PERMANENT GENETIC RESOURCES

Isolation and characterization of microsatellite loci in the western long-fingered bat, *Miniopterus magnater*

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Abstract

We isolated and characterized 10 microsatellite loci in the western long-fingered bat, *Miniopterus magnater*. These loci were tested on 48 individuals from Anhui Province of China, and all loci were highly polymorphic. The mean number of observed alleles per locus was 13.6 (range from six to 27). Observed and expected heterozygosity values ranged from 0.364 to 0.957, and from 0.676 to 0.951, respectively. After Bonferroni correction, four loci deviated significantly from Hardy–Weinberg equilibrium. No pairs of loci were in linkage disequilibrium. These polymorphic markers will be used to examine population structure and genetic diversity in this species.

Keywords: genetic diversity, microsatellites, *Miniopterus magnater*

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The western long-fingered bat (*Miniopterus magnater*) is an insectivorous and gregarious species, widely distributed over northeast India, southeast China, Myanmar, Thailand, Laos, Vietnam, Malaysia, Sumatra, Java, Timor (Indonesia), Borneo, McLucas and New Guinea, including the Bismarck Arch (Simmons 2005). In China, this species has been found in Anhui, Hainan, Fujian, Guangxi and Guizhou Provinces, among others (Zhang 1999). Field surveys indicate that these bats usually congregate in moist and dark places, such as caves or man-made structures. Coronavirus has been isolated from this species (Poon *et al*. 2005), which has implications for human health in regions where humans may come into contact with bats. However, to date, very few studies have been carried out on this species, and so little is known about its population structure, genetic diversity, and migration and roosting habits. Development of appropriate molecular markers will greatly facilitate such research. Here, we describe 10 microsatellite loci that were isolated from *M. magnater,* in order to study population structure and genetic diversity of this species.

Samples of *M. magnater* were collected from a mine cave (31°32′N, 116°08′E) of Jinzhai County in Anhui Province,

East China. Muscle biopsies were taken from five bats and preserved in 95% alcohol. Genomic DNA was extracted with the DNeasy Tissue Kit (QIAGEN). The enrichment method used to construct a partial genomic DNA library for isolating microsatellite loci of (AC) dinucleotide repeats was based on Kandpal *et al*. (1994) and Karp *et al*. (1998), with some additional modifications (Hua *et al*. 2006).

Genomic DNA samples from five animals were pooled and digested with the restriction enzyme *Mbo*I (TaKaRa) overnight. The digested products were electrophoresed through an agarose gel, and fragments of 400–800 bp were excised and recovered using the agarose gel purification kit (TaKaRa).

The purified fragments were then ligated to a blunt-end adapter (adapter A, 5′-GCGGTACCCGGGAAGCTTGG-3′ and adapter B, 5′-GATCCCAAGCTTCCCGGGTACCGC-3′) with T4 DNA ligase (TaKaRa) for 18 h at 16 °C. The ligation products were amplified using the adapter A sequences as forward and reverse primers with the annealing temperature 67 °C in a PTC-220 thermal cycler (Bio-Rad). The recovered polymerase chain reaction (PCR) products were denatured by incubation at 95 °C for 5 min, and hybridized to a biotin-labelled dinucleotide repeat $(CA)_{15}$ probe (Sangon) in 0.5 M sodium phosphate and 0.5% sodium dodecyl sulphate (pH 7.4) for 16 h at 50 °C. The hybridization mixture was incubated with VECTREX Avidin D (Vector Laboratories)

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Table 1 Characterization of 10 microsatellite loci isolated from *Miniopterus magnater*

Locus	Primer sequence (5′–3′)	Fluorescent dye	Allele size range (bp)	Repeat motif	No. of alleles	T_a (°C)	H_E	H_O	GenBank Accession no.
CH37*	F: GAAATCAGATGGAAATGCCTCA	FAM	100–146	$(TG)_3CG(TG)_{12}$	18	62	0.912	0.487	EU010361
	R: CTCCAGCCACATGGTAATGC								
CHD2	F: GGAGGCTGATAAGTTGGTCTA	FAM	207–231	$(AC)_{24}$	13	56	0.900	0.822	EU010362
	R: GCACTTGGCAATGGATGTA								
CHB6*	F: CATGGCTTTGTGTCTCTGTCC	HEX	373–389	$(AC)_9$	9	56	0.846	0.447	EU010363
	R: TGTTATCTGCTATGCGACCCT								
CH87	F: ATGGCACCCAGTAGGTTTCC	FAM	140–152	$(TG)_6CG(TG)_6$ $(CG)_3(TG)_3TA(TG)_3$	6	59	0.676	0.660	EU010364
	R: TTCCCACAGAGCCTGCCTGT								
CHB12	F: GGGGATGTGAGATATGAGT	FAM	244–262	$(TG)_{11}$	10	54	0.780	0.792	EU010365
	R: ACAGCTCATCATGTTATAGC								
CHD3	F: CCAGTGGCAACTTTCTTGTT	HEX	290–314	$(TG)_{14}$	10	62	0.732	0.558	EU010366
	R: AGCCCAGTTTGTCACATCTC								
CHA14	F: ATGAATGGCAGGTAGGTGAC	TAMARA	237–283	$(TG)_{19}$	15	58	0.733	0.625	EU010368
	R: CTTGCTACTTAGGCTTTCTCC								
CH13	F: GGGAAAGAATAAAAGAGGAGC	FAM	166–230	$(TG)_{15}$	27	58	0.951	0.957	EU010369
	R: ATTCGCAGTTTCACTATGGC								
CH42*	F: TGCTTCCTAGCATCCCTCT	HEX	272–294	$(TG)_{11}$	12	58	0.845	0.489	EU010371
	R: CCATTCAACGCACATAAAC								
CH85*	F: GCTGGGTTTGTGCCAAGGAC	TAMARA	328–370	$(AC)_{17}$	16	63	0.880	0.364	EU010372
	R: GATGTAAAGCGGGGCGTGAA								

