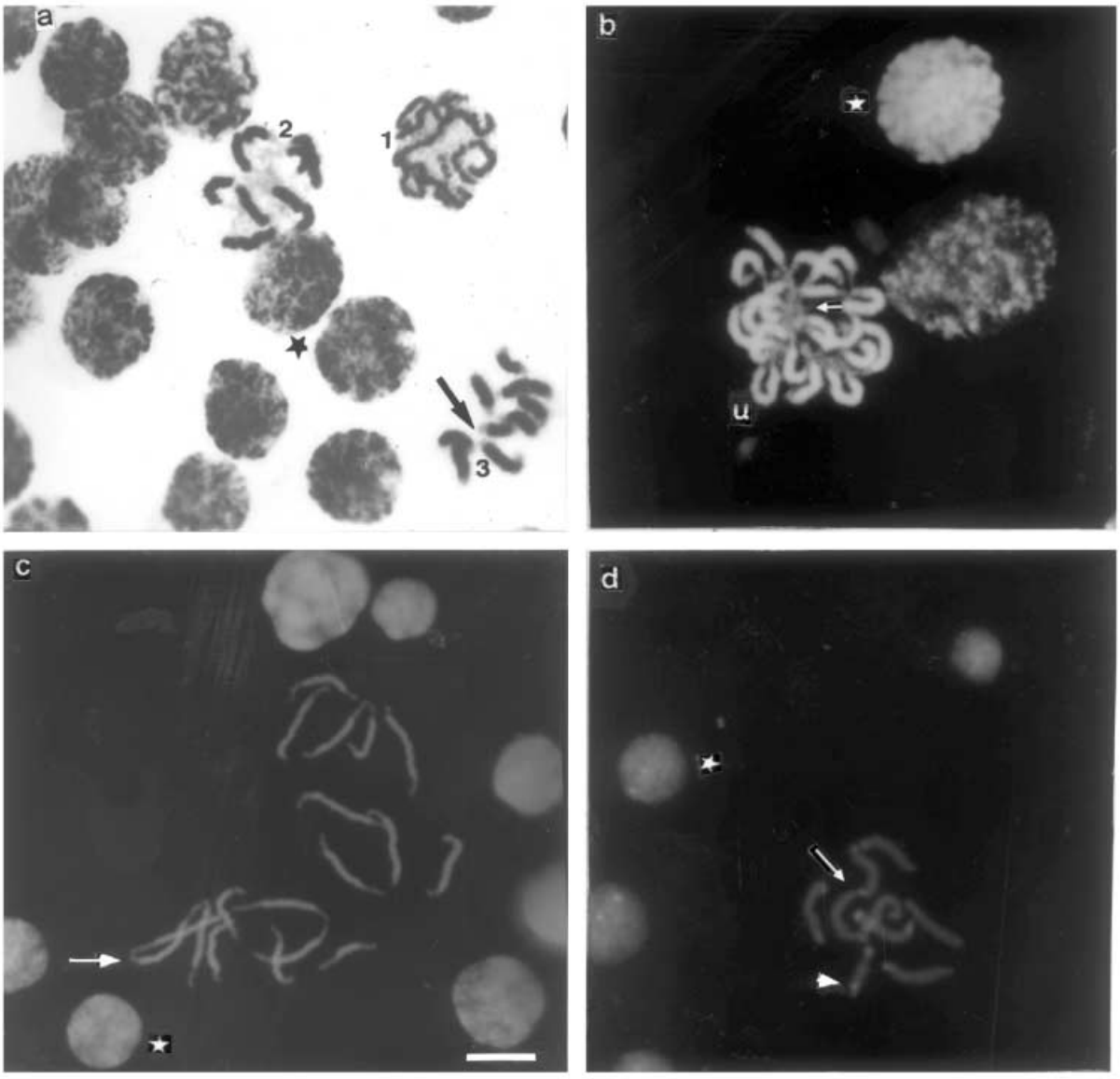

Fig. 5. Mitotic cells of *Melipona* in different division stages. **a** – 1, 2 and 3, cells of males of *M. seminigra fuscopilosa* in successive division stages after C-banding protocol. DAPI-stained prometaphase cells of female of *M. seminigra fuscopilosa* (u indicates U-shaped chromosome) **b**; metaphase of female *M. scutellaris* stained with DAPI **c**; metaphase of male *M. capixaba* stained with CMA_3 **d**. Arrowhead indicates CMA_3^+ region, * indicates interphasic nucleus; arrows indicate euchromatic associations. Scale bar = 5 μm.

