Detection of diploid males in a natural colony of the cleptobiotic bee *Lestrimelitta* sp (Hymenoptera, Apidae)

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Abstract

When working at quantifying the genome size of stingless bees, it was observed that males of *Lestrimelitta* sp possessed the same amount of nuclear DNA as the females. Thus, we used flow cytometry (FCM) and cytogenetic analysis to confirm the ploidy of these individuals. The males analyzed proved to be diploid, since, through cytometric analysis, it was demonstrated that the mean genome size of both males and females was the same (C = 0.463 pg), and, furthermore, cytogenetic analysis demonstrated that both had 2n = 28 chromosomes.

Key words: cytogenetic, flow cytometry, genome size, karyotype, stingless bee.

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In Hymenoptera, sex is determined by haplodiploidy and in several species it is regulated by a single, multiallelic sex locus (sl-CSD) (Beye et al., 2003). In this model, hemizygous individuals will become males (haploid drones), whereas diploid individuals that are heterozygous at the sex locus will develop into females and those homozygous into diploid males.

In general, diploid males are less viable (Whiting, 1943; Rothenbuhler et al., 1968), and either sterile (El Agoze et al., 1994; Duchateau and Marien, 1995; Krieger et al., 1999) or produce diploid sperm which leads to triploid offspring (Naito and Susuki, 1991; Liebert et al., 2005; de Boer et al., 2007), thereby imposing a genetic load on the population as a whole. In only one of the species studied so far, *Euodynerus foraminatus*, fertility is normal in diploid males, with the production of diploid, rather than triploid female offspring (Cowan and Stahlhut, 2004).

Diploid males have been detected in more than 60 species of Hymenoptera, this including several species of bees (both social and solitary), wasps, ants, sawflies and parasitoids. Among the stingless bees, they have been observed only in *Melipona compressipes*, *M. quadridifaciata*, *Scaptotrigona postica*, *Trigona carbonaria* and *Tetragona quadrangula* (van Wilgenburg et al., 2006, Heimpel and de Boer, 2008), but not so in the genus *Lestrimelitta*.

**Lestrimelitta** is an essentially cleptobiotic (robber) stingless bee that exploits the resources of other bees by stealing food from their nests, instead of collecting it from flowers (Sakagami and Laroca, 1963; Bego et al., 1991; Sakagami et al., 1993). The genus occurs in the Neotropical region (Michener, 2000), and is represented in Brazil by at least fourteen species (Marchi and Melo, 2006).

When dealing with the quantification of genome size in stingless bees, it was noted that, in a colony of *Lestrimelitta* sp obtained in Domingos Martins/Espírito Santo (20°21’48” S; 40°39’33” W), nuclear DNA content proved to be the same in both males and females. As diploid males had not been previously noted in this species, individual ploidy was thereupon confirmed by flow cytometry (FCM) and cytogenetic analysis.

For the FCM analysis, the nuclear DNA content of *Lestrimelitta* sp male and female larvae was measured by using the C DNA content (0.42 pg) of *Scaptotrigona xantotricha* as internal standard, as described by Lopes et al. (2009). Brain ganglion nuclei of the standard and sample were excised in physiological saline solution (0.155 mM NaCl). The material was simultaneously crushed 10 times with a pestle in a tissue grinder (Kontes Glass Company®, 100 g in microcentrifuge tubes for 5 min.

The pellet was then incubated for 10 min in 100 µL of OTTO-I lysis buffer (Otto, 1999) containing 0.1 M citric acid (Merck), 0.5% Tween 20 (Merck) and 50 µg mL⁻¹ of RNase (Sigma-Aldrich), pH 2.3. The suspension was adjusted to 1.0 mL with the same buffer, filtered through a 30 µm nylon mesh (Partec) and centrifuged at 100 g in microcentrifuge tubes for 5 min.

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50 µg mL⁻¹ of RNase, pH = 7.8. The nuclear suspension was filtered through a 20 µm diameter mesh nylon filter and maintained in the dark for 5-40 min.

