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 6 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 MboI (TaKaRa) overnight. The digested products were electrophoresed on 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′-GCGGTACCCTGGAAGCTTGG-3′ and adapter B, 5′-GATCCCCAGCTTCCCCGGGTACC-3′) with T4 DNA ligase (TaKaRa) for 18 h at 16 °C. The ligation products were electrophoresed through an agarose gel, and fragments of 400–800 bp were excised and recovered using the agarase gel purification kit (TaKaRa).

The purified fragments were then ligated to a blunt-end adapter (adapter A, 5′-GCGGTACCCTGGAAGCTTGG-3′ and adapter B, 5′-GATCCCCAGCTTCCCCGGGTACC-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 30 °C. The hybridization mixture was incubated with VECTREX Avidin D (Vector Laboratories)
ments were eluted with dH₂O after incubating at 65 °C.

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

| Locus | Primer sequence (5′–3′) | Fluorescent dye | Allele size range (bp) | Repeat motif | No. of alleles | _T_α (°C) | _H_β | _H_O | GenBank Accession no. |
|-------|-------------------------|-----------------|------------------------|--------------|---------------|-----------|------|------|----------------------|
| CH35* | F: GAAATCAGTGTGAAAGACTTCCA | FAM | 100–146 | (TG)_3(CG(TG))_12 | 18 | 62 | 0.912 | 0.487 | EU010361 |
| CHD2 | F: GGGCTGTTAGTTGTTGTTCTTA | FAM | 207–231 | (AC)_34 | 13 | 56 | 0.900 | 0.822 | EU010362 |
| CHB6* | CH2 F: CAGGCTCTTTATCCCTGCCC | HEX | 373–389 | (AC)_9 | 9 | 56 | 0.846 | 0.447 | EU010363 |
| CH87 | F: ATGACCAGATTGATTTCCC | FAM | 140–152 | (TG)_3(CG(TG))_6 | 6 | 59 | 0.676 | 0.660 | EU010364 |
| CHB12 | F: GGGATATGCTGATAAGTTGTA | FAM | 244–262 | (TG)_11 | 10 | 54 | 0.780 | 0.792 | EU010365 |
| CHD3 | F: CCAGTGGCAACTTTCTTGTT | HEX | 290–314 | (TG)_14 | 10 | 62 | 0.732 | 0.558 | EU010366 |
| CHA14 | F: AGCACTCTTGTGCAGCCTGAC | TAMARA | 237–283 | (TG)_19 | 15 | 58 | 0.733 | 0.625 | EU010368 |
| CH13 | F: GAGAAAAGATAAAGAGGAGGC | TAMARA | 166–230 | (TG)_15 | 27 | 58 | 0.951 | 0.957 | EU010369 |
| CHH4* | TACCTCTTCAGATCTCCCTT | HEX | 272–294 | (AC)_7 | 12 | 58 | 0.845 | 0.489 | EU010371 |
| CHH5* | F: GCTTGCTTGGCCCAAGGAGC | TAMARA | 328–370 | (AC)_16 | 16 | 63 | 0.880 | 0.364 | EU010372 |

Abbreviations used: F: forward primer, 5′-labelled (FAM, TAMARA or HEX); R: reverse primer; _T_α, annealing temperature of the primer pairs; _H_β, observed heterozygosity; _H_β, expected heterozygosity; *indicates significant deviation from Hardy–Weinberg equilibrium after Bonferroni correction for multiple comparisons (adjusted α = 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 dH₂O 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 (Tianang).

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 MgCl₂) 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, CHH4 and CHH5) 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

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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.

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References
Hua PY, Chen JP, Zhang LB, Liang B, Rossiter SJ, Zhang SY (2006) Isolation and characterization of microsatellite loci in the flat-headed bat (\( Tylonycteris \ pachypus \)). Molecular Ecology Notes, 7, 486–488.
Kandpal RP, Kandpal G, Weissman SM (1994) Construction of libraries enriched for sequence repeats and jumping clones, and hybridization selection for region-specific markers. Proceedings of the National Academy of Sciences, USA, 91, 88–92.
Karp A, Isaac PG, Ingram DS (1998) Molecular Tools for Screening Biodiversity. Chapman & Hall, London, UK.
van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4, 535–538.
Poon LLM, Chu DKW, Chan KH et al. (2005) Identification of a novel coronavirus in bats. Journal of Virology, 79, 2001–2009.
Raymond M, Rousset F (1995) genepop (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86, 248–249.
Simmons NB (2005) Order Chiroptera. In: Mammal Species of the World: a Taxonomic and Geographic Reference (eds Wilson DE, Reeder DM). The Johns Hopkins University Press, Baltimore, Maryland.
Worthington Wilmer J, Barratt EM (1996) A non-lethal method of tissue sampling for genetic studies of chiropterans. Bat Research News, 37, 1–3.
Zhang YZ (1999) Zoogeography of China. Science Press, Beijing, China.