Interlaboratory Standardization of Coast-wide Chinook Salmon Genetic Data for International Harvest Management

Final Report

A progress report from the Genetic Analysis of Pacific Salmonids (GAPS) consortium[1] to the Chinook Technical Committee of the Pacific Salmon Commission, FY2004, FY2005
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[1] Only a single representative from each laboratory is listed here due to the very large number of authors and participants in this study. For a complete list, please see Appendix 4.

Abstract

This report describes the results of a 2-year project to create a coast-wide microsatellite database for use in Chinook salmon harvest management. The most important feature of this data set, beyond its geographical breadth, is the fact that these microsatellite markers were standardized among nine principal salmon genetics laboratories on the West Coast of North America. Thus, all participating laboratories can contribute to and use these data. This collaboration involved representatives of tribal, university, state, and federal agencies. Multinational participation in this effort is a testament to the importance of standardizing genetic data for international fishery management. Our goal was to provide a tool that would augment the current CWT program and especially to overcome problems related to total marking of hatchery fish and the relative inability to monitor wild stocks. The standardization phase of this project demonstrated consistent genotyping among laboratories despite a number of errors that were revealed. Results of population genetic analysis of the new baseline data were highly concordant with previous allozyme studies and strongly supported the genetic reporting groups recognized earlier. Simulated mixture analyses suggest that this new data set has remarkable power for accurate estimates of stock contributions to mixed-stock, Pacific Salmon Treaty fisheries. Indeed, 32 of 41 reporting groups partitioned more than 95% of 100% simulated "mixtures" to the correct group. Anticipated applications of this new management tool include independent stock composition estimates for multiple Pacific Salmon Treaty fisheries, improved evaluation of index stocks used to estimate wild stock contributions, and a wide range of other research, conservation, and recovery efforts.

Introduction

Programmatic background

The Pacific Salmon Treaty was ratified in 1985 and renegotiated in 1999 between the United States and Canada. Through the Treaty, the two nations agreed to cooperate in the management, research, and enhancement of Pacific salmon. Pacific salmon migrate long distances during their marine period and are routinely intercepted in fisheries beyond the jurisdiction of the government in whose waters they spawn. The Pacific Salmon Treaty through the Pacific Salmon Commission (PSC) serves as a means to coordinate management of the salmon resource and conduct conservation actions as required.

Chinook salmon are harvested throughout the year by commercial and sport fishers in the waters of Southeast Alaska, British Columbia, and the Pacific Northwest. Fisheries typically harvest highly mixed stocks of Chinook salmon and are therefore under the jurisdiction of the Pacific Salmon Treaty. Quotas are specified by the PSC and are dependent on the projected abundance of Chinook salmon forecasted by the Chinook Technical Committee (CTC) using the Chinook salmon model. The Chinook salmon model uses catch, escapement, coded-wire tag recovery, and recruitment information to forecast relative abundance in treaty fisheries. Stock composition estimates from coded-wire tag data and the Chinook salmon model may not be completely reliable; they rely on

data that are subject to error from lack of coded-wire tags on all stocks contributing to the fishery or poor estimates of escapement or terminal run size. Further, how well the index stocks within the model accurately represent wild stocks is unknown. An independent mechanism to estimate stock composition and the validity of the index stock approach is therefore an important priority.

History and need for Genetic Stock Identification

Genetic stock identification methods can provide stock-specific catch estimates, and this information can be used as an independent mechanism to validate the performance of the model and to assess the ability of the various index stocks to accurately represent the contribution of wild populations. Beginning in the 1980's, genetic analyses using an allozyme database were used extensively to estimate the stock contribution of fisheries in the Columbia River, coastal Washington, and Strait of Juan de Fuca (e.g., Marshall et al. 1991; Miller et al. 1993; Shaklee et al. 1999). Collaborative work by multiple state, provincial, and federal agencies led to the establishment of a shared and standardized coast-wide database (Shaklee and Phelps 1990). During the 1990s, effort was directed towards enlargement of the database, particularly to include populations from central British Columbia and Alaska (e.g., Seeb et al. 1995; Teel et al. 2000; Crane et al. 2000). Funding for this database expansion was provided in part by the US Chinook Technical Committee (Crane et al. 2000). The current allozyme database grew to include comprehensive coverage of populations ranging from California through Alaska with representative populations from Russia (Teel et al. 1999).

Although extremely comprehensive, and used by multiple laboratories, a number of limitations led researchers to replace the standardized allozyme database with a comparable DNA baseline. Limitations of allozymes included the requirements for lethal sampling, fastidious cryopreservation, and a relatively limited number of loci with modest levels of variability. Markers based on DNA have also demonstrated utility for resolving Chinook population structure and for conservation applications (Scribner et al. 1998; Banks et al. 2000; Banks 2005). The most widely used DNA markers have been microsatellites and baselines evolved rapidly within single laboratories. The large number of available microsatellite loci resulted in little overlap among researchers (see below). Microsatellite markers have proven their effectiveness, yet the lack of standardization among laboratories had become a significant limitation to full implementation as a tool for international fishery management.

Standardization of Chinook salmon microsatellite markers

Two principal challenges were faced at the outset for this project. First, very few laboratories were using common genetic markers (microsatellite loci) (Fig. 1A). Without a common set of markers there can be no standardization and very limited comparison among studies from different laboratories. Second, microsatellite alleles are identified based on size. Electrophoretic instruments used to size fragments are extremely precise but not necessarily very accurate (LaHood et al. 2003; Moran et al. 2006). Absolute size estimates among laboratories using different electrophoretic instruments may differ by 2 or 4 or more base pairs (Fig. 1B), although run-to-run variation within instruments is tenths of a base pair.

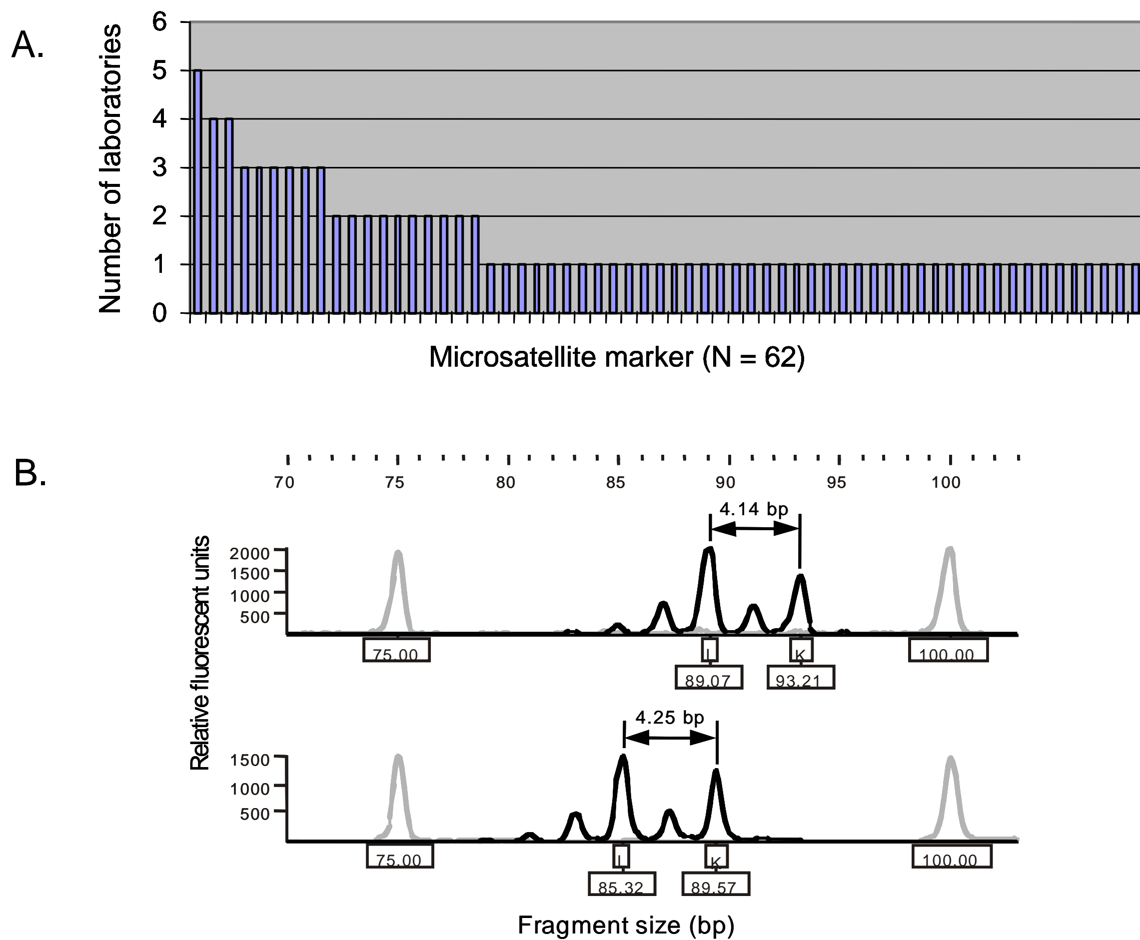

Figure 1. At the beginning of this project most markers were being assayed routinely in only a single laboratory (A.)—indeed only 3 loci were used in more than half of the labs. The "electropherograms" in the lower panel (B.) represent genotypes for the exact same Chinook salmon individual assayed on two different instruments. The same size standard and sizing algorithm were used, yet dramatic differences in size are seen that relate to fundamentally different physics between platforms (slab gel versus capillary). The figure illustrates a significant difference in the estimated size of the alleles, in addition to a different relative size of the repeat unit (i.e., 4.14 versus 4.25 bp). Comparison of fragment sizes among laboratories is complicated by the fact that genotyping instruments are extremely precise, but not necessarily very accurate.

