Extension of the Chinook Salmon Microsatellite Baseline

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by

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Summary:

Genetic stock identification is a tool with great potential to meet the needs of mixed fisheries stock assignments. The two key components to successful genetic stock identification are statistical power and thorough baseline representation. The objective of this project was to fill holes in the coverage initially provided in the GAPS (Genetic Analysis of Pacific Salmonids) baseline. We identified 12 additional baseline populations based upon ESA status and genetic distinction. Genetic distinction of populations was determined from the Interior Columbia Basin Technical Recovery Team report (TRT 2003). A total of 1695 individuals representing 12 populations of Chinook salmon were genotyped with standardized protocols for contribution to the baseline. We successfully completed this objective and closely followed the anticipated time schedule from the proposal. This additional data will now be incorporated into the genetic baseline with plans to post all baseline genotypes to an online database (currently under construction by the GAPS consortium).

Introduction

Current GAPS Baseline Highlights and Limitations

The current Chinook salmon microsatellite baseline constructed by the GAPS (Genetic Analysis of Pacific Salmonids) consortium for the CTC provides a powerful tool for evaluating mixed stock fisheries. The initial baseline (version 1.1) is a broad coastwide coverage of 110 populations (~16,000 samples; Appendix A; Figure 1) that have been genotyped with 13 highly polymorphic microsatellite loci (~500 alleles). Initial power analyses indicate that 35 of 37 reporting groups in the baseline have at least 90% accuracy in 100% mixture simulations (Moran et al. 2005). Further baseline expansion and maintenance planned in 2006 by GAPS laboratories will lead to a microsatellite baseline with even more dense geographic coverage (approximately 200 populations). Thus, a highly effective tool for mixed stock analysis of Chinook salmon is now in place and GAPS plans to make it available through an online database.

Despite large, representative sample sizes from many populations and very high microsatellite allelic diversity, the resolution of specific stocks and populations in the baseline is limited in some cases. For example, fall Chinook salmon in the Columbia River are closely related and remain difficult to distinguish even with this powerful set of 13 microsatellite markers. Several other closely related populations in the baseline are similarly difficult to distinguish and thus have been pooled into a single reporting unit for GSI applications. In some cases (i.e., Washington coast troll fishery), a finer level of stock discrimination is necessary for management of fisheries. Additional baseline coverage

should increase stock assignment reliability when greater resolution is required (Banks 2005).

Current Project Objectives

As a contributing GAPS laboratory, CRITFC recognized the need to add critical and distinct stocks to the baseline since assignments of unknown origin fisheries are limited to populations included in the baseline. The objective of this project was to genotype 12 additional Chinook salmon populations for inclusion in the microsatellite baseline. The additional populations focus on endangered Snake River fall Chinook salmon and genetically distinct stocks of spring/summer Chinook salmon from the Snake River. The 12 populations are as follows: Clearwater River (fall Chinook), Nez Perce Tribal Hatchery (fall Chinook), Lostine River (spring/summer Chinook), Catherine Creek (spring/summer Chinook), Dworshak Hatchery (spring/summer Chinook), South Fork Clearwater broodstock (spring/summer Chinook), Clearwater Creek (spring/summer Chinook), Newsome Creek (spring/summer Chinook), Sawtooth Hatchery (spring/summer Chinook), Big Creek (spring/summer Chinook), Johnson Creek (spring/summer Chinook), and Pahsimeroi River (spring/summer Chinook).

Methods

The objective of this project was to fill holes in the coverage initially provided in the GAPS (Genetic Analysis of Pacific Salmonids) baseline. We identified 12 additional baseline populations based upon ESA status and genetic distinction. For each of the 12 populations, 96 to144 individuals were genotyped with CTC standardized microsatellite loci (Moran et al. 2005). The process included DNA extraction from tissue samples, polymerase chain reaction (PCR) to amplify 13 microsatellite loci (Table 1), and fragment analysis to detect fluorescently labeled PCR products. Raw genotype data was converted to standardized CTC alleles. The converted data will be incorporated to the microsatellite baseline for improved mixed fishery genetic stock identification. In order to maintain sample exchange among GAPS labs, an aliquot of DNA from each new sample will be distributed to NWFSC for distribution under guidelines established by the GAPS “mega-swap”.