cies of mammals. Our data for species of Group II clearly indicate that this pattern of distribution of heterochromatin, as reported in interphasic nuclei is also extended to other stages of the cell cycle (Fig. 5).

Another striking behavior of the chromatin of Group II species was its strong tendency for euchromatic associations (Fig. 2 and 5). As an apparent consequence of the spatial segregation of heterochromatin and euchromatin within the nucleus, we observed a close proximity among the euchromatic regions of the chromosomes in successive stages from late prophase to metaphase (Fig. 5). This association was observed either as an interchromosomal phenomenon when chromosomes were arranged as links of a chain or as an intrachromosomal event, which determined its "U" configuration (Fig. 2, 5b and 5c). Many hypotheses have been advanced to explain this chromosomal association, albeit involving heterochromatic regions (SUMNER 1990). In these species of *Melipona*, the nature of chromatin association seems related to euchromatic regions instead, and the conspicuous associations are provisionally explained by the rather restricted location of euchromatic regions isolated by vast extensions of heterochromatin.

Karyotypic evolution

Phylogenies based on morphologic data of *Melipona* are controversial. The genus has been considered either as the sister group of all Meliponini (MICHENER 2000) or alternatively, as the sister group of most Neotropical stingless bees (CAMARGO and PEDRO 1992). On a higher systematic level ROIG-ALSINA and MICHENER (1993) placed *Melipona* as the sister group of *Partamona* with *Apis* and *Bombus* as putative more inclusive sister groups. In *Apis* and *Partamona*, and in other genera of Meliponini, heterochromatin is predominantly located on one of the chromosome arms (POMPOLO 1992, 1994; HOSHIBA and IMAI 1993; POMPOLO and CAMPOS 1995; BRITO and POMPOLO 1997; BRITO et al. 1997; MENEZES 1997; MOREIRA and POMPOLO 1997; CAIXEIRO et al. 1998; ALVES and POMPOLO 1999; BRITO-RIBON et al. 1999a,b; CAIXEIRO and POMPOLO 1999; MAMPUMBU et al. 1999) fitting IMAI's minimum-interaction hypothesis (1986).

It has been postulated (KERR 1969) that Meliponini karyotypes evolved by alterations of ploidy levels. However, POMPOLO (1992, 1994) argued that the minimum interaction hypothesis (IMAI et al. 1986) was necessary and sufficient as an explanatory model of karyotypic evolution for the Meliponini. Accordingly, one should expect that ancestral species showed low chromosome numbers, which would increase by recurrent fissions, with a later increase of heterochromatin in one of the arms of the chromosomes. Although this pattern is observed in most genera of Meliponini, *Melipona* does not fit this model because its low chromosome number, and the position of the heterochromatin (at least in Group I species), which is pericentromeric or it is present on the short arm of the chromosomes. On the other hand, in species of Group II the heterochromatin is present along most of the chromosome length and the euchromatin is restricted to the distal end of the chromosomes.

To determine character polarity of heterochromatin contents within the genus, we considered as an outgroup the meliponine *Leurotrigona muelleri* (*Hypotrigona* sensu MICHENER 1990), $n = 8$, $2n = 16$ (POMPOLO and CAMPOS 1995). This species has a diploid number close to the species of *Melipona*, but its heterochromatin content and distribution resembles the ones observed in species of Group I and its chromatin does not show euchromatic associations. Based on these patterns of character distribution we hypothesized that high heterochromatin contents and unique chromatin (euchromatic) association, resulting in "U" or chain configuration are derived characters that characterize Group II species as a natural group or clade within *Melipona*. The increase in heterochromatin content may have evolved either via amplification of heterochromatic segments, or by heterochromatin addition. Within Meliponini, Group I does not display unique derived characters and it may be also evolutionarily more diverse, in contrast to the monophyletic Group II; mitochondrial DNA data also supports this hypothesis (Salomão unpubl.).

