Molecular Ecology Notes (2004) **4**, 119–121 doi: 10.1111/j.1471-8286.2004.00588.x

PRIMER NOTE

Microsatellite loci for the Siberian flying squirrel, *Pteromys volans*

JODIE N. PAINTER,* VESA SELONEN and ILPO K. HANSKI
Department of Ecology and Systematics, University of Helsinki 00014, Finland

Abstract

We report the isolation and characterization of polymorphic microsatellite loci for the Siberian flying squirrel *Pteromys volans*. The seven most useful loci had between six and 11 alleles and expected heterozygosities ranging from 0.477 to 0.866. We also tested the utility of these loci in other squirrel species, northern flying squirrels (*Glaucomys sabrinus* and *G. volans*) and the common red squirrel (*Sciurus vulgaris*). Three of the Siberian flying squirrel loci were polymorphic in other squirrel species, suggesting a limited potential for cross-species use.

Keywords: cross-species amplification, microsatellites, Siberian flying squirrel

Received 16 September 2003; revision received 5 November 2003; accepted 3 December 2003

The Siberian flying squirrel is a nocturnal, arboreal rodent resident in spruce-dominated boreal forests in Eurasia. We have been studying home ranges, space use, movements, natal dispersal and mating behaviour of the flying squirrel in Finland using radio telemetry from 1996 to 2002 (Hanski 1998; Hanski *et al*. 2000b; Selonen 2002). Male flying squirrels have home ranges five to 10 times larger than those of females, which usually overlap the ranges of several females. The females seem to live in separate home ranges, whereas the ranges of males overlap with each other (Hanski *et al*. 2000a and unpublished). Due to their nocturnal habits many aspects of flying squirrel behaviour, such as mating and copulations, are difficult to observe. We developed microsatellite loci for the Siberian flying squirrel in order to investigate questions of paternity and the mating system and also the distribution of genetic variation amongst flying squirrel populations.

DNA for the construction of genomic libraries was extracted from muscle tissue using a standard phenol–chloroform extraction procedure. Two different methods were used in the construction of the genomic libraries used to screen for microsatellite repeat motifs. The first method approximately followed the protocol of Painter *et al*. (1997). Briefly, 2 μg of DNA were digested with two restriction enzymes, *Sau*3AI and *Rsa*I (Amersham). The digested DNA was ligated into pUC19 vector (Amersham) and then transformed into ELECTROMAX™ DH10B™ cells (Gibco BRL). Approximately 10 000 colonies were screened for microsatellite repeat motifs by hybridization at 55 °C overnight using the following γP^{33} end-labelled probes: $(AC)_{15}$, $(AG)_{15}$, $(AAT)_{15}$, $(CCT)_{15}$, $(AAAG)_8$ and $(ATAG)_8$. Thirty positive clones were polymerase chain reaction (PCR) amplified and sequenced with both the pUCF23 and pUCR24 primers, using BIGDYE terminators (ABI PRISM). Most inserts contained AC repeats. Primers were designed for 16 loci that contained repeat motifs longer than 10 repeat units in length and checked for self and pair complementarity using PRIMER3.0 (Rozen & Skaletsky 2000). Five of the loci were polymorphic (Table 1).

The second method was a modification of the enrichment protocol of (Fleischer & Loew 1995; Hamilton & Fleischer 2001; Hamilton *et al*. 1999). Briefly, 2 μg of genomic DNA were digested with *Sau*3AI, *Hae*III and *Nhe*I. Single-stranded overhangs were removed using S1 nuclease (MBI Fermentas) and, following column purification, the 5′ phosphates were removed using calf intestinal alkaline phosphastase (MBI Fermentas). This DNA was then ligated to the SNX linkers and the 'library' enriched by hybridizing a biotinylated $(GA)_{10}$ oligonucleotide to the insert DNA at 55 °C overnight. The oligonucleotide–DNA complexes were isolated using streptavidin MagneSphere paramagnetic particles (Promega) according to the manufacturer's instructions. After PCR amplification and the subsequent removal

Correspondence: J. N. Painter. *Present address: Folkhälsan Institute of Genetics and Department of Medical Genetics, BioMedicum Helsinki P.O. Box 63, University of Helsinki 00014, Finland. Fax: + 358 919125073; E-mail: jodie.painter@helsinki.fi

