DNA CHARACTERIZATION OF THREE COLLECTIONS OF SNAKE RIVER FALL CHINOOK (02GL, 02GK, & 02PH)

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Introduction

In 2002, scales from all unmarked/untagged (possibly "natural") fish trapped and processed at Lyons Ferry Hatchery were collected to document the occurrence of hatchery and naturally reared fish in the collection. The unmarked/untagged fish trapped at Lyons Ferry Hatchery are thought to be predominantly from returns of unmarked/untagged fish (subyearlings) from the Nez Perce Tribe acclimation sites (Lyons Ferry Hatchery origin fish). If so, it would seem reasonable to use some of these fish in the Lyons Ferry Hatchery broodstock. Reading scales allows biologists to differentiate hatchery-produced from naturally produced ("wild") fish but will not determine the specific origin of those hatchery fish because of similar sizes at release. This item is of great concern for fish management in the Snake River and Columbia River basins.

Beginning with subyearling releases by the Nez Perce Tribe in 1999, unmarked/untagged fall Chinook have been released above Lower Granite Dam. Some of these releases have associated CWT groups and some do not. Based on the tagging history of fish recovered from trapping efforts at Lyons Ferry Hatchery, biologists believe the majority of unmarked/untagged fish trapped at Lyons Ferry Hatchery are Snake River origin hatchery-produced fish that should not be genetically different than the Lyons Ferry Hatchery CWT groups.

In the future, it would be beneficial to compare the Umatilla stock and other Columbia River stocks to the Lyons Ferry Hatchery broodstock and the naturally produced Snake River stock to assess if the Lyons Ferry Hatchery program is maintaining the genetic integrity of the Snake River stock. In 2001 and 2002, the run of fall Chinook at Lower Granite Dam, in conjunction with large steelhead runs, effectively shut down the adult trap at times, which allowed stray fish to pass the dam. As a result, it is unknown at what level strays have been infused into natural production. These are all questions that may be answered in future studies.

The present study was undertaken to address two questions:

1) Can scale samples from adults be successfully used for microsatellite DNA characterization of Snake River fall Chinook?

2) How genetically similar are the following three groups of fish: a) unknown hatchery adults from subyearling releases (unmarked/untagged but verified as subyearling hatchery via scale analysis) volunteering into Lyons Ferry Hatchery, b) known Lyons Ferry Hatchery adults (broodstock), and c) known naturally produced adults of unknown origin sampled at Lower Granite Dam in 2002?

This memo summarizes the results of the microsatellite DNA analysis of three collections (02GL, 02GK, and 02PH) of fall-run Chinook salmon in the Snake River.

Materials & Methods

The 02GL collection included 130 samples of known Lyons Ferry Hatchery Chinook, 02GK included 255 subyearling Chinook trapped at Lyons Ferry Hatchery, and 02PH included 119 samples collected at Lower Granite Dam trap from presumed wild Chinook (Table 1).

Table 1. Collections analyzed in the present study

Stock	Collection Code	Total Number Sampled	Number Analyzed for DNA	Sample type
Snake River Stock (LFH broodstock)	02GL	130	96	Fin
Unmarked/untagged adults trapped at LFH (subyearling hatchery component trapped at LFH)	02GK	255	96	Fin
Unmarked/untagged adults released at Lower Granite Trap (expect natural Snake R. stock based on scales verified by John Sneva.)	02PH	119	70	Scales

Genomic DNA was extracted from tissues using a chelex resin/proteinase-K protocol (Small et al., 1998). Microsatellite alleles at 14 loci were amplified using fluorescently

labeled primers and the polymerase chain reaction (PCR) and the resulting products were run on an Applied Biosystems 3100 automated sequencer. Alleles were sized (basepairs, bp) using an internal lane size standard (GS500 by Applied Biosystems), using the Applied Biosystems Genemapper ver. 3.0 computer program. The raw allele size calls from Genemapper were imported into MS Excel where final allele calling was accomplished using bins defined based on the primary classes observed in plotted distributions of the Genemapper size calls for each locus and the presumed minimum repeat motif of each microsatellite. Allele bins were centered on median values, with bin boundaries defined to leave inter-bin spaces equal to one-half the interval between adjacent bins. This was done to ensure that all DNA fragments (alleles) falling within an allele bin were more similar in size to each other than any was to a fragment in another recognized allele bin. Single-locus genotypes that included any raw size calls falling into an inter-bin space were zeroed to remove ambiguous allele scores from the data set. Details of PCR conditions, the loci screened, and the alleles observed in this study are presented in Appendix 1.

Results & Discussion

We extracted DNA from fin tissue from 96 samples from both the 02GL and 02GK collections. We attempted to extract DNA from 70 of the 02PH scale samples; however, twelve individuals from that collection did not have any uncleaned scales in the scale envelopes and we were unable to extract DNA from the dried mucus remaining in the scale envelopes for these samples. Therefore, only a total of 58 samples from the 02PH collection were successfully analyzed.

Table 2 summarizes the numbers of fish that were not successfully scored at each microsatellite DNA locus in each of the three collections. Overall, the scale samples (collection 02PH) performed about as well as the fin clip samples (collections 02GL and 02GK), 2.5% missing scores vs. 2.0% and 4.5%, respectively. This result indicates that minimally invasive, non-lethal sampling (of scales) can provide suitable samples for DNA analysis.

