DNA CHARACTERIZATION OF LYONS FERRY HATCHERY FALL CHINOOK BROODSTOCK (04NM)

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Introduction

In 2004, scales from Lyons Ferry Hatchery fall Chinook broodstock were collected to address the following questions:

1) How genetically distinct are the 2004 Lyons Ferry Hatchery broodstock from: a) Lyons Ferry Hatchery broodstock collected in 2002 and 2003, b) Lyons Ferry Hatchery volunteers identified as yearlings collected in 2002 and 2003, c) Lyons Ferry Hatchery volunteers identified as sub-yearlings collected in 2002 and 2003, d) known naturally produced adults of unknown origin sampled at Lower Granite Dam in 2002 and 2003, and e) a collection of fall Chinook collected from Umatilla Hatchery broodstock in 2003?

Results for the comparisons between the 2002 and 2003 samples are available in Kassler and Shaklee (2003) and Kassler et al. (2004). This memo; therefore focuses on the microsatellite DNA analysis of the fall-run Chinook salmon from Lyons Ferry Hatchery broodstock (04NM) and the comparison to the earlier samples.

Materials & Methods

Genomic DNA was extracted from 100 samples by digesting scales using silica membrane based kits obtained from Machery-Nagel. Microsatellite alleles at 13 loci were amplified using fluorescently labeled primers and the polymerase chain reaction (PCR) and the resulting products were run on an Applied Biosystems 3730 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 MicrosatelliteBinner v.1.h (available from S.F. Young, WDFW).

The genetic interrelationships among all the collections was addressed using pairwise genotypic tests of population differentiation (Table 1). The tests were calculated using the program GENEPOP version 3.4 (Raymond and Rousset 1995) while tests from

Kassler et al. (2004) were performed using FSTAT version 2.9.3.1 (Goudet 2001). Pairwise calculations by FSTAT (Goudet 2001) only use individuals with complete genotypes while GENEPOP (Raymond and Rousset 1995) uses individuals with missing data. Pairwise genotypic tests calculated by GENEPOP in this analysis may therefore vary slightly from Kassler (2004) due to the differences in the programs used. Collections with little to no missing data will be similar while results for collections with missing data may vary.

Bonferroni correction for multiple testing (Rice 1989) was used for final estimate of statistical significance between comparisons. Bonferroni correction was used to provide the most conservative approach to estimate the significant difference between two collections being analyzed. There is an increased probability of finding a statistically significant difference between comparisons when calculating multiple tests, as compared with a single test. The probability of finding two or more individual P-values or alpha values that are less than or equal to 0.05, for example, is about 7% with five comparisons versus a probability of 5% for one comparison (Rice 1989). Therefore, to maintain an error rate of 0.05 over all comparisons, the P-value for each individual comparison must be reduced. This ensures that the Type I error (error associated with incorrectly showing statistical significance) remains constant through the entire series of tests. A Bonferroni correction minimizes the potential for a significant difference by reducing the accepted P-value (usually 0.05) revealing only differences that are highly significantly different.

Results & Discussion

Results of these pairwise genotypic tests were mostly consistent with those of the earlier results (Kassler and Shaklee 2003, Kassler et al. 2004) on samples from Lyons Ferry Hatchery. Results of this analysis revealed the 04NM samples were significantly different from the unmarked/untagged hatchery yearling volunteers to Lyons Ferry Hatchery (2002 and 2003 samples were combined), the Umatilla Hatchery broodstock, and the unmarked/untagged adults collected at Lower Granite Dam in 2002.

The 04NM samples (Lyons Ferry Hatchery broodstock) were not significantly different from the 2002 or 2003 broodstock collections, the unmarked/untagged hatchery sub-yearling volunteers to Lyons Ferry Hatchery from 2002 and 2003, or the unmarked/untagged adults collected at Lower Granite Dam in 2003.

There were two inconsistent results from the present analysis to that in Kassler et al. (2004). The first came from comparisons of Lyons Ferry Hatchery broodstock and unmarked/untagged adults collected at Lower Granite Dam. The Lyons Ferry Hatchery broodstock from 2004 was not significantly different from the unmarked/untagged adults collected at Lower Granite Dam in 2003 while the broodstock collections in 2002 and 2003 were significantly different. The second was the comparison between the Lyons Ferry Hatchery unmarked/untagged hatchery sub-yearling volunteers to Lyons Ferry Hatchery from 2002 to the Umatilla broodstock 2003.

