

PRIMER NOTE

Development and characterization of 11 microsatellite markers in the rock sparrow, *Petronia petronia*

ALESSANDRO GRAPPUTO,* FEDERICA BARBISAN,† MIRKO DE GIROLAMO,‡
ANDREA PILASTRO† and LORENZO ZANE†

*Department of Biological and Environmental Science, PO Box 35, FIN-40014, University of Jyväskylä, Finland, †Dipartimento di Biologia, Università di Padova, I-35121 Padova, Italy, ‡Departamento de Oceanografia e Pescas, Universidade dos Açores, PT-9901-862 Horta, Portugal

Abstract

Eleven polymorphic microsatellite loci were isolated in the passeriform *Petronia petronia* using the enrichment protocol FIASCO (fast isolation by AFLP of sequences containing repeats). We detected three to 13 alleles per locus in 25 specimens collected from an Italian population. The level of expected heterozygosity ranged from 0.439 to 0.856. One locus is sex linked to the Z chromosome. The total exclusionary probabilities using these loci for the first and the second parent were 0.978 and 0.999, respectively. These are the first microsatellite loci characterized from the rock sparrow that can be used for estimating population structure and for large-scale parentage analysis.

Keywords: FIASCO, microsatellites, paternity analysis, *Petronia petronia*

Received 7 March 2006; revision received 20 March 2006; accepted 12 April 2006

Over the last 20 years, new research has produced a revolution in our views of avian reproductive behaviour. Advances in genetic techniques, in particular the development of microsatellite markers, have dramatically enhanced our ability to assess the real paternity of offspring revealing that extra-pair paternity is widespread among birds (Westneat & Stewart 2003).

The rock sparrow, *Petronia petronia*, is a monomorphic species. It breeds in small, loose colonies in warm temperate Mediterranean steppe, but in some regions, the bird favours alpine meadows, grassy or shrubby riversides and human settlements. A north Italian alpine population, in the Upper Susa Valley, is the subject of a long-term ecology and conservation study (Tavecchia *et al.* 2002; Griggio *et al.* 2005). This population presents a wide array of mating patterns, including monogamy, polygyny and sequential polyandry (Pilastro *et al.* 2001). Males and females can cooperate in parental care or can desert the brood and females can lay a new clutch in the nest of another male (Griggio *et al.* 2003). A previous study on the mating system of this species had found that rock sparrow males engaging in polygyny do not have higher breeding success than

monogamous males (Pilastro *et al.* 2002). However, the study employed fingerprinting analysis allowing only the exclusion of paternity; thus, markers that can determine paternity are needed to estimate male reproductive success.

In this note, we report 11 microsatellites isolated using the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol (Zane *et al.* 2002a) with slight modifications. Genomic DNA was isolated from blood using phenol extraction (Kocher *et al.* 1989). Approximately 250 ng of DNA were simultaneously digested with *MseI* and ligated to *MseI* amplified fragment length polymorphism (AFLP) adapters. Only the AFLP *MseI* adaptor-specific primers, *MseI*-T, *MseI*-G and *MseI*-C were used since the *MseI*-A primer produced a single over-amplified band. The enrichment was carried out by hybridization with 10 µM of a biotinylated probe; either (AC)₂₂ or (CAG)₁₁. DNA, probe and 100 µg of magnetic beads (Roche) were incubated either at room temperature [probe (AC)₂₂] or at 40 °C [probe (CAG)₁₁] for 30 min. The beads–probe–DNA complex was precipitated using a magnetic particle separator. Three nonstringent and three stringent washes were carried out as described in Zane *et al.* (2002b). All washes were carried out either at room temperature [(AC)₂₂] or at 40 °C [(CAG)₁₁]. The precipitate was finally eluted with 13 µL of NaOH 0.1 M, neutralized with 0.16 M of acetic acid and

