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Timing and Origin of Chinook Salmon Stocks in the Copper River using DNA Markers

Report to the Alaska Board of Fisheries December 1-7, 2008, Cordova, Alaska

by

William D. Templin,

Nick Decovich,

Andy Barclay,

Christian T. Smith,

and

Lisa W. Seeb

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Alaska Department of Fish and Game Divisions of Sport Fish and Commercial Fisheries

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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, χ^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

SPECIAL PUBLICATION NO. 08-14

TIMING AND ORIGIN OF CHINOOK SALMON STOCKS IN THE COPPER RIVER USING DNA MARKERS REPORT TO THE ALASKA BOARD OF FISHERIES DECEMBER 1-7, 2008, CORDOVA, ALASKA

by
William D. Templin, Nick Decovich, Andy Barclay, Christian T. Smith, and Lisa W. Seeb
Alaska Department of Fish and Game, Division of Commercial Fisheries, Gene Conservation Laboratory, Anchorage

Alaska Department of Fish and Game
Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska, 99518-1565

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William D. Templin, Nick Decovich, Andy Barclay, Christian T. Smith, and Lisa W. Seeb
Alaska Department of Fish and Game, Division of Commercial Fisheries,
333 Raspberry Road, Anchorage, AK 99518, USA

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TABLE OF CONTENTS

Page

LIST OF TABLES ii

LIST OF FIGURES ii

ABSTRACT 1

INTRODUCTION 1

METHODS 2

Sample Collection 2

Laboratory Analysis 3

Statistical Analyses 3

- Diversity within Populations 3
- Population Structure 4

Analysis of Run-Timing 4

RESULTS 6

Sample Collection 6

Laboratory Analysis 6

Statistical Analyses 6

- Diversity within Populations 6
- Population Structure 7

Analysis of Run-Timing 8

DISCUSSION 8

Genetic Diversity 9

Spatial Analysis 10

Marker Comparison 10

Inriver Migration 11

Standardization 12

CONCLUSIONS 12

ACKNOWLEDGEMENTS 13

REFERENCES CITED 14

TABLES AND FIGURES 19

LIST OF TABLES

Table **Page**

1. Chinook salmon collections from the Copper River drainage. 20
2. Genetic markers assayed in Copper River Chinook salmon. 21
3. Measures of within population diversity in populations of Chinook salmon in the Copper River, Alaska. 24
4. Mean reporting group allocations of simulated mixtures of Copper River Chinook salmon from the baseline of 45 SNPs and 13 microsatellite markers. 25
5. Simulated mixtures of individual populations of Copper River Chinook salmon from the baseline of 45 SNPs and 13 microsatellite markers. 26
6. Stock composition estimates and 90% credibility intervals from two mixture samples from sport-caught Chinook salmon in the Copper River drainage using the BAYES model. 27
7. Relative proportion of reporting groups of Chinook salmon sampled approximately weekly from the Baird Canyon fish wheel, 2005. 28

LIST OF FIGURES

Figure **Page**

1. Collection locations for Chinook salmon from the Copper River drainage. 29
2. Multidimensional scaling plot based on genetic distances calculated from 45 SNP loci. 30
3. Multi-dimensional scaling plot based on genetic distances calculated from 13 microsatellite loci 31
4. Allelic richness for Copper River Chinook salmon populations measured with microsatellite and SNP loci. 32
5. Isolation by distance relationship based on pairwise comparison of genetic and geographic distances between Copper River Chinook salmon populations using 45 SNPs. 33
6. Weekly stock composition estimates of Chinook salmon captured by the Baird Canyon fish wheel, 2005. 34
7. Cumulative return for five regional groups of Chinook salmon in samples taken at the Baird Canyon fish wheel, 2005. 35

PREFACE

This report was written to provide a preview of the current status of a genetic study on the population structure and migration timing of Chinook salmon in the Copper River and was presented to the Board of Fisheries in December, 2008, at the meeting in Cordova, Alaska. This study was funded by the U.S. Fish and Wildlife Service, Office of Subsistence Management, through the Fisheries Resource Monitoring Program as Project 04-507. The contents of this report were extracted from the more comprehensive analysis presented in the final report for Project 04-507. Reference to results presented in this report should be cited as:

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This report should not be cited without the author's permission.

ABSTRACT

The objectives of this study were to delineate major geographic and temporal stocks of Chinook salmon within the Copper River, determine the potential of genetic markers to distinguish among stocks within the Copper River drainage, and to investigate run timing within the Copper River. The system exhibits significant genetic divergence both within and among its major drainages. With some exceptions, populations adhere to an isolation-by-distance model in that populations closest geographically are also closest genetically. The broad groups include a heterogeneous collection of populations in the Upper Copper River, a homogeneous group from the Gulkana River drainage, and a diverse set of Lower Copper River glacial lake populations from the Tazlina, Klutina, Tonsina, and Chitina river drainages. Within the Lower Copper River group, two single collections were particularly divergent, Tebay River from the Chitina River drainage and Mendeltna Creek from the Tazlina River drainage. This genetic structure was adequate to allow for the discrimination of five stocks within stock mixtures with high accuracy and precision. The inriver collections from Baird Canyon showed that the Upper Copper River stocks contributed early followed by the Gulkana River then Mendeltna Creek. Stocks from the Lower Copper River were the last to pass through Baird Canyon. Extension of genetic stock identification to the commercial fishery on the Copper River delta will require combining these data with the baseline developed by the Pacific Salmon Commission and including Chinook salmon systems from the rest of the Gulf of Alaska.

Key words: Chinook salmon, *Oncorhynchus tshawytscha*, microsatellite, single nucleotide polymorphism, population structure, Copper River, run timing.

INTRODUCTION

Chinook salmon *Oncorhynchus tshawytscha* from the Copper River provide opportunities for commercial, subsistence, personal use, and sport harvests. Chinook salmon from the Copper River have been harvested commercially since the late 1800s (Moser 1899). Today, commercial harvests occur in an ocean drift gill net fishery in the Copper River District (in and around the mouth of the Copper River). The number of Chinook salmon that return to the Copper River has varied markedly. The most current management report for Copper River Chinook salmon reviews the 2005 season in detail (Hollowell et al. 2007) and reports that the total run was 65,949 with 52.5% harvested commercially, 7.2% harvested by personal use and subsistence users, and 6.2% harvested by upriver sport users with the remaining 32.8% (21,604) from 2005 contributing to spawning escapement. Preliminary numbers for 2007 indicate that an estimated 39,456 Chinook salmon were harvested in the Copper River District (ADF&G 2007).

In recent years, a number of comprehensive studies of the abundance, spawning distribution, and run-timing of Chinook salmon from the Copper River have been conducted using radiotelemetry methods (Savereide 2005; Wuttig and Evenson 2001). In these studies, returning adult Chinook salmon were radio-tagged near Baird Canyon and tracked to upriver destinations using ground-based receiving stations and aerial tracking techniques. Chinook salmon were tracked to six major tributaries: Gulkana, Tonsina, Klutina, Tazlina, Chitina, and East Fork Chistochina rivers (Figure 1). Although run-timing patterns varied over time, upriver stocks returned earlier than downriver stocks (Savereide 2005).

Life history diversity of Copper River Chinook salmon has long been recognized both for temporal divergence in run-timing as well as phenotypic diversity. Chinook salmon, along with sockeye salmon *O. nerka*, have been the mainstay of the Ahtna who have inhabited the region for at least a millennium (Workman 1976). Recent studies based on Ahtna environmental knowledge (Simeone and Valentine 2007) highlight this diversity, which is reflected in a large number of descriptive traditional names.

