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TECHNICAL NOTE

Isolation and characterization of fourteen microsatellite loci for striped field mouse (*Apodemus agrarius*)

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Abstract We describe the isolation and characterization of 14 microsatellite loci in the striped field mouse from genomic DNA-enriched libraries in this paper. The 14 new loci were tested in 24 individuals from four populations in Southwest China. These loci were highly polymorphic with numbers of alleles per locus ranging from 6 to 14 and expected heterozygosities from 0.789 to 0.925. All loci followed Hardy–Weinberg expectations except SFM4, SFM11 and SFM13 loci. No significant linkage association was found among all loci. The 14 novel polymorphic microsatellite loci will be useful in studying phylogeography and population genetics of the striped field mouse.

Keywords Striped field mouse (*Apodemus agrarius*) · Microsatellites · Heterozygosity · Phylogeography · Population genetics

The striped field mouse (*Apodemus agrarius*) is an important member of the *Apodemus* group. Due to its strong adaptive abilities, the striped field mouse is wildly distributed in the Temperate Zone of Palearctic and Oriental regions (Musser and Carleton 1993). However, the limited dispersal abilities of striped field mouse make it subject to influence by geologic and climatic events, and form numerous local specific populations (Serizawa et al. 2000, 2002; Liu et al. 2004). Consequently, the striped field mouse is an excellent model for studying phylogeography and population genetics of organisms.

Microsatellite markers are ideal for phylogeography and population genetics analysis because of their abundance, high polymorphism content, co-dominance, and bi-parentally inherited characteristics (Zhang and Hewitt 2003). Therefore, although several microsatellite markers have been developed for striped field mouse (Makova et al. 1998), more microsatellite loci are still needed for phylogeography and population genetics studies on this species. In this paper, we report the isolation and characterization of 14 novel microsatellite loci in the striped field mouse.

Microsatellites were isolated as the enrichment protocols by Hamilton et al. (1999) with slight modifications as follows. Whole genome DNA was extracted from a male and female mouse samples preserved in 95% ethanol using standard phenol–chloroform procedures (Sambrook et al. 1989). Approximately 10 μg of genomic DNA was digested with *Mbo*I restriction enzyme (TaKaRa) and ligated to an adaptor generated by annealing together oligo A and oligo B (Refseth et al. 1997). Then the 300–1,000 bp DNA fragments were isolated from the ligated products and enriched by polymerase chain reaction (PCR) (5 min 72°C, then 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 67°C and 2 min extension at 72°C) using Oliga A as the PCR primer. Enrichment was carried out using $(CA)_{20}$ biotin-labelled probe and streptavidincoated magnetic

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beads (Promega). First, 6μg of the adaptor-ligated size fractionated genomic DNA was hybridized to 2μg of probe in 6× SSC at 50°C overnight, and the complex was added to 600 μl of streptavidin coated beads and the two were mixed for 1 h at 43°C. The unbound DNA was washed away using three washes each of 300 μl of 2× and 1× SSC. The enriched fragments that released from the probe were amplified to double-stranded form, and ligated into pMD18-T vector (TaKaRa) and transformed into competent Top10 cells. Then cells were plated onto LB agar plates containing ampicillin, IPTG and X-gal. Approximately 496 recombinant colonies were screened by PCR amplification directly from bacterial colonies using CA RPT and universal M13 primers (Lunt et al. 1999). 173 positive clones were sequenced using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and ABI 3730 Genetic Analyser.

Sixty-one sequences having adequate flanking regions for primer designing and showing no homology to microsatellite markers previously published for this species were selected for primer design. Primer pairs were designed using the software Primer 3 (http://www.genome.wi.mit.edu/genome_software/other/primer3.html.). Unlabelled primers were used to determine optimal amplification conditions. PCR amplifications were performed in 10 μl reaction volume containing approximately 20 ng of template DNA, 0.2 mM of each dNTP, 0.15 μM of each primer, 1.5 mM Mg^{2+}, 1× PCR buffer and 0.15 U of *Taq* DNA polymerase (TaKaRa). The amplification conditions were as follows: 95°C for 5 min, 35 cycles at 94°C for 30 s, Ta for 30 s, 72°C for 45 s and a final extension at 72°C 10 min. All reactions were amplified using an MBS Satellite thermal cycler (Thermo Electron Corp., USA). Finally, a total of 27 primers that had the efficiency of PCR

Table 1 Characteristics of fourteen polymorphic microsatellite loci in the striped field mouse (*Apodumus agrarius*)

