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TECHNICAL NOTE

# Isolation and characterization of microsatellite loci in the Indian false vampire bat *Megaderma lyra*

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**Abstract** We isolated and characterized eight microsatellite markers for Indian false vampire bat *Megaderma lyra.* These loci were tested on 60 individuals representing four populations, and all loci were highly polymorphic. The mean number of observed alleles per locus was 13.1 (range from 5 to 24). Observed heterozygosity values range from 0.876 to 0.982, and expected heterozygosity value were ranged from 0.986 to 1.0. Out of eight loci, only two loci deviated significantly from Hardy–Weinberg equilibrium, and no pairs of loci were in linkage disequilibrium. These polymorphic markers will be useful to examine population structure, mating and dispersal behaviour, including monitoring the effect of habitat fragmentation and parental analysis.

**Keywords** Chiroptera · *Megaderma lyra* · Microsatellite · Polymorphism

The Indian false vampire bat, *Megaderma lyra*, belongs to a heterogeneous group of echolocating bat called gleaners. Gleaning bats prefer to capture prey from ground and water surfaces. They consume larger arthropods and smaller vertebrates such as frogs, geckoes, lizards, fish, mice, birds and even on smaller bat species (Advani 1981). This species is broadly distributed from Afghanistan to southern China, Pakistan, India, Sri Lanka and Malaysia (Bates and Harrison 1997). Like many tropical bats, individuals of *M. lyra* (30–50 bats) use their roost constantly for several years and exhibit no seasonal migration or hibernation. It roosts in caves, temples, old buildings and artificial underground (Brosset 1962). The period of partiturition fell between January and May, and produces a single young in a reproductive season (Emmanuvel Rajan and Marimuthu 1999). Recent studies have reported that it's reproductive success as well as the population growth adversely affected by many factors such as climate, urbanization process, modernization in agriculture, disturbances of suitable roosting sites and foraging habitats (Sripathi et al. 2004). Molecular genetics techniques provide easy way to understand the complex behavior, genetic structure and parentage analysis (Sugg et al. 1996; Hughes 1998). Here, we report the isolation and characterization of microsatellite marker for *M. lyra,* that will be a reliable tool to assess population structure, gene flow, and dispersal pattern and, useful to generate valuable guideline for conservation strategies.

From individuals of *M. lyra*, a 3-mm wing membrane biopsy punch was taken and stored in TENS (Tris Cl pH7.5, 10 mM EDTA, 125 mM NaCl, 1.0% SDS) extraction buffer, following the method described by Wilmer and Barratt (1996). Genomic DNA was isolated from tissue samples according to proteinase K, and phenol–chloroform protocol (Sambrook et al. 1989). A microsatellite library was constructed based on PCR isolation of microsatellite arrays (PIMA) method proposed by Lunt et al. (1999). This method takes advantage of the fact that the RAPD fragments contain microsatellite repeats more frequently than random genomic library clones (Cifarelli et al. 1995). The amplified RAPD-PCR products were size selected (200–700 bp) then purified using spin column (Invitrogen Inc),

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and ligated to pTZ57R/T cloning vector (Fermentas International Inc). Freshly prepared *Echerichia coli* DH5α competent cells were transformed with ligation mixture to construct DNA library. Single recombinant colonies were taken from LB agar Amp + plates that contained X-gal (40 mg/ml) and IPTG (30 mg/ml) by blue/white selection.

Plasmids containing microsatellite repeats were detected by hybridization of biotin-11-dUTP (DecaLabel DNA Labeling Kit, Fermentas International Inc) labeled probes $(GA)_{12}$, $(CT)_{13}$, $(GTCAC)_5$ (Sigma Genosys, India) with southern hybridization of PCR amplified plasmid inserts (Glenn et al. 2000). Specific hybridization of microsatellite oligonucleotide probe with plasmid inserts was visualized using streptavidin conjucated-alkaline phosphatase (AP) and chromogenic detection (Fermentas International Inc). Among the 320 recombinant clones, 44 positive clones were identified as positive, which were sequenced with M13 primers (Bangalore GeNei, India). We obtained 17 sequences containing microsatellite repeats, seven of these did not have sufficient flanking region for primer design. Primers were designed for the best 10 microsatellite sequences using Primer 3 software (Rozen and Skaletsky 2000). Initially, primers were tested for amplification and polymorphism across 15 individuals from a population, located at Tiruchirappalli (10°10′N, 78°15′E). The optimal annealing temperature and $MgCl_2$ concentrations for each primer pair was standardized (Table 1) on a gradient thermal cycler (MJ Mini Thermal Cycler, Bio-Rad, USA). Two primer pairs failed to detect polymorphisms and were not screened further in *M. lyra*, eight primer pairs were amplified consistently and used to test the polymorphism of the loci.

