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TECHNICAL NOTE

Characterization of small microsatellite loci isolated in endangered Indiana bat (*Myotis sodalis*) for use in non-invasive sampling

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Abstract Primers for 10 microsatellite loci were developed specifically to amplify low quantity and quality DNA in the endangered Indiana Bat (*Myotis sodalis*). In a screen of 20 individuals from a population in Missouri, the 10 loci were found to have levels of variability ranging from seven to 18 alleles. No loci were found to be linked, although two loci revealed significant departures from Hardy–Weinberg equilibrium. These microsatellite loci will be applicable for population genetic analyses and for use in mark-recapture studies that utilize DNA collected non-invasively from fecal pellets, which will ultimately aid in management efforts.

Keywords Indiana bat · Microsatellite · *Myotis sodalis* · Non invasive sampling

Introduction

The Indiana bat (*Myotis sodalis*) was listed as an endangered species in 1967. Despite protection, populations continue to decline, particularly in the southern portion of the range (Clawson 2002). Declines are likely the result of multiple factors including modification and disturbance of hibernacula (Richter et al. 1993), loss of summer habitat (Kurta 2005; Sparks et al. 2005), and impacts of pesticides (O'Shea and Clark 2002). Although many factors have been identified as contributing to these declines, virtually no recent information is available about population demographics. This lack of information is primarily a result of limitations associated with traditional monitoring techniques that do not provide researchers with the ability to regularly track individual bats throughout a field season.

Advances in molecular genetic techniques have made it possible to uniquely identify animals using DNA as an individual mark for mark-recapture studies (Ernest et al. 2000; Taberlet et al. 1997). Although DNA has been successfully extracted from feces and used in this manner for a number of other mammals, it has only recently been attempted on bat fecal pellets (Vege and McCracken 2001; Zinck et al. 2004; Puechmaille et al. 2007; Zielinski et al. 2007). Because accurate demographic and relatedness information is critical for the management and recovery of the Indiana bat, we isolated and designed primers for microsatellites in this species.

We isolated microsatellites specifically from Indiana bats using the technique developed by St John and Quinn (2008). Captured fragments were amplified and cloned using PCR-Script® Amp cloning kit (Stratagene) following manufacturer's protocol. The isolation yielded 74 clones that were subsequently sequenced.

A colony touch protocol was used to isolate the plasmid DNA. Using a sterile pipette tip, the colony was transferred to a tube containing 100 µl T.E (10 mM Tris, 0.1 mM EDTA, pH 8.0) and heated to 100°C for 10 min. A 1 µl aliquot was used as the template in a 25 µl polymerase chain reaction (PCR) as in St John et al. (2005). The PCR products were purified for sequencing by the addition of 5 U exonuclease I (USB) and 0.5 U shrimp alkaline phosphatase (USB) and a subsequent 37°C incubation for 30–45 min. These two enzymes were denatured by a 15 min 80°C incubation. Sequencing was performed using 4 µl prepared template and a Quickstart kit (Beckman Coulter) following manufacturer's protocol except using half reaction volumes (10 µl). Sequencing reactions were

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run on the CEQ8000 XL DNA Analysis System (Beckman Coulter).

Primers were designed for 22 loci using the software PRIMER 3 (Rozen and Skaletsky 2000). Eighteen of the 22 loci consistently produced products of the expected size and subsequently were screened for polymorphism using DNA previously isolated from 20 Indiana bats from Missouri. Ten of these primer pairs were polymorphic and easy to score. Screening PCRs were performed in 10 μL reactions containing 100μM of each dNTP, 1X GoTaq Flexi Buffer (Promega), 1.25 mM $MgCl_2$, 1X BSA, 0.5 μM of each primer (dye-labeled forward), and 1 U of *Taq* DNA polymerase (Promega). The amplification conditions were as follows: 94°C for 2 min, then 94°C for 30 s, annealing temp (Table 1) for 30 s, 72°C for 30 s for 30 cycles, then 72°C for 10 min and a final extension at 60°C for 45 min. The PCR products were run on the CEQ8000 XL DNA Analysis System (Beckman Coulter). All loci were run with the S400 size standard (Beckman Coulter) and analyzed using the Frag 3 default method associated with the CEQ8000 XL DNA Analysis System.

