

A Distinctive Microsatellite Locus That Differentiates Ocean-Type from Stream-Type Chinook Salmon in the Interior Columbia River Basin

SHAWN R. NARUM*

Columbia River Inter-Tribal Fish Commission,
3059F National Fish Hatchery Road, Hagerman, Idaho 83301, USA

MADISON S. POWELL

University of Idaho, Center for Freshwater and Salmonid Species at Risk,
3059F National Fish Hatchery Road, Hagerman, Idaho 83301, USA

ANDRÉ J. TALBOT

Columbia River Inter-Tribal Fish Commission,
729 NE Oregon Street, Suite 200, Portland, Oregon 97232, USA

Abstract.—Chinook salmon *Oncorhynchus tshawytscha* display two life history strategies that are referred to as ocean type and stream type. Ocean-type Chinook salmon typically differ from stream-type fish in juvenile migration timing, adult spawning location, and run timing. Spatial and temporal separation during spawning can lead to reproductive isolation and genetic divergence between the two life history strategies. We identified a distinctive microsatellite locus, *OtsG474*, capable of distinguishing ocean-type from stream-type Chinook salmon in 93% of the samples collected from various rivers within the interior Columbia River basin (east of the Cascade Mountains). Allele frequencies at *OtsG474* revealed major differences in the dominant allele as well as in the number of alleles detected in each type. This distinctive marker may be highly useful as part of a suite of microsatellite loci, allowing managers to detect the two types of Chinook salmon in the Columbia River basin. Further research is necessary to determine the ability of this locus to discriminate Chinook salmon types in the lower Columbia River and other river basins coastwide.

The Chinook salmon *Oncorhynchus tshawytscha* is a diverse species with alternative life history characteristics. Differences in the timing of adult spawning migration and utilization of freshwater habitat, along with distinctive juvenile morphology and behavior, have produced two races of Chinook salmon (ocean type and stream type) in the Pacific Ocean (Healey 1991). Ocean-type adult Chinook salmon begin their freshwater migration in early fall, spawning in warm water near tide-waters or the lower sections of large rivers. Conversely, stream-type adult Chinook salmon begin

migrating in spring, utilizing colder headwater tributaries for spawning. Further, due to their early emergence and rapid growth, ocean-type juveniles migrate to estuaries within 3 months, while stream-type juveniles postpone migration for 1 year or more.

The management of Chinook salmon is often intensive, with guidelines to provide protection for stocks listed as evolutionarily significant units (ESUs) under the Endangered Species Act. In the Columbia River basin, healthy Chinook salmon stocks mix with listed stocks, thereby providing a challenge to harvest managers. Currently, genetic techniques using allozyme electrophoresis (Marshall et al. 2000) and randomly amplified polymorphic DNA (RAPD; Rasmussen et al. 2003) have been identified to segregate ocean- and stream-type Chinook salmon. However, the current markers of choice for many population genetic studies remain microsatellite loci.

Often multiple loci are capable of detecting genetic divergence between sample groups, but costs limit their use. Ideally, a small number of diagnostic markers would lead to cost-effective and informative genetic information. The purpose of this study was to test ocean- and stream-type Chinook salmon for genetic divergence using a putatively diagnostic microsatellite locus.

Methods

Sample collections.—Fin clip samples of Chinook salmon were collected throughout various tributaries of the interior Columbia River basin from 1998 to 2002 (Figure 1). These include stream-type Chinook salmon sampled from Johnson Creek ($n = 95$; 2000) and Lolo Creek ($n =$

