Molecular Ecology Notes (2002) **2**, 17–19

PRIMER NOTE

Characterization of microsatellite loci in chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids

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Abstract

Previous studies of population genetic structure of fall-run chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley have either not focused on or have been unable to resolve intertributary differences within the San Joaquin River basin. The authors describe the isolation, the polymerase chain reaction conditions, and characterize the cross-species amplification of 17 microsatellite loci in six species of salmonids. Fourteen of these loci are polymorphic in fall-run chinook from the San Joaquin River drainage. These results indicate the potential utility of microsatellite markers developed for one species, for both congenerics and species within a closely related genus.

Keywords: chinook, microsatellite, *Oncorhynchus tshawytscha*, primers

Received 5 July 2001; revision accepted 15 August 2001

Previous studies have sought to assess the level of genetic variation of chinook salmon within California's Central Valley. Early allozyme studies (Bartley & Gall 1990; Gall *et al*. 1992) demonstrated an association between genetic similarity and major river drainages. Nielsen *et al*. (1994) tested whether mitochondrial DNA (mtDNA) could be used to characterize the four temporal spawning runs of chinook. Banks *et al*. (1999, 2000) showed that microsatellites allowed the distinction of endangered stocks from less threatened conspecific stocks. However, these studies have either not focused on or have been unable to provide within-basin resolution of populations in the San Joaquin River system. In addition, allozyme analyses by Myers *et al*. (1998) yielded equivocal results regarding population structure of chinook within the San Joaquin River basin. The molecular markers reported here will be used to examine the population genetic structure of fall-run chinook salmon within and between streams of the San Joaquin River basin of California's Central Valley. While some microsatellite loci have already been developed for chinook salmon (Banks *et al*. 1999; Nelson & Beacham 1999), the isolation and characterization of additional loci should increase the power for population discrimination on the geographical scale of a single river basin.

A TAGA tetranucleotide repeat enriched genomic DNA library was constructed (Jones *et al*. 2000) by Genetic Identification Services Inc. (Chatsworth, California, USA) using genomic DNA extracted from muscle tissue of *Oncorhynchus tshawytscha* by standard methods (Sambrook *et al*. 1989). In brief, the method of Jones *et al*. (2000) is as follows: DNA was restricted with seven blunt end cutting enzymes, and the 300–750 bp fragments (resolved by gel electrophoresis) were excised, purified, ligated to adaptors, then exposed to magnetic bead capture to enrich for TAGA repeat containing fragments. Captured molecules were amplified and then restricted with *Hin*dIII to remove the adaptors. The resulting fragments were ligated into pUC19/*Hin*dIII.

Approximately 490 clones were sequenced (BigDye Dye Terminator chemistry, Perkin Elmer/ABI) and examined for tandem repetitive sequences (microsatellites). Duplicate sequences were eliminated and 69 primer pairs were designed using Primer Select (DNAStar, Inc.) (Table 1). Seventeen microsatellite loci were tested on five to nine individuals each of six species of salmonids, Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), Paiute cutthroat trout (*O. clarki*), and steelhead (*O. mykiss*)

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Table 1 Repeat motif, PCR primer sequences, optimal annealing temperature (T_a), $MgCl_2$ concentration for amplification and preliminary population characteristics (based on nine individuals) for 17 polymorphic chinook salmon microsatellite loci

