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Isolation and characterization of 14 microsatellite loci for the Chevrier's field mouse (*Apodemus chevrieri*)

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Abstract

We describe the isolation and characterization of 14 microsatellite loci in the Chevrier's field mouse from genomic DNA-enriched libraries. The 14 loci were highly polymorphic with numbers of alleles per locus in 24 individuals ranging from three to 13, observed heterozygosity from 0.381 to 0.867 and expected heterozygosity from 0.448 to 0.931. All loci followed Hardy-Weinberg expectations except for CFM3, CFM10 and CFM12 loci. No significant linkage association was found among all these loci. The 14 polymorphic microsatellite loci will be useful in studying phylogeography and population genetics of the Chevrier's field mouse.

Keywords: Chevrier's field mouse (*Apodemus chevrieri*), heterozygosity, microsatellites, phylogeography, population genetics

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Table 1 Characteristics of fourteen polymorphic microsatellite loci in the chevrier's field mouse (*Apodemus chevrieri*)

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.
CFM1	F: CCTTGGCTCGGCAAACAG R: GCAGAGGCTAAATGTCAATA	$(CA)_{22}$	TAMRA	50	11	104–130	0.700	0.877	EU442520
CFM2	F: ATCAGCTTCTGGCATCTACAC R: TCCTGGTCTCCTTAACATTAC	$(CA)_{22}$	HEX	52	13	175–221	0.682	0.879	EU442521
CFM3*	F: CTCAACTGAAATACCCTA R: CTTGTTTGGAAGTGATGT	$(GT)_{21}$	TAMRA	52	8	166–188	0.381	0.720	EU442522
CFM4	F: TTTCTCCAGGAAGCAACA R: CCTCACTCACAGCAGGTATT	$(GT)_{20}$	TAMRA	54	3	156–160	0.500	0.448	EU442523
CFM5	F: ACTGAGAAAGATGACAAAGGTT R: CCTCACAGTGCTGGTGTT	$(CA)_{18}$	TAMRA	52	7	199–213	0.737	0.797	EU442524
CFM6	F: CCCCAAATCCCTATCTTAC R: CTACCCACTCTGCTATCCTC	$(CA)_{21}$	HEX	54	8	198–234	0.545	0.693	EU442525
CFM7	F: CCCTCAATCCCTGCTAAG R: TCCAATTCCTGCAACGAC	$(TC)_{16}$	HEX	50	12	231–281	0.867	0.931	EU442526
CFM8	F: GGGATAGCAAATGGTCAAAT R: CACTCATAACCTCAGCGTCA	$(GT)_{21}$	FAM	48	13	280–318	0.650	0.787	EU442527
CFM9	F: CCACCACATCTCGGCTATTT R: TACGGCTAAGCTGTCCTCTG	$(GT)_{19}$	TAMRA	52	8	174–208	0.737	0.852	EU442528
CFM10*	F: TGCAGCTAACAGAGGACTG R: TCTTGATTGCTATGGTGGT	$(GT)_{18}$	HEX	52	10	191–229	0.647	0.866	EU442529
CFM11	F: ACCCGAGTTAGTCAATAAGA R: CAGTAGATTCCCTGTGGC	$(GT)_{18}$	FAM	52	13	242–292	0.762	0.892	EU442530
CFM12*	F: CTGCTGCTTTCTGACATTAC R: ACATTGCTGGATTTGGAT	$(GT)_{16}$	FAM	52	7	244–258	0.522	0.739	EU442531
CFM13	F: ACAAGGTCATTCTCAGGT R: ATCTGCCTTGAGGATTAT	$(CA)_{17}$	HEX	48	4	233–241	0.545	0.589	EU604081
CFM14	F: TTCAGCTCAAGGTCACAATC R: TAATCTGGGATATGGGCTAG	$(CA)_{23}$	FAM	54	11	258–284	0.790	0.869	EU604082

T_a, annealing temperature; A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; *significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$).

The Chevrier's field mouse (*Apodemus chevrieri*) is distributed in the west central China (Carleton & Musser 1993). The limited dispersal abilities of Chevrier's field mouse make it subject to influence by geological and climatic events (Liu *et al*. 2004). Consequently, the Chevrier's field mouse is an excellent model for studying phylogeography and population genetics of organisms. Microsatellite marker is an ideal tool to analyse phylogeography and population genetics (Hewltt 2000). Therefore, we report in this paper the isolation and characterization of 14 microsatellite loci in the Chevrier's 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 two mouse muscle samples using standard phenol–chloroform procedures (Sambrook *et al*. 1989). Approximately 6 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 to 1000-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 oligo A as the PCR primer. Enrichment was carried out using $(CA)_{20}$ biotin-labelled probe and streptavidin-coated magnetic beads (Promega). 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. Approximately 496 recombinant colonies were screened by PCR amplification directly from bacterial colonies using CA RPT and universal M13 primers (Lunt *et al*. 1999). One hundred and ten positive clones were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and ABI PRISM 3730 Genetic Analyser.

Forty-four sequences having adequate flanking regions for primer designing 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, T_a 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.). Finally, a total of 21 primers were selected, and one of each of the 21 primer pairs was labelled with one of the fluorescent dye (FAM, TAMRA or HEX) for polymorphism detection. PCRs were performed as above but using the optimal annealing temperatures (Table 1). Genotyping of the 21 microsatellite loci for each individual was carried out 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 Southwest China. Population genetic parameters were estimated in GenePop version 3.4 (Raymond & Rousset 1995). The number of alleles per locus ranged from three to 13 (Table 1). The observed heterozygosity ranged from 0.381 to 0.867 and the expected heterozygosity from 0.448 to 0.931 (Table 1). All loci followed Hardy–Weinberg expectations except for CFM3, CFM10 and CFM12 loci. No significant linkage association was found among all these loci. The 14 polymorphic microsatellite loci will be useful in studying phylogeography and population genetics of the Chevrier's field mouse.

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Isolation and characterization of microsatellite markers from *Robinia pseudoacacia* L.

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Abstract

Microsatellite markers were isolated from *Robinia pseudoacacia* L. using an enrichment method. Eleven of the 23 primer pairs designed successfully amplified unambiguous and polymorphic single loci among 39 individual *R. pseudoacacia* L. from northeastern Japan. The observed and expected heterozygosities of the 11 microsatellite markers ranged from 0.333 to 0.821 and from 0.489 to 0.867, respectively. The polymorphisms observed at the 11 microsatellite loci are useful genetic data for forest ecological studies involving *R. pseudoacacia* L.