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Characterization and inheritance of seven microsatellite loci from Dolly Varden, *Salvelinus malma*, and cross-species amplification in Arctic char, *S. alpinus*

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Dolly Varden (*Salvelinus malma*) is a dominant freshwater species in arctic and subarctic eastern Russia, Alaska, and Western Canada and an important food source for indigenous people in these regions (Jarvela and Thorsteinson 1997). Dolly Varden exhibit a diverse array of life history types, including anadromous, residual, and resident forms. Anadromous Dolly Varden show highly complex migration patterns. They typically feed in marine waters in the summer, home to spawn in their natal streams, and overwinter in natal and non-natal freshwater lakes and rivers in mixed aggregates (Armstrong and Morrow 1980). We developed seven microsatellite loci to estimate population structure and identify the origin of Dolly Varden sampled from overwintering areas as a method to document their movement patterns. We also tested the loci for amplification in the closely related Arctic char (*S. alpinus*). Arctic char often occur in multiple morphological, ecological, and behavioral forms within a single watershed (Johnson 1980) and are a model species for evaluating the mechanisms of sympatric speciation (Hindar et al. 1986; Volpe and Ferguson 1996). Increasing the number of microsatellite loci that amplify in Arctic increases the power to detect reproductive isolation among sympatric forms (Gislason et al. 1999).

Genetic Identification Services, Inc. (GIS; Chatsworth, California, USA) constructed di-, tri-, and tetranucleotide microsatellite libraries using genomic DNA isolated from a single Dolly Varden following the methods described by Peacock et al. (2002), with two exceptions. Magnetic bead capture technology was used to enrich libraries for CA, ATG, CATC, and TAGA repeat motifs, and clones were prepared by ligating capture fragments into the HindIII site of the pUC19 vector and electroporated into *Escherichia coli* DH5α. Clones (N = 112) were sequenced for the presence of usable microsatellites and primer sequence for 34 potential loci were designed using Designer PCR™ version 1.03 (Research Genetics, Huntsville, Alabama). Seven primer pairs yielded products in the expected size range with little or no stutter (Table 1).

Dolly Varden from 16 localities in western Alaska (N = 2615) distributed from the Beaufort Sea to Bristol Bay and Arctic char from four localities in western and southcentral Alaska and one locality in Ireland (N = 49) were used to evaluate locus polymorphism. Total genomic

Table 1. Locus name, forward and reverse primer sequences, repeat motif, GenBank Accession number, annealing temperature (°C), sample size (N), number of alleles per locus (A), range in allele size, and observed and expected heterozygosity for microsatellite loci isolated from Dolly Varden screened in Dolly Varden and Arctic char

Locus	Primer sequence (5′–3′)	Repeat motif	GenBank no.	°C	Dolly Varden					Arctic char				
					N	A	Size	H_o	H_e	N	A	Size	H_o	H_e
Smm-3	F: Tgg CTC AAA TTA AgA TCC TAC R:AgC CAT TAT gCA TTA CTT gTT C	$(CA)_3Cg(CA)_{11}$	AY327124	58	2490	4	120–126	0.461	0.477	32	3	110–128	0.133	0.158
Smm-5	F: AgA TgT gTg ATA AAC TCA gCC TC R: AgT TgT TTA AAT Agg gCg gAT Ag	$(CA)_7C(CA)_7$	AY327125	55	2509	2	90–92	0.001	0.001	45	1	90	0.000	0.000
Smm-10	F: AAA ATg TCT CCC CTC CCT CTC R: TCC CTA ACA TAA CAA gTT TTC ATC CT	$(TCCA)_{16}$	AY327126	55	2506	28	140–252	0.817[a]	0.849	43	8	140–172	0.653	0.530
Smm-17	F: AAg gAT ggT gAg gAC AAT ACA R: ACC TTg AgA AAT CTA TAT gTg gTC TA	$(CA)_{29}$	AY327127	56	2450	21	81–141	0.745	0.761	45	12	101–129	0.693	0.628
Smm-21	F: ggC TgT TCA CCA CAT AgA gTA AT R: TTA AgA Tgg gAT gCA TAT TCA gT	$(TC)_4TTTC(T\text{-}C)_{21}$	AY327128	56	2460	20	105–153	0.732	0.725	48	2	103–105	0.160	0.133
Smm-22	F: CCC AAT gCA gAT AAg ACC TT R: TCT ATA ggC TTA TTT gAA Tgg AAT	$(TAgA)_{19}$	AY327129	55	2497	28	148–264	0.907	0.908	45	23	160–260	0.986	0.893
Smm-24	F: CAT TgA TCA AgA AgC CAg TgC R: TgT ATT Tgg CCA ATA TAA CAC AgC	$(TATC)_{33}$	AY327130	56	2464	44	154–338	0.902[b]	0.945	28	14	178–242	0.856	0.804

[a] $F_{IS} > 0$ for one of 16 collections of Dolly Varden (Arctic char were not tested).
[b] $F_{IS} > 0$ for four of 16 collections of Dolly Varden.