Locus	Repeat motif of original clone*	Primer sequence (5′–3′)†	Allele size range (bp)	No. of alleles	Heterozygosity‡			Accession no.
					H_O	H_E	EMBL H–W	
OtsG3	$(GAAT)_8$-GATAGATTAATA-$(GATA)_{11}$-GATTAATAGAGA-$(GATA)_{26}$	F: GGACAGGAGCGTCTGCTAAATGACTG R: GGATGGATTGATGAATGGGTGGG	146–246	5	0.33	0.37	ns	AF393184
OtsG13	$(GATA)(TAGA)_{20}(TGGATAGA)_4$-$(TGGA)_2(TAGA)_{10}$	F: GGTTCCTCTCACATAGAA R: GCCTAGTTAAATAAAGGTAAA	292–436	11	0.66	0.95	***	AF393185
OtsG43	$(GACA)_{13}(GATAGACA)_2(GATA)_{25}$	F: AACTCCCGTTGACAATTTACTGTTG R: TTTTGGCAAAGTTGGCTACTCTG	ML§	—	—	—	—	AF393186
OtsG68	$(GATA)_{30}(TAGA)_1$	F: TATGAACTGCAGCTTGTTATGTTAGT R: CATGTCGGCTGCTCAATGTA	184–296	12	0.88	0.97	ns	AF393187
OtsG78b	$TAGA(TATA)_2$-N_{12}-$(TAGA)_{31}$	F: GTCCCTTGAATTGAATTGATTAGA R: CAGCCTACTGCAGTTCAATAGACT	216–356	13	0.88	0.95	ns	AF393188
OtsG83b	$(TGTC)_7$-N_{51}-$(TATC)_{34}$	F: TAGCCCTGCACTAAAATACAGTTC R: CATTAATCTAGGCTTGTCAGCAGT	155–303	15	1.0	0.98	ns	AF393189
OtsG85	$(GATA)_{19}$	F: CCATGTCAGCACTGACTTAAT R: GGATGTTGTTCCTAATGTTTT	ML§	—	—	—	—	AF393190
OtsG243	$(TAGA)_{63}(CAGA)_{12}(GACA)_7(GA)_{22}$	F: TTATTAAACTGCACTGTCTAACTACA R: GTATGCAGCAAGCCAGGTG	190–466	12	1.0	0.96	ns	AF393191
OtsG249	$(TAGA)_{19}$	F: TTCTCAGAGGGTAAAATCTCAGTAAG R: GTACAACCCCTCTCACCTACCC	192–310	13	1.0	0.95	ns	AF393192
OtsG249b	$(TAGA)_{19}$	F: ATGGCAGTTAAGAGAACAAAAGTT R: GTACAACCCCTCTCACCTACCC	—	—	—	—	—	AF393192
OtsG253	$(GACA)_{10}$ $(GATA)_{14}$	F: GAGCAGGCCGAGCAGGTGTCT R: GGAGCATTCCAATCAAGCCACTG	—	—	—	—	—	AF393193
OtsG253b	$(GACA)_{10}$ $(GATA)_{14}$	F: GAGCAGGCCGAGCAGGTGTCT R: AATTGGGTCATTAAGGCTCTGTGG	141–301	12	1.0	0.96	ns	AF393193
Ots311	$(GATA)_{30}$-GACA-$(GATA)_2$-$(GAGTGATA)_7$-GATA	F: TGCGGTGCTCAAAGTGATCTCAGTCA R: TCCATCCCTCCCCCATCCATTGT	278–374	12	0.88	0.95	ns	AF393194
OtsG401	$(GATA)_{12}$	F: CTGCCCTGAGAAGCTGGAGTGCTC R: TTGCCCCACCCTTGCATCTATCCA	Mono-morphic	—	—	—	—	AF393195
OtsG409	$(GA)_9(TAGA)_6$-GGTA-$(GATA)_{16}$	F: GTAGCCATTTGTGTCACCATCATT R: CATTCTCCTGCCTCACAGAGTTTA	116–282	10	0.77	0.91	ns	AF393196
OtsG422	$(GATA)_{24}$	F: GCTTGCTCGCTCAATCTTCTTATT R: GAGGCAATGAGGGAGGATGGTGAG	264–414	15	1.0	0.97	ns	AF393197
OtsG423	$(GATA)_{41}$	F: AGGCCTGCCAGGCACTAAAGGTAT R: GCAAGCAAACATGTAGCTTCATGG	ML§	—	—	—	—	AF393198
OtsG432	$(GATA)_3$-GGAT-$(GATA)_8$	F: TGAAAAGTAGGGGAAACACATACG R: TAAAGCCCATTGAATTGAATAGAA	122–202	12	0.88	0.95	ns	AF393199
OtsG474	$(GATA)_6$	F: TTAGCTTTGGACATTTTATCACAC R: CCAGAGCAGGGACCAGAAC	155–191	6	0.66	0.75	ns	AF393200

*N = any nucleotide.

†F and R are forward and reverse primers, respectively.