Abbreviations used: F, forward primer, 5′-labelled (FAM, TAMARA or HEX); R, reverse primer; T_a, annealing temperature of the primer pairs; H_O, observed heterozygosity; H_E, expected heterozygosity; *indicates significant deviation from Hardy–Weinberg equilibrium after Bonferroni correction for multiple comparisons (adjusted $\alpha = 0.005$).

in binding buffer (100 mM Tris, 150 mM NaCl, pH 7.5) at 37 °C and then washed four times with binding buffer at increasing temperatures to remove unbound fragments (37 °C for 30 min, 50 °C for 30 min, 65 °C for 30 min, and finally 65 °C for 30 min in 0.1× binding buffer). Bound fragments were eluted with dH_2O after incubating at 65 °C for 30 min, and then recovered by PCR amplification as described above. These targeted fragments were used to construct the (AC) repeat genomic library by ligating with a pMD19-T vector (TaKaRa), which was then used to transform DH5α competent cells (Tiangen).

We identified 67 positives out of 127 recombinant colonies using the M13 universal primers. They were selected for sequencing with M13 primers (Invitrogen). We identified 43 sequences containing CA/TG repeats, 24 of which were selected to design primers for the microsatellite flanking regions using the software PRIMER PREMIER 5.0 (Premier Biosoft International). Ten primer pairs (Table 1) amplified polymorphic microsatellite loci and these were used to screen genetic variation.

We collected wing membrane biopsies (Worthington Wilmer & Barratt 1996) from 48 *M. magnater* individuals and preserved them in 95% alcohol. Genomic DNA was extracted from the biopsies with the DNeasy Tissue Kit (QIAGEN). PCRs were carried out in a PTC-220 thermal cycler (Bio-Rad) using the following conditions in a 15-µL

reaction volume: 50–100 ng genomic DNA, 0.2 µM 5′-labelled (FAM, TAMARA or HEX) forward primer (Table 1), 0.2 µM unlabelled reverse primer, 0.2 U HotStar*Taq* DNA Polymerase (QIAGEN), 1× PCR buffer (containing 1.5 mM $MgCl_2$) and 0.2 mM of each dNTP. The PCR cycling was based on following conditions: initial denaturation at 95 °C for 15 min followed by 35 cycles of 30 s at 94 °C, 30 s at the relevant annealing temperature (Table 1) and 30 s at 72 °C, with a final extension step of 20 min at 72 °C. The PCR products were genotyped on an ABI 3730 sequencer (Applied Biosystems) and analysed with GENESCAN 3.7 (Applied Biosystems) and GENOTYPER 3.6 software (Applied Biosystems). Calculation of the expected and observed heterozygosities, and tests for both Hardy–Weinberg equilibrium and linkage disequilibrium were performed with GENEPOP version 3.4 software (Raymond & Rousset 1995).

We identified a total of 136 alleles from the 10 microsatellite loci. The mean number of alleles per marker was 13.6 (range: 6–27, Table 1). Observed and expected heterozygosity values ranged from 0.364 to 0.957, and from 0.676 to 0.951, respectively. After Bonferroni correction, four loci (CH37, CHB6, CH42 and CH85) showed significant heterozygote deficit ($P < 0.005$, $k = 10$). The presence of null alleles, stuttering and large allele dropout of the four loci was detected with MICRO-CHECKER version 2.2.3 software (van Oosterhout *et al*. 2004). None of them showed stuttering or

large allele dropout, but all of the four loci showed the presence of null alleles ($P < 0.05$). No pairs of loci displayed linkage disequilibrium (adjusted $\alpha = 0.00111$, $k = 45$). We anticipate that the microsatellite markers reported here will be useful to evaluate genetic variability and the gene flow of *M. magnater* in the near future.

Acknowledgements

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