New polymorphic microsatellite markers in the greater false vampire bat *Megaderma lyra* (Chiroptera: Megadermatidae)

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Abstract In this study, we present 10 new microsatellite markers for a carnivorous bat, *Megaderma lyra* (family Megadermatidae). Out of 10 loci, only one locus showed a statistically significant value ($P < 0.05$) for F_{IS} coefficient, and one was found to be X-linked. These loci were tested on 189 individuals representing four populations from southern India, and all loci were highly polymorphic. The mean number of observed alleles per locus was 10.1 (range from 6 to 16). Expected heterozygosity values ranged from 0.54 to 0.91. These polymorphic markers will be used to examine population structure, mating and dispersal behaviour of *M. lyra* in the field.

Keywords Chiroptera · *Megaderma lyra* · Microsatellites · Polymorphism

Megaderma lyra is a gleaning, carnivorous bat feeding on large arthropods and small vertebrates such as frogs, geckoes, lizards, fish, mice, birds, and even smaller bat species. It has a wide distributional range, from Afghanistan, Pakistan, India, Sri Lanka to Myanmar, South China, Malaysia and the Philippines (Bates and Harrison 1997). It

forms small colonies, including both males and females, and roosts in caves, attics of houses, and artificial underground tunnels (Brosset 1962). Evidently, this is a fascinating species of bat, however little is known about its genetic structure and diversity. Integrating genetic data into the research on this species is essential for understanding complex processes in its biology and ecology and to design conservation strategies. Recently, Rajan et al. (2009) published a paper describing 8 highly polymorphic nuclear microsatellite loci, but this number of markers may not be sufficient to infer detailed genetic comparisons. Koskinen et al. (2004) postulated that at least 10 polymorphic loci are needed for non-erroneous inference in a genetic population. In the present paper we describe 10 additional microsatellite loci and now the combination of both data sets will provide an ideal tool for the study of genetic relationships in *M. lyra*.

To obtain sequences of microsatellite loci and their flanking regions we constructed two genomic libraries enriched for common vertebrate microsatellite motives (TG and ATCT), using the strategy of Estoup and Martin (1996). Total DNA was extracted from the wing membrane biopsy punch (see Whortington Wilmer and Barratt 1996) of a single male of *M. lyra* using DNeasy Tissue Kit (Qiagen) and digested with RSAI restriction enzyme (Promega). Fragments of 500–900 bp length were extracted from the agarose gel, ligated to RSA21 and RSA25 adaptors (Edwards et al. 1996), and amplified using RSA21 oligos as PCR primers. Biotinylated oligo probes (TG)₁₀ and (ATCT) were hybridized to the diluted PCR product and the DNA molecules containing respective motives were selected using streptavidin magnetic particles (Promega). Enriched DNA was PCR amplified, ligated to a plasmid vector (Promega) and transformed into JM109 competent cells (Promega). Ca. 500 clones for each library were transferred

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Table 1 Primer sequences and characterization of 10 microsatellite loci developed for *M. lyra* (GenBank accession numbers JF907000–JF907009)

