Chinook Baseline Expansion with SNP Markers

Final Report to the Pacific Salmon Commission
March 14, 2011

by

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Summary:

The objective of this project was to genotype collections of Chinook salmon from populations in the Columbia River Basin using single nucleotide polymorphism (SNP) loci to add to the existing genetic baseline. Of the 96 loci employed, 75 have been adopted by the Genetic Analysis of Pacific Salmon (GAPS) consortium; GAPS is a multiple state and federal agency collaborative effort aimed at establishing a central data base for evaluating and characterizing salmon species including Chinook salmon from California to Alaska.

In this multi-year project, a total of 52 collections had previously been genotyped with a panel of 96 SNP markers. In the expansion effort for 2010, 32 collections were selected from areas in the Columbia River Basin represented with minimal coverage in the existing baseline from 2009. Of the total 2,640 samples analyzed in the 2010 project year, 1,373 (16 of 32 total collections) were processed with the support of Pacific Salmon Commission funding (Table 1). Among these current efforts each of the three major genetic lineages are represented with: 8 populations from the lower Columbia River, 6 from the interior ocean-type lineage (i.e., fall-run), and 18 from the interior stream-type lineage (i.e, spring-run). This genetic data will be added to the shared GAPS database for use by various agencies.

Introduction

The use of SNPs is becoming increasingly popular for population analyses that were previously dominated by (neutral) μSAT markers. Many studies have compared the relative utility of both marker types (Liu et al. 2005; Morin et al. 2009, Smith and Seeb 2008, Hess et al. 2010), and in most observations SNPs perform equal that of μSATs, though a larger number of SNP loci are necessary to reach the same level of resolution. The use of SNPs provides many advantages over μSAT's, in that they are more prolific in the genome, with greater coverage for linkage analyses (Moen et al. 2008). More importantly, because SNPs may be located within functional genes, they are candidates for detecting positive selection or selective divergence shaping population differences. Chinook salmon in the CRB have been studied in great detail (Waples et al. 2004; Beacham et al. 2006; Narum et al. 2010), and our efforts are likely to provide additional information that will benefit and expound on the characterization the species (Matala et al. 2010). SNP discovery and assay development has provided the resources (e.g., Campbell and Narum 2008a) to include additional genetic markers to the existing GAPS microsatellite baseline (Seeb et al. 2007). SNPs are relatively easily amplified and scored, even with poor quality tissue source or DNA extract (Campbell and Narum 2008b), and with advances in analysis platforms they are currently amenable to superior high throughput capabilities. Using this technology, 96 SNP markers were genotyped in 32 collections of Chinook salmon from near the Columbia River estuary to the Snake River; these will eventually be submitted to the GAPS baseline once it can accommodate such data. These additional markers are expected to greatly increase power for Genetic Stock Identification (GSI) of mixed stock fisheries. Of the total 2,640 samples analyzed in the 2010 project year, 1,373 (16 of 32 total collections) were processed with the support of Pacific Salmon Commission funding (Table 1). This study was intended to improve upon the existing genetic baseline, and follows from Recommendation 12 in the PSC Expert Panel Report (Hankin et al. 2005), that there is a need for support of an "immediate evaluation of a coordinated transition for all salmon species from GSI based on the use of microsatellite markers to GSI based on SNP markers."

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Current Project Objectives

The specific objective of this project was to genotype SNPs in multiple collections of Chinook salmon from the Columbia River to add to coastwide and regional genetic baselines. Current microsatellite markers do not adequately distinguish populations of summer/fall run Chinook salmon in the Columbia River, but inclusion of SNP markers used in tandem with existing microsatellite data will not only improve the resolution of stocks for GSI, but will also improve accuracy of stock assignments (Hess et al. 2010). Greater coverage of the entire Columbia River Basin will benefit evaluation of coastal mixed stock fisheries by providing an improved and more resolved genetic 'signature' of inclusive stocks and stock proportions.