Table 2. Numbers of unscored fish at each locus.

Locus	Lyons Ferry Hatchery broodstock (02GL) N = 96 fin	Unmarked & untagged adults at Lyons Ferry Hatchery (02GK) N = 96 fin	Unmarked & untagged adults released at Lower Granite Dam (02PH) N = 58 scales
Ogo-2	1	5	7
Ogo-4	0	8	1
One-8	2	3	2
Ocl-1	0	1	0
Ots-107	2	2	2
Ots-100	5	6	5
Ots-2M	2	1	0
Ots-101	2	5	1
Ots-3M	2	0	1
Ssa-197	2	2	1
Ots-1	2	1	0
Omy-1001	0	7	0
Omm-1142	0	4	0
Omy-1135	7	15	0
Total unscored	27	60	20
% unscored	2.0%	4.5%	2.5%

The second question, (What are the genetic interrelationships among the three collections?), was addressed using pairwise genotypic tests of population differentiation. The tests were calculated using the program GENEPOP, version 3.3 (Raymond and Rousset 1995).

Results of this analysis revealed that the 02GK and 02GL samples (unmarked, untagged adults trapped at Lyons Ferry Hatchery and Lyons Ferry Hatchery broodstock) were not significantly different while the 02PH collection (unmarked, untagged natural-origin adults released at the Lower Granite Dam trap) was significantly different from both the 02GK and 02GL collections (Table 3). This result is consistent with the possibility that the Lower Granite Dam trap collection consisted of fish from two or more populations. However, tests of Hardy-Weinberg equilibrium indicated that no loci deviated significantly from expected genotypic proportions in the Lower Granite

Dam trap collection (after correction for multiple testing; see Rice, 1989) as might have been expected if this were a mixture of stocks. It should be noted that the 02PH collection occurred between 17August and 5 September and did not span the duration of the run, which ended on 14 November 2002. Thus, it is possible these samples may not represent a full cross section of the fall Chinook passing Lower Granite Dam in 2002. All loci in the other two collections were also in Hardy-Weinberg equilibrium.

Table 3. Pairwise genotypic differentiation tests of three fall Chinook collections from the Snake River. Uncorrected P-values shown for each pairwise comparison were calculated using GENEPOP.

	Lyons Ferry Hatchery broodstock (02GL)	Unmarked & untagged adults at Lyons Ferry Hatchery (02GK)	Unmarked & untagged adults released at Lower Granite Dam (02PH)
02GL	-		
02GK	0.17301	-	
02PH	**0.00003**	**0.01047**	-

Conclusions

The fourteen loci screened exhibited high levels of variation (observed heterozygosity and numbers of alleles) that should provide considerable power to discriminate populations (cf. Appendix 1). Scale samples obtained non-lethally proved to be a suitable source for DNA extraction and amplification; as good as fin-clip samples. The collection of unmarked and untagged adults volunteering to the Lyons Ferry Hatchery and the collection of known (tagged) Lyons Ferry Hatchery broodstock were not significantly different from each other, whereas both of these were significantly different from the collection of unmarked and untagged adults made at Lower Granite Dam.

Acknowledgements

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Appendix 1. Microsatellite DNA multiplexes, loci, three measures of variability, and PCR conditions used to analyze the three collections of fall-run Chinook from the Snake River.

Multiplex *Locus*	Repeat length (bp)	Ho[b] (observed heterozygosity)	Number of alleles observed	Allele size range[a] small	Allele size range[a] large	Dye label	Primer concen. (µM)	Annealing temp.	# of cycles
OtsJ1									
Ogo-2	2	0.863	16	205	241	6fam	0.06	58	32
Ogo-4	2	0.761	14	125	165	hex	0.05	58	32
One-8	2	0.858	18	151	187	ned	0.05	58	32
OtsJ2									
Ocl-1	2	0.703	11	143	170	6fam	0.05	50	35
Ots-107	4	0.953	33	176	327	hex	0.06	50	35
Ots-100	2	0.961	47	215	389	ned	0.07	50	35
OtsK									
Ots-2M	2	0.841	16	133	168	ned	0.03	49	36
Ots-101	4	0.943	27	157	278	hex	0.08	49	36
Ots-3M	2	0.765	10	127	153	6fam	0.04	49	36
Ssa-197	4	0.947	30	163	280	6fam	0.05	49	36
Ots-1	2	0.508	8	181	197	ned	0.06	49	36
OtsL									
Omy-1001	4	0.973	54	213	345	6fam	0.08	52	35
Omm-1142	4	0.900	24	141	204	hex	0.12	52	35
Omm-1135	2	0.813	11	199	225	ned	0.06	52	35

[a] Size estimates in base pairs from the Applied Biosystems 3100 DNA sequencer. Although the genetic data set used in this analysis is internally consistent, no attempt has been made to standardize these allele size estimates for the three Snake River fall Chinook collections in this study with allele size estimates obtained for other Chinook populations using an Applied Biosystems 3730 DNA sequencer. Furthermore, no correction has been made to the estimates to make the interval between alleles fit the repeat motif models (i.e., a dinucleotide locus would have 2 bp intervals).

[b] = Observed heterozygosity was calculated using MSA (Dieringer, D. and C. Schlotterer. 2003. MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. Mol. Ecol. Notes 3:167-169.)