

PRIMER NOTE

Identification and characterization of 14 polymorphic microsatellite loci for a member of the Herpestidae (*Mungos mungo*)

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Abstract

In most cooperative breeders, reproductive skew is high. However, in the banded mongoose, *Mungos mungo*, multiple females reproduce concurrently within a social group, indicating that skew is relatively low in this species. In order to evaluate the degree of reproductive skew in the banded mongoose, we identified 14 polymorphic microsatellite loci for parentage analysis. Markers were found to have low levels of variability in this cooperative breeder, and several loci were found to be polymorphic in another member of the Herpestidae, the slender-tailed meerkat (*Suricata suricatta*). For the banded mongoose, this suite of polymorphic loci provides a paternity exclusion of over 90%.

Keywords: Herpestidae, meerkat, microsatellites, mongoose, *Mungos mungo*, polymorphic

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Kin selection theory predicts that cooperative breeding would evolve in populations in which dispersal is limited and localized inbreeding occurs (Hamilton 1964). Consequently, levels of genetic variability might be expected to be low in cooperative breeders (e.g. Pope 2000). Microsatellite markers are ideal for the study of such populations, because their high mutation rates make them highly polymorphic (Bruford & Wayne 1993; Nauta & Weissing 1996). Moreover, conservation in the sequences flanking the microsatellite repeats means that primers developed for one taxa may amplify variable regions in a wide range of divergent taxa.

The banded mongoose is a cooperative breeding species that occurs in social groups comprising 5–40 individuals of different ages and both sexes. In contrast to other social mongooses such as the meerkat (*Suricata suricatta*), in which reproductive skew is high (Clutton-Brock *et al.* 2001), multiple, concurrent pregnancies are known within banded mongoose social units. We were interested in assessing the degree of reproductive skew in the banded mongoose by identifying parentage and examining kinship levels within

and between social groups. Initially, we selected and screened a selection of 45 microsatellite loci developed for other taxa. These included some developed for another Herpestidae, the slender-tailed meerkat, *Suricata suricatta* (Griffin 1999), and several for the Fissipedia (*Felis* and *Canis*; Ostrander *et al.* 1995; Menotti-Raymond *et al.* 1999). Of those screened that produced identifiable target products, only three were polymorphic, all of which were derived from the slender-tailed meerkat (Ss7.1; Ss11.12; Ss10.1). However, differential amplification was observed among the alleles and consequently, priming sites were repositioned further from the microsatellite repeat region, in both the upstream and downstream directions. Modified priming sites successfully improved amplification of all alleles (Table 1). To identify additional microsatellite loci for parentage testing, we constructed a phagemid library specific for the banded mongoose, screening it for (TG)_n(GATA)₆(AAAC)_n and (AAT)₁₀ repeats.

Genomic DNA for library construction was extracted from organ tissue provided by the Oregon Zoo (Portland, USA). DNA was extracted using a DNeasy Tissue kit (Qiagen) and approximately 5 µg of the genomic DNA were digested with *Sau3aI* (Gibco-Invitrogen). Fragments were size separated on a 2% agarose gel and isolated by

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Table 1 Characteristics of polymorphic loci in the banded mongoose. Values are based on a sample of 15 individuals for *Mungos mungo*, all of which were selected from different groups and were presumed to be unrelated, using the programs CERVUS (Marshall *et al.* 1998) and GENEPOP (Raymond & Rousset 1995)