SNP Genotyping Methods

Tissue samples from each individual were processed with Qiagen DNeasy® kits to extract DNA from fin clips stored in 100% ethanol. Isolated DNA from each sample was genotyped for 96 SNP markers (appendix 1) with Taqman chemistry (Applied Biosystems) and Fluidigm 96.96 dynamic array chips (reaction volumes of ~6nl) for SNP genotyping. Since genotyping in small reaction volumes reduces the average starting copy number to a range where genotyping accuracy becomes less reliable (Campbell and Narum 2008b), a pre-amplification protocol was used to increase the number of starting copies. The cycling regime and PCR conditions for the pre-amp step were as follows: one initial cycle of 95°C for 15 min, 14 cycles of 95°C for 15 seconds, 60°C for four minutes, and a final dissociation step. For each data collection run, a panel of 96 SNP loci were arrayed with 96 samples using a Fluidigm® microfluidic 96.96 chip (including one genotype indicator and one no-template control sample) to generate high throughput genotyping. Sample cocktails included: 3.4µl GTXpress Taqman (Applied Biosystems), 0.30µl GT load buffer (inclusing taq polymerase), 0.30µl H2O and 2.0µl pre-amp DNA template. Single SNP assays were prepared in a 5.0µl reaction mix (per sample), containing the following reagents: 2.5µl DA load buffer, 0.25µl Rox dye, 1µl H2O, and 1.25µl primer/probe. Microfluidic chips were loaded with assay cocktail dispensed at 4.5µl per well, and sample cocktail dispensed at 5.0µl per well. Chip loading and amplification was completed following standard manufacturers protocol on a Fluidigm IFC controller. Amplification conditions using a fast-cycling protocol were; 70°C for 30 min, 25°C for 10 minutes, and 95°C for one minutes, followed by 50 cycles of 95°C for 5 seconds, and 50°C for 25 seconds, and a final cool down step of 25°C for 10 minutes. Chips were imaged and scored on a Fluidigm EP1 imager using Fluidigm SNP Genotyping Analysis Software version 2.1.1. Carcass samples often provide poor quality and/or quantity of viable DNA relative to fresh tissue, and our final sample sizes were pared based on individual genotyping success.

Results & Discussion

In 2010, we genotyped 2,640 individual Chinook salmon from 32 collections in the Columbia River using 96 SNP loci. The SNP markers were variable throughout the region with an unbiased heterozygosity range of 0.0041-0.4772 (appendix 1).

Markers were tested for deviation from Hardy-Weinberg in each population with GENEPOP (Raymond and Rousset 1995). In some cases, deviations from Hardy-Weinberg expectations were shown to be population specific, indicating that the data representing these collections in the baseline is affected by how the collections were partitioned, temporal variations, laboratory errors, etc. These

along with some locus specific deviations were further scrutinized for possible null alleles. Some individuals and loci will ultimately be excluded from the baseline.

The collections in this project year combined with the 2009 effort represent the majority of populations from the Columbia River, with geographic coverage extending throughout the basin. However, future efforts to expand the number of loci employed and still finer geographic scale of inclusive collections will undoubtedly contribute to our ability to differentiate productivity units and population distinctions valuable for determining stock origins in downstream fisheries.

Quality Control

Genetic data was tested under standard quality control procedures in CRITFC's genetic laboratory. This includes confirmation of assay genotypes, repetitive genotyping, positive and negative controls, and automated allele conversion. Successful genotyping for a given sample was defined proportionally as less than 10% missing data (i.e. fewer than nine SNP loci for Chinook salmon).

Project Benefits / Monitoring and Evaluation

The objective of this study was intended to improve both regional and coastwide GSI applications. The additional SNPs that were genotyped (beyond the GAPS 75) will benefit all agencies as these markers are available for the entire genetics community.

Acknowledgements

We thank Vanessa Morman and Jeff Stephenson for genotyping effort and assistance in the genetics lab. Samples and DNA for genotyping were provided by multiple agencies including NOAA, IDFG, WDFW, and USFWS.

Table 1. Columbia River collections of Chinook salmon genotyped with SNP markers in 2010 for baseline expansion. Collections analyzed with Pacific Salmon Commission funding are identified (**PSC**).