* Corresponding author: nars@critfc.org

Received July 29, 2003; accepted January 20, 2004

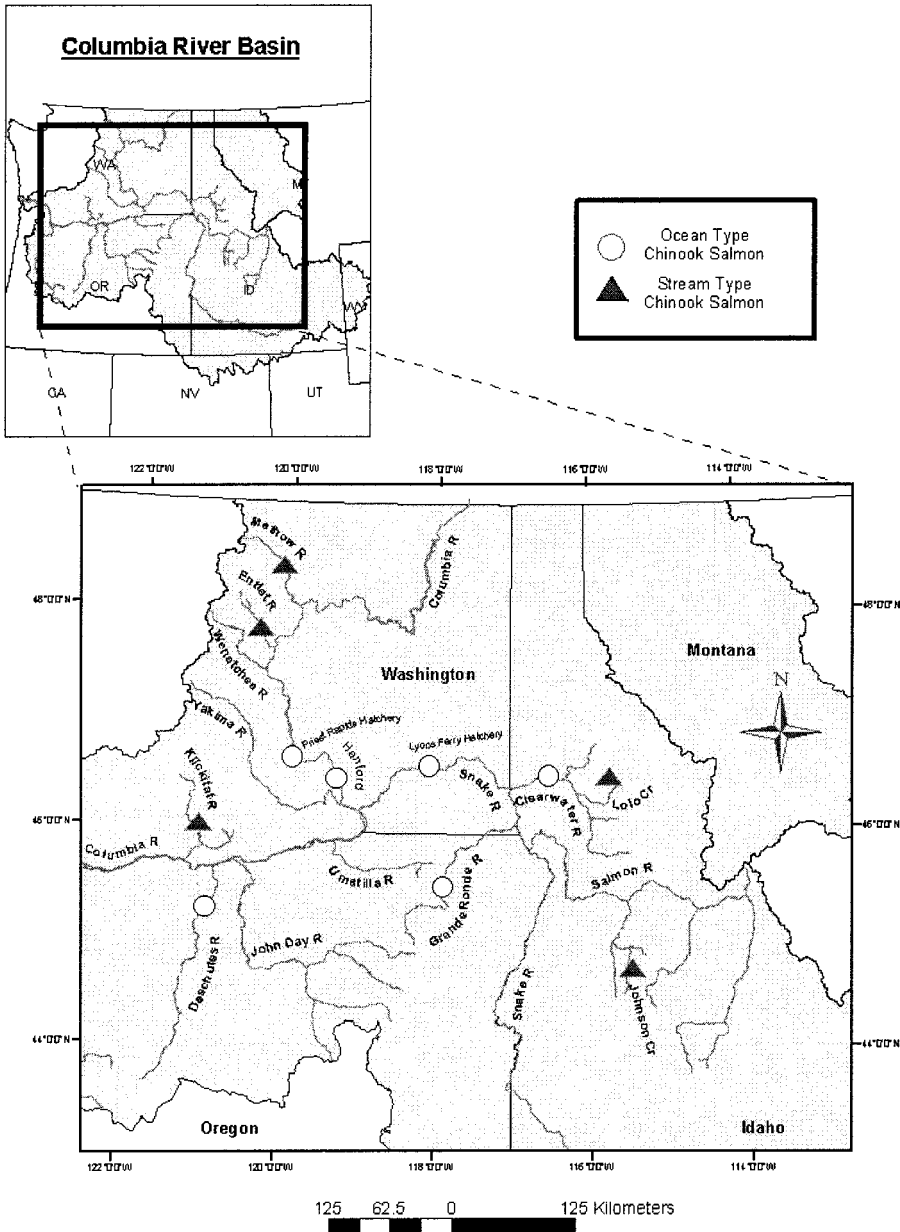


FIGURE 1.—Map of the Columbia River Basin showing the sites from which samples of ocean-type and stream-type Chinook salmon were obtained.

68; 2001, 2002) in Idaho and the Klickitat River ($n = 73$; 2002), the Entiat River ($n = 80$; 2002), and the Methow River ($n = 58$; 2001) in Washington. Ocean-type samples were taken from the Clearwater River ($n = 64$; 1998) and the Grande Ronde River ($n = 21$; 1998) in Idaho, Lyons Ferry Hatchery ($n = 85$; 2000), Priest Rapids Hatchery ($n = 141$; 1998–2000), and Hanford Reach of the

Columbia River ($n = 166$; 1998, 1999) in Washington, and the Deschutes River in Oregon ($n = 34$; 1999). All collections were comprised of adult fish sampled either as carcasses or as fish that had passed tributary weirs, with the exception of juveniles collected from Lyons Ferry Hatchery, Priest Rapids Hatchery, and Hanford Reach (1999). In rivers supporting both life history types,

