

PRIMER NOTE

Isolation of 18 polymorphic microsatellite loci from the North American red squirrel, *Tamiasciurus hudsonicus* (Sciuridae, Rodentia), and their cross-utility in other species

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Abstract

We isolated 18 polymorphic microsatellite loci to be used for pedigree analysis in a wild population of North American red squirrels, *Tamiasciurus hudsonicus*. Allelic diversity and observed heterozygosity ranged from six to 13 and 0.39 to 0.89, respectively, in a sample of 93 individuals. Up to 13 sets of primers also amplify in other rodent species.

Keywords: cross-species amplification, microsatellite, North American red squirrel, Rodentia, Sciuridae, *Tamiasciurus hudsonicus*

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The North American red squirrel, *Tamiasciurus hudsonicus*, is a widespread inhabitant of the boreal forest. As part of a study to investigate the genetic response to climate change in a population in the Yukon, Canada (Réale *et al.* 2003), we have developed a set of microsatellite markers for paternity analysis. Loci were isolated using slightly different methods in two laboratories: the University of Alberta (UofA) and at the Sheffield Molecular Genetics Facility (SMGF).

At the UofA, genomic DNA was extracted from muscle tissue of a single, female *T. hudsonicus* individual using a standard method (Sambrook *et al.* 1989). An unenriched genomic library was created as described in Davis & Strobeck (1998). Briefly, 200–500 bp *Sau3AI* DNA fragments were ligated into M13mp18RF-*Bam*HI/SAP (dephosphorylated with shrimp alkaline phosphatase) and transformed into *Escherichia coli* DH5alpha F'. In total, 10 000 unenriched transformant clones were screened by hybridization to the biotinylated sequence (GT)₁₁ using a nonradioactive kit (Gibco BRL), which identified approximately 80 positives, of which 18 were sequenced. Eleven primer pairs were designed. Three primer pairs amplified a scoreable, polymorphic product (Thu03, Thu08, Thu14, Table 1).

In order to isolate sufficient markers for paternity analysis, a second genomic library was created at the SMGF. An

unenriched library was created using the protocol at <http://www.shef.ac.uk/misc/groups/molecol/smgf.html>. DNA was extracted from one *T. hudsonicus* individual (sex unknown) using an ammonium acetate–ethanol precipitation technique (Nicholls *et al.* 2000) (<http://www.shef.ac.uk/misc/groups/molecol/protocols.html>). DNA was digested with *Mbo*I (New England Biolabs). Following size selection, 100–600 bp *Mbo*I DNA fragments were ligated into pUC19 *Bam*HI/BAP (Amersham Pharmacia Biotech) and transformed into *Escherichia coli* XL1-Blue MRF' competent cells (Stratagene). Approximately 10 000 unenriched transformant clones were screened by hybridization to the alternating copolymers Poly(dA-dC)·Poly(dG-dT) or Poly(dA-dG)·Poly(dC-dT) (Amersham Biosciences) radiolabelled with [α^{32} P]-dCTP, which identified 200 positives. Sequencing was performed with BigDye Terminator 1.1 (Applied Biosystems) on an ABI PRISM 3730 Sequencer using HPLC-purified M13–21 Universal forward and M13 reverse primers (Sigma). Forward and reverse sequences were compared to create consensus sequences for 50 positives. Thirty primer pairs were designed using PRIMER 3 (Rozen & Skaletsky 2000). Fifteen primer pairs amplified a scoreable, polymorphic product (Table 1).

The sequences isolated from both genomic libraries were checked for duplication and for pUC19 or M13mp18RF vector contamination, using GENEJOCKEY sequence processor software (Taylor 1996). All sequences were confirmed to be

Table 1 Characterization of microsatellite loci in the American red squirrel, *Tamiasciurus hudsonicus*. The concentration of MgCl₂, the annealing temperature (T_a), the number of alleles (A) and allele size range (in bp), the observed (H_O) and expected (H_E) heterozygosities and the number of individuals tested (n) are given for each locus. All squirrels tested originate from Yukon, Canada. Fluorescent labels are underlined and 'pigtail' modifications (GTTTCTT, Brownstein *et al.* 1996), where used, are shown in parentheses

Locus	EMBL Accession nos†	Repeat motif in clone* (5'-3')	Primer sequences (5'-3')	T _a (°C)	MgCl ₂ (mM)	n	A	Exp. allele size (bp)*	Allele sizes (bp)	H _O	H _E
Thu03S	AJ585770	(TG) ₅ GG(GT) ₁₉	F TELT-GTTTTCAAAAAGCCATTTCAGG R TACAGACAGACAGATGGGCAATATTAC	59	1	85	12	228	221-245	0.74	0.79
Thu08S	AJ585775	(GT) ₂₀	F HEXC-CTCAATTCCTCCCTGCTTC R ACAGACCTTTCTTTCCCTTGAA	64	1.5	76	8	199	182-208	0.77	0.78
Thu14S	AJ585779	(GT) ₂₂ and (GA) ₂₂	F NED-TAGCTAGCAAGCTGCTCTC R AAAAGACTTTTATTTAAAGGTGCATTTTTC	62	1.5	85	9	270	268-286	0.86	0.79
Thu21+**	AJ841898	(GT) ₄ AT(GT) ₁₉	F FAM- <u>A</u> ATGAGAGGGCTCCACAGAG R AACTCCACCTTCTCAGTCTGTTTC	64	1.5	86	10	156	150-168	0.61	0.71
Thu23+	AJ841900	(AT) ₈ GCAT (AC) ₂₁	F FAM-GCTTACCCACTGTGACCCAAAC R TGTGACCATGGAACAAGATGC (PIGTAIL)	62	1.5	83	11	188	181-203	0.82	0.83
Thu25+	AJ841902	(AC) ₃ (TG) ₁₆	F NED- <u>A</u> CCCTGCTGATTTAGGACACAC R GATGTACCTTAAATGGTGGACGAC	64	1.5	86	10	188	182-202	0.83	0.78
Thu31+	AJ841908	(GT) ₂₄	F VIC-GGGCCCTTTTCCATGATGC R AAAAGAGTGAACCTCTGAGC (PIGTAIL)	64	1.5	85	7	136	131-147	0.85	0.76
Thu32+	AJ841909	(CA) ₂₃	F PET-GCATTTGGCTGGTACGTTATG R CCCCTTTTAAACAATGACTGTTAC (PIGTAIL)	64	1.5	82	9	282	265-289	0.70	0.78
Thu33+	AJ841910¶	(AC) ₁₉	F PET-AGGATTAATAAGAGCGTTGATGC R TCTAATCTGGCCTTGAAGAATAGTG	64	1.5	86	9	150	145-161	0.80	0.83
Thu37+	AJ841914	(CAC) ₂ (AC) ₈ AA (AC) ₅	F VIC-GATTCCTCCCTCCACTTC R AGTGGGGGTGTGACTCTGTGG	60	1.5	89	6	161	155-182	0.39	0.41
Thu38+	AJ841915	(AC) ₁₂	F VIC-GATTCCTCCCTCCACTTC R GCATTTTCTTCCGTTGACTG (PIGTAIL)	64	1.5	85	6	299	294-306	0.48	0.56
Thu40+	AJ843221	(TG) ₂ GG(TG) ₂₆	F PET-TCTCAACTGACATCAAAAGAG R TTGCGCAGGCTGTTCTCAC (PIGTAIL)	64	1.5	78	13	387	376-400	0.89	0.87
Thu41+	AJ843222	(CA) ₁₁	F NED- <u>C</u> AAACCAAGCTCATTTGACAGC R TCGAACAATTTCAACAATAAAGATTG	64	1.5	84	7	230	238, 255-265	0.70	0.77
Thu42+	AJ843223	(GT) ₁₈ (GA) ₄	F FAM-TTGAACCTGCCAATCAAAAGG R ACAAGTTGACAGCAATCTTG	64	1.5	83	9	246	232-260	0.75	0.75
Thu49+	AJ843230	(TG) ₅ CATGCA(TG) ₂ CA(TG) ₁₅ TA(TG) ₅ TA (TG) ₃ (AG) ₆ GG(AG) ₅ AA(AG) ₆ GG(AG) ₁₄	F FAM-TTGGAGCCTGCTGATTTCC R TCAGTATGTCACACTCTCTACCTGAC (PIGTAIL)	64	1.5	82	9	188	188-208	0.72	0.76
Thu50+	AJ843231	(CA) ₁₆	F FAM-ATTTCCACCCCGCAAA R TCGAACCCTTTGTTTATCTGTTTTCAA	64	1.5	83	8	290	273-293	0.63	0.65
Thu55+	AJ843236	(CA) ₁₇	F FAM-TGTCAACCAAGTGTCAACG R CTGCACTGTCCCTTTTCTTG (PIGTAIL)	64	1.5	83	9	258	262-280	0.77	0.80
Thu59+**	AJ843240	(GC) ₈ (AC) ₁₈	F FAM-GATCTCCCGCGCAGAT R GGCCCTTGGCCGACTGTGTTTCT†	64	1.5	81	10	113	92-112	0.52	0.77

