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PRIMER NOTE

Isolation and characterization of microsatellite loci in the black rat snake (*Elaphe obsoleta*)

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Abstract

We obtained molecular markers useful for population level studies of the black rat snake (*Elaphe obsoleta*) by screening genomic DNA libraries enriched for dinucleotide, tetranucleotide, and pentanucleotide microsatellite repeats. Following sequencing of the positive clones, 11 pairs of primers were designed for polymorphic loci and their variability assessed in > 350 individuals from four populations in North America. The loci had between 9 and 40 alleles and observed heterozygosities ranged from 0.071 to 0.87. Some of these pairs of primers also successfully amplified DNA from two other snake species.

Keywords: black rat snake, DNA loci, *Elaphe obsoleta*, microsatellite

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The use of highly variable genetic markers has transformed the studies of kinship, behaviour and population biology of many vertebrates, but their use in snakes remains limited (Gibbs & Weatherhead 2001). Here we describe the isolation of microsatellite DNA loci from the black rat snake (*Elaphe obsoleta*) for the purpose of detecting fine-scale patterns of genetic structuring and for assigning parentage. This species has already been the subject of considerable study at the population level (Lougheed *et al*. 1999; Blouin-Demers *et al*. 2002).

We isolated nuclear microsatellite DNA loci following two established protocols. To obtain dinucleotide loci, we followed Gibbs *et al*. (1998) and screened a plasmid library containing 300–400 bp inserts of genomic DNA that had been digested with *Alu*I, *Hae*III, and *Rsa*I with dinucleotide polymers (CA_n, GC_n, and CT_n [Pharmacia]) that had been radioactively labelled via random priming. Positive clones were then manually cycle-sequenced (AmpliCycle; Perkin Elmer). To obtain loci consisting of pentanucleotide repeats, we generated a plasmid library containing genomic DNA inserts that had been enriched for 18 distinct 5 bp repeats via a hybridization capture procedure developed by T. Glenn (Savannah River Ecology Laboratory, University of Georgia) which is a modification of the protocol described by Hamilton *et al*. (1999). Clones containing inserts were then sequenced, with no additional screening, using BigDye termination (Applied Biosystems) and the products resolved on an ABI 3100 sequencer. Pairs of primers were made from the sequence region flanking the microsatellite repeat in each clone.

Variation at these loci was then analysed via polymerase chain reaction (PCR) for more than 350 individuals (Table 1) from four regional populations: Arkansas, Maryland, south-western Ontario, and eastern Ontario (Lougheed *et al*. 1999). The PCR cocktail consisted of 1 × of PCR buffer (Invitrogen Life Technologies), 200 μM of dNTPs, 0.1 μM of un-labelled forward primer, 0.1 μM of labelled forward primer, 0.2 μM of reverse primer, 2500 μM of magnesium chloride, 0.25 U of *Taq* DNA Polymerase (Invitrogen Life Technologies), and 100 ng of template DNA brought to a total reaction volume of 10 μL with doubly distilled water. For the dinucleotide loci (Eobμ1–4, 10, 13, 16, and 34) the forward primer was end-labelled with γ^{33}P-ATP whereas the pentanucleotide loci were end-labelled with one of the ABI fluorescent dyes 6-FAM, HEX, and NED. The PCR was conducted using a PTC-100 thermal cycler (MJ Research) and consisted of 5 min of denaturation at 94 °C, 35 cycles of 20 s annealing at 56 °C, 45 s extension at 72 °C and 20 s of melting at 94 °C, followed by 5 min of final extension at 72 °C. Radioactively labelled products were resolved on 6% denaturing polyacrilamide gels and scored via reference to a known size clone while fluorescently labelled products were run on an ABI 310 sequencer with an internal size marker and analysed using GENESCAN software.

