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PERMANENT GENETIC RESOURCES

Isolation of 10 tetranucleotide microsatellite loci in the blackcap (*Sylvia atricapilla*)

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Abstract

We isolated 10 polymorphic microsatellite DNA loci from the blackcap (*Sylvia atricapilla*) and optimized them for future studies of population differentiation in populations with different migration strategies in southwestern Germany. The loci were screened for polymorphism using 178 individuals from two populations in Germany and Spain. The primers amplified highly variable loci characterized by two to 19 alleles per locus and their observed and expected heterozygosities range from 0.47 to 0.81 and from 0.50 to 0.91, respectively.

Keywords: primer, *Sylvia atricapilla*, tetranucleotide microsatellites

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The blackcap (*Sylvia atricapilla*) is an abundant Eurasian passerine and a model species for studying avian biology and the evolution of migratory behaviour (Helbig 1996; Berthold 1998; Coppack *et al*. 2001). Blackcaps exhibit a wide diversity of migratory strategies including nonmigratory populations, partial migrants and populations with different migratory orientation and distances. Because the entire range from sedentariness to long-distance migration can be found within a single species, this species illustrates the flexibility of migratory behaviour in an evolutionary sense.

Apart from studies demonstrating differences in migratory behaviour, the genetic diversification of migration patterns has also been studied (Perez-Tris *et al*. 2004). With regard to breeding areas, the species is currently divided into five subspecies which show differences in physiology, morphology and life-history traits (Shirihai *et al*. 2001; Fiedler 2005). Particularly interesting is the recent rapid microevolution of a new migratory direction within the blackcap in west-central Europe (Berthold *et al*. 1992). The proportion of birds migrating with northwestern direction, wintering in Britain, has increased from 0% to 10–15% in the last 50 years. This change in migratory direction has a genetic basis (Berthold *et al*. 1992). Assortative mating driven by differential arrival times in spring may lead to an increasing isolation of populations wintering in Britain and in the Mediterranean (Bearhop *et al*. 2005). The blackcap is therefore a model system to investigate the genetic structure of different populations to study microevolutionary processes leading to different migratory strategies in avian species.

Microsatellites were isolated using magnetic bead capture enrichment (Glenn & Schable 2005) from the genomic DNA of 178 individuals. A genomic library was made after double enrichments for the motifs $(AACT)_8$ $(AAGT)_8$ $(ACAT)_8$ and $(AGAT)_8$. Total DNA was digested with *Rsa*I (New England Biolabs), and fragments were ligated to doublestranded SuperSNX24 linkers. Fragments were hybridized to biotinylated oligonucleotides and captured with magnetic streptavidin beads (Invitrogen). Enriched DNA was amplified using the polymerase chain reaction (PCR) primer forward SuperSNX24. Cloning was conducted using TOPO-TA Cloning Kit (Invitrogen). Clones with

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inserts between 300-bp and 700-bp length were purified using QIAquick PCR Purification Kit (QIAGEN) and sequenced. Sequences from both strands were assembled and edited in BIOEDIT (Hall 1999) and microsatellites located using TANDEMREPEATSFINDER (http://tandem.bu.edu/trf/trf.html) and confirmed by eye. Primers were designed from the flanking sequences of the tandem repeats using PRIMER 3 software. Twenty-one out of the 32 sequenced clones contained microsatellites. Eighteen PCR primers were designed using PRIMER 3 (http://frodo.wi.mit.edu/primer3/input.htm) and were tested for amplification on 1.2% agarose gels. Ten of the tested primer pairs amplified high-quality PCR products that showed polymorphism across 10 selected individuals and were further genotyped in a larger sample.

We caught 118 birds at Radolfzell (47°N, 8°E), southwestern Germany, during spring 2006 and 60 individuals in Southern Spain (37°N, 5°W). Genomic DNA was extracted from blood samples using the DNeasy Blood and Tissue Kit (QIAGEN). PCR amplifications were performed in a 10-µL volume consisting of 1 × QIAGEN PCR buffer (contains Tris-Cl, KCl and $(NH_4)_2SO_4$ at unspecified concentrations), 0.025 mM of each primer, 3 mM $MgCl_2$, 0.40 mM of each dNTP and 0.5 U *Taq* DNA Polymerase (QIAGEN) and 1-µL template using an Eppendorf Mastercycler Gradient. A Touchdown thermal cycling programme encompassing a 10 °C span of annealing temperatures ranging between 60 °C and 50 °C was used for the amplification. Following an initial denaturation step of 95 °C for 3 min, cycling parameters were 20 cycles at 95 °C for 30 s, 60° annealing temperature (decreased 0.5 °C per cycle) for 30 s and 72 °C for 40 s and 15 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 40 s and a final extension step of 72 °C for 5 min. PCR products were run on Elchrom Spreadex EL 400 gels run in an Elchrom SEA 2000 apparatus and sized with M3 size standard (Elchrom, Switzerland).