Two factors important for sustained productivity of salmon are the maintenance of genetic diversity and population structure (NRCC 1996). In Bristol Bay, Alaska, Hilborn et al. (2003) hypothesized that the sustainable fisheries are supported by several hundred discrete spawning populations with diverse life history characteristics and local adaptations in spawning and rearing habitat. They concluded that the biocomplexity has enabled the aggregate of populations to sustain overall productivity despite major changes in climatic conditions in freshwater and marine environments and fluctuations in abundance of individual populations.

Numerous population genetic studies have documented the diversity of Chinook salmon from throughout their range and have demonstrated the existence of multiple genetic lineages and a high level of genetic diversity within the species. Allozyme studies provided the first descriptions of the population genetic structure (Crane et al. 1996; Gharrett 1987; Templin et al. 2005; Utter et al. 1987; Waples et al. 2004) and demonstrated the high level of diversity among life history types of Chinook salmon. Studies based on microsatellite DNA markers have confirmed the allozyme results and provided details in many areas of the range (Beacham et al. 2008; Seeb et al. 2007). In addition, genetic databases and the techniques of genetic stock identification (GSI) have been shown to be useful management tools in many different salmon fisheries, including Chinook salmon fisheries on the Yukon and Kuskokwim rivers, in areas across Alaska, and the Pacific Northwest (e.g., Utter et al. 1987; Templin et al. 2005; Smith et al. 2005c). Recently, studies based on single nucleotide polymorphisms (SNPs) have provided additional insights provided by putative adaptive marker loci (Smith et al. 2005c; Smith et al. 2007; Narum et al. 2008).

Despite this wealth of genetic data, the diversity of Chinook salmon of the Copper River is poorly described, and only a few representative populations have been included in the previous surveys. This lack of information prevents the inclusion of genetic considerations in management or conservation decisions and the use of genetic stock identification applications within the drainage. Further, the lack of genetic data prevents the identification of Copper River-origin stocks in marine or high-seas analyses.

Here we report the use of molecular genetics techniques to describe genetic diversity of Chinook salmon populations within the Copper River. This information is then applied in GSI analyses to monitor in-river migration and run-timing. These estimates provide one year of information on stock-specific run timing of Copper River spawners past the Baird Canyon fish wheel, demonstrating the potential use of genetic stock identification for investigating the migration of Chinook salmon within the Copper River drainage.

METHODS

SAMPLE COLLECTION

During the field seasons of 2004–2006, fin tissue, axillary processes, or intact juveniles were collected from the Copper River drainage by personnel from the Native Village of Eyak (NVE), National Park Service (NPS), Alaska Department of Fish and Game (ADF&G), and other local collaborators (Figure 1; Table 1). With the exception of the juvenile samples, tissues were collected non-lethally without regard to size, sex, or condition. Sites were accessed using a combination of methods depending on the river system including fixed and rotary-wing aircraft, boats, and road vehicles. Adults were captured on or near spawning grounds by hook and line or

by seine, sampled, and released live. Non spawning-ground samples were collected from sport fishing guides on the Klutina and Gulkana rivers and from minnow traps that attracted juveniles on the Tonsina River.

In addition to these spawning collections, tissue samples from radio-tagged Chinook salmon were collected by NVE and ADF&G as part of two studies, FIS Study 01-020 (Feasibility of using fish wheels for long-term monitoring of Chinook salmon escapement on the Copper River) (Smith 2004) and FIS Study 02-015 (Inriver abundance, spawning distribution and run timing of Copper River Chinook salmon, 2002–2004) (Savereide 2005). Fish were captured at the Baird Canyon fish wheel site, tagged with a radio transmitter, and located periodically during their upstream migration to spawn. At the end of the study, the upriver destinations of the tagged individuals were determined and assigned to the appropriate tissue sample. Radio-tagged individuals returning to the mainstem Tonsina River were included in the baseline because of the difficulty in obtaining returning adults on the spawning grounds. Radio-tagged individuals returning to the Chitina River were not included in the baseline because this collection was composed of small numbers of individuals tracked to a number of widely distributed tributaries within the drainage. This set of individuals could not be considered to represent a single population without further corroboration.

In 2005, the crew operating the Baird Canyon fish wheel as part of FIS Study 04-503 (Estimating Chinook salmon escapement on the Copper River) (Smith and van den Broek 2005) sampled Chinook salmon over a 2 month period (May 12–July 14, 2005; weeks 20–26) to provide a comprehensive set of samples to evaluate stock-specific run-timing. Samples were divided into approximately weekly samples for analysis. Weeks 26–29 were combined to achieve a sufficient sample size.

LABORATORY ANALYSIS

Specific information on the conditions and methods used for laboratory analysis are described in Seeb et al. (In review). Baseline collections were genotyped for 51 SNPs in nuclear DNA and one SNP in mitochondrial DNA (*Ots_C3N3*; Table 2). Data were collected for the 13 microsatellite loci currently included in the coastwide standardized database (Table 2; Seeb et al. 2007) following the procedures outlined.

Genotypes collected for both datasets were entered into the Gene Conservation Laboratory Oracle database, *LOKI*. Quality control measures included reanalysis of 8% of each collection for all markers to insure genotypes were reproducible and to identify laboratory errors and rates of inconsistencies. Genotypes were assigned to individuals using a double-scoring system. Two observers independently produced allele scores for an entire project before the two data sets were compared. Discrepancies between the two sets of scores were then resolved with one of two possible outcomes: 1) one score was accepted and the other rejected, or 2) both scores were rejected and the score was excluded.

STATISTICAL ANALYSES

Diversity within Populations

Although some populations were sampled in multiple years (e.g. Bone Creek, Tebay River) sample sizes were not adequate from individual years to test for temporal variability with sufficient power. Samples were pooled across years following the recommendations of Waples

(1990). Non-spawning ground samples including juveniles and samples from guides were not used in the baseline. Samples from radio tagged individuals were evaluated for inclusion in the baseline based on the number of individuals in the sample from a final location and conformance to Hardy-Weinberg equilibrium (HWE).

Genepop V4 (Rousset 2008) was used to perform exact tests for genotypic ratios that departed from HWE expectations and Fisher's tests for genotypic linkage disequilibrium between each pair of loci across samples within collections. Critical values for both tests were adjusted for multiple tests (Rice 1989) using an experiment-wise critical value of $\alpha = 0.05$ for each locus and adjusting for the number of possible tests within a locus. For pairs of loci where linkage was detected, the least polymorphic locus was excluded from further analyses. Mean expected and observed heterozygosities by locus over all populations were calculated using GenAlEx (Peakall and Smouse 2006). The presence of null alleles (alleles that cannot be detected using the current methods) in each population and locus, was tested using the ML-Null program (Kalinowski and Taper 2006) and critical values were adjusted for multiple tests as described above.

Population Structure

Genetic diversity as measured by F_{ST} was calculated pairwise between populations and overall populations for every locus and then over all loci for both SNPs and microsatellites using Genepop V4. The multi-locus estimates were calculated following the method of Rousset (2007) where additional weight is given to loci with larger sample sizes. Mean expected and observed heterozygosities by population over all loci were calculated using GenAlEx.

To infer the genetic relationship between sample collections, pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated among sites using PHYLIP for each marker-type dataset (Felsenstein 2004). Genetic chord distances for each marker type were then used to construct multidimensional scaling (MDS) plots to visualize patterns of similarity between populations.

Allelic richness, a measure of the number of alleles independent of sample size, was calculated for all loci for all populations to compare levels of genetic diversity using FSTAT v2.9.3.2 (Goudet 1995; Goudet 2001). Allelic richness was calculated across populations and loci and then averaged over samples and loci for regional groups. A permutation test was performed to test significance with 1,000 permutations in FSTAT. We evaluated three regional groups based on the larger drainage systems: Upper Copper River, Middle Copper River (Gulkana River), and Lower Copper River (Tazlina, Klutina, Tonsina, and Chitina rivers).

