SUPPLEMENTARY MATERIAL

Development of species-specific microsatellite primers

Effects of forest fragmentation on the morphological and genetic structure of a dispersal-limited, understory bird

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DNA was extracted from the blood samples of the Ecuadorian Tapaculos (*Scytalopus robbinsi*) using the DNeasy Blood and Tissue Kit (Qiagen, Hilden). 50 µl of extracted DNA from three museum samples collected between September 1990 and December 1991 (from the Tissue collection at the Zoological Museum Copenhagen; sample numbers 125058, 125070 and 126057) were pooled and sequenced (at MICROSYNTH, Switzerland). We used the software MSATCOMMANDER (Faircloth 2008) to look for repetitive motives out of a pool of 35,057 DNA sequences. We found a total of 203 sequences containing di-, tri- or tetranucleotid repeats and created a set of 49 primers using the software PRIMER3 (Rozen and Skaletsky 2000). The sequences were amplified in a Mastercycler Gradient Thermocycler (Eppendorf, Hamburg, Germany). The PCRs were conducted for each locus separately. Each reaction was carried out in a 10µl volume containing 1 µl DNA extract, 6 µl 10 µM forward primer, 6 µl 10 µM reverse primer, 12 µl 10 x TopTaq buffer, 12 µl Coral Load buffer, 0.72 µl Taq polymerase (all from Qiagen, Hilden), 3.6 µl 10 mM dNTPs and 68.4 µl distilled water. A touchdown temperature profile was used for the PCR (5 min at 95 °C; 20 cycles of 30 s at 94 °C, 30 s at 62 °C, 70 s at 72 °C; 15 cycles of 30 s at 94 °C, 30 s at 52 °C, 40 s at 72 °C; 5 min at 72 °C, storage at 5 °C). 4 µl of each PCR product were transferred on an agarose gel (1.2 %) to check via gel

electrophoresis (Elchrom SEA 2000) whether amplification was successful. Only polymorphic loci were used for further analysis. We tested for linkage disequilibrium between the loci using the program GENEPOP ON THE WEB 4.2 (Rousset 2008). Moreover, we determined the number of alleles, observed and expected heterozygosity using the program GENALEX 6.5 (Peakall and Smouse 2012), as well as the polymorphic information content and the null allele frequency for each locus with the program CERVUS 3.0.3 (Marshall et al. 1998).

After excluding loci that did not amplify or were monomorphic, we compiled a final set of 10 polymorphic microsatellite primers (Table 1). The number of alleles per locus ranged from 4 to 7, mean polymorphic information content was 0.573, mean observed heterozygosity was 0.597 and mean expected heterozygosity 0.636. No linkage disequilibrium was found between any primer pair. Two individuals had to be excluded from the analysis due to failure of amplification during PCR in two loci.

References

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Table 1. Microsatellites of the polymorphic loci in *Scytalopus robbinsi* (n = 33).

Locus	Accession no.	Repeat motif	Primer sequences (5'-3')	Range (bp)	Dye	PIC	NA	Ho	He	Null alleles
ScyRo1	KT266563	(ACCTTATAC)$(GT)_{11}$GCATGTTGAGG	AGCAGTGTCATCCCAGAGC ATGCCATGTGGTTGCTGAC	175-183	FAM	0.580	4	0.636	0.658	+0.0061
ScyRo2	KT266564	$(CA)_3$(CT)$(CA)_{11}$(CT)$(CA)_3$CTCA	TCCATAACCTGCCAGCGAC TGAGCTGGAGGCCTGATTG	209-280	HEX	0.472	7	0.455	0.519	+0.0447
ScyRo4	KT266565	CAGTCTTTCA$(TA_{11}$TTTGATACCAT	GGGTACCTTGTGCATTGGC GCGTTGTTGGAGGAGATGC	178-205	FAM	0.701	5	0.576	0.757	+0.1324
ScyRo6	KT266566	TTTTCTGAAA$(CA)_{14}$CTTCT$(AC)_2$ATC	GAAGGCTGAACTTCCCTGC ACCTGTGCATTGCTGGTTC	292-298	FAM	0.520	4	0.636	0.571	-0.0781
ScyRo8	KT266568	TGGT$(TGG)_2(TTTG)_{11}$TGGTTCTTTG	TCACAATAGGCTGTACGCAG GTAGAACAGCAAGGTCAGGC	350-366	FAM	0.619	4	0.606	0.696	+0.0618
ScyRo9	KT266569	GGAGCTGGA$(GT)_{13}$CTAGGCA$(G)_3$GC	TGTCAGCCCTTGGATCACC TGGCAAACGCATGTTCAGG	255-275	HEX	0.554	5	0.636	0.629	-0.0069
ScyRo10	KT266570	GC$(AGA)_2(TGGT)_{10}$TTCTGGGCTGCA	GGGACTCACATGGGCAGG TGGAGAATGGGTTGGGAGC	212-224	FAM	0.562	4	0.667	0.643	-0.0209
ScyRo11	KT266571	ACTT$(CA)_2$CAG$(ATTT)_8(C)_4$ACTCATC	TCACCGCACCACAAATGAG ATGGGAGAGAAGGCAGGTC	222-242	FAM	0.533	5	0.515	0.598	+0.0498
ScyRo12	KT266572	$(A)_3$GTGGAGG$(GATG)_7$GACAGACTGG	GCCTGGTACAGGTAGGCTC GAGAGGCCAGAGGTGGAAC	374-410	FAM	0.517	6	0.636	0.552	-0.0987
ScyRo13	KT266573	T$(AC)_3$GT$(AC)_{24}$CG$(TG)_3$TAA	GCAGTCAGATGCCCTACTTC CTCTGCAAGAACCTATGCCC	261-283	HEX	0.672	6	0.606	0.733	+0.0885

PIC = polymorphic information content, NA = Number of alleles, Ho = observed heterozygosity, He = expected heterozygosity