Microsatellite loci for stingless bees

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The stingless bees are a eusocial, pantropical group of more than 400 species (Kerr 1969; Sakagami 1982; Roubik 1989). Similar to honeybees, they usually have single queens, store honey, and have morphologically distinct workers and queens. However, they differ from honeybees in their social organization. Queens are produced in substantial numbers, then imprisoned, detained or killed if a vigorous queen is already present in the nest (Imperatriz Fonseca & Zucchi 1995). Workers of most species may lay either male-producing or trophic eggs, often as part of a complex, highly ritualized provisioning and oviposition process which sometimes results in two eggs laid in a cell capable of supporting only one, so one larva cannibalizes the other (Zucchi 1993). Whether behavioural conflict has a basis in genetic conflicts of interest is not known, partly because of the lack of genetic markers. Among the most desirable markers are microsatellites because of their high levels of polymorphism and codominant Mendelian inheritance (Queller *et al.* 1993). This report presents 33 novel microsatellite loci for stingless bees obtained from *Melipona bicolor bicolor*.

Table 1 Microsatellite loci and primers for *Melipona bicolor* and related taxa. For each species (*M. bicolor* = *Melipona bicolor*, eight unrelated females; *M. quad.* = *Melipona quadrifasciata*, two females each from two colonies; *T. clav.* = *Tetragona clavipes*, four unrelated females; *S. post.* = *Scaptotrigona postica*, four unrelated females) data are observed heterozygosity (number of alleles). GenBank accession numbers are AF002824–AF002848

Locus	Size*	Ta (°C)	Repeat*	*M. bicolor*	*M. quad.*	*T. clav.*	*S. post.*	PO	Primers (5'-3')
Mbi11CAC	152	55	$(AAC)_8ATC(GCC)_5$	0.63(5)	0.00(2)	0.00(1)	0.00(1)	F R	CGTTCGTTCTTCCCAAT GATCGAATTTAACCGGC
Mbi13GAC	129	57.5	$(GTC)_{10}$	0.88(4)	NP	NP	NP	F R	CCCCTAACAGCCAAAGCTAT CGATGCCCACTTTCGAC
Mbi28AAG	108	57.5	$(TCC)_6ACC(TCC)_3$	0.25(3)	0.00(1)	0.00(1)	0.00(1)	F R	TTTTATCGCTCCTATCCTCC AATCCAACAGGACGGTGT
Mbi32GAG	154	57.5	$(GGA)_4(GGAGAA)_5$	0.63(4)	0.00(1)	0.00(1)	0.00(1)	F R	CTTTATCCGGTGCGTCGAA GAAGGCATTCCGGGTTGTT
Mbi33AAG	140	60	$TTC(TCC)_2TCTTCC(TCT)_2(TCC)_3$	0.00(1)	0.00(1)	0.00(1)	0.00(1)	F R	ATCACCTAACTTGGCATCCC GATCAAGGGCCAAGAGGA
Mbi34GAC	156	57.5	$(TCG)_6(AG)_5$	0.12(2)	NP	NP	NP	F R	GGGCTCGTTTCCTACCAG CCTTCGCGACCAATGTG
Mbi36GAG	125	60	$(CTC)_{10}TTTCTCC(TTCCT)_2TTC$	0.75(5)	NP	NP	NP	F R	GCATCGGCGTCAGCCAG GCGTGTCAGGCGGTGAAG
Mbi85GAC	127	60	$TCGACG(TCG)_5$	0.00(1)	0.00(1)	0.00(1)	NP	F R	CGAACTCGAGAGCCAGC GATCCGAACGACGAAGG
Mbi88AAAGA	94	57.5	$(GAAA)_2GGAAG(AAAGA)_4$ $(AAGGA)_2AAAGAAAC(GAG)_2$	0.25(2)	0.00(1)	NP	NP	F R	GCCGCCGTACGTTCTGA GCGCTCGAGCAGCGTT
Mbi103AAT	175	50	$(ATT)_6$	0.00(1)	NP	NP	NP	F R	CCCCACAGTGTAACCAGAAAG TTATGGTGATAAACGGCGAAG
Mbi105AAT	146	57.5	$(AAT)_2AAC(AAT)_8$	0.38(3)	NP	NP	NP	F R	GGCCTTGATTTTCAACAAAGA CTCCTACTTAGCTCTCGCGTC
Mbi201AAG	152	60	$(CTT)_{10}CTC(CTT)_5CCT(CTT)_2$	0.75(5)	0.75(2)	0.00(1)	0.00(1)	F R	GTTTAATCGCCCAAAGAGGC GTTGACGAGAAGGAGCACG
Mbi213AAG	123	62.5	$(CTT)_8$	0.75(6)	NP	NP	NP	F R	CCCTCATCCCTTCGTTTCTT CGTGACAGGAAGTCCGTCTAA
Mbi215AAG	92	57.5	$(TTC)_6$	0.50(3)	0.00(1)	0.00(1)	0.00(1)	F R	AGAGACGAAAAGTGGCGG GATAGCGGCGGAAGAGATT
Mbi218AAG	131	60	$(CCT)_3(TCT)_7$	0.12(3)	0.25(2)	†	†	F R	CTCGACTTAATTTCCATCGGC GCAATTTCAATCGCGACC
Mbi219AAT	151	57.5	$(AAT)_4$	0.00(1)	0.00(1)	0.00(1)	0.00(1)	F R	GCAGCGAAAGACTAATTTACC GACGTTGGAATGGAAACTC