Locus	Repeat motif	Primer sequence (5'–3')	T _m (°C)	No. alleles	Size range (bp)	H _O	H _E	F _{IS}
MIA10	(TG)15	F: AAGTCCCTTGGGTGACTC R: GAAGTCAGCTGCTTTTCAGT	57	11	153–175	0.820	0.823	0.005
MID1	(TG)13C(TG)11	F: TAAGAACACTGGGAAACCAC R: GGAGACTTGTCTTCTGTGGAG	57	10	207–237	0.724	0.765	0.053
MID11	(TG)21	F: ACAAATGTGAATCAAAGCA R: CGCTCAGTCATTTTCAGTTT	57	11	177–227	0.675	0.726	0.070
MID12	(TG)15	F: CTTTAGGGACCTCCTCTCTG R: TGCCATTCTGAAGTAGAACA	57	10	215–237	0.702	0.691	–0.016
MIE6	(TG)15	F: AAAATGAGGGCATTATGG R: TTTAAGGCTCTTCATTGCTC	55	11	158–178	0.820	0.875	0.063 ^b
MIF6	(TG)8CA(TG)8	F: TTACGTGAAGATGGCTACAA R: GCACATACCACACACGTAAG	55	8	163–177	0.526	0.563	0.066
MIG3	(TG)17	F: CTACTIONACTGTTGCTGACA R: CACTCTGTGCTACTAAGTCTGC	55	11	144–164	0.824	0.845	0.025
MIG11	(ATCT)12	F: TTTAGATACAATTGACACATAGCA R: TGGAAAATGATGATCACAAG	55	7	172–192	0.634	0.677	0.063
MIA3	(TG)9T(GA)15	F: ACACGAGCATGTGCCTAT R: AGCTCCCTCCAAGTCTTTAC	55	6	150–162	0.486	0.537	0.095
MIA4 ^a	(TC)20(TG)5C(TG)4	F: ATAGGGGGTATCAGGACAAT R: GCATGCATATTGGAGTAGGT	55	16	184–220	0.837	0.912	0.083
Mean				10.1		0.675	0.741	0.090

T_m annealing temperature, No. alleles number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} fixation index

^a Locus MIA4 is located on the X chromosome. H_O and H_E are calculated for females only

^b Significant value of F_{IS} ($P < 0.05$)

onto Hybond-N + membranes (Amersham) and screened using digoxigeninend-labelled (TG)₁₀ and (ATCT)₅ probes. The 96 positive clones were sequenced in MacroGen (South Korea). Sequences were checked with BIOEDIT ver. 7.0.5. (Hall 1999) for regular microsatellite motives with flanking regions of sufficient length. Primers were designed using PRIMER 3 v. 0.4.0 (Rozen and Skaletsky 2000) and tested for 15 loci, of which 10 polymorphic loci were chosen for further analyses.

We amplified these loci in 189 individuals of *M. lyra* from four colonies from southern India. Conditions for the PCR reaction were as follows: 96°C for 3 min, 35 cycles at 96°C for 30 s, 55°C/57°C for 45 s, 72°C for 55 s, and final cycle 94°C for 30 s, 55°C/57°C for 1 min. 72°C for 5 min. Amplifications were carried out in 15 µl with 0.5 µl of DNA (10–50 ng), 7.5 RedTag Ready Mix (Sigma–Aldrich), 5 pmol of each primer and 5 µl of PCR water. Each forward primer was fluorescent labelled with WellRED dyes (Beckman Coulter, Inc.). Loci MIA10, MID11, MID12 and MIG3 were labelled with D2, loci MID1, MIE6 and MIF6 were labelled with D3, loci MIG11, MIA3 and MIA4 were labelled with D4. We run samples in two post-PCR multiplex sets, first with loci MIA10, MID12, MIF6, MIA3, and

MIA4, and second with loci MID1, MID11, MIE6, MIG3, and MIG11. Allele lengths were scored on a CEQ 8000 sequencer (Beckmann-Coulter), with internal lane standard (DNA Size Standard Kit—400, Beckmann-Coulter). Levels of heterozygosity were calculated in Arlequin ver 3.11 (Excoffier et al. 2005) and F_{IS} coefficients were obtained using F_{STAT} ver. 2.9.3.2 (Goudet 1995, 2001).

All analysed loci were highly polymorphic with the number of alleles ranging from 6 to 16. The mean observed heterozygosity was equal to 0.675 (Table 1). One of these loci (MIE6) showed a statistically significant value ($P < 0.05$) for F_{IS} based on the randomization test implemented in F_{STAT} (1000 randomizations performed), probably resulting from the presence of null alleles. In one locus (MIA4) we observed that all males (41 individuals) were homozygotes. This indicates that we scored a marker located on the X chromosome. These 10 new markers, together with those previously developed (Rajan et al. 2009), give a unique opportunity for performing detailed genetic studies of *M. lyra*, and will add new information to other areas of research such as classical capture-recapture (Sripathi et al. 2004) or population structure studies (Raghuram et al. 2006).

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