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

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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 This set of nine partly unpublished markers have previously been used for parentage testing on 298 juvenile Siberian jays from which feather or blood samples were collected during a long-term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 MboI and BamHI 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)₁₂bp simultaneously and in a separate batch with (AT)₁₅, (AAT)₁₀ and (AAAT)₁₂bp. The probes were endlabeled with [γ³²-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 (GGCCCT₉ + GTTCCT₉), 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).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequence (5′→3′)</th>
<th>T_a (°C)</th>
<th>Repeat motif (seq. clone)</th>
<th>Size, bp b</th>
<th>Size, bp c</th>
<th>No. of alleles c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ck.1B5D</td>
<td>F/R: TARR and Fleischer 1998</td>
<td>61</td>
<td>(GT)_15</td>
<td>83</td>
<td>83, 85</td>
<td>2</td>
</tr>
<tr>
<td>Ck.2A5A</td>
<td>F/R: TARR and Fleischer 1998</td>
<td>53</td>
<td>(GT)_11, (TG)_23</td>
<td>139</td>
<td>132–192</td>
<td>16</td>
</tr>
<tr>
<td>(Ck.5A5F)</td>
<td>F: TARR and Fleischer 1998</td>
<td></td>
<td>(AT)_3, (GT)_14</td>
<td>147</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CkL5</td>
<td>* F: ATACCCAGAGTCC-TATAAACCA</td>
<td>54</td>
<td>(AT)_1, (AAAT)_5</td>
<td>168–194</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>* R: TGTTCCTCTCAAGACAC-CTGTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(LTR7)</td>
<td>F/R: McDonald and Potts, unpubl.</td>
<td>61</td>
<td>(TG)_6</td>
<td>128, 130</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>LTML7</td>
<td>* F: GCTTTCCAAGTGACCT-GTGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>* R: ACCCTCCACCTTTTT-TACGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(LTR8)</td>
<td>F/R: McDonald and Potts 1994</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTML8</td>
<td>* F: TGTTAACATTTCAT-GTGC</td>
<td>54</td>
<td>(AC)_17</td>
<td>140–148</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>* R: AGCATTTCTGATAAT-GCTTCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MJG1</td>
<td>* F/R: Li et al. 1997</td>
<td>54</td>
<td>(AAAG)_6</td>
<td>143–330</td>
<td>157–163</td>
<td>2</td>
</tr>
<tr>
<td>Per1</td>
<td>* F: CTGGGAAAGACCCAT-GTGC</td>
<td>61</td>
<td>(GGCCTC)_6</td>
<td>150–190</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>* R: TGGAGTTGTGTCT-GCAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ppi1</td>
<td>* F/R: Martínez et al. 1999</td>
<td>60</td>
<td></td>
<td>241–247</td>
<td></td>
<td>4</td>
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<tr>
<td>Ppi2</td>
<td>* F/R: Martínez et al. 1999</td>
<td>54</td>
<td></td>
<td>263–271</td>
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<td>5</td>
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</tbody>
</table>

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 MgCl₂ 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 [γ³²-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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ck.1B5D</th>
<th>Ck.2A5A</th>
<th>(Ck.5a5F)</th>
<th>(LRT7)</th>
<th>(LRT8)</th>
<th>MJG1</th>
<th>Per1</th>
<th>Ppi1</th>
<th>Ppi2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perisoreus infausus</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Garrulus glandarius</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>3?</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3?</td>
</tr>
<tr>
<td>Acrocephalus arundinaceus</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>1</td>
<td>1</td>
<td>1?</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Apus apus</td>
<td></td>
<td></td>
<td></td>
<td>1?</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1?</td>
<td>–</td>
</tr>
<tr>
<td>Calidris alpina</td>
<td></td>
<td></td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1?</td>
<td>–</td>
</tr>
</tbody>
</table>

= no amplification, \( x \) = several non-specific bands or a smear, \( ? \) = unclear amplification product.