Table 1 Characteristics of 11 microsatellite loci of the rock sparrow obtained from the analysis of 25 individuals from the Upper Susa Valley, Italy. Reported are: locus name, GenBank Accession no., repeat motif, sequences of forward (F) and reverse (R) primers, PCR annealing temperatures [T_a (°C)], PCR product sizes, number of alleles (k), observed (H_O) and expected heterozygosity (H_E), probability of Hardy–Weinberg equilibrium (HW_ P) and individual exclusion probability in both absence (Excl1) and presence (Excl2) of genetic information from one sire

Locus	Accession no.	Repeat motif	Primer sequence 5'–3'	T_a (°C)	Dye	Size (bp)	k	H_O	H_E	HW_ P	Excl1	Excl2
PP38	DQ389082	(AC) ₈	F: TCTCATAGGAAAGGCTTGT R: AGTGCCAAGGACTTCAACTA	55	IDR-700	154–170	5	0.84	0.693	0.977	0.253	0.417
PP14	DQ389083	(TG) ₈	F: GTGTGTGGCTGTGAGTGAG R: GCTGCCCTAGCTCAGTCTTT	55	IDR-800	202–206	3	0.48	0.538	0.323	0.139	0.267
PP15	DQ389084	(AC) ₂₂	F: TAGCTCTTTTCTGGAGGTGA R: TTTTGTAGATCAAGCAGGAGCAT	55	IDR-700	132–166	13	0.92	0.855	0.932	0.522	0.689
PP01	DQ389085	(TG) ₂₃	F: GCATACAGGAACATCTGACC R: TGTAAAGTCCCTTCCCAAATA	55	IDR-700	82–104	7	0.88	0.762	0.973	0.359	0.542
PP11	DQ389086	(TA) ₇ (TG) ₇ (TA) ₃ (TG) ₃ (TA) ₆	F: TTGACCTGGATAAAGACAG R: CCTAAAGGAGACAGAACCAA	55	IDR-800	174–198	8	0.72	0.764	0.364	0.357	0.538
PP1813*	DQ389087	(CA) ₁₃	F: AGCTCAAGATGTCCATCAGG R: TGAAAGAAAAGCAGAACACGTTG	52	ABI-PET	124–132	5	0.32	0.696	0.005		
PP18117	DQ389088	(AC) ₉	F: TTAGCTCGCTCCTGTTGGAT R: AAACCTGAATGGGCTCCTGA	52	ABI-VIC	140–148	4	0.6	0.641	0.373	0.209	0.366
PP18114	DQ389089	(AC) ₂₂	F: GGCATCAGATCTTCAGCACA R: TAGGGCACAAGACAGGAACC	52	ABI-PET	190–240	10	0.76	0.856	0.146	0.515	0.683
PP18113	DQ389090	(TG) ₁₀	F: AGAGTCTCCCTTCCAGCTCA R: CCACGGGCAATAAAGATTTTT	52	ABI-6-FAM	103–123	6	0.72	0.749	0.4	0.335	0.513
PP18112	DQ389091	(CA) ₁₀	F: AAGTCTGCATCCCATCAAGG R: TGTGTTTCAGGAGCTGCATTC	52	ABI-6-FAM	252–270	7	0.64	0.669	0.436	0.26	0.442
PP18111	DQ389092	(TA) ₈ (CA) ₈	F: TAAGGATGGGCACCAAAGTG R: TTGAGATGCTGGGTGTCCA	52	ABI-NED	112–121	5	0.48	0.439	0.909	0.1	0.25

*Sex-linked locus (avian Z chromosome).

brought to 50 μ L with TE pH 7.4. Two microlitres of eluted DNA were subject to polymerase chain reaction (PCR) amplification and 1 μ L of the PCR product was cloned using the QIAGEN PCR cloning kit. The resulting white colonies were boiled in 50 μ L of H₂O and 1 μ L was subjected to PCR using the M13 primers. PCR products bigger than 500 bp were subsequently sequenced. Optimal PCR primer pairs were designed using the software PRIMER 3 (Rozen & Skaletsky 2000).