Table 1. Microsatellite loci standardized for Chinook salmon

Locus	Primer Sequence (5'→ 3') **F** > Forward, **R** > Reverse	Citation	Curator Agency[1]
Ots201b	F- CAGGGCGTGACAATTATGC R- TGGACATCTGTGCGTTGC	OSU unpublished[2]	ADFG
Ots208b	F- GGATGAACTGCAGCTTGTTATG R- GGCAATCACATACTTCAACTTCC	Grieg et al. 2003	CRITFC
Ots211	F - TAGGTTACTGCTTCCGTCAATG R - GAGAGGTGGTAGGATTTGCAG	Grieg et al. 2003	ADFG
Ots212	F- TCTTTCCCTGTTCTCGCTTC R- CCGATGAAGAGCAGAAGAGAC	Grieg et al. 2003	OSU
Ogo4	F- GTCGTCACTGGCATCAGCTA R- GAGTGGAGATGCAGCCAAAG	Olsen et al. 1998	WDFW
Ogo2	F- ACATCGCACACCATAAGCAT R- GTTTCTTCGACTGTTTCCTCTGTGTTGAG	Olsen et al. 1998	ADFG
Ots3M	F- TGTCACTCACACTCTTTCAGGAG R- GAGAGTGCTGTCCAAAGGTGA	Banks et al. 1999	WDFW
Ots213	F- CCCTACTCATGTCTCTATTTGGTG R- AGCCAAGGCATTTCTAAGTGAC	Grieg et al. 2003	OSU
Omm1080	F- GAGACTGACACGGGTATTGA R- GTTATGTTGTCATGCCTAGGG	Rexroad et al. 2001	SWFSC
Ssa408UOS	F- AATGGATTACGGGTACGTTAGACA R- CTCTTGTGCAGGTTCTTCATCTGT	Cairney et al. 2000	NWFSC
Ots9	F- ATCAGGGAAAGCTTTGGAGA R- CCCTCTGTTCACAGCTAGCA	Banks et al. 1999	DFO
OtsG474	F- TTAGCTTTGGACATTTTATCACAC R- CCAGAGCAGGGACCAGAAC	Williamson et al. 2002	CRITFC
Oki100	F- CCAGCACTCTCACTATTT R- CCAGAGTAGTCATCTCTG	DFO unpublished	DFO

[1]Laboratory abbreviations: OSU, Oregon State University; SWFSC, Southwest Fisheries Science Center – National Marine Fisheries Service; DFO, Department of Fisheries and Oceans Canada; NWFSC, Northwest Fisheries Science Center – National Marine Fisheries Service; CRITFC, Columbia River Inter-Tribal Fish Commission; ADFG, Alaska Department of Fish & Game; WDFW, Washington Department of Fish & Wildlife.

Results

A total of 1695 Chinook salmon samples were genotyped from 12 populations for addition to the microsatellite baseline. Across all 13 loci, 367 alleles were observed with five new alleles not previously included in the baseline. Those new alleles are being verified among GAPS labs to ensure standardized nomenclature. Of 156 tests for Hardy-Weinberg equilibrium, 6 were statistically significant after Bonferroni corrections. Populations with loci that deviated from equilibrium were: Pahsimeroi River with three significant loci (OMM1080 – heterozygote deficit, Ots201b – heterozygote excess, and Ots213 - heterozygote excess), SF Clearwater R. (Ssa408 – heterozygote deficit), and Big Creek (Ots208b – heterozygote excess, Ots213 – heterozygote deficit).

Genetic diversity as measured by observed and expected heterozygosity, total alleles, average alleles per locus, and allelic richness was consistently higher in collections of ocean-type than collections of stream-type Chinook salmon (Table 2).

Table 2. Estimates of genetic diversity for 12 populations of Chinook salmon added to the genetic baseline.

Population	Sample size	Unbiased Hz	Obs Hz	Total allels	Avg alleles/locus	Allelic Richness
Johnson Cr.	144	0.777	0.776	209	16.1	14.1
Catherine Cr.	144	0.775	0.775	217	16.7	15.0
Newsome Cr.	144	0.768	0.760	207	15.9	14.4
Clearwater R.	144	0.855	0.854	298	22.9	20.5
Newsome Cr.	144	0.866	0.865	310	23.8	20.3
Pahsimeroi Hat.	144	0.778	0.788	192	14.8	13.6
Sawtooth Hat.	192	0.790	0.792	229	17.6	15.3
Dworshak Hat.	96	0.793	0.792	222	17.1	16.0
Clearwater Cr. stock	89	0.795	0.795	215	16.5	15.6
SF Clearwater R.	192	0.785	0.784	230	17.7	15.2
Lostine R.	118	0.754	0.761	180	13.8	12.9
Big Cr.	144	0.763	0.774	205	15.8	14.0
Average	1695	0.791	0.793	226.2	17.4	15.6

Tests of genetic differentiation with pairwise F_{ST} indicated that all collections were significantly different from one another ($P < 0.0008$) with the exception of natural origin fall Chinook from the Clearwater River and hatchery broodstock of fall Chinook from Nez Perce Tribal Hatchery ($P = 0.1412$). Overall, population relationships were consistent with expectations and suggest the data can be merged with the existing GAPS baseline.