We also used spatial analyses to evaluate patterns of genetic structure. Mantel tests were used to estimate the significance of genetic isolation by distance (fluvial) among sites for each marker-type. These tests involve the regression of pairwise genetic distances (calculated as $F_{ST}/(1-F_{ST})$) on geographic distance (calculated as the river distance (km) between the mouths of spawning tributaries) to determine significance of this relationship (Smouse and Long 1992).

ANALYSIS OF RUN-TIMING

Simulations were conducted to evaluate the statistical power of the microsatellites and SNPs to proportionally assign unknown mixtures to regional groups and evaluate composition of the run through time. Populations were assigned into five reporting groups based on geographic proximity and population structure (from MDS plots) for genetic stock identification analyses (GSI). Two of the groups were similar to those described above: 1) Upper Copper River

drainages, and 2) Gulkana River. The large Lower Copper River group was split into three groups: 3) Mendeltna Creek, 4) Tonsina, Klutina, and Tazlina lakes (collectively referred to as "Lakes"), and 5) Chitina River. The reporting groups were evaluated using 100% simulations. Simulated mixtures were first constructed with SPAM (SPAM version 3.7b, Debevec et al. 2000; Reynolds et al. 2001) using parametric bootstrapping with replacement (PB-R). The simulations were based on 400 individuals using population-specific allele frequencies from every population within each reporting group and an equal number of fish were generated from each population within a reporting group. This process was repeated 1,000 times for each reporting group, and the mean and central 90% of the distribution of estimates were reported as the estimate and the 90% confidence interval. Simulated mixtures were analyzed using a maximum likelihood model. A critical level of 90% mean correct allocation was used to determine if the reporting group was acceptably identifiable.

We also conducted simulations for both SNPs and microsatellites using the newly described method of Anderson et al. (2008). This method addresses bias in the predicted accuracy of GSI by accounting for sampling error in baseline allele frequencies which may be significant in closely related populations and may increase as more genetic data (loci and/or alleles) are added to the analysis. The method is based on a leave-one-out cross validation (CV-GC) and yields unbiased estimates of GSI accuracy (Anderson et al. 2008). We conducted the simulation through the program ONCOR (http://www.montana.edu/kalinowski) with the parameters set for 1,000 simulations and a sample size of 400. Simulated baseline sample sizes were the same as in the actual baseline.

Next, the collections taken from Chinook salmon captured by sportfishing guides operating in the Klutina and Gulkana rivers (Table 1) were used as another test of baseline performance. These tests, termed "proof tests", were performed to further examine the utility of the baseline using both maximum likelihood (SPAM) and a Bayesian method for mixed stock analysis (BAYES, Pella and Masuda 2001). Proof tests allow evaluation of the baseline using data that are independent of the baseline. Based on the geographic locations of the sport fisheries within the rivers, it was assumed that all fish captured were expected to spawn within the particular drainage, and no fish were strays or were "nosing in." This was the most challenging test of the method because fish may have originated from populations not represented in the baseline. For BAYES, the estimation was run using a single chain without thinning with a Markov Chain Monte Carlo sample size of 10,000. Inference was based on the posterior distribution derived from a combined set of the last 7,500 steps of the chain. The mean of the posterior distribution is reported as the best estimate, and the central 90% of the distribution was reported as the 90% credibility interval. An uninformative prior was used for the BAYES analyses in which the Dirichlet prior distribution parameters for all stock proportions were equal (1/N).

Estimates of stock composition and their 90% credibility intervals for the Baird Canyon collections were generated using the Bayesian analysis. The estimation for a single chain was run without thinning with a Markov Chain Monte Carlo sample size of 10,000. Three chains were run beginning with different starting conditions. Inference was based on the posterior distribution based on a combined set of the last 5,000 steps of each chain. The mean of the posterior distribution is reported as the best estimate, and the central 90% of the distribution was reported as the credibility interval. As previously, an uninformative prior was used.

RESULTS

SAMPLE COLLECTION

Extensive efforts were made during the summer field seasons to sample spawning populations of Chinook salmon from throughout the drainage. The goal of sampling a minimum of 100 Chinook salmon per spawning population was achieved for the majority of the collections from the Upper Copper River. Target sample sizes were not consistently achieved for collections from Klutina, Tazlina, and Gulkana river drainages. The lowest success rates were realized for the Chitina and Tonsina river drainages, although each spawning location was visited multiple times within years, and many were sampled in multiple years (Table 1). Sampling over multiple years is often the only means of attaining large sample sizes for species such as Chinook salmon, and multiple years sampling can actually improve the estimates of allele frequencies (Waples 1990). Samples across years within locations were pooled for Bone, Indian, Sinona, and Manker creeks and the Little Tonsina and Tebay rivers.

Three types of non-spawning samples were also taken: samples from guides of sport fishing trips in the mainstem Gulkana and Klutina rivers, juvenile samples from the Little Tonsina River, and individuals radio-tagged at Baird Canyon and tracked to river system (Savereide 2005). After review of the collection information and preliminary genetic data, river guide, juveniles, and the Chitina River radio tag samples were excluded as the uncertainty associated with mixed-stock origins was high. The Tonsina River radio-tagged individuals were included, however, because of the reported relatively high abundance of mainstem spawners (Savereide 2005) and the very low collecting success from traditional on-the-ground sampling.

LABORATORY ANALYSIS

Genomic DNA was extracted from 3,309 Chinook salmon (Table 1) including 1,644 individuals from spawning populations and 1,665 individuals sampled from the Baird Canyon fish wheel (including radio tags). Laboratory analysis was completed as described, but for the Baird Canyon samples individual genotypes were only assayed at the SNP loci.

STATISTICAL ANALYSES

Diversity within Populations

Four SNP loci with known polymorphisms in Chinook salmon, *Ots_arf-188*, *Ots_HGFA-446*, *Ots_PSMB1-197,* and *Ots_LEI-292*, were found to be monomorphic in the Copper River drainage (Table 2) and were omitted from further analyses. The one mitochondrial SNP, *Ots_C3N3,* was polymorphic only in Mendeltna Creek. Several other SNP loci also exhibited low frequency variation over all populations with allele frequencies of the most common allele greater than 0.980 (Table 2; *Ots_Ikaros-250*, *Ots_Ots2, Ots_RFC2-558, Ots_TAPBP, Ots_u211-85*). Private alleles were observed in Chistochina River at *Ots_RFC2-558* (relative frequency = 0.004), Gulkana Middle Fork at *Ots_GST-375* (relative frequency = 0.007), Kaina Creek at *Ots_TAPBP* (relative frequency = 0.013), and Manker Creek at *Ots_ZNF330-181* (relative frequency = 0.016). The mean expected heterozygosity (H_E)for SNP loci varied from 0.001 (*Ots_GST-375* and *Ots_RFC2-558)* to 0.478 (*Ots_SWS1op-182*) (Table 2).

All microsatellite loci were polymorphic in every population and the widest range of allele frequency for the most common allele was 0.217 to 0.926 at *Ots9* (Table 2). The number of

observed alleles in all populations ranged from two (*Ots9)* to 47 (*Omm1080*). The mean H_E ranged from 0.340 (*Ots9*) to 0.915 (*Ots208b*).

Over all loci and populations, all SNP loci conformed to HWE after adjustments for multiple tests. For microsatellites, 176 possible tests were performed; three tests were significant after adjustment for multiple tests ($\alpha = 176/0.05 = 0.0003$). When a test of the alternative hypothesis of heterozygote deficiency was performed, 12 of the 14 tests at *Ssa408* were significant prior to adjustments for multiple tests; six were significant at $P < 0.05$ and the remaining six were significant at $P < 0.01$. This suggests the presence of a null allele at *Ssa408*. This locus was retained for the remainder of the analysis.

