Molecular Ecology Notes (2005) **5**, 387–389 doi: 10.1111/j.1471-8286.2005.00936.x

PRIMER NOTE

Tetranucleotide, trinucleotide, and dinucleotide loci from the bobcat (*Lynx rufus*)

BRANT C. FAIRCLOTH,* ALLISON REID,* TERESA VALENTINE,* SOO HYUNG EO,* THERON M. TERHUNE,* TRAVIS C. GLENN,†‡ WILLIAM E. PALMER,§ CAMPBELL JOSEPH NAIRN* and JOHN P. CARROLL*

**D. B. Warnell School of Forest Resources, University of Georgia, Athens, GA, 30602, USA, †Savannah River Ecology Laboratory, University of Georgia, P.O. Drawer E, Aiken, SC 29802, USA, ‡Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA, §Tall Timbers Research Station, 13093 Henry Beadel Drive, Tallahassee, FL 32312, USA*

Abstract

We describe primers and polymerase chain reaction (PCR) conditions to amplify four dinucleotide, one trinucleotide, and three tetranucleotide microsatellite DNA loci from the bobcat (*Lynx rufus*). The primers were tested on 22 individuals collected from a population located within southwestern Georgia (USA). The primer pairs developed in this study yielded an average of 7.4 alleles per locus (range four to 10), an average observed heterozygosity of 0.60 (range 0.40 to 0.76), and an average polymorphic information content of 0.70 (range 0.51 to 0.78).

Keywords: bobcat, dinucleotide repeats, *Lynx rufus*, microsatellites, primers, SSRs, tetranucleotide repeats, trinucleotide repeats

Received 14 December 2004; revision accepted 26 January 2005

Bobcats (*Lynx rufus*) are the most widely distributed North American felid, ranging throughout most of the United States and Canada into southern Mexico (Lariviére & Walton 1997). They are solitary carnivores with few social interactions other than those resulting from mating and parentage (Anderson 1987). Bobcat population structure and spatial organization are largely dependent on the establishment and inhabitance of individual home ranges. Home ranges exhibit sex-specific differences: males inhabit larger home ranges with varying degrees of intra- and intersexual overlap, whereas females maintain smaller, primarily exclusive, home ranges (Anderson 1987; Conner *et al*. 1999). It is hypothesized that (i) genetic relatedness may influence degrees of intra- and intersexual home range overlap, and (ii) competition for mating may occur in areas where several male ranges overlap that of female (Anderson & Lovallo 2003). Additionally, little is known regarding multiple mating, multiple paternity, or sperm competition in the bobcat beyond the belief that bobcats are polygynous (Provost *et al*. 1973).

In succeeding discussions, we describe the development of a panel of species-specific microsatellite markers that will enable study of the relationship between kinship, dispersal, and spatial distribution in the bobcat in addition to facilitating future research on population genetics, population structure, and mating system(s) of these organisms.

Correspondence: Brant C. Faircloth, Fax: 706 542 8356, E-mail: brant@uga.edu

We extracted DNA from tissue obtained from a bobcat of unknown sex using a DNeasy tissue kit (QIAGEN). We digested DNA with *Bst*UI (New England Biolabs) and enriched for dinucleotide, trinucleotide, and tetranucleotide repeats (Glenn & Schable in press). Product was ligated into polymerase chain reaction (PCR) 2.1-TOPO vector and used to transform OneShot Top 10 chemically competent *Escherichia coli* cells (TOPO TA Cloning Kit, Invitrogen). Colonies were screened for inserts using the β-galactosidase gene. Positive (white) colony PCR products of 500–1000 bp were sequenced using BIGDYE version 3.0 (PE Applied Biosystems) chemistry and an ABI 3700 sequencer. Sequences were assembled and edited in SEQUENCHER version 4.2 (Gene Codes) and exported into EPHEMERIS 1.0 (http://www.uga.edu/srel/DNA_Lab/programs.htm) to search for microsatellites. Primers were developed and an M13-reverse or CAG tag was added to the 5′ end of one of each primer pair using OLIGO version 4.0 (Molecular Biology Insights) to facilitate fluorescent size detection on an ABI 377 (Schable *et al*. 2002). GTTT 'pigtails' were added to the 5′ end of each primer, lacking either

