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Original article

Genome size diversity in stingless bees (Hymenoptera: Apidae, Meliponini)

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Abstract – The first studies on the genome size of stingless bee species showed a range from 0.27 pg (*Melipona subnitida* and *Melipona quadrifasciata*) to 1.38 pg (*Melipona capixaba*). Considering this variation, we quantified the DNA content of 26 species of Meliponini, in order to provide input for future comparative studies in this tribe. Haploid genome size (1C) estimates, using flow cytometry analyses (FCM), ranged from 0.26±0.003 pg (*Paratrigona subnuda*) to 0.98±0.023 pg (*Melipona flavolineata*), with an average of 0.54±0.17 pg. FCM analyses also demonstrated a small difference in the haploid genome size between males and females of the same species, with the males generally having a smaller genome than females. Our data also evidenciated that variations in the genome size of stingless bees do not correlate with changes in chromosome number and that in some genera the DNA content is more variable than in others.

C-value / DNA content / flow cytometry / meliponines

1. INTRODUCTION

The stingless bees (Meliponini) exhibit a pantropical distribution, covering most of the Neotropical regions. More than 500 species and 50 genera have been described, with more than 300 species being found in Central and South America (Michener, 2000).

The stingless bees differ from other bees by particular characteristics such as atrophy of the sting and reduced wing venation (Michener, 2000). They also are distinguished by their great diversity of social behavior and its important role in pollinating of native species.

The first studies on the genome size of these species have been published recently by Lopes et al. (2009) and Tavares et al. (2010a, b). In total, the genome size of 19 stingless bee species was estimated, most of them belonging to the genus *Melipona*. At the same time, the genomes of 89 other Hymenoptera species were quantified (Ardila-Garcia et al., 2010). These studies helped to expand the genome size Hymenoptera dataset, which until then had data for only 50 species.

Together, these results showed a range in the haploid genome size (1C) of Hymenoptera from 0.10 pg (*Aphidius colemani*) to 1.38 pg (*Melipona capixaba*). Considering this variation it is evident that additional information can provide important data on the evolution of genome size of this order and their associations with rates of development, body size, and levels of sociality. Genome size determination can also guide the choice of genomes to be sequenced (Hardie et al., 2002; Gregory, 2005; Geraci et al., 2007). The aim of this study therefore was to quantify the genome size of 26 species of Meliponini, in order to

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provide input for future comparative studies in this tribe and in the order Hymenoptera.

2. MATERIALS AND METHODS

2.1. Biological material

The present study analyzed 26 species of stingless bees, belonging to 15 genera. This material was kindly provided by researchers from different educational institutions or bee keepers that sent us combs containing individuals at different developmental stages. Species were identified by Dr. Lucio Antonio de Oliveira Campos (Universidade Federal de Viçosa—UFV).

2.2. Flow cytometry analysis

The flow cytometry (FCM) analyses were carried out at the Laboratory of Cytogenetics and Cytometry, Department of General Biology, UFV. Analyses were performed using the cerebral ganglion of female pupae. In a complementary way, male pupae in the same stage of development were analyzed when present in the combs. *Melipona quadrifasciata* diploid males (see Table I) were obtained from inbred colonies originated from a brother–sister mating and identified by cytogenetic analysis according to Imai et al. (1988).

The nuclear DNA content of pupae was measured using as internal standard the C DNA content value of a female of *Scaptotrigona xantotricha* as described by Lopes et al. (2009). For preparation of FCM nuclei suspensions, brain ganglion nuclei of the standard and sample were excised in physiologic solution (0.155 mM NaCl). The materials were simultaneously crushed 10 times with a pestle in a tissue grinder (Kontes Glass Company®) with 100 μL OTTO-I lysis buffer (Otto, 1990) containing 0.1 M citric acid (Merck®), 0.5 % Tween 20 (Merck®), and 50 μg mL^{-1} RNAse (Sigma-Aldrich®), pH=2.3. The suspension was adjusted to 1.0 mL with the same buffer, filtered through 30 μm nylon mesh (Partec®) and centrifuged at 100×*g* in microcentrifuge tubes for 5 min. The pellet was then incubated for 10 min in 100 μL OTTO-I lysis 100 buffer and stained with 1.5 mL OTTO-I:OTTO-II (1:2) solution (30 min; Loureiro et al., 2006a, b) supplemented with 75 μM propidium iodide (PI Sigma®—excitation/emission wavelengths, 480–575/550–740 nm; Shapiro, 2003) and 50 μg mL^{-1} RNAse (Sigma-Aldrich®), pH=7.8. The nuclear suspension was filtered through 20 μm diameter mesh nylon filter (Partec®) and maintained in the dark for 5–40 min. The suspension was analyzed with a Partec PAS® flow cytometer (Partec®) equipped with a Laser source (488 nm). The equipment was calibrated for linearity and aligned with microbeads and standard solutions according to the manufacturer's recommendations. FlowMax® software (Partec®) was used for data analyses. Three independent replications were done, and histograms with coefficient of variation above 5 % were rejected.