Genotypic disequilibrium was not detected at any microsatellite loci, but the two SNPs at *Ots_FGF6* (*Ots_FGF6A* and *Ots_FGF6B)* were significantly linked to each other as were the two *Ots_HSP90B* SNPs (*Ots_HSP90B-100* and *Ots_HSP90B-385*). *Ots_FGF6B* and *Ots_HSP90B-385* were dropped from subsequent analyses. Significant genotypic disequilibrium was also detected between O*ts_MHC-2* and *Ots_LWSop-638*. Both loci were retained for further analyses as the structural relationship between these loci is uncertain, and significant disequilibrium between them was not detected by Smith et al. (2007).

Population Structure

Pairwise tests for significant differences between populations based on SNPs detected no significant difference for any of the three collections in the Gulkana River drainage or between Indian Creek and Chistochina River. In the lower portion of the drainage, no significant differences for SNPs were detected between any populations in the Lakes reporting group (Kaina River, Manker River, Tonsina River, or Tonsina River Radio Tags). All other tests were significant. For microsatellites, all pairwise tests were significant with the exception of tests between Gulkana Middle Fork and Gulkana Mainstem and between two Tonsina River spawning-ground populations (Greyling Creek and Little Tonsina River). Because no difference was found between them for either marker and the sample sizes were small, the Little Tonsina River and Greyling Creek collections were combined for further analysis.

When genetic diversity was measured by F_{ST}, calculated for every locus, values for SNP loci ranged from a low of 0.001 for *Ots_GST-375* to a high of 0.452 for *Ots_MHC2* with an overall value of 0.068 across the entire dataset (Table 2). The F_{ST} values for microsatellites ranged from a low of 0.023 for *Ots208b* to a high of 0.237 for *Ots9* with an overall value of 0.054 across the entire dataset.

Comparison of the pairwise population chord distance matrices was highly significant with positive correlation suggesting broadly concordant patterns between marker classes (R^2=0.78). The MDS plots from the two marker sets were highly concordant and showed five distinct clusters: 1) Upper Copper River, 2) Gulkana River, 3) Mendeltna Creek, 4) Lakes (Tonsina, Klutina, and Tazlina lakes), and 5) Chitina River (Figures 2 and 3). In each plot Mendeltna Creek was intermediate to the Gulkana River and Lakes groups, indicating partial affinity for each group. The Chitina River (Tebay River) sample was plotted near to the Lakes group, but remained separate.

Tests indicated that significant differences exist among populations in levels of allelic richness. When arranged into three groups: Upper Copper River, Gulkana River, and Lower Copper River, the average richness among groups was significantly different for both SNPs ($P < 0.01$) and

microsatellites ($P < 0.02$) (Table 3; Figure 4). Averages by regions for SNPs were 1.60, 1.68, and 1.74 and for microsatellites 9.51, 11.65, and 13.27 for Upper Copper River, Gulkana River, and Lower Copper River respectively. For both marker sets, allelic richness was lowest in the Upper Copper River and increased for middle and lower-river populations. In addition, the allelic richness of Mendeltna Creek (and to some extent Chitina River) appeared to be more similar to Gulkana River collections than other populations in the Lower Copper River group.

Significant positive correlation was found between genetic and geographic distances between population pairs for both markers, SNPs (R^2 = 0.362; Figure 5) and microsatellites (R^2 = 0.353). Because similar patterns were found for genetic distance values for the two markers (Figures 2 and 3), the isolation by distance relationships were highly concordant between the marker sets.

ANALYSIS OF RUN-TIMING

Results for the 100% simulations were tabulated for the five reporting groups using the parametric bootstrap resampling method (PB-R) of SPAM (Table 4): 1) Upper Copper River, 2) Gulkana River, 3) Mendeltna Creek, 4) Tonsina, Klutina, and Tazlina lakes (collectively referred to as "Lakes"), and 5) Chitina River. Results indicate these groups are highly identifiable in mixtures with the mean of 1,000 bootstrap iterations ranging from 0.93 to 0.99 for SNPs and 0.94 to 0.99 for microsatellites. Chitina River, one of the two reporting groups with only a single population characterized by 68 individuals, had the lowest level of correct assignment for both marker sets, but was still above the 90% level commonly used in fishery analyses (Seeb et al. 2007). From simulations using the unbiased cross-validation method over gene copies (CV-GC), the resulting correct proportional assignments to regions of the CV-GC analysis were similar to the PB-R method for both marker types, ranging from 0.967 to 1.000 for SNPs and 0.959 to 1.000 for microsatellites (Table 5).

When fish sampled from the sport fisheries in the Gulkana and Klutina rivers were used as mixtures, the conditional maximum likelihood method demonstrated an ability to correctly allocate Chinook salmon to reporting groups (>90% correct allocation) with the SNP baseline (results not shown). When the Bayesian method was applied, the accuracy and precision improved to almost complete identifiability (99% or better correct allocation) (Table 6).

Estimates of stock composition of Chinook salmon passing the Baird Canyon fish wheel were made for approximately weekly periods from mid-May to mid-July, 2005. Sample sizes for each estimate varied from 65 to 274 depending on the availability of the fish. Stock composition in the first two weeks was heavily weighted towards stocks higher up in the drainage as represented by the Upper Copper River and Gulkana River reporting groups (Table 7, Figure 6). In the first three sampling periods (May 12 through May 28), these stocks represented over 80% of the Chinook salmon passing the fish wheel. Beginning in the fifth sampling period (June 5) stocks from the Lakes group predominated, and the Upper Copper River group declined precipitously. Migratory-timing profiles for each of the reporting groups show the cumulative proportional contribution of each reporting group through time (Figure 7).

DISCUSSION

The goals of this study were to develop genetic markers to delineate major geographic and temporal stocks on the Copper River and then to use the markers to determine the potential of

genetic markers to distinguish among stocks within the Copper River drainage. Then the results would be used to investigate run timing. Collections analyzed spanned the entire drainage representing the known spawning areas of the river. Copper River Chinook salmon exhibit significant genetic divergence both within and among the major drainages. This genetic divergence translated to differences in allele frequencies among stocks that were adequate to determine stock contributions of mixtures within the Copper River drainage. In-river collections from Baird Canyon showed the Upper Copper River stocks contributing early followed by the Gulkana River and Lower Copper River populations.

GENETIC DIVERSITY

Significant divergence among populations was detected across the major drainages of the Copper River. The Upper Copper River region is an area of non-glaciated lakes and upland highlands with small populations. These populations exhibit significant differences in allele frequency for both microsatellites and SNPs as supported by significant pairwise tests between every pair for both markers and regional F_{ST} values of 0.026 and 0.027 for microsatellites and SNPs, respectively. This area clearly exhibits a high level of diversity with multiple genetically diverse populations contributing to the region. The populations are located at the extreme upper end of the Copper River drainage and exhibit the lowest range of allelic richness, indicative of smaller, isolated populations.

The Gulkana River drainage flows through an area of rolling hills and upland highlands. Collections from the Gulkana River drainage were obtained from three geographically close reaches and exhibited a high level of similarity among the three samples for both marker types. F_{ST} values among collections from this region were low (mean = 0.003) for both marker types. All pairwise test results were non-significant for SNPs and significant for microsatellites only between Gulkana River Middle Fork and Gulkana River Mainstem. However, the samples were taken in relatively close proximity and additional divergence, not represented by the current sampling, could exist within the drainage.

