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PRIMER NOTE

Microsatellite loci in the European bee-eater, *Merops apiaster*

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Abstract

Twelve polymorphic microsatellite markers have been developed for the European bee-eater, *Merops apiaster* (Coraciiformes: Meropidae). Screening of eight individuals at these loci showed that the average allelic diversity was 5.8, with a range of two to 11 alleles per locus. The loci reported here will provide insight into the levels of extra-pair parentage, kin selection and dispersal in this species, which has co-operative breeding and nests in large colonies.

Keywords: bee-eater, Coraciiformes, Meropidae, *Merops apiaster*, microsatellite

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The European bee-eater, *Merops apiaster*, is a migrant species breeding in Europe, North Africa and Asia to more than 80° E and wintering in Africa (Fry 1984; Cramp 1985). The species has cooperative breeding with about 20% of the broods having one or more helpers-at-the-nest. Helpers are usually males whose breeding attempts have failed earlier in the year (Lessells 1990). Multilocus minisatellite markers have previously been used to estimate the proportion of chicks with extra-pair parentage and the relatedness of helpers to the breeding pair (Jones *et al*. 1991), but microsatellite markers will allow increased resolution of relationships, including the identification of the true parents in cases of extra-pair parenthood. European bee-eaters breed in colonies ranging up to several hundred pairs although most colonies are much smaller and they may breed solitarily (Fry 1984). Natal dispersal is female biased and breeding philopatry is high (Lessells *et al*. 1994), however, very little is known about intercolony dispersal. Polymorphic microsatellite markers are required to assess the amount of population structuring and the importance of immigration and emigration in driving observed changes in colony size. Initial work involved the screening of 19 passerine primers (GenBank Accession nos AF127385, AF041465, AF041466, U59113, U59117, Y15125, AJ279804, AJ279805, AJ279806, AJ279809, AJ279810, AJ279811, U82388, AJ272375, X84087, X84092, X77082, X77078 and PAT MP 2–43) (Otter *et al*. 1998). However, this did not prove to be an efficient strategy for identifying variable microsatellites, with only five (26%) producing visible bands on an agarose gel, none of which were polymorphic. In comparison, subsets of the same 19 primers amplified heterogeneric DNA in 83% ($n = 12$) of cases in great tits (*Parus major*) and 100% of cases in both blue tits (*P. caeruleus*) ($n = 6$) and Seychelles magpie robins (*Copsychus sechellarum*) ($n = 12$). Consequently, we elected to clone a panel of microsatellite loci ourselves.

Microsatellite loci for the European bee-eater were isolated using a modification of the enrichment protocol followed in Armour *et al*. (1994). PUREGENE DNA purification kits (Gentra Systems) were used to extract and purify genomic DNA from the blood of *M. apiaster*. Approximately 5 μg of DNA from eight individuals was pooled and digested with the restriction enzyme *Sau*3AI. *Sau* linkers were ligated to the ends of the digested DNA, fragments in the size range 300–700 bp were excised from an agarose gel and purified using QIAquick gel extraction spin columns (QIAGEN). This DNA was diluted and subjected to 10 cycles of amplification by polymerase chain reaction (PCR). The amplified DNA was enriched for AC repeat motifs by overnight hybridization at 65 °C to Hybond-N membranes (Amersham Biosciences) to which poly(dA-dC).poly(dG-dT) oligonucleotides (Amersham Biosciences) had been bound. After stripping the enriched DNA from the filters, it was amplified by PCR. The PCR products were purified using MinElute PCR purification spin columns (QIAGEN), ligated into p-GEM T-Easy

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Table 1 Characteristics of *Merops apiaster* microsatellite loci

Locus	Primer sequence (5′–3′)	Repeat motif in clone	Size of clone (bp)	T_a (°C)	No. of alleles	H_E	H_O	EMBL Accession no.
Be24	F: TTCCAACTGTGGGTGGAAAG R: CAATCGCAATCCTTCTCTGG	$(AC)_{11}$	156	61	6	0.86	0.57	AJ630044
Be48	F: CATCAACCCACAGCTTCCTC R: GCGTTACTTCCCCTTTAAGC	$(AC)_{10}$	158	61	3	0.27	0.29	AJ630045
Be72	F: CACTAATGATGAAAGGCTCA R: GATCTATAAACACATCTGCAT	$(AC)_{19}$	228	61	8	0.91	0.78	AJ630046
Be2.16	F: TCAGCAAGTTGGAAGACTGC R: ACCAACCACACTCGTTCTCC	$(AC)_{12}$	195	61	2	0.50	0.75	AJ630047
Be2.31	F: CTTCAGGCAAGTGACCACAG R: CAGAGGGACACCAGAGCTTC	$(AC)_{13}$	178	61	6	0.84	0.88	AJ630048
Be2.52	F: GGAGTCATCTAGGCCCATCC R: TTTCCCGAGGCAGTGTAAAG	$(AC)_{19}$	233	61	8	0.89	1.00	AJ630049
Be2.33	F: CAGGAATGCTGTTGAACCTG R: ACTGTGCCTTGCTCACATTG	$(AC)_7$	174	59	2	0.33	0.38	AJ630050
Be2.46	F: AATGGCTGTAAGTGGTCATGG R: TGATTTCATCCCAGATGTGC	$(AC)_5n_3(AC)_3n_8(AC)_8$	186	59	2	0.13	0.13	AJ630051
Be3.24	F: GATCACATTATCCTGCATGTG R: TTATGAAAGTCTACTTATTATGTGTCC	$(AC)_{21}$	165	59	11	0.96	1.00	AJ630052
Be3.9	F: GGATCTATAAACACATCTGCAT R: AAGGAATTACCTGCCCCTTA	$(AC)_{19}$	188	59	8	0.84	0.88	AJ630053
Be1.29	F: TTTTCTCTGGGAGGTGGTTG R: GCTTGAAGGGGATTATGATAGC	$(AC)_{14}$	157	59	6	0.87	0.88	AJ630054
Be19.2	F: GTCAAGTGGGCTGTTGGAG R: AAGAGGGGCTACTTCCAAGC	$(AC)_9$	173	59	7	0.82	0.75	AJ630055