Locus (AN)	Primers (5'–3')	Repeat	<i>M. mungo</i>					<i>S. suricatta</i>
			T_a (°C)	Size range	No. of alleles	H_E	PIC	No. of alleles
Ss7.1*	F gCTACCTGTTTTCAAATATGCC R cCAAAGCCTCAGAAATCCC	(GT) ₁₄ (AG) ₁₇	52	181	4	0.66	0.61	1
Ss10.1*	F GTGAGTAGATACTTTATCATCC R ACCAGGAACATTGAAGCC	(CA) ₂₄	52	154	4	0.64	0.57	3
Ss11.12*	F CTCATTTTCAGGAAATTTTCATCC R TCATCATTTCTTTCCCTAGC	(GT) ₂₂	54	153	8	0.68	0.63	4
Mm10–7 AY142693	F CTATGAATGAAGGGGAGCAG R AGACAGGCTGGGTCAAAGTGA	(TG) ₁₀ A(TG) ₇	57	195	3	0.46	0.39	1
Mm7–5 AY142694	F GGAGGCAGGAAATGAGATG R GGGTGAGGTGGCACTCTTG	(GT) ₇ A(TG) ₅	57	137	2	0.14	0.13	2
Mm6 AY142695	F CCGTGGAGGTTTCGACAGC R GAAGACAAATCTCGCAGGAG	(CACACG) ₆	55	129	2	0.10	0.08	1
Mm18–1 AY155580	F CTACAAGATGTTTGATTATATTG R CAGAAGGTGTATTAATTAGCTG	(C) ₁₂	57	144	3	0.40	0.31	3
Mm2–10 AY142696	F CTTCTCGTGTGCCCAAGTCCT R CTGCCAGATGGGGTGACAAC	(TG) ₂ GA(GT) ₁₀	56	173	2	0.32	0.26	2‡
Mm2–3 AY142697	F GATTTGGAGGAGTAGGTGG R TTGGATCAGCTCTGGGACC	(TG) ₂ (GT) ₃	54	157	2	0.40	0.30	2‡
Mm18–2 AY142698	F TTGTTGCTGATTGTC TTC R CAAGGTTTCAGAACTATGGA	(TG) ₁₈	55	208	3	0.47	0.37	1
Mm18–3 AY146696	F TTCTTAGAAGACAGTGACGCC R CAGTGTGTTTCTCCATGTCTCC	(C) ₁₈	59	183	2	0.30	0.24	1
Mm19 AY142700	F GAACACCTTTCATCACTACT R GCCACTATTCCAAGTCAG	(TG) ₁₉	57	173	4	0.36	0.33	1
MmAAAC6 AY142702	F CAACATGGAGCCTCCTTGGG R TCTAAACCAGACTAGAAAGTG	(AAAC) ₆	58	105	3	0.54	0.43	2
MmAAAC5 AY142703	F GTTGGCCTTTCAGACTGACA R GAAGAATGGACCCTACAG	(AAAC) ₅	58	214	3	0.49	0.40	3

**Suricata suricatta* sequences courtesy of A. Griffin, University of Edinburgh. †Sample of 6 individuals screened. ‡All heterozygotes.

centrifugation of excised gel fragments into two size categories: 200–350 bp and 350–550 bp. Digested DNA was then ligated into Lambda Zap Express (Stratagene) as outlined in the manufacturer's guidelines in a 2 : 1 DNA to vector ratio. Cloning methods followed those outlined by Hughes & DeLoach (1997). Lifts were carried out using Hybond N (Amersham-Biosciences) filters and approximately 500 000 plaques were screened using (TG)_n, (AAAC)_n, (GATA)₆ and (AAT)₁₀ oligos, labelled by incorporation (Oligolabelling kit, Amersham-Biosciences).

Collectively, 186-(TG)_n, 10-(AAT)_{10,000}, 11-(AAAC)_n and six-(GATA)_{6,000} positive plaques were identified. One hundred and forty-five inserts were recovered using Ex-Assist helper phage (Stratagene). These were sequenced using an ABI sequencing machine at the Institute for Molecular Biology and Biotechnology (McMaster University) and the DNA sequencing facility at Cambridge University. Primers

were developed for 82 of the sequences using the software package PRIMER v3 (Rozen & Skaletsky 2000). DNA was then extracted from tail tips of individually identifiable banded mongooses from our study population in Queen Elizabeth Park, Uganda, using the DNeasy tissue extraction kit (Qiagen) or 10% Chelex 100™. (with 0.5 mg/mL proteinase K, 200 mM dithiothreitol). A subset of 14–30 randomly selected individuals was used to screen for polymorphic loci. Polymerase chain reactions (PCRs) were carried out in 10 µL reaction volumes in a buffer of 100 mM Tris (pH 8.3), 500 mM KCl, 0.1% Tween 20, 0.1% gelatin, 0.1% NP40, 15 mM MgCl₂, 0.1 mM dATP, DTTP, dGTP and 0.01 mM dCTP. Each reaction used 400 nM of each primer, 0.1 µCi [α^{32} P]-dCTP, and approximately 0.2 U *Taq*. Alleles were run on a 6% polyacrylamide gel and visualized using autoradiographs. Collectively, nine of the 82 species-specific loci and three cross-species loci were polymorphic

with easily recognizable phenotypes (Table 1). Some of the loci derived from the banded mongoose were also found to be variable in the meerkat (Mm7-5; Mm18-1; MmAAAC₅; MmAAAC₆; Mm2-10; Mm2-3; Table 1).

As predicted, levels of variability were low in this cooperative breeding species. Polymorphic loci identified had between two and eight alleles, with an average heterozygosity of 0.46 (range 0.14 to 0.68). Nevertheless, paternity analysis based on these polymorphic loci provide an assignment probability of over 90%. Using these 12 loci, parentage analysis will be used to confirm the maternity of pups to known pregnant females, and to evaluate the extent of reproductive skew among male group members for the banded mongoose.

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