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Scientific note

A scientific note on the characterization of microsatellite loci for *Melipona mondury* (Hymenoptera: Apidae)*

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Microsatellites are a class of markers much used in population, phylogenetic and parentage studies because of their high degree of polymorphism, codominance and high reproducibility (De la Rúa et al., 2001; Oliveira et al., 2006). Microsatellite sequences are amplified by PCR, using primers that flank the repeat sequences of nuclear DNA. The development of these primers, however, is the limiting factor for the use of these markers. Because of this difficulty, there are microsatellite primers developed for only three species of stingless bee, *Melipona bicolor* (Peters et al., 1998), *Scaptotrigona postica* (Paxton et al., 1999) and *Trigona carbonaria* (Green et al., 2001). However, considering the diversity of stingless bee species and their importance as pollinating agents in natural and cultivated environments (Kerr et al., 1994, 1996; Heard, 1999) the design of further species-specific primers for stingless bees has become urgent.

Thus the objective of this study was to identify and characterize microsatellite primers specific to *Melipona mondury* Smith, 1863, a stingless bee species considered an important pollinator that is distributed throughout the Atlantic Rainforest in the states of Bahia, Espírito Santo, Rio de Janeiro, Minas Gerais, São Paulo, Paraná and Santa Catarina (Brazil) (Melo, 2003). The primers were developed using the methodology proposed by Nest et al. (2000), that is based on the amplification of sequences located between two inversely oriented microsatellites (ISSR - *Inter-simple sequence repeats*). Because it is a simpler and quicker procedure when compared to other techniques used for this purpose, this methodology has been widely used to design microsatellite primers (Kanchanaprayudh et al., 2002; Francisco-Candeira et al., 2007; Provan and Wilson, 2007).

To develop the primers, the DNA extracted from an *M. mondury* individual (Waldschmidt et al., 1997), was amplified using the following ISSR primers: $(GA)_8C$, $(CT)_8T$, $(CA)_8G$, $(AC)_8T$, $(AC)_8G$, $(AG)_8YC$, $(GA)_8YG$, CCGA CTCGAG$(N)_6$ATGTGG, $(AC)_8RG$, $(AC)_8YA$, $(TG)_8RC$, $(AC)_8G$, $(CCG)_6$, $(CTC)_6$ and CATG-GTGTTGGTCATTGTTCCA. The amplification program consisted of a first denaturing step at 94 °C (3 min), followed by 40 cycles at 92 °C (1 min), 55 °C (2 min) and 72 °C (2 min) with a final extension at 72 °C (7 min). The amplifications were carried out in volumes of 25 μL consisting of 0.2 μM primer, 8 μM dNTP, 0.5 U *Taq* DNA polymerase (Phoneutria), 1X of the PCR buffer and 20 ng genomic DNA. The ISSR fragments were purified and linked in the pGEM T-Easy vector (Promega) according to the manufacturer's instructions. After transformation in *E.coli* DH5α, 55 positive clones were sequenced.

The presence of microsatellite sequences was verified in the Simple Sequence Repeat Identification Tool - SSRIT (Temnykh et al., 2001). Results showed that some sequenced fragments presented microsatellites corresponding to the ISSR primers at the 5' and 3' extremities as well as internal microsatellites. The analyses of these sequences allowed the design of 10 pairs of microsatellite primers for *M. mondury* using the program

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Table I. Characterization of the microsatellite loci developed for *Melipona mondury*. GenBank accession numbers are given below locus names.