T_a, annealing temperature; H_E, expected heterozygosity; H_O, observed heterozygosity.

vectors (Promega) and used to transform DH5α subcloning efficiency competent cells (Invitrogen). About 100 transformed colonies were picked and transferred onto new reference Luria-Bertani agar plates for future use and also into 50 µL TE. To identify colonies containing AC repeat motifs in the vector inserts, the plasmids were released from the bacteria transferred to the TE by boiling for 5 min and the insert amplified by PCR using the *Sau*LA primer. The amplification products were bound to Hybond-N membranes and subjected to overnight hybridization at 65 °C to ^{32}P-labelled AC probes. About 50 candidate colonies for sequencing were grown from the reference plate and the plasmid DNA extracted by alkaline lysis. The insert was sequenced using the M13 reverse primer and BigDye 3.1 terminators (Applied Biosystems). Sequencing was carried out on an ABI PRISM 377 sequencer. Twenty sequences had sufficient flanking DNA around the AC repeat region to design primer pairs using PRIMER3 (Rozen & Skaletsky 2000). These sequences have been submitted to the EMBL database (Accession nos AJ630044–AJ630063).

Primer pairs were used to amplify microsatellites in eight bee-eater samples collected close to the Camargue (Provence, France). Approximately 5 ng of template DNA was amplified in 10-µL reaction volumes containing 100 mM Tris-HCl, pH 8.0, 50 mM KCl, 2.0 mM $MgCl_2$, 0.01% Tween 20, 0.01% gelatine, 0.01% IGepal, 0.2 mM each of dATP, dTTP and dGTP, 0.05 mM dCTP, 400 nM of each primer, 0.25 U *Taq* polymerase (MEB) and 0.1 µCi [α^{32}P]-dCTP. The PCR programme used was 3 min denaturing at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at the annealing temperature (see Table 1) and 25 s at 72 °C, ending with a 20 min final elongation stage at 72 °C. The PCR products were resolved on polyacrylamide gels, visualized by autoradiography and the alleles scored manually. Twelve loci were found to be polymorphic (Table 1), while eight primer pairs amplified monomorphic loci, loci with a high null allele frequency or failed to amplify a consistent product. Using GENEPOP 3.4 (Raymond & Rousset 1995) the 12 polymorphic loci were found to be in Hardy–Weinberg equilibrium (α = 0.05) and there was no evidence of linkage disequilibrium between the loci. Mean expected heterozygosity for the 12 loci was 0.69. These polymorphic loci are currently being used to study population structure and dispersal in populations of *M. apiaster* in Provence.

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References

Armour JAL, Neumann R, Gobert S, Jeffreys AJ (1994) Isolation of human simple repeat loci by hybridization selection. *Human Molecular Genetics*, **3**, 599–605.

Cramp S (1985) *Handbook of the Birds of Europe, the Middle East and North Africa*, Vol. 4. *Terns to Woodpeckers*. Oxford University Press, Oxford.

Fry CH (1984) *The Bee-Eaters*. T & AD Poyser, Calton.

Jones CS, Lessells CM, Krebs JR (1991) Helpers-at-the-nest in European Bee-eaters (*Merops apiaster*): a genetic analysis. In: *DNA Fingerprinting* (eds Burke T, Dolf G, Jeffreys A, Wolff R), pp. 169–192. Birkhauser-Verlag, Basel.

Lessells CM (1990) Helping at the nest in European bee-eaters: who helps and why? In: *Population Biology of Passerine Birds, an Integrated Approach* (eds Blondel J, Gosler A, Lebreton JD, McCleery R), pp. 357–368. NATO ASI series. Springer-Verlag, Berlin.

Lessells CM, Avery MI, Krebs JR (1994) Non-random dispersal of kin: why do bee-eater brothers nest close together? *Behavioral Ecology*, **5**, 105–113.

Otter K, Ratcliffe L, Michaud D, Boag PT (1998) Do black-capped chickadees prefer high-ranking males as extra-pair partners? *Behavioural Ecology and Sociobiology*, **43**, 25–36.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rozen S, Skaletsky HJ (2000) PRIMER3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, NJ.