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PRIMER NOTE

Extremely variable di- and tetranucleotide microsatellite loci in Brazilian free-tailed bats (*Tadarida brasiliensis*)

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Abstract

We present three dinucleotide and six tetranucleotide microsatellite loci that were developed for the Brazilian free-tailed bat, *Tadarida brasiliensis* (Chiroptera, Molossidae). Ninety-one individuals from two populations were scored at each locus, revealing extremely high levels of polymorphism (15–55 alleles per locus). These loci provide genetic markers for studying gene flow, migration and mating behaviour.

Keywords: bats, Chiroptera, microsatellites, Molossidae, *Tadarida brasiliensis*

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Tadarida brasiliensis (Chiroptera, Molossidae) is a New World bat that is capable of long-distance dispersal and is migratory in portions of its range (Cockrum 1969). While promiscuous in its mating behaviour (McCracken & Wilkinson 2000), studies of captive populations have revealed two distinct mating strategies in males (French & Lollar 1998). We present nine highly variable microsatellite loci for *T. brasiliensis*. These markers should prove useful for analysing dispersal patterns and for studying the evolution and maintenance of multiple mating strategies in single populations.

Genomic DNA was isolated from approximately 1 g of wing tissue from two Brazilian free-tailed bats from the Eckert James River Cave colony in Mason County, Texas, USA, using the phenol–chloroform method of Sambrook *et al*. (1989). The DNA was pooled and sent to Genetic Identification Services (http://www.genetic-id-services.com; Chatsworth, California, USA) for the construction of eight genomic libraries that were enriched for inserts containing $(GA)_n$, $(AAT)_n$, $(GAC)_n$, $(CATC)_n$, $(TACA)_n$, and $(GATA)_n$ repeats. Library construction followed the method of Jones *et al*. (2002), and resulted in at least 10 000–12 000 recombinant cells per library. Seven to ten colonies were sequenced per library, and primers for 19 microsatellite loci identified from these sequences were designed using the GENEFISHER software (Giegerich *et al*. 1996).

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Genomic DNA was isolated from 3 mm wing tissue biopsies (Worthington Wilmer & Barratt 1996) of 48 *T. brasiliensis* from Rosario, Argentina and 43 *T. brasiliensis* from Huntsville, Texas, USA, using the phenol–chloroform method of Sambrook *et al*. (1989). Polymerase chain reactions (PCRs) were performed in 12 μL volumes containing 1.2 μL 10× buffer (contains 15 mM $MgCl_2$; QIAGEN), 1.5–2 mM $MgCl_2$ (total concentration), 0.1 mM dNTPs, 50 ng fluorescent-labelled and nonlabelled primers (see Table 1 for dyes used), 0.1 U HotStarTaq™ DNA polymerase (QIAGEN) and 10 ng genomic DNA. PCR cycling parameters were as follows: denaturation at 95 °C for 15 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing for 30 s (see Table 1 for specific temperatures) and elongation at 72 °C for 45 s, with a final extension at 72 °C for 7 min. Differently labelled loci were combined for genotyping with a ROX500 size standard (ROX1000 standard for locus TabrH2; Applied Biosystems), using 1 μL of each PCR and 0.5 μL of the size standard, and then brought to 15 μL with Hi-Di Formamide (Applied Biosystems). PCR fragments were denatured (95 °C for 5 min) and separated by capillary electrophoresis in an ABI PRISM 3100 genetic analyser (Applied Biosystems). Raw allelic peak data were analysed using GENESCAN version 3.1 software (PerkinElmer). Five of the 19 identified loci were not amplifiable in the PCR, and another five were unscorable due to the presence of multiple ambiguous peaks. All individuals were genotyped for the nine successful loci for a minimum of 2× coverage.

The nine loci surveyed were extremely polymorphic, having 15–55 alleles per locus (average 36.7; Table 1).

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Table 1 Characteristics of microsatellite loci isolated from *Tadarida brasiliensis*, including GenBank Accession nos, primer sequences, PCR conditions and observed (H_O) and expected (H_E) heterozygosity values for populations from Huntsville, Texas ($n = 43$) and Rosario, Argentina ($n = 48$). Significant deviations from Hardy–Weinberg equilibrium are indicated (*, $P < 0.001$; †, $P < 0.05$). Fluorescent dyes used are indicated at the end of the primer sequence (‡, NED; §·, HEX; ¶, 6-FAM)

