Molecular Ecology Notes (2007) 7, 833–835 doi: 10.1111/j.1471-8286.2007.01719.x

PRIMER NOTE

Microsatellite markers isolated from barn swallows (*Hirundo rustica*)

OLGA V. TSYUSKO,* MAUREEN B. PETERS,* CRIS HAGEN,* TRACEY D. TUBERVILLE,* TIMOTHY A. MOUSSEAU,† ANDERS P. MØLLER‡ and TRAVIS C. GLENN*†

**The University of Georgia, Institute of Ecology, Savannah River Ecology Laboratory, PO Drawer E, Aiken, SC 29802, USA, †Department of Biological Sciences, University of South Carolina, Columbia, SC 29802, USA, ‡Laboratoire de Parasitologie Evolutive, Université Pierre et Marie Curie, FR-75252 Paris, Cedex 05, France*

Abstract

Fifteen polymorphic microsatellite DNA loci were isolated from barn swallow (*Hirundo rustica*) and optimized for future studies of radiation-induced mutations in populations from Ukraine. The loci were screened for variability among 25 individuals from two populations. The primers amplified loci with relatively high numbers of alleles ranging from five to 32 alleles per locus and polymorphic information content from 0.481 to 0.951. Observed heterozygosity varied from 0.458 to 0.960. None of the loci showed deviations from Hardy–Weinberg equilibrium in either population.

Keywords: *Hirundo rustica*, microsatellites, mutation, PCR, primer, radiation

Received 2 December 2006; revision accepted 8 January 2007

Microsatellites from barn swallows (*Hirundo rustica*) have been used in several classic studies, including putative radiation-induced germline mutations in wildlife near Chornobyl (Ellegren *et al*. 1997). Given the heterogeneous responses in the germline mutation rates observed between two microsatellite loci used in previous barn swallow studies (Ellegren *et al*. 1997; Brohede *et al*. 2002), further work in this species requires additional loci to be developed. Here we describe the development and optimization of polymorphic microsatellite DNA loci that can be used to study barn swallows and the many other species from which their primers are known to work (e.g. Primmer *et al*. 1996).

Blood was collected using heparinized capillaries, which were immediately transferred to cryovials with a citrate buffer, and then stored at –70 °C. DNA from two individuals was extracted from blood using a DNeasy Kit (QIAGEN) and was serially enriched twice for microsatellites using three probe mixes [mix 2: $(AG)_{12}$, $(TG)_{12}$, $(AAC)_6$, $(AAG)_8$, $(AAT)_{12}$, $(ACT)_{12}$, $(ATC)_8$; mix 3: $(AAAC)_6$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACAG)_6$, $(ACCT)_6$, $(ACTC)_6$, $(ACTG)_6$; mix 4: $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$, $(AGAT)_8$] following Glenn & Schable (2005). Briefly, the DNA was digested with restriction enzyme *Rsa*I (New England Biolabs) and simultaneously ligated to double-stranded SuperSNX linkers (SuperSNX24 forward 5′-GTTTAAGGCCTAGCTAGCAGCAGAATC and SuperSNX24 reverse 5′-GATTCTGCTAGCTAGGCCTTAAACAAAA). Linker-ligated DNA was denatured and hybridized to biotinylated microsatellite oligonucleotide mixes, which were then captured on magnetic streptavidin beads (Dynal). Unhybridized DNA was washed away and the remaining DNA was eluted from the beads, amplified in polymerase chain reactions (PCR) using the forward SuperSNX24 as a primer, and cloned with TOPO-TA Cloning Kits (Invitrogen). The positive white clones with inserts were detected using β-galactosidase gene. A total of 192 positive clones were isolated by transferring each colony to an individual well of a deep-well plate containing LB broth and the inserts were amplified with M13 forward and reverse primers. Total of 96 plasmids were sequenced using the BigDye Terminators version 3.1 (Applied Biosystems) and ABI 3130xl capillary sequencer. Sequences from both strands were assembled and edited in SEQUENCER 4.1 (Genecodes) and exported to EPHEMERIS 1.0 (available at http://www.uga.edu/srel/DNA_Laboratory/programs.htm) for microsatellite searching. Ninety-two out of the 96 sequenced clones contained microsatellites. Twenty-four PCR primers were designed using OLIGO 6.67 (Molecular Biology Insights) and one primer in each pair was modified on the 5′ end with an engineered sequence (CAG tag

