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

Received 22 April 2004; revision accepted 18 May 2004

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 heterogenic 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 (Genta 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...
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 SauLA primer. The amplification products were bound to Hybond-N membranes and subjected to overnight 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.

Acknowledgements

We would like to thank the Conservatoire de l’Espace Littoral for permission to carry out field work at Les Marais du Vigueirat and the Centre de Recherches sur la Biologie des Populations d’Oiseaux for permission to capture and ring bee-eaters.
References