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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).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Original species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aar1, Aar2, Aar3, Aar7, Aar8 (new primer for Esc6-locus)</td>
<td>Acrocephalus arundinaceus</td>
<td>Hansson et al. 2000</td>
</tr>
<tr>
<td>Ck.1B5D, Ck.1B6G, Ck.2A5A, Ck4A3G, Ck.4B6D, Ck.5A4B, Ck.5A4D, (Ck.5A5F) CkL5</td>
<td>Corvus kubaryi</td>
<td>Tarr and Fleischer 1998</td>
</tr>
<tr>
<td>Esc2, Esc4, Esc6, FhU2 (previously PTC3)</td>
<td>Emberiza schoeniclus</td>
<td>Hanotte et al. 1994</td>
</tr>
<tr>
<td>FhU3</td>
<td>Ficedula hypoleuca</td>
<td>Ellegren 1992</td>
</tr>
<tr>
<td>G7B</td>
<td>Ficedula hypoleuca</td>
<td>Primmer et al. 1996a,b, 1997a</td>
</tr>
<tr>
<td>Gf01, Gf12, Gf14</td>
<td>Acrocephalus orientalis</td>
<td>I. Nishiumi, unpubl.</td>
</tr>
<tr>
<td>HrU1, HrU2, HrU3, HrU5, HrU7</td>
<td>Geospiza fortis</td>
<td>Petren 1998</td>
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<tr>
<td>HrU10</td>
<td>Hirundo rustica</td>
<td>Primmer et al. 1995</td>
</tr>
<tr>
<td>LS2</td>
<td>Hirundo rustica</td>
<td>Primmer et al. 1996b</td>
</tr>
<tr>
<td>LTR6, (LTR8) LTML8, LTR15 (LTR7) LTML7, LTR9, LTR16</td>
<td>Lanius ludovicianus</td>
<td>Mundy and Woodruff 1996</td>
</tr>
<tr>
<td>Mcy4</td>
<td>ChIROXIPHA linearis</td>
<td>McDonald and Potts 1994</td>
</tr>
<tr>
<td>MJG1, MJG3, MJG4, MJG7, MJG8</td>
<td>ChIROXIPHA linearis</td>
<td>McDonald and Potts, unpubl.</td>
</tr>
<tr>
<td>Mme12</td>
<td>Maturus cyanus</td>
<td>Double et al. 1997</td>
</tr>
<tr>
<td>PCAµ2, PCAµ7, PCAµ9</td>
<td>Aphelocoma ultramarina</td>
<td>Li et al. 1997</td>
</tr>
<tr>
<td>Pdou5</td>
<td>Melospiza melodia</td>
<td>Jeffery et al. 2001</td>
</tr>
<tr>
<td>Phtr2, Phtr3, Phtr4</td>
<td>Parus caeruleus</td>
<td>Hanotte et al., unpubl.</td>
</tr>
<tr>
<td>Pocc4</td>
<td>Passer domesticus</td>
<td>Griffith et al. 1999</td>
</tr>
<tr>
<td>Ppi1, Ppi2, Ppi3</td>
<td>Phylloscopus trochilus</td>
<td>Fridolfsson et al. 1997</td>
</tr>
<tr>
<td>SJ1, SJ3, SJ4, SJ6, SJ13, SJ21, SJ25, SJ31</td>
<td>Phylloscopus occipitalis</td>
<td>Bensch et al. 1997</td>
</tr>
<tr>
<td>SJ133</td>
<td>Pica pica</td>
<td>Martinez et al. 1999</td>
</tr>
<tr>
<td>4be5, 4b1</td>
<td>Aphelocoma coerulescens</td>
<td>McDonald and Potts, unpubl.</td>
</tr>
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<td></td>
<td>Aphelocoma coerulescens</td>
<td>McDonald and Potts 1994</td>
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<td>Aphelocoma coerulescens</td>
<td>McDonald and Potts, unpubl.</td>
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