This study reports the development and evaluation of a standardized microsatellite database for Chinook salmon for use within the PST area. Construction and evaluation of the database were carried out through the collaborative efforts of a multi-agency work group, the Genetic Analysis of Pacific Salmonids collaborators (GAPS). Guiding principles included that the databases would be: 1) subject to review by scientists from all interested agencies, 2) freely available to all researchers managing or studying Chinook salmon, 3) covering the range of Chinook salmon at a geographic scale appropriate to the management objectives of the PSC, 4) dynamic in terms of adding new baseline populations and new genetic markers, and 5) sufficiently documented and organized to facilitate the "certification" of data from new laboratories. This database can provide independent fishery estimates for management actions or comparison to the CTC model.

In addition, the database will allow for a wide variety of additional management and research applications on all life history stages over much of the species range in both the freshwater, marine, and estuarine environments

Materials and Methods

Locus selection

From the original field of 63 microsatellite loci used most widely in the GAPS laboratories, 13 were selected for coast-wide interlaboratory standardization (Table 1). The markers were chosen based primarily on the robustness and reliability of the polymerase-chain-reaction amplification (PCR) and genotyping under differing laboratory conditions.

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	Greig et al. 2003	CRITFC
Ots211	F - TAGGTTACTGCTTCCGTCAATG R - GAGAGGTGGTAGGATTTGCAG	Greig 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	Greig 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.

No effort was made to standardize laboratory methods, chemistry, PCR amplification, or electrophoresis. Instead individual laboratories were encouraged to use the methods to which they were accustomed, and standardization focused only on the final genotype. Details of individual microsatellite loci were recorded in the "Curator Documents," materials that describe the original literature citation, Genbank accession number, primer sequence, successful PCR and electrophoresis conditions, number of alleles and size range, example images (electropherograms), heritability information, and general comment about robustness. Curator documents are currently hosted by ADFG.

Standardized genotyping

Interlaboratory genotyping standardization was achieved through exchange of reference samples for each locus that represented all known Chinook salmon microsatellite alleles ("sanctioned" by individual laboratories serving as "Locus Curators"). For every sanctioned allele, there is a specific fish DNA sample that is identified as the "holotype" (the first documented observation of a given allele in the course of this study). Following the same taxonomic convention, other DNA samples were identified as paratypes, alleles assumed to be identical to the holotype.

Two blind tests of genotyping concordance among laboratories provided a measure of success in standardization. Concordance of scores across laboratories or "percent accuracy" for each locus for each laboratory was based on the mode allele score. Percent accuracy took into account only the scored data relative to the mode allele score (i.e., missing data were not included in the calculation). This measurement of accuracy has limitations such as the possibility of samples with two modes or samples with modes of zero (failed reaction, no data). It is also possible that the mode is incorrect and that labs with outlier scores analyzed the samples correctly. In practice, these situations were rare, and percent accuracy is a reasonable measurement of data standardization across laboratories.

Performance on the blind tests also served to "certify" laboratories to contribute data to the CTC microsatellite baseline. Certain criteria were established to meet certification including at least 95% overall genotyping accuracy (see above), no lower than 90% accuracy at any given locus and no more than 25% missing data (relaxed on Blind Test 2 because of DNA quality, see Results). Blind test samples (i.e., unknown genotypes) came from diverse mixed fisheries (west coast of Vancouver Island and southeast Alaska) and included both known and unknown alleles. Samples were genotyped by individual laboratories without consultation and the data submitted to one of the collaborators to hold results in confidence until all data were in. Following evaluation of the blind results, attention focused on interlaboratory concordance values that fell below the certification criteria set by the group. Results of Blind Test 2 were reinterpreted after the correction of two critical record-keeping errors that most dramatically affected the overall

concordance. Thus, although the initial test phase was blind, the reconciliation phase that followed Blind Test 2 was no longer blind (see Results). Additional, less significant errors were also corrected if it was believed that similar errors had also been corrected and would not recur in the baseline data.

Baseline construction

In the second year of this project, data provided by the GAPS collaborators were combined to create a large baseline data set suited to mixed stock analysis of fisheries managed under the PST. 220 sample collections totaled 16,394 individual fish and represented 110 putative populations (Appendix 1 lists baseline populations with region, runtime and other collection data). All individual fish were assayed across all 13 loci; however, PCR failures at particular loci sometimes resulted in incomplete multilocus genotypes. A target was set of 144 individuals per population with at least 120 genotypes per locus. In some cases, more than 144 individuals were assayed to obtain 120 genotypes at most loci in most populations. In some populations, however, fewer than 120 individuals were available, so sample sizes were reduced accordingly. The number 144 individual fish per population and subsequently 120 genotypes per locus represented a compromise. Some collaborators, accustomed to allozyme variation, felt baseline samples needed to be >200, whereas others felt baseline samples of 48 – 96 were adequate, based on simulations and empirical results of microsatellite analyses.

Baseline populations were selected to represent all recognized genetic lineages of Chinook from the southern end of the species range north to Southeast Alaska with focus on major production areas and likely contributors to Pacific Salmon Treaty fisheries (Fig. 2). Laboratories distributed the genotyping effort on a regional basis, such that most samples analyzed in a given laboratory came from the geographic region of their most immediate interest. Baseline data were compiled by a single collaborator (P. Moran, NWFSC) to facilitate error trapping, corrections, and version control. The latest working copy of the database was kept posted to a server available to all the collaborators. Different versions of the baseline were carefully identified to assure standardized analyses among laboratories.

The hope for the baseline populations was that these sites would become reference populations or genetic index sites for ongoing studies, including expanded temporal sampling and characterization of new genetic markers. Most DNA samples that were used for baseline genotyping were distributed among all the GAPS collaborators. In some cases DNAs were in limited quantity and were depleted, but all laboratories cooperated in the so called “megaswap,” and, with the exception of those limited samples that are now slated for replacement, a nearly complete set of samples is available to each lab.

Figure 2. Collection locations for Chinook salmon populations that contributed to the CTC baseline (populations are listed by reporting group in Appendix 1).

The first step in analysis of this multi-laboratory data set was a preliminary round of population genetic analysis to identify potential errors, problem samples, or problem loci (these loci were well-vetted by this time, but it was still possible that problems might crop up in new regions, e.g., null alleles, imperfect and single-base repeats, or other anomalies that might complicate standardized genotyping). Descriptions of typical quality control (QC) measures employed in individual laboratories are provided in Appendix 2. QC analyses for the combined data presented here focused on examination of outliers for genetic distance or other population genetic metrics and parameter estimates. Populations that showed significant heterozygote deficits or clustered in an unexpected way were singled out for extra scrutiny. Similarly, each locus was examined both within and across populations for pervasive or extreme departures from expected genotypic frequencies (Hardy-Weinberg expectations).

After correction of two obvious record-keeping errors (see Results), data analysis proceeded on two separate tracks. First, descriptive population genetic analyses were conducted to evaluate expectations and further elucidate Chinook population genetic structure, demographics, and evolutionary history (including estimation of fixation indices, genetic distance and diversity estimates, and multivariate analysis). This analysis of basic biology reinforces and provides a foundation for the second aspect of our data analysis; simulations for power analysis of mixed-stock fisheries. A first step in the power analysis was the delineation and scaling of reporting groups. The reporting groups are the genetic/regional aggregates of population samples that represent putative contributors to the mixed-stock fisheries of interest.

The goals of our preliminary analyses were to determine how well the microsatellite data reflected the known population genetic structure of Chinook salmon estimated from several decades of allozyme analysis and more recent DNA-based studies of Chinook salmon population structure, as well as how the accuracy and bias of the two techniques compare. New information or unexpectedly high or low power to apportion mixtures might require adjustment of the reporting groups.

Mixture simulations

Reporting groups were defined based on a combination of genetic similarity, geographic features and management applications. The results of previously described analyses of population structure and genetic similarity formed the basis of developing reporting groups. In general, these reporting groups (Table 4, Appendix 1) are similar to the groups defined for use with the coast-wide allozyme baseline (Teel et al. 2000); changes consist of increasing the resolution of stock estimates by splitting large groups based on general locations, behavioral traits, and genetic similarity, into smaller groups based on more specific biological and management distinctions (see Discussion).

The utility of these reporting groups for genetic stock identification of mixtures of Chinook salmon harvested in Treaty fisheries was evaluated through a series of simulations. These simulations were designed to assess whether the baseline of microsatellite allele frequencies provides sufficient information to identify stocks or stock groups (reporting groups) in hypothetical mixtures. Simulations were performed using

the Statistical Package for Analyzing Mixtures (SPAM version 3.6, Debevec et al. 2000; Reynolds 2002) to estimate the composition of a hypothetical mixture of predetermined stock proportions. This process involved 1000 iterations during which the mixture genotypes and baseline frequencies were randomly generated for each iteration from the known baseline allele frequencies assuming Hardy-Weinberg equilibrium. Mean estimates of mixture proportions and 90% confidence intervals were derived from the results of 1000 iterations. The lower and upper bounds of the confidence intervals were determined by sorting the estimates and selecting the 51st and 950th result.

The first set of simulations involved mixtures (N = 400) entirely composed (100%) of a single reporting group; repeated for each reporting group. When a reporting group mixture was simulated, all baseline populations in the group contributed equally to the mixture. Reporting groups with mean correct estimates of 90% or better are considered highly identifiable in potential mixtures from Treaty fisheries. Reporting groups with mean correct estimates lower than 90% can still be considered identifiable in mixtures, but sources of misallocation should be considered when interpreting the results.

In addition to the 100% simulations, three additional simulations were run in which the mixture compositions were similar to proportions potentially seen in Treaty fisheries. Stock group proportions were selected based on stock compositions observed in the Southeast Alaska troll fishery (Templin et al. 2005), Strait of Juan de Fuca net fishery (Marshall et al. 1991), and Columbia River net fishery (Shaklee et al. 1998). All populations in each reporting group contributed equally to the makeup of the group contribution. Mean estimated allocation to stock group provided an indication of the ability for mixed stock analysis using this baseline in non-homogenous mixtures.