The mean genome size (in picograms) of each sample was measured according to the formula adapted from Dolezel and Bartos (2005) and will be presented here as C-values in picograms and mega-base pairs (1 pg=978 Mbp; Dolezel et al., 2003). A standard Student's *t* test ($P<0.05$), as implemented in the GENES program (Cruz, 2011), was used to determine significant differences in genome size between sexes, for the 20 species for which it was possible to obtain estimates of genome size for both sexes (Table I).

3. RESULTS

Genome size (1C) estimates for the analyzed females ranged from 0.26±0.003 pg (*Paratrigona subnuda*) to 0.98±0.023 pg (*Melipona flavolineata*), with an average of 0.54±0.17 pg (Table I). Analyses also showed that the genome size of haploid males was slightly smaller than the 1C content of diploid females (Table I). The results of the *t* standard test showed statistically significant differences ($P<0.05$) for *Celetrigona longicornis*, *Scaptotrigona bipunctata*, *Nanotrigona testaceicornis*, *Partamona rustica*, *Melipona mondury*, *Melipona quinquefasciata*, *Melipona asilvai*, and *Melipona mandacaia*. Diploid males of *M. quadrifasciata*, however, presented exactly the same genome size of females. Variations in the genome size of *Frieseomelita varia* and *Plebeia droryana* according to their geographical origins were also observed. Samples of *F. varia* from Ribeirão Preto and Bocaiúva, for instance, showed genome sizes of 0.46±0.000 and 0.48±0.004 pg, respectively, while the genome size of *P. droryana* samples

Table 1. Haploid genome size estimates for 26 stingless bee species, their origin, number of individuals analyzed (N) and chromosome number.

Species	Origin	Haploid genome size (pg)±SE (Mbp)		*N*	Chromosome number
		Female	Male		
Friesella schrottkyi	Pedregulho/SP	0.44±0.007 (430.32)	0.42±0.005 (410.76)	3	$2n=34^{a}$
Frieseomelita varia	Ribeirão Preto/SP	0.46±0.000 (449.88)	–	3	$2n=30^{a}$
F. varia	Bocaiúva/MG	0.48±0.004 (469.44)	0.47±0.005 (459.66)	3	$2n=30^{a}$
Frieseomelita sp.	Jequié/BA	0.44±0.006 (430.32)	–	3	ND
Celetrigona longicornis	Nova Xavantina/AC	0.49±0.004 (479.22)	0.46±0.005 (449.88)	3	$n=15^{a}$
Cephalotrigona sp.	Urbano Santos/MA	0.55±0.007 (537.90)	–	3	ND
Scaptotrigona bipunctata	Ribeirão Preto/SP	0.44±0.005 (430.32)	0.40±0.010 (391.20)	3	ND
S. depilis	Ribeirão Preto/SP	0.41±0.008 (400.98)	0.39±0.008 (381.42)	3	$2n=34^{a}$
S. tubiba	Urbano Santos/MA	0.45±0.006 (440.10)	–	3	ND
S. xantotricha[b]	Viçosa/MG	0.44±0.004 (430.3)	0.42±0.005 (410.76)	3	$2n=34^{a}$
Nanotrigona testaceicornis	Viçosa/MG	0.53±0.009 (518.34)	0.49±0.004 (479.22)	3	$2n=34^{a}$
Nanotrigona sp.	Xapuri/AC	0.45±0.025 (440.10)	–	3	ND
Paratrigona subnuda	Viçosa/MG	0.26±0.003 (254.28)	–	3	$2n=34^{a}$
Trigona spinipes	Viçosa/MG	0.44±0.010 (430.32)	0.42±0.007 (410.76)	3	$2n=34^{a}$
T. fulviventris	Urbano Santos/MA	0.70±0.013 (684.60)	0.68±0.012 (665.04)	3	$2n=32^{c}$
T. pallens	Urbano Santos/MA	0.81±0.027 (792.18)		3	$2n=34^{d}$
Plebeia droryana	Viçosa/MG	0.49±0.009 (479.22)	0.48±0.008 (469.44)	3	$2n=34^{a}$
P. droryana	Ribeirão Preto/SP	0.52±0.007 (508.56)	–	3	$2n=34^{a}$
P. lucii	Viçosa/MG	0.43±0.003 (420.54)	–	3	$2n=34^{e}$
Plebeia sp.	Xapuri/AC	0.44±0.005 (430.32)	0.43±0.007 (420.54)	3	ND
Tetragonisca angustula	Viçosa/MG	0.90±0.015 (880.20)	–	3	$2n=34^{a}$
Partamona helleri	Viçosa/MG	0.55±0.006 (537.90)	–	3	$2n=34^{a}$
P. rustica	Januária/MG	0.59±0.010 (577.02)	0.57±0.007 (557.46)	3	$2n=34^{f}$
P. chapadicola	Urbano Santos/MA	0.63±0.027 (616.14)	–	3	$2n=34^{g}$
Scaura latitarsis	Ribeirão Preto/SP	0.44±0.005 (430.32)	0.43±0.013 (420.54)	3	$2n=34^{a}$
Schwarziana sp.	Domingos Martins/ES	0.65±0.008 (635.70)	–	3	ND
Leurotrigona muelleri	Ribeirão Preto/SP	0.32±0.003 (312.96)	0.31±0.007 (303.18)	3	$2n=16^{a}$
Melipona fasciculata	Urbano Santos/MA	0.82±0.005 (801.96)	–	3	$2n=18^{h}$
M. flavolineata	Urbano Santos/MA	0.98±0.023 (958.44)	–	3	$2n=18^{h}$
M. mondury[b]	Itamarandiba/MG	0.95±0.014 (929.10)	0.94±0.010 (919.32)	3	$2n=18^{i}$
M. quinquefasciata[b]	CE	0.70±0.011(684.60)	0.67±0.011 (655.26)	3	$2n=20+Bs^{a}$
M. asilvai[b]	São João Sabugi/PB	0.29±0.003 (283.62)	0.26±0.007 (254.28)	3	$2n=18^{a}$
M. bicolor[b]	Viçosa/MG	0.28±0.004 (273.84)	0.25±0.002 (229.83)	3	$2n=18^{a}$
M. mandacaia[b]	Uauá/BA	0.35±0.004 (342.30)	0.33±0.007 (322.74)	3	$2n=18^{a}$
M. quadrifasciata[b]	Viçosa/MG	0.27±0.002 (264.06)	0.25±0.003 (229.83—male, *n*) and 0.27±0.005 (264.06—male, 2*n*)	3	$2n=18$; $n=9^{a}$

