Hereditas 137: 157–160 (2002)

Brief report
Isolation and cross-species amplification of microsatellite loci in the Siberian jay (*Perisoreus infaustus*)

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(Received October 14, 2002. Accepted October 20, 2002)

Microsatellites are superior compared to other genetical markers for parentage determination, because they can be analysed from tiny and partially degraded DNA-samples extracted from e.g. hairs or bird feathers (ELLEGREN 1992). However, bird genomes contain relatively few microsatellite loci (LONGMIRE et al. 1999; PRIMMER et al. 1997b). It is therefore a tedious process to isolate a set of markers that is sufficient for conclusive parentage analyses. Here we report on nine microsatellite markers that are polymorphic in the Siberian jay (*Perisoreus infaustus*), a resident family dwelling species occurring throughout the Eurasian taiga (HELLE and LILLANDT 1997). The markers were found using two methods; (1) isolating new microsatellite sequences from a size-selected Siberian jay genomic library, and (2) exploring 64 heterologous microsatellite markers isolated in other mostly passerine species, and modifying primer sequences if necessary. This set of nine partly unpublished markers have previously been used for parentage testing on 298 juvenile Siberian jays from which feather or blood samples were collected during a long-term study in Finland 1976–1998. Methods for data analysis and parentage determinations were described in LILLANDT et al. (2001). These markers will be used in analyses of family structure, dispersal behaviour and fitness consequences of genetic similarity within pairs of the Siberian jay (B.-G. LILLANDT et al., in prep.).

Microsatellite isolation followed the procedure used by HANSSON et al. (2000). Siberian jay DNA extracted from blood of an adult female was digested with *Mbo*I and *Bam*HI separately and electrophoresed in a 0.8 % agarose gel. DNA fragments in the size range 500–1200 bp were excised from the gel and extracted using JETsorb (Genomed Inc.), according to the manufacturer's instructions. DNA fragments mixed from both digestions were ligated into the vector M13mp18 and electro-transformed into *E. coli* DH5αF′ cells. Filter prints taken from agar plates were hybridized with probes $(CA)_{15}$, $(GA)_{15}$ and $(GACA)_{7+2bp}$ simultaneously and in a separate batch with $(AT)_{15}$, $(AAT)_{10}$ and $(AAAT)_{7+2\ bp}$. The probes were endlabelled with [γ^{32}-P]ATP. Positive clones were sequenced using standard protocols for an ABI PRISM 310 automated sequencer. From ten sequenced clones we obtained six different sequences of which only three contained any microsatellite repeat sequence, and all of these were short (5–10 repeats). However, one of these three clones also contained a compound 6 bp repeat totally different from the used probes ($GGCCCT_9 + GGTCCT_4$), located 490 bp from the repeat we aimed for. We designed primers for all colonies containing at least some kind of repeated sequence. Out of a total of four microsatellites tested on 11 unrelated individuals, only the 6 bp-repeat was found to be polymorphic (locus Per1, 6 alleles in 419 individuals, Table 1).

We also screened Siberian jay DNA with 64 microsatellite primers isolated from other species (Appendix). PCR-reactions were performed following the same protocol as in microsatellite typing (see below). In the reactions we mostly used annealing temperatures in the range 0–5 degrees below the optimal temperature given for the original species. In three cases (Ck.5A5F, LTR7, LTR8) the tested primer pair amplified a polymorphic product that was difficult to evaluate because of stutter bands and co-amplification of non-specific fragments. To be able to use these primers we sequenced the amplified fragment using a standard TA-cloning kit (Invitrogen) according to the manufacturer's instructions, and designed totally or partly new internal primers, closer to the repeat sequence. Altogether 8 of 64 tested primer pairs gave polymorphic products in the Siberian jay. The number of polymorphic loci found among primers designed for other corvid species was six out of 27 tested (22.2 %), compared to only two out of 37 (5.4 %) from other passerines. This is in agreement with the results obtained by PRIMMER et al. (1996a) and GALBUSERA et al. 2000, showing that primers from more closely related species are more likely to amplify polymorphic loci than primers from more distantly related species.