To quantify the level of polymorphism, a total 60 individuals of *M. lyra* representing four populations (Tirunelveli: 08°50′N, 77°67′E; Sattur: 09°50′N, 77°06′E; Madurai: 09°58′N, 78°10′E; Tiruchirappalli 10°10′N, 78°15′E) were genotyped for each loci. PCR amplifications were carried out with the following profile: 94°C for 3.00 min, 35 cycles of 94°C for 1.00 min, the optimized annealing temperature (Table 1) for 1.00 min and 72°C for 1.00 min; final extension of 72°C for 10.00 min. Each PCR mixure (20 µl) contained 50 ng of genomic DNA, 2.0 pmol of each primer, 0.2 mM each dNTP, X mM $MgCl_2$ (X was 1.0 or 2.0) using 1.0 unit of *Taq* DNA polymerase (Promega) and 1× PCR buffer.

The PCR products were resolved in 6.0% polyacrylamide gel and bands were visualized by silver staining (Neilan et al. 1994). Size of the alleles was estimated according to 100 bp DNA ladder (RBC Bioscience, Taiwan). Gel images were scanned and the number of alleles was determined with Quantity One software (Bio–Rad, USA). Levels of observed ($H_O$) and expected heterozygosities ($H_E$) were calculated using Genetic Data Analysis (Ver 1.0) software (Lewis and Zaykin 2001).

**Table 1** Primer sequences and characterization of the eight microsatellite markers isolated from *Megaderma lyra* through genotyping 60 individuals

| Locus | GenBank Accession no. | Repeat motif | Primer sequence (5′–3′) | $T_a$ (°C) | $MgCl_2$ (mM) | $N_A$ | Size range (bp) | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|---|---|
| MlyraE1 | GQ240802 | $(TG)_6$ | F: TTAAACGGGTGTGCAGAGTG | 45.5 | 1.5 | 12 | 105–218 | 0.982 | 1.000 |
| | | | R: AGTCACCCAAACCTGAAGGA | | | | | | |
| MlyraE2 | GQ465848 | (TG)7 | F: CTCCGCTAGCACAGAGAGGT | 49.0 | 2.0 | 24 | 101–204 | 0.923 | 0.986 |
| | | | R: TGTAGGCCGGCTCATTTATT | | | | | | |
| MlyraE3 | GQ465849 | (TAA)7 | F: GTCACTGTGCTGCTTCATCC | 45.5 | 1.5 | 14 | 101–267 | 0.910 | 0.989 |
| | | | R: CAGGTTCGTGGAGTTGACCT | | | | | | |
| MlyraE4 | GQ240803 | $(CG)_3$ | F: GACATGTTCAAGCCACCCTT | 47.6 | 1.0 | 13 | 117–269 | 0.956 | 1.000 |
| | | | R: ACAGGCTGGGACATTGTGAT | | | | | | |
| MlyraE5 | GQ240804 | $(GA)_4$–$(TGA)_3$ | F: ATCCGGAAGGAAGAGCCTTA | 47.6 | 1.0 | 5 | 176–385 | 0.932 | 1.000 |
| | | | R: GATGAGAAAAATGGGGCTCA | | | | | | |
| MlyraE6 | GQ240805 | $(TAT)_3$–$(ATG)_3$ | F: CATGGCTAGTCAGGGACCAT | 45.0 | 1.0 | 15 | 100–171 | 0.876* | 1.000 |
| | | | R: CCCTGGACTCAGTTTCCTCA | | | | | | |
| MlyraE7 | GQ240806 | $(TGA)_3$ | F: CACCACGTGACACTGCCTAC | 46.1 | 1.0 | 10 | 110–306 | 0.885* | 1.000 |
| | | | R: TGGTTGCACCTTCCATCATA | | | | | | |
| MlyraE8 | GQ240807 | $(CA)_4$ | F: GTGACTAGGCCCATGCTCTC | 46.1 | 1.0 | 12 | 128–289 | 0.974 | 1.000 |
| | | | R: AATAAGGGCATTTGGTGCAG | | | | | | |

$T_a$ primer annealing temperatures, $N_A$ number of alleles, $H_E$ expected heterozygosity, $H_O$ observed heterozygosity

* Significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$)

Tests for deviation from Hardy–Weinberg (HW) equilibrium and linkage disequilibrium (LD) at each locus were performed with GENEPOP 3.3 (Raymond and Rousset 2002).

Eight primer pairs were successfully amplified the genomic DNA of *M. lyra* and showed polymorphism in all the tested individuals. The observed number of alleles ranged from 5 to 24, with an average of 13.1 alleles per locus (Table 1). Observed heterozygosities ranged from 0.876 to 0.982, whereas expected heterozygosities were ranged from 0.986 to 1.000. Two of these loci showed the evidence of departure from Hardy–Weinberg equilibrium ($P < 0.05$), and no significant linkage disequilibrium was observed between any pairs of loci. The polymorphic microsatellite loci reported here is invaluable in studies such as population structure, mating and paternity studies and dispersal patterns.

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