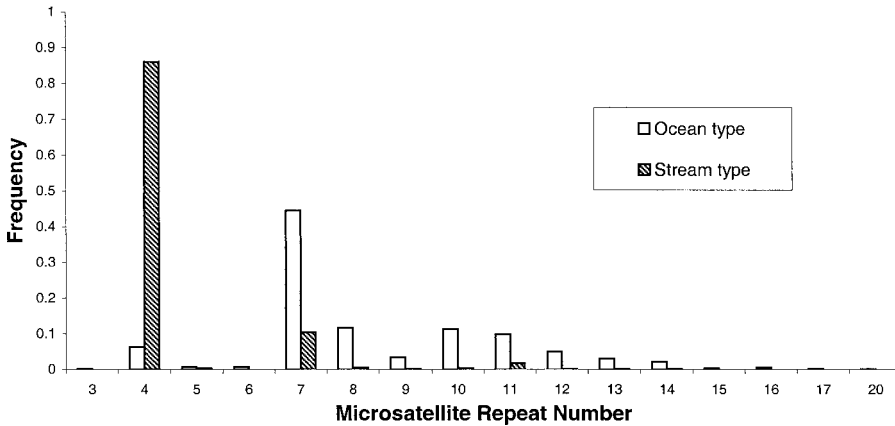


FIGURE 2.—Allele frequencies of microsatellite locus *OtsG474* from 511 ocean-type and 374 stream-type Chinook salmon from the interior Columbia River basin.

run timing was used to identify individuals to ocean or stream type a priori. Sampling during run times of potential ocean-stream-type overlap was avoided. Juveniles collected at Hanford Reach in 1999 were sampled by beach seining soon after fry had emerged from redds.

Laboratory analysis.—Fin clips were digested and DNA extracted with Qiagen DNeasy in conjunction with a Qiagen 3000 robot. Genomic DNA was quantified and arrayed into 96-well plates for high-throughput genotyping.

Preliminary data pointed to *OtsG474* (Williamson et al. 2002) as a potential diagnostic microsatellite locus between ocean- and stream-type Chinook salmon. Initial locus screening was performed on samples from Clearwater River ocean-type and Johnson Creek stream-type Chinook salmon. The polymerase chain reaction (PCR) was used to amplify the tetranucleotide microsatellite locus *OtsG474*. Amplifications were performed using the AmpliTaq Reagent System (Applied Biosystems) in an MJ Research PTC-100 thermal cycler following the manufacturer's protocols, with approximately 25 ng of template genomic DNA in a 15- μ L total volume. Cycling conditions included an initial denaturation of 5 min at 96°C followed by 30 cycles of 30 s at 94°C, 30 s at 59°C, and 30 s at 72°C. Final extension was carried out for 10 min at 72°C. The forward primer was fluorescently labeled with 6-FAM (Applied Biosystems), and PCR products were genotyped according to the manufacturer's protocols with an Applied Biosystems Model 3100 genetic analyzer.

Statistical analysis.—Genotypes were binned using GeneMapper (Applied Biosystems 2002) and allele frequencies generated using GENEPOP

(Raymond and Rousset 1995). Exact-significance testing methods (GENEPOP) with sequential Bonferroni corrections (Rice 1989) were used to evaluate conformance to Hardy-Weinberg equilibrium for all individual collections. To estimate the level of within-population (i.e., within life history type) genetic diversity, the expected heterozygosity (H_E), observed heterozygosity (H_O), number of alleles per locus, and allele range were calculated. Differences in heterozygosity between ocean-type and stream-type fish were evaluated by means of the Wilcoxon signed rank test in SyStat (SyStat 1998). The proportion of genetic variance between ocean-type and stream-type fish was calculated from allele frequencies (F_{ST} ; Weir and Cockerham 1984) using GENEPOP (Raymond and Rousset 1995). Assignment tests were performed with GeneClass (Cornuet et al. 1999) by self-classifying reference data using the Bayesian method and the leave-one-out option.

Results

All individual collections conformed to Hardy-Weinberg equilibrium with the exception of the 1998 Hanford Reach collection. Allele frequencies at *OtsG474* reflected strong genetic divergence between ocean- and stream-type Chinook salmon (Figure 2). Comparisons of H_E (ocean = 0.76; stream = 0.25), H_O (ocean = 0.65; stream = 0.21), and the number of alleles at *OtsG474* (ocean = 16; stream = 10) confirm highly significant differences in genetic variance at this locus. While a single allele (repeat number 4) comprised 86% of the alleles found in stream-type populations, the second most common allele (repeat number 7) in stream-type populations was the most common al-

lele found in ocean-type populations (Figure 2). The average F_{ST} between the ocean and stream types equaled 0.42 and was highly significant ($P < 0.00001$). Assignment success over all samples was 93%. However, the Klickitat stream-type sample group was assigned much less accurately (75.3%) than other stream-type sample groups (range = 95.6–100%), and the Hanford Reach ocean-type sample group was assigned less accurately (78.6%) than other ocean-type groups (range = 84.4–100%).