Collection (map) ID	BPA Region	(n)	Lat	Long	Lineage	Run	Origin	Year	Age
01). White Salmon R.	Big White Salmon	93	45.744	-121.525	LC	spring/fall	NOR	2008	juv
02). Cowlitz R. **(PSC)**	Cowlitz	92	46.513	-122.635	LC	spring	HOR	2004	adult
03). North Fork Lewis R. **(PSC)**	Lewis	85	45.867	-122.724	LC	late fall bright	NOR	2004	adult
04). North Fork Lewis R. **(PSC)**	Lewis	94	45.867	-122.724	LC	early fall bright	NOR	2004	adult
05). Sandy R. **(PSC)**	Sandy	92	45.563	-122.395	LC	spring	NOR	2006	adult
06). Sandy R.	Sandy	112	45.563	-122.395	LC	fall	NOR	2002	adult
07). Kalama R. **(PSC)**	Kalama	90	46.017	-122.733	LC	spring	HOR	2004	adult
08). Elochoman R.	Elochoman	86	46.261	-123.298	LC	fall	NOR	1995-97	adult
09). Tumwater & Dryden **(PSC)**	Wenatchee	93	47.542	-120.559	OT	summer	NOR	1993	adult
10). Lower Yakima R. **(PSC)**	Yakima	62	46.312	-119.473	OT	fall	NOR	1998	adult
11). White Salmon R.	Big White Salmon	91	45.744	-121.525	OT	fall	NOR	2008	juv
12). Entiat R.	Entiat	64	47.696	-120.321	OT	summer	NOR	2008	adult
13). Little White Salmon R.	Little White Salmon	94	45.722	-121.641	OT	fall	HOR	2007	Juv
14). Lower Crab Creek	Crab	93	46.828	-119.874	OT	fall	NOR	2009	adult
15). Middle Fork John Day R. **(PSC)**	John Day	91	44.913	-119.301	ST	spring	NOR	2006	adult
16). North Fork John Day R.	John Day	111	45.012	-119.007	ST	spring	NOR	2006	adult
17). Leavenworth-NFH **(PSC)**	Wenatchee	93	47.559	-120.672	ST	spring	HOR	2005	adult
18). Cle Elum R. **(PSC)**	Yakima	90	47.178	-120.999	ST	spring	HOR	1997	?
19). Shitike Creek **(PSC)**	Deschutes	93	44.764	-121.238	ST	spring	NOR	2004	juv
20). Peshastin Creek **(PSC)**	Wenatchee	87	47.558	-120.575	ST	spring	NOR	2005	juv
21). Entiat R. **(PSC)**	Entiat	93	47.696	-120.321	ST	spring	NOR	2006	juv
22). American R. **(PSC)**	Yakima	78	46.976	-121.158	ST	spring	NOR	2003	adult
23). Warm Springs R.	Deschutes	94	44.861	-121.244	ST	spring	HOR	2004	adult
24). Little White Salmon R. **(PSC)**	Little White Salmon	92	45.722	-121.641	ST	spring	HOR	2007	juv

25).	Chamberlain Creek	Salmon	45	45.454	-114.933	ST	spring	NOR	2009	juv
26).	Wenaha R. **(PSC)**	Grande Ronde	48	45.946	-117.455	ST	spring	NOR	2006	juv
27).	John Day R.	John Day	119	44.76	-119.65	ST	spring	NOR	2000	juv/adult
28).	Chiwawa R.	Wenatchee	44	47.789	-120.659	ST	spring	HOR	2000	adult
29).	Lostine R. weir	Grande Ronde	56	45.535	-117.451	ST	early spring	HOR	2009	adult
30).	Lostine R. weir	Grande Ronde	56	45.535	-117.451	ST	late spring	HOR	2009	adult
31).	Lostine R. weir	Grande Ronde	57	45.535	-117.451	ST	early spring	NOR	2009	adult
32).	Lostine R. weir	Grande Ronde	52	45.535	-117.451	ST	late spring	NOR	2009	adult