For each polymorphic locus, we calculated observed heterozygosity (H_O), expected heterozygosity (H_E) and null allele frequencies using CERVUS 1.0 (Marshall et al. 1998). GENEPOP version 3.4 (Raymond and Rousset 2000) was used to test for evidence of linkage disequilibrium and deviations from Hardy–Weinberg equilibrium. The number of alleles per locus ranged from seven to 18, and single locus heterozygosities ranged from 0.500 to 0.900 (Table 1). Significant deviations from Hardy–Weinberg equilibrium were observed at 2 loci and high null allele frequencies were detected at 4 loci (Table 1). We tested 45 pairwise comparisons and found no evidence for genotypic linkage disequilibrium between any set of paired loci after a sequential Bonferroni was applied ($P < 0.0002$). Each primer pair was tested on multiple DNA samples extracted from fecal pellets and amplified successfully (data not shown) using the multiplex pre-amplification method described by Piggott et al. (2004). These loci will enable researchers to gather information regarding the population structure and relatedness of Indiana bats from molecular tagging studies including those utilizing non-invasively collected DNA from fecal pellets.

Table 1 Characterization of 10 polymorphic microsatellite loci developed for the Indiana Bat

Locus	GenBank accession No.	Primer sequence	T_A (°C)	Repeat of cloned allele	A	Allele size range	H_E	H_O
IBat CA5	HQ127370	F-CTCTGCCTCTTCATGCTGCT R-GGAGGCAACCAATTGATGTT	60	$(CA)_{18}$	14	185–217	0.921	0.800†
IBat CA11	HQ127371	F-AGAACCCAGTGCCCTTCTTT R-ATGAGAGGGGGATGTGACAG	58	$(CA)_{20}$	10	165–185	0.859	0.900
IBat CA13	HQ127372	F-TGGTTTGCTATCTGCTTCCA R-TTGCCAGGTATGTGAAGTCTG	58	$(CA)_{22}$	16	107–155	0.938	0.500*†
IBat CA19	HQ127373	F-TCTCTCTCCCTCTCCCTTCC R-GACCTTAGCAAAAATGCCACA	60	$(CA)_{17}$	13	104–132	0.901	0.850
IBat CA33	HQ127374	F-TCTCTCTCTCTTCCCCCTCTC R-GCCTCTCCTGTAAATGCTTACC	58	$(CA)_9GA(CA)_5$	8	132–152	0.692	0.700
IBat CA38	HQ127375	F-GCATCTTTCCCAAGCCATTA R-GCCTCCTGCAAGAGTTGTTC	60	$(CA)_{16}$	15	157–189	0.914	0.800†
IBat CA47	HQ127376	F-ACAAGTGCCTGTGGCTTCTT R-GTAGGCGGCTTCATCCTACA	58	$(CA)_{19}$	16	101–149	0.932	0.850
IBat M7	HQ127377	F-TCCCAGTCTCCTGGTCAAAC R-CCAAGGATAAGACAGGAAAGTCT	58	$(GATA)_{12}$	7	94–134	0.723	0.800
IBat M22	HQ127378	F-GCAAAGCCTCCCTCAAATAA R-GGAGCATTTCTGCCCATCTA	58	$(GATA)_{23}$	17	77–333	0.930	0.526*†
IBat M23	HQ127379	F-ATCCTGGGTTTTGGGTTCAT R-TCATGTAAATTTCAAAAACAGCAAA	58	$(GATA)_{14}$	18	150–222	0.933	0.900

Annealing temperature (T_A), number of alleles (A), expected (H_E) and observed (H_O) heterozygosities are reported. Loci were screened in 20 individuals except for locus M22 which was run on 19 individuals

* Significant deviation from Hardy–Weinberg equilibrium ($\alpha = 0.05$)

† Frequency of null alleles > 0.05

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