Table 1. *Characterization of microsatellite loci polymorphic in the Siberian jay (Perisoreus infaustus). The original primers that were modified to fit the Siberian jay in parenthesis. The LTR loci were previously named LTMR (McDonald and Potts 1994, Hansson et al. 2000). More details about the variability in these nine loci were given in Lillandt et al. (2001)*

Locus		Primer sequence (5′→3′)	T_a °C	Repeat motif (seq. clone)	Size, bp [b]	Size, bp [c]	No. of alleles [c]
Ck.1B5D	* F/R	Tarr and Fleischer 1998	61	$(GT)_{15}$ [b]	83	83, 85	2
Ck.2A5A	* F/R	Tarr and Fleischer 1998	53	$(GT)_{11}$ [b] $(TG)_{23}$ [c]	139	132–192	16
(Ck.5A5F)	F:	Tarr and Fleischer 1998		$(AT)_4(GT)_{14}$ [b]	147		
	* R:	Tarr and Fleischer 1998					
CkL5	* F:	ATACCAGAGGTCC-TATAAACCA	54	$(AT)_{11}(AAAT)_5$ $(AT)_{13}(GT)_8$ [c]	168–194		11
	R:	TTGTTCTCTCAAGACAC-CTGTT					
(LTR7)	F/R	McDonald and Potts, unpubl.					
LTML7	* F:	GCTTTCCAAGTGACTCT-GTGC	61	$(TG)_9$ [c]		128, 130	2
	* R:	ACCCTCCACCTTGTTTT-TACTG					
(LTR8)	F/R	McDonald and Potts 1994			140–148		
LTML8	* F:	TGTTAACCATTTTCCAAT-GTGC	54	$(AC)_{17}$ [c]		101–135	14
	* R:	AGCATTTCTGATAAT-GCTTCCA					
MJG1	* F/R	Li et al. 1997	54	$(AAAG)_n$ [b]	143–330	157–163	2
Per1	* F:	CTGGGAACAGCCATG-GTC	61	$(GGCCCT)_9$ $(GGTCCT)_4$ [c]	150–190		6
	* R:	TGCAGTGGTTTGTCT-GCAG					
Ppi1	* F/R	Martinez et al. 1999	60			241–247	4
Ppi2	* F/R	Martinez et al. 1999	54			263–271	5

T_a = annealing temperature optimal for the Siberian jay, [b] in the original species (if published), [c] in the Siberian jay, * = primer pairs used for typing (Ck.5A5F R and CkL5 F combined).

All PCR amplifications were performed on a Perkin Elmer 9600 thermal cycler using AmpliTaq PCR-kit (Perkin Elmer). Reactions of 10 µl included 25 ng genomic DNA from blood samples or 1–3 µl from the total amount of 25 µl DNA dilution from one tail-feather (DNA extraction described in Lillandt et al. 2001). The reaction volume contained 0.5 U AmpliTaq DNA polymerase, 0.125 mM of each nucleotide, 1.5 mM $MgCl_2$ and 0.4 µM forward and reverse primer. The general PCR-profile consisted of 28–35 cycles of 94°C for 30 s, 30 s at an annealing temperature specific for every primer (Table 1), and 30 s at 72°C. Before the cycles there was a 2 min incubation at 94°C and after completion of the cycles a 10 min incubation at 72°C.

Because of amplification problems when using DNA-samples extracted from old feather samples, we tested different methods to visualize the PCR-products. Primers were labelled either with [γ^{32}-P]ATP or fluorescein, or the amplification product stained by ethidium bromide. In reactions with radioactive labelling we used 0.2 µM unlabelled, 0.06 µM labelled forward primer and 0.4 µM unlabelled reverse primer. After PCR-cycling 5 µl loading dye was added and 3–10 µl of each sample was electrophoresed in a 6–8 % denaturing polyacrylamide gel. The locus MJG1 was run with unlabelled primers on a nondenatured 8 % polyacrylamide gel, followed by ethidium bromide staining, because of the large size difference (6 bp) between the two alleles. The gels containing radioactively labelled PCR-products were transformed to Whatman-paper, dried and exposed to X-ray film overnight, or longer if necessary. Samples labelled with fluorescein or stained with ethidium bromide were scanned on a Vistra FluorImager.

The degree of polymorphism in our nine loci ranged between two and 16 alleles per locus (Table 1). The length of the alleles was determined by running reac-

Table 2. *Cross-species amplification of nine microsatellites polymorphic in the Siberian jay (Perisoreus infausus), numbers indicate the number of alleles found. Tests performed on common jay (Garrulus glandarius, n = 2), great reed warbler (Acrocephalus arundinaceus, n = 3, except in Ppi2 (n = 242), HANSSON et al. 2000), swift (Apus apus, n = 2) and dunlin (Calidris alpina, n = 2). Primers modified for the Siberian jay (original name in parenthesis) were used for amplification in three loci*