Locus GenBank Accession no.	Repeat motif	$MgCl_2$ (mM)	T_a	No. of alleles	Size range	Texas		Argentina		Primer sequences (5′–3′)
						H_O	H_E	H_O	H_E	
TabrA10	GA	1.5	65	20	226–268	0.821	0.931	0.756†	0.934	F: AAGTGGTTGGGCGTTGTC‡
AY954900										R: GCGATGCACTGCCTTGAGA
TabrA30	GA	2.0	57	27	193–281	0.821†	0.946	0.326	0.333	F: AGTCGCGGGTTTGATTCCAGTTA
AY954901										R: ACCCCTTCCCTTTGTTCCTTCAG§
TabrD10	GATA	2.0	50	15	308–376	0.900†	0.801	0.773	0.818	F: CCCCACTCATTTATCCATCCACA‡
AY954902										R: ATCTCGCAGCTATTGAAGTA
TabrD15	GATA	1.5	58	29	235–395	0.974	0.946	0.872†	0.945	F: AGTCCTGGCTCCTATTCTCATTG¶
AY954903										R: CTATCCGTCTACCTGTCCGTCTAT
TabrE9	GA	1.5	52	36	349–461	0.925	0.953	0.814†	0.952	F: GTTTGTCTTCCCCACTGA
AY954904										R: CTTAGGACAGGAGAAGTCA¶
TabrH2	TAGA	2.0	60	55	403–735	0.842†	0.968	0.696*	0.968	F: AGTGGGTCAGTGGGTCA¶
AY954905										R: ATGGCATTCTACCGGACA
TabrH3	TAGA	1.5	55	47	140–304	0.775*	0.953	0.698*	0.942	F: ATGGACATAGAGGAGAGACA¶
AY954906										R: TGTCTCTGTCTCTCTGTGA
TabrH6	TAGA	1.5	55	47	187–357	0.923	0.933	0.936	0.952	F: ATCTCTCCAGTCCTTACCA§
AY954907										R: TTTACCCTCCACAGTCTCA
TabrH12	TAGA	2.0	57	54	126–366	0.795*	0.972	0.846†	0.959	F: CCATGTGAGCCAATTCCTA§
AY954908										R: GTCAGGACTCTCCAGAGA

Heterozygosity values, deviations from Hardy–Weinberg equilibrium (HWE) and patterns of linkage disequilibrium (LD) were assessed using the GENEPOP version 3.4 software package (Raymond & Rousset 1995). Every locus but one (TabrH6) showed a significant deviation from HWE in at least one population (Table 1). This is undoubtedly due, at least in part, to the presence of null alleles. Locus TabrH2, for example, failed to amplify in a significant portion of both populations (11.6% of the Texas population and 52.1% of the Argentina population). There is also evidence of heterozygote excess at locus TabrD10 in the Texas population. The higher incidence of heterozygote deficiency in the Argentina population (six loci, vs. four loci in the Texas population) is likely due to the fact that the source DNA for the genomic libraries originated in North America. Previous studies have shown that South American populations of *T. brasiliensis* are significantly differentiated from North American populations (Russell & McCracken in press), so it is not unexpected that null alleles would be more common in the South American population. Observed heterozygosity was higher in the North American than in the South American population at seven of the nine loci. In addition to the apparently higher incidence of null alleles in South American bats, this may reflect the substantially larger effective population size of North American *T. brasiliensis* (Russell & McCracken in press). We found no consistent signal of LD between any pair of loci.

These highly variable loci should prove invaluable in studies requiring the identification of individuals, such as mating and paternity studies and analyses of long-distance dispersal patterns.

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References

Cockrum EL (1969) Migration in the guano bat, *Tadarida brasiliensis*. *Miscellaneous Publications, University of Kansas Museum of Natural History*, **51**, 303–336.

French B, Lollar A (1998) Observations on the reproductive behavior of captive *Tadarida brasiliensis mexicana* (Chiroptera: Molossidae). *Southwestern Naturalist*, **43**, 484–490.

Giegerich R, Meyer F, Scheiermacher C (1996) GENEFISHER — software support for the detection of postulated genes. In: *Proceedings of the Fourth International Conference on Intelligent Systems for Molecular Biology*, pp. 68–77. AAAI Press, Menlo Park.

Jones KC, Levine KF, Banks JD (2002) Characterization of 11 polymorphic tetranucleotide microsatellites for forensic applications in California elk (*Cervus elaphus canadensis*). *Molecular Ecology Notes*, **2**, 425–427.

McCracken GF, Wilkinson GS (2000) Bat mating systems. In: *Reproductive Biology of Bats* (eds Crichton EG, Krutzsch PH), pp. 321–362. Academic Press, New York.

Raymond M, Rousset F (1995) GENEPOP (version 3): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Russell AL, McCracken GF (in press) Population genetic structure of very large populations: the Brazilian free-tailed bat, *Tadarida brasiliensis*. In: *Functional and Evolutionary Ecology of Bats* (eds Zubaid A, McCracken GF, Kunz TH). Oxford University Press, New York.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Worthington Wilmer J, Barratt E (1996) A non-lethal method of tissue sampling for genetic studies of chiropterans. *Bat Research News*, **37**, 1–4.