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Microsatellite loci for the Chinese bamboo rat *Rhizomus sinensis*

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Abstract

We describe 19 polymorphic microsatellite loci for the bamboo rat *Rhizomus sinensis*. When tested with 20 samples from a single population, these loci exhibited a mean of 4.5 alleles per locus and a mean expected heterozygosity of 0.612. All loci were in Hardy–Weinberg equilibrium and no evidence for linkage disequilibrium was detected between any loci. These loci will be useful for studying population genetic diversity and differentiation in the Chinese bamboo rat.

Keywords: Chinese bamboo rat, microsatellites, population genetics, *Rhizomus sinensis*

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The bamboo rat, *Rhizomus sinensis*, is a medium-sized, fossorial muroid rodent, approximately 700 g in weight and 40 cm in body length. The species is a bamboo specialist and its main distribution area comprises the large bamboo forests found between 1200 and 4000 m at altitude in central and southern China, Myanmar and Vietnam (Hu & Wang 1999). As part of an ongoing comparative population genetic and phylogeographic study of animal and plant species associated with this ecosystem, we report on the isolation of microsatellite markers for this important subterranean species.

Tissue samples were stored in 99.7% ethanol at ambient temperature in the field and at –20 °C in the laboratory. Total DNA from four samples, comprising three from Sichuan and one from Yunnan Province, China, was extracted using standard phenol–chloroform methods (Sambrook *et al.* 1989). We constructed a genomic library following the protocol of Glenn & Schable (2005).

DNA was digested overnight with *Rsa*I and *Stu*I (New England BioLabs), respectively, and the digested DNA was subsequently ligated to Super SNX24 linkers. We electrophoresed the ligated products in a 2% agarose gel and exercised fragments between 300 and 800 bp and re-extracted the DNA from the gel using the QIAquick Gel Extraction Kit (Qiagen). We then captured the fragments using a set of biotinylated microsatellite-containing oligonucleotides (mix 2 in Glenn & Schable 2005), and the biotinylated probe-DNA complex was enriched by hybridization to streptavidin-coated magnetic beads (Dynabeads M-280, Invitrogen). Nonspecific DNA was removed by

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Table 1 Characteristics of 19 polymorphic microsatellite loci isolated for the Chinese bamboo rat

Locus	Primers (F, forward; R, reverse)	Motif	5′-Label	T_a (°C)	A	Size range (bp)	H_O	H_E	GenBank ID
BR5	F: ATGTCCCCAGGCTTCGTTG R: TGGAGATCAAATATGCAGAAGAGC	$(TG)_{16}$	HEX	62	5	255–265	0.600	0.587	FJ664554
BR11	F: TCTTGGGGGTTTTCTGACATT R: ATTCCCAAAAGCTACCCTCTGAC	$(TG)_{16}$	FAM	58	3	171–177	0.650	0.559	FJ664555
BR16	F: TCACTACTGAGCTTTCTTTAGGTC R: TGGAACAATAAATGAGTAACGGAAC	$(TG)_{17}$	FAM	62	2	214–232	0.600	0.513	FJ664556
BR17	F: TGTATTGGGCATCAGCA R: CTGTCAAAAGTAAGGGAAATCAT	$(AG)_{23}$	FAM	54	7	124–133	0.850	0.809	FJ664557
BR18	F: AGACTGGCTGTTGAGGGC R: CAGTCCCAGGGGTGGAAAG	$(TG)_{30}(AG)_{11}$	FAM	62	5	204–230	0.700	0.680	FJ664558
BR23	F: GGATCCCTGAGCTGTGACTGTGA R: TGAGAAAGATGCCCAAAGGTGA	$(TG)_{11}(TG)_{14}$	FAM	62	2	140–144	0.500	0.431	FJ664559
BR30	F: TTCATTGCACCTGCTTGCC R: TGTGACTTCCCCACCACAG	$(AG)_{17}$	HEX	62	3	205–219	0.650	0.537	FJ664560
BR33	F: GGCAGCTACCTCCTACCTCAGT R: AACCTATATCCCCAGCCTTTTA	$(AG)_{31}(AG)_9(AG)_6$	HEX	62	7	143–203	0.650	0.800	FJ664561
BR42	F: AGTGGTGGCTCTGGTATTGG R: ATTGACCTCGCTGCTTTTG	$(TG)_{18}$; $(TG)_{13}$	HEX	54	4	250–256	0.650	0.571	FJ664562
BR2	F: GTCAGAGTTCAGGCTTGGATGTC R: CTAATGCTGGTGAGGTGGAGATG	$(TG)_{25}$	TAMRA	62	5	191–201	0.600	0.610	FJ664553
BR6	F: GGAATTGCTGCCATATAAAGAGTT R: TGTAGAAGGCCAGATGAGATG	$(TG)_{21}$	FAM	63	4	105–119	0.650	0.588	FJ664563
BR9	F: GGAAACCAACGGTGCCAAG R: TGGACACAACAGACAGGTAGG	$(ATCT)_{11}$	HEX	62	4	210–222	0.800	0.700	FJ664564
BR19	F: AGCATGGAAGTTAGAAGACCAAAG R: GGCTTCAACACAGCACCAC	$(TG)_{18}$	FAM	62	7	165–195	0.550	0.764	FJ664565
BR29	F: TGGAAACTCACATCGTAGGC R: ATTGACCTCGCTGCTTTTG	$(TG)_{19}$	HEX	62	3	158–166	0.550	0.573	FJ664566
BR31	F: AGGAACCACATCTGCCAATC R: ACAGAAGCCATGAGGGTGG	$(AG)_{11}$;$(AG)_{10}$; $(TG)_{16}$	FAM	62	9	242–278	0.550	0.571	FJ664567
BR36	F: AAAGTTGGGGTGTTTTTAGTTA R: GTGTGCAGGCCTGTTGAGTT	$(TG)_{20}$	FAM	54	3	177–181	0.550	0.504	FJ664568
BR20	F: GGGGGCTGTTGTTGTTT R: TTTTTGCGAAGATGCTAA	$(TG)_{27}$	TAMRA	62	3	262–266	0.750	0.527	FJ664569
BR38	F: CACACACCTCCATGGCTTC R: ACTGGGGAGGCAGAAACAG	$(TG)_{16}$	TAMRA	62	3	225–237	0.350	0.555	FJ664570
BR40	F: AAGCCACAGATTATTTTAG R: GACTCCCTCATGTTATCCT	$(TG)_{20}$	HEX	50	6	128–138	0.700	0.741	FJ664571

