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PRIMER NOTE

Isolation and characterization of polymorphic microsatellite loci in Bornean treeshrews (*Tupaia* spp.)

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Abstract

In this study, we developed five microsatellite loci from an enriched genomic library constructed for the pygmy treeshrew (*Tupaia minor*), and adapted another two from a previous study on the common treeshrew (*Tupaia glis*), for use in studying mating and dispersal patterns in Bornean treeshrews. We screened 32 plain treeshrew (*Tupaia longipes*) and 54 large treeshrew (*Tupaia tana*) individuals at these loci. Polymorphism ranged from two to 13 alleles, and heterozygosity ranged from 0.29 to 0.88. These results indicate the general utility of these microsatellites for genetic analyses in other *Tupaia* spp.

Keywords: Borneo, cross-species amplification, microsatellite, Scandentia, treeshrew, *Tupaia*, Tupaiidae

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The treeshrews (Tupaiidae, Scandentia) are little-known but common mammalian inhabitants of the Indomalayan tropics. Their close phylogenetic affinity with primates (Sargis 2004) and relatively rare behavioural traits of absentee maternal care and social monogamy (Emmons 2000) have recently attracted attention from researchers. Male-female treeshrew pairs defend joint territories against same-sex conspecifics, but individuals typically have access to extra-pair mates at the edges of their territorial boundaries, especially when ecological conditions are favourable (Munshi-South *et al.* unpublished manuscript). We developed five new polymorphic microsatellites from a genomic library created from pygmy treeshrew (*Tupaia minor*) DNA, and then adapted them for a study of mating and dispersal patterns in the large treeshrew (*Tupaia tana*) and plain treeshrew (*Tupaia longipes*) in Sabah, Malaysia (NE Borneo). We also designed six primer pairs for microsatellite loci previously sequenced from the common treeshrew (*Tupaia glis*, Srikwan *et al*. 2002), but only two produced polymorphic polymerase chain reaction (PCR) products from both *T. tana* and *T. longipes* DNA (SKTg19 and SKTg22, Table 1).

After digesting *T. minor* DNA with *Nhe*I, *Xmn*I, *Alu*I and *Bam*HI (New England Biolabs (NEB)), we created a genomic library enriched for a dinucleotide repeat motif using the standard protocol of Hamilton *et al*. (1999). The enriched library was cloned into *Xba*I-digested P-bluescript SK+ plasmid vectors (Stratagene), and transformed into *Escherichia coli* Supercompetent cells (Stratagene) for cloning. Positive colonies were picked and heated for 10 min at 100 °C in 200 μL TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). PCR of cloned DNA contained the following in a total volume of 30 μL: 50–100 ng DNA from each colony, 0.5 U Vent polymerase (NEB), 1× Thermopol buffer (NEB), 0.2 mM of each dNTP, and 8 μM of T3 and T7 primers. We used a PCR profile of 96 °C for 5 min followed by 30 cycles at 96 °C for 45 s, 51 °C for 1 min and 72 °C for 2 min. PCR products were run in 2% ethidium bromide agarose gels to identify genomic DNA inserts of 70–1000 bp. We cleaned PCR products using the QIAquick PCR purification kit (QIAGEN) and sequenced in one direction using the ABI BigDye ready reaction kit (Applied Biosystems). If clones contained microsatellites with at least seven dinucleotide repeats, then we sequenced them in the reverse direction and examined the resulting sequences in SEQUENCHER 4.1 (Gene Codes).

We designed flanking primers for 18 sequences containing microsatellites using the PRIMER 3 program (Rozen & Skaletsky 2000). We optimized primers for PCR amplification in *T. tana* and *T. longipes* using either a gradient or touchdown cycle on a PTC-200 Programmable Thermal Cycler (MJ Research). The annealing temperature in the touchdown program began at 65 °C and then decreased

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Table 1 Characteristics of microsatellite loci amplified from two species of Bornean treeshrews