‡H_O, observed heterozygosity; H_E, expected heterozygosity (Nei's unbiased); H–W, test for conformance to Hardy–Weinberg equilibrium (ns = not significant, $P > 0.05$; ***$P = 0.030$); pseudo-exact tests performed using GENEPOP v3.1 population genetics software (Raymond & Rousset 1995).

§two loci detected — presumed duplicate pair reflecting the tetraploid origin of the salmonid genome (Ohno 1970).

PCR conditions for all primer pairs are 52 °C annealing temperature and 2.0 mM $MgCl_2$.

(Table 2). The forward primer of each pair was 5′ labelled with FAM, HEX, or TET dyes (Perkin Elmer/ABI) to facilitate visualization of DNA bands. Polymerase Chain Reaction (PCR) products were sized with internal lane standards (ROX, Perkin Elmer/ABI) on an MJ Research BaseStation. PCR reactions were carried out using 40 ng genomic DNA, 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 μM of each PCR primer, and 0.25 Units of *Taq* DNA Polymerase

Table 2 PCR product size range and observed number of alleles (in parentheses) for cross species comparison of 17 microsatellite loci. An X means that no amplicon was detected. A ML means multiple loci amplified

Locus	Atlantic	Brook	Chinook	Coho	Cutthroat	Steelhead
OtsG3	X	121	137–141 (2)	137	151	146–246 (5)
OtsG13	X	X	292–436 (10)	X	316	ML
OtsG43	X	ML	ML	ML	ML	149–169 (5)
OtsG68	174–250 (4)	131–135 (2)	184–294 (12)	232–348 (5)	151–155 (2)	X
OtsG78b	X	X	217–357 (12)	148–186 (6)	X	X
OtsG83b	ML	71	154–302 (15)	110–310 (8)	109	125–157 (5)
OtsG85	X	124–180 (5)	ML	X	280	131–243 (5)
OtsG243	94	186	190–470 (13)	110	110	110–130 (4)
OtsG249	ML	108	192–312 (13)	108–144 (3)	131	
OtsG249b						123–187 (6)
OtsG253						161–193 (4)
OtsG253b	ML	108–184 (5)	143–301 (12)	151–291 (5)	111–178 (2)	
OtsG311	ML	X	279–374 (12)	ML	109	X
OtsG401	173	209	164	X	175	168–208 (4)
OtsG409	86	84	188–356 (13)	102–108 (2)	82–94 (2)	ML
OtsG422	162	142	264–414 (15)	256–406 (10)	213–221 (2)	ML
OtsG423	X	94–166 (7)	ML	ML	70	88–124 (8)
OtsG432	191	127–434 (7)	124–202 (11)	444	111–241 (4)	ML
OtsG474	151–177 (2)	X	154–190 (6)	155	155	155

all in 20 mM Tris, pH 8.5 and 50 mM KCl in 10 μL volumes. Amplifications were performed in MJ Research PTC100 thermal cyclers under the following conditions: one denaturation cycle at 94 °C for 90 s, 35 amplification cycles of 95 °C for 60 s, 52 °C for 30 s, 72 °C for 60 s, and an extension cycle of 72 °C for 30 s.

Heterozygosities were determined for each of the loci in fall-run chinook salmon (Table 1), but not for the cross-species tests (Table 2). Not all loci could be amplified in all species under the given set of PCR conditions, and the degree of polymorphism at each locus differed between the species examined (Table 2). All but four (OtsG43, 85, 401, and 423) of the 17 loci described here may be used to investigate population structure of fall-run chinook salmon. These four loci are either monomorphic (OtsG401) or amplify multiple loci (OtsG43, 85, 423) in chinook salmon. The primer sets OtsG249b and OtsG253b are redesigns of the original primer sets OtsG249 and OtsG253, respectively. The redesigned primer sets allow better amplification in steelhead (OtsG249b) and the other five salmonids tested (OtsG253b) (Table 2). These results indicate the potential utility of microsatellite markers developed for one species, for both congenerics and species within a closely related genus.

Acknowledgements

We thank J. Navicky and associates of the California Department of Fish and Game Rancho Cordova Salmonid Tissue Archive for making samples available. Funding for this project has come from California Department of Fish and Game project P9940011 through CALFED.

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