Results

This study succeeded in creating a powerful baseline data set for partitioning groups of fish taken in mixed-stock harvest in all Pacific Salmon Treaty fisheries. The genotyping and allele designations are standardized across laboratories so that data can be combined seamlessly, and documentation was created to facilitate the standardization of new laboratories. Preliminary analyses showed that the results are highly consistent with previous genetic data and offer further resolution of fine scale relationships. Mixture simulations demonstrated excellent power even relative to much larger allozyme data sets (i.e., with many more populations and individuals).

Standardization

Overall, interlaboratory genotyping concordance in the most recent blind test (Blind Test 2) was 95.5% (Table 2A); however a number of low-concordance values for specific loci immediately revealed important errors in the data from two of the laboratories. One lab obtained a concordance of only 44% for *Ogo*4, whereas other loci in the same lab were 98% or more. Another lab had 0% concordance at six loci. Again, data for other loci from the same lab were highly concordant and close to the overall average. Cursory checks in respective laboratories immediately revealed record-keeping errors that explained most of the discrepancies. The first lab had inadvertently used an outdated set

of bin definitions (allele size categories) that resulted in incorrect allele names being exported. The second lab used a set of lookup tables that were mismatched to the primers used for amplification of those six loci. This error resulted in genotypes that were perfectly concordant except six base pairs larger. After correction of these two errors the overall concordance for the GAPS labs at these loci was over 99.3%. Other labs also made more subtle errors in bin definitions for less common alleles, manual transcription errors, and other record-keeping errors that are now corrected and not expected to be an issue in the data that were submitted to the baseline. Errors such as upper allele drop out and unexplained genotype mismatches were not corrected because such errors are expected to creep into the baseline data themselves (see Appendix 3 for a complete explanation of errors from individual laboratories).

Table 2. Proportional genotyping accuracy by laboratory and locus for Blind Test 2, including averages across loci and laboratories. A. Results as submitted. B. Results after correction for record-keeping errors in Lab 1 and Lab 6 (see text).

A) Initial submission

Locus	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Average
Ogo2	0.987	1.000	1.000	1.000	0.993	0.000	1.000	0.993	1.000	0.886
Ogo4	0.439	1.000	1.000	0.995	1.000	0.000	0.995	0.994	0.990	0.824
Oki100	0.978	1.000	1.000	1.000	1.000	1.000	1.000	0.970	1.000	0.994
OMM1080	1.000	1.000	0.995	1.000	1.000	0.000	1.000	0.994	1.000	0.995
Ots201b	0.984	1.000	1.000	1.000	1.000	0.993	0.995	0.985	1.000	0.990
Ots208b	0.994	1.000	1.000	1.000	1.000	1.000	0.995	0.970	0.995	0.996
Ots211	1.000	1.000	0.994	1.000	0.993	0.955	0.994	0.985	0.994	0.884
Ots212	0.989	1.000	1.000	1.000	1.000	0.989	0.995	0.994	1.000	0.886
Ots213	0.987	1.000	0.982	1.000	0.985	0.000	1.000	1.000	1.000	0.999
Ots3M	1.000	1.000	0.988	0.994	1.000	0.000	1.000	1.000	0.995	0.980
Ots9	1.000	1.000	1.000	1.000	1.000	0.823	1.000	1.000	1.000	0.991
OtsG474	0.995	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999
Ssa408	0.987	0.929	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.991
Average	0.949	0.995	0.997	0.999	0.998	0.597	0.998	0.991	0.998	0.955

B) Partially corrected

Locus	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Average
Ogo2	0.987	1.000	1.000	1.000	0.993	0.988	1.000	0.993	1.000	0.996
Ogo4	0.994	1.000	1.000	0.995	1.000	0.968	0.995	0.994	0.990	0.993
Oki100	0.978	1.000	1.000	1.000	1.000	1.000	1.000	0.970	1.000	0.994
OMM1080	1.000	1.000	0.995	1.000	1.000	0.938	1.000	0.994	1.000	0.995
Ots201b	0.984	1.000	1.000	1.000	1.000	0.993	0.995	0.985	1.000	0.990
Ots208b	0.994	1.000	1.000	1.000	1.000	1.000	0.995	0.970	0.995	0.996
Ots211	1.000	1.000	0.994	1.000	0.993	0.955	0.994	0.985	0.994	0.994
Ots212	0.989	1.000	1.000	1.000	1.000	0.989	0.995	0.994	1.000	0.992
Ots213	0.987	1.000	0.982	1.000	0.985	0.994	1.000	1.000	1.000	0.999
Ots3M	1.000	1.000	0.988	0.994	1.000	0.949	1.000	1.000	0.995	0.980
Ots9	1.000	1.000	1.000	1.000	1.000	0.979[2]	1.000	1.000	1.000	0.991
OtsG474	0.995	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999
Ssa408	0.987	0.929	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.991
Average	0.992	0.995	0.997	0.999	0.998	0.981	0.998	0.991	0.998	0.994

Quality Control and error trapping

Potential error in the baseline were identified and corrected during preliminary descriptive population genetic analyses. First, a mixture sample was inadvertently included in baseline submission and produced an extreme F_{IS} value (CTC03 baseline v1.0, FSTAT output). Second, subsets of genotypes were swapped between two populations, again producing a high F_{IS} value. Third, upper allele drop out problems were identified through examination of heterozygote deficits and corrected. Finally, some DNA samples appeared to have identical multilocus genotypes for all loci scored

[2] Value from Blind Test 1 accepted for purposes of certification, as agreed by GAPS standardization collaborators.

and therefore almost certainly represented duplicates of the same individual fish. Such duplications are not uncommon and can arise at multiple stages between field sampling and data analysis. Although these putative duplications will be investigated further and some samples will undoubtedly be removed, their effect is negligible in our primary application (see below).

Population genetics and biogeography

The loci selected for standardization in this project capture a great deal of genetic variation among these 110 populations. The average number of alleles per locus was 38 but individual values varied markedly across loci, ranging from 9 (*Ots*9) to 71 (*Omm*1080) (Table 3). Across all loci and all individuals, 489 alleles were observed. Holotype samples for each of the officially sanctioned alleles were identified and distributed among all collaborators. For some alleles, insufficient DNA was available for a particular holotype, so a paratype was substituted (an individual sample determined by the Locus Curator to express the same mobility as the holotype). Average heterozygosity (Nei 1987) was relatively high at 0.85 across loci (Fig. 3). Values ranged from 0.52 (*Ots*G474) to 0.95 (*OMM*1080). Average F_{ST}, a measure of the variation within relative to among populations, was 0.071. F_{ST} estimates ranged from 0.03 (*Oki*100) to 0.22 (*Ots*G474) (Fig. 3). It is interesting to note that *Ots*G474 had the highest F_{ST} and the lowest heterozygosity of all loci studied. This potential departure from neutrality is the subject of further investigation. Non-neutrality is not necessarily a problem for mixture analysis and might actually improve results in some cases. However, it is important for other applications to know which loci conform to neutral expectation and which do not.

Examination of temporal variation within populations is still in progress. Likewise, comprehensive N_e and N_m estimates required significant computer processing time and were not available at the time this document was prepared. More complete population genetic analyses will be included in a manuscript for peer-reviewed publication.

Table 3. Observed number of alleles and allelic size ranges for standardized microsatellite loci.[3]

Locus	Alleles observed	Size range (bp)
*Ots*201b	51	133 - 342
*Ots*208b	53	142 - 378
*Ots*211	41	196 - 337
*Ots*212	36	123 - 263
*Ogo*4	20	132 - 170
*Ogo*2	27	200 - 258

[3] These values reflect the numbers of alleles actually observed in the current coast-wide baseline data set (v1.1). A slightly larger number of alleles is officially recognized by the GAPS curators based on other sample sets (e.g., blind test samples and reference samples sets).

Locus	Alleles observed	Size range (bp)
*Ots*3M	19	122 - 170
*Ots*213	51	178 - 378
*Omm*1080	71	162 - 458
*Ssa*408UO*S*	39	180 - 320
*Ots*9	9	99 - 115
*Ots*G474	19	144 - 220
*Oki*100	46	164 - 353
Total	482	99 – 378

Cursory examination and statistical testing of these data did not reveal pervasive departure from H-W expectation at any of the loci assayed here. There were some population samples that showed significant heterozygote deficits (Battle Creek, Lyons Ferry Hatchery, Siuslaw, Stuart, and Swift rivers). Two additional cases (Torpy and Babine river samples) turned out to involve data processing errors that were corrected in the baseline data, as described above.