Correspondence: Olga Tsyusko, Fax: 803 725 3309;
E-mail: tsyusko@srel.edu

Table 1 Characterization of 15 polymorphic microsatellite loci for *Hirundo rustica*. Size indicates the range of observed alleles in bp; *k* is number of alleles; *n* is the number of the genotypes obtained; H_O and H_E are expected and observed heterozygosity and PIC is polymorphic information content; null alleles are estimated from CERVUS 2.0. A total of 25 individuals from two populations (eight from Dityatki and 17 from Boryspil, Ukraine) were genotyped. Initial annealing temperature was 55 °C for all amplifications

Locus Accession no.	Primer sequence 5′–3′	Repeat motif	Size (bp)	*k*	*n*	H_O	H_E	PIC	Null alleles
Hir 4 EF120606	U: FAMGCAAGGACCGATCACA L: AAAAACCCAAACAATCAAC	$(GTTT)_{10}$	286–310	8	24	0.458	0.828	0.786	0.270
Hir 5 EF120607	U: GTGTGCTGAAAAGTGTAT L: FAMACAGGCATGTGTGAGT	$(GTTT)_5$	226–246	6	23	0.652	0.713	0.653	0.045
Hir 6 EF120608	U: FAMGACGGCCTGGGGGTAGA L: AAGAGCATGACCACCAGAGAT	$(TCTA)_{16}$	202–230	9	25	0.720	0.804	0.758	0.051
Hir 7 EF120609	U: FAMCTTGCGCAGAAAGTAT L: GCTCTGGGATCTCTAG	$(CT)_{14}$	247–284	18	25	0.760	0.942	0.918	0.098
Hir 8 EF120610	U: TAAAAGCAAGGAAGATCT L: FAMGACACCGTCACAGTAGA	$(CTT)_{11}CTA(CTT)_{14}$	218–367	24	24	0.792	0.952	0.928	0.082
Hir 10 EF120611	U: FAMGGACAAGGGGAGTCTT L: ATTCAGCCAGCCTCTAAT	$(GTTT)_9$	180–206	8	24	0.917	0.868	0.832	– 0.039
Hir 11 EF120612	U: AACACCTGAAAACCTACAC L: FAMCTTTGAGCAAAATGAGTG	$(GATA)_{14}$	208–248	10	25	0.960	0.850	0.812	– 0.076
Hir 15 EF120613	U: NEDTTGTGGTGGCTGACTT L: CTGACGAGATGCAATTAC	$(ATGT)_7$	236–252	7	24	0.750	0.706	0.647	– 0.066
Hir 16 EF120614	U: NEDCTTCAGGCAGGTAAG L: GAAAACCATTCCTGTTAA	$(ATGT)_6 \ldots (GT)_{11}$	301–329	12	24	0.542	0.892	0.861	0.237
Hir 17 EF120615	U: NEDATGCCATGCTTCAGAT L: CTGTCATGCCTAAGTATCA	$(CTAT)_{10}TTAT(CTAT)_3$	215–267	16	25	0.920	0.935	0.910	–0.003
Hir 19 EF120616	U: NEDGCTCACAACCAGCTAGAC L: ATAGCCACAGGGAAAGTCT	$(ATGG)_4ACGG(ATGG)_{15}$	153–202	10	24	0.875	0.848	0.809	–0.029
Hir 20 EF120618	U: GAAGTTGGAGAAAGATTAG L: NEDTTATTGCTCTGGGTATGT	$(ATAG)_{14}$	259–287	11	25	0.800	0.860	0.826	0.016
Hir 21 EF120617	U: AAATTGCCAATTTTGTGTC L: FAMTGGTGGCGAAACATTAA	$(CTTT)_{19}CCTT(CTTT)_8$	273–426	32	24	0.667	0.973	0.951	0.180
Hir 22 EF120619	U: NEDATCCGCACCTAATGT L: GATTCACATATCCCATCTAG	$(ATCT)_7$	267–303	14	24	0.750*	0.868	0.836	0.076
Hir 24 EF120621	U: NEDCTCACCAAAGCGTGTGA L: ACTCCACGGTTTCAGAGA	$(AGTG)_{12}$	218–247	13	25	0.520	0.895	0.865	0.256

*Significant deviations from Hardy–Weinberg equilibrium are indicated at $P < 0.05$. Primers with CAG tag (5′-CAGTCGGGCGTCATCA-3′) are indicated with superscript FAM or NED, which was used as the fluorescent dye for genotyping.