Locus	Repeat	Size (bp)	*Ta* (°C)	Primer sequence (5'-3')	*A*	*H*
Mmo03 (EU735180)	$(TAAA)_3$	133	50°	*F*: GGTGAAAAGGGGATGAAG *R*: ACTGCGTAAAACGAAGAC	1	0.0
Mmo08 (EU735181)	$(CTTT)_2CT(CTTT)_3$	104	59°	*F*: CGAGCTGAAAGGGCGTT *R*: TCCTCTCTTTTGGTGGTC	2	0.38
Mmo10 (EU735182)	$(GAA)_5$	106	60.5°	*F*: AGAGAGACGAAGCGAAG *R*: ATGACAACCGATCACGA	2	0.32
Mmo11 (EU735183)	$(AG)_3GG(AG)_6$	116	57°	*F*: AGAGAGAGACAGAGAGAA *R*: CGACCCATATTTTCCGAG	2	0.32
Mmo15 (EU735184)	$(GAAC)_5$	100	54°	*F*: GCAAAATGAGCAAACGGACA *R*: GAGAGAGAGGGAGGTTC	2	0.49
Mmo19 (EU735185)	$(GT)_{17}$	107	62°	*F*: AGCGATTTAACTGAACGAT *R*: TATTCCTCTCTTCCTACAC	6	0.67
Mmo20 (EU735186)	$(TCG)_6$	121	60.5°	*F*: GAGGGCGACGATTACGAC *R*: AACGGCATTGTGTAAACAGA	2	0.26
Mmo21 (EU735187)	$(GA)_5$	168	57°	*F*: AACGTGGAACAGTCGGAC *R*: AGCGATTCGTTCAATTCG	5	0.77
Mmo22 (EU735188)	$(AG)_{16}$	191	61.5°	*F*: TGTAATCCTAACCCGATT *R*: CTTTGCAGTTTTCACACG	5	0.79
Mmo24 (EU735189)	$(AG)_4(AG)_3(GAAAA)_2$	180	52°	*F*: TCAGCGATTAATGAACAC *R*: TGGAAATGTTTTACCTGT	3	0.10

F and *R*, forward and reverse primers, respectively; *Ta*, annealing temperature; *A*, number of alleles; *H*, gene diversity.

Oligo Explorer Version 1.2 (Gene Link Inc, www.genelink.com).

These primers were tested by amplifying the DNA of 20 workers, representing a single population of *M. mondury* and 20 individuals of *M. quadrifasciata, M. bicolor, M. rufiventris* and *Partamona helleri*. The amplifications were made in reactions of 10 μL containing 12.5 ng genomic DNA, 1X Promega *Taq* PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl), 0.5 or 0.25 μM of each forward and reverse primer, 0.1 mM dNTP, 1.5 or 1.0 mM $MgCl_2$, and 1 U *Taq* DNA polymerase (Promega). The conditions for the PCR were: 94 °C (3 min), followed by 40 cycles at 92 °C (30 s), annealing temperatures specific for each primer (Tab. I) (1 min) and 72 °C (30 s) with a final extension step at 72 °C (5 min). The amplification products were separated in 8% polyacrylamide gel and the POPGENE 1.32 program (Yeh et al., 1999) was used to calculate the Nei (H) genetic diversity, test the deviation of the loci for the Hardy-Weinberg equilibrium and the linkage disequilibrium.

Nine of the 10 loci tested were polymorphic (90.0%) and the number of the alleles ranged from 1 to 6 (Tab. I). The genetic diversity values ranged from 0.0 to 0.79 (mean = 0.41). Only the Mmo15 locus did not deviate from the HW equilibrium at the level of 5% and no linkage disequilibrium was observed.

These 10 microsatellite primers were tested in *M. quadrifasciata, M. bicolor*, *M. rufiventris* and *P. helleri* (Tab. II) and the success of the results obtained showed their usefulness in genetic studies with other stingless bee species.

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Note scientifique sur la caractérisation de microsatellites pour *Melipona mondury* (Hymenoptera : Apidae).

Eine wissenschaftliche Notiz über die Charakterisierung von Mikrosatelliten-Loci für *Melipona mondury* (Hymenoptera: Apidae).

Table II. Number of microsatellite alleles observed in four stingless bee species, using microsatellite primers designed for *Melipona mondury*. NA denotes a locus which failed to amplify in a given species.

