Sociobiology 61(3): 313-317 (September 2014) DOI: 10.13102/sociobiology.v61i3.313-317

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

Genetic Variability of Stingless Bees *Melipona mondury* Smith and *Melipona quadrifasciata* Lepeletier (Hymenoptera: Apidae) from a Meliponary

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Article History

Edited by
Astrid Kleinert, USP, Brazil
Received 13 March 2014
Initial acceptance 24 April 2014
Final acceptance 26 June 2014

Keywords
conservation, heterospecific primers, Meliponini, microsatellites

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Abstract

The species of stingless bees *Melipona mondury* Smith and *Melipona quadrifasciata* Lepeletier are native to the Atlantic Forest. These species are sensitive to environmental changes and due to habitat loss they are endangered in several Brazilian states. This study aimed to evaluate the genetic variability of populations of these two species at the meliponary of the Regional University of Blumenau through the use of heterospecific microsatellite primers. We collected one worker from 19 colonies of *M. mondury* and from 25 colonies of *M. quadrifasciata*. We found low levels of genetic variability for both species, which may be explained by queen philopatry, intraspecific reproductive parasitism, and/or artificial maintenance of hives. If natural populations of these species are also presenting low genetic variability they might be endangered.

Introduction

The strictly Neotropical stingless bee genus *Melipona* (Hymenoptera: Apidae) comprises more than sixty species in Brazil (Camargo & Pedro, 2013) and is one of the most important insects for pollination in natural and in cultivated areas (Heard, 1999; Slaa et al., 2006). The species *Melipona* (*Michmelia*) *mondury* Smith, 1863 inhabits the Atlantic Forest biome, from Bahia to Rio Grande do Sul states (Camargo & Pedro, 2013). The species *Melipona* (*Melipona*) *quadrifasciata* Lepeletier, 1836 has a wide distribution in southern and southeastern Brazil (Camargo & Pedro, 2013) where it is widely cultivated and especially valued in tomatoes production (Santos et al., 2009; Sarto et al., 2005). In addition, its propolis has medicinal properties (Mercês et al., 2013). Both species are endangered in several Brazilian states (Machado et al., 1998; Marques et al., 2002; Mikich & Bérnils, 2004; Santa Catarina, 2011) due to habitat loss caused by deforestation (Brosi et al., 2007; Brown & Oliveira, 2014).

Nowadays, the Atlantic Forest is extremely fragmented, and bee species suffer from the negative impacts of the interruption of gene flow and decrease in genetic diversity (Freiria et al., 2012). One way to prevent local extinction of these bees is maintaining hives in free-foraging wooden boxes in meliponaries (apiaries for stingless bees). Meliponiculture for crop pollination and for honey extraction has been an encouraging economic practice aligned with sustainable development and educational purposes in several countries (Cortopassi-Laurino et al., 2006).

However, little is known about the genetic variability of hives of native bees in meliponaries (Carvalho-Zilse et al., 2009). Such knowledge is essential for the development of conservation strategies and rational exploitation of native species (Cortopassi-Laurino et al., 2006; Alves et al., 2011). Our aim was to characterize the genetic variability of *M. mondury* and *M. quadrifasciata* maintained in the meliponary of the Regional University of Blumenau.

Material and Methods

We collected one worker from each of 19 colonies of *M. mondury* and from 25 colonies of *M. quadrifasciata*

Open access journal: http://periodicos.uefs.br/ojs/index.php/sociobiology
ISSN: 0361-6525

acquired from beekeepers from Vale do Itajaí region, in the Santa Catarina state, Brazil and maintained currently at the meliponary of the Regional University of Blumenau (mRUB) (Fig. 1) localized at 26° 54’ 21.81” S 49° 04’ 48.53” W (Fig.2). Colonies of *M. quadrifasciata* have been maintained at mRUB since 1998 and colonies of *M. mondury* since 2003. These hives were extracted directly from nature in geographically close cities and have not been artificially divided.

Figure 1. Meliponary of the Regional University of Blumenau (mRUB).

