**Fishery Data Series No. 06-20**

# Progress in Development of a DNA Baseline for Genetic Identification of Chinook Salmon Stocks of the Copper River Basin, Alaska

**by**

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**April 2006**

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**Weights and measures (metric)**

| | |
|---|---|
| centimeter | cm |
| deciliter | dL |
| gram | g |
| hectare | ha |
| kilogram | kg |
| kilometer | km |
| liter | L |
| meter | m |
| milliliter | mL |
| millimeter | mm |

**Weights and measures (English)**

| | |
|---|---|
| cubic feet per second | $ft^3/s$ |
| foot | ft |
| gallon | gal |
| inch | in |
| mile | mi |
| nautical mile | nmi |
| ounce | oz |
| pound | lb |
| quart | qt |
| yard | yd |

**Time and temperature**

| | |
|---|---|
| day | d |
| degrees Celsius | °C |
| degrees Fahrenheit | °F |
| degrees kelvin | K |
| hour | h |
| minute | min |
| second | s |

**Physics and chemistry**

| | |
|---|---|
| all atomic symbols | |
| alternating current | AC |
| ampere | A |
| calorie | cal |
| direct current | DC |
| hertz | Hz |
| horsepower | hp |
| hydrogen ion activity (negative log of) | pH |
| parts per million | ppm |
| parts per thousand | ppt, ‰ |
| volts | V |
| watts | W |

**General**

| | |
|---|---|
| Alaska Administrative Code | AAC |
| all commonly accepted abbreviations | e.g., Mr., Mrs., AM, PM, etc. |
| all commonly accepted professional titles | e.g., Dr., Ph.D., R.N., etc. |
| at | @ |
| compass directions: | |
| east | E |
| north | N |
| south | S |
| west | W |
| copyright | © |
| corporate suffixes: | |
| Company | Co. |
| Corporation | Corp. |
| Incorporated | Inc. |
| Limited | Ltd. |
| District of Columbia | D.C. |
| et alii (and others) | et al. |
| et cetera (and so forth) | etc. |
| exempli gratia (for example) | e.g. |
| Federal Information Code | FIC |
| id est (that is) | i.e. |
| latitude or longitude | lat. or long. |
| monetary symbols (U.S.) | $, ¢ |
| months (tables and figures): first three letters | Jan,...,Dec |
| registered trademark | ® |
| trademark | ™ |
| United States (adjective) | U.S. |
| United States of America (noun) | USA |
| U.S.C. | United States Code |
| U.S. state | use two-letter abbreviations (e.g., AK, WA) |

**Measures (fisheries)**

| | |
|---|---|
| fork length | FL |
| mideye-to-fork | MEF |
| mideye-to-tail-fork | METF |
| standard length | SL |
| total length | TL |

**Mathematics, statistics**

| | |
|---|---|
| *all standard mathematical signs, symbols and abbreviations* | |
| alternate hypothesis | $H_A$ |
| base of natural logarithm | $e$ |
| catch per unit effort | CPUE |
| coefficient of variation | CV |
| common test statistics | (F, t, $\chi^2$, etc.) |
| confidence interval | CI |
| correlation coefficient (multiple) | R |
| correlation coefficient (simple) | r |
| covariance | cov |
| degree (angular ) | ° |
| degrees of freedom | df |
| expected value | $E$ |
| greater than | > |
| greater than or equal to | ≥ |
| harvest per unit effort | HPUE |
| less than | < |
| less than or equal to | ≤ |
| logarithm (natural) | ln |
| logarithm (base 10) | log |
| logarithm (specify base) | $\log_2$, etc. |
| minute (angular) | ' |
| not significant | NS |
| null hypothesis | $H_O$ |
| percent | % |
| probability | P |
| probability of a type I error (rejection of the null hypothesis when true) | α |
| probability of a type II error (acceptance of the null hypothesis when false) | β |
| second (angular) | " |
| standard deviation | SD |
| standard error | SE |
| variance | |
| population | Var |
| sample | var |

*FISHERY DATA SERIES NO. 06-20*

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April 2006

This project was approved by the Federal Subsistence Board, managed by the U.S. Fish and Wildlife Service, Office of Subsistence Management, and funded by the U.S. Forest Service under agreement number 53-0109-4-0036.

