Molecular Ecology 1997, 6, 399–400

PRIMER NOTE

Characterization of microsatellite loci in the Northern Idaho ground squirrel *Spermophilus brunneus brunneus*

B. MAY,*† T. A. GAVIN,† P. W. SHERMAN‡ and T. M. KORVES*
* *Department of Animal Science, Meyer Hall, University of California, Davis, CA 95616, USA*†*Department of Natural Resources, Fernow Hall, Cornell University, Ithaca, NY 14853, USA,* ‡*Section of Neurobiology and Behavior, Mudd Hall, Cornell University, Ithaca, NY 14853, USA*

The Idaho ground squirrel *Spermophilus brunneus* is the rarest sciurid in North America. A northern (*S. b. brunneus*) and a southern subspecies (*S. b. endemicus*) have recently been described (Yensen 1991). The former is currently found in only 18 populations in Adams and Valley counties (west–central Idaho); the largest population is < 300 animals and the subspecies consists of < 1000 animals (Yensen & Sherman, in press). Reductions in *S. b. brunneus*'s range and population sizes are apparently due to major alterations of the habitat in the last 100 years. Many of the resultant small disjunct populations have recently become extinct, and others are in imminent danger of extinction. Previously we used allozymes to study mating behaviour (Sherman 1989) and population structure (T. A. Gavin *et al*. unpublished data). Recently we developed microsatellite markers in *S. b. brunneus* to enhance the resolving power of our data for understanding the extant and historical population structuring of *S. b. brunneus*, prior to restorative efforts.

Samples of *S. b. brunneus* consisted of (i) blood taken from the suborbital sinus or (ii) buccal scrapings taken with a toothpick and placed in 1.5-mL cryovials and either air-dried or immersed in 0.5 mL of lysis buffer (White & Densmore 1992). DNA was extracted from 150 to 200 μL blood or buccal scrapings using QIAGEN Blood Kits.

Genomic libraries were constructed from the DNA from a single individual by digesting with *Sau*3AI, ligating into pUC 18, and transforming DH5α competent cells (see May *et al*. in press for more detail). One library of 196 colonies (Library I) was dot-blotted and screened with total genomic DNA. A second library of 53 000 colonies (Library II) was lifted and screened with trimer and tetramer oligonucleotides from BIOS Laboratories (AAAT, AAAG, AAAC, CCG, AAG, AAT, AAC, and CAG). Total genomic DNA and oligos were end labeled (tailed) with either the Oligonucleotide 3′-End Labeling Kit or the Oligonucleotide Tailing Kit from Boehringer Mannheim. Positives were detected with the Lumi-Phos Chemiluminescent Kit or the colorimetric (chromogenic) method using the NBT/BCIP Kit (Boehinger Mannheim). Initially positive colonies were screened two more times for confirmation. Tertiary positives with inserts of 300–1500 bp were sequenced on an ABI 373A automated sequencer. Microsatellite sequences were located in 8/8 positives from Library I and 17/26 positives from Library II, and primers were designed for five and 15 of the sequences, respectively.

Polymerase chain reaction amplifications were carried out in an MJR PTC-100 thermocyler in 50-μL reactions containing 3–10 ng DNA, 1.5 mM $MgCl_2$, 0.4 μM each primer, 175 μM dNTPs, and 1 unit of GIBCO *Taq*. Amplification conditions were 94 °C for 3 min; 34 cycles of 94 °C for 1 min, 52° (57 °C for IGS-BM1) for 30 s, and 72 °C for 30 s; and a final extension of 5 min at 52 °C (or 57 °C). Amplified samples were concentrated from 25 μL down to 10 μL in a vacuum centrifuge and run on 4% 1 × TBE MetaPhor (FMC) agarose gels (Fig. 1) at 460 V (17 V/cm), with the circulating buffer (0.5 × TBE) at 12 °C for the first 5 min and the remainder of the run at 20 °C. Gels were run for 45 min to 1.5 h depending on size of PCR product (about 15 min per 25 base pairs). Gels were stained with ethidium bromide.

The amplification effectiveness of the 20 primer pairs was tested against the clone and three individual squirrel DNA extracts. Thirteen pairs produced a resolvable amplicon in all four samples. The other seven amplified only in the clone or did not produce sufficient product to be useful. Each of the useable 13 primer pairs was subsequently tested against 10 individuals from across the range of *S. b. brunneus*. Primer sequences, GenBank accession numbers, repeat motif, clone amplicon size, and number of alleles detected for these 13 loci are presented in Table 1. The first three loci were from Library I and the latter 10 loci were from Library II.