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Appendix 1. List of a) 96 SNP loci and b) descriptive statistics for 32 Chinook salmon collections. Column headings are: (n) mean sample size by locus or population, (A) mean number of observed alleles, (He) Expected Heterozygosity, (Ho) Observed Heterozygosity, (F_{is}) Fixation Index, ($F_{st\ (mean)}$) among-collection variation per locus, (# Dev.) number of populations or loci that deviate from HWE, and (%P) percentage of polymorphic loci.

a)

SNP locus	(n)	Ho	He	F_{is}	# Dev.	$F_{st\ (mean)}$
Ots-102414-395	81.8	0.4854	0.4645	-0.0451		0.0685
Ots-105105-613	81.8	0.4018	0.3933	-0.0214		0.1950
Ots-106747-239	81.2	0.4762	0.4526	-0.0522		0.0619
Ots-110064-383	82.1	0.4092	0.4161	0.0165		0.1673
Ots-nramp-321	82.3	0.1211	0.1512	0.1989	(9)	0.6849
Ots-113242-216	81.3	0.3303	0.3286	-0.0053		0.1835
Ots-113457-40R	82.0	0.3080	0.3074	-0.0018		0.3366
Ots-123048-521	82.2	0.0977	0.0992	0.0151		0.0734
Ots-128757-61R	82.0	0.3130	0.3103	-0.0085		0.1543
Ots-94857-232R	81.9	0.4779	0.4567	-0.0464		0.0789
Ots-94903-99R	82.2	0.4528	0.4579	0.0112		0.0826
Ots-96222-525	82.4	0.2692	0.2818	0.0450		0.2229
Ots-96500-180	81.7	0.4226	0.4285	0.0139		0.1274
Ots-96899-357R	82.3	0.1170	0.1211	0.0338		0.0729
Ots-97077-179R	82.3	0.1901	0.1959	0.0299		0.2179
Ots-AldB1-122	81.8	0.2023	0.2097	0.0357		0.0569
Ots-aldb-177M	78.7	0.2312	0.2246	-0.0295		0.2604
Ots-ARNT	82.1	0.2361	0.2387	0.0109	(4)	0.5178
Ots-arp-436	79.4	0.2070	0.2059	-0.0054		0.3289
Ots-AsnRS-60	82.1	0.3065	0.3018	-0.0156		0.0425
Ots-aspat-196	82.2	0.1430	0.1412	-0.0130		0.1668
Ots-C3N3	82.3	0.0000	0.2380	1.0000		0.3972
Ots-Cath_D141	82.3	0.0377	0.0392	0.0386		0.0405
Ots-CCR7	82.3	0.0430	0.0431	0.0034		0.0739
Ots-CD59-2	82.2	0.4604	0.4589	-0.0033		0.0245
Ots-CD63	82.2	0.3034	0.2965	-0.0235		0.2461
Ots-cox1-241	82.1	0.2481	0.2695	0.0793		0.4510
Ots-CRB211	81.9	0.0409	0.0464	0.1192		0.0564
Ots-E2-275	82.0	0.3913	0.4084	0.0418		0.1800
Ots-EndoRB1-486	77.6	0.2195	0.2603	0.1568	(10)	0.0995
Ots-EP-529	82.1	0.1067	0.1037	-0.0291		0.0377
Ots-ETIF1A	82.1	0.3875	0.3820	-0.0146		0.2346
Ots-FARSLA-220	81.9	0.1246	0.1466	0.1500	(4)	0.6957
Ots-FGF6A	82.0	0.3850	0.3838	-0.0034		0.1278