The tributaries draining Klutina and Tonsina lakes as well as Kaina Creek in the Tazlina River drainage represent glacial systems with similar genetic profiles. The populations of these lakes cluster closely on the MDS plots for both marker sets (Figures 2 and 3). Further, no significant differences in pairwise tests were found between these populations in the SNP analysis. However, evidence does exist that suggests timing differences may exist between mainstem and tributary spawners within these lakes. For example, radio-tagging studies (Savereide 2005) have suggested early- and late-components to the Klutina and Tonsina river systems with tributary spawners the first to arrive followed by mainstem spawners. Such segregation could promote divergence of populations within the lakes that was not detected in this study. More comprehensive sampling would be needed to evaluate genetic diversity associated with timing and tributary vs. mainstem spawning. Sampling in the Tonsina River drainage was particularly challenging. Only 16 spawners were obtained from the upper portion of the drainage at Greyling Creek, although 61 were collected from the Little Tonsina River. Despite the difficulty in collecting genetic samples, radio-tag information indicates that the Tonsina drainage can produce a significant portion (estimated 27% in 2002, Savereide 2005) of the Copper River escapement.

Based on escapement and radio-tag results, Chitina River contributes from 22% to 34% of the spawners over the period 2002-2004 (Savereide 2005), and spawners are distributed both in the mainstem and the tributaries. Tebay River, at 29 km from the mouth of the Chitina River, was

the only successful collection (N=68) in this study. Float trips on the mainstem Chitina River were unsuccessful. Additional diversity within the Chitina River system is highly possible and should be investigated.

SPATIAL ANALYSIS

Spatial or landscape analyses have become an increasingly valuable tool to place the genetic data into a geographic context and to better understand how geography shapes the diversity of populations (Manel et al. 2003; Scribner et al. 2005). We conducted several analyses reflecting spatial structure.

Isolation by distance tests are commonly applied to explore the relationship between genetic and geographic distances. For both marker types, a significant correlation between distances was found suggesting a rate of gene flow proportional to distances between populations (see Figure 5 for SNPs). Although significant, isolation by distance analysis cannot account for other variables or barriers that shape spatial structure. For example, divergence within drainages that may reflect stream capture or other barriers cannot be adequately described by a model based on isolation by distance. Mendeltna and Kaina creeks, both tributaries to Tazlina Lake, exhibit such a relationship.

We also detected a spatial relationship with levels of allelic diversity as represented by allelic richness. For both marker sets, allelic richness increased from upstream to downstream populations suggesting that the Upper Copper River populations are the smallest and/or the most isolated within the drainage (Figure 4).

MARKER COMPARISON

Microsatellites have been extensively applied to population and conservation genetic studies over the last decade due to their high variability and power to resolve population structure. However, several properties of microsatellites including complicated mutation rates, presence of null alleles, high potential genotyping error rate, and low throughput have led salmonid researchers to seek alternative markers (Narum et al. 2008; Smith et al. 2005a,b). Currently, baselines are being constructed using SNPs to take advantage of their lower error rates, increased automation of sample processing, potential for genome-wide scans of selectively neutral or adaptive variation, and facilitation of data sharing (Brumfield et al. 2003; Morin et al. 2004; Morin et al. *In review*; Seeb et al. 2007).

Several recent studies have compared the ability of SNPs and microsatellites to reveal population structure (Morin et al. *In review*; Narum et al. 2008; Ryynanen et al. 2007; Smith et al. 2007). Narum et al. (2008) in an analysis of Chinook salmon for 37 SNPs (a subset of loci analyzed in this study) and the same set of 13 microsatellites as used here, evaluated the utility of the two marker sets for information content and population structure analysis. Their set of 29 populations was broadly distributed from northern Southeast Alaska to California. They found that information content *(In)* was highest for microsatellites, but standardized gene diversity analyses (G'_{ST}) ranked SNPs at the top. Similar to this study, pairwise tests had similar results. Narum et al. (2008) indicated that closely related populations were better differentiated with microsatellites than SNPs, but that using all markers provided the highest accuracy. In a recent analysis using simulations of SNPs for population structure and conservation, Morin et al. (*In review*) found that approximately 30 SNPs were sufficient to detect moderate ($F_{ST} = 0.01$) levels of differentiation, studies aimed at detecting demographic independence (e.g. $F_{ST} < 0.005$) would

require 80 or more SNPs. These simulation results are consistent with the findings of this study, where significant pairwise differences were not detected among Gulkana River drainage spawning aggregates (F_{ST} = 0.003) with SNPs. Cumulatively, these results suggest that additional SNPs would improve fine-scale resolution and individual assignments in the Copper River drainage.

Departures from HWE were found in microsatellites, but not at the SNP loci. These departures are most often caused by 1) analysis of an admixed sample (i.e. Wahlund effect), 2) departure from the evolutionary model assumed for HWE, or 3) existence of null alleles or other errors leading to the miscalled genotypes. The detection of heterozygote deficiencies *(P* < 0.05) at the microsatellite locus, *Ssa408,* in 12 of the 14 populations suggests the presence of a null allele at this locus. The locus was ascertained from Atlantic salmon (*Salmo salar*) (Cairney et al. 2000) and found both useful and within HWE in the southern portion of the range (e.g. Snake River (Narum et al. 2007; Neville et al. 2007)). However, there may be ascertainment bias (see Smith et al. (2007)) which is manifested in null alleles when this locus is applied in the northern portion of the range.

The similarity in results of population structure analyses using each set of markers (SNPs and microsatellites) has been demonstrated previously in analyses involving multiple markers in Pacific salmon species (e.g. Allendorf and Seeb 2000; Templin et al. 2004; Narum et al. 2008).

INRIVER MIGRATION

The results from the simulations using two different methods (PB-R and CV-CG) indicate that five reporting groups could be accurately identified in the Copper River drainage. Of the two methods, the more conservative method, CV-CG, showed the highest levels of correct assignment. This conclusion is further supported by the results of the proof tests, in which mixtures of Chinook salmon caught during guided trips on the Gulkana and Klutina rivers were correctly detected as originating from the appropriate river.

In the mixture analyses, two reporting groups were represented by single populations. In these cases, the underlying assumption is that unsampled populations spawning within these drainages are more similar to the population representing the reporting group than to populations in any other reporting group. This assumption is likely valid for Mendeltna Creek, which is relatively short and not many other spawning locations were unsampled in the Tazlina River drainage. This assumption may not be valid for Tebay River in the Chitina River drainage, which is large and has many, widely separated tributaries with spawning Chinook salmon (Savareide 2005).

The Baird Canyon results delineate stock-specific differences in entry patterns with the Upper Copper River and Gulkana River populations entering first followed by populations from the lower glacial-lake systems. These results confirm the previous observations from radio-tagging studies that the earliest entry is by stocks in the upper drainages. The proportional contribution of the Chitina River reporting group is consistently low in this study (1.4% to 8.1%) and differs from the results of the radio-tag studies in which estimates as large as 34% were reported (Savereide 2005). This may suggest that the limited baseline from Tebay River is not capturing the diversity and genetic characteristics of the Chitina River drainage. Alternatively, this could be accounted for by annual variation and/or over-estimation of the Chitina River escapement by the radio-tagging study. The Chitina River drainage is a large and potentially diverse system. A more complete baseline for Chitina River is definitely desirable and would be required to evaluate the discrepancies between the studies.

Radio-tag results also suggested the potential existence of early- and late-timing stocks for the Tonsina and Klutina lakes. Assuming the current baseline adequately characterizes both runtimes, there was no clear signal of early- and late-components in the analysis of cumulative proportions (Figure 6). However, the Lakes reporting group represents three drainages possibly confounding run-timing signals. To better evaluate genetic diversity associated with run-timing, more comprehensive baseline sampling, designed to characterize early and late components within drainages, would be needed.

