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PRIMER NOTE

Isolation and characterization of microsatellite loci from the Arctic cisco (*Coregonus autumnalis*)

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Abstract

Eight polymorphic microsatellite loci were isolated and characterized for the Arctic cisco, *Coregonus autumnalis*. Loci were evaluated in 21 samples from the Colville River subsistence fishery. The number of alleles per locus ranged from two to 18. Observed heterozygosity of loci varied from 0.10 to 1.00, and expected heterozygosity ranged from 0.09 to 0.92. All eight microsatellite markers were in Hardy–Weinberg equilibrium. The loci presented here will be useful in describing population structure and exploring populations of origin for Arctic cisco.

Keywords: Arctic, *Coregonus autumnalis*, microsatellite, source population, subsistence

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The Arctic cisco, *Coregonus autumnalis*, is an important commercial and subsistence resource occurring along the North coast of Alaska (Gallaway *et al*. 1983; Moulton *et al*. 1991). Previous genetic investigations using DNA restriction analysis and allozymes have suggested that Arctic cisco caught in Alaskan waters originate primarily from spawning populations in the Mackenzie River system (Bickham *et al*. 1989; Morales *et al*. 1993). Results using these and other techniques including flow cytometry (Lockwood & Bickham 1991; Lockwood & Bickham 1992) have been unable to assign individuals to natal tributaries. In this study, we present eight microsatellite loci developed in *C. autumnalis* that may be useful in identifying putative source populations.

Microsatellite loci were developed using a partial genomic library derived from *C. autumnalis* based on the procedure reported in Kandpal *et al*. (1994). Genomic DNA was extracted from fin and muscle tissue using the DNeasy Tissue kit (QIAGEN), digested with the *Sau*3A1 restriction enzyme (New England Biolabs), and size fractioned in a 1.2% agarose gel. Fragments ranging from 400 to 1500 bp were purified and ligated to *Sau*3A linkers. Linker-ligated products were amplified using a single oligonucleotide primer and probed with $(CA)_{13}$ $(GA)_{13}$ and $(GATA)_{10}$ biotinylated repeats (Operon Technologies). Hybridized fragments were captured and retained on a Vectrex Avidin D matrix (Vector Laboratories). Enriched fragments were eluted from the matrix, ligated into the lacZα gene of the pCR 2.1 cloning vector, and transformed into One Shot INVaF′ chemically competent *Escherichia coli* cells (Invitrogen). Transformed cells were grown on Luria-Bertani (LB) agar containing ampicillin (100 mg/mL) and X-Gal (20 mg/mL). A total of 192 positive *E. coli* colonies were individually subcultured in LB–ampicillin broth and cloning vectors were isolated using the Wizard Plus Miniprep DNA Purification System (Promega). Isolated cloning vectors were bidirectionally sequenced using a SequiTherm EXCEL II DNA Sequencing Kit (Epicentre Biotechnologies). Sequences were visualized on a LI-COR Model IR2 automated fluorescent DNA sequencer, aligned using ALIGNIR software (LI-COR), and assessed for microsatellite repeats. Forty-three clones were identified as containing microsatellite repeat regions. Nineteen clones were not pursued because of the small size of the repeat region or the close proximity of repeat regions to the cloning site, thus providing inadequate data for polymerase chain reaction (PCR)-primer design. Primer sets were synthesized for 24 remaining prospective microsatellite loci and assessed for polymorphism.

