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A scientific note on the isolation and characterization of microsatellite loci of *Frieseomelitta varia* (Hymenoptera, Apidae, Meliponini)

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The environmental changes mediated directly or indirectly by humans represent a threat for many species. Most of the bee species build their nest in hollow of trees, so deforestation represents a great loss both in terms of number of species and in number of individuals (nests). The reduction of forest to fragments surrounded by urban areas may represent a severe barrier to gene flow leading to inbreeding and loss of genetic variability (Frankham et al. 2004). The assessment of the genetic structure of populations depends on the availability of reliable molecular markers. Studies aiming at characterizing stingless bee populations have been traditionally performed by using heterospecific microsatellite primers. It has been shown, however, that this strategy is not sensitive enough to detect polymorphism to perform robust population analysis (Francisco et al. 2006).

The genus *Frieseomelitta* has a large geographic distribution ranging from Mexico to southern Brazil. It consists of 16 valid species from which ten are present in Brazil (Silveira et al. 2002). The species *Frieseomelitta varia* (Meliponini) has drawn attention by the presence of several nests in buildings housing the University of São Paulo facilities (in Ribeirão Preto, SP). This area is surrounded by residential areas or sugar cane crop where nests have not been found. Several questions concerning inbreeding, gene flow, genetic variability can be addressed using this small population as a model. Thus, aiming to answer these questions we isolated and characterized microsatellite loci from *F. varia*'s genome, designed primers, and standardized PCR conditions.

Genomic DNA from a pool of ten females was obtained following a phenol/chloroform protocol. The microsatellite enriched genomic library was built following the procedure described by Billotte et al. (1999). Approximately 5 µg of genomic DNA were digested with 50 U of *Rsa*I. Fragments were linked to single-strand adaptors (10 µM each): *Rsa*21 (5'-CTCTTGCTTACGCG TGGACTA-3') and *Rsa*25 (5'-

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Table 1. Name of locus, flanking primers, optimal annealing temperatures (Ta), number of alleles (K), allele size range, observed (H_O) and expected (H_E) heterozygosity estimated from 19 unrelated workers.

Locus	Repeat motif	Primer sequence (5'-3')	Ta (°C)	K	Allele size range (bp)	H_O	H_E	Genbank accession number
Friv1	(GA) ₁₄	F: CACAGCCTAGCCGAATAC R: GGGAAACATCTTTGGTTCA	64	13	210–236	0.67	0.85	HM222393
Friv2	(GA) ₁₀	F: GACGAATTAATCAGTCTGGAG R: GCGAGACGAGCCTCTGTT	56	6	202–220	0.12	0.58	HM222394
Friv4	(TC) ₈	F: CGATGACACGAGCAITGACT R: TCCGTCAATGAATGCCAATAG	60	1	135	–	–	HM222395
Friv5	(AG) ₁₁	F: TAGACGTAGCCAGCGAAG R: TCGATCCGTCCTTTGGTAA	60	2	138–140	0.15	0.14	HM222396
Friv9	(TCAC) ₇ TCAT(TC) ₁₅ TG(TTTC) ₂	F: GTCGAGACGAACTTGGACGA R: CGCCGCTAGTTCCCAATAC	57.5	8	198–224	0.39	0.77	HM222397
Friv10	TT(TC) ₁₆	F: GGAACGAATCGATCTGCATTA R: TATCGATCCAGGATCCACT	63	7	276–302	0.50	0.77	HM222398
Friv11	(GT) ₃ GC(GT) ₁₀	F: AGAACGAGATCTGCCACGAC R: TCACGAAATCCGCTGAACAC	60	2	222–224	1.00	0.50	HM222399
Friv12	(CA) ₁₇	F: TTGCCAATCGGTCTAATCG R: GACCGTATCGTTGCAGAACT	60	7	172–192	0.78	0.76	HM222400
Friv14	(AG) ₁₇ (G) ₃ (AG)	F: TAATCCACGTGTGCGTGAT R: CAATCAGGACGACCCGTATG	61	11	140–190	0.11	0.83	HM222401
Friv15	(AG) ₁₈	F: CGTACATACATCTGCGAAGAACG R: GCTGGCTCCTCATACGAG	63	15	132–174	0.58	0.89	HM222402
Friv16	(AG) ₁₈	F: GCTTCGGGTGTCAGCTC R: GGCCCTCTCGTTTCCITACT	61	11	138–202	0.42	0.87	HM222403
Friv18	(AC) ₂ (GA) ₆ (AAAA)(GA) ₉	F: ATCCCAAGGACGTGCTAC R: CTCGTGTAGTCGGCTGAA	60	16	170–204	0.63	0.87	HM222404

TAGTCCACGC GTAAGCAAGAGCACA-3'). Those fragments were amplified by using the Rsa21 adaptor as primer. The PCR products were screened for repeat motifs through hybridization with four biotinylated probes [(CT)₈, (GT)₈, (GA)₈, and (AGA)₅]. This selection was mediated by metallic micro beads (Streptavidin MagneSphere®-Promega). The selected fragments were cloned into pGem®-T vector (Promega) and used to transform *E. coli* DH5α cells. Positive clones (71) were sequenced in an automated sequencer (ABI Prism 3100). The sequences were visualized and screened for the presence of microsatellites using the BioEdit v. 7.0 software (Hall 1999). Seventeen primer pairs were designed (see Table I) using the Primer3 software (Rozen and Skaletsky 1998).