washing twice with 2× SSC, 0.1% SDS, twice with 1× SSC, 0.1% SDS at room temperature and twice again with 1× SSC, 0.1% SDS at 50 °C. After recovery by PCR using the forward SuperSNX-24 primer, the enriched library was constructed using a TA Cloning Kit according to the manufacturer's instructions (Invitrogen). Positive colonies were amplified using universal M13 forward and reverse primers (M13F: 5′-GTAAAACGACGGCCAG-3′; M13R: 5′-CAGGAAACAGCTATGAC-3′) and fragments between 500 and 1200bp were selected to be sequenced using the ABI BigDye terminator kit, following the manufacturer's instructions and were electrophoresed using a 3130XL Genetic Analyzer (Applied Biosystems).

Sequences were assembled and edited in MEGA 4.0 (Tamura *et al*. 2007) and visually checked for microsatellite repeats. Of the 192 colonies analysed, 161 were successfully sequenced and 86 had repeats (53%). Unique sequences with sufficient flanking DNA and more than 10 repeat units were selected for primer design using the PRIMERSELECT (DNAStar) and Primer 3 in MSATCOMMANDER (Faircloth 2008). Forty-one pairs of primers were designed with melting temperatures between 50 and 66 °C and length of PCR products between 100 and 300 bp.

Twenty bamboo rat DNA templates were extracted from samples collected from a population in Labahe Nature Reserve, Sichuan, China. Five extracts were used to optimize the PCR conditions of unlabelled primers. PCR amplifications were performed in a GeneAmp® PCR System 9700 (Applied Biosystems) in a 10-μL reaction volume containing 0.5 μL of template DNA, 0.2 μM of each primer, 5 μL of *Premix Taq* (TaKaRa, 0.25 μL *Takara Ex Taq*, 0.2 mM each dNTP, 1× *Ex Taq* Buffer) and 0.2 μg/μL of BSA

(New England BioLabs). The amplification conditions were as follows: 94 °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 for 10 min. A total of 27 primers, which produced a single target band, were obtained and each forward primer was labelled with one of the fluorescent dyes (5′-FAM, TAMRA or HEX; Invitrogen). All 20 DNA extracts were used to test the polymorphism of the loci. PCR reactions were performed as mentioned earlier but using the optimal annealing temperatures (Table 1). Amplification products were scanned together with the GS-400 HD Rox standard in an ABI Prism 3700 Genetic Analyzer (Applied Biosystems), and fragment lengths were scored using GENESCAN v3.7 and GENOTYPER v3.7 (Applied Biosystems).

Nineteen polymorphic microsatellite loci with reliable and consistent results were identified (Table 1). All loci were successfully genotyped on all the 20 DNA samples. The mean number of alleles was 4.5 (range 2–9). The observed and expected heterozygosities for each locus were calculated using ARLEQUIN v3.1 (Excoffier & Schneider 2005). The expected heterozygotisty was 0.612 on average (range 0.431–0.809) and observed heterozygosity was 0.626 on average (0.5–0.8). Exact Hardy–Weinberg probabilities were tested using GENEPOP (Raymond & Rousset 1995) with default parameters and ARLEQUIN v3.1 with 100 000 Markov chain steps. Significance levels were adjusted using Bonferroni corrections for multiple testing ($P < 0.003$ in our dataset). All loci were in Hardy–Weinberg equilibrium. ARLEQUIN, GENEPOP and FSTAT v2.9.3 (Goudet 2001) were used to test linkage disequilibrium between loci. No consistent evidence for linkage disequilibrium between any pair of loci was found. These 19 microsatellite loci will be useful for the future study of population genetic diversity and differentiation in the bamboo rat.

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