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SHORT COMMUNICATION

Isolation and characterization of ten microsatellite loci in stingless bee *Trigona spinipes* (Apidae: Meliponini)

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Abstract

Stingless bees are the most abundant pollinators of Brazilian tropical flora. *Trigona spinipes* has some of the largest colonies of any stingless bee species found in several types of environment. This work describes the isolation and characterization of microsatellite loci for this species. A microsatellite-enriched genomic library was constructed and ten primer pairs were designed for *T. spinipes*. The primers were tested in 20 unrelated individuals. The mean number of alleles was 8.10 and mean observed and expected heterozygosity were 0.655 and 0.680, respectively. Primers were also tested in cross-species amplification and five loci were successfully amplified in *Trigona chanchamayoensis*, *Trigona hyalinata*, *Tetragonisca angustula*, *Partamona mulata* and *Frieseomelitta varia*. The microsatellite primers described herein will be useful for evaluating genetic variability and gaining a better understanding of the population structure of *T. spinipes* as well as other species of stingless bees.

Key words: amplification, genomic library, pollinators, primers.

Trigona spinipes is a generalist stingless bee species whose colonies contain up to 180 000 individuals (Michener 1974). It is widely distributed in Brazil, Argentina, Paraguay, Colombia, Guyana and Peru (Camargo & Pedro 2012). The species nests in natural as well as rural and urban areas. Due to its habit of severing the corolla tube of some fruit species it is often regarded as a pest (Kerr *et al.* 1981). However, it is promoted as a potential pollinator of other crops (Almeida & Laroca 1988). Here we report on the isolation and characterization of ten microsatellite loci from *T. spinipes*.

Total DNA of 20 workers from three different nests was extracted from the thoraces using a phenol–chloroform protocol. Construction of the enriched genomic library was performed according to Billotte *et al.* (1999), with modifications (Brito *et al.* 2009). From the 32 clones sequenced, 23 of the sequences contained short tandem repeats. We used the software Primer3 (Rozen & Skaletsky 2000) to select the best sequences for the design of primers. We designed primers to ten loci and tested these primers on 20 unrelated workers of *T. spinipes*, collected in 20 different nests located in Itirapina Ecological Station, Brazil (22°11′ to 22°15′S, 47°51′ to 48°00′W). DNA extraction from individuals followed the Chelex method (Walsh *et al.* 1991). Polymerase chain reaction (PCR), visualization and genotyping were performed according to Francisco *et al.* (2011). The program Micro-Checker v2.2.3 (Van Oosterhout *et al.* 2004) was used to detect the presence of null alleles and scoring errors. PCR was conducted with initial denaturation of 94°C for 8 min, followed by 35 cycles of denaturation (94°C/1 min); annealing (1 min, temperature according to Table 1); and elongation (72°C/1 min), and an extension of 72°C

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Table 1 General information for microsatellite loci characterized for *Trigona spinipes*

Locus	Repeat motif	GenBank accession number	Primer sequence 5′-3′	Ta (°C)	*k*	ASR	H_O	H_E
Trig1B	$AGA(GG)_4(AG)_7GG(AG)_2$	KC633950	F: TGGACTAACCGGCTGTGG R: ATTCTCTCGCTCGCGTCT	53	2	195–197	0.250	0.219
Trig1D	$(CT)_4CC(CT)_{12}$	KC833472	F: CGCAAGTCACACGCCATC R: CTTCTCGTCCGACCGAACT	54	15	151–188	0.750	0.884
Trig2A	$(GA)_{22}$	KC833473	F: TTGAGAGGCCACCGTATTG R: ACGTCTGGCTCTTCAGGTT	48	12	149–174	0.800	0.760
Trig2F	$(GA)_{14}(AGG)_2$	KC833474	F: AAGGATGTTTGGGTGATCC R: CCGAGTGATGCGAGTTGA	54	10	201–217	0.750	0.740
Trig2H	$(TC)_{14}(TGTC)_3$	KC833475	F: GGCATCTTCGAACCGTGA R: CACCTTTGTGAACCAGATGC	48	10	154–188	0.850	0.836
Trig3F	$(GA)_2AA(GA)_{18}$	KC833476	F: TGGACTAACTCGGACGAAGAA R: CTGAAATCCGGCACGACA	60	7	195–207	0.550	0.699
Trig3G	$(CT)_2(AA)_8$	KC833477	F: GCATTCCCCATTGGATTT R: GCGGACGTCTTCTTGATTG	54	6	182–200	0.700	0.720
Trig4D	$(GA)_8AA(GA)_{10}$	KC833478	F: GAAAGCGATTAACGCTCAAA R: CGTTCAACCACACCGACA	60	9	111–137	0.600	0.725
Trig4E	$AT(TA)_3T(TA)_2(TT)_2CC(TA)_2$	KC833479	F: GGACCAGGATCGACAACAGT R: GATGAAATGGGAATTGGTGA	43–48†	8	201–241	0.500	0.735
Trig4F	$CTCC(CT)_6G(TT)_2CCTTT(CT)_3$	KC833480	F: GACCAACGCAAGATGGATG R: AACGTTCGTGTCTTCATGTTG	48	2	190–194	0.800	0.480

