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PRIMER NOTE

Isolation of polymorphic tetranucleotide microsatellite markers for the silky short-tailed bat *Carollia brevicauda*

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Abstract

We isolated 10 polymorphic microsatellite markers for the silky short-tailed bat, *Carollia brevicauda*, from genomic libraries enriched for $(AAGG)_n$ repetitive elements. The number of alleles ranged from six to 25 per locus with the observed heterozygosity ranging from 0.29 to 0.95. These markers will be useful for analysis of questions concerning population genetic structure and models of speciation. Results of cross-species amplification in *Carollia castanea* and *Carollia perspicillata* are also reported.

Keywords: bat, Carollia, microsatellite, Phyllostomidae, tetranucleotide

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Short-tailed bats in the genus *Carollia* are distributed throughout tropical America, from southern Mexico to southern Brazil, with three species (*C. brevicauda*, *C. castanea* and *C. perspicillata*) occurring on both sides of the Andes and two (*C. castanea* and *C. perspicillata*) present north and south of the Isthmus of Panama (Pine 1972; Hoffman & Baker 2003). Extended geographical distribution in a region supporting record levels of bat diversity (Kalko *et al*. 1996; Simmons & Voss 1998) and occurrence across two major biogeographic boundaries make short-tailed bats excellent candidates for phylogeographic and population genetic analyses. Likewise, high intrageneric diversity (five recognized species; Simmons 2005) and the recent identification of divergent mitochondrial lineages that may represent cryptic species (Hoffman & Baker 2003), suggest that comparative population genetic studies of *Carollia* species will provide insight into the evolutionary processes driving diversification in Neotropical bats. As part of an integrative project designed to test alternative models of speciation in tropical vertebrates, we developed 10 polymorphic microsatellite markers for *C. brevicauda*.

Microsatellite loci were isolated using a biotin-capture method, as previously described (Bardeleben 2004; Bardeleben *et al*. 2004). Genomic DNA was extracted from liver preserved in 95% ethanol using the QIAGEN Tissue Extraction Kit. Approximately 3 μg of genomic DNA from a single individual was restricted with *Sau3A* (NEBiolabs) and ligated to an adaptor generated by annealing together oligo A and oligo B (Refseth *et al*. 1997) and size fractionated on a 1% agarose/TAE gel. DNA in the 0.5–1.5 kilobase range was excised from the gel and the DNA purified by UltraClean (Mcfrugal). In addition, approximately 3 μg of genomic DNA was restricted with *Mse*I (NEBiolabs), ligated to an adaptor generated by annealing together oligo A and oligo C (Bardeleben 2004) and size-fractionated as above. Each hybridization was carried out separately with approximately 1 μg of the adaptor-ligated size fractionated genomic DNA mixed with 50 nM biotin-labelled oligo probe 5′-$(AAGG)_6$GCA(Biotinyl-C)A-3′ in 6× SSC (1 M NaCl, 0.1 M NaCitrate) at 65 °C, overnight. Addition and incubation of M280 streptavidin-coated magnetic beads (Dynal), washes, elution, polymerase chain reaction (PCR) amplification of eluates and a second round of hybridization were carried out as previously described (Bardeleben 2004; Bardeleben *et al*. 2004, 2005). A PCR-based method described previously was used to screen 176 clones (Bardeleben *et al*. 2004). Plasmid DNA was prepared using the QIAprep spin Miniprep kit (QIAGEN) from the 87 clones that were positive for a repetitive element and subsequently sequenced using either ABI PRISM BigDye Ready mix or ABI PRISM dGTP BigDye Ready mix (Applied Biosystems) with either the M13(−20) forward or the M13-alt reverse primer (Bardeleben *et al*. 2005). Sequences were run on an

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Table 1 Microsatellite loci for *Carollia brevicauda* including primer sequence, repeat motif, annealing temperature (T_a); *N*, number of individuals successfully genotyped out of 54 samples; size range of alleles; number of alleles; H_O, observed heterozygosity, H_E, expected heterozygosity; GenBank Accession number; base pair (bp)

Locus	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	*N*	Size range (bp)	No. of alleles	H_O	H_E	GenBank Accession no.
*AAGG-1**†	ATTTGGATTCAGGAAGAGATG¶ TTTTACGGACAAAAGCACTG	Imperfect‡	55	49	132–241	25	0.40	0.94	DQ442865
AAGG-3	TGGCCTGGTTTCCCCTTT†† TGGCCTGGAATATGACCAAT	$(CCTT)_4(TCTT)(CCTT)_4$	55	54	146–178	10	0.59	0.82	DQ442866
AAGG-7	GCGTCTGGCATATTAGTCC†† TTGACCATGTAGAGCAAGGTT	$(AAGG)_{11}$	55	52	129–182	15	0.84	0.87	DQ442867
AAGG-91	CATGAAACAATCCACTGGC‡‡ CGTGAGCAAAACAACCAAGA	Imperfect§	50	54	143–179	8	0.29	0.41	DQ442868
AAGG-98	TATTCAAGAAATAAATGAAGGGCGGG†† TGGTGGCTACTGCTTAGTGC	$(AAGG)_{12}(AAG)(AAAG)_{12}$	61	54	179–249	17	0.95	0.89	DQ442869
AAGG-112	GGGAAACCACACAAAGTTGG§§ TTGGAACAGGATAGGGGAGA	$(AAGG)_7(AATG)_{11}$	50	54	130–187	14	0.73	0.88	DQ442870
AAGG-117	GACTAAGATAGATAAATTGATGGATAA‡‡ CAAAATGCTTTAGTTTCCTGAAT	$(AAGG)_{16}$	55	54	136–189	14	0.77	0.88	DQ442871
AAGG-119	TGTAAATCCGTGAGGACAGAAA†† CGTTGGAAGAGACCACCTG	$(AAGG)_{12}$	61	54	185–229	10	0.74	0.86	DQ442872
*AAGG-140**	GATCCCTGTTGCCTATA¶ ATTGGCGACAGAGCAAGG	$(AAGG)_{18}$	55	52	136–185	11	0.62	0.86	DQ442873
AAGG-143	GATCATTTCTCAAAAGTTTCTT‡‡ ACTGACATGTACTAAGGTTAT	$(CCTT)_3(CCTA)(CCTT)_6$	50	53	141–182	6	0.44	0.43	DQ442874