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*This document should be cited as:*
*Seeb, L. W., D. Moore, C. T. Smith, and W. D. Templin. 2006. Progress in development of a DNA baseline for genetic identification of Chinook salmon stocks of the Copper River Basin, Alaska. Alaska Department of Fish and Game, Fishery Data Series No. 06-20, Anchorage.*

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# ABSTRACT

This report serves as a first-year summary of progress of a contract study intended to document the timing and origins of Chinook salmon *Oncorhynchus tshawytscha* stocks in the Copper River and adjacent ocean fisheries using DNA markers. The project is intended to investigate the genetic structure of Chinook salmon from the Copper River drainage using both microsatellite and single nucleotide polymorphisms (SNPs). Identification of genetic stocks of Chinook salmon within the Copper River drainage and knowledge of their run timing and migration patterns will provide valuable information to optimize management and provide for sustainable fisheries. The first objective of this contract study is to develop a DNA database to delineate major geographic and temporal stocks of Chinook salmon within the Copper River. This objective requires collection of tissue samples as well as the laboratory DNA analyses of those tissues. A second objective is to investigate run timing and entry patterns within the Copper River through the analysis of radio-tagged and fishwheel samples from Baird Canyon. A third objective is to characterize the timing of Copper River stocks in the ocean fisheries and estimate the contribution to this fishery by stocks of non-Copper River origin. Finally, these data will be standardized and contributed to a coastwide DNA database so that Copper River Chinook salmon can be tracked throughout their marine migration. This report reviews the first year of the study which concentrated on Objective 1. A total of 1,272 individual Chinook salmon were sampled from the Upper Copper, Gulkana, Tazlina, Klutina, Tonsina, and Chitna river drainages for the baseline collections. In addition, 477 and 496 radio-tagged individuals were collected at the Baird Canyon fishwheel in 2003 and 2004, respectively, in collaboration with another contracted study, FIS01-020 (*Feasibility of using fishwheels for long-term monitoring of Chinook salmon escapement on the Copper River*). Analyses of the baseline collections for 25 SNP markers have been completed. Preliminary results indicate diversity among the major drainages of Chinook salmon of the Copper River at a scale that should prove useful for in-river stock identification studies. Analyses of the baseline collections for microsatellite markers are underway. Baird Canyon and ocean fisheries samples will be analyzed once the baseline has been completed.



Key words: Chinook salmon, *Oncorhynchus tshawytscha*, Copper River, salmon genetics, single nucleotide polymorphisms


# INTRODUCTION

Sustained productivity of salmon is thought to be dependent on the maintenance of genetic diversity and population structure (NRCC 1996; Hilborn et al. 2003). This can only be accomplished through careful and informed management of the resource. Identification of genetic stocks of Chinook salmon within the Copper River drainage and knowledge of their run timing and migration patterns is important to enable optimal management and sustainable fisheries.

Population genetic studies of Chinook salmon in Alaska have demonstrated the existence of several genetic lineages (Gharrett et al. 1987; Crane et al. 1996; Templin et al. 2005; Smith et al. 2005a, b). In addition, genetic databases have been shown to be useful management tools in many different salmon fisheries, including Chinook salmon fisheries on the Yukon and Kuskokwim rivers, in other areas of Alaska, and the Pacific Northwest (e.g., Utter et al. 1987; Marshall et al. 1991; Templin et al. 2005; Smith et al. 2005b). Population genetic studies of salmon in Alaska have demonstrated the importance of examining many loci when estimating genetic differentiation and patterns of genetic exchange among populations or when estimating the contributions of individual populations in mixed stock aggregations (Scribner et al. 1998; Allendorf and Seeb 2000). In this study, we will use two DNA marker types: microsatellites and single nucleotide polymorphisms (SNPs) to assay populations in the Copper River drainage. This report reviews the results to date on SNP markers. Analyses of microsatellite markers are currently underway in our laboratory.

SNPs are the most common form of genetic variation known to exist in eukaryotic genomes and are thus being intensively studied as part of several genome projects. Genotyping assays developed for SNPs are much quicker and cheaper than assays available for alternative DNA markers such as microsatellites (Landegren et al. 1998; Hirschhorn et al. 2000; Ranade et al. 2001; Fujimura et al. 2002, also see recent review in Melton 2003). Furthermore, SNP data are, unlike

allozyme and microsatellite data, completely reproducible and combinable among all laboratories without the need for standardization. Both of these attributes have contributed to the success of recent population genetic studies based on SNP markers (Batley and Hayes 2003; Smith et al. 2005b) and offer many advantages for stock identification studies of Pacific salmon.

Data gathered from the two DNA marker types will be used to develop a baseline for Chinook salmon populations within the Copper River drainage. The baseline can be used to investigate run timing and entry patterns within the river, estimate stock composition of adjacent ocean fisheries, and ascertain the effectiveness of management actions for the conservation of the resource. These data will be contributed to a Pacific Rim genetic database for Chinook salmon, aiding the identification of Copper River salmon in studies of migration patterns of juveniles and sub-adults on the high seas and in interception fisheries.