Locus	Species			
	M. quadrifasciata	*M. bicolor*	*M. rufiventris*	*P. helleri*
Mmo03	1	2	2	NA
Mmo08	2	3	1	NA
Mmo10	1	1	1	3
Mmo11	1	4	2	NA
Mmo15	-	1	3	2
Mmo19	-	-	4	NA
Mmo20	1	1	1	1
Mmo21	-	-	3	1
Mmo22	6	3	6	NA
Mmo24	-	2	2	1

REFERENCES

De la Rúa P., Galián J., Serrano J., Moritz R.F.A. (2001) Molecular characterization and population structure of the honeybees from the Balearic Islands, Apidologie 32, 417–427.

Francisco-Candeira M., González-Tizón A., Varela M.A., Martínez-Lage A. (2007) Development of microsatellite markers in the razor clam *Solen marginatus* (Bivalvia: Solenidae), J. Mar. Biol. Assoc. U. K. 87, 977–978.

Green C.L., Franck P., Oldroyd B.P. (2001) Characterization of microsatellite loci for *Trigona carbonaria*, a stingless bee endemic to Australia, Mol. Ecol. Notes 1, 89–92.

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

Kanchanaprayudh J., Lian C., Zhou Z., Hogetsu T., Sihanonth P. (2002) Polymorphic microsatellite markers of a *Pisolithus* sp. from a *Eucalyptus* plantation, Mol. Ecol. Notes 2, 263–264.

Kerr W.E., Carvalho G.A., Nascimento V.M. (1996) Abelha Uruçu. Biologia, Manejo e Conservação, Coleção Manejo da Vida Silvestre, No. 2. Acangaú, Belo Horizonte.

Kerr W.E., Nascimento V.A., Carvalho G.A. (1994) Há salvação para os meliponíneos? Anais II Encontro sobre abelhas, Ribeirão Preto, pp. 60–65.

Melo G.A.R. (2003) Notas sobre meliponíneos neotropicais (Hymenoptera, Apidae), com a descrição de três novas espécies, in: Melo G.A.R., Alves-dos-Santos I. (Eds.), Apoidea Neotropica, Homenagem aos 90 anos de Jesus Santiago Moure, UNESC, Santa Catarina, Brasil, pp. 85–92.

Nest M.A.V.D., Steenkamp E.T., Wingfield B.D., Wingfield M.J. (2000) Development of simple sequence repeat (SSR) markers in *Eucalyptus* from amplified inter-simple sequence repeats (ISSR), Plant Breeding 119, 433–436.

Oliveira E.J., Pádua J.G., Zucchi M.I., Vencovsky R., Vieira M.LC. (2006) Origin, evolution and genome distribution of microsatellites, Genet. Mol. Biol. 29, 294–307.

Paxton R.J., Weibschuh N., Quezada-Euán J.J.G. (1999) Characterization of dinucleotide microsatellite loci for stingless bees, Mol. Ecol. 8, 690–692.

Peters J.M., Queller D.C., Imperatriz-Fonseca V.L., Strassmann J.E. (1998) Microsatellite loci for stingless bees, Mol. Ecol. 7, 784–792.

Provan J., Wilson P.J. (2007) Development of microsatellites for the peat moss S*phagnum capillifolium* using ISSR cloning, Mol. Ecol. Notes 7, 254–256.

Temnykh S., DeClerck G., Lukashova A., Lipovich L., Cartinhour S., McCouch S. (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential, Genome Res. 11, 1441–1452.

Waldschmidt A.M., Salomão T.M.F., Barros E.G., Campos L.A.O. (1997) Extraction of genomic DNA from *Melipona quadrifasciata* (Hymenoptera: Apidae, Meliponinae), Braz. J. Genet. 20, 421–423.

Yeh F.C., Yang R., Boyle T. (1999) POPGENE version 1.32: Microsoft Windows-based freeware for population genetic analysis, Quick User Guide.