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REFERENCES

Almeida MG, (1981). Estudo sobre o número de cromossomos e contagem de espermatozóides na abelha Melipona scutellaris, Latreille,1811. Ciênc. Cult. 33: 539–542.

Alves VS and Pompolo SG, (1999). Análise citogenética comparativa de quatro gêneros de abelhas sem ferrão (Hymenoptera, Apidae, Meliponini). Genet. Mol. Bio. 22: 28.

Bella JL, Serrano L, Hewitt GM and Gonsálvez J, (1993). Heterochromatin heterogeneity and rapid divergence of the sex chromosomes in Chorthippus parallelus parallelus and C. perythropus (Orthoptera). Genome 36: 542–547.

Brito RM and Pompolo SG, (1997). C; G patterns and fluorochromes staining with DAPI and CMA_3 in Trigona spinipes (Jurine, 1807) (Hymenoptera, Apidae, Meliponinae). Rev. Bras. Gen. 20: 62.

Brito RM, Costa MA and Pompolo SG, (1997). Characterization and distribution of supernumerary chromosomes in 23 colonies of Partamona helleri (Hymenoptera, Apidae, Meliponinae). Braz. J. Gen. 20: 185–188.

Brito-Ribon RM, Pompolo SG, Martins MF, Barros EG and Sakamoto-Hojo ET, (1998). Estudo da origem de cromossomos supranumerários em Partamona helleri (Hymenoptera, Apidae) por meio de hibridação in situ fluorescente e coloração com fluorocromos. Anais do 3° Encontro sobre Abelhas 3: 213–218.

Brito-Ribon RM, Miyazawa CS and Pompolo SG, (1999a). First karyotype characterization of four species of Partamona (Friese, 1980) (Hymenoptera, Apidae, Meliponinae) in Mato Grosso State, Brazil. Cytobios 100: 19–26.

Brito-Ribon RM, França K and Miyazawa CS, (1999b). First cytogenetic data of eight stingless bee species (Hymenoptera, Apidae, Meliponinae) from cerrado areas of Mato Grosso State-Brazil. Gen. Mol. Biol. 22: 30.

Bruvo B, Plohl M and Ugarkovic D, (1995). Uniform distribution of satellite DNA variants on the chromosomes of tenebrionid species Alphitobius diaperinus and Tenebrio molitor. Hereditas 123: 69–75.

Caixeiro APA and Pompolo SG, (1999). Caracterização citogenética da heterocromatina constitutiva e sua impli-

cação na evolução do cariótipo do gênero Plebeia (Hymenoptera: Apidae, Meliponinae). Gen. Mol. Biol. 22: 31–32.

Caixeiro APA, Brito-Ribon RM and Pompolo SG, (1998). Cytogenetic study of Partamona peckolti by C, GTC, NOR, DA/DAPI and DA/CMA3 banding (Hymenoptera, Apidae, Meliponinae). Gen. Mol. Biol. 21: 48.

Camacho JPM, Viseras E, Navas J and Cabrero J, (1984). C-heterochromatin content of supranumerary chromosome segments of grasshoppers: detection of an euchromatic extra segment. Heredity 53: 167–175.

Camacho JPM, Cabrero J, Viseras E, López-León MD, Navas-Castilho J and Alche JP, (1991). G banding in two species of grasshoppers and its relationship to C, N and fluorescence banding techniques. Genome 34: 638–643.

Camargo JMF and Pedro SRM, (1992). Sistemática de Meliponinae (Hymenoptera, Apidae): sobre a polaridade e significado de alguns caracteres morfológicos. Naturalia Ed. Esp. 45–51.