# OBJECTIVES

Specific objectives associated with the multi-year contract between the United States Forest Service and the Alaska Department of Fish and Game (ADF&G) are to:

1. Develop a DNA database of genetic markers to delineate major geographic and temporal stocks within the Copper River.
2. Investigate run timing and entry patterns within the Copper River through the analysis of radio-tagged and fishwheel samples from Baird Canyon.
3. Characterize the timing of Copper River stocks in the ocean fisheries and estimate the contribution to this fishery by stocks of non-Copper River origin.
4. Standardize and contribute the Copper River data to a coastwide DNA database.

The objective of this report is to provide a summary of progress to date in development of a DNA baseline representing spawning stocks of Chinook salmon in the Copper River drainage.

# METHODS

## SAMPLE COLLECTION

During the 2004 field season, fin tissue, axillary processes, or intact juveniles were collected from the Copper River drainage by personnel from the Native Village of Eyak, National Park Service, ADF&G, and other local collaborators (Figure 1). A total of 1,272 fish, primarily adults, were sampled (Table 1). The goal of sampling a minimum of 100 Chinook salmon per spawning population was achieved for the Upper Copper, Klutina, Tazlina, and Gulkana drainages. Although each drainage was visited multiple times, the full sampling goal was not achieved for the Chitina and Tonsina drainages. A second year of sampling was included in the contract, and additional fish from these two areas will be sampled in 2005, Year 2 of the study. Sites were accessed using a combination of techniques depending on the system including fixed and rotary-wing aircraft, float trips, and sampling along the road system. Adults were captured by hook and line, sampled, and released live. Minnow traps were used for juveniles from the Tazlina and Tonsina rivers.

In addition to the spawning collections, 477 and 496 Chinook salmon were collected by Native Village of Eyak (NVE) and ADF&G at the Baird Canyon fish wheel/tagging project during 2003 and 2004, respectively. The radio-tag information from these individuals will be evaluated to determine the ultimate spawning location of the tagged individuals and where appropriate, those fish will be added to the baseline in Year 2.

## LABORATORY ANALYSIS

Genomic DNA was extracted from each tissue sample using DNAeasy 96 columns (QIAGEN, Valencia, CA). Twenty five SNP loci were genotyped following the methods described by Smith et al. (2005a, c). SNP assays were developed at the ADF&G laboratory and included those described in Smith et al. (2005a, c)