© 2005 Blackwell Publishing Ltd

Table 1 Characterization of primer pairs amplifying microsatellite loci from *Lynx rufus*

Locus	Primer sequence (5′–3′)	GenBank Accession no.	Dye	Annealing start temp. (°C)	Repeats in cloned allele	Clone size (bp)	*N*	*A*	Size	H_O	H_E	P_{HW}	PIC
BC1AT U	GTTTAAGAAAGATGGAGCAGTCAG	AY771652	HEX	50	$(AAAG)_{13}$	318	22	7	309–333	0.73	0.78	0.49	0.72
BC1AT L	CAGTCGGGCGTCATCATAAAGAAAAGGGAAGTAACC												
BCB12D U	GTTTCTCCATTTCCCTTTGTAGTA	AY771651	FAM	50	$(AC)_9$	275	19	8	282–352	0.63	0.81	0.01	0.77
BCB12D L	GGAAACAGCTATGACCATACTTTTGTCTCCCTTTGTTA												
BCD1T U	CAGTCGGGCGTCATCAGCTTATACTTAGGGACTTAC	AY771645	HEX	50	$(AAC)_9$	260	22	4	282–288	0.41	0.56	0.04	0.51
BCD1T L	GTTTCAAGGTATGTAGATTTCAGT												
BCD8T U	GTTTCCTGCTGCTACTTGATAC	AY771648	FAM	60	$(ATCT)_6 \ldots (ATCT)_5$	185	22	10	158–269	0.55	0.56	0.63	0.54
BCD8T L	GGAAACAGCTATGACCATTCCAGACTGCTAACACTTT												
BCE5T U	CAGTCGGGCGTCATCATAGCTCTCCAGAGAAACATA	AY771646	HEX	50	$(AGAT)_8$	261	21	7	260–278	0.76	0.82	0.14	0.77
BCE5T L	GTTTAAACCAAAAGGCTAAGTAAG												
BCG3D U	GTTTGTTGACTGTTTCCACCTGAG	AY771647	HEX	60	$(AG)_{16}$	221	22	8	220–240	0.59	0.82	0.01	0.77
BCG3D L	CAGTCGGGCGTCATCAAGCTGAGGTAAAAGACATT												
BCG8T U	CAGTCGGGCGTCATCATAGGCTGGGTAGAAGTGAGT	AY771649	HEX	50	$(AC)_{19}$	265	20	9	278–300	0.65	0.81	0.25	0.77
BCG8T L	GTT TCAAGACACATTCAAGCATAA												
BCH6T U	CAGTCGGGCGTCATCAGACAAGGACAATCAGAACAG	AY771650	HEX	55	$(AC)_{21}$	162	20	6	176–186	0.50	0.83	0.01	0.78
BCH6T L	GTTTGGTATAGTCCCAGTCATTTA												

Sequences used to introduce sites for the universal primer are in italics.
Underlined bases indicate sharing of nucleotides between CAG (5′–CAGTCGGGCGTCATCA–3′) or M13R (5′–GGAAACAGCTATGACCAT–3′) tag and the locus specific primer binding site.
Clone size refers to the predicted size of the PCR product amplified from the clone used to develop each locus; *N*, number of individuals genotyped at each locus; *A*, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; P_{HW}, probability that genotype proportions conform to HWE; and PIC, polymorphic information content.

CAG or M13-reverse tag, to facilitate the nontemplated addition of adenosine by *Taq* polymerase (Brownstein *et al*. 1996).

Primer pairs were optimized using bobcat DNA samples obtained from wild individuals at the Joseph W. Jones Ecological Research Center (JWJERC), Newton, Georgia, USA. PCR amplifications were performed in 10-μL volumes using MyCytler thermal cyclers (Bio-Rad Laboratories). Final concentrations for optimizing reactions were 10 mM Tris pH 8.4, 50 mM KCl, 0.5 μM 'pigtailed' primer, 0.05 μM CAG or M13-reverse tagged primer (CAG or M13-reverse + primer), 0.45 μM dye labelled tag (HEX or FAM + CAG or M13-reverse), 1.5 mM $MgCl_2$, 0.5 mM dNTPs, 0.5 U *Taq* DNA polymerase, and 56 ng DNA. M13 and CAG universal primers were labelled with FAM or HEX fluorescent dyes. Reactions were optimized with seven individuals using four touchdown thermal cycling programs (Don *et al*. 1991), each encompassing a 7.5-°C span of annealing temperatures (ranges: 50–42.5 °C, 55.0–47.5 °C, 60–52.5 °C, 65.0–57.5 °C). Cycling parameters were: 15 cycles at 95 °C for 20 s; highest annealing temperature for 20 s minus 0.5 °C per annealing cycle; and 72 °C for 30 s, followed by 15 cycles of 95 °C for 20 s; 42.5, 47.5, 52.5, or 57.5 °C, respectively, for 30 s; 72 °C for 30 s; and a final extension period of 10 min at 72 °C.

