Molecular Ecology Notes (2005) **5**, 469–471 doi: 10.1111/j.1471-8286.2005.00947.x

PRIMER NOTE

Development and characterization of seven novel di-, tri-, and tetranucleotide microsatellite markers in Atka mackerel (*Pleurogrammus monopterygius*)

INGRID B. SPIES, SANDRA LOWE, YVONNE HONG and MICHAEL F. CANINO
NOAA Fisheries, Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle, WA 98115

Abstract

We describe the enrichment for and characterization of seven *Pleurogrammus monopterygius* microsatellite loci. We used $(GACA)_4$ as an oligonucleotide enrichment probe and screened 484 clones from the enriched library. The seven microsatellite loci include five tetranucleotide, one dinucleotide, and one trinucleotide motif. These markers can potentially be useful tools for use in population genetics studies, parentage analyses, and examining other life history questions.

Keywords: Atka mackerel, enrichment libraries, enrichment, microsatellites, *Pleurogrammus monopterygius*, population genetics

Received 6 October 2004; revision received 8 December 2004; accepted 20 January 2005

Atka mackerel (*Pleurogrammus monopterygius*) is one of the most abundant groundfish species in the Aleutian Islands of Alaska, where they sustain an important commercial fishery and play a pivotal role in the Aleutian Islands ecosystem as a forage species for piscivorous groundfish, seabirds, and marine mammals. Despite their significance, many aspects of Atka mackerel life history and ecology are poorly understood. In particular, little is known about their population structure, migration patterns, and recruitment dynamics. Here we report on seven novel microsatellite markers isolated from Atka mackerel that will be used for population genetic analyses and interpreted in terms of spatial distribution, degree of genetic connectivity among putative stocks, and hydrographic features influencing patterns of gene flow. This information adds to the current knowledge of Atka mackerel stock structure, which in turn should help improve resource assessments and harvest management strategies.

Microsatellite enrichment for *P. monopterygius* was performed using a variation of the method described in Hamilton *et al*. (1999) in which the restriction of genomic DNA is combined with the ligation of a universal 'SNX' linker in an overnight incubation step. The reaction mixture consisted of 1 mM rATP, 1.95 μM double-stranded SNX linker, 100 μg/mL BSA, 20 U *Hin*cII (NEB, New England Biolabs) 40 U *Xmn*I, 800 U T4 DNA Ligase, (1:10) NEBuffer 2, 250 ng/mL genomic DNA in a total volume of 60 μL. Enzymes and buffers, unless otherwise specified, were purchased from NEB. The thermal profile consisted of 22 cycles (37 °C for 10 min and 16 °C for 30 min), followed by 65 °C for 20 min. The restriction enzyme *Hin*cII was chosen for use in the protocol, because its restriction site (GTYRAC where R = A or G and Y = C or T) cuts genomic DNA to the desired size (200–1000 bp), but is not found within the linkers or in the targeted microsatellite repeat motif. A microsatellite enrichment library was then constructed using the biotin-labelled oligonucleotide probe $(GACA)_4$ at a hybridization temperature of 48 °C. Enriched DNA fragments were amplified directly from the beads via polymerase chain reaction (PCR) and cloned with the TOPO TA Cloning Kit (Invitrogen) according to manufacturer's instructions.

A total of 484 clones from the enrichment library were sequenced. The first 100 were screened for insert size and sequenced from plasmid minipreps (QIAprep Spin Miniprep Kit, QIAGEN) on a LI-COR 4300S DNA analyser, using the ThermoSequenase II cycle sequencing kit (Amersham Biosciences). The remaining 384 clones were PCR amplified using M13 primers, treated with ExoSAP-IT® (USB Corp.) to degrade unincorporated primers and deoxynucleotides, and then sequenced directly using the same sequencing chemistry and sequencing platform. Sequences

Correspondence: Ingrid B. Spies, Fax: 206 526 6723; E-mail: ingrid.spies@noaa.gov

Table 1 Characteristics of Atka mackerel microsatellite loci, including GenBank Accession no., repeat motif, primer sequences, range of observed alleles (*R*), number of alleles (*A*), observed and expected heterozygosities (H_E and H_O), and probability (*P*) values for exact tests of Hardy–Weinberg equilibrium