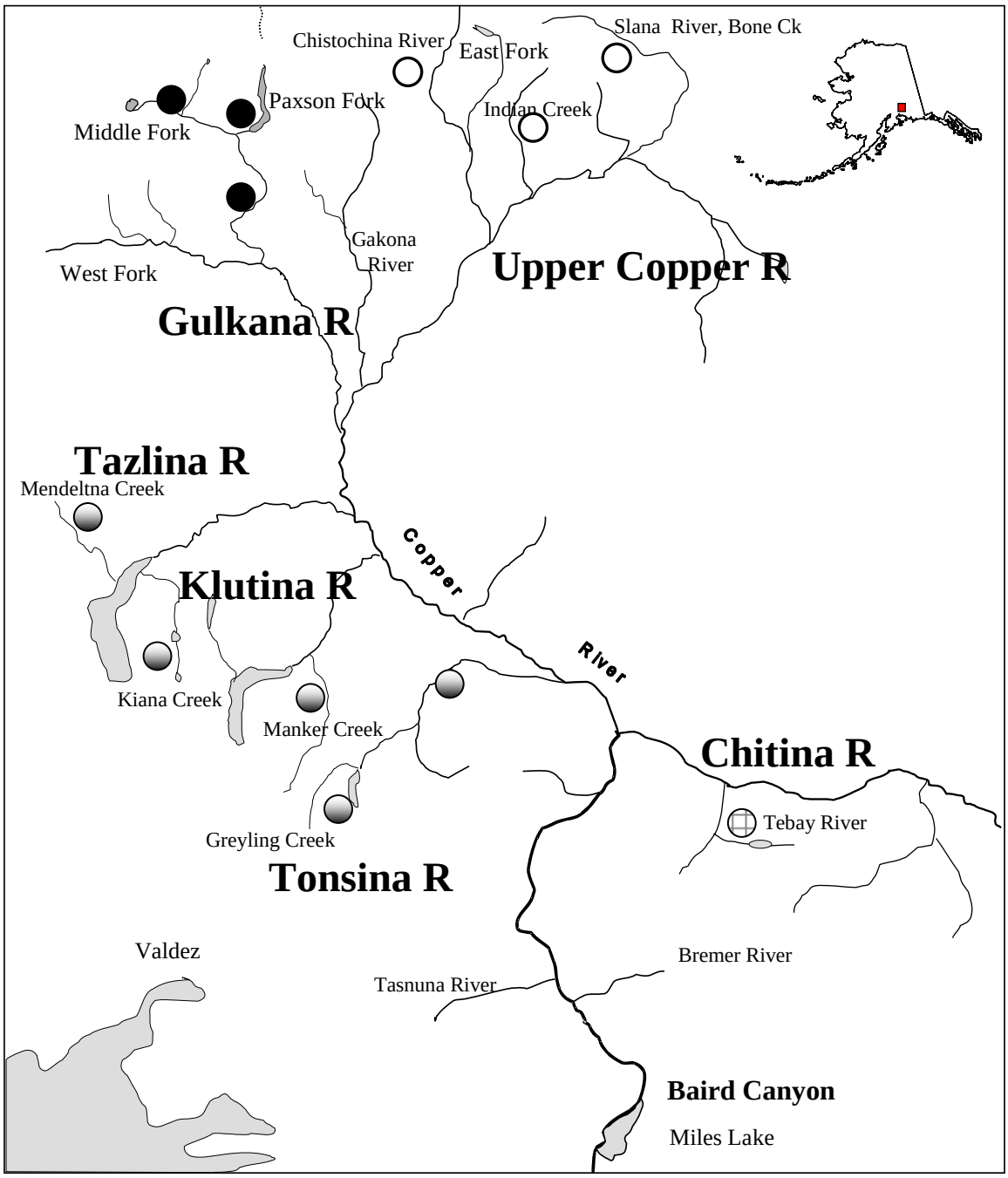


**Figure 1.–**Map of the Copper River drainage with the locations of collections indicated by dots. Color of dot indicates major drainage.

**Table 1.**–Chinook salmon collections from the Copper River drainage and fisheries.

| Drainage Location | n | Life Stage | Year |
|---|---|---|---|
| Baseline | | | |
| Chitna | | | |
| Tebay River – lake outlet | 27 | Adult | 2004 |
| Tonsina | | | |
| Little Tonsina River [a] | 26 | Juvenile | 2004 |
| Little Tonsina River | 31 | Adult | 2004 |
| Greyling Creek | 16 | Adult | 2004 |
| Klutina | | | |
| Klutina River (guides)[a] | 168 | Adult | 2004 |
| Manker Creek | 41 | Adult | 2004 |
| Tazlina | | | |
| Kaina Creek [a] | 133 | Juvenile | 2004 |
| Kaina Creek | 75 | Adult | 2004 |
| Mendeltna Creek | 144 | Adult | 2004 |
| Gulkana | | | |
| Middle Fork | 79 | Adult | 2004 |
| Mainstem | 46 | Adult | 2004 |
| Paxson Fork | 88 | Adult | 2004 |
| Gulkana (guides)[a] | 130 | Adult | 2004 |
| Upper Copper | | | |
| Bone Creek | 70 | Adult | 2004 |
| East. Fork Chistochina River | 145 | Adult | 2004 |
| Indian Creek | 43 | Adult | 2004 |
| Sinona Creek[a] | 7 | Adult | 2004 |
| Slana River, Ahtell Creek[a] | 3 | Adult | 2004 |
| Radio Tagged Individuals | | | |
| Baird Canyon[a] | 477 | Adult | 2003 |
| Baird Canyon[a] | 496 | Adult | 2004 |
| Fishwheel Collections | | | |
| 6 weeks @200/week (end of May to late July) | 1,200 | Adult | 2005 |
| Fishery Collections | | | |
| 4 weeks @300/week (May to June) | 1,200 | Adult | 2005 |

[a] Excluded from Year 1 analyses.

Amplification was conducted in 384-well thermal cycler blocks on either a DNA Engine Tetrad (MJ Research, Waltham, MA) or an ABI7900 real-time PCR instrument (Applied Biosystems Inc., Foster City, CA). End point analysis of each 384-well plate was performed on an ABI7900. Sequence Detection Software 2.1 (Applied Biosystems Inc.) was used to score alleles. Each run was scored independently by two researchers in ensure data integrity.

### DATA ANALYSIS

Non-spawning ground samples including juveniles, samples from guides, and radio tagged individuals were not included in the Year 1 data analyses. After the Year 2 sampling effort is complete and the extent of the spawning ground samples known, the non-spawning samples will be evaluated and included in the baseline as technically appropriate.

Evaluation criteria will include both the number and distribution of the spawning ground samples as well as statistical tests of the non-spawning samples to determine their genetic composition and whether they are representative of the spawning populations.

Analyses conducted to date were based on 12 populations (Table 1). Estimates of the population frequency of individual alleles for each locus were calculated from the observed frequency of the allele in the representative sample. Observed and expected heterozygosity for each locus were calculated as well as genetic diversity ($F_{ST}$ values) across all populations (Table 2) using FSTAT version 2.8 (Goudet 1999) and *GENEPOP*. Chord distances (Cavalli-Sforza and Edwards 1967) were calculated to summarize allele frequency differences between pairs of populations. Population structure was visualized using multidimensional scaling (MDS) as implemented in *NtSYS* (Exeter Software, Setauket, NY) to reduce the dimensionality of the interpopulation chord distances to three-dimensional space. Patterns observed using this method reflect the genetic distinctions between the populations in the analysis.