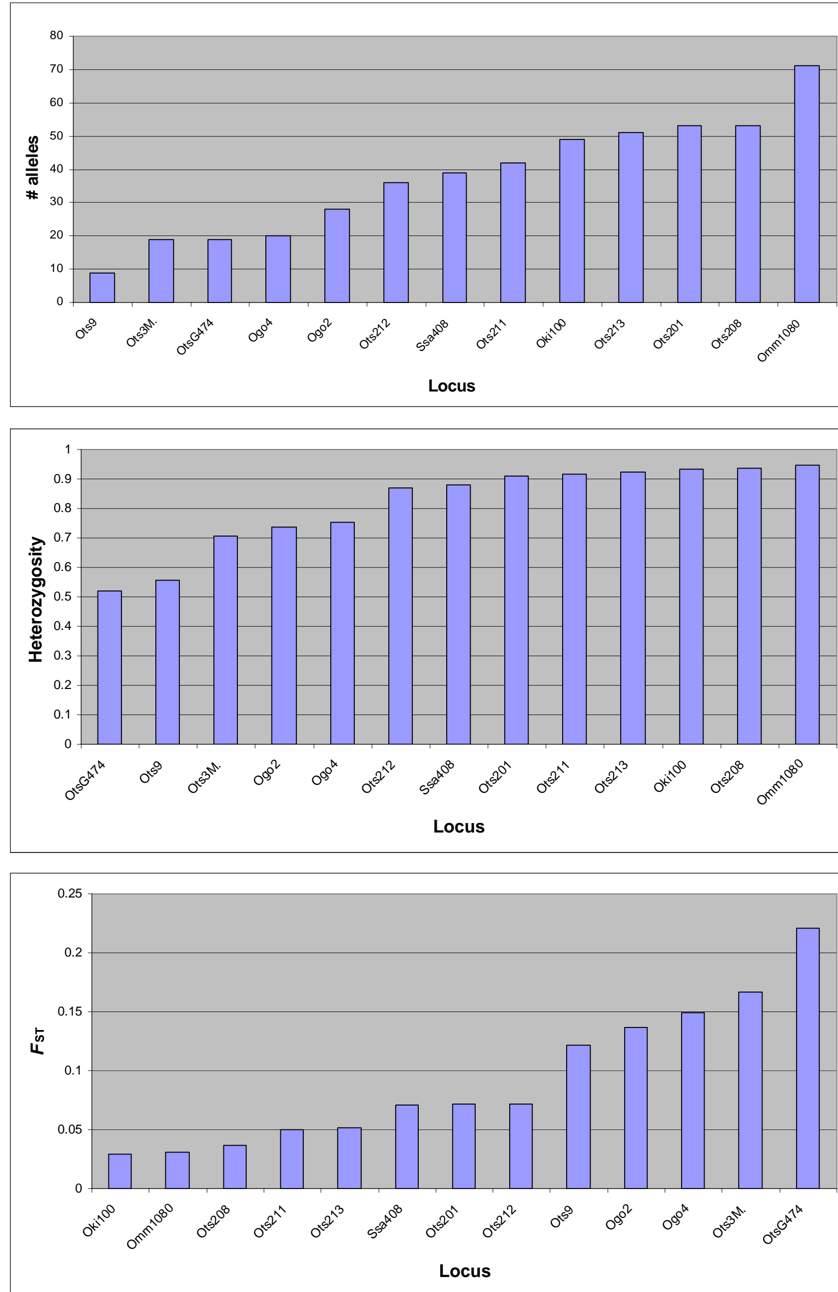

Figure 3. Over 480 alleles were identified, and high levels of variability were revealed within and among population samples.

Multivariate analyses were used to evaluate the integrity of the regional reporting groups that were derived from a combination of previous genetic and ecological data, life-history information, and geography. The resulting cluster diagram, based on chord distance, shows a close concordance with geography, largely consistent with isolation by distance (Fig. 4). Some distinct exceptions are evident that were already well-known, e.g., different life-history forms in the Columbia River show highly divergent allele frequencies with allozymes, mitochondrial DNA and microsatellite loci.

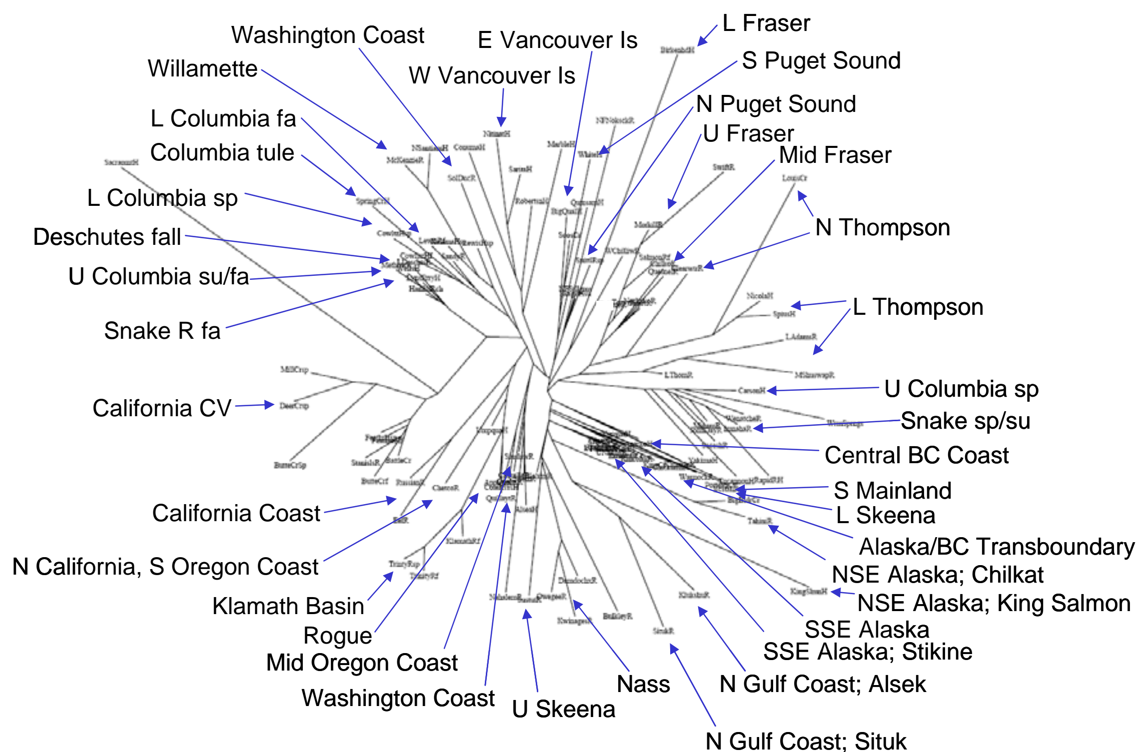

Figure 4. Neighbor-joining cluster analysis based on genetic chord distance (Cavalli-Sforza-Edwards et al. 1967) shows a general pattern of monophyly among reporting groups (i.e., all member populations cluster together to the exclusion of non-member populations).

Several genetic outliers were identified, especially Sacramento Hatchery winter run, but also Birkenhead Hatchery spring run, Louis Creek, and King Salmon Hatchery. Despite the distinctiveness of each of these populations from all others, each clustered with geographically proximate locations in the same reporting group.

Mixture simulations

Forty-one reporting groups were defined based on genetic similarity, geographic features, and management applications (Appendix 1). When each of these reporting regions was the sole contributor to a hypothetical mixture almost all reporting groups were found to be highly identifiable with mean correct allocations above 90% (Table 4). The exceptions were the Deschutes Fall and Upper Stikine River reporting groups which had 89.5% and 84.4% mean correct allocations, respectively. The largest misallocations of the Deschutes Fall reporting group were 4.8% to the Snake River Fall and 4.5% to the Upper Columbia River Summer/Fall reporting groups. For the Upper Stikine River reporting group the reporting group accounting for the greatest portion of the misallocation was the Taku River (11.2%). In addition, the lower bound of the 90% confidence interval for each of the 41 reporting groups was above the 90% threshold, with the exception of the Deschutes Fall, Upper Stikine River, Lower Stikine River, and Taku River reporting groups.

Table 4. Results of 100% "mixture" simulations for 41 Chinook salmon reporting groups ("Regions") including point estimates as well as upper and lower bootstrap confidence limits (90%).

Region #	Region	Estimate	Confidence Interval	
			Lower	Upper
1	Central Valley fall	0.946	0.917	0.972
2	Central Valley spring	0.936	0.907	0.963
3	Central Valley winter	0.989	0.980	0.998
4	California Coast	0.986	0.974	0.995
5	Klamath R Basin	0.984	0.971	0.995
6	North CA, South Oregon coast	0.972	0.955	0.986
7	Rogue River	0.941	0.912	0.966
8	Mid Oregon Coast	0.944	0.918	0.969
9	North Oregon Coast	0.956	0.932	0.977
10	Lower Columbia spring	0.970	0.952	0.986
11	Lower Columbia fall	0.973	0.955	0.987
12	Willamette River	0.982	0.969	0.994
13	Mid Columbia tule fall	0.969	0.950	0.985
14	Mid and Upp Columbia spring	0.966	0.946	0.984
15	Deschutes fall	0.895	0.857	0.931
16	Upper Columbia Summer fall	0.962	0.936	0.986
17	Snake Fall	0.945	0.912	0.972
18	Snake River spring/summer	0.966	0.945	0.985
19	Washington Coast	0.951	0.930	0.972
20	South Puget Sound	0.988	0.977	0.996
21	North Puget Sound	0.971	0.954	0.985
22	Lower Fraser	0.985	0.974	0.994
23	Lower Thompson River	0.978	0.964	0.990
24	North Thompson River	0.978	0.964	0.991
25	Mid Fraser River	0.979	0.964	0.991
26	Upper Fraser River	0.969	0.949	0.985
27	East Vancouver Island	0.979	0.964	0.990
28	West Vancouver Island	0.989	0.980	0.997
29	South BC Mainland	0.975	0.961	0.987
30	Central BC Coast	0.960	0.940	0.977
31	Lower Skeena River	0.949	0.927	0.970
32	Upper Skeena River	0.949	0.925	0.970
33	Nass River	0.938	0.913	0.961
34	Upper Stikine River	0.844	0.794	0.893
35	Taku River	0.919	0.884	0.951
36	Southern Southeast Alaska	0.969	0.951	0.985
37	Southeast Alaska, Stikine River	0.916	0.882	0.945
38	King Salmon River	0.987	0.976	0.996
39	Chilkat River	0.988	0.978	0.996
40	Alsek River	0.979	0.965	0.991
41	Situk River	0.977	0.963	0.990

When mixtures were simulated that were based on stock compositions observed previously, the estimated reporting group proportions were very close to the simulated proportions (Table 5). The largest discrepancy between the simulated proportion and the mean of the estimates was 1.2% (Willamette River, Mixture 3). In general, the mean estimates were within 0.3% of the simulated proportion. The largest misallocation to a reporting group that did not contribute to the mixtures was 0.6% (Lower Columbia spring, Mixture 3).