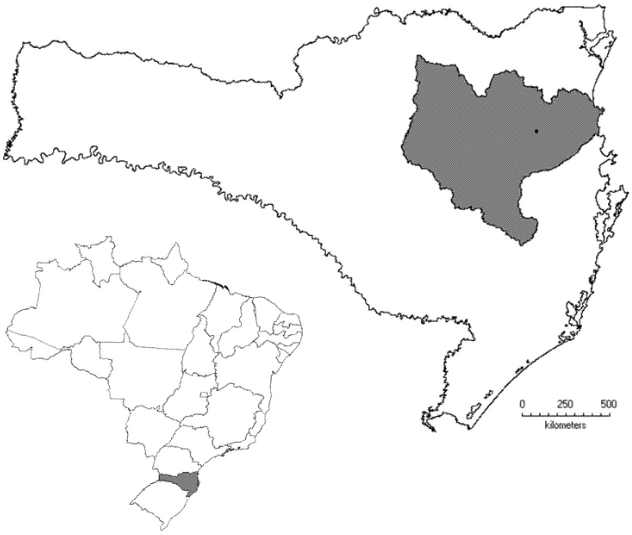

Figure 2. Localization of Santa Catarina state showing the Vale do Itajaí region. Meliponary localization (●).

DNA extraction was performed according to Anderson and Fuchs (1998). For both species, we used 10 microsatellite primer pairs developed for the species *M. bicolor* (Mbi11, Mbi28, Mbi32, Mbi33, Mbi88, Mbi218, Mbi233, Mbi254, Mbi259 and Mbi522) (Peters et al., 1998) and 11 primer pairs developed for *M. mondury* (Mmo03, Mmo06, Mmo08, Mmo10, Mmo11, Mmo15, Mmo19, Mmo20, Mmo21, Mmo22 and Mmo24) (Lopes et al., 2010b). PCR reactions and the annealing temperatures were performed according to Peters et al. (1998) for the Mbi primers and according to Lopes et al. (2010b) for the Mmo primers. PCR products were separated by electrophoresis in 12% polyacrylamide gels and stained with silver nitrate for visualization.

The program Arlequin v.3.5.1.3 (Excoffier & Lischer, 2010) was used to calculate allelic richness (Â), observed heterozygosity (H_O), expected heterozygosity (H_E), percentage of polymorphic loci (PPL) and F_{IS} with 10,000 permutations. Hardy-Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD) were computed using Genepop v.4.1.4 (Rousset, 2008). *P*-values were adjusted with Bonferroni correction (Rice, 1989). The frequencies of null alleles were computed using Cervus 3.0.6 (Kalinowski et al., 2007).

Results

Genetic variability was low for *M. mondury,* but no inbreeding was detected (Table 1). Loci Mbi254 and Mmo19 deviated from HWE even after Bonferroni correction ($P< 0.0167$). LD was detected between Mbi254 and Mmo19 after Bonferroni correction ($P= 0.0062$). The presence of null alleles with a frequency of 29% was found in the locus Mbi254, a heterospecific primer (Table S1). The loci Mmo19 and Mmo21, both specific primers for *M. mondury*, were not affected by null alleles (Table S1).

Melipona quadrifasciata also showed low genetic variability although higher than *M. mondury* (Table 1). Significant inbreeding was detected. Primers developed for *M. bicolor* were more polymorphic in *M. quadrifasciata* than those developed for *M. mondury* (Table S2). Deviation from HWE was detected for the loci Mbi218, Mbi233 and Mmo21 after Bonferroni correction ($P = 0.0000$). LD was not detected (all $P > 0.05$). For *M. quadrifasciata*, the frequency of null alleles was higher than 25% in the following heterospecific primer pairs: Mbi218, Mbi233, Mmo03, Mmo11 and Mmo21 (Table S2). The loci Mbi11, Mbi88, Mbi254 and Mbi259 were not affected by null alleles (Table S2).

Table 1. Genetic variability for *Melipona mondury* and *Melipona quadrifasciata* based on microsatellite data. Â: allelic richness; F_{IS}: inbreeding coefficient; H_E: expected heterozygosity; H_O: observed heterozygosity; N: sample size; PPL: percentage of polymorphic loci.