Simulations were conducted to evaluate the potential application of genetic stock identification to mixtures of Chinook salmon from the Copper River. These simulations may be used to help assess whether the baseline of allele frequencies at SNP and microsatellite markers provides sufficient information to identify individual stocks or groups of stocks (reporting groups) in hypothetical mixtures.

Reporting groups were defined based on a combination of genetic similarity, geographic features, and management applications. Simulations were performed using the Statistical Package for Analyzing Mixtures (SPAM version 3.6, Debevec et al. 2000). Baseline and mixture genotypes were randomly generated from the baseline allele frequencies assuming Hardy-Weinberg equilibrium. Each simulated mixture (N=400) was composed of 100% of the stock or reporting group under study. When a reporting group mixture was simulated, all stocks in the stock group contributed equally to the mixture. Average estimates of mixture proportions and 90% confidence intervals were derived from 1,000 simulations. Reporting groups with mean correct estimates of 90% or better are considered highly identifiable in fishery applications (e.g. Seeb et al. 2000). 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.

## RESULTS

All 25 SNPs examined were polymorphic although the variant alleles at three nuclear SNPs (*Ots_GPDH, Ots_Ikaros-250, Ots_u211-85)* loci were expressed at an average frequency of <0.05 (Table 2). Expected heterozygosity per locus varied from 0.003 to 0.477, and $F_{st}$ values ranged from near zero to a high of 0.183 at *Ots_u211-85.*

Chord distances were calculated between every pair of populations and used to construct an MDS diagram (Figure 2). The MDS analysis divided the Chinook salmon samples among 5 regional groupings corresponding to the major drainages: Chitna, Tonsina, Klutina/Tazlina, Gulkana, and Upper Copper. Diversity is apparent within the Upper Copper drainages between Bone Creek and collections from Chistochina River and Indian Creek. Conversely, little differentiation is apparent

between collections originating from the Klutina and Tazlina drainages.

Simulations were performed to assess the ability of the SNP data to accurately assign regions. We performed 100% simulations for Chinook salmon from the four major regions or drainages identified in the MDS: Chitna, Middle Lakes (Tonsina, Klutina, and Tazlina), Gulkana, and Upper Copper. Simulated fish were correctly assigned to region/drainages 89–98% of the time (Table 3). We also performed a 100% simulation for Bone Creek Chinook salmon populations which was significantly divergent from the other populations in the Upper Copper drainage. The results for Bone Creek indicate that these Chinook salmon are also highly identifiable; simulated fish were correctly assigned to Bone Creek 96% of the time.

**Table 2.–**Single nucleotide polymorphisms assayed in Copper River Chinook salmon. Observed range of most common allele, observed heterozygosity (Ho), expected heterozygosity (Hs), and genetic diversity (FST) values are given..