Gomes LF, Brito RM, Pompolo SG, Campos LAO and Peruquetti RC, (1998). Karyotype and C- and G-banding patterns of Eufriesea violacea (Hymenoptera, Apidae, Euglossinae). Hereditas 128: 73–76.

Heard TA, (1999). The role of stingless bees in crop pollination. Annu. Rev. Entomol. 44: 183–206.

Hoshiba H, (1988). Karyological analysis of a stingless bee, Melipona favosa (Apidae, Hymenoptera). Cytologia 53: 153–156.

Hoshiba H and Imai HT, (1993). Chromosome evolution of bees and wasps (Hymenoptera, Apocrita) on the basis of C-banding pattern analyses. Jpn. J. Ent. 61: 465–492.

Howell WM and Black DA, (1980). Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014–1015.

Hsu TC, (1975). A possible function of constitutive heterochromatin: the bodyguard hypothesis. Genetics 79: 137–150.

Imai HT, Maruyama T, Gojobori T, Inoue Y and Crozier RH, (1986). Theoretical bases for karyotype evolution. The minimum-interaction hypothesis. Amer. Nat. 128: 900–920.

Imai HT, Taylor RW, Crosland MWJ and Crozier RH, (1988). Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. Jpn. J. Genet. 63: 159–185.

John B, King M, Schweizer D and Mendelak M, (1985). Equilocality of heterochromatin distribution and heterochromatin heterogeneity in acridid grasshoppers. Chromosoma 91: 185–200.

Juan C and Petitpierre E, (1989). C-banding and DNA content in seven species of Tenebrionidae (Coleoptera). Genome 32: 834–839.

Kerr WE, (1948). Estudos sobre o gênero Melipona. Anais da E. S. A. "Luiz de Queiroz" 5: 182–276.

Kerr WE, (1952). A variação do número de cromosomas na evolução dos Hymenoptera. Sci. Genet. 4: 182–190.

Kerr WE, (1969). Some aspects of the evolution of the social bees. Evol. Biol. 3: 119–175.

Kerr WE, (1972). Numbers of chromosomes in some species of bees. J. Kans. Entomol. Soc. 45: 11–122.

Kerr WE and Silveira ZW, (1972). Karyotype evolution of bees and corresponding taxonomic implications. Evolution 26: 197–202.

Kerr WE, Carvalho GA and Nascimento VA, (1996). Abelha Uruçu – Biologia, Manejo e Conservação. Fundação Acangaú, Belo Horizonte.

King M, (1980). C-banding studies on Australian hylid frogs: secondary constriction structure and the concept of euchromatin transformation. Chromosoma 80: 191–217.

Lorite P, Aránega AE, Luque F and Palomeque T, (1997). Analysis of the nucleolar organizing regions in the ant Tapinoma nigerrimum (Hymenoptera, Formicidae). Heredity 78: 578–582.

Maffei EMD, Pompolo SG, Silva-Junior JC, Caixeiro APA, Rocha MP and Dergam JA, (2001). Silver staining of nucleolar organizer regions (NORs) in some species of Hymenoptera (bees and parasitic wasp) and Coleoptera (lady-beetle). Cytobios 104: 119–125.

Mampumbu AR, Aleixo LA, Maffei EMD and Pompolo SG, (1999). Análise da estrutura cariotípica de Friesella schrottkyi (Hymenoptera, Apidae, Meliponini), da região de Viçosa Minas Gerais. Gen. Mol. Biol. 22: 29.

Mandrioli M, Manicardi GC, Bizarro D and Bianchi U, (1999). NOR heteromorphism within a parthenogenetic lineage of the aphid Megoura viciae. Chrom. Res. 7: 157–162.

Martínez-Lage A, González-Tizóz A and Méndez J, (1994). Characterization of different chromatin types in Mytilus galloprovincialis L. after C-banding, fluorochrome and restriction endonuclease treatments. Heredity 72: 242–249.