Locus	GenBank Accession no.	Repeat motif	Primer sequences (5′–3′)	*R*	*A*	H_E/H_O	*P*
Pmo69	AY677369	$(GT)_{11}$	F: CCCGAGGCATTAACTGTCAT R: TCTGAGACCAGAGCCGAGTT	174–214	16	0.796/0.809	0.791
Pmo70	AY677368	$(GTCT)_{14}$	F: TCTGAACTTTGCATCAACTCG R: AATGATCCAGCAGAGGGTTG	128–278	21	0.894/0.921	0.305
Pmo152	AY677367	$(CAGA)_{11}$	F: AGTTTCGGGACTGCATCTTG R: AAGAAATAACCAGCCGCATC	114–362	36	0.945/0.893	0.319
Pmo164	AY677371	$(CTGT)_{10}$	F: TGATGAAGCTTTAGTTCGTCTCA R: GAGCAGAGTGTTTGGGTTCC	188–244	14	0.823/0.902	0.041
Pmo268	AY677372	$(CCT)_6$	F: GCTGGGCTGGACATGTTTAT R: TGCTCCTTCATCTCCTCCAC	152–176	3	0.268/0.296	0.687
Pmo367	AY677373	$(GTCT)_8$	F: TATGCCTCTGCAGCACAAAC R: AATTCCCTCGAAGCGTTCTT	134–358	47	0.961/0.934	0.092
Pmo399	AY677374	$(CTGT)_{15}$	F: GGAGTTCCTCCGGACCTCTA R: AAAACCCCGATCACACAGAG	149–185	10	0.781/0.862	0.086

were analysed using E-SEQ software (LI-COR Biosciences). Aligned sequence contigs were constructed using SEQUENCHER software version 4.0.5 (Gene Codes). Approximately 40% of the clones sequenced contained microsatellites, a quarter of which contained sufficient flanking sequence for designing oligonucleotide primers for amplification via PCR. Primers were designed using the PRIMER 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), then screened on a sample of 96 Atka mackerel collected off Akun Island, Alaska in 2004.

Genomic DNA was extracted using the QIAGEN DNeasy Tissue Extraction Kit according to the manufacturer's protocol. All loci were amplified using a nested PCR method (Schuelke 2000). Instead of a 5′ dye-labelled primer, an M13F(−21) sequence is added to the 5′ end of each forward primer and, as a result, DNA fragments produced have the M13(−21) sequence. To produce labelled DNA fragments, labelled M13F(−21) was added to the reaction.

PCR amplifications were conducted in 10-μL volumes containing *c.* 100 ng template DNA, 10 mM Tris-HCl (pH 8.3), 50 μM KCl, 2.5 mM $MgCl_2$, 1.5 mM dNTPs, 5 pm of each primer (F primer 5′ end M13 tag), 0.5 pmol of fluorescently labelled M13 primer [either IRD700 or IRD800 (LI-COR], and 0.5 U Promega *Taq* polymerase. A 'touchdown' thermal cycle was performed in an MJ Research PTC-100 thermal cycler. An initial denaturation step at 95 °C (2 min) was followed by 5 cycles of 95 °C (1 min), 60 °C [1 min (−1°/cycle)], 72 °C (1 min), and then by 20 cycles of 95 °C (30 s) 55 °C (30 s) and 72 °C (30 s) for all loci. Automated genotyping was performed on the LI-COR 4300S and analysed using LI-COR SAGAGT software.

Seven loci were tested for conformance to expected Hardy–Weinberg equilibrium frequencies using exact tests implemented in GENEPOP (Raymond & Rousset 1995) to assess their potential as population genetic markers. Linkage disequilibrium and heterozygosities were calculated using GENETIX version 4.03 (Belkhir *et al*. 2000).

The loci exhibit a wide range of polymorphism (expected heterozygosity 0.268–0.961), and did not depart significantly from Hardy–Weinberg expectations in the screened sample after Bonferroni correction was performed (adjusted *P* value = 0.00714; Table 1). Two of 21 tests for linkage disequilibrium (*Pmo70* vs. *Pmo164* and *Pmo70* vs. *Pmo399*) were significant at the tablewide α level (*P* = 0.05) corrected for multiple tests using sequential Bonferroni correction (Rice 1989), a result not much greater than could have occurred by chance alone (0.05 ∗ 21 tests = 1.05). Subsequent tests for disequilibrium between these locus pairs were not significant after screening 192 additional samples (data not given). We anticipate that these loci will provide unbiased and robust estimation of population differentiation in Atka mackerel and may also be suitable as population markers in other hexagrammid species.

Acknowledgements

The authors gratefully acknowledge the Resource Assessment and Conservation Engineering Division at the Alaska Fisheries Science Center (AFSC) for sample collection. The Resource Ecology and Fisheries Management group at the AFSC funded this work. This manuscript represents contribution FOCI-0511 to NOAA's Fisheries-Oceanography Coordinated Investigations.

References

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2000) *GENETIX 4.02, logiciel sous Windows pour la genetique des populations*.

Montpellier (France), Laboratoire Genome, Populations, Interactions: CNRS UPR 9060, Universite de Montpellier, II.

Hamilton MB, Pincus EL, DiFiore A, Fleischer RC (1999) Microsatellite-enhanced genomic library construction streptavidin-biotin bench version SNX linker. *BioTechniques*, **27**, 500–507.

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

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, **18**, 233–234.