PCR products were checked for amplification and scored using an ABI Prism 377–96 sequencer with GENESCAN Rox500 fluorescent size standard (Applied Biosystems). Results were analysed using GENESCAN and GENEMAPPER software (Applied Biosystems) and the optimal touchdown cycling schemes were identified. Following optimization, 15 additional individuals were genotyped. We calculated observed and expected heterozygosities and polymorphic information content for each locus using CERVUS version 2.0 (Marshall *et al*. 1998). GENEPOP version 3.4 (Raymond & Rousset 1995) was used to test for Hardy–Weinberg equilibrium (HWE) and genotypic linkage disequilibrium (LD), and a posteriori sequential Bonferroni correction was conducted according to the method of Rice (1989).

Table 1 summarizes the characteristics of eight primer pairs developed from the bobcat. The number of alleles per locus ranges from four to 10, averaging 7.4. Total exclusionary power with both unknown parents is 0.98. No significant LD was detected after sequential Bonferroni correction. Three loci (*BCH6T*, *BCB12D*, *BCG3D*) deviated from HWE (P values < 0.01) but are not significant following Bonferroni correction.

Acknowledgements

This research was supported by: Tall Timbers Research Station's Quail Research Initiative; Daniel B. Warnell School of Forest Resources (WSFR); McIntire–Stennis project GEO-0100-MS, and the JWJERC. This research benefited from the University of Georgia (UGA) Plant Center's 2003 Microsatellite Workshop which was supported by the UGA Office of the Vice President of Research, WSFR, UGA Department of Plant Biology, and award DE-FC09–96SR18546 to Savannah

River Ecology Laboratory from the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research, US Department of Energy. Capture, handling, and sedation of bobcats at JWJERC were approved by the Georgia Department of Natural Resources (permit: 29-WMB-04–84) and the UGA Institutional Animal Care and Use Committee (approval: A99-0159).

References

Anderson EM (1987) *A Critical Review and Annotated Bibliography of Literature on the Bobcat*. Special Report No. 62, Colorado Division of Wildlife, Terrestrial Wildlife Research, Ft. Collins.

Anderson EM, Lovallo MJ (2003) Bobcat and lynx. In: *Wild Mammals of North America: Biology, Management, and Conservation*, 2nd edn (eds Feldhamer GA, Thompson BC, Chapman JA). Johns Hopkins University Press, Baltimore.

Brownstein MJ, Carpenter JD, Smith JR (1996) Modulation of non-templated nucleotide addition by *Taq* polymerase: primer modifications that facilitate genotyping. *BioTechniques*, **20**, 1004–1010.

Conner LM, Plowman B, Leopold DB, Lovell C (1999) Influence of time-in-residence on home range and habitat use of bobcats. *Journal of Wildlife Management*, **63**, 261–269.

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research*, **19**, 4008.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. In: *Methods in Enzymology 395, Molecular Evolution: Producing the Biochemical Data, Part B* (eds Zimmer EA, Roalson EH). Academic Press, San Diego.

Larivière S, LR.Walton (1997) *Lynx rufus*. *Mammalian Species*, **563**, 1–8.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Provost EE, Nelson CA, Marshall AD (1973) Population dynamics and behaviour in the bobcat. *World's Cats*, **1**, 42–67.

Raymond M, Rousset F (1995) GENEPOP version 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analysing tables of statistical tests. *Evolution*, **43**, 223–225.

Schable NA, Fischer RU, Glenn TC (2002) Tetranucleotide microsatellite DNA loci from the dollar sunfish (*Lepomis marginatus*). *Molecular Ecology Notes*, **2**, 509–511.