†one nucleotide differences observed in *C. brevicada*, but not in *C. castanea*.
‡$(AAGG)_3(GAGG)(AAGG)_{14}(AAAGGAAAG)_2(AAAGGAAG)(AAAG)_2$.
§$(AAGG)_3(G)(AAGG)_3(AGAA)_2(GAGG)(AAGG)_{12}$.
¶labelled with VIC.
††labelled with 6FAM.
‡‡labelled with NED.
§§labelled with PET.
*significantly out of Hardy–Weinberg equilibrium in the Shell-Mera population ($P < 0.01$).

automated DNA Sequencer (ABI PRISM 377, PE, Applied Biosystems).

Primers were designed using PRIMER 3 (Rozen & Skaletsky 2000) from the flanking sequences of 35 unique clones to amplify the repetitive element. PCR conditions were optimized for 10 primer sets and each locus was evaluated for polymorphism and heterozygosity in 54 individuals captured from five locations in Ecuador including Shell-Mera, Pastaza Province (19 samples), Comunidad Santo Domingo, Napo Province (14), Laguna San Jose de Chamanal, Orellana Province (5), Sacha Lodge, Sucumbios Province (11) and Estación Científica Yasuní, Orellana Province (5). The forward primer was labelled with fluorescent dye (either 6FAM, NED, PET or VIC). The conditions for PCR were 10 mM Tris pH 8.3, 50 mM KCl, 2.0 mM $MgCl_2$, 188 μM each dNTP, 0.4 μM each primer and 1.0 U *Taq* Gold DNA polymerase (Applied Biosystems), in a final reaction volume of 16 μL. Fifty nanograms of extracted DNA was used as template. The following thermal profile was repeated for 35 cycles with an initial denaturation step at 95 °C for 5 min and a final extension at 72 °C for 2–5 min: 94 °C for 30 s, annealing temperature (T_a) for 45 s (Table 1), and 72 °C for 50 s in a PerkinElmer 9700 . Amplified products were run on an Applied Biosystems 3100 automated sequencer along with GENESCAN 500 LIZ Size Standard (Applied Biosystems); allele size was estimated in GENEMAPPER (version 3.7) and edited by eye.

Ten polymorphic loci were found. The number of alleles ranged from six to 25 per locus. Observed and expected heterozygosity were calculated using FSTAT (version 2.9.3.2; Goudet 1995). Observed heterozygosity ranged from 0.29 to 0.95 (Table 1). We tested for departures from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium in the Shell-Mera population ($n = 19$) using GENEPOP version 3.4 (Raymond & Rousset 1995). Two of the 10 loci, *AAGG-1* and *AAGG-140*, were found to be significantly ($P < 0.01$) out of HWE. Both loci exhibited a significant heterozygote deficiency suggesting the presence

Table 2 *Carollia* cross-species amplification: locus, no. of alleles, size range, H_O, observed heterozygosity, H_E, expected heterozygosity

	*C. perspicillata**				*C. castanea*†			
Locus	No. of alleles	Size range (bp)	H_E	H_O	No. of alleles	Size range (bp)	H_E	H_O
AAGG-1	NA				18	121–213	0.94	0.91
AAGG-3	NA				12	124–170	0.87	0.78
AAGG-7	16	125–178	0.79	0.81	9	121–178	0.83	0.81
AAGG-91	16	133–209	0.72	0.54	NA			
AAGG-98	NA				12	178–231	0.91	0.71
AAGG-112	12	126–198	0.81	0.77	NA			
AAGG-117	18	149–196	0.89	0.76	9	157–192	0.87	0.78
AAGG-119	12	181–221	0.86	0.63	9	176–212	0.91	0.68
AAGG-140	17	128–200	0.91	0.48	2	115–119	0.54	0.48
AAGG-143	11	158–198	0.55	0.42	5	154–177	0.55	0.42

**N* = 51–53.
†*N* = 18–23.
NA, no amplification.

of null alleles. However, analysis of samples from four additional populations (*n* = 5–14) indicated that only *AAGG-1* was significantly out of HWE in three of the populations. None of the loci displayed significant linkage disequilibrium after applying a Bonferroni correction ($\alpha = 0.001$). Seven and eight of the 10 loci cross-amplify in *C. perspicillata* and *C. castanea*, respectively (Table 2). An additional seven loci were isolated in *C. brevicauda* and amplified reliably in this species but were not characterized for polymorphism; primer information is available from the Correspondence.

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