| Locus or Temporary Name | Source | Range of Common Allele | Observed ($H_o$) | Expected ($H_s$) | ($F_{ST}$) |
|---|---|---|---|---|---|
| *Ots_C3N3* | Smith et al. 2005a | 0.924 - 1.000 | - | - | 0.524 |
| *Ots_E2-275* | Smith et al. 2005a | 0.625 - 0.964 | 0.203 | 0.203 | 0.043 |
| *Ots_FGF6A* | NWFSC[a] Unpublished | 0.500 - 1.000 | 0.292 | 0.292 | 0.037 |
| *Ots_FGF6B* | NWFSC[a] Unpublished | 0.757 - 1.000 | 0.131 | 0.135 | 0.005 |
| *Ots_GH2* | Smith et al. 2005a | 0.441 - 0.729 | 0.464 | 0.455 | 0.027 |
| *Ots_GnRH-271* | Smith et al. 2005c | 0.941 - 1.000 | 0.037 | 0.037 | 0.000 |
| *Ots_GPDH* | Smith et al. 2005c | 0.962 - 1.000 | 0.012 | 0.011 | 0.000 |
| *Ots_IGF-I.1-76* | Smith et al. 2005c | 0.374 - 0.765 | 0.472 | 0.466 | 0.072 |
| *Ots_Ikaros-250* | Smith et al. 2005c | 0.984 - 1.000 | 0.003 | 0.003 | 0.000 |
| *Ots_ins-115* | Smith et al. 2005c | 0.821 - 1.000 | 0.112 | 0.110 | 0.011 |
| *Ots_LWSop-638* | Smith et al. 2005c | 0.810 - 1.000 | 0.110 | 0.115 | 0.057 |
| *Ots_MHC1* | Smith et al. 2005a | 0.259 - 0.852 | 0.395 | 0.385 | 0.073 |
| *Ots_Ots2* | Smith et al. 2005a | 0.929 - 1.000 | 0.007 | 0.007 | 0.000 |
| *Ots_P450* | Smith et al. 2005a | 0.484 - 0.707 | 0.490 | 0.472 | 0.017 |
| *Ots_P53* | Smith et al. 2005a | 0.339 - 0.685 | 0.443 | 0.460 | 0.039 |
| *Ots_Prl2* | Smith et al. 2005a | 0.500 - 0.845 | 0.438 | 0.414 | 0.070 |
| *Ots_SClkF2R2-135* | Smith et al. 2005c | 0.443 - 0.756 | 0.470 | 0.470 | 0.030 |
| *Ots_SL* | Smith et al. 2005a | 0.477 - 0.742 | 0.487 | 0.470 | 0.029 |
| *Ots_SWS1op-182* | Smith et al. 2005c | 0.357 - 0.687 | 0.546 | 0.477 | 0.016 |
| *Ots_Tnsf* | Smith et al. 2005a | 0.744 - 0.963 | 0.285 | 0.281 | 0.060 |
| *Ots_u202-161* | Smith et al. 2005c | 0.707 - 0.976 | 0.227 | 0.225 | 0.082 |
| *Ots_u211-85* | Smith et al. 2005c | 0.987 - 1.000 | 0.004 | 0.004 | 0.183 |
| *Ots_u4-92* | Smith et al. 2005c | 0.859 - 0.993 | 0.100 | 0.101 | 0.000 |
| *Ots_u6-75* | Smith et al. 2005c | 0.750 - 0.993 | 0.099 | 0.109 | 0.000 |
| *Ots_Zp3b-215* | Smith et al. 2005c | 0.926 - 1.000 | 0.026 | 0.025 | 0.000 |