Table 5. Simulations of three potential fishery mixtures with expected and estimated proportions.

Region #	Region	Mixture 1		Mixture 2		Mixture 3	
		Expected	Estimated	Expected	Estimated	Expected	Estimated
1	Central Valley fall		0.001	0.05	0.047		0.001
2	Central Valley spring		0		0.003		0
3	Central Valley winter		0		0		0
4	California Coast		0		0		0
5	Klamath R Basin		0		0		0
6	North CA, South Oregon coast	0.10	0.096		0		0
7	Rogue River		0.001		0.002		0
8	Mid Oregon Coast		0.003	0.10	0.099		0.001
9	North Oregon Coast		0	0.10	0.096		0
10	Lower Columbia spring	0.10	0.098	0.10	0.099		0.006
11	Lower Columbia fall		0.003	0.20	0.197	0.20	0.193
12	Willamette River	0.10	0.096		0	0.40	0.388
13	Mid Columbia tule fall		0		0		0
14	Mid and Upper Columbia spring		0		0		0
15	Deschutes fall		0.002		0.001		0.003
16	Upper Columbia summer fall	0.10	0.103		0.003	0.20	0.206
17	Snake R fall	0.10	0.099		0.001	0.20	0.198
18	Snake R spring/summer		0		0		0
19	Washington Coast		0.001		0.001		0.001
20	South Puget Sound	0.05	0.050	0.25	0.246		0
21	North Puget Sound		0.002		0.002		0.001
22	Lower Fraser		0	0.20	0.197		0
23	Lower Thompson River	0.05	0.049		0.001		0
24	North Thompson River		0		0		0
25	Mid Fraser River		0.001		0		0
26	Upper Fraser River		0		0		0
27	East Vancouver Island	0.10	0.096		0.001		0
28	West Vancouver Island		0		0		0
29	South BC Mainland		0		0		0
30	Central BC Coast		0.002		0.001		0

31	Lower Skeena River	0.05	0.047	0	0
32	Upper Skeena River		0.001	0	0
33	Nass River		0.001	0	0
34	Upper Stikine River		0.001	0	0
35	Taku River		0.002	0	0
36	Southern Southeast Alaska	0.25	0.242	0.001	0
37	SE Alaska, Stikine River		0.002	0	0
38	King Salmon River		0	0	0
39	Chilkat River		0	0	0
40	Alsek River		0	0	0
41	Situk River		0	0	0

Discussion

This project succeeded in developing a powerful data set for mixed fishery analysis. Moreover, the genotypic data are fully standardized among nine West Coast salmon laboratories. Any of those laboratories may use the standardized data for independent analyses and may add to the coast-wide data to support more detailed analyses of particular fisheries. Microsatellite data, the prevalent class of data in current salmon ecological genetics, are now essentially independent of the laboratory in which they were generated, thus dramatically facilitating management and research collaborations.

Interlaboratory standardization

Initially, seven laboratories were involved as formal collaborators. A total of nine labs are now "certified[4]" to submit data (ADFG, CDFO, CRITFC, IDFG, NWFSC, OSU, SWFSC, USFWS, and WDFW). Two rounds of blind genotyping tests were conducted and most labs passed certification in the first test. At the conclusion of the second test, all labs had demonstrated their ability to produce concordant microsatellite data for the 13 selected markers, reliably discriminating nearly 500 officially sanctioned alleles. A number of errors were revealed in this process, however, by the end if the second test nearly all genotyping discrepancies were limited to data processing or record-keeping errors. For example, two labs used binning algorithms (for converting raw fragment sizes to allele designations) for incorrect primer pairs, resulting in a consistent offset to the correct allele designation. In another case a lab made an error in the code that extracts and assembles the final genotypes such that subsets of genotypes were mixed among individuals.

Although these data processing errors initially compromised, or potentially compromised, the actual baseline data, as well as the blind tests, none of the most recent errors were related to the chemistry or instrumentation of inter-laboratory standardization. Far more errors throughout this study arose through data processing rather than some inherent limitation of the genetic methods. Most errors would have compromised data integrity within those labs. Moreover, all the major errors discovered in the last blind test would have been immediately apparent in either intralab or interlab quality control associated with population genetic analysis. Based on careful quantification of error rates within the NWFSC lab (E. Berntson, NWFSC, unpubl.) the level of interlab standardization achieved in this study rivals intralab repeatability for most loci.

The principal limitation of the current coast-wide microsatellite set now appears to be tissue quality for DNA extraction rather than interlaboratory data standardization. Because most of these microsatellite loci are relatively large in size, they often don't work reliably in compromised tissue such as old scale samples, decomposed carcasses,

[4] "Certification," for the purpose of this report is defined by concordance with mode allele designation in a multi-laboratory blind genotyping test. Specifically, labs must show average concordance across loci >95%, no single locus <90%, and at least 75% data density (<25% missing genotypes—this last requirement was suspended in Blind Test 2 due to DNA quality issues experienced in multiple labs). One laboratory was below the 90% criterion at a single locus (82.3%), and although the GAPS collaborators chose to provisionally accept baseline data for that locus, this discrepancy is being investigated.

bones from scat, etc. However, with the exception of baseline collection (essentially a one-time effort), DNA quality should not be a limitation in most mixed fishery applications.

The laboratories involved in the microsatellite standardization project have demonstrated that standardization of allele designations has been achieved. This large-scale effort could not have been achieved without dedicated funding that allowed multiple agencies to contribute the substantial time that was required to produce a high-quality product (i.e., a truly robust locus set). Prior to the initiation of this project, different laboratories had applied generally non-overlapping sets of microsatellite loci in order to investigate population structure and estimate stock compositions in mixed-stock fisheries. Success in the current project required that individual laboratories needed to compromise in the choice of microsatellites to include in a standardized baseline. In most cases laboratories were required to adopt new markers, which required that old samples be run for new loci to update existing databases. This compromise represented a significant "hidden cost" of this large-scale standardization project.

Error trapping and QC

Blind tests followed by reconciliation of low concordance values identified two critical errors that were corrected immediately. All the collaborators re-examined genotypes they submitted that deviated from the mode score, and several laboratories made corrections and refinements that improved their accuracy (Appendix 3). There has been no final blind test, but the collaborators are satisfied that the data in the current baseline are free of the record-keeping errors that appeared in Blind Test 2. With provisional acceptance of corrections for the two errors identified above in Results (errant lookup table in one case, and incorrect—outdated—bin definitions in the other) all labs have met the self-imposed certification requirements for all loci (Table 2B). It is important to note that blind tests were initially considered without correction of any errors. Subsequently, we only fixed errors that could be corrected with confidence had they occurred in the baseline data. Thus, binning and transcription errors were corrected, but upper allele drop out or other unexplained genotype mismatches were not (see Appendix 3).

The QC procedures for the combined baseline data set also revealed several errors: 1) a mixture sample was inadvertently included in baseline submission and produced an extreme F_{IS} value, 2) a record-keeping error resulted in blocks of multilocus genotypes swapped between populations, 3) upper allele drop out problems were revealed and corrected through examination of heterozygote deficits, and 4) some DNA samples represented duplicates of the same individual fish. Nevertheless, some high F_{IS} values remain, indicative of additional heterozygote deficits. Likewise, putatively duplicate samples will not be removed until the data are more fully analyzed (some of the identical multilocus genotypes could be chance observations). Despite the certainty that subtle errors remain in the baseline, the general results show high internal consistency and concordance with previous genetic studies. Moreover, the simulated mixture analyses presented here suggest that the data set has unprecedented power in spite of any remaining errors. Ongoing work will test whether empirical results from actual mixtures are comparable to the outstanding results presented here from simulations (see below).

Population genetics and biogeography

The microsatellite data collected and analyzed for this study were highly concordant with earlier Chinook salmon allozyme and DNA studies. As in those previous studies, much of the genetic population structure revealed by cluster analysis (Fig. 4) appears to have a geographic basis (Utter et al. 1989). Nearly all populations are genetically most similar to other populations that are geographically proximate. And, consistent with earlier allozyme results, populations of differing run time within drainages and regions cluster together (Utter et al. 1989, Waples et al. 2004). For example spring-run populations within the Sacramento, Rogue, lower Columbia, and lower Fraser rivers are genetically distinct from fall-run populations in those drainages. However, spring- and fall-run populations in the same basin are more similar to each other than to populations of the same run time in other regions. A notable exception to this pattern, and also consistent with previous genetic studies, spring- and spring/summer-run populations in the interior Columbia River Basin are genetically distinct and not closely related to nearby fall- or summer-run populations (Utter et al. 1995, Waples et al.2004, Narum et al. 2004).

Many of the major geographic boundaries delineating genetic groups that have been identified in previous genetic studies are also readily apparent in our results. For example, strong shifts in genetic affinities among populations are associated with features such as Cape Blanco on the Oregon coast (Bartley et al. 1992), the Cascade Crest in the Columbia River Basin (Myers et al. 1998) and the Fraser Canyon in the Fraser River (Teel et al. 2000, Nelson et al. 2001).

The clustering of Alaskan samples in the tree was also in good agreement with that from the allozyme study of Guthrie and Wilmot (2004). Samples from Alaska/BC transboundary populations (Taku and Little Tahltan rivers) and from southern Southeast Alaska (Keta and Stikine rivers) all grouped together. Samples from northern Southeast Alaska (King Salmon and Chilkat rivers) and the north gulf coast (Alsek and Situk rivers) were all genetically differentiated from other Alaskan samples and from each other.

Several individual populations were genetically very distinct, yet clustered with others in the same drainage or region. This observation was also concordant with earlier genetic studies. For example, our study supports previous results reported for Sacramento River winter-run Chinook salmon (Banks et al. 2000), Birkenhead River in the lower Fraser River (Teel et al. 2000, Beacham et al. 2003), Louis River in the Thompson (Beacham et al. 2003), possibly a genetically bottlenecked population with an estimated M value of 0.64, and King Salmon River in Southeast Alaska (Guthrie and Wilmot 2004).