The genetic data provide the first indication that Mendeltna Creek is a highly divergent system. It may also have an earlier runtime than other populations returning to Tazlina, Klutina, and Tonsina lakes based on the results of the cumulative proportions (Figure 6). Since Kaina Creek was pooled in the Lakes reporting group, a specific comparison of run-timing between the two Tazlina River tributaries, Kaina and Mendeltna creeks, is not possible with the current dataset.

STANDARDIZATION

The microsatellite data were standardized following the protocols outlined in Seeb et al. (2007) and are being contributed to the coastwide Chinook salmon database housed at the Northwest Fisheries Science Center of the National Marine Fisheries Service in Seattle, Washington (http://www.nwfsc.noaa.gov/research/divisions/cbd/standardization.cfm). The SNP markers are standardized by definition (Smith et al. 2007) and can also be contributed to the growing coastwide Chinook salmon SNP database (e.g. Narum et al. 2008).

Through the use of the standardized markers and methods that are used by other laboratories contributing to the Coastwide Chinook salmon database (Seeb et al. 2007), the genetic data collected enable the extension of this baseline beyond its previous northernmost extension at the Situk River to include the Copper River. Further extension to the west will eventually include populations of Chinook salmon spawning in Cook Inlet, Kodiak Island, and the Alaska Peninsula. The presence of Chinook salmon tagged in Cook Inlet in the commercial harvest on the Copper River Delta indicates that this extension is necessary prior to the use of these stock-contribution results for allocation or management purposes.

CONCLUSIONS

This study provides the first comprehensive analysis of the genetic diversity of Chinook salmon within the Copper River drainage, a highly valued and productive system. Chinook salmon spawning within the Copper River exhibit significant genetic divergence both within and among its major drainages, likely contributing to the productivity and sustainability of the populations. With some exceptions, populations adhere to an isolation-by-distance model in that populations closest geographically are also closest genetically. The broad groups include a heterogeneous collection of populations in the Upper Copper River, a homogeneous group from the Gulkana River drainage, and a diverse collection of Lower Copper River glacial lake populations from the Tazlina, Klutina, Tonsina, and Chitina river drainages.

Within the Lower Copper River group, two single collections were particularly divergent, Tebay River from the Chitina River drainage and Mendeltna Creek from the Tazlina River drainage. Tebay River is the sole representative from the Chitina River drainage, a large, complex drainage that is apparently productive for Chinook salmon. Mendeltna Creek is one of two geographically

close, but genetically divergent populations in the Tazlina River drainage. Additional fine-scale differentiation likely exists within individual drainages beyond that revealed by this study, e.g. radio tag evidence of possible run-timing differences in the Klutina and Tonsina river drainages. Improving the resolution and documentation of genetic diversity within the Copper River would allow investigation of potential genetic differences between early- and late-runs within major tributary drainages and evaluation of differences between estimates of escapement based on radio-tags and genetic markers.

Results for both marker sets, SNPs and microsatellites, were very consistent giving similar signals for population structure. For both marker sets, allelic richness was the lowest in the Upper Copper River and increased downriver, suggesting that the Upper Copper River drainage consists of populations with lower effective population sizes and reduced diversity as compared to those in the lower drainages. Similarly, both marker sets revealed the diversity within the Tazlina River system with large allele frequency differences between the collections from the Kaina and Mendeltna creek collections.

The in-river collections from Baird Canyon in the Copper River Delta consistently showed the Upper Copper River stocks contributing early followed by the Gulkana and Lower Copper river populations. This pattern is probably also reflected in the commercial fishery on the Copper River Delta. Genetic stock identification of these harvests can proceed once the data from this study are added to the larger baseline developed by the Pacific Salmon Commission to the south and east and extended to include the rest of the Gulf of Alaska.

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TABLES AND FIGURES

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

Collection Number	Drainage Location	Number collected by Year	Year	Total Genotyped
	Baseline			
	Upper Copper			
1	Bone Creek	70, 8	2004, 2005	78
2	Otter Creek	128	2005	128
3	Indian Creek	43, 7	2004, 2005	50
4	East Fork Chistochina River	145	2004	145
5	Sinona Creek	7, 152	2004, 2005	159
	Slana River, Ahtell Creek [a]	20, 1	2004, 2005	21
	Gulkana			
6	Mainstem	46	2004	46
7	Middle Fork	79	2004	79
8	Paxson Fork	88	2004	88
	Gulkana River (guides) [a]	130	2004	130
	Tazlina			
9	Mendeltna Creek	144	2004	144
10	Kaina Creek	75	2004	75
	Klutina			
11	Manker Creek	41, 21	2004,2005	62
	Klutina River (guides) [a]	168	2004	168
	Tonsina			
	Little Tonsina River (juveniles) [a]	20	2004	20
12	Little Tonsina River [b]	31, 1, 29	2004, 2005, 2006	61
12	Greyling Creek [b]	16	2004	16
13	Tonsina Radio Tags	106	2003,2004	106
	Chitina			
14	Tebay River–lake outlet	27, 34, 7	2004, 2005, 2006	68
	Radio Tagged Individuals			
	Baird Canyon	477, 496	2003, 2004	283 [c]
	Baird Canyon fish wheel			
	May 12–July 14, 2005	1,382	2005	1,382

[a] Not included in analysis of baseline.

[b] Collections pooled into single population

[c] Does not include the Tonsina radio tagged individuals.

Table 2.–Genetic markers assayed in Copper River Chinook salmon.

Locus	Reference	Observed Number of Alleles	Range of Most Frequent Allele			H_E	H_O	F_{ST}
Single nucleotide polymorphisms								
Ots_arf-188	Smith et al. 2005a	1	1.000	-	1.000	-	-	-
Ots_AsnRS-60	Smith et al. 2005a	2	0.311	-	0.740	0.462	0.459	0.077
Ots_C3N3 [a]	Smith et al. 2005b	2	0.924	-	1.000	-	-	0.064
Ots_ETIF1A	Narum et al. 2008	2	0.592	-	0.896	0.351	0.352	0.044
Ots_FARSLA-220	Smith et al. 2007	2	0.744	-	0.975	0.211	0.217	0.039
Ots_FGF6A	Narum et al. 2008	2	0.561	-	1.000	0.302	0.303	0.136
Ots_FGF6B [b]	Unpublished	2						
Ots_GH2	Smith et al. 2005b	2	0.441	-	0.712	0.457	0.465	0.025
Ots_GnRH-271	Smith et al. 2005a	2	0.934	-	1.000	0.036	0.036	0.029
Ots_GPDH-338	Smith et al. 2005a	2	0.973	-	1.000	0.013	0.014	0.011
Ots_GPH318	Smith et al. 2007	2	0.577	-	0.972	0.247	0.243	0.108
Ots_GST-207	Smith et al. 2007	2	0.927	-	1.000	0.033	0.031	0.032
Ots_GST-375	Smith et al. 2007	2	0.993	-	1.000	0.001	0.001	0.001
Ots_GTH2B-550	Narum et al. 2008	2	0.276	-	0.745	0.453	0.459	0.097
Ots_HGFA-446	Smith et al. 2005a	1	1.000	-	1.000	-	-	-
Ots_hnRNPL-533	Smith et al 2007	2	0.702	-	1.000	0.208	0.209	0.060
Ots_HSP90B-100	Smith et al. 2007	2	0.927	-	1.000	0.057	0.059	0.022
Ots_HSP90B-385 [c]	Smith et al. 2007	2						
Ots_IGF-I.1-76	Smith et al. 2005a	2	0.374	-	0.765	0.468	0.480	0.056
Ots_Ikaros-250	Smith et al. 2005a	2	0.992	-	1.000	0.002	0.002	0.002
Ots_il-1racp-166	Smith et al. 2005a	2	0.383	-	0.858	0.441	0.486	0.104
Ots_LEI-292	Smith et al. 2007	1	1.000	-	1.000	-	-	-
Ots_MetA	Unpublished	2	0.898	-	1.000	0.056	0.049	0.037
Ots_MHC1	Smith et al. 2005b	2	0.246	-	0.852	0.394	0.410	0.112
Ots_MHC2	Smith et al. 2005b	2	0.233	-	1.000	0.226	0.229	0.452
Ots_NOD1	Narum et al. 2008	2	0.619	-	0.827	0.420	0.431	0.016

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Table 2.–Page 2 of 3.