The summary statistics (Table 1) for the 11 loci were obtained from 25 adult individuals collected in 1997 in the Upper Susa Valley, Italy. The amplifications were carried out with the use of a 1 \times PCR buffer (Biotools), 1.5 mM of MgCl₂, 5 pmol of each primer (1/10 of the F primer was labelled) (see Table 1), 200 μ M of each dNTPs, 0.5 U of *Taq* polymerase (Biotools) and 20–50 ng of DNA. PCR profile was as follows: 94 °C for 5 min, then 30 cycles of 94 °C for 30 s, T_a for 30 s, 72 °C for 45 s, followed by a final extension of 72 °C for 5 min. Products of amplifications labelled with IDR dyes were run in a LI-COR autosequencer; alleles were scored by eye and the sequence of the plasmid pUC18 was used as a size standard. Products labelled with ABI dyes were run in an ABI 3100 autosequencer using the

GS500-LIZ size standard (Applied Biosystems); alleles were scored using GENEMAPPER version 3.7 (Applied Biosystems). Analyses were performed with CERVUS (Marshall *et al.* 1998). The total number of alleles (k) ranged from 3 to 13 and the mean number of alleles per locus was 6.6 ± 2.9 . Expected heterozygosity (H_E) ranged from 0.439 to 0.856. We tested Hardy–Weinberg equilibrium (HWE) using the randomization test on F_{IS} implemented in FSTAT (Goudet 1995). Only the locus *PP1813* showed significant departure from HWE. The analysis of seven families showed that this locus is sex-linked to the Z chromosome. All the females were homozygote and all females' offspring did not carry the mother allele. No linkage disequilibrium between each pair of loci was detected using FSTAT.

Per-locus exclusion probability, calculated using CERVUS, in both the absence (Excl1) and presence (Excl2) of the genetic information from the other parent ranged from 0.100 to 0.522 and from 0.250 to 0.689, respectively. The high combined exclusionary power (0.978 for the first parent and 0.999 for the second one, given that the first parent is known) of these polymorphic microsatellites suggests that they can be a useful tool for large-scale parentage analysis in rock sparrows.

Acknowledgements

We thank Sari Viinikainen and Paula Sarkkinen for their technical assistance. This research was funded by the University of Padova (Ex-60% grant) and by the MIUR (COFIN 2001 grant) to A.P., and by the Centre of Excellence in Evolutionary Ecology at the University of Jyväskylä to A.G.

References

- Goudet J (1995) FSTAT (version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.
- Griggio M, Matessi G, Pilastro A (2005) Should I stay or should I go? Female brood desertion and male counterstrategy in rock sparrows. *Behavioral Ecology*, **16**, 435–441.
- Griggio M, Tavecchia G, Biddau L, Mingozzi T (2003) Mating strategies in the rock sparrow *Petronia petronia*: the role of female quality. *Ethology, Ecology and Evolution*, **15**, 389–398.
- Kocher TD, Thomas WK, Meyer A *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, USA*, **86**, 6196–6200.
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.
- Pilastro A, Biddau L, Marin G, Mingozzi T (2001) Female brood desertion increases with number of available mates in the rock sparrow. *Journal of Avian Biology*, **32**, 68–72.
- Pilastro A, Griggio M, Biddau L, Mingozzi T (2002) Extrapair paternity as a cost of polygyny in the rock sparrow: behavioural and genetic evidence of the 'trade-off' hypothesis. *Animal Behaviour*, **63**, 967–974.
- Rozen S, Skaletsky HJ (2000) PRIMER 3 on WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Ecology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, New Jersey.
- Tavecchia G, Pradel R, Lebreton JD, Biddau L, Mingozzi T (2002) Sex-biased survival and breeding dispersal probability in a patchy population of the rock sparrow *Petronia petronia*. *Ibis*, **144**, E79–E87.
- Westneat DF, Stewart IRK (2003) Extra-pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 365–396.
- Zane L, Bargelloni L, Patarnello T (2002a) Strategies for microsatellite isolation: a review. *Molecular Ecology*, **11**, 1–16.
- Zane L, Patarnello T, Ludwig A, Fontana F, Congiu L (2002b) Isolation and characterization of microsatellites in the Adriatic sturgeon (*Acipenser naccarii*). *Molecular Ecology Notes*, **2**, 586–588.