[a] Northwest Fisheries Science Center, NOAA Fisheries, Seattle, WA

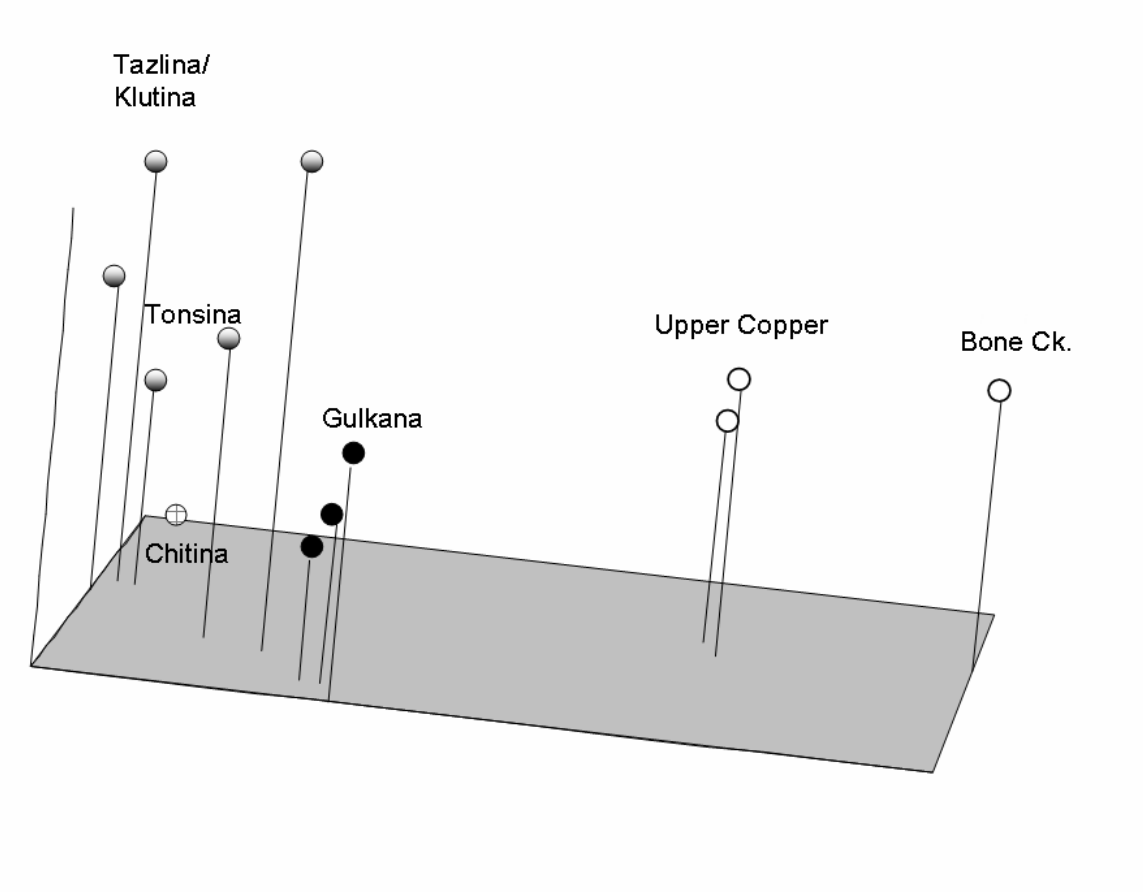


**Figure 2.–**Multidimensional scaling of pairwise genetic distances (Cavalli-Sforza and Edwards 1967) between Chinook salmon populations in the Copper River calculated from allele frequencies at 25 SNP loci. Color of dots indicate major drainages.

**Table 3.–**Results of simulated mixtures of Copper River Chinook salmon with 1,000 bootstrap resamples and a simulated sample size of 400 in which each of four drainages comprised 100% of the hypothetical mixture. Bold type indicates the allocation to the correct drainage(s).

| | Mixture | Upper Copper | Gulkana | Middle Lakes | Chitina |
|---|---|---|---|---|---|
| 1 | Upper Copper | **0.986** | 0.007 | 0.005 | 0.001 |
| 2 | Gulkana | 0.008 | **0.976** | 0.015 | 0.001 |
| 3 | Middle Lakes | 0.010 | 0.030 | **0.950** | 0.007 |
| 4 | Chitina | 0.005 | 0.002 | 0.142 | **0.852** |

# DISCUSSION

This report reviews the results for the first year of a three year study. In this first year, we primarily addressed Objective 1, to develop a DNA database of genetic markers to delineate major geographic and temporal stocks within the Copper River. This objective included field work to develop a comprehensive tissue collection from the Copper River as well as laboratory analyses. This project will survey both microsatellite and SNP loci. Results to date include only SNP loci. The microsatellite loci have now been finalized by the GAPS group (Table 4), and analyses are currently underway.

Extensive efforts were made during the summer field season to sample spawning populations of Chinook salmon from the drainage. Overall effort was successful although the project was more successful than anticipated in the upper Copper River drainage, but experienced sampling difficulties in the lower river (Chitina and Tonsina drainages). However, the project includes two years of field collections to ensure areas that were unrepresented in Year 1 could be resampled in Year 2. Plans are currently underway to augment sample sizes in the underrepresented areas.

During this first year, we were able to assay all the adults collected from the spawning grounds for 25 SNP markers. SNP markers are an appealing management tool as they are easily automated and standardized among laboratories and platforms. The results of the SNP analyses are highly encouraging. These loci alone identified Chinook salmon from the major drainages of the Copper River as well as considerable diversity within the Copper River at a scale that should prove very useful for in-river analyses. These 25 SNPs did not, however, differentiate between populations originating from the Klutina and Tazlina rivers. Additional markers may differentiate among these populations or, alternatively, provide further evidence for the similarity and probable connectivity between the Klutina and Tazlina rivers.

Tissue collections were also made at the Baird Canyon fishwheel in 2003 and 2004 from radio-tagged fish in cooperation with project FIS01-020 (*Feasibility of using fishwheels for long-term monitoring of Chinook salmon escapement on the Copper River*). As appropriate, these fish will be used to augment the baseline and/or identify underrepresented populations. In 2005, the fishwheel will be sampled weekly over a 6-week period to assess Objective 2, run timing and entry patterns into the river.

Comparisons to the run-timing results generated in Project 02-215 [*Migratory timing and spawning distribution of Chinook salmon in the Copper River;* Savereide (2004)] will also be conducted to compare the relationship between genetic lineages and run-timing. For example, Savereide (2004) found that the run timing of Chinook salmon bound for the tributaries of the Tonsina and Klutina rivers was earlier than other mainstem drainages.

Consistent with the proposed timeline, no work was planned for Objective 3 in Year 1. This objective is to characterize the timing of Copper River stocks in the ocean fisheries and estimate the contribution to this fishery by stocks of non-Copper River origin. Sampling is underway for the ocean fisheries in 2005. The GAPS coastwide database will be required to complete this objective. The first contributions to the database of approximately 100 populations ranging from California to Yakutat will be made in June 2005. Through other ADF&G studies, additional spawning collections from the Gulf of Alaska will be contributed to the GAPS database including representative populations from Cook Inlet and Kodiak Island. Inclusion of these populations will be necessary before compositional estimates can be generated for the ocean fisheries.