Table 1 *Pteromys volans* microsatellite primer pairs

Locus name	Repeat motif in clone	Primer sequence	No. alleles	Size range (bp)	H_O	H_E	Annealing temp. (°C)	GenBank Accession no.
Pvol1	$(AC)_{17}CT(AG)_{23}$ $(AC)_{15}$	F: GCCACCAGTCCTTATCTATCCA R: TCATTCTTTCTTGGCCTCTCA	3	188–194	N/A	N/A	50	AY277560
Pvol3	$(AC)_{15}$	F: GCCTGTTAGTTCCTTTGCCTA R: GGGAAGCTGGTGTTGAAATC	3	205–209	N/A	N/A	55	AY277561
Pvol10	$(AC)_{14}$	F: GTCATAACATCAGTCTTTGG R: ATCACAAAAAAATAAATAAAAGTC	10	118–136	0.781	0.824	50	AY277562
Pvol41	$(AG)_{38}$	F: AGGAAATAGGTCTAGTATATGG R: TGGAGTATATAATTTTTCCTG	11	126–144	0.798	0.811	50	AY277563
Pvol74	$(AG)_{14}(n \times 40)(AG)_{20}$	F: TGGCATGCTGATATGCTGAT R: GGGCAGAAAGGAAGCAAAAT	8	165–179	0.730	0.731	TD 55–45	AY277564
PvolE1	$*(AG)_{16}(GAAA)_{14}$	F: CAGGACTCAAGGGGGAAAA R: GCAGAAGCCATTCTACTGGA	11	307–329	0.832	0.866	50	AY277565
PvolE5	$(AG)_{22}$	F: GCACAATTTCAGCTGCTTACC R: TGAGCTAGGGACTACATGATATGG	7	153–169	0.642	0.688	50	AY277566
PvolE6	$(AG)_{21}$	F: TCCTTACTAATGTGAACCCTGACA R: CAGTCTTCAAGCACACTTCCT	7	188–200	0.446	0.477	50	AY277567
PvolE10	$(AG)_{19}$	F: GGGTCTATAATTTGAAAAGAGAAAGA R: TGTTCTGGTGGTGAGAAGCA	6	132–142	0.612	0.688	50	AY277568
PvolE25	$(AG)_{21}$	F: AGCCATTATTTGATGTGTCCTG R: CGTAGCATTTCTTCTTCTTGCA	4	135–153	N/A	N/A	55	AY277569

*Longest uninterrupted repeat motifs at the end of a compound repeat.
The letter E in the primer name denotes the loci that were found using the enrichment protocol. H_O, observed heterozygosity; H_E, expected heterozygosity; N/A, not available; TD, touchdown.

of the linkers by digestion with *Nhe*I, the insert DNA was ligated into pUC18 (Amersham) previously digested with *Xba*I and dephosphorylated. The ligated DNA was then transformed as above. Only 116 white colonies were obtained and, of these, 21 contained a microsatellite motif of more than 10 repeat units. Primers were designed for 13 loci as there was too little or no flanking sequence on one side of the microsatellite in the remaining inserts. Again, five of the loci were polymorphic (Table 1).

DNA for the amplification of microsatellite loci was extracted from flying squirrel hair follicles by incubating 1 cm pieces (including the follicle) of approximately 20 hairs in 5% Chelex 100 (Bio-Rad) with 20 μg proteinase K at 56 °C for 1 h, followed by 10 min at 95 °C. The extractions were subsequently stored at 4 °C. For each primer pair the forward primer was 5′ end-labelled with either HEX or FAM fluorescent dyes (DNA Technology). The ratios of the two primers in the PCR mix were dependent on the dye, 1 : 2 labelled : unlabelled for HEX and 1 : 10 labelled : unlabelled for FAM. Each 10 μL PCR mix contained 1× PCR buffer plus $NHSO_4$ (MBI Fermentas), 1.5 μM $MgCl_2$, 100 μM each dNTP, 1 pmol (FAM) or 5 pmol (HEX) of labelled primer, 10 pmol of unlabelled primer, 10 μg bovine serum albumin, 0.5 U *Taq* and 1 μL of DNA. The PCR profile consisted of denaturation for 2 min at 92 °C, 30 s annealing (see Table 1 for temperatures) and extension at 72 °C for 30 s, followed by 32 cycles of 92 °C for 30 s, annealing for 30 s and 72 °C for 30 s. During the final cycle the extension time was extended to 10 min. The Pvol74 products were then incubated at 60 °C for 1 h to allow additional time for the *Taq* enzyme to add an A to the fragments.

Ten of the 29 microsatellite loci for which primers were designed were polymorphic (Table 1). However, we have used only seven of these markers for the paternity analyses as for two loci (PvolI1 and PvolI3) almost all individuals were homozygous for one common allele, whilst a third locus (PvolE25) was often difficult to interpret due to stuttering and requires further optimization. The seven most useful loci had between six and 11 alleles and expected heterozygosities ranging from 0.477 to 0.866.