ND not determined

[a] Revision in Rocha et al. (2003)

[b] Female DNA content estimated previously by Tavares et al. (2010a, b)

[c] Domingues et al. (2005)

[d] Praça-Fontes et al. (2010)

[e] Caixeiro (1998, referred as *Plebeia* sp2)

[f] Fernandes et al. (2012) personal communication

[g] Fernandes et al. (2009)

[h] Lopes et al. (2011)

[i] Lopes et al. (2009)

from Viçosa and Ribeirão Preto was 0.49±0.009 and 0.52±0.007 pg, respectively (Table I).

The cytometry analyses also demonstrated that in some bee genera, the genome size is more variable than in others. However, there are no defined limits for genome size among genera (Table I).

4. DISCUSSION

The data obtained in the present study substantially extend knowledge about stingless bees' genome size. Their mean haploid genome size (0.54 pg±0.17) is within the range previously described for *S. xantotricha* (Lopes et al., 2009), 17 *Melipona* (Lopes et al., 2009; Tavares et al., 2010a), and one *Lestrimelitta* species (Tavares et al., 2010b). It should be noted, however, that when all the 45 stingless bees that have had their genome size estimated are analyzed together, their average genome size rises to 0.61±0.27 pg. The average genome size of stingless bees, therefore, varies about five times and is considerably higher than the average found for the other Apidae or other Hymenoptera non-Apidae analyzed so far (0.40±0.18 and 0.37±0.18 pg, respectively; Gregory, 2011). In any case, it remains within the limit of 2 pg proposed for holometabolous insects (Gregory, 2002). However, considering the lower limit, other *Paratrigona* species need to have their genome size estimated in order to confirm if the small genome size of *P. subnuda* is an exception or a characteristic of the entire genus.

A possible explanation for this variation is the existence of some *Melipona* species with a high genome size (range 0.7–1.38 pg; Tavares et al., 2010a). However, relatively large genomes are not unique to *Melipona* once *Partamona chapadicola*, *Schwarziana* sp., *Trigona fulviventris*, *Trigona pallens*, and *Tetragonisca angustula* also have genomes with more than 0.63 pg (range 0.63–0.90 pg; Table I). Variations caused by different numbers of pseudogenes, transposons, intergenic sequences, introns, and microsatellites could also explain the differences found. Nevertheless, as the genome of any stingless bee has been completely sequenced, such information is not yet available.