Locus	Motif	Primer sequence (5′–3′)	Size range (bp)	No. of typed treeshrews	No. of alleles	H_O	H_E	Annealing temp. (°C)	Accession no.
JS22	$(GT)_3GC$	F: CAATGTCCTGGTGGTTATGG	*Tl*: 156–180	*Tl*: 32	*Tl*: 4	*Tl*: 0.88	*Tl*: 0.57	35 cycles;	DQ334277
	$(GT)_{14}$	R: GAAGTGGTCACTCTGCAATCC	*Tt*: 157–183	*Tt*: 48	*Tt*: 10	*Tt*: 0.79	*Tt*: 0.60	65–0.5/cycle	
JS132	$(GT)_{15}$	F: GCCAACCACAGTTTGAGTCC	*Tl*: 219–259	*Tl*: 29	*Tl*: 5	*Tl*: 0.79	*Tl*: 0.66	54	DQ334278
		R: TCTTTATTGGGAAGGCATGG	*Tt*: 256–260	*Tt*: 48	*Tt*: 2	*Tt*: 0.29	*Tt:* 0.37		
JS183	$(GT)_{15}$	F: GAAACAATAAGCCAGACTTCAGC	*Tl*: 115–153	*Tl*: 25	*Tl*: 12	*Tl*: 0.73	*Tl*: 0.89	35 cycles;	DQ334279
		R: TCACGAGTAACCTACGATAGCC	*Tt*: 132–166	*Tt*: 54	*Tt*: 12	*Tt*: 0.60	*Tt*: 0.79	65–0.7/cycle	
JS188	$(CA)_{13}$	F: ACACACACAAAACTCATTTTATCC	*Tl*: 170–200	*Tl*: 30	*Tl*: 11	*Tl*: 0.77	*Tl*: 0.86	57	DQ334280
		R: TCTACACGAATGTGCCAACC	*Tt*: 176–190	*Tt*: 51	*Tt*: 8	*Tt*: 0.66	*Tt:* 0.63		
JS196	$(GT)_{19}$	F: ACCTCCTGGTGGCTTGC	*Tl*: 139–151	*Tl*: 32	*Tl*: 3	*Tl*: 0.62	*Tl*: 0.48	56	DQ334281
		R: TAATTGCAGGATGCTTCAGG	*Tt*: 227–233	*Tt*: 48	*Tt*: 4	*Tt*: 0.54	*Tt:* 0.44		
SKTg19	$(CA)_7TA$	F: AAACCCCTCCCTAAAGGAAC	*Tl*: 167–197	*Tl*: 28	*Tl*: 11	*Tl*: 0.81	*Tl*: 0.82	51	AY064163
	$AA(CA)_8$	R: ACCCGCCCTATAGAAACCTC	*Tt*: 158–186	*Tt*: 53	*Tt*: 6	*Tt*: 0.62	*Tt*: 0.48		
SKTg22	$(CA)_8A$	F: GAGTGCACTTGCCCTGTAAC	*Tl*: 133–187	*Tl*: 29	*Tl*: 13	*Tl*: 0.53	*Tl*: 0.90	57	AY064165
	$(CA)_{10}$	R: TCCTGAACCTGGTGGCTAAC	*Tt*: 160–176	*Tt*: 47	*Tt*: 7	*Tt*: 0.78	*Tt:* 0.76		

F, forward primer; R, reverse primer; *Tl*, *Tupaia longipes*; *Tt*, *Tupaia tana*.

0.5 °C per cycle to a final annealing temperature of 47.5 °C. We selected five primer pairs that showed evidence of length variation for use in our study of Bornean treeshrews, and the forward primer was fluorescently labelled with 6-FAM or HEX. We used the QIAGEN DNeasy Tissue Kit (QIAGEN) to extract DNA from ear-clips stored in 95% ethanol from 54 *T. tana* and 32 *T. longipes* individuals trapped at two different sites in Sabah. PCRs were performed in 9 μL volumes containing 1 μL template DNA, 0.125 U *Taq* polymerase (Invitrogen), 1× PCR buffer (Invitrogen), 0.3 mM of each dNTP, 2.5 mM $MgCl_2$ and 0.55 μM of each primer.

Fluorescently labelled alleles were separated on an Applied Biosystems 3100 DNA Analyser and sized and scored using GENOTYPER 2.5 (Applied Biosystems). All seven primer pairs amplified PCR products in both *T. longipes* and *T. tana* (Table 1). Expected heterozygosity, tests of genotypic linkage disequilibrium and deviations from Hardy–Weinberg equilibrium (HWE) were calculated using FSTAT (Goudet 2001). Mean observed heterozygosity (H_O = 0.73 for *T. longipes*, H_O = 0.61 for *T. tana*) was not significantly different from mean expected heterozygosity (H_E = 0.74 for *T. longipes*, H_E = 0.58 for *T. tana*) for either species. No loci were found to be in linkage disequilibrium for either species ($P > 0.05$). Loci were in HWE in both species except SKTg22 in *T. longipes* and JS183 in both species ($P < 0.01$). These two loci showed low observed heterozygosities relative to expected values. Polymorphism in these seven microsatellites was generally moderate but ranged widely from two to 13 loci. These results from two evolutionarily divergent *Tupaia* spp. (Olson *et al*. 2005) indicate the general suitability of these primers for microsatellite analyses within this taxonomic group.

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