The Sol Duc in the Washington Coast region is a different class of "outlier." Not only does it create a long branch in the dendrogram indicating a general distinctiveness from all other populations, but Sol Duc does not cluster with other populations in the same region. This population has a complicated hatchery stocking record which may somehow contributes to its distinctiveness or there may be some cryptic genetic substructure in this

region. This is consistent with previous observation (D. Teel, NWFSC, pers. comm.), however the explanation remains unclear.

However, our depiction of genetic relationships among populations shows at least one important difference from earlier findings. Although previous studies (Beacham et al. 1996, Teel et al. 2000) found that Chinook salmon in mainland southern British Columbia were genetically most similar to nearby Vancouver Island populations, our samples from Porteau Cove and Klinaklini River clustered with populations from further north in British Columbia including those along the central coast and in the lower Skeena River. The significance of this difference in the results from earlier data sets is not yet clear.

The results of the simulations demonstrate that the standardized baseline presented here can achieve high levels of accuracy and precision for identification of reporting group contributions in simulated mixtures. The 100% simulations used are considered to be conservative tests of the baseline, because bias and variability in the estimates are greatest when true contributions are near the bounds, 0% and 100% (Pella and Masuda 2001). While two of the reporting groups (Deschutes Fall and Upper Stikine River) did not meet the 90% threshold, misallocations in simulated mixtures were predominantly associated with closely related reporting groups. The Deschutes Fall has previously been grouped with Snake River Fall (Teel et al. 1999) and for most analyses could continue to be pooled with this group. In the same way, the Little Tahltan River population (currently the sole member of the Upper Stikine River reporting group) was previously grouped with Taku River populations (Teel et al. 1999, Guthrie and Wilmot 2004) and might continue to be grouped with these populations. For more fine scale applications, these two groups can be used in providing composition estimates, but the source of potential misclassification should be considered when interpreting results.

Simulations of heterogeneous mixtures showed accurate identification with little misallocation to non-contributing groups. This is a preliminary investigation of the behavior of the baseline for estimating mixture components. Correct proportional assignment of mixture components when many groups contribute can indicate the ability for genetic stock identification techniques to find the correct stock proportions in mixtures which are not dominated by a single reporting group. One limitation of this technique is the requirement that the simulated mixtures are generated from the baseline data and thus assume that the baseline frequencies are well-known and can contain no novel information. The next step will be to evaluate mixtures of known composition with genotypes not generated from the baseline.

Future priorities

The participating geneticists will publish the genotypic data collected in this 2-year study in peer-reviewed literature thereby providing public access to the baseline. Currently, all collaborating labs have access to the baseline data set presented here, which is complete and available for mixture analysis. All geographic regions and Chinook salmon lineages likely to contribute to fisheries of CTC interest are represented, and these baseline data should be appropriate for complex fishery mixtures that include diverse populations from

widespread locations. Although the current baseline is broad, it is not comprehensive. Efforts are currently underway to increase local coverage. Expanded baseline data will improve accuracy of allocation to the regional reporting groups presented here and, in at least some cases, provide a finer scale of reporting (e.g., sub-basins within major river systems). Fine-scale reporting allows more precise information on harvest and run reconstruction. In addition to improved management, current expansion of the coast-wide Chinook baseline will be used to better understand the basic biology of the species. These data provide information about effective population size, evolutionary and demographic history, and population boundaries. Fine-scale geographic allocation of mixtures and potentially assignment of individual fish to population-of-origin will also provide important biological and life-history information, including migration timing and pathways, and juvenile habitat use (Lundrigan et al. 2004).

The population genetic analyses presented here were only preliminary and were only intended to focus on two goals, 1) quality control and error trapping and 2) evaluation of the reporting groups on which subsequent mixture analyses would depend. Much more comprehensive characterization of the distribution of genetic diversity within and among Chinook salmon populations is currently in progress. We estimated long-term evolutionarily effective population size (Nei 1987) and M-ratios (Garza and Williamson 2001), and although those results suggest that some of the genetic outliers, most notably Sacramento winter-run, are due to recent bottlenecks rather than long periods of isolation and limited gene flow (data not shown), exploration of these parameter estimates is beyond the mixed fishery emphasis of this project.

Several immediate priorities present themselves for the current baseline. A comprehensive power analysis is underway that includes extensive simulations and CWT test samples (i.e., known-origin). Individual assignment and proportional estimation are scheduled to occur as another multi-laboratory collaboration in FY06. In the coming year, additional baseline samples will permit more extensive power analyses and the addition of a suite of single-nucleotide-polymorphism (SNP) markers will provide valuable comparisons with this important new class of markers. Simultaneously, plans are being made for a long-term, safe and secure repository for current and rapidly accumulating data (full proposals requested by the Northern and Southern Boundary Funds). The recognition that virtually all genotyping effort on Chinook salmon coast-wide is generating standardized data for the same set of genetic markers demands attention toward the logistics of storage and access.

Finally, the collaborators have given considerable thought and discussion to the issue of long-term QC and curatorship of the data, beyond the IT issues of physical storage, database maintenance, and connectivity. Who would serve as a gate-keeper in receiving new data, screening the data for format errors or incomplete data? Scripts will do much of this work eventually, but the group agreed that some genetics lab should serve as a contact and liaison to the IT group. Which group should assume that role will become clear in the coming weeks of October 2005 as proposals for IT support are drafted for the PST Northern and Southern Boundary funds.

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Appendices

Appendix 1. Chinook salmon populations analyzed in this study including run time, hatchery (H) or wild (W) origin, life stage, collection data, and analysis laboratory are given.

Region #	Region	Population	Run time[1]	Origin	Life Stage	Collection Date	Analysis Laboratory[2]
1	Central Valley fall	Battle Creek	Fa	W	Adult	2002, 2003	SWFSC
		Feather Hatchery fall	Fa	H	Adult	2003	SWFSC
		Stanislaus River	Fa	W	Adult	2002	SWFSC
		Tuolumne River	Fa	W	Adult	2002	SWFSC
2	Central Valley spring	Butte Creek	Sp	W	Adult	2002, 2003	SWFSC
		Deer Creek spring	Sp	W	Adult	2002	SWFSC
		Feather Hatchery spring	Sp	H	Adult	2003	SWFSC
		Mill Creek spring	Sp	W	Adult	2002, 2003	SWFSC
3	Central Valley winter	Sacramento River winter	Wi	W/H	Adult	1992, 1993, 1994, 1995, 1997, 1998, 2001, 2003, 2004	SWFSC
4	California Coast	Eel River	Fa	W	Adult	2000, 2001	SWFSC
		Russian River	Fa	W	Juvenile	2001	SWFSC
5	Klamath River	Klamath River fall	Fa	W	Adult	2004	SWFSC
		Trinity Hatchery fall	Fa	H	Adult	1992	SWFSC
		Trinity Hatchery spring	Sp	H	Adult	1992	SWFSC
6	N California/S Oregon Coast	Chetco	Fa	W	Adult	2004	OSU
7	Rogue River	Applegate	Fa	W	Adult	2004	OSU
		Cole Rivers Hatchery	Sp	H	Adult	2004	OSU
8	Mid Oregon Coast	Coquille	Fa	W	Adult	2000	OSU

Region #	Region	Population	Run time[1]	Origin	Life Stage	Collection Date	Analysis Laboratory[2]
		Siuslaw	Fa	W	Adult	2001	OSU
		Umpqua	Sp	W	Adult	2004	OSU
9	North Oregon Coast	Alsea	Fa	W	Adult	2004	OSU
		Nehalem	Fa	W	Adult	2000, 2002-1, 2002-2	OSU
		Siletz	Fa	W	Adult	2000	OSU
10	Lower Columbia R. spring	Cowlitz H. spring	Sp	H		2004	CRITFC
		Kalama H. spring	Sp	H		2004	CRITFC
		Lewis H. spring	Sp	H		2004	CRITFC
11	Lower Columbia R. fall	Cowlitz H. fall	Fa	H		2004	CRITFC
		Lewis fall	Fa	W	Adult	2003	WDFW
		Sandy	Fa	W	Adult	2002, 2004	OSU
12	Willamette River	McKenzie	Sp	H	Adult	2002, 2004	OSU
		North Santiam	Sp	H	Adult	2002, 2004-1, 2004-2	OSU
13	Mid Columbia R. tule fall	Spring Creek	Fa	H		2001, 2002	CRITFC
14	Mid and Upper Columbia R. spring	Carson H.	Sp	H		2001, 2004	CRITFC
		John Day	Sp	W	Juvenile, Adult	2000-1, 2000-2, 2000-3, 2000-4, 2000-5, 2000-6, 2004	OSU
		Upper Yakima	Sp	H	Adult, Mixed	1998, 2003	WDFW
		Warm Springs Hatchery	Sp	H		2002, 2003	CRITFC

Region #	Region	Population	Run time[1]	Origin	Life Stage	Collection Date	Analysis Laboratory[2]
		Wenatchee spring	Sp	W	Adult	1993, 1998, 2000	WDFW
15	Deschutes River fall	Lower Deschutes R.	Fa	W		1999-1, 1999-2, 2001, 2002	CRITFC
16	Upper Columbia R. summer/fall	Hanford Reach CR	Su/Fa	W		1999, 2000-1, 2000-2, 2000-3,2001-1, 2001-2, 2001-3	CRITFC
		Methow R. summer	Su/Fa	W		1992, 1993, 1994	CRITFC
		Wells Dam	Su/Fa	H		1993-1, 1993-2	CRITFC
17	Snake River fall	Lyons Ferry	Fa	W	Adult	2002-1, 2002-2, 2003-1, 2003-2	WDFW
18	Snake River spring/summer	Imnaha R.	Sp/Su	W		1998, 2002, 2003	CRITFC
		Minam R.	Sp/Su	W		1994, 2002, 2003	CRITFC
		Rapid River H.	Sp/Su	H		1997, 1999, 2002	CRITFC
		Sesech R.	Sp/Su	W		2001, 2002, 2003	CRITFC