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Table 1 Eleven microsatellite loci isolated from black rat snakes (*Elaphe obsoleta*) with primer sequences, repeat motif and size from the original cloned allele, the number and size range of alleles, the observed (H_O) and expected (H_E) heterozygocities [calculated using GENEPOP: Raymond & Rousset (1995)] and the GenBank accession number. In all cases, the forward primer was labelled. In total, 392 individuals were genotyped at loci 2, 3, and 4, whereas 1227 individuals were genotyped at the other loci. Annealing temperatures for all loci were 55 °C

Locus	Primer sequences (5′ to 3′)	Motif	Size of clone	No. of alleles (size range)	H_O	H_E	GenBank no.
Eobμ1	F: ATCAGTAGGAGTGAGAGCAACT R: CTGCATACTCTTCCAGAACC	$(TG)_{21}$	158 bp	17 (136–190 bp)	0.664	0.628	AF544651
Eobμ2	F: CTTGGGGAGAAAGTGTCAT R: TGGCTGGATTCTTACAAGT	$(AG)_{10}$	156 bp	9 (150–170 bp)	0.413	0.663	AF544652
Eobμ3	F: ATTTGGTAGCCATCACATC R: CAGTCCTAAATGTTCTGTTGA	$(AC)_{17}$	123 bp	18 (115–176 bp)	0.071	0.239	AF544653
Eobμ4	F: TTCAGGTTTGGTTGAATTATGA R: AATGCTGACTCAAAGGCATG	$(TG)_{18}$	155 bp	18 (127–169 bp)	0.408	0.803	AF544654
Eobμ10	F: ATTGACTTCATAGCACAATGTCA R: CAGAGTCTCCTTGGTGAGAAG	$(AC)_{15}$	130 bp	18 (112–152 bp)	0.853	0.838	AF544655
Eobμ13	F: TGATCTGAGTCTCTTTCTGG R: CAATTCAAATCCATTGGTTT	$(AC)_{20}$	149 bp	15 (133–165 bp)	0.738	0.317	AF544656
Eobμ16	F: AGACCACAAGTTTGCACTCC R: AGCATTTTCCTAGCAAAGGAT	$(AC)_{27}$	179 bp	40 (139–249 bp)	0.868	0.914	AF544657
Eobμ34	F: CTCTGAGTTATTCAGGGTTGGATC R: GCAGTCCCAAAGAAAGAACG	$(TC)_{22}(AC)_{14}$	125 bp	34 (95–191 bp)	0.763	0.792	AF544658
Eobμ358	F: CAAATATGTTGGTCTCTAAGCA R: CAACCAACCAACCAACTAAC	$(GATG)_8$	193 bp	9 (83–229 bp)	0.742	0.695	AF544659
Eobμ366	F: ACCGTGTCACATTACCAAAC R: GGTGTAGAGTTTCCTGCCTA	$(GAATA)_{14}$	204 bp	12 (175–255 bp)	0.846	0.788	AF544660
Eobμ373	F: GAGACCATATGCACCAAGAC R: GGCTGAAGTTTACTGGTCTG	$(CTAAT)_{17}$	193 bp	10 (158–223 bp)	0.830	0.859	AF544661

Eleven loci were successfully amplified and showed high levels of polymorphism and heterozygosity (Table 1). The number of alleles ranged from 9 to 40 with observed heterozygosities from 0.071 to 0.868. Two of the microsatellites loci showed anomalous patterns. Eobμ3 was invariant in the two Canadian populations while, for Eobμ4, the reduction in the observed number of heterozygotes relative to the expected value suggests the presence of null alleles. The applicability of these microsatellite loci to other snake species was determined by testing the markers on two individuals of three other snake species and looking for a clear band approximately the same size as the *Elaphe obsoleta* band on starch gels stained with ethidium bromide. All primer pairs except Eobμ3 amplified template DNA from eastern fox snakes (*Elaphe gloydi*). Eobμ1, Eobμ2, Eobμ3, Eobμ10, and Eobμ13 primer pairs amplified template DNA from northern water snakes (*Nerodia sipedon*). No primer pair amplified template DNA from eastern massasauga rattlesnakes (*Sistrurus catenatus*).

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