†Touch-up cycle. In °C: 43, 44, 45, 45.5, 46, 47, and 48 (6× each). Ta, annealing temperature; *k*, number of alleles; ASR, allele size range (bp) excluding the size of the labeled primer; H_O and H_E, observed and expected heterozygosity, respectively.

for 10 min. The program Genalex v6.5 (Peakall & Smouse 2006, 2012) was used to calculate the number of alleles (*k*), the observed and expected heterozygosity from Hardy–Weinberg proportions (H_O and H_E, respectively), and the percentage of polymorphic loci. Exact tests for Hardy–Weinberg equilibrium (HWE) and log likelihood ratio statistics for linkage disequilibrium were computed using Genepop v4.2.1 (Rousset 2008). Sequential Bonferroni correction (Holm 1979) was applied when multiple comparisons were performed.

All ten loci were polymorphic. The number of alleles varied from two (Trig1B and Trig4F) to 15 (Trig1D), with an average of 8.10 ± 1.29 (Table 1). Average values of H_O and H_E were 0.655 ± 0.058 and 0.680 ± 0.061, respectively. Null alleles were found at the Trig4E locus only. After Bonferroni correction, two loci (Trig4E, $P = 0.015$; and Trig4F, $P = 0.049$) were found to deviate significantly from the HWE. No linkage disequilibrium was detected after Bonferroni correction (all $P > 0.05$). Five loci (Trig1B, Trig1D, Trig2F, Trig3G and Trig4E) were successfully amplified in five species of stingless bee, *Trigona chanchamayoensis*, *Trigona hyalinata*, *Tetragonisca angustula*, *Partamona mulata*, and *Frieseomelitta varia*, by the PCR conditions described above. Three loci (Trig2A, Trig3F and Trig4D) were amplified in four species and two (Trig2H and Trig4F) failed in all attempts (Table 2).

The microsatellite primers described herein will be useful for evaluating genetic variability and gaining a better understanding of the population structure of *T. spinipes* as well as other species of stingless bees. These primers will allow the development of new approaches in behavior ecology besides the classical mark-recapture methodology, mainly for aggressive species and for those that are difficult to keep in the laboratory. Indeed, this primer set has already been applied to study reproduction strategies in *T. spinipes* (Jaffé *et al.* 2014).

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Table 2 Cross-species amplification of ten microsatellite loci from *Trigona spinipes* in five stingless bee species

Locus	*Trigona chanchamayoensis*	*Trigona hyalinata*	*Tetragonisca angustula*	*Partamona mulata*	*Frieseomelitta varia*
Trig1B	+	+	+	+	+
Trig1D	+	+	+	+	+
Trig2A	+	+	+	+	–
Trig2F	+	+	+	+	+
Trig2H	–	–	–	–	–
Trig3F	+	+	–	+	+
Trig3G	+	+	+	+	+
Trig4D	+	–	+	+	+
Trig4E	+	+	+	+	+
Trig4F	–	–	–	–	–

+, successful amplification; –, no product or multiple bands.

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