A sample of nineteen unrelated individuals of *F. varia* collected in different Brazilian regions was analyzed. Moreover, for detection of cross-specific amplifications, one individual of each of the following species was analyzed: *Frieseomelitta flavicornis*, *Frieseomelitta doederleini*, *Frieseomelitta trichocerata*, *Paratrigona* sp., *Scaptotrigona postica*, and *Schwarziana quadripunctata*. PCR reactions were carried out in a final volume of 10 μL containing: 5.45 μL of deionized H₂O; 2 μL of betaine 5 M; 1X reaction buffer; 0.15 μL of MgCl₂ 50 mM; 0.2 μL of each primer 10 μM; 1 U of *Taq* polymerase (Invitrogen); and 1 μL of template DNA obtained through phenol/chlorophorm extraction from one

individual. Amplifications were performed in a GeneAmp® PCR System 9700 (Applied Biosystems) for 4 min at 94°C, followed by 35 cycles of 40 s at 94°C, 50 s at specific annealing temperature (see Table I), and 40 s at 72°C with a final elongation step of 10 min at 72°C. The amplified fragments were resolved by electrophoresis in 9% nondenaturing polyacrylamide gels and visualized by silver staining. A 100 bp DNA Ladder (Invitrogen) was used to estimate fragment sizes. Observed heterozygosity (*Ho*), expected heterozygosity (*He*), and number and frequency of alleles were calculated using FOFpop v.2.0 (Francisco 2009). As this study was performed with individuals sampled in geographically distant regions, tests for Hardy Weinberg and linkage disequilibrium were not applied.

The amplification analysis showed that 12 out of 17 primer pairs successfully amplified the respective loci, with 11 being polymorphic (Table I). So far, the average number of alleles found in this study (8.08) was the second highest in Meliponini. Higher values (8.94) were only described for the species *Tetragonisca angustula* (Brito et al. 2009). In contrast, studies applying ISSR methodology to isolate species specific microsatellite loci found only low levels of allelic richness, 3 and 2.63 for *Melipona mondury* and *Melipona rufiventris*, respectively (Lopes et al. 2009, 2010). Therefore, the methodology of an enriched genomic library seems to be more efficient, even though the low level of

Table II. Results from cross-species amplification tests: (+) amplification and (-) no amplification.

Locus	<i>Frieseomelitta flavicornis</i>	<i>Frieseomelitta doederleini</i>	<i>Frieseomelitta trichocerata</i>	<i>Paratrigona</i> sp.	<i>Scaptotrigona postica</i>	<i>Schwarziana quadripunctata</i>
Friv1	+	+	+	+	+	+
Friv2	-	-	-	-	-	-
Friv4	+	+	+	+	+	+
Friv5	+	+	+	+	+	+
Friv9	+	+	+	+	+	+
Friv10	+	+	+	-	+	+
Friv12	+	+	+	+	+	-
Friv14	+	+	+	+	+	+
Friv15	+	+	+	+	-	+
Friv16	+	+	+	+	-	+
Friv18	+	+	+	+	+	+

polymorphism detected by Lopes et al. (2009, 2010) may be related to their sampling strategy. The authors analyzed 20 individuals from a single population of each species, whereas in our study we analyzed 19 unrelated individuals collected in different localities.

Expected and observed heterozygosities ranged from 0.14 to 0.89 (mean 0.69) and from 0.00 to 1.00 (mean 0.48), respectively. In general, the observed heterozygosity was lower than the expected, except for the loci Friv5, Friv11, and Friv12 (Table I).

The primer set presented herein is suitable for studies aiming at characterizing *F. varia* with respect to genetic diversity, relatedness, inbreeding levels, nest aggregations, intraspecific variation, and genetic mapping. Also, as the cross-species amplification tests revealed PCR fragments of expected size also for the other species analyzed we are confident that, with few exceptions (Table II), these loci will be suitable and informative in studies of other stingless bee species, and thus will contribute to a better understanding of the biology of *F. varia*, and for stingless bees in general.

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Note scientifique sur l'isolation et la caractérisation de microsatellites de *Frieseomelitta varia* (Hymenoptera, Apidae, Meliponini)

Eine wissenschaftliche Notiz über die Isolierung und Charakterisierung von 112 Mikrosatelliten-Loci von *Frieseomelitta varia* (Hymenoptera, Apidae, Meliponini)

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