Usually only the primary amplicons were observed, as well as one or two heteroduplex bands in heterozygous individuals, demonstrating that amplification was

Keywords: microsatellite, primer, ground squirrel, *Spermophilus*, MetaPhor agarose

Received 9 July 1996; revision accepted 26 October 1996

Correspondence: Bernie May. Tel.: +1-916-754 8123, Fax: +1-916-752-0175. E-mail: bpmay@ucdavis.edu

Locus	Primer Sequence 5′-3′	GenBank no.	Motif	Size (bp)	Alleles
IGS-1	ATAACAGCACCCTGCTCCAC AATCCATCCTCTACCTGTAATGC	U73691	$(CA)_{20}$	103	3
IGS-4	CAGTAACCTTCCCAACTCTGG GGGAGGAAATATACCTGGCC	U73692	$(AAAAAC)_4$	140	2
IGS-6	GGGCATTAATTCCAGGACTT GGGCTGGAATTAAAGGTATCA	U73693	$(CA)_{28}$	137	3
IGS-9d	CAAACATTTATTAAACCGTAAAG GGATTTGAAAGAAGTGACATC	U73694	$(AAC)_{12}$	145	2 (4†)
IGS-24d	CCCTCAGATTAAATTGAATTGG GCCCTGCATGAAACCTTG	U73695	$(GTT)_8$	140	2
IGS-31c	TGCGCGATCCACTGACTTAC AGTGGGCTGTGCTCAATTGTC	U73696	$(AAC)_7$	135	1
IGS-92b	CTTGTGGTTCCTGCATAAGCAC AGCAGCTTGCAGANCTCAGTGG	U73697	*	138	1
IGS-110b	CCATGGAAGCATGTCTGGTG TGCTTCCTGATTTCAAAGTTGC	U73698	$(TGC)_9$	131	4
IGS-204a	GGGTAAGACAAGATAACACAAG GCCTTTCTAGTGTCTGATGTG	U73699	$(AGGGG)_5$	138	2
IGS-BM1	TGCCTGGCAAGATAAACC TTGTTTCCCTTGCTATCAAG	U73700	$(AAAC)_2(AAC)_7$	169	3
IGS-BP1	ATCATCAGGCTCCTACACCC AAGAACTCCTTTGAGAACCTGG	U73701	$(GCA)_{10}$	101	3
IGS-CQ1	GGGTTTCTACATNTTNTTGTTG AGATGAATCTGTCTTCCTCTAG	U73702	$(GTT)_9$	67	3
IGS-CK1	AAGTCGGATCAATCAGCTAAAG AGATTGATTCCTAAGCATTTGG	U73703	$(GTTT)_6$	91	2

Table 1 Primer sequences, GenBank Accession No., repeat motif, clone amplicon size, and number of alleles for 13 microsatellite loci in *S. b. brunneus*, the Northern Idaho ground squirrel

*The repeat array in the clone has the sequence:
$(CCCT)_3CTGGATACACAGCTTCCAAGG(CTG)_5T(CTC)_5(CTCTTC)_3$
†Two additional alleles were found in examining several hundred additional individuals.

conservative (very few artefact bands) and that MetaPhor agarose (FMC) can achieve high resolution of single repeat differences between alleles. While we were able to resolve 2 bp differences between amplicons, we found scoring to be most effective using MetaPhor (FMC) gels for microsatellite loci with motifs of trimers (or larger) for alleles of 60–220 bp (see also May *et al*. in press).

Many authors have reported on the evolutionary conservation of flanking regions around microsatellite loci (e.g. four species within the cat family Felidae, Menotti-Raymond & O'Brien 1995; 30 species within the order Cetacea, Valsecchi & Amos 1996). Initial amplification tests of the primers described in this paper using DNA from *S. townsendii* (M. Antolin and B. May, unpublished data), *S. columbianus* (B. May *et al*. unpublished data), and *S. beecheyi* (L. Cody and B. May, unpublished data) produced amplicons that were as bright, resolved, and polymorphic as in *S. b. brunneus*. These results suggest that these primers should amplify well in other members of this widespread genus (52 species world-wide) and perhaps reasonably well in related sciurid genera (e.g. *Ammospermophilus*, *Cynomys* and *Tamias*).

Acknowledgements

We thank Steve Bogdanowicz, Wayne Gottlieb, Rebecca Sawyer, and Krista Van Zwieten for laboratory assistance.

References

May B, Krueger CC, Kincaid H (in press) Genetic variation at microsatellite loci in sturgeon: primer sequence homology in *Acipenser* and *Scaphirhynchus*. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**.

Menotti -Raymond MA, O'Brien SJ (1995) Evolutionary conservation of ten microsatellite loci in four species of Felidae. *Journal of Heredity*, **86**, 319–322.

Sherman PW (1989) Mate guarding as paternity insurance in Idaho ground squirrels. *Nature*, **338**, 418–420.

Valsecchi E, Amos W (1996) Microsatellite markers for the study of cetacean populations. *Molecular Ecology*, **5**, 151–156.

White PS, Densmore IIILD (1992) Mitochondrial DNA isolation. In. *Molecular Genetic Analysis of Populations*. (ed. Hoelzel AR), pp. 29–58. Oxford University Press, New York.

Yensen E (1991) Taxonomy and distribution of the Idaho ground squirrel. *Spermophilus brunneus Journal of Mammalogy*, **72**, 583–600.

Yensen E, Sherman PW (in press) *Spermophilus brunneus*. Mammalian species. *American Society of Mammalogists*.