Menezes MBF, (1997). Caracterização citogenética e análise da heterocromatina constitutiva em Tetragonisca angustula angustula Latreille, 1811 (Hymenoptera, Apidae, Meliponinae). Thesis for the degree of Magister os Science, Universidade Federal de Viçosa. Viçosa. Brazil.

Michener CD, (1990). Classification of the Apidae (Hymenoptera). Univers. Kansas Scien. Bul. 54: 75–164.

Michener CD, (2000). The bees of the world. The Johns Hopkins University Press, Baltimore and London.

Moreira CMLC and Pompolo SG, (1997). Caracterização cariotípica de espécies do gênero Frieseomelitta Ihering, 1912 (Hymenoptera, Apoidae, Meliponinae). Rev. Brasil. Gen. 20: 61.

Panzera FCO, Alvarez F, Sanchez-Rufas J, Pérez R, Suja JA, Scvortzoff E, Dujardin JP, Estramil E and Salvatella R, (1992). C-heterochromatin polymorphism in holocentric chromosomes of Triatoma infestans (Hemiptera: Reduviidae). Genome 35: 1068–1074.

Pardue ML and Gall JG, (1970). Chromosomal localization of mouse satellite DNA. Science 168: 1356–1358.

Plohl M, Lucijanic-Justic V and Ugarkovic D, (1993). Satellite DNA and heterochromatin of the flour beetle Tribolium confusum. Genome 36: 467–475.

Pompolo SG, (1992). Estudos citogenéticos em Meliponinae. Naturalia Ed. Esp. 62–66.

Pompolo SG, (1994). Análise dos cariótipos de 19 gêneros de abelhas da subfamília Meliponinae. Anais do 1° Encontro sobre Abelhas 1: 143–146.

Pompolo SG and Campos LAO, (1995). Karyotypes of two species of stingless bees, Leurotrigona muelleri and Leurotrigona pusilla (Hymenoptera, Meliponinae). Rev. Brasil. Genet. 18: 181–184.

Rocchi A, (1982). On the heterogeneity of heterochromatin. Caryologia 35: 169–189.

Rocha MP and Pompolo SG, (1998). Karyotypes and heterochromatin variation (C-bands) in Melipona species (Hymenoptera, Apidae, Meliponinae). Gen. Mol. Biol. 21: 41–45.

Roig-Alsina A and Michener CD, (1993). Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). Univ. Kansas Sci. Bul. 55: 123–173.

Schmid M, (1980). Chromosome banding in Amphibia IV. Differentiation of GC- and AT-rich chromosome regions in Anura. Chromosoma 77: 83–103.

Schmid M, Steinlein C, Feichtinger W, Almeida CG and Duellman WE, (1988). Chromosome banding in amphibia. XIII Sex chromosomes, heterochromatin and meiosis in marsupial frogs (Anura, Hylidae). Chromosoma 97: 33–42.

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

Sentis C, Santos J and Fernandez-Piqueras J, (1986). C-heterochromatin polymorphism in Baetica ustulata: intraindividual variation and fluorescence banding patterns. Chromosoma 94: 65–70.

Sumner AT, (1990). Chromosome banding. Unwin Hyman, London.

Tambasco AJ, Giannoni MA and Moreira LMA, (1979). Analyses of G-bands in chromosomes of the Melipona quadrifasciata anthidioides Lepeletier (Hymenoptera, Apidae, Meliponinae). Cytologia 44: 21–27.

Viegas-Péquignot E, (1992). In situ hybridization to chromosomes with biotinylated probes. In: In situ hybridization: a practical approach (ed. D Wilkison). Oxford University Press, p. 137–158.

Vitturi R, Colomba MS, Caputo V, Sparacio I and Barbieri R, (1997). High heterochromatin content in somatic chromosomes of two unrelated species of diplopoda (Myriapoda). Chromos. Res. 5: 407–412.

Warchalowska-Sliwa E and Bugrov AG, (1997). C-heterochromatin variation in Deracantha onos (Pall.) (Deracanthini, Bradyporinae, Tettignoniidae, Orthoptera). Cytologia 62: 7–12.