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PRIMER NOTE

Isolation and characterization of microsatellite markers in hoary marmots (*Marmota caligata*)

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Abstract

Microsatellite loci were developed from hoary marmots (*Marmota caligata*) to aid in the investigation of the social structure and mating system of this species. Seven of the microsatellite loci developed were found to be moderately polymorphic with between two and seven alleles per locus. In addition to the microsatellites developed in hoary marmots we also tested markers developed for other scuirids, namely European alpine marmots (*M. marmota*), Columbian ground squirrels (*Spermophilus columbianus*) and European ground squirrels (*S. citellus*). Of these markers, 13 were polymorphic when amplified in hoary marmots with between two and nine alleles per locus.

Keywords: hoary marmot, *Marmota caligata*, mating system, microsatellite, parentage, Scuiridae

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Behavioural observations of copulation, dominance and other fitness correlates have often proven to be inadequate predictors of true parentage (Avise 1994; Hughes 1998). The use of highly variable molecular markers to identify parentage has led to challenges to long-held paradigms in behavioural ecology such as the presumed monogamy of many avian species (e.g. Gibbs *et al*. 1990; Birkhead & Moller 1998) and specific behavioural measures of reproductive success in polygynous ungulates (Coltman *et al*. 1999).

Hoary marmots are gregarious, normally existing in colonies that consist of a resident dominant adult male and female, subordinate males and females, yearlings and infants (Barash 1974). Polygyny has been visually observed in southern populations of hoary marmots (Wasser & Barash 1983). In contrast, Holmes (1984) described northern populations as being monogamous in response to the severe ecological conditions of northern environments with limited hibernacula and food. Here we have developed microsatellite loci to aid in assigning parentage and determining the social and mating system of hoary marmot populations by molecular means.

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Microsatellite libraries were constructed using a method similar to that described by Paetkau & Strobeck (1994). Approximately 50 μg of genomic DNA was isolated from muscle tissue by standard methods (Sambrook *et al*. 1989) and completely digested with *Sau*3A1. The digested DNA was electrophoresed on a 1% agarose gel. Fragments between 200 and 500 bp were excised and isolated from the gel using QIAquick™ spin columns (QIAGEN). Fragments were then ligated into *Bam*HI-digested dephosphorylated M13mp18RF (Gibco BRL) and transfected into *Escherichia coli* DH5aF′ by electroporation (*E. coli* Pulser™; Bio-Rad). Approximately 2000 recombinant clones were screened by plaque lifts and hybridization to a biotinylated $(GT)_{12}$ oligonucleotide probe, allowing for detection with a non-radioactive detection kit (Gibco BRL). Following secondary screening, genomic inserts from 30 clones were amplified by PCR, gel purified, sequenced using ABI dRhodamine chemistry and resolved on a 377 ABI sequencer. Primers were designed using OLIGO (version 4.0; National Biosciences Inc.) with one primer of each set fluorescently labelled with 6-FAM, TET or HEX (ABI) for products containing microsatellite repeats. Microsatellite PCR for all loci was performed as follows: approximately 10 ng of DNA was used as template in 15-μL PCR reactions also containing 0.16 μM (2.4 pmol) of each primer, 120 μM dNTPs, 2 mM $MgCl_2$, 0.3 U of *Taq* DNA polymerase (isolated as per Engelke *et al*. 1990) and 1 × PCR buffer (10 mM Tris buffer, pH 8.8, 0.1% Triton X-100, 50 mM KCl and 0.16 mg/mL bovine serum

Table 1 Hoary marmot primer sequences (5′–3′) for microsatellite loci and variation (*A*, number of alleles) at each locus based on $n = 29$ from one population from the southwest Yukon, Canada

Locus	Microsatellite sequence	Primer sequence	Size (bp)	*A*	H_O	H_E	GenBank Accession no.
2g2	$(GT)_{17}$	F: TGAACTGGGTCTTGAGGTCT R: GTCTGCTCTGCTCTCCATCA	103–127	7	0.41	0.46	AY702712
2h10	$(TG)_{17}CGTG(CG)_5$	F: GAATCCGAGTTGCCCAGTCC R: CAGACAGGGACACGCACACG	143–154	6	0.62	0.55	AY702708
2g4	$(TG)_{13}$	F: TAAGGCTGAATAATATTCCTCT R: CAAACAACCCGAGTAGACAT	142–146	3	0.52	0.57	AY702709
2h15	$(GT)_{19}$	F: TGGTTACGAAGATGGGAGAC R: CTGGGCTGGAAGAACTGG	250–264	5	0.69	0.74	AY702710
2h6a	*	F: TAACTACACATTTGACTTTCTGC R: ATCCCCAGCACCACATAC	153–161	3	0.48	0.54	—
2h6b	$(GT)_{18}$	F: TAACTACACATTTGACTTTCTGC R: ATCCCCAGCACCACATAC	186–202	3	0.41	0.35	AY702711
3b1	$(CA)_{10}$	F: AACAAAACAACTAAAGTCCT R: ACAAAATGGTCAAAGTAGAG	350–360	2	0.34	0.47	AY702707

H_E, expected heterozygosity; H_O, observed heterozygosity.
*Primer pair amplified two loci that were not found to be linked by a test of genotypic disequilibrium.