Discussion

The objective of this project was to genotype 12 Chinook salmon populations with standardized microsatellite loci to extend the genetic baseline. We successfully completed this objective and closely followed the anticipated time schedule from the proposal. This additional data will now be incorporated into the genetic baseline with plans to post all baseline genotypes to an online database (currently under construction).

Quality Control

Genetic data was tested under standard quality control procedures in CRITFC's genetic laboratory. This includes confirmation of raw genotypes through repetitive genotyping, positive and negative controls, and automated allele conversion. Further, data was compared to initial baseline data generated in our laboratory to ensure consistency of new baseline data.

Project Benefits

This project was intended to enhance PSC genetic stock identification of mixed stock fisheries since this is essential information related to the Pacific Salmon Treaty. The baseline populations genotyped in this project add critical fall Chinook salmon populations from the Snake River, and remove erroneous stock assignments by including distinct populations. We expect the microsatellite baseline to be a long standing tool for genetic stock identification of mixed fisheries under the Pacific Salmon Treaty. All agencies and organizations that utilize the microsatellite baseline for genetic stock identification will

benefit from a more complete baseline. The CRITFC genetics lab is currently one of the members of the coast-wide Chinook salmon microsatellite standardization effort supported by the PSC.

Future directions for the Genetic Baseline

In order to achieve the high level of precision and accuracy desired for mixed stock analysis, many loci are required (Kalinowski 2004) and this can be done most cost effectively for the CTC using SNP markers in tandem with existing microsatellite data. Stock assignments and Full Parental Genotyping (FPG) will likely require a dramatic increase in the number of loci, although the actual number is still under investigation. In order to achieve incremental increases in discrimination power, we will need to add significantly more SNPs than the number of microsatellites now included in the current baseline data. In summary, SNP discovery efforts followed by baseline genotyping of these SNP markers will supplement the baseline to provide a significant increase in power for mixed fisheries analysis (Hankin et al. 2005).

References

Banks, M.A. 2005. Stock identification for the conservation of threatened or endangered species. *In*: Stock identification methods Eds: Cadrin, S.X., K.D. Friedland and J.R. Waldman. Elsevier Press. Pgs. 609-629.

Hankin, D.G., Clark, J.H., Deriso, R.B., Garza, J.C., Morishima, G.S., Riddell, B.E., Schwarz, C., Scott, J.B. 2005. Report of the expert panel on the future of the coded wire tag recovery program for Pacific salmon. Final Report to the Pacific Salmon Commission. 243pp. Available from: www.psc.org/info_codedwiretagreview.htm

Kalinowski, S. 2004. Genetic polymorphism and mixed stock fisheries analysis. Canadian Journal of Fisheries & Aquatic Sciences 61:1075-1082.

Moran, P., and 27 other authors. 2005. Interlaboratory Standardization of Coast-wide Chinook Salmon Genetic Data for International Harvest Management. LOA Technical Report prepared for the Pacific Salmon Commission Chinook Technical Committee. 44 pp.

Appendix 1. Chinook salmon populations originally included in baseline Version 1.1. Run time (Fa = Fall, Wi = winter, Su = summer, Sp = spring), hatchery (H) or wild (W) origin..

Region	Population	Run time[1]	Origin
Lower Columbia R. spring	Cowlitz H. spring	Sp	H
	Kalama H. spring	Sp	H
	Lewis H. spring	Sp	H
Lower Columbia R. fall	Cowlitz H. fall	Fa	H
	Lewis fall	Fa	W
	Sandy	Fa	W
Willamette River	McKenzie	Sp	H
	North Santiam	Sp	H
Mid Columbia R. tule fall	Spring Creek	Fa	H
Mid and Upper Columbia R. spring	Carson H.	Sp	H
	John Day	Sp	W
	Upper Yakima	Sp	H
	Warm Springs Hatchery	Sp	H
	Wenatchee spring	Sp	W
Deschutes River fall	Lower Deschutes R.	Fa	W
Upper Columbia R. summer/fall	Hanford Reach CR	Su/Fa	W
	Methow R. summer	Su/Fa	W
	Wells Dam	Su/Fa	H
Snake River fall	Lyons Ferry	Fa	W
Snake River spring/summer	Imnaha R.	Sp/Su	W
	Minam R.	Sp/Su	W
	Rapid River H.	Sp/Su	H
	Sesech R.	Sp/Su	W
	Tucannon	Sp/Su	H

Figure 1. Map indicating coastwide sample sites for populations included in the initial version 1.1 of the genetic baseline (from Moran et al. 2005).