Ots-FGF6B_1	81.8	0.4116	0.4104	-0.0031		0.1766
Ots-GDH-81x	82.0	0.3759	0.3936	0.0449		0.1063
Ots-GH2_1	82.4	0.0303	0.0298	-0.0175		0.0200
Ots-GnRH-271	82.1	0.0146	0.0143	-0.0219		0.0142
Ots-GPDH-338	82.4	0.0408	0.0419	0.0259		0.0249
Ots-GPH-318	82.4	0.2002	0.2050	0.0236		0.0720
Ots-GST-207	82.4	0.0916	0.0930	0.0149		0.0624
Ots-GST-375	82.5	0.0195	0.0205	0.0497		0.0371
Ots-GTH2B-550	81.9	0.3339	0.3522	0.0519		0.2443
Ots-hsc71-3'-488	81.8	0.2915	0.2993	0.0262		0.4010
Ots-hsc71-5'-453	81.9	0.2600	0.2696	0.0354		0.2491
Ots-hsp27b-150	82.3	0.2342	0.2357	0.0063		0.1555
Ots-HSP90B-100	82.3	0.2293	0.2467	0.0705		0.5021
Ots-IGF-I.1-76	82.5	0.1534	0.1529	-0.0034		0.1088
Ots-Ikaros-250	82.0	0.2126	0.2299	0.0752		0.5351
Ots-IL11	82.4	0.1067	0.1033	-0.0329		0.0831
Ots-IL8R_C8	82.2	0.2965	0.3024	0.0195		0.3036
Ots-mapK-3'-309	82.0	0.4484	0.4665	0.0389		0.0625
Ots-mapKpr-151	81.5	0.2803	0.2965	0.0547		0.1174
Ots-MHC1	82.2	0.2129	0.2375	0.1035	(4)	0.4646
Ots-MHC2	81.9	0.3735	0.3627	-0.0297		0.1167
Ots-mybp-85	82.2	0.3140	0.3185	0.0139		0.2615
Ots-Myc-366	82.3	0.0103	0.0106	0.0285		0.0185
Ots-myo1a-384	82.3	0.2005	0.2094	0.0429		0.1161
Ots-myoD-364	82.0	0.2928	0.2969	0.0137		0.3341
Ots-nkef-192	82.0	0.2766	0.2872	0.0371		0.3597
Ots-NOD1	81.7	0.3100	0.3138	0.0119	(4)	0.3588
Ots-LWSop-638	82.4	0.0471	0.0488	0.0355		0.0898
Ots-Ots311-101x	79.3	0.1849	0.1892	0.0228		0.3484
Ots-P450	82.4	0.1485	0.1724	0.1382	(6)	0.6403
Ots-P53	82.0	0.3893	0.3803	-0.0234		0.1468
Ots-PGK-54	82.3	0.2299	0.2432	0.0544		0.4321
Ots-Prl2	82.1	0.4348	0.4184	-0.0392		0.1532
Ots-RAG3	81.9	0.3280	0.3369	0.0264		0.3091
Ots-RAS1	82.3	0.0112	0.0115	0.0277		0.0248
Ots-RFC2-558	82.2	0.2264	0.2284	0.0089		0.3116
Ots-S7-1	81.9	0.4237	0.4309	0.0167		0.0910
Ots-SClkF2R2-135	82.0	0.4207	0.4202	-0.0011		0.0277
Ots-SL	82.3	0.1494	0.1751	0.1471		0.6253
Ots-SWS1op-182	82.1	0.4206	0.4055	-0.0374		0.1882
Ots-TAPBP	82.0	0.3313	0.3381	0.0202		0.3232
Ots-TGFB	82.3	0.1954	0.1941	-0.0066		0.0967

Ots-TLR3	82.0	0.3642	0.3714	0.0196		0.2556
Ots-TNF	79.5	0.0368	0.0344	-0.0676		0.6430
Ots-Tnsf	82.2	0.2949	0.3066	0.0383		0.1470
Ots-u07-07.161	81.7	0.4845	0.4778	-0.0140		0.0431
Ots-u07-17.135	82.3	0.1416	0.1423	0.0052		0.0576
Ots-u07-18.378	82.2	0.3046	0.3008	-0.0125		0.1852
Ots-u07-20.332	82.4	0.0522	0.0517	-0.0087		0.0652
Ots-u07-25.325	81.7	0.2779	0.2789	0.0035		0.2380
Ots-u07-49.290	81.9	0.4143	0.4232	0.0212		0.1199
Ots-u07-53.133	82.0	0.3057	0.3160	0.0326		0.2682
Ots-u07-57.120	81.8	0.1709	0.1907	0.1038	(5)	0.6124
Ots-u07-64.221	82.5	0.0053	0.0052	-0.0238		0.0206
Ots-u202-161	81.8	0.2304	0.2588	0.1099	(4)	0.4242
Ots-u211-85	82.3	0.2905	0.3055	0.0491		0.3141
Ots-u4-92	82.1	0.1093	0.1178	0.0719		0.0632
Ots-u6-75	82.1	0.1334	0.1359	0.0182		0.0334
Ots-unk526	81.8	0.2627	0.2636	0.0033		0.0512
Ots-zP3b-215	82.4	0.0003	0.0003	-0.0055		0.0053
Ots-ZR-575	81.9	0.2096	0.2495	0.1599	(8)	0.4933
Ots-SEXY1	77.9	0.4437	0.3331	-0.3320	(11)	0.0352
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All	81.9	0.2442	0.2502	0.0305		0.2079