Locus	Primer sequence (5′–3′) (F, forward; R, reverse)	Repeat motif	Labelling dye	T_a (°C)	A	Size range (bp)	H_O	H_E	GenBank accession no.
SFM1	F: GAGCTTAACTAGCCCTGAC	$(CA)_{19}$	TAMRA	52	9	181–201	0.750	0.878	EU430642
	R: ATGTGCCTCTGTTTGTGG								
SFM2	F: GCTTCGGATCTACCTACCTTT	$(CA)_{29}$	HEX	52	8	216–236	0.826	0.803	EU430643
	R: GCCTGCCTTTGACTCTGTTT								
SFM3	F: GGCTCCATCCTTCACTCC	$(GT)_{15}$	FAM	52	9	270–286	0.857	0.877	EU430644
	R: ATCCACCAACCACTTCTTTA								
SFM4	F: CGAGCAGGTCAATCAAGT	$(TC)_{20}$	TAMRA	50	9	211–231	0.435	0.894	EU430645
	R: TTTATCCATCAAGCCATC								
SFM5	F: CCAGCATTCATGCAGAGG	$(GT)_{14}$	HEX	52	7	218–234	0.652	0.861	EU430646
	R: TCGGGCGATGCTACCTTA								
SFM6	F: AGCCACCACATTTGGAAGAG	$(GA)_{28}$	FAM	54	10	294–312	0.895	0.872	EU430647
	R: CTACAGCCAGCAACAACAGG								
SFM7	F: CTTCGGATCATAACTCTTTC	$(GT)_{24}$	HEX	50	14	234–266	0.778	0.911	EU430648
	R: TTTCCCAACAATTAACTCAC								
SFM8	F: CTCAAGCGAGATGGAAAG	$(CA)_{17}$	HEX	48	9	207–235	0.625	0.789	EU430649
	R: GAAGCAGCACTAAGAAAC								
SFM9	F: AAGCCAAGCCCGAAGAAA	$(GT)_{21}$	FAM	52	10	266–288	0.600	0.886	EU430650
	R: TAGAGCCAGCCACCGTTA								
SFM10	F: AAAGTCTGTGGCGAGCATAA	$(GT)_{24}$	HEX	52	9	216–244	0.864	0.867	EU430651
	R: CCTAAAGAAGAACATCCCAAGTA								
SFM11	F: TTGTTTTCTTACCTCCAT	$(GA)_{24}$	TAMRA	48	10	204–226	0.350	0.815	EU430652
	R: ACCAACCAGCTATACTTT								
SFM12	F: TTTTCTCATAAGCACCCT	$(GT)_{15}$	HEX	48	8	234–250	0.714	0.843	EU430653
	R: CATCCATGCAGGCAAAGC								
SFM13	F: TTGTAATCCTCCTTCTTCAGC	$(CA)_{22}$	FAM	54	6	255–267	0.222	0.876	EU430654
	R: ACTTCACAGGGCTCGTCT								
SFM14	F: ATGCACATATCAACCCAA	$(CA)_{26}$	FAM	50	13	234–260	0.857	0.925	EU430655
	R: AGCAAACGACCTTACTCC								

T_a, annealing temperature; A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity

amplification were selected, and one of each of the 27 primer pairs was labelled with one of the fluorescent dye (FAM, TAMRA or HEX) for polymorphism detection. PCR reactions were performed as above but using the optimal annealing temperatures (Table 1). Genotyping of the 27 microsatellite loci for each individual was done by electrophoresis on an ABI Prism 3700 Genetic Analyser (Applied Biosystems), and fragment length was determined in comparison to an internal size standard (GeneScan ROX 400, Applied Biosystems) using GeneScan version 3.1.2 and Genotyper version 2.5 (Applied Biosystems).

Fourteen polymorphic loci were tested in 24 individuals from four populations at Southwest China, and were easily and consistently scored without ambiguity. Population genetic parameters were estimated in Genepop version 3.4 (Raymond and Rousset 1995). The number of alleles per locus ranged from 6 to 14 (Table 1). The observed heterozygosity ranged from 0.222 to 0.895 and the expected heterozygosity from 0.789 to 0.925 (Table 1). All loci followed Hardy–Weinberg expectations except SFM4, SFM11 and SFM13 loci. No significant linkage association was found among all loci. The 14 novel polymorphic microsatellite loci will be useful in studying phylogeography and population genetics of the striped field mouse.

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