Region #	Region	Population	Run time[1]	Origin	Life Stage	Collection Date	Analysis Laboratory[2]
		Tucannon	Sp/Su	H	Adult	2003-1, 2003-2, 2003-2	WDFW
19	Washington Coast	Queets	Fa	W	Adult	1996, 1997	WDFW
		Quillayute/ Bogachiel	Fa	W	Adult	1995-1, 1995-2, 1995-3, 1996-1, 1996-2	WDFW
		Sol Duc	Sp	H	Adult	2003	WDFW
20	South Puget Sound	Soos Creek	Fa	H	Adult	1998-1, 1998-2, 2004	WDFW
		White River	Sp	H	adult	1998-1, 1998-2, 2002	WDFW
21	North Puget Sound	NF Nooksack	Sp	H/W	adult	1999	WDFW
		NF Stilliguamish	Su	H/W	adult	1996, 2001-1, 2001-2	WDFW
		Skagit summer	Su	W	adult	1994, 1995	WDFW
		Suiattle (Skagit)	Sp	W	adult	1989, 1998, 1999	WDFW
22	Lower Fraser River	Birkenhead River	Sp	H	Adult	1996, 1997, 1999, 2001, 2002, 2003	SWFSC
		WChilliwack	Fa	H	Adult	1998, 1999	DFO
23	Lower Thompson River	Lower Adams	Fa	H	Adult	1996	DFO
		Lower Thompson	Fa	W	Adult	2001	DFO

Region #	Region	Population	Run time[1]	Origin	Life Stage	Collection Date	Analysis Laboratory[2]
		M.Shuswap	Fa	H	Adult	1997	DFO
		Nicola	Sp	H		1998, 1999	OSU
		Spius River	Sp	H	Adult	1996, 1997, 1998	SWFSC
24	North Thompson River	Clearwater	Fa	W	Adult	1997	DFO
		Louis River	Fa	W	Adult	2001	DFO
25	Mid Fraser River	Chilko	Fa	W	Adult	1995, 1996, 1999, 2002	DFO
		Nechako	Fa	W	Adult	1996	DFO
		Quesnel	Fa	W	Adult	1996	DFO
		Stuart	Fa	W	Adult	1996	DFO
26	Upper Fraser River	Morkill River	Fa	W	Adult	2001	DFO
		Salmon River (Fraser)	Sp	W	Adult	1997	SWFSC
		Swift	Fa	W	Adult	1996	DFO
		Torpy River	Fa	W	Adult	2001	DFO
27	East Vancouver Island	Big Qualicum	Fa	H	Adult	1996	DFO
		Quinsam	Fa	H	Adult	1996, 1998	DFO
28	West Vancouver Island	Conuma	Fa	H	Adult	1997, 1998	DFO
		Marble at NVI	Fa	H	Adult	1996, 1999, 2000	DFO
		Nitinat	Fa	H	Adult	1996	DFO
		Robertson	Fa	H	Adult	1996, 2003	DFO
		Sarita	Fa	H	Adult	1997, 2001	DFO
29	S BC Mainland	Klinaklini	Fa	W	Adult	1997	DFO
		Porteau Cove	Fa	H	Adult	2003	DFO
30	Central BC Coast	Atnarko	Fa	H	Adult	1996	DFO
		Kitimat	Fa	H	Adult	1997	DFO
		Wannock	Fa	H	Adult	1996	DFO

Region #	Region	Population	Run time[1]	Origin	Life Stage	Collection Date	Analysis Laboratory[2]
31	Lower Skeena River	Ecstall	Fa	W	Adult	2000, 2001, 2002	DFO
		Lower Kalum	Fa	W	Adult	2001	DFO
32	Upper Skeena River	Babine	Fa	H	Adult	1996	DFO
		Bulkley	Fa	W	Adult	1999	DFO
		Sustut	Fa	W	Adult	2001	DFO
33	Nass River	Damdochax	Fa	W	Adult	1996	DFO
		Kincolith	Fa	W	Adult	1996	DFO
		Kwinageese	Fa	W	Adult	1996	DFO
		Owegee	Fa	W	Adult	1996	DFO
34	Upper Stikine River	Little Tahltan River	Sp	W	Adult	1989, 1990	OSU
35	Taku River	Kowatua Creek (Taku)		W	Adult	1989, 1990	ADFG
		Nakina River (Taku)		W	Adult	1989, 1990	ADFG
		Tatsatua Creek (Taku)			Adult	1989, 1990	ADFG
		Upper Nahlin River (Taku)		W	Adult	1989, 1990, 2004	ADFG
36	Southern Southeast Alaska	Chikamin River (West Behm Canal)		W	Adult	1990, 1993	ADFG
		Clear Creek (Unuk)		W	Adult	1989, 2003, 2004	ADFG
		Cripple Creek (Unuk)		W	Adult	1988, 2003	ADFG
		Keta River (Boca de Quadra)		W	Adult	1989, 2003	ADFG
		King Creek (West Behm Canal)		W	Adult	2003	ADFG
37	Southeast Alaska Stikine R.	Andrews Creek (Stikine)		W	Adult	1989, 2004	ADFG
38	N. Southeast Alaska	King Salmon River		W	Adult	1989, 1990, 1993	ADFG

Region #	Region	Population	Run time[1]	Origin	Life Stage	Collection Date	Analysis Laboratory[2]
39	Chilkat River	Big Boulder Creek		W	Adult	1992, 1995, 2004	ADFG
		Tahini River		W	Adult	1992, 2004	ADFG
40	Alsek River	Klukshu River		W	Adult	1989, 1990	ADFG
41	Situk River	Situk River		W	Adult	1988, 1990, 1991, 1992	ADFG

[1] Run time abbreviations: spring (Sp), summer (Su), fall (Fa), and winter (Wi)

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

Appendix 2. Quality control procedures for genetic data collection in individual laboratories

CDFO (email from Janine Supernault, 10/21/05)
Quality Control Report
-all samples were documented (life history/collection date/collection site/tissue type) and data maintained in a database. Stocks were given unique identifiers as were individual samples. All extractions were done using a 96 well format with a negative control placed in well H6.
-after extraction, samples were archived to maintain individual identity relative to this project
-Loci were optimized and subsequently run on MJR thermocyclers.
-all samples were run out on ABI 377 sequencers, using standard gel conditions
-during the standardization of these loci samples in which genotyping did not agree with the other labs were re-amplified and re-analyzed to confirm amplification and scoring of individuals.
-samples with a high failure rate were re-run or were replaced with "new" samples. In cases where trays were re-amplified/re-run genotyping was compared to the first run for that tray to ensure that scoring was consistent
-genotyping was performed using ABI Genotyper software and all trays were scored by two technicians
-all data (raw and scored) was retained in a data archive
-H-W tests both by individual locus and for all loci was tested in addition to being tested by population

CRITFC (email from Shawn Narum, 9/30/05)
Our standard QC measures for usat data include:
-sample inventory
-unique sample ID assigned
-tracking system for each sample ID (plate/well)
-bulk PCR cocktails for consistency
-dilution tests for each amplified locus prior to genotyping run (to avoid "blown-out" peaks)
-double scoring of all peaks by two observers
-repeat genotyping of 5-10% of samples to confirm genotypes
-statistical tests such as HWE to highlight het. deficiencies that may be due to scoring errors (e.g., allele drop-out, alleles outside of the range, etc...)

NWFSC (contributed by Paul Moran, 10/11/05)
QA/QC procedures for microsatellite analysis of population samples
-All collection data are collated and logged into the CBD Tissue Archive database as soon as possible after sample receipt.

-Each sample collection is assigned a unique 5-digit accession number that is retained from DNA extraction through to data analysis. The accession number is related to all associated metadata, location, collector, date, method, etc. The accession number is also related to any electronic or hard-copy collection information from the collector.
-Individual ID numbers from the field are retained in the DNA samples and carried through to analysis.
-Standardized record-keeping and data logging facilitate sample tracking and minimize the chance of multilocus genotyping errors (samples are invariably handled in groups of 12, 16, 96, and 384 further reducing errors).
-All raw and intermediate data are retained and catalogued in a way that permits checking and data verification at each step where errors might occur (all these data are archived in duplicate).
-Microsatellite loci are carefully optimized before full-scale data collection and bulk PCR cocktails are used to increase consistency among amplifications.
-Double scoring of genotypes for some (but not all) studies. Reconciliation of any repeated genotyping. Automated genotyping features of ABI software are used, but every genotype is examined by eye.
-Binning almost invariably involves a continuous set of allele categories ~2 or 4 bp from center to center and 1 – 1.6 bp in width. This creates an important buffer between categories such that any allele with an unusual mobility will be flagged as "unknown." Those relatively narrow categories and their uniform distribution are possible because each run (96 – 384 samples) is scored individually with offset categories as needed. Thus run-to-run variation is isolated, even over years. Uniform distribution facilitates the interpretation of new alleles relative to the apparent repeat motif of the locus.
-Repeat spot genotyping (sometimes from DNA extraction forward).
-Tests for internal consistency: H-W tests by individual locus, across loci, by population, and across populations (e.g., a homozygote for a rare or unique allele gets more scrutiny that a heterozygote for two common alleles).
-Multivariate analysis of allele frequencies to evaluate against previous genetic data or geographic distance. If data are collected in multiple sets (e.g., two or three multiplex sets or in multiple years) then the resulting data sets are evaluated for consistency.