Species	Ck.1B5D	Ck.2A5A	(Ck.5a5F) CkL5	(LTR7) LTML7	(LTR8) LTML8	MJG1	Per1	Ppi1	Ppi2
Perisoreus infaustus	2	16	11	2	14	2	6	4	5
Garrulus glandarius	1	3?	2	2?	1	1?	2	3?	4?
Acrocephalus arundinaceus	1	x	1	1	1	1?	1	1	23
Apus apus	1?	–	1	1	–	?	1?	1?	–
Calidris alpina	–	–	–	–	–	–	1	1?	–

– = no amplification, x = several nonspecific bands or a smear,? = unclear amplification product.

tions from a few individuals beside a DNA fragment of known length, and the results were compared to length information from sequencing if available. The primers that amplified polymorphic loci in the Siberian jay were also tested on four other species to find out their suitability for cross-species amplification. In these tests we used the same PCR-conditions as for the Siberian jay samples. All of the nine microsatellite loci found to be polymorphic in the Siberian jay also amplified a specific product in the common jay (*Garrulus glandarius*), and eight of them in the great reed warbler (*Acrocephalus arundinaceus*). Five loci gave a specific but monomorphic product in one or both of the two non-passerine species tested, the swift (*Apus apus*) and the dunlin (*Calidris alpina*) (Table 2). The ability for cross-species amplification of these primers suggests that many of them can be useful in other corvid species.

ACKNOWLEDGEMENTS

We thank Kerstin Persson and other members of the Molecular Population Biology Lab. at Lund University for help during the labwork. Terry Burke, David McDonald and Isao Nishiumi provided unpublished microsatellite primers, and Tom Reuter gave valuable comments on the manuscript. The labwork was supported by grants from Kungliga Fysiografiska Sällskapet i Lund, NorFa and Svensk-Österbottniska Samfundet (to B-GL). This is report no. 3 from Tjöck Skrikebo Jay Centre.

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Appendix. List of 64 microsatellite primers isolated from other species, that were tested in the Siberian jay (*Perisoreus infaustus*). The original primers that were modified to fit the Siberian jay in parenthesis. The LTR loci were previously named LTMR and SJ were SJR (McDonald and Potts 1994; Hansson et al. 2000).

Locus	Original species	Reference
Aar1, Aar2, Aar3, Aar7, Aar8 (new primer for Escμ6-locus)	*Acrocephalus arundinaceus*	Hansson et al. 2000
Ck.1B5D, Ck.1B6G, Ck.2A5A, Ck4A3G, Ck.4B6D, Ck.5A4B, Ck.5A4D, (Ck.5A5F) CkL5	*Corvus kubaryi*	Tarr and Fleischer 1998
Escμ2, Escμ4, Escμ6,	*Emberiza schoeniclus*	Hanotte et al. 1994
FhU2 (previously PTC3)	*Ficedula hypoleuca*	Ellegren 1992
FhU3	*Ficedula hypoleuca*	Primmer et al. 1996a,b, 1997a
G7B	*Acrocephalus orientalis*	I. Nishiumi, unpubl.
Gf01, Gf12, Gf14	*Geospiza fortis*	Petren 1998
HrU1, HrU2, HrU3, HrU5, HrU7	*Hirundo rustica*	Primmer et al. 1995
HrU10	*Hirundo rustica*	Primmer et al. 1996b
LS2	*Lanius ludovicianus*	Mundy and Woodruff 1996
LTR6, (LTR8) LTML8, LTR15	*Chiroxiphia linearis*	McDonald and Potts 1994
(LTR7) LTML7, LTR9, LTR16	*Chiroxiphia linearis*	McDonald and Potts, unpubl.
Mcyμ4	*Malurus cyaneus*	Double et al. 1997
MJG1, MJG3, MJG4, MJG7, MJG8	*Aphelocoma ultramarina*	Li et al. 1997
Mme12	*Melospiza melodia*	Jeffery et al. 2001
PCAμ2, PCAμ7, PCAμ9	*Parus caeruleus*	Hanotte et al., unpubl.
Pdou5	*Passer domesticus*	Griffith et al. 1999
Phtr2, Phtr3, Phtr4	*Phylloscopus trochilus*	Fridolfsson et al. 1997
Pocc4	*Phylloscopus occipitalis*	Bensch et al. 1997
Ppi1, Ppi2, Ppi3	*Pica pica*	Martinez et al. 1999
SJ1, SJ3, SJ4, SJ6, SJ13, SJ21, SJ25, SJ31	*Aphelocoma coerulescens*	McDonald and Potts, unpubl.
SJ133	*Aphelocoma coerulescens*	McDonald and Potts 1994
4be5, 4b1	*Aphelocoma coerulescens*	McDonald and Potts, unpubl.