Three independent replicates of each suspension were analyzed with a Partec PAS flow cytometer (Partec) equipped with a Laser source (488 nm).

The mean genome size (pg) of each bee sample was measured according to a formula adapted from Dolezel and Bartos (2005).

For cytogenetic analysis, metaphase chromosomes were obtained from cerebral ganglia and testes of male larvae, and from cerebral ganglia of females in the final defecation stage (Imai et al., 1988). On an average, 5 females and 15 males, with ten metaphases per individual, were analyzed. Conventional Giemsa staining, using a 0.06 M Sörensen buffer, pH 6.8, was carried out according to Rocha and Pompolo (1998).

A 12-bit CoolSNAP-Pro cf (Roper Scientific) video camera, assembled on an OlympusTM BX-60 microscope with a 100x objective, was used for capturing chromosome images. The frame was digitized using an Image Pro-Plus analysis system (Media Cybernetics). A Power Macintosh G4 computer was employed for image analysis, with freely available Image SXM software (Barrett, 2002). This is a spin-off of the public domain image analysis application NIH Image which was developed by Rasband (1998). The karyotype was mounted by pairing chromosomes in the order of decreasing size.

Cytometry analysis of nuclei suspensions stained with PI demonstrated that the mean genome size of both males and females was the same (C = 0.463 pg) (Table 1 and Figures 1a and 1c), thereby indicating that the males were diploid.

Cytological analysis confirmed female and male chromosome content to be 2n = 28 (Figures 1b and 1d), as already described for Lestrimelitta limao females (Rocha et al., 2003). Neither cytometry nor cytogenetic analysis revealed haploid males among those analyzed.

The presence of diploid males is likely to generate high fitness costs for individual colonies and their queens, since there is a potential reduction in the proportion of workers performing essential tasks for colony survival (Green and Oldroyd, 2002). In colonies of stingless bees, workers construct and mass provision the cells prior to ovipositing. Thereafter, the queen lays her eggs in the cells, which the workers then seal (Sakagami, 1982). This prevents the detection and early removal of diploid males. In fact, it was noted that diploid males of Lestrimelitta sp presented normal viability in the larval and pupa phases, and fully developed into imagos. Nevertheless, several workers were seen attacking young diploid males inside the colony. Furthermore, the colony was weak, presenting several brood cells with dead progeny, with numerous mites attacking the larvae. This colony perished only a few days after being opened in the laboratory. Likewise, in colonies of Bombus atratus (Plowright and Pallet, 1979) and Solenopsis invicta (Ross and Fletcher, 1986), the production of diploid males also retarded colony growth, with consequential high mortality.

Diploid male production has been attributed to habitat fragmentation, the loss of sex allele diversity by drift in small, isolated populations, and the mating of parents sharing a sex allele in common, i.e., matched matings (revision in Cowan and Stahlhut, 2004). For Lestrimelitta in particular, the active human destruction of its colonies, in order to countering pillage of other stingless bee colonies, could have reduced species population size, thus favoring in-

![Image](image-url)

**Table 1** - Estimation of genome size of cerebral ganglia of Lestrimelitta sp.

|                     | Mean genome size (1C; pg) |
|---------------------|--------------------------|
|                     | *R1 | *R2 | *R3 | Mean ± SD  |
| Lestrimelitta sp (diploid males) | 0.460 | 0.465 | 0.465 | 0.463 ± 0.003 |
| Lestrimelitta sp (females) | 0.455 | 0.465 | 0.470 | 0.463 ± 0.008 |

*R1, *R2 and *R3: independent replicates.
breeding, with diploid males as the possible outcome of matched mating. Consequently, since Lestrimelitta females mate with a single male (Peters et al., 1999), it may be inferred that sex in Lestrimelitta is controlled by a single multiple-allelic locus.

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Internet Resources

Rasband W (1998) NIH Image is a public domain program developed at the U.S. National Institutes of Health. http://rsb.info.nih.gov/nih-image (October 19, 2009).

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