b)

Chinook salmon by population

Population	(n)	Ho	He	F_{is}	# Dev.	%P
01). White Salmon R.	92.8	0.2270	0.2300	0.0558	(7)	0.9063
02). Cowlitz R.	91.7	0.2680	0.2706	0.0137		0.8854
03). North Fork Lewis R.	84.3	0.2857	0.2897	0.0122		0.9063
04). North Fork Lewis R.	93.4	0.2859	0.2875	0.0116		0.9583
05). Sandy R.	91.1	0.2653	0.2893	0.0854	(9)	0.9479
06). Sandy R.	111.4	0.2853	0.2901	0.0131		0.9479
07). Kalama R.	89.1	0.3040	0.3152	0.0370	(4)	0.9583
08). Elochoman R.	85.2	0.2636	0.2751	0.0484	(5)	0.9375
09). Tumwater & Dryden	92.6	0.2439	0.2504	0.0114		0.9375
10). Lower Yakima R.	61.3	0.2550	0.2632	0.0312	(10)	0.9167
11). White Salmon R.	90.5	0.2546	0.2630	0.0193	(4)	0.9271
12). Entiat R.	62.9	0.2388	0.2994	0.1938	(22)	0.9271
13). Little White Salmon R.	93.9	0.2539	0.2752	0.1114	(15)	0.9583

14).	Middle Fork John Day R.	89.8	0.2278	0.2332	0.0461		0.8750
15).	North Fork John Day R.	109.4	0.2313	0.2353	0.0247		0.8854
16).	Leavenworth-NFH	92.4	0.2297	0.2234	-0.0077		0.8646
17).	Cle Elum R.	89.6	0.2744	0.2734	0.0006		0.8542
18).	Shitike Creek	92.8	0.2269	0.2231	0.0020		0.8229
19).	Peshastin Creek	86.8	0.2270	0.2267	0.0271	(6)	0.8125
20).	Entiat R.	92.9	0.2327	0.2609	0.1397	(15)	0.9271
21).	American R.	76.6	0.2155	0.2147	-0.0101		0.7917
22).	Warm Springs R.	93.6	0.2379	0.2351	-0.0022	(4)	0.8854
23).	Little White Salmon R.	91.8	0.2302	0.2319	0.0154		0.8333
24).	Chamberlain Creek	44.9	0.1843	0.1781	-0.0334		0.6458
25).	Wenaha R.	47.9	0.2475	0.2641	0.0838		0.8646
26).	John Day R.	118.3	0.2416	0.2490	0.0494	(7)	0.8646
27).	Chiwawa R.	43.7	0.2204	0.2176	-0.0105		0.8229
28).	Lostine R. weir	55.2	0.2279	0.2229	-0.0090		0.8125
29).	Lostine R. weir	55.9	0.2116	0.2178	0.0186		0.8229
30).	Lostine R. weir	56.6	0.2227	0.2216	0.0376	(6)	0.8229
31).	Lostine R. weir	51.9	0.2422	0.2301	-0.0138	(5)	0.8854
32).	Lower Crab Creek	89.8	0.2514	0.2492	-0.0135		0.8542
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	Mean	81.9	0.2442	0.2502	0.0326		0.8770