If a Bonferroni correction had not been applied, both of the comparisons that were different would still have been significant. The application of a Bonferroni correction in determining statistical significance reduces the possibility of Type I errors, but also reduces the power of the test to determine truly significant or subtle differences. The change in statistical significance for this comparison, therefore, results from the choice of the correction that was applied and in this case does not necessarily reflect the relationship between the collections.

Literature Cited

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Table 1. Pairwise comparisons of fall-run Chinook salmon collected from Lyons Ferry Hatchery, Lower Granite Dam, and Umatilla Hatchery broodstock calculated using GENEPOP v.3.4. Pairwise comparisons that were significantly different are highlighted in black with white type. Pairwise comparisons were defined as significant after implementation of Bonferonni correction for multiple tests (Rice 1989; 36 comparisons; alpha = 0.05/36 = 0.0014). Pairwise comparisons that were not significantly different after Bonferroni correction, but reflect large genetic differences and should be considered as genetically different are highlighted in grey.

	LFH V02/03 Y	LFH V02 SY	LFH B02	LGD 02	LFH V03 SY	LFH B03	LGD 03	LFH B04
LFH V02 SY	0.0000							
LFH B02	0.0000	0.0390						
LGD 02	0.0004	0.1519	0.0000					
LFH V03 SY	0.0000	0.1812	0.0872	0.0089				
LFH B03	0.0000	0.3416	0.0368	0.0000	0.5113			
LGD 03	0.0003	0.3656	0.0000	0.6501	0.0157	0.0001		
LFH B04	0.0000	0.6843	0.3426	0.0000	0.0338	0.2366	**0.0065**	
Umatilla	0.0487	**0.0015**	0.0000	0.0412	0.0000	0.0000	0.0976	0.0000

LFH V02/03 Y - unmarked/untagged fish trapped at LFH, scales indicate hatchery yearling. We anticipated these fish were strays.

LFH V02 SY - unmarked/untagged fish trapped at LFH, scales indicate hatchery subyearling. We anticipated these fish were the unmarked/untagged portion of LF origin hatchery fish released upstream of LGR Dam.

LFH B02 - random sample of broodstock from fish collected at LFH and LGR Dam, broodstock consisted of fish verified as LF origin based on CWT or VIE (did not use any unmarked/untagged fish in broodstock). We anticipated these fish would be significantly different than Umatilla.

LGD 02 - Unmarked/untagged naturally produced fish based upon scale analysis, fish collected at beginning of run (Aug 17-Sept 5) at LGR Dam, not a full representation of run, n=70. We anticipated these fish would be similar to LF hatchery origin fish but would have out-of-basin influence from Umatilla, Hanford, and Priest Rapid stocks.

LFH V03 SY - unmarked/untagged fish trapped at LFH, scales indicate hatchery subyearling. We anticipated these fish as the unmarked/untagged portion of LF origin hatchery fish released upstream of LGR Dam.

LFH B03 - random sample of broodstock from fish collected at LFH and LGR Dam, broodstock consisted of fish verified as LF origin based on CWT or VIE, and unmarked/untagged females trapped at LFH that had scales indicating subyearling hatchery production (did not use any unmarked/untagged fish from LGR Dam broodstock), two naturally produced fish (trapped at LFH) were included in broodstock

Table 1. Continued.

LGD 03 - Unmarked/untagged naturally produced fish based upon scale analysis, fish collected throughout run at LGR Dam. Preliminary mixture analysis indicated 6-20% of the parentage of these fish consisted of Hanford Reach wilds, Priest Rapids hatchery, or Umatilla hatchery. It was anticipated these fish would be similar to LF origin fish but would have some stray influence as well.

LFH B04 - random sample of broodstock from fish collected at LFH and LGR Dam, broodstock consisted of fish verified as LF origin based on CWT or VIE, and unmarked/untagged females trapped at LFH and LGR Dam that had scales indicating subyearling hatchery production or Snake River natural origin (subyearling or reservoir reared scales). Included in broodstock were 130 Snake River natural origin fish (127 females). The LFH B04 contains the same proportion of natural fish that were used in broodstock.

Umatilla B03 - random sample of fish collected at 3 Mile Dam that were used as broodstock at Umatilla Hatchery. Some of the fish in this sample may be of LF origin since they do not remove wire from fish unless they are adipose clipped. Umatilla Hatchery releases blank wire tagged fish that are not adipose clipped while the NPT releases CWT tagged fish that are not adipose clipped. At spawning it is assumed these wire tagged fish contain BWTs so we do not know to what extent LF origin fish are included in their broodstock. In 2002 wire was dissected from 50 snouts from fish not adipose clipped, and 1 CWT was decoded indicating LF origin.