Table 1 *Continued*

Locus	Size*	Ta (°C)	Repeat*	*M. bicolor*	*M. quad.*	*T. clav.*	*S. post.*	PO	Primers (5'-3')
Mbi221AAG	160	60	$(AGA)_2TGGCT(AGA)_5$	0.00(1)	0.00(1)	0.00(1)	NP	F	CATTTCGCCGCTCTCTATCT
								R	CTTGAATCTTTTGACACAACGTCT
Mbi232AAG	128	50	$(CTT)_{13}$	0.88(4)	1.00(4)	0.75(2)	0.00(1)	F	TTTTTCTCTTAAATTTTCTTCT
								R	CTTACTCGACGACTTTATTT
Mbi233AAG	119	57.5	$(GAA)_{15}$	0.88(6)	0.00(1)	NP	NP	F	ACGAGCACGGGCCATAA
								R	GATCCATCGACCGCTTCTT
Mbi254AAG	213	55	$(AAG)_{11}$	0.38(3)	1.00(3)	0.00(1)	0.50(5)	F	CAATCGTTGGAAGGGAAC
								R	GGACCTATACCCAAGTCCAT
Mbi256AAG	127	57.5	$(AGA)_9$	0.50(4)	1.00(3)	NP	NP	F	GGATTCAAGCAGACGATA
								R	GATCAAAGTCCCCATCTT
Mbi259AAG	183	57.5	$(AGG)(AGA)_5(GGA)_2GAA(GGA)_2$	0.12(2)	0.50(2)	0.75(3)	0.50(3)	F	CGACGTTAACATTTCGCTA
								R	CTGTTCGACTGTTCTCACTCT
Mbi278AAG	113	60	$CTT(CTC)_2CTTCTCTGCTTCC$ $(TCT)_9CCTTCG(TCT)_2$	0.86(5)	0.00(1)	0.25(2)	0.25(2)	F	GTTCGTGTTTCGTGGTGAAT
								R	GTTGCGAGAACTCTGACGAT
Mbi305CAT	106	60	$(CAT)_3(CAG)(CAT)_2(CCT)_3$ $(TCT)(CTT)_2(CCT)(CTC)$	0.43(3)	0.50(2)	NP	0.75(3)	F	GATCCGCTGCGCGAGAC
								R	GGACGAGGCTGAGGCATG
Mbi522CAG	136	60	$(TGC)_4TGT(TGC)_3$	0.00(1)	0.00(1)	0.00(1)	0.00(1)	F	CCCTGGACAAATACAAACGTA
								R	GAACAATGCTCTTCTCCGAA

*, Sequenced allele; Ta, annealing temperature; NP, no product; †, Not attempted; PO, primer orientation.