OSU Quality Control Report (Reneé Bellinger)
September 30, 2005

Reaction conditions
PCR is performed on a MJ thermal cycler. 0.2 to 0.5 ul of each PCR product was run on an ABI 3730 XL capillary system. CTC Bins were automatically assigned unless RFU's were less than 100. Data were scored manually in Genemapper; scored Genemapper files were added to the database and labeled by date. All raw scores are retained in original files with a subset of binned data used for final database. We used the following primer multiplex combinations: Ots208b/213, Ots9/201b, Ots211/212, Ogo4/OtsG474, SSA408*UOS*/Ogo2, and Oki100/Ots3. OMM 1080 was amplified alone.

Extraction and PCR Negatives

One negative was included in each n = 96 well Qiagen extraction. This negative was used as the PCR negative in subsequent reactions. In no cases was the extraction contaminated. However, there were several cases where after the extraction was confirmed clean, subsequent PCRs indicated that contamination of PCR reagents had occurred.

PCR for each 96 or 384 well plate was performed with bulk master mix. In all cases that the PCR negative indicated contamination, all data for that gel were discarded and the PCR was repeated and re-run on the ABI. The majority of PCR was conducted in 384 well plates with multiple negative controls (2 to 4).

Gel Quality

Any gel that had approximately 15% failure was rerun. Scores from the first run were retained and compared against the second run.

Each gel was first hand scored and then scanned to double check results. During the second scan, homozygous fishes were double checked to verify that smaller alleles were not missed.

Individual Samples and Scores

Any DNA sample that displayed an extra band was considered contaminated and data were discarded. However, if the extra band could be attributed to bleedover or bleedthrough, the data were retained if the source of the extra band was evident. "Evident" means that the adjacent capillary had a sample with high RFU's (> 20,000) and was obviously the source of the extra band.

DNA extracted from tissue obtained by carcass surveys was problematic due to sample degradation. In over 100 fish samples "extra" alleles were observed that indicated contamination. However, DNA was re-extracted from ~50 fish, and results indicated that samples remained contaminated despite strict prevention measures. I question if this was actual contamination, or rather the result of highly degraded samples producing fragmented PCR products. All of these fish were eliminated from the baseline and replaced with suitable samples.

Generally, RFU's on Genemapper for both homozygotes and heterozygotes were between 1000-5000. However, in many cases the second allele was much smaller than the first allele. Sometimes the second allele had RFU's less than 100, which could not be scored by Genemapper software. In this case, the entire fishes' score for this locus was hand-recorded and added to data after it was transferred to an excel file.

Two loci, Oki 100 and Ots 3 had very weak signals. For these two loci, RFU's less than 100 were generally discarded unless the sample was clearly a heterozygote and the genotype was not the same as an adjacent cell. 50% of all CTC baseline samples were run twice at locus Oki 100. OMM 1080 was PCR amplified using two different dye sets due to low fluorescence signal.

Sub-samples from 384-well plates (approximately 15% of the final baseline) were re-run using multiple loci (at least four) to spot check and compare against the final genotypes submitted to the database.

Appendix 3. Explanation from individual laboratories of genotyping mismatches relative to the modal allele designation across labs.

ADFG (email from Nick Decovich 9/23/05)
The following is a brief statement on the sources of ADF&G's deviation from the group on blind test number two. I have also attached a PowerPoint file with the original traces from Blind 2. These traces represent points 1-3 below. In the two cases where allelic dropout was to blame for our error, the overall signal was weak and these samples probably should have been blanked for Ots213 and Ots211. In the future we will take a more conservative approach to scoring these loci.

1. Ogo2- #17, our upper allele was on the upper edge of a bin. All other labs placed it into the next highest CTC bin. I think this was just a shifty allele and no cause to adjust our bins. Cause: unknown.

2. Ots213- #93 one sample deviates. Cause: upper allele dropout

3. Ots211- #43 one sample deviates. Cause: upper allele dropout

4. Ots3m- I'm not sure where we deviate on this one. On Shawn's blind 2 summary we have a score of 99.4%, but on his mode calculation sheet we have 100% accuracy at this locus.

CRITFC (email from Shawn Narum, 9/30/05)
We had three errors in the blind2 test. All three were samples with low signal that we probably shouldn't have attempted to score.

OSU (email from Reneé Bellinger 9/30/05)
OSU had a total of six errors in Blind Test 2.

1. OMM 1080. Allelic dropout of first allele. KWTRL04AL_58 was scored as a heterozygote (262/266in other labs), but my data shows a clear 266/266 score.

2. Ots 213. Allelic dropout of second allele at KWTRL04AL_57.

3. Ots 213. Typographical error that occurred while manually substituting placeholder scores with CTC bins.

4., 5. Ots 3: Two errors, both due to missing the conversion of the old CTC Bin (143) to the new bin (142) (KWTRL04AL_5, KWTRL04AL_79).

6. Ots 211. One error where the second allele of KWTRL04AL_9 was manually entered wrong. This individual's PCR amplification was low quality and the ABI didn't automatically score it.

USFWS-AK:
Hi Shawn,
I attached a spreadsheet with our corrected scores (we had 4 typos). There are two tabs in the spreadsheet...one is the data in two column format and the other is one column format and the tabs are labeled as such. Thank you,
Ora Stefanowski
Geneticist
USFWS Conservation Genetics Lab
Anchorage, Alaska

CDFO:
Here is our corrected table. I only made changes to the highlighted samples for Ssa408. Let me know if this is not the format you would like.

Thanks for doing this Shawn.

Janine Supernault
MGL, Pacific Biological Station
Dept. Fisheries and Oceans
3190 Hammond Bay Rd.
Nanaimo, B.C
V9T-6N7
ph. 250-756-3357, fax 250-756-7053

SWFSC:
Hi Shawn,

I haven't been able to contact Carlos about this, as he is away, but I thought I should check with you to make sure you have the right data from us. I apologize if Carlos has already sent you updated data regarding the correction of aberrant /Ogo/ 4 upper allele bins.

I have attached a spreadsheet with revised /Ogo/ 4 data, where standardization was conducted using the proper upper bins. Regarding all other discrepancies, I cannot countenance any changes in data for genotypes "correctly" scored here that subsequently were determined discrepancies. Yet, we did "miss" one upper allele at /Ssa/ 408 for individual #20, as it was above our expected allele size range. I have

changed the genotype for #20, adding the high allele. All other discrepancies from the original submission of the Blindtest 2 data remain, excluding revised/ Ogo/ 4 genotypes.

If the data I have submitted to you is not in the format you want let me know.
scott

NWFSC: (notes from Tricia Lundrigan and Melissa Baird 10/11/05)
Five mistyped alleles, details in the table below.

Locus	**Sample #**	**Mode**	**NWFSC**	**Explanation**
Ogo4	84	132/136	136/136	Both labels were present originally, apparent transcription error
Ots201B	5	190/190	186/190	Extreme signal, distorted peak shape
Ots208B	89	262/358	262/362	Bin definitions needed refinement
Ots211	60	212/284	212/212	Upper-allele dropout
Ots212	83	131/211	131/131	Weak signal, upper-allele dropout

Consistent with the approach of other labs, we suggest changing our scores for Ogo4 (transcription error) and Ots208B (bin definition refinements) in the modified Blind 2 results.

Table 6. Genotyping concordance among laboratories after final reconciliation of Blind Test 2 results (see Table 2A for initial uncorrected values from this test) [5]

Locus	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Average
Ogo2	0.987	1.000	1.000	1.000	0.993	0.988	1.000	0.993	1.000	0.996
Ogo4	0.994	1.000	1.000	0.995	1.000	0.968	1.000	1.000	0.990	0.994
Oki100	0.978	1.000	1.000	1.000	1.000	1.000	1.000	0.976	1.000	0.995
OMM1080	1.000	1.000	0.995	1.000	1.000	0.938	1.000	0.994	1.000	0.992
Ots201b	0.984	1.000	1.000	1.000	1.000	0.993	0.995	0.993	1.000	0.996
Ots208b	0.994	1.000	1.000	1.000	1.000	1.000	1.000	0.970	0.995	0.995
Ots211	1.000	1.000	1.000	1.000	0.993	0.955	0.994	0.985	0.994	0.991
Ots212	0.989	1.000	1.000	1.000	1.000	0.989	0.995	0.982	1.000	0.995
Ots213	0.987	1.000	0.988	1.000	0.985	0.994	1.000	1.000	1.000	0.995
Ots3M	1.000	1.000	1.000	0.994	1.000	0.949	1.000	1.000	0.995	0.993
Ots9	1.000	1.000	1.000	1.000	1.000	0.979[6]	1.000	1.000	1.000	0.980
OtsG474	0.995	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999
Ssa408	0.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999
Average	0.992	1.000	0.999	0.999	0.998	0.981	0.999	0.992	0.998	0.995

Appendix 4. Complete list of authors and collaborators on this project with their respective affiliations.

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Bill Ardren, US Fish and Wildlife Service, Abernathy
Melissa Baird, Northwest Fisheries Science Center
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Eric Kretschmer, US Fish and Wildlife Service, Anchorage
David Kuligowski, Northwest Fisheries Science Center
Tricia Lundrigan, University of Washington

[5] This table presents the final reconciliation results of Blind Test 2. The difference between initial genotype submissions (Table 3A) and post correction accuracy presented here was largely attributable to two factors: 4.0% of the 4.3% improvement in overall concordance of the reconciled Blind Test 2 result was due to the correction of record-keeping errors in two labs. The remaining 0.3% differences between "initial" and "corrected" percent accuracy reflected refinements and corrections in bin definitions and correction of transcription errors in multiple labs (see notes from collaborators). The errors identified and reconciled after Blind Test 2 are no longer expected to be a problem in the current baseline.

[6] Uncorrected concordance in this cell was 0.823 (Table 3A). Apparently that low value was also due to a binning problem that was subsequently corrected but not available at the time of this report. In Blind Test 1, this value was 0.979, and that is the value that was used for certification purposes, as agreed by the GAPS collaborators (see also Table 1 and associated footnote).

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