We tested the utility of the seven most useful primers for cross-species amplification in three additional Sciuridae species, the common red squirrel *Scurius vulgaris* and two species of northern flying squirrels, *Glaucomys sabrinus* and *G. volans*. The PCR conditions were as for Siberian flying squirrels except that a touchdown amplification protocol (55–45 °C) was employed. Siberian flying squirrels are relatively distantly related to other squirrel species. In limited trials we were unable to successfully amplify microsatellite loci using a subset of the primers that have been designed for other squirrel species: from Columbian ground squirrels (*Spermophilus columbians*) GS14, GS17, GS22, GS25 and

Table 2 Cross-species amplification of *Pteromys volans* microsatellite loci

	Pvol10	Pvol41	Pvol74	PvolE1	PvolE5	PvolE6	PvolE10
Sciurus vulgaris (5)	NP	1	1	1	1	5	NP
Glaucomys sabrinus (10)	NP	4	1	1	1	4	MB
Glaucomys volans (4)	NP	3	4	1	1	1	MB

NP, no product for a particular locus; MB, multiple bands were obtained using the current polymerase chain reaction conditions, amongst which a microsatellite locus could not be discerned.

GS26 (Stevens *et al*. 1997); from northern Idaho ground squirrels (*S. brunneus brunneus*) IGS-1 and IGS-6 (May *et al*. 1997) and from northern flying squirrels (*G. sabrinus*) GS-2, GS-8, GS-10, GS-13 and GS-16 (Zittlau *et al*. 2000). In our cross-species trials a resolvable product was obtained for five of the Siberian flying squirrel loci in all three species (Table 2). Three of the loci were polymorphic in at least one additional species, with allele sizes within the ranges seen in Siberian flying squirrels. Hence, the microsatellite loci that we designed for the Siberian flying squirrel may have limited utility for use in other squirrel species.

Acknowledgements

This research was supported by grants from the Academy of Finland to Ilkka Hanski, from Maj and Tor Nessling Foundation, Emil Aaltonen Foundation, Ella and Georg Ehrnrooth Foundation, Ministry of Environment and the Finnish Forest and Park Service to I.K.H. and Ministry of Education (LUOVA graduate school) to V.S. *Glaucomys sabrinus* samples were kindly donated by Winston Smith and on loan from the University of Alaska museum (AF nos 12526, 13577, 24774, 28866 and 33797). The *G. volans* samples were also on loan from the University of Alaska museum (AF nos 14471–14474).

References

Fleischer RC, Loew S (1995) Construction and screening of microsatellite-enriched genomic libraries. In: *Molecular Zoology; Advances, Strategies and Protocols* (eds Ferraris J, Palumbi S), pp. 459–468. Wiley-Liss, New York.

Hamilton MN, Fleischer RC (2001) Microsatellite-enhanced genomic library construction streptavidin-biotin bench version. http://www.mnh.si.edu/GeneticsLab/TechnicalPage/Protocols/Microsat/beadmicro.pdf.

Hamilton MN, Pincus EL, DiFiore A, Fleischer RC (1999) A universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. *Biotechniques*, **27**, 500–507.

Hanski IK (1998) Home ranges and habitat use in the declining flying squirrel, *Pteromys volans*. *Wildlife Biology*, **4**, 33–46.

Hanski IK, Mönkkönen M, Reunanen P, Stevens P (2000a) Ecology of the Eurasian flying squirrel *Pteromys volans* in Finland. In: *Biology of Gliding Mammals* (eds Goldingay R, Scheibe J), pp. 67–86. Filander Verlag, Furth, Germany.

Hanski IK, Stevens P, Ihalempiä P, Selonen V (2000b) Home-range size, movements, and nest-site use in the Siberian flying squirrel, *Pteromys volans*. *Journal of Mammology*, **81**, 798–809.

May B, Gavin TA, Sherman PW, Korves TM (1997) Characterization of microsatellite loci in the Northern Idaho ground squirrel. *Spermophilus brunneus brunneus*. *Molecular Ecology*, **6**, 399–400.

Painter JN, Crozier RHC, Crozier C, Clarke MF (1997) Characterization of microsatellite loci for a cooperatively breeding honeyeater. *Molecular Ecology*, **6**, 1103–1105.

Rozen S, Skaletsky HJ (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, NJ.

Selonen V (2002) Spacing behaviour of the Siberian flying squirrel — effects of landscape structure. PhD Thesis (available at http://www.ethesis.helsinki.fi). University of Helsinki, Finland.

Stevens S, Coffin J, Strobeck CO (1997) Microsatellite loci in Columbian ground squirrels *Spermophilus columbians*. *Molecular Ecology*, **6**, 493–495.

Zittlau KA, Davis CS, Strobeck C (2000) Characterization of microsatellite loci in northern flying squirrels (*Glaucomys sabrinus*). *Molecular Ecology*, **9**, 817–829.