Molecular Ecology Notes (2003) doi: 10.1046/j.1471-8286.2003.00498.x

PRIMER NOTE

Isolation and characterization of 16 microsatellite loci in the Great Tit *Parus major*

VERENA SALADIN, DANIELLE BONFILS, THOMAS BINZ and HEINZ RICHNER
Department of Evolutionary Ecology, Zoological Institute, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland

Abstract

Six dinucleotide, three trinucleotide and seven tetranucleotide microsatellite loci developed for the great tit *Parus major* are presented. Thirty individual birds were screened at each locus. Loci were polymorphic (four to 19 alleles per locus). These markers provide a system to study paternity, genetic diversity in natural populations, gene flow, dispersal and inbreeding.

Keywords: great tit, microsatellite, Parus, paternity, population genetics

Received 28 April 2003; revision received 29 June 2003; accepted 29 June 2003

Microsatellites have become one of the most important tools for studying paternity, genetic diversity in natural populations, segregation and linkage of loci in families, gene flow, dispersal and inbreeding. In this report, we describe the isolation of 16 microsatellite DNA loci in *Parus major* and the development of appropriate polymerase chain reaction (PCR) primers.

Genomic DNA from blood cells of 50 great tit individuals was isolated using the Wizard Genomic DNA Isolation Kit (Promega, Switzerland). The DNA was pooled (50 μg in total) and sent to the Genetic Identification Services (GIS, http://www.genetic-id-services.com; Chatsworth, California, USA), to develop four enriched microsatellite libraries containing inserts with $(CA)_n$ $(GA)_n$ $(TGA)_n$ and $(TAGA)_n$ repeats, respectively (Peacock *et al*. 2002). Cloning and sequencing procedures were as previously described (Binz *et al*. 2000). Primers for amplification of microsatellite loci were designed with the help of PRIMER 3 software (Rozen & Skaletsky 1996, 97). Genomic DNA from 30 individual great tits originating from three different populations around Bern, Switzerland, was isolated using the Wizard Genomic DNA Isolation Kit (Promega, Switzerland). PCR reactions were carried out in a 10-μL volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 U of Ampli*Taq* Gold DNA Polymerase (Applied Biosystems, Rotkreuz, Switzerland) 200 μM dNTPs, 0.5 μM of locus-specific fluorescent-labelled forward primer (fluorescent dyes were 6-FAM, HEX and NED) and nonlabelled reverse primer (see Table 1). Between 5 and 50 ng of genomic DNA were used as template.

PCR cycling parameters were the following (in a Geneamp 9700 Thermocycler, Applied Biosystems): 10 min at 95 °C, 33 cycles at 95 °C for 30 s, 57 °C for 30 s and 72 °C for 75 s, followed by a final step at 72 °C for 7 min. Fluorescent PCR fragments were visualized by capillary electrophoresis on an ABI 3100 Genetic Analyser (Applied Biosystems).

All 16 loci analysed were polymorphic and their characteristics are summarized in Table 1. Heterozygosity values were calculated using the CERVUS software package (Marshall *et al*. 1998). The allele frequencies of all loci except PmaGAn28 did not deviate significantly from Hardy–Weinberg equilibrium ($P < 0.05$) when using the exact test provided in the GENEPOP version 3.1d (Raymond & Rousset 1995). For the locus PmaGAn28, only one allele per individual was detected in females whereas males where mostly heterozygous. When correcting the female genotypes of the locus PmaGAn28 with null alleles in the CERVUS software, allele frequencies corresponded to Hardy–Weinberg equilibrium, indicating that the observed significant deviation from Hardy–Weinberg equilibrium detected by the exact test is probably due to a presence of null alleles in females (Table 1). These may be located on the W chromosome, as this occurs in the female and not in the male birds. All 16 loci tested positive for mendelian segregation in three families analyzed.

Correspondence: V. Saladin. Fax: + 41 31 631 30 08; E-mail: verena.saladin@esh.unibe.ch

Acknowledgements

We gratefully acknowledge financial support by the Swiss National Science Foundation (grant #31–53956.98).

Table 1 Primer sequences, repeat unit structure, expected allele size deduced from sequence, observed (H_O) and expected (H_E) heterozygosities, and GenBank accession numbers