Table 2 Amplification of interspecific microsatellites in *Marmota caligata* based on $n = 29$ from one population from the southwest Yukon, Canada

Species microsatellite developed in	Locus	Size (bp)	*A*	H_O	H_E	GenBank Accession no.	Reference
Marmota marmota	SS-Bibl4	182–186	3	0.29	0.34	*	Goossens *et al.* (1998)
Marmota marmota	SS-Bibl18	132–136	3	0.45	0.48	*	Goossens *et al.* (1998)
Marmota marmota	SS-Bibl25	136–144	3	0.63	0.21	*	Goossens *et al.* (1998)
Marmota marmota	SS-Bibl31	154–164	5	0.77	0.76	*	Goossens *et al.* (1998)
Marmota marmota	MS6	152–164	6	0.79	0.90	AF259372	Hanslik & Kruckenhauser (2000)
Marmota marmota	MS47	177–193	9	0.79	0.75	AF259375	Hanslik & Kruckenhauser (2000)
Marmota marmota	MS53	131–139	5	0.33	0.34	AF259376	Hanslik & Kruckenhauser (2000)
Spermophilus citellus	ST10	127–135	3	0.57	0.52	AF254436	Hanslik & Kruckenhauser (2000)
Spermophilus columbianus	GS12	145–163	2	0.26	0.24	U63149	Stevens *et al.* (1997)
Spermophilus columbianus	GS14	243–249	3	0.58	0.59	U63150	Stevens *et al.* (1997)
Spermophilus columbianus	GS17	156–160	3	0.61	0.71	U63151	Stevens *et al.* (1997)
Spermophilus columbianus	GS22	172–186	4	0.49	0.45	U63153	Stevens *et al.* (1997)
Spermophilus columbianus	GS25	106–128	3	0.32	0.34	U63154	Stevens *et al.* (1997)

A, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity.
*Sequence not present in GenBank.

albumin). Cycling was performed in a Perkin Elmer™ 9600 thermal cycler under the following conditions: 1 min at 94 °C, three cycles of 30 s at 94 °C, 20 s at 49 °C and 5 s at 73 °C, 33 cycles of 15 s at 94 °C, 20 s at 50 °C and 1 s at 72 °C and a final extension at 72 °C for 30 min. PCR products were resolved on a model 377 ABI sequencer and analysed using GENESCAN™ (version 3.1) and GENOTYPER (version 2.0) software.

A total of 15 primer pairs amplified strong interpretable banding patterns. Six primer pairs amplified microsatellites with more than two alleles among 29 hoary marmots sampled from southwest Yukon, Canada (Table 1). One primer pair amplified two loci that were subsequently listed as 2h6a and 2h6b. Observed and expected heterozygosities ranged from 0.35 to 0.74 (Table 1). All PCR reactions were optimized for an annealing temperature of 54 °C.

In addition to the microsatellites developed in this study, we also screened microsatellites developed for other closely related species (Table 2). All microsatellite primer pairs from Goossens *et al*. (1998), Hanslik & Kruckenhauser (2000) and Stevens *et al*. (1997) were screened. Of the markers screened, 13 microsatellites developed in Alpine marmots (*Marmota marmota*) (Goossens *et al*. 1998, SS-Bibl4, SS-Bibl18, SS-Bibl25 and SS-Bibl31; Hanslik & Kruckenhauser 2000, MS6, MS47 and MS53), European ground squirrels (*Spermophilus citellus*) (Hanslik & Kruckenhauser 2000, ST10) and Columbian ground squirrels (Stevens *et al*. 1997, GS12, GS14, GS17, GS22 and GS25) were identified that had

clear banding patterns and two or more alleles. All PCR reactions were optimized for an annealing temperature of 54 °C.

All loci were screened for deviations from Hardy–Weinberg equilibrium (HWE) and genotypic disequilibrium using GENEPOP (version 3.3; Raymond & Rousset 1995). Locus SS-Bibl25 was found to deviate from HWE from a heterozygote deficit. Several departures from genotypic equilibrium were also observed, including GS25/GS14, GS14/SS-Bibl18, 2h15/SS-Bibl18, GS12/SS-Bibl25, SS-Bibl14/MS47 and SS-Bibl/MS47. Overall, we have identified 20 microsatellite loci to help more clearly identify parentage in this species.

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