Discussion

The level of genetic divergence observed between ocean- and stream-type Chinook salmon at locus *OtsG474* indicates the strong reproductive isolation of these types in the interior Columbia River basin. This locus may be very useful in future genetic studies to distinguish the run timing and co-occurrence of the two life history types. The information could be highly influential in setting harvest limits and detecting ESU stocks in the Columbia River. Microsatellite locus *OtsG474* complements distinctive allozyme (Utter et al. 1995; Marshall et al. 2000) and RAPD (Rasmussen et al. 2003) markers and provides researchers a suite of markers to choose from in discerning ocean- and stream-type Chinook salmon. This could be useful because concordant results from multiple types of molecular markers are statistically powerful (Rasmussen et al. 2003).

Further investigation of *OtsG474* with Chinook salmon samples from lower Columbia River, Alaskan, Canadian, and California stocks is necessary to determine whether this locus is distinctive between types throughout the species' full range in the northeastern Pacific Ocean or this marker's utility is limited to the types within the interior Columbia River basin. As evidenced by stream-type samples from the Klickitat River, lower Columbia River stocks may contain more complexity in their genotypes than upriver stocks or may have experienced introgression from large transplants of hatchery fish. Further, Alaskan, Canadian, and California stocks need investigation because ocean-type Chinook salmon are predominate south of 56°N and stream-type fish are most abundant above 56°N (Healey 1991).

Additional studies with this locus may provide information as to the origins of Columbia River Chinook salmon stocks and the transition zones between types along the North American coastline (Teel et al. 2000). However, there is complexity (e.g., the Klickitat River samples) that is not ad-

equately captured by a single locus. Multilocus analysis is needed to characterize and identify individuals to populations with a high degree of certainty. Locus *OtsG474* is a good candidate for studies utilizing suites of microsatellite loci to determine specific stock identification (e.g., Beacham et al. 2003) as well as the origins of Chinook salmon populations or their transition zones.

Acknowledgments

We thank John Whiteaker, Doug Hatch, Bill Arnsberg, and Jason Vogel for providing genetic samples, Vanessa Jacobson for performing genetic laboratory work, and Joyce Faler and Robin Schrock for genetic data from the Entiat River. Randy Henry provided Figure 1. Allele frequencies are available at http://www.critfc.org/tech/tech_rep.html.

References

- Applied Biosystems. 2002. ABI PRISM GeneMapper software, version 3.0: user's manual. Applied Biosystems, Foster City, California.
- Beacham, T. D., J. R. Candy, K. J. Supernault, M. Wetklo, B. Deagle, K. Labaree, J. R. Irvine, K. M. Miller, R. J. Nelson, and R. E. Withler. 2003. Evaluation and application of microsatellites for population identification of Fraser River Chinook salmon (*Oncorhynchus tshawytscha*). U.S. National Marine Fisheries Service Fishery Bulletin 101:243–259.
- Cornuet, J. M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153:1989–2000.
- Healey, M. C. 1991. Life history of Chinook salmon (*Oncorhynchus tshawytscha*). Pages 311–395 in C. Groot and L. Margolis, editors. Pacific salmon life histories. UBC Press, Vancouver.
- Marshall, A. R., H. L. Blankenship, and W. P. Connor. 2000. Genetic characterization of naturally spawned Snake River fall-run Chinook salmon. *Transactions of the American Fisheries Society* 129: 680–698.
- Rasmussen, C., C. O. Ostberg, D. R. Clifton, J. L. Holloway, and R. J. Rodriguez. 2003. Identification of a genetic marker that discriminates ocean and stream-type Chinook salmon in the Columbia River Basin. *Transactions of the American Fisheries Society* 132:131–142.
- Raymond, M., and F. Rousset. 1995. GENEPOP, version 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248–249.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.
- SyStat. 1998. SyStat, version 9: instruction manual. SyStat, Waterloo, Ontario.
- Teel, D. J., G. B. Milner, G. A. Winans, and W. S. Grant. 2000. Genetic population structure and origin of life history types in Chinook salmon in British Co-

- lumbia, Canada. Transactions of the American Fisheries Society 129:194–209.
- Utter, F. M., D. W. Chapman, A. R. Marshall. 1995. Genetic population structure and history of Chinook salmon of the Upper Columbia River. Pages 149–165 in J. Nielsen and D. A. Powers, editors. Evolution and the aquatic ecosystem. American Fisheries Society, Symposium 17, Bethesda, Maryland.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating F -statistics for the analysis of population structure. Evolution 38:1358–1370.
- Williamson, K. S., J. F. Cordes, and B. May. 2002. Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Molecular Ecology Notes 2:17–19.