Species	N	Â	H_O	H_E	PPL	F_{IS}	*P-value*
M. mondury	19	1.60	0.105	0.102	15%	-0.00629	0.569307
M. quadrifasciata	25	2.22	0.129	0.189	50%	0.33493	0.000000

This article has supplementary material published and available on line

DOI: 10.13102/sociobiology.v61i3.313-317.s435

URL: http://periodicos.uefs.br/ojs/index.php/sociobiology/article/view/315

Discussion

Our results indicate low genetic variability in the populations of *M. mondury* and *M. quadrifasciata* maintained at the mRUB. Low genetic variability in native stingless bees is also documented in studies using molecular markers such as RAPD (Tavares et al., 2001), mitochondrial polymorphism (Brito et al., 2013), ISSR (Inter Single Sequence Repeats) (Nascimento et al., 2010; Miranda et al., 2012), and microsatellites (Francisco et al., 2006; Tavares et al., 2007; Carvalho-Zilse et al., 2009; Francini et al., 2009; Alves et al., 2011; Duarte et al., 2011).

A first explanation of this low variability could be the presence of null alleles. In this study, we found high values of null alleles only in one out of three polymorphic loci for *M. mondury* (Mbi254) and in five out of nine polymorphic loci for *M. quadrifasciata* (Mbi218, Mbi233, Mmo03, Mmo11 and Mmo21). In addition, null alleles might be responsible for the high number of monomorphic loci found in both species.

The use of heterospecific primers may also be related to low genetic diversity. In the stingless bee *Plebeia remota*, the same samples analyzed with heterospecific (Francisco et al., 2006) and specific (Francisco et al., 2013) primers showed high divergent values. The same result was found in *Melipona* bees (Lopes et al., 2010a). Cross-species amplification is one of the advantages of microsatellites, and low variability should always be interpreted with caution when using heterospecific primers. However, data obtained from other populations/species with the same primer pairs we used show higher levels of genetic diversity (Tables S3 and S4) suggesting that the low diversity we observed in this work are not due to heterospecific primers only.

Low variability may also be explained by the species natural biology. Queens of most stingless bee species mate with a single male (monandric) (Peters et al., 1999; Palmer et al., 2002) and are known to nidify near maternal nests (Nogueira-Neto, 1954). Their low dispersion increases genetic drift and inbreeding within sub-populations (Hartl & Clark, 2007). A special concern is the maintenance of alleles of the complementary sex determination system in small populations, because inbreeding can lead to the production of diploid males (Cook & Crozier, 1995; Zayed, 2009; Alves et al., 2011). For other stingless bee species, males are the dispersing sex (Cameron et al., 2004; Carvalho-Zilse & Kerr, 2004; Francisco et al., 2013), but no data is available for *M. mondury* and *M. quadrifasciata*. Nevertheless, the location of mRUB near forest fragments might allow the mating of queens from the meliponaries with males from native forests in the surroundings, decreasing their inbreeding probability.

Managed populations of local bees can be considered a reservoir of genetic diversity if they can interbreed with wild populations (Alves et al., 2011). However, to introduce bees from distant populations or another species that could hybridize with local populations might cause outbreeding depression (Lynch, 1991; Waser et al., 2000).

Recent data from Wenseleers et al. (2011) showed intraspecific reproductive parasitism in *M. scutellaris*. If we speculate that this behavior occurs in other *Melipona* species, colonies that were previously unrelated could become related if sibling queens take over these colonies. In these cases, genetic variability would decrease.

Another explanation to account for the low variability may be related to the artificial maintenance of hives through founder events and bottlenecks as already reported for *Apis mellifera* (Sheppard, 1988; Schiff & Sheppard, 1996; Moritz et al., 2007; Delaney et al., 2009; Jaffé et al., 2010; Meixner et al., 2010) and *M. scutellaris* (Carvalho-Zilse et al., 2009; Alves et al., 2011). However, despite low variability, populations can be successfully maintained if a strong care is dispensed over the nests (Alves et al., 2011).

If natural populations of these species are also presenting low genetic variability they might be endangered. Adaptation to environmental changes is dependent on the genetic variations that exist among members of a population. Without variation, populations are more prone to extinction. Therefore, the analysis of natural populations of these species in south Brazil is crucial. Detecting the male flight range is also important since they can prevent genetic isolation in fragmented populations. If meliponaries were able to assist in maintaining the genetic variability of natural populations, they could be used for research and reintroduction programs.

Acknowledgments

We thank the Universidade Regional de Blumenau-FURB for technical assistance. JR Koser was supported by a scholarship from Pipe/Art 170.

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