Table 2. Inheritance tests for six microsatellite loci in four families of Dolly Varden. (a) Mutations observed in two tetranucleotide-repeat loci: bold alleles were not observed in parents and underlined alleles are parental alleles closest in size, (b) *P*-values for Chi-square tests of single-locus segregation (asterisk indicates significant value after Bonferroni correction), and (c) LOD scores for linkage and recombination rates (*r*) for *Smm-17* and *Smm-24*

Locus	Family	Dam	Sire	Progeny genotypes	Genotypes not matching parents (number of progeny with genotype in parentheses)
(a)					
Smm-24	B	226/246	194/254	194/226	194/**238** (1)
				194/246	
				226/254	
				246/254	
	C	226/278	238/238	238/262	**242**/278 (1)
				238/278	
	D	250/274	230/270	230/250	230/**254** (1)
				230/274	**234/**250 (1)
				250/270	
				270/274	
Smm-22	C	192/192	200/216	192/200	**188/**200 (1)
				192/216	
	D	184/212	196/224	184/196	**188/**196 (1)
				184/224	
				196/212	
				212/224	

(b)

	Family			
Locus	A	B	C	D
Smm-3	0.3544	0.0889	0.4615	1.0000
Smm-10	0.1364	0.0045*	0.4187	0.6469
Smm-17	0.2157	0.0836	0.0135	0.0828
Smm-21	0.9270	0.1441	0.4054	0.3942
Smm-22	0.4048	0.6974	0.4054	0.2693
Smm-24	0.1421	0.3916	0.6381	0.4463

(c)

	Parental genotype		Progeny counts for gametic class						
		Smm-17	*Smm-24*						
Family	Parent	Aa	Bb or BB	AB	Ab	aB	ab	LOD	*r*
A	Dam	121/127	226/246	26	42	19	28	0.09	0.47
	Sire	125/129	194/222	56	3	54	2	25.7	0.04
B	Dam	121/127	226/246	18	28	27	29	0.14	0.46
	Sire	125/127	194/254	47	0	0	55	30.7	0
C	Dam	127/129	262/278	29	34	25	24	0.07	0.47
	Sire	121/127	238/238	70		42		–	–
D	Dam	125/127	250/274	25	44	20	25	0.37	0.44
	Sire	127/129	230/270	0	59	55	0	34.3	0

DNA was isolated from fin tissue using a quick lysis procedure. PCR amplification of microsatellite loci was carried out in 10 μl reaction volumes: approximately 100 ng DNA, 1.5 mM $MgCl_2$, 8 mM dNTPs, 0.5 U Taq DNA polymerase (Promega), 0.4 μM unlabeled/labeled forward primer, and 0.4 μM reverse primer, using an MJResearch™ DNA Engine™ PCT-200. Cycling conditions were 2 min at 92° C; 30 cycles of 15 s at 92° C, 15 s at T_a (see Table 1), and 30 s at 72° C; with a final extension for 10 min at 72° C. Microsatellites were separated and visualized on 64-well denaturing polyacrylamide gels using a Li-CorIR$^{2®}$scanner and scored with Li-Cor Saga™ GT ver. 2.0 software (Lincoln, NE). Li-Cor 50–350 or 50–500 bp size standards were loaded in the first and last lanes at intervals of 14 lanes or less across each gel. Positive controls, consisting of 2–10 alleles of predetermined size, were loaded in three lanes distributed evenly across the gels to ensure consistency of allele scores. Two researchers scored alleles independently. Samples with score discrepancies between researchers were re-amplified at the loci in question and rescored. Observed and expected heterozygosity were calculated using FSTAT version 2.9.3 (Goudet 2001). Deviation of genotypic frequencies from Hardy-Weinberg expectation for Dolly Varden samples was evaluated by testing for a deficit of heterozygotes, $F_{IS} > 0$, in FSTAT version 2.9.3 (Goudet 2001). Significance for each locus was evaluated by adjusting the table-wide $\alpha = 0.05$ for 16 multiple tests using the sequential Bonferroni technique (Rice 1989).

Six of the seven loci were polymorphic in both species (Table 1). The average number of alleles per locus was 21 (range = 2–44) for Dolly Varden and 9 (range = 1–23) for Arctic char. The average observed heterozygosity was 0.66 (range = 0.001–0.91) for Dolly Varden and 0.50 (range = 0–0.99) for Arctic char. Significant deviations of genotypic frequencies from Hardy–Weinberg expectation were detected for *Smm-10* and *Smm-24* for Dolly Varden samples.

Mendelian inheritance of polymorphic loci was tested using 120 progeny from each of four controlled matings of Dolly Varden; two were half-sibling families with the same dam. Mutations were observed at two loci: two alleles for *Smm-22* and four alleles for *Smm-24* were observed in progeny that were not observed in the parents. Correct parentage of these individuals was verified by the other five loci. All mutant alleles were observed in the parent population (USFWS, unpublished data); further, *Smm-22*188* descended from different alleles (Table 2a) indicating a potentially high degree of homoplasy at these loci (Blankenship et al. 2002, Steinberg et al. 2002). Mutation rates were calculated following Steinberg et al. (2002), and were 2.1×10^{-3} for *Smm-22* and 4.4×10^{-3} for *Smm-24*. Random assortment within loci was tested using a Chi-square test. After applying a Bonferroni correction for four simultaneous tests for each locus (Rice 1989), one significant deviation from expected Mendelian ratios was observed (Table 2b). We tested for linkage and calculated recombination rates for each sex using LINKMFEX (R. G. Danzmann, www.uoguelph.ca/˜rdanzman/software/LINKMFEX). A linkage relationship was detected between *Smm-17* and *Smm-24* using the sire as the mapping parent; LOD scores in three families with heterozygous sires ranged from 25.7 to 34.3 (Table 2c). Recombination rates for males were near zero and for females were greater than 40% (Table 2c). Higher recombination rates in females are consistent with findings from other linkage studies for salmonids (e.g., Allendorf and Thorgaard 1984; Johnson et al. 1987; Sakamoto et al. 2000) and suggest that these loci map close to the centromere (Sakamoto et al. 2000).

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