Each locus was tested for polymorphism and heterozygosity using 178 individuals from two different locations (Spain, Western Germany). Characteristics of the 10 working primer pairs are given in Table 1. We estimated the number of alleles per locus, observed and expected heterozygosity, polymorphic information content and frequency of null alleles and tested for deviations from Hardy–Weinberg-equilibrirum using CERVUS version 3.0 (Marshall *et al*. 1998). Loci syl3, syl4 and syl10 deviated significantly from HWE only when all samples were analysed, but not when each population was analysed alone. No linkage was detected among all 45 paired loci comparisons ($P < 0.05$; GENEPOP version 3.4, Raymond & Rousset 1995).

Overall the high numbers of alleles per locus, high PIC values and heterozygosity and paternity exclusion probabilities of 0.99 demonstrate the potential of the blackcap microsatellite primers for a variety of questions like kinship analysis and population differentiation.

Table 1 Microsatellite loci in *Sylvia atricapilla* including GenBank Accession number, primer sequence, repeat motif, size of cloned allele in base pair, number of alleles (*k*); H_E, expected heterozygosity; H_O, observed heterozygosity; and PIC, polymorphic information content and frequency of null alleles

Locus accession number	Primer sequence (5′–3′)	Repeat motif	Size (bp)	*k*	H_E (H_O)	PIC	Null allele frequency
Syl1	TTGCTTTTGGCAAAGATATAGATG	$(GATA)_{14}$	150	13	0.86 (0.73)	0.841	0.08
EU304338	CCTGGGTTGTTTTACCAGATTC						
Syl2	GTCCCACTAATGGGTTTTCC	$(CTAT)_7$	158	12	0.86 (0.81)	0.837	0.03
EU304344	GTTGTCAGAATTCAACTACTTACTGC	CGATCTAAT$(CTAT)_5$					
Syl3	CACGTTACCGTCTTTATATTCCATC	$(GATA)_2(GAGA)_3$GATA	197	6	0.66 (0.52)*	0.603	0.11
EU304336	TTTTTCACATGGGTGCAGTC	GAGG$(GATA)_2(GACA)_3$GATA					
Syl4	ACCCATATGGAATAAATTGTGG	$(GTAT)_{10}$	200	14	0.87 (0.49)*	0.849	0.28
EU304337	GAGCTGGCAGGGAATATTTG	$(CTAT)_{12}$					
Syl5	CAAAAATTTGGTATAAACTTCCAAC	$(CTAT)_3$AT$(CTAT)_{14}$	195	15	0.89 (0.54)	0.887	0.24
EU304341	GCCCTTGAAAAACAAACACC						
Syl6	TCAGCAGCAACAAGATGAGC	GATACATA$(GATA)_{10}$	192	19	0.89 (0.64)	0.878	0.16
EU304342	GGAACTTGCAAACTGTGAATTG	GAAA$(GATA)_3$					
Syl7	CTTGTTCTTTCTTCTGCATTGG	GATAGATTTA	189	2	0.50 (0.47)	0.375	0.03
EU304339	GGAGTTTTGGCTTTTGTTGG	$(GATA)_2$GATGTA$(GATA)_2$					
Syl8	CAATGCCCATCTACCCATCT	$(CTAT)_2(GTATCTAT)_2$	193	17	0.91 (0.48)	0.897	0.31
EU304340	TTTCTAAATATGCACAGGTGCTG	$(CTAT)_2(GTATCTAT)_2(CTAT)_9$					
Syl9	AGGCATTTAAAGAAGGCAGTG	$(CTAT)_{12}$	190	17	0.77 (0.65)	0.749	0.08
EU304343	GCCAAGAAAGAGGGAGGTTC						
Syl10	TCTTCAGAGGTGAAGGTGTGC	$(CTAT)_{11}$	185	13	0.85 (0.49)*	0.833	0.27
EU304345	GCTGGGGAGTGTAAAAGTGG						

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