**Table 4.–**Microsatellite loci for Chinook salmon adopted by the Genetic Analysis of Pacific Salmonids (GAPS) group and funded by the Chinook Technical Committee of the Pacific Salmon Commission. Copper River Chinook salmon spawning populations will be surveyed at each of these loci.

| Locus | Primer Sequence (5'→ 3') **F** > Forward, **R** > Reverse | Curator Agency[a] |
|---|---|---|
| *Ots201b* | F- CAGGGCGTGACAATTATGC<br>R- TGGACATCTGTGCGTTGC | ADF&G |
| *Ots208b* | F- GGATGAACTGCAGCTTGTTATG<br>R- GGCAATCACATACTTCAACTTCC | CRITFC |
| *Ots211* | F - TAGGTTACTGCTTCCGTCAATG<br>R - GAGAGGTGGTAGGATTTGCAG | ADF&G |
| *Ots212* | F- TCTTTCCCTGTTCTCGCTTC<br>R- CCGATGAAGAGCAGAAGAGAC | OSU |
| *Ogo4* | F- GTCGTCACTGGCATCAGCTA<br>R- GAGTGGAGATGCAGCCAAAG | WDFW |
| *Ogo2* | F- ACATCGCACACCATAAGCAT<br>R- GTTTCTTCGACTGTTTCCTCTGTGTTGAG | ADF&G |
| *Ots3M* | F- TGTCACTCACACTCTTTCAGGAG<br>R- GAGAGTGCTGTCCAAAGGTGA | WDFW |
| *Ots213* | F- CCCTACTCATGTCTCTATTTGGTG<br>R- AGCCAAGGCATTTCTAAGTGAC | OSU |
| *Omm1080* | F- GAGACTGACACGGGTATTGA<br>R- GTTATGTTGTCATGCCTAGGG | SWFSC |
| *Ssa408uos* | F- AATGGATTACGGGTACGTTAGACA<br>R- CTCTTGTGCAGGTTCTTCATCTGT | NWFSC |
| *Ots9* | F- ATCAGGGAAAGCTTTGGAGA<br>R- CCCTCTGTTCACAGCTAGCA | CDFO |
| *OtsG474* | F- TTAGCTTTGGACATTTTATCACAC<br>R- CCAGAGCAGGGACCAGAAC | CRITFC |
| *Oki100* | F- CCAGCACTCTCACTATTT<br>R- CCAGAGTAGTCATCTCTG | CDFO |

[a] Laboratory abbreviations: OSU, Oregon State University; SWFSC, Southwest Fisheries Science Center – NOAA Fisheries; CDFO, Canadian Department of Fisheries and Oceans; NWFSC, Northwest Fisheries Science Center – NOAA Fisheries; CRITFC, Columbia River Inter–Tribal Fish Commission; ADF&G, Alaska Department of Fish and Game; WDFW, Washington Department of Fish and Wildlife.

## CONCLUSIONS AND RECOMMENDATIONS

1. Significant genetic structure exists among populations of Chinook salmon from the Copper River drainage. At least five separate genetic lineages were detected in the drainage associated with many tributaries. Populations spawning in the Upper Copper River are particularly genetically divergent.
2. Genetic analyses based solely on SNP loci provide adequate distinction within the Copper River to estimate composition and run timing of regional groups in the river and from collections made at Baird Canyon. Microsatellite and additional SNP loci are currently under analysis and should provide increased resolution.
3. Collections from Baird Canyon and ocean fisheries are underway in 2005.
4. Once a coastwide DNA baseline is complete, analysis of the ocean fisheries targeting Copper River stocks can be initiated.

## ACKNOWLEDGEMENTS

A large number of researchers and local residents assisted with the collection of tissue samples. We thank Judy Berger, Nick Decovich, Andy Barclay, Bruce Whelan, Anton Antonovich, Eric Lardizabal, Jim Seeb, Heather Hoyt, and David Robinson from the ADF&G Gene Conservation Laboratory. The assistance of Tom Taube, Mark Stadtmiller, and James Savereide from ADF&G Sport Fish was especially valuable in locating spawning populations and collecting radio-tagged individuals. We are also thankful for the valuable assistance of Mike Lambert, Keith Van den Broek, and Jake Ferguson from the Native Village of Eyak, Jason Smith from LGL, Allen LaMaster from Klutina River Guides, Charlie David and Kathryn Martin from Mentasta Village Council, Jay Capps from Slana, Shawn Sanford from the village of Mentasta, and Joeneal Hicks from Chistochina Village Council. Bruce Whelan, Andy Barclay, David Robinson, and Nick Decovich performed the laboratory analyses. This project was approved by the Federal Subsistence Board, managed by the U.S. Fish and Wildlife Service, Office of Subisistence Management, and funded by the U.S. Forest Service under Agreement Number 53-0109-4-0036.

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