In this sense, our data indicate that *M. quadrifasciata* and *M. bicolor* (which have been widely studied from a biological standpoint), and *Melipona subnitida*, *Melipona marginata*, and *P. subnuda* are the stingless bee species best suited to have their genomes completely sequenced, once they have small genome sizes. Data on the genome organization of these species may be used in future studies on the organization and evolution of Hymenoptera genomes.

Male ganglion cerebral cells of eight stingless bee species demonstrated a smaller genome than the haploid genome size from females. Lopes et al. (2009) had already observed this when analyzing the genome size of males and females of *S. xantotricha*. Geraci et al. (2007) also observed significant differences between the genome size of males and females of the ticks *Amblyomma americanum*, *Amblyomma cajannense*, *Amblyomma maculatum*, and *Dermacentor andersoni*. In these cases, the differences were justified by the XX:XO sex determination system of these species. Additionally, in the first three species, the presence of a relatively large X chromosome compared to autosomes has already been detected. This explanation, however, does not seem to apply to stingless bees, where males are haploid and females are diploid. On the other hand, for some stingless bee species analyzed herein, the differences in the haploid genome size of males and females were not significant, as has been reported for *Apis mellifera* (Honeybee Genome Sequencing Consortium, 2006).

It is worth noting that, as expected, diploid males of *M. quadrifasciata* had exactly the same genome size than females of this species. This result, together with data from *Lestrimelitta* sp. (Tavares et al., 2010b), suggests that the technique of flow cytometry can be used to distinguish between haploid and diploid stingless bees males.

Variations in the genome size of stingless bees do not correlated with changes in chromosome number. For example, *Leurotrigona muelleri* has 0.32 pg of DNA and $2n=16$ chromosomes, while

the estimated genome of *P. subnuda* is 0.26 pg and this species has $2n=34$ chromosomes. Likewise, the genome size of 18 species of *Melipona* with $2n=18$ chromosomes ranged from 0.27 to 1.38 pg (Tavares et al., 2010a; this study). However, some species of stingless bees, such as *Partamona helleri* (Costa et al., 1992), *M. quinquefasciata* (Rocha, 2002), *Melipona rufiventris* (Lopes et al., 2008), and *Partamona cupira* (Marthe et al., 2010) may have B chromosomes and their presence can interfere in the estimation of genome size of species (unpublished data).

Although variations in the genome sizes according to the geographical origin of samples, as observed in the present study for *F. varia* and *P. droryana*, had already been observed in ants (Tsutsui et al., 2008, Ardila-Garcia et al., 2010), wasps, and bees (Ardila-Garcia et al., 2010), its causes are not clear. As discussed above, it can be correlated with differences in the amount of heterochromatin between populations. Thus, the FCM technique could be evidenciating inter-population differences in the amount of heterochromatin in these species. The association of flow cytometry with cytogenetic techniques that permit monitor the condensation degree of chromosomes might show if this correlation is also observed in other stingless bees genera.

The FCM analysis also showed that, while the genomes size of *Frieseomelita* and *Scaptotrigona* showed little variation, the values found for the three species of the genus *Trigona* ranged from 0.44 pg (*T. spinipes*) to 0.70 pg (*T. fulviventris*) and 0.81 pg (*T. pallens*). This high variation corroborates the phylogenetic relationships between these three species, because as evidenced in the analysis conducted by Rasmussen and Camargo (2008) and Rasmussen and Cameron (2010), *T. spinipes* and *T. fulviventris*/*T. pallens* are in different clades.

On the other hand, our results differ from the phylogenetic proposal of Rasmussen and Cameron (2010) in relation to the four subgenera of *Melipona*. Considering the genome size, it is possible to verify that *Melipona*/*Eomelipona* and *Melikerria*/*Michmelia* form two distinct groups. However, according to Rasmussen and Cameron (2010), despite the four genera form a single clade, *Eomelipona* and *Michmelia* are the two closer subgenera.

In summary, the data obtained in this study indicate that the genome size of the stingless bees differs significantly between species. Therefore, in more detailed studies involving other genera, a greater number of species and species with different levels of sociality may reveal important features about the evolution of the genome of this group of insects and if there are precise limits between the genome sizes of different genera of stingless bees.

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Diversité dans la taille du génome chez les abeilles sans aiguillon (Hymenoptera: Apidae, Meliponini)

Valeur C / contenu de l'ADN / cytométrie en flux / meliponines

Variation der Genomgrösse bei Stachellosen Bienen (Hymenoptera: Apidae, Meliponini)

C-Wert / DNA-Gehalt / Durchflusszytometrie / Meliponinen

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