5′-CAGTCGGGCGTCATCA-3′; see http://www.uga.edu/srel/DNA_Laboratory/protocols.htm) to allow use of a third primer in the PCR (CAG) that is fluorescently labelled for detection on the ABI 3130xl.

The 24 PCR primer pairs were tested for amplification using eight individuals across four different Ukrainian populations. PCR amplifications were performed in a 25-μL volume [10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/mL BSA, 0.4 μM unlabelled primer, 0.04 μM tag-labelled primer, 0.36 μM universal dye-labelled primer, 3 mM $MgCl_2$, 0.15 mM dNTPs, 0.5 U JumpStart *Taq* DNA Polymerase (Sigma), and 50 ng DNA template] using an Applied Biosystems thermalcycler (GeneAmp PCR System 9700). Touchdown thermal cycling programs (Don *et al*. 1991) encompassing a 10 °C span of annealing temperatures, ranging between 65–55 °C, 60–50 °C or 55–45 °C, were used for the amplification. Cycling parameters were 21 cycles at 96 °C for 20 s, highest annealing temperature (decreased 0.5 °C per cycle) for 20 s, and 72 °C for 30 s; and 15 cycles of 96 °C for 20 s, lowest annealing temperature for 20 s, and 72 °C for 30 s. PCR products were run on an AB-3130xl sequencer and sized with Naurox size standard prepared as described in DeWoody *et al*. (2004). Results were analysed using GENEMAPPER version 4.0 (Applied Biosystems). Fifteen of the tested primer pairs successfully amplified PCR product of high quality.

Blood samples from 25 individuals from two populations were used to test for microsatellite variability across the 15 loci. The PCRs were performed in 11.5 μL volume and touchdown 55 program encompassing a 10 °C span of annealing temperatures between 55 °C and 45 °C was used. Characteristics of the 15 working primer pairs are

given in Table 1. We estimated the number of alleles per locus (k), observed and expected heterozygosity (H_O and H_E), and polymorphic information content (PIC) and tested for deviations from Hardy–Weinberg equilibrium (HWE) using CERVUS version 2.0 (Marshall *et al*. 1998). There was one locus (Hir 22) that deviated significantly from HWE only when all samples were analysed, but not when each population was analysed alone. No linkage was detected among 122 paired loci comparisons ($P < 0.05$; GENEPOP version 3.4; Raymond & Rousset 1995). BLAST searches of the barn swallow sequences indicate partial homology with two loci from other birds [110 bp of Hir 6 matched partially to accession AY366081 (*Garrulax canorus*) and 205 bp of Hir19 matched partially to accession XM416761 (*Gallus gallus*)].

Overall, the high numbers of alleles per locus, high PIC values and heterozygosities demonstrate the potential of the developed microsatellite primers for a variety of purposes, including detection of genetic effects of radiation in *H. rustica* populations from contaminated environments.

Acknowledgements

This research was supported by the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research, US Department of Energy, through Financial Assistance Award no. DE-FC09-96-SR18546 to the University of Georgia Research Foundation. The financial support also came from grants to T.A.M. and A.P.M. from the National Geographic Society, the Civilian Research Development Foundation, and the Samuel Freeman Charitable Trust.

References

Brohede J, Primmer C, Møller A, Ellegren H (2002) Heterogeneity in the rate and pattern of germline mutation at individual microsatellite loci. *Nucleic Acids Research*, **30**, 1997–2003.

DeWoody AJ, Schupp J, Kenefic L *et al*. (2004) Universal method for producing ROX-labeled size standards suitable for automated genotyping. *BioTechniques*, **37**, 348–350.

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research*, **19**, 4008.

Ellegren H, Lindgren G, Primmer CR, Møller AP (1997) Fitness loss and germline mutations in barn swallows breeding in Chernobyl. *Nature*, **389**, 593–596.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202–222.

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

Primmer CR, Møller AP, Ellegren H (1996) A wide-ranging survey of cross-species microsatellite amplification in birds. *Molecular Ecology*, **5**, 365–378.

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