Locus	Reference	Observed Number of Alleles	Range of Most Frequent Allele			H_E	H_O	F_{ST}
Ots_ZNF330-181	Smith et al. 2005a	2	0.984	-	1.000	0.002	0.002	0.011
Ots_LWSop-638	Smith et al. 2005a	2	0.829	-	1.000	0.122	0.118	0.055
Ots_SWS1op-182	Smith et al. 2005a	2	0.514	-	0.687	0.478	0.545	0.011
Ots_Ots2	Smith et al. 2005b	2	0.980	-	1.000	0.009	0.008	0.006
Ots_P450	Smith et al. 2005b	2	0.449	-	0.698	0.474	0.491	0.023
Ots_P53	Smith et al. 2005b	2	0.339	-	0.748	0.460	0.436	0.050
PGK54	Narum et al. 2008	2	0.648	-	0.960	0.190	0.188	0.051
Ots_Prl2	Smith et al. 2005b	2	0.500	-	0.847	0.420	0.442	0.063
Ots_ins-115	Smith et al. 2005a	2	0.833	-	0.997	0.106	0.108	0.048
Ots_PSMB1-197	Smith et al. 2007	1	1.000	-	1.000	-	-	-
Ots_RAG3	Narum et al. 2008	2	0.830	-	1.000	0.119	0.124	0.059
Ots_RFC2-558	Smith et al. 2005a	2	0.996	-	1.000	0.001	0.001	0.000
Ots_S7-1	Narum et al. 2008	2	0.238	-	0.709	0.473	0.468	0.043
Ots_SClkF2R2-135	Smith et al. 2005a	2	0.443	-	0.742	0.468	0.464	0.018
Ots_SERPC1-209	Smith et al. 2007	2	0.867	-	0.993	0.119	0.117	0.016
Ots_SL	Smith et al. 2005b	2	0.374	-	0.733	0.466	0.479	0.049
Ots_TAPBP	Narum et al. 2008	2	0.987	-	1.000	0.002	0.002	0.008
Ots_Tnsf	Smith et al. 2005b	2	0.746	-	0.942	0.288	0.287	0.016
Ots_u202-161	Smith et al. 2005a	2	0.724	-	0.967	0.222	0.226	0.049
Ots_u211-85	Smith et al. 2005a	2	0.989	-	1.000	0.004	0.004	0.002
Ots_U212-158	Smith et al. 2005a	2	0.879	-	1.000	0.056	0.054	0.076
Ots_u4-92	Smith et al. 2005a	2	0.859	-	0.987	0.100	0.099	0.017
Ots_u6-75	Smith et al. 2005a	2	0.795	-	0.993	0.118	0.108	0.034
Ots_E2-275	Smith et al. 2005a	2	0.770	-	0.997	0.207	0.207	0.044
Ots_Zp3b-215	Smith et al. 2005a	2	0.926	-	1.000	0.025	0.027	0.034
							Average	0.068

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Table 2.–Page 3 of 3.

Locus	Reference	Observed Number of Alleles	Range of Most Frequent Allele	H_E	H_O	F_{ST}
Microsatellites						
Ogo2	Olsen et al. 1998	9	0.152 - 0.699	0.642	0.624	0.095
Ogo4	Olsen et al. 1998	13	0.118 - 0.642	0.685	0.680	0.095
Oki100	Narum et al. 2008	30	0.022 - 0.313	0.892	0.901	0.035
Omm1080	Rexroad et al. 2001	47	0.051 - 0.298	0.915	0.916	0.026
Ots201b	Grieg et al. 2003	23	0.007 - 0.296	0.898	0.878	0.026
Ots208b	Grieg et al. 2003	34	0.008 - 0.250	0.915	0.919	0.023
Ots211	Grieg et al. 2003	32	0.011 - 0.628	0.831	0.832	0.086
Ots212	Grieg et al. 2003	22	0.256 - 0.522	0.803	0.811	0.031
Ots213	Grieg et al. 2003	30	0.052 - 0.275	0.891	0.907	0.024
Ots3M	Grieg and Banks 1999	5	0.317 - 0.599	0.612	0.598	0.030
Ots9	Banks et al. 1999	2	0.217 - 0.936	0.383	0.384	0.237
OtsG474	Williamson et al. 2002	6	0.641 - 0.919	0.340	0.336	0.050
Ssa408	Cairney et al. 2000	23	0.063 - 0.380	0.838	0.759	0.030
				Average		0.054

Note: Observed number of alleles, range of most common allele, mean expected heterozygosity (H_E), mean observed heterozygosity (H_O), and genetic diversity (F_{ST}) values are given.

[a] Heterozygosity cannot be calculated because this locus is in mitochondrial DNA.

[b] Dropped from the analysis because of linkage to *Ots_FGF6A*.

[c] Dropped from the analysis because of linkage to *Ots_HSP90B-100*.

Table 3.–Measures of within population diversity in populations of Chinook salmon in the Copper River, Alaska.

	SNPs					Microsatellites				
Collection	N	M	A	H_O	H_E	N	M	A	H_O	H_E
Bone Creek	78	1.60	1.59	0.19	0.18	77	10.54	9.60	0.69	0.70
Otter Creek	126	1.71	1.68	0.20	0.20	126	10.69	9.42	0.74	0.73
Indian Creek	49	1.58	1.58	0.19	0.19	48	10.38	10.22	0.72	0.73
Chistochina River	132	1.67	1.61	0.18	0.18	129	12.08	10.39	0.71	0.72
Sinona Creek	154	1.58	1.54	0.18	0.17	154	9.15	7.90	0.70	0.71
Gulkana Mainstem	46	1.69	1.69	0.22	0.21	46	11.69	11.55	0.74	0.74
Gulkana Middle Fork	76	1.69	1.67	0.20	0.20	77	13.69	12.06	0.73	0.74
Gulkana Paxson Fork	87	1.71	1.68	0.20	0.20	87	12.77	11.34	0.71	0.73
Mendeltna Creek	143	1.71	1.67	0.23	0.23	141	13.77	11.28	0.75	0.76
Kaina Creek	74	1.77	1.73	0.22	0.22	74	15.23	13.74	0.79	0.79
Manker Creek	61	1.83	1.79	0.24	0.23	60	14.92	13.90	0.77	0.77
Little Tonsina/Greyling	75	1.79	1.76	0.22	0.22	73	15.77	14.27	0.77	0.78
Tonsina Radio Tags	105	1.75	1.74	0.22	0.22	104	17.54	14.86	0.75	0.77
Tebay River	61	1.73	1.71	0.21	0.20	67	12.54	11.48	0.71	0.72

Note: number successfully genotyped (N), observed mean number of alleles (M), allelic richness (A), expected and observed heterozygosity (H_E, H_O) for nuclear SNPs and microsatellites.

Table 4.–Mean reporting group allocations of simulated mixtures of Copper River Chinook salmon from the baseline of 45 SNPs and 13 microsatellite markers.

	SNPs		Microsatellites	
Region	Mean	90% CI	Mean	90% CI
Upper Copper	0.994	(0.982–1.000)	0.988	(0.976–0.998)
Gulkana	0.955	(0.919–0.989)	0.973	(0.955–0.990)
Mendeltna	0.954	(0.915–0.987)	0.977	(0.960–0.991)
Lakes	0.986	(0.964–1.000)	0.991	(0.980–1.000)
Chitina	0.931	(0.881–0.973)	0.945	(0.919–0.969)

Note: Each set of mixtures (N=400) was created from a single reporting region based on allelic frequencies for that region. The results reported are the mean and bounds of the middle 90% (CI) of correct allocations from 1,000 bootstrap iterations calculated using parametric bootstrap resampling (PB-R) as implemented in SPAM.

Table 5.–Simulated mixtures of individual populations of Copper River Chinook salmon from the baseline of 45 SNPs and 13 microsatellite markers.

	Region			
	SNPs		Microsatellites	
Collection	AVG	SD	AVG	SD
Bone	1.000	0.000	1.000	0.001
Otter	0.997	0.004	1.000	0.001
Indian	1.000	0.001	0.998	0.002
Chistochina	0.999	0.001	0.999	0.002
Sinona	1.000	0.001	1.000	0.000
Gulkana Mainstem	0.967	0.017	0.997	0.003
Gulkana Middle Fork	0.995	0.006	0.985	0.008
Gulkana Paxson Fork	0.996	0.005	0.996	0.004
Mendeltna	0.979	0.013	0.992	0.006
Kaina	0.976	0.015	0.995	0.004
Manker	0.974	0.016	0.997	0.003
Little Tonsina/Greyling	0.997	0.004	0.995	0.004
Tonsina Radio Tags	0.994	0.007	0.989	0.006
Tebay	0.977	0.014	0.959	0.013

Note: Each set of mixtures (N=400) was created from a single population based on allelic frequencies for that region. The results are based on leave-one-out cross validation (CV-GC) that follows the method of Anderson et al. (2008) to provide unbiased estimates of GSI accuracy. Mean estimates to population and region are given.

Table 6.–Stock composition estimates and 90% credibility intervals from two mixture samples from sport-caught Chinook salmon in the Copper River drainage using the BAYES model.

Region	Klutina Guides		Gulkana Guides	
	Estimate	90% CI	Estimate	90% CI
	N=169		N=130	
Upper Copper	0.001	(0.000–0.007)	0.002	(0.000–0.011)
Gulkana	0.002	(0.000–0.012)	0.989	(0.962–1.000)
Mendeltna	0.009	(0.000–0.041)	0.005	(0.000–0.028)
Lakes	0.961	(0.911–0.995)	0.002	(0.000–0.010)
Chitina	0.027	(0.000–0.069)	0.002	(0.000–0.010)

Note: These analyses were based on the 45-SNP baseline.

Table 7.–Relative proportion of reporting groups of Chinook salmon sampled approximately weekly from the Baird Canyon fish wheel, 2005.

		Week (Dates)						
		20	21	22	23	24	25	26-29
		(5/12–5/14)	(5/15–5/21)	(5/22–5/28)	(5/29–6/04)	(6/5–6/11)	(6/12–6/18)	(6/19–7/14)
Region								
1	Upper	0.404	0.359	0.293	0.147	0.073	0.006	0.003
2	Gulkana	0.430	0.485	0.571	0.440	0.287	0.215	0.033
3	Mendeltna	0.018	0.035	0.007	0.018	0.038	0.044	0.014
4	Lakes	0.095	0.106	0.115	0.375	0.545	0.655	0.869
5	Chitina	0.054	0.015	0.014	0.020	0.058	0.080	0.081
	N	65	243	265	274	234	193	107

Note: Sampling dates and sample sizes are given; analysis is based on 45 SNP loci using BAYES.

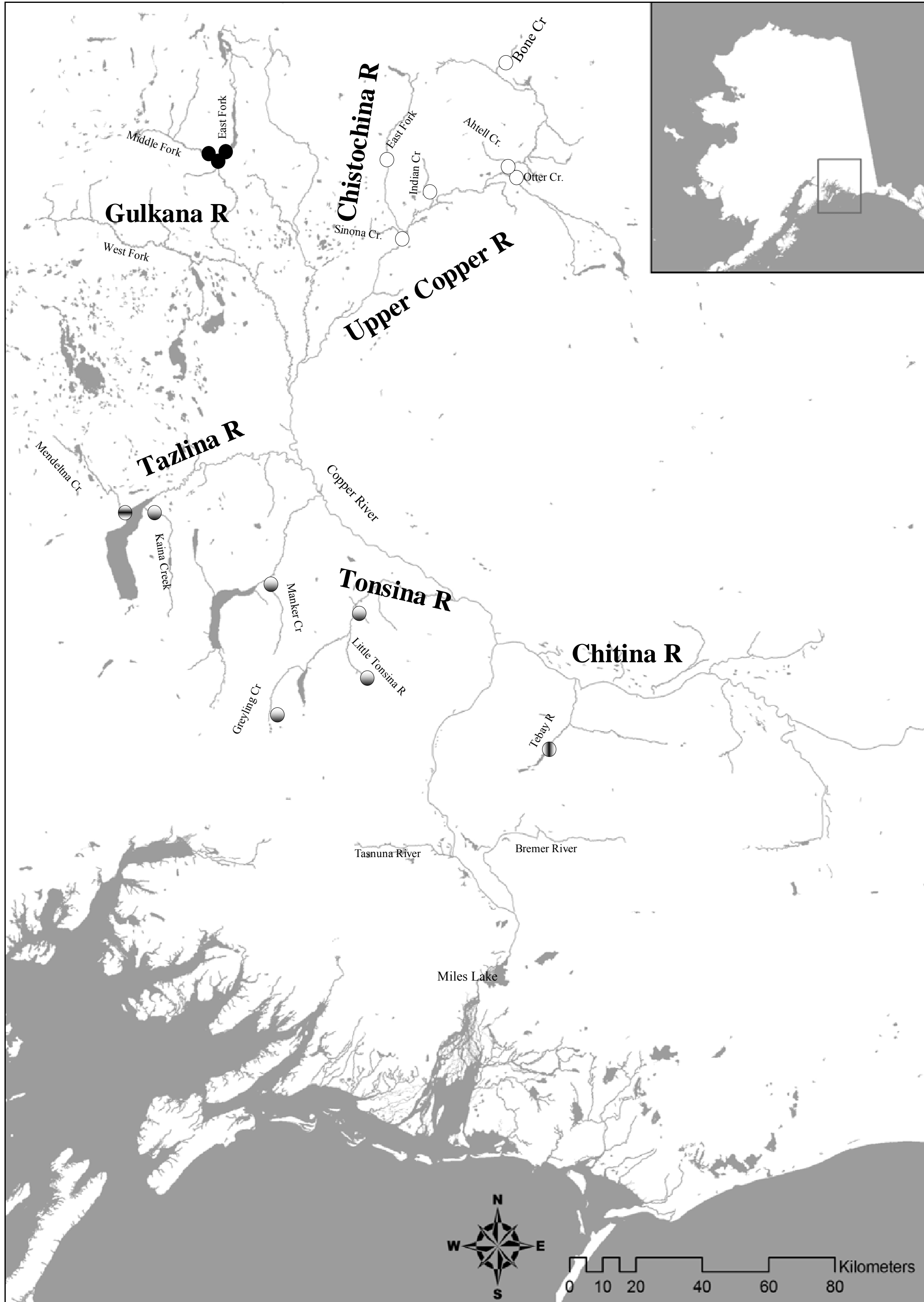

Figure 1.–Collection locations for Chinook salmon from the Copper River drainage.

Note: Three larger regions are identified: Upper Copper (open circles), Gulkana (black circles), Lower Copper (shaded circles). Two subregions within the Lower Copper are identified by shaded circles with a horizontal or vertical line, for Mendeltna and Chitina, respectively.

