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PRIMER NOTE

Characterization of microsatellite loci in fulvous fruit bat *Rousettus leschenaulti*

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Abstract

***Rousettus leschenaulti* is an abundant species in many countries of South-East Asia, including south China. We isolated seven microsatellite loci in *R. leschenaulti* from genomic DNA enriched for CA repeats with the enriched library method. A total of 56 samples from a population in the Guangxi Province of China were tested with these microsatellite markers. The polymorphism ranged from seven to 16 alleles, and the observed heterozygosity was 84–94%. It is the first time microsatellite markers were characterized from *R. leschenaulti*, and these markers can be an important tool for analysing population structure and genotypic diversity.**

Keywords: microsatellite loci, polymorphism, *Rousettus leschenaulti*

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The fulvous fruit bat, *Rousettus leschenaulti*, is distributed over Sri Lanka, Pakistan, Vietnam, south China, Java and Bali. It prefers to roost in dark places, such as caves or man-made structures (Khan 1985). The main food of *R. leschenaulti* is fruit, e.g. litchi and longan (Fujita & Tuttle 1991). Occasionally, it also eats orchards, which has resulted in the killing of bats in large scale in China, and the population size has decreased dramatically. Moreover, some kinds of virus such as west Nile virus, Parainfluenza and SARS like coronaviruses were found in *R. leschenaulti* (Paul *et al*. 1970; Pavri *et al*. 1971; Li *et al*. 2005), it has a direct effect on human health. Although it has a close relationship with human, few researches on *R. leschenaulti* involve its population studies by molecular means. In order to study the genetic structure of *R. leschenaulti* with effective molecular markers, we isolated microsatellite loci from a DNA library enriched for $(CA)_n$ repeats. This study reports the isolation and characterization of these loci and the genetic polymorphism in a population from Guangxi, China.

The specimens of *R. leschenaulti* were collected from the cave of Yiling in Guangxi, south China. Genomic DNA was extracted from the muscle tissue with the DNeasy Tissue Kit (QIAGEN). The enrichment method for isolating CA repeats was based on Kandpal *et al*. (1994) and Karp *et al*. (1998) with some modifications. The genomic DNA extracted from *R. leschenaulti* was digested with *Mbo*I, and the fragments between 400 and 800 bp were cut out from an agarose gel and purified with DNA Purification Kit (TaKaRa). The selected fragments were ligated to linker sequences and amplified by using linker specific primers. The polymerase chain reaction (PCR) product was denatured and hybridized with biotin-labelled dinucleotide repeat oligonucleotides $(CA)_{15}$ probe. The hybridization mixture was mixed with VECTREX Avidin D (Vector Laboratories). After successively washing for four times with buffer A (150 mM NaCl/100 mM Tris, pH = 7.5) at different temperatures to remove the unbound fragments, the matrix was washed with dH_2O to elute the bound fragments and the supernatant was retained. A PCR was performed to amplify the targeted genomic fragments and the product was ligated to pMD19-T vector (TaKaRa). *Escherichia coli* DH5α competent cells were transformed with the ligation product to construct the $(CA)_n$-enriched DNA library. Out of 135 recombinant colonies, 53 positive clones were identified using linker primers and M13 universal primers amplification. They were selected for sequencing with M13 primers (Invitrogen). According to the microsatellite insert sequences, primers flanking microsatellite repeats for PCR amplification were designed using the PRIMER PREMIER 5.0

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Table 1 Listed locus, primer sequences, repeat motifs, annealing temperatures, number of alleles, size range, H_E (expected) and H_O (observed) heterozygosities from *Rousettus leschenaulti*

Locus	Primer sequence	Repeat motif	T_m (°C)	Number	Size range of alleles (bp)	H_E	H_O	GenBank Accession no.
M3-8	F: TGACCCAGTAGCATGAGCAG R: TGATGCCCAGGGAGCTATATT	$(GT)_{20}$	65	13	176–206	0.917	0.839	DQ389096
M3-6	F: ACACGCTACCAGTTCTGTGA R: TTAAGGACCCAGGAGACAGC	$(GT)_{16}$	65	16	172–210	0.943	0.911	DQ389097
M4-2	F: ATTCCACTATAAGTGAGT R: GAGCCTAAGTATCCATC	$(CT)_{10}(CA)_9$ TA$(CA)_{10}$	53	12	225–263	0.920	0.893	DQ389098
M3-120	F: CGCATTCTGCCTTTAGGTGT R: GACTAGAAGTCTGTTGTGCTG	$(CA)_{13}$	61	14	176–206	0.899	0.946	DQ389099
M3-121	F: TAGGAAGCAGCCAGAGCAGT R: CCCACCCTAAGCAGAATGAG	$(GT)_{15}$	65	8	168–194	0.877	0.929	DQ389100
M3-3	F: TTCGGGAGGTATGAGATGAGA R: CACAGTTGTTCCAACCAGGTACT	$(CA)_{15}$	65	9	175–205	0.890	0.839	DQ389101
M3-1	F: AGTTATAGGCCACACCGAAA R: TCAGGAGTTCTTAACTTTGAAT	$(CA)_{14}C(CA)_2$	61	7	197–215	0.848	0.839	DQ389102

software (PREMIER Biosoft International) and PRIMER 3 (Rozen & Skaletsky 2000) (see Table 1). The PCR conditions were optimized through gradient PCR amplification.

A total of 56 samples collected from Yiling of Guangxi, China, were genotyped. Genomic DNA was extracted using the conventional proteinase K/phenol–chloroform extraction protocol (Sambrook & Russell 2001). PCRs were carried out in a PTC-200 (MJ Research) thermal cycler using the following conditions in a total of 10 μL volume: 50–100 ng genomic DNA, 0.25 μM of each primer and 1× PCR buffer containing 2 mM $MgCl_2$, 0.4 mM of each dNTP and 0.25 U *Taq* DNA polymerase (Premix *Taq*, TaKaRa). The reactions were performed using the following conditions: denaturation at 95 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, annealing temperature for 30 s (see Table 1), 72 °C for 40 s, with a final extension step at 72 °C for 5 min. The PCR products were separated on 10% polyacrylamide gels (29:1 acrylamide : bis-acrylamide, 1× TBE, 120 V for 12–14 h) and displayed with silver nitrate staining (Allen & Budowle 1989). The pUC19-*Msp*I digest DNA marker used in electrophoresis was purchased from MBI Fermentas. The length of alleles was identified with QUANTITYONE 4.5.1 software (Bio-Rad).

For all seven microsatellite loci of *R. leschenaulti*, a total of 79 alleles were found and the average number was 11.3 alleles per locus, ranged from seven (M3-1) to 16 (M3-6). Expected and observed heterozygosities, Hardy–Weinberg equilibrium test and linkage disequilibrium (LD) were analysed with GENEPOP version 3.4 software (Raymond & Rousset 1995). The results showed that expected heterozygosity ranged from 0.85 (M3-1) to 0.94 (M3-6). The mean observed heterozygosity (0.885) was not significantly different from the mean expected heterozygosity (0.899). No locus pair showed significant LD ($P < 0.05$) among the seven loci.

Based on the results of the high heterozygosity and large number of alleles, we conclude that these loci can serve as an effective molecular tool in population genetic analyses and population biology studies of *R. leschenaulti.* This has potential significance for further studying the relationship between *R. leschenaulti* and human health.

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