Microsatellite loci were amplified using the PCR in 10-μL volumes containing 21–50 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.5–1.0 μM unlabelled primers, 1.0–1.5 μM labelled tail primer, 2 mM dNTP and 0.3–0.5 U of Promega *Taq* DNA polymerase. The PCRs were carried out in MJ Research (Bio-Rad) or MWG thermocyclers (MWG Biotech Inc.) with an initial denaturation time of 2 min at 94 °C followed

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Table 1 Characterization of eight microsatellite loci from *Coregonus autumnalis*

Locus	Cloned repeat motif	Primer sequence (5′–3′)	T_a (°C)	N	N_A	Allele size range (bp)	H_O	H_E	P value (HWE)	GenBank Accession no.
Aut062	$(GA)_5AA(GA)_6$	F: GGGGGAGGAATGTATGTG R: AGCGTCTAGGGGCAAC	50	21	3	128–138	0.76	0.57	0.23	EF192593
Aut066	$(CA)_6(TA)_3(CA)_{55}$	F: TGTACAGTGCCTCCAG R: GACTGTTGGATTCTGAC	50	21	15	120–186	1.00	0.90	0.82	EF192597
Aut122	$(CA)_{32\ \text{imperfect}}$	F: GGCTCAGGACAAGGTA R: AGTGCAGGGTTGTATG	50	21	11	372–420	0.95	0.87	0.29	EF433767
Aut128	$(CA)_7(GA)_2(CA)_2$	F: TGAGCCCTGACAGAA R: GTTGTTATGTGAGCCC	50	21	2	105–107	0.10	0.09	1.00	EF433768
Aut135	$(GA)_2TA(CA)_5$	F: CGACCCTGCAGACAGAAA R: CAGTCTAGCAAGCGAGGACC	50	21	2	148–150	0.33	0.28	1.00	EF192594
Aut139	$(CA)_{41}$	F: GGGTAAAGCAGAATGAC R: GGGAGTGTGTTTGTCT	50	21	14	131–213	1.00	0.89	0.40	EF192595
Aut151	$(CA)_{27\ \text{imperfect}}$	F: GACCAGTTGTTTATGAAGC R: GTAGCATAGGGGGTTTTA	50	21	13	96–166	1.00	0.88	0.83	EF192596
Aut167	$(GT)_{21}CT(GT)_{10}$	F: GTCACTGTCATTATGGG R: GTTGTCAGGAAGGTAGG	50	21	18	236–418	1.00	0.92	0.60	EF433769

T_a, annealing temperature; N, number of samples; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; P value, significance value from Hardy–Weinberg equilibrium exact test.

by 40 cycles of 94 °C for 15 s, 50 °C for 15 s, 72 °C for 30 s and a final 30-min elongation step at 72 °C. PCR products were separated on 6% polyacrylamide gels using a LI-COR Model IR2 automated fluorescent DNA sequencer. Forward primers were synthesized with universal tails on the 5′ ends and visualized by adding fluorescently labelled primers complementary to the universal tail sequence to the PCR (Oetting *et al*. 1995). Allele sizes for specific samples at each locus were determined relative to the M13 phage single nucleotide ladder. These samples were later used as internal size standards in subsequent gels. GENE IMAGIR version 4.05 (Scanalytics) was used to assign allele scores.

Seventeen of 24 primer sets produced unambiguous PCR products of expected size. Amplification conditions were optimized for eight polymorphic microsatellite markers and amplified in genomic DNA from 21 *C. autumnalis* samples collected from the Colville River subsistence fishery. Observed and expected heterozygosities per locus were calculated using ARLEQUIN version 3.01 (Excoffier *et al*. 2005). Probability tests for Hardy–Weinberg equilibrium and genotypic linkage disequilibrium were made using GENEPOP version 3.4 (Raymond & Rousset 1995). The number of alleles at each locus ranged from two to 18 (Table 1). Locus-specific observed heterozygosity varied from 0.10 to 1.00, and expected heterozygosity ranged from 0.09 to 0.92. All eight loci conformed to Hardy–Weinberg equilibrium. Linkage disequilibrium was detected in one pair of loci (Aut062 and Aut135) after Bonferroni correction ($P < 0.0018$). These loci were not linked in subsequent analysis with a larger sample population ($n = 86$; unpublished data). Isolation and characterization of these polymorphic microsatellite markers from *C. autumnalis* will be useful in describing population structure and exploring populations of origin for Arctic cisco.

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