We followed published protocols (Strassmann *et al.* 1996), but used a positive-selection plasmid (pZErO-2.1, Invitrogen) which eliminated the need for plasmid dephosphorylation. We cut genomic DNA with *Sau*3AI then ligated the resulting fragments in the 400–500 bp size class into pZErO-2.1 plasmids. We transformed TOP10F′ cells to obtain a 10 000 clone library which was plated onto nylon. Probing library replicates with oligonucleotides of all 10 trinucleotide motifs yielded 237 positives. Southern blots of plasmid DNA confirmed 64 positives which were sequenced using an ABI automated sequencer. We identified 29 trinucleotide microsatellites consisting of four or more repeats, and two longer, complex microsatellites. Of the 31 primer pairs we designed, 25 yielded scoreable genetic markers (Table 1). We also give GenBank accession numbers for eight dinucleotide microsatellites for which we did not design primers (Table 2).

PCR was performed in 10 µL volumes containing 50 mM KCl, 10 mM Tris pH 9.0, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 2.5 mM of each dNTP, 2.5 µM of each primer, 10 ng of target DNA, 2 µCi [α-^{35}S]-dATP, and 0.5 units of *Taq* DNA polymerase (Promega, MJResearch Thermocycler). We denatured at 94 °C for 3 min, then ran 40 cycles of 30 s at 92 °C, 1 min at the annealing temperature, and 30 s at 72 °C followed by a final 5-min extension at 72 °C. Samples were electrophoresed through 4% acrylamide/8 M urea sequencing gels.

We investigated the utility of these markers by genotyping adult workers collected from captive hives at the University of São Paulo (Table 1). Nineteen of 25 loci were polymorphic in *M. bicolor*. Only 19 loci yielded a scoreable PCR product for *M. quadrifasciata* and only eight of these were polymorphic. For the other two genera approximately half of the loci yielded scoreable PCR products but only three or four were variable. Mean heterozygosities of polymorphic loci for all species ranged from 0.49 to 0.63. Most loci with more than five perfect repeats were polymorphic in *M. bicolor* as were three loci with complex or interrupted repeats. Trinucleotide repeat loci with seven or fewer perfect repeats in the cloned allele showed a mean heterozygosity in *M. bicolor* of 0.13 while those with more than seven perfect repeats showed a significantly higher heterozygosity of 0.70 ($P < 0.0001$, two-tailed t-test).

These loci represent the first microsatellite markers for stingless bees. They should prove useful in furthering our understanding of stingless bee societies. The amplification of scoreable polymorphic products in the distantly related genera *Tetragona clavipes* and *Scaptotrigona postica* suggests that these markers will be useful in many neotropical genera of the Meliponini.

Table 2 Dinucleotide microsatellite loci for *Melipona bicolor*

Clone	Repeat	GenBank accession no.
Mbi24AC	$(TG)_8CG(TG)_3$	AF002849
Mbi210AC	$(AC)_{10}$	AF002850
Mbi217AC	$(CA)_{13}\ldots(TC)_5$	AF002851
Mbi220AC	$(TG)_{14}$	AF002852
Mbi263AC	$(CA)_{12}TA(CA)_4AACG(CA)_4CT(CA)_3$	AF002853
Mbi270AC	$(CA)_{11}$	AF002854
Mbi295AG	$(AC)_8\ldots(GA)_{16}$	AF002855
Mbi297AC	$(CG)_4(CA)_2TA(CA)_9(TA)_4\ldots(CA)_9$	AF002856

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