Locus	Primer name	Sequence (5′–3′)	Repeat type	Expected allele size (bp)	Observed alleles (bp)	H_O	H_E	GenBank accession no.
PmaC25	PmaC25	F°: CGTCCTGCTGTTTGTATTTCTG R°: CCATGAACCATTTTTAGGGTG	$(CAT)_{11}$	323	309, 315, 318, 321, 324, 326, 327, 330, 332, 333, 336	0.800	0.900	AY260526
PmaD22	PmaD22	F°: GATCAGAGCTTGCCTCAACAC R°: TCTGGGCTGAAATACCTACCC	$(CTAT)_{15}(CCAT)_{12}$	403	392, 396, 400, 404, 408, 412, 416, 420, 424, 428, 432, 436, 440, 448, 452	0.933	0.920	AY260527
PmaD105	PmaD105	F°: CAAATCACACAGTTGCTGCC R°: CCTGGTATAAGACTGGTCAAAACAG	$(GTCT)_3(ATCT)_{12}$	404	377, 385, 397, 401, 405, 409, 413, 417, 421, 425	0.833	0.819	AY260528
PmaD130	PmaD130	F°: TGAGTGGAAAGATGCTGGC R°: CCCTATAAAAACCGAGGCTG	$(TAGA)_{17}$	438	379, 391, 395, 419, 423, 427, 431, 435, 439, 443, 447, 451	0.700	0.751	AY260529
PmaCAn1	PmaCAn1	F°: CTCTTTTCCCAGCCTCCAG R°: TATGTTTTGTCTGCTCGGGG	$(CA)_{15}(CT)(CA)_4$	118	105, 107, 109, 119, 122, 124, 126, 128, 130, 132, 134	0.900	0.842	AY260530
PmaGAn11	PmaGAn11	F°: GCTTCTGCCTCCATTAAGAGTC R°: GAAAAATCACCCACTCAGCC	$(GA)_9$	105	100, 102, 104, 106	0.267	0.295	AY260531
PmaGAn27	PmaGAn27	F°: TATAAACCACAGCCACACGC R°: CACAACCACAGAGGCATGAG	$(CAT)_{16}$	202	201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 234, 237, 243	0.833	0.926	AY260532
PmaGAn28	PmaGAn28	F°: GTTGGTGCAGCGGTCTACTC R°: CATGTTGGGACAGCAGTTTG	$(GA)_{16}$	199	190, 192, 202, 204, 206, 208, 228	0.400*	0.812	AY260533
PmaGAn30	PmaGAn30	F°: GTTTCTGCCCAAATGGTGTC R°: TCAGACCTTTCCAATGATGG	$(GA)_{10}$	305	300, 302, 304, 306, 308, 310	0.500	0.573	AY260534
PmaGAn31	PmaGAn31	F°: TGTTCTAATATGGAAGCAAGGG R°: TCATGCCAGAGAAAGCTGTG	$(GA)_{13}$	88	77, 83, 85, 87, 89	0.367	0.328	AY260535
PmaGAn40	PmaGAn40	F°: CGTTCCTCCTTTGCTTTCTG R°: AATGGCACAACACCTTCTCC	$(GA)_{10}$	416	414, 416, 418, 420, 422	0.567	0.529	AY260536
PmaTAGAn71	PmaTAGAn71	F°: TCAGCCTCCAAGGAAAACAG R°: GCATAAGCAACACCATGCAG	$(TAGG)_6(TAGA)_{11}$	186	174, 178, 182, 186, 190, 194, 198, 202, 206	0.733	0.784	AY260537
PmaTAGAn86	PmaTAGAn86	F°: AAAACAAGGCCACTTAGAGCTG R°: ACTCCTCCAGGTCACACAGG	$(TAGA)_{21}$	196	140, 156, 164, 168, 172, 176, 180, 184, 188, 192, 196	0.767	0.856	AY260538
PmaTGAn33	PmaTGAn33	F°: TTCCCCAAGTATCCTGCATC R°: AAACCATATCACCCAGTGCC	$(GATA)_{14}GAT(GATA)_8$	305	268, 272, 273, 276, 280, 284, 285, 288, 289, 292, 293, 296, 297, 300, 305, 306, 309, 313, 322	0.867	0.935	AY260539
PmaTGAn42	PmaTGAn42	F°: ACTTCCACATGCCAGTTTCC R°: TGTTAAGGCAGAGAGGTGGG	$(TCCA)_{15}$	285	246, 264, 266, 270, 274, 278, 282, 290	0.767	0.732	AY260540
PmaTGAn45	PmaTGAn45	F°: CCCCTGGCTCTTTCATATCC R°: GACAGGTGTTGGCACAAGG	$(TGA)_{10}$	307	290, 300, 303, 306, 309, 313	0.700	0.762	AY260541

*significant deviation from H-W equilibrium ($P < 0.05$).

 Molecular Ecology Notes, 10.1046/j.1471-8286.2003.00498.x

References

Binz T, Reusch TBH, Wedekind C, Schärer L, Sternberg J, Milinski M (2000) Isolation and characterization of microsatellite loci from the tapeworm *Schistocephalus solidus*. *Molecular Ecology*, **9**, 1926–1927.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Peacock MM, Kirchoff VS, Merideth SJ (2002) Identification and characterization of nine polymorphic microsatellite loci in the North American pika, Ochotona princeps. *Molecular Ecology Notes*, **2**, 360–362.

Raymond M, Rousset F (1995) GENEPOP Version 1.2: population genetics software for exact tests and ecumenism. *Journal of Heredity*, **86**, 248–249.

Rozen S, Skaletsky HJ (1996–97) PRIMER 3. *Code available at: http://www-genome.wi.mit.edu/genome_software/other/primer3/html.*