†An additional 29 primer sets were monomorphic (EMBL Accession nos AJ585769S, AJ585772S, AJ841897T, AJ843228T, AJ843232T/AJ843237T/AJ843242T) or produced no amplification or amplified unscorable products (EMBL Accession nos AJ585771S, AJ585773S, AJ585776S, AJ585777S, AJ585778S, AJ585780S, AJ585781S, AJ841899T, AJ841901T, AJ841903T, AJ841907T, AJ841911T, AJ841912T, AJ843220T, AJ843225T, AJ843226T, AJ843227T, AJ843231T, AJ843234T, AJ843235T/AJ843229T, AJ843238T). Accession nos divided by '/' are identical clones or alleles of the same locus.

* of sequenced clone; †, SMGF library; ‡, UoFA library; ¶, an identical clone of this locus was also found (EMBL accession number AJ841906); **, locus departs significantly from Hardy-Weinberg equilibrium.

Table 2 Number of alleles observed for 18 *Tamiasciurus hudsonicus* microsatellite loci when tested in six other species, as analysed on an ABI PRISM 3730 Sequencer (allele size ranges are given in parentheses)

Locus	Order: Rodentia						
	Sciuridae Sciurinae			<i>Spermophilus</i> <i>parryi</i> (<i>n</i> = 2)	Muridae Sigmodontinae	Muridae Arvicolinae	
	<i>Tamiasciurus</i> <i>hudsonicus</i> (expected allele size)	<i>Sciurus</i> <i>vulgaris</i> (<i>n</i> = 18)	<i>Sciurus</i> <i>carolinensis</i> (<i>n</i> = 6)		<i>Peromyscus</i> <i>leucopus</i> (<i>n</i> = 2)	<i>Clethrionomys</i> <i>glareolus</i> (<i>n</i> = 7)	<i>Clethrionomys</i> <i>rutilus</i> (<i>n</i> = 2)
Thu03	228	2 (223–231)	1 (192)	x	x	x	x
Thu08	199	x	1 (192)	1 (180)	x	1 (130)	x
Thu14	270	8 (241–296)	3 (261–267)	x	x	x	x
Thu21	156	x	5 (148–157)	2 (156–158)	x	1 (179)	x
Thu23	188	2 (159–189)	1 (186)	1 (181)	x	x	x
Thu25	188	3 (196–202)	2 (187–189)	x	x	x	x
Thu31	136	2 (137–141)	x	1 (122)	1 (128)	1 (121)	2 (119–121)
Thu32	282	1 (245)	1 (262)	x	x	x	x
Thu33	150	6 (125–157)	2 (149–154)	x	x	x	x
Thu37	161	3 (144–181)	7 (141–174)	x	x	1 (179)	x
Thu38	299	x	x	x	x	x	x
Thu40	387	1 (356)	2 (374–395)	x	x	x	x
Thu41	230	6 (218–265)	1 (227)	x	x	x	x
Thu42	246	3 (248–253)	x	x	x	1 (254)	x
Thu49	188	x	x	x	x	1 (178)	x
Thu50	290	5 (275–289)	1 (273)	x	x	x	x
Thu55	258	5 (262–272)	x	x	x	1 (276)	x
Number of loci amplifying		13	12	4	1	7	1
Number of loci polymorphic		11	6	1	0	0	1

n, number of individuals tested; x, no amplification. Species which failed to amplify when tested with *Tamiasciurus* loci included: Rodentia; *Tamias amoenus*, *Mus musculus*, *Muscardinus avellanarius* (hair sample), *Acomys cahirinus*, *Microtus agrestis*, *Microtus pennsylvanicus*, Lagomorpha; *Lepus americanus*, Chiroptera; *Rhinolophus ferrumequinum* (wing punch), *Hipposideros cervinus* (wing punch), *Myotis nattereri* (wing punch), *Myotis daubentonii* (wing punch), *Murina florium* (wing punch), *Kerivoula papillosa* (wing punch), Primates; *Homo sapiens*. In some cases, amplification failure may be due to the low concentrations of DNA available for testing especially when DNA extraction was from wing punches or hair samples.

vector free using BLASTN 2.2.4 software (Altschul *et al.* 1997) and then submitted to EMBL (Accession nos in Table 1). Primers were initially tested on 15 individuals to determine polymorphism, using fluorescently labelled primers. Each 10- μ L polymerase chain reaction (PCR) contained about 10 ng of genomic DNA, 1.0 μ M of each primer and 0.05 μ L BioTaq DNA polymerase (Bioline) in the manufacturer's buffer [final concentrations 20 mM (NH₄)₂SO₄, 75 mM Tris-HCl pH 9.0, 0.01% (w/v) Tween], including 1/1.5 mM MgCl₂ (Table 1) and 0.2 mM of each dNTP. PCR amplification was performed in an MJ Research DNA Engine Tetrad. The PCR profile used was 94 $^{\circ}$ C 2 min, followed by 30 cycles of 94 $^{\circ}$ C 1 min; annealing temperature (between 59 and 64 $^{\circ}$ C Table 1) 30 s, 72 $^{\circ}$ C 30 s. PCR amplification was confirmed using 1% agarose gels with ethidium bromide staining. Polymorphism was assessed on an ABI PRISM 3730, using the G5 dye set with Liz-500 as a size standard (Applied Biosystems). The forward primer of each primer pair was fluorescently labelled with 6-FAM,

VIC, NED or PET (Table 1), and polymorphism was analysed using GENEMAPPER 3.5 software.

For the 18 polymorphic loci, the number of alleles in 93 individuals ranged from six to 13 (Table 1). All individuals tested were from a single population in the Yukon, Canada. Tests for Hardy-Weinberg and linkage disequilibrium were implemented in GENEPOP version 3.4 (Raymond & Rousset 1995). Thu21 and Thu59 showed significant heterozygote deficiency ($P = 0.0007$, $P = 0.0001$, respectively) as a result of null alleles. Null alleles were confirmed in four known mother-offspring pairs. Loci Thu14 and Thu40 showed significant ($P < 0.0003$) linkage disequilibrium after Bonferroni correction (Rice 1989), perhaps as a result of physical linkage, assortative mating or nonrandom sampling of individuals from the same family groups.

The 18 *T. hudsonicus* loci were tested in several other species as shown in Table 2. Thirteen loci amplified in *Sciurus vulgaris*, of which 11 were polymorphic. Twelve loci

amplified in *Sciurus carolinensis*, of which six were polymorphic. Cross-amplification suggests that these markers will be useful for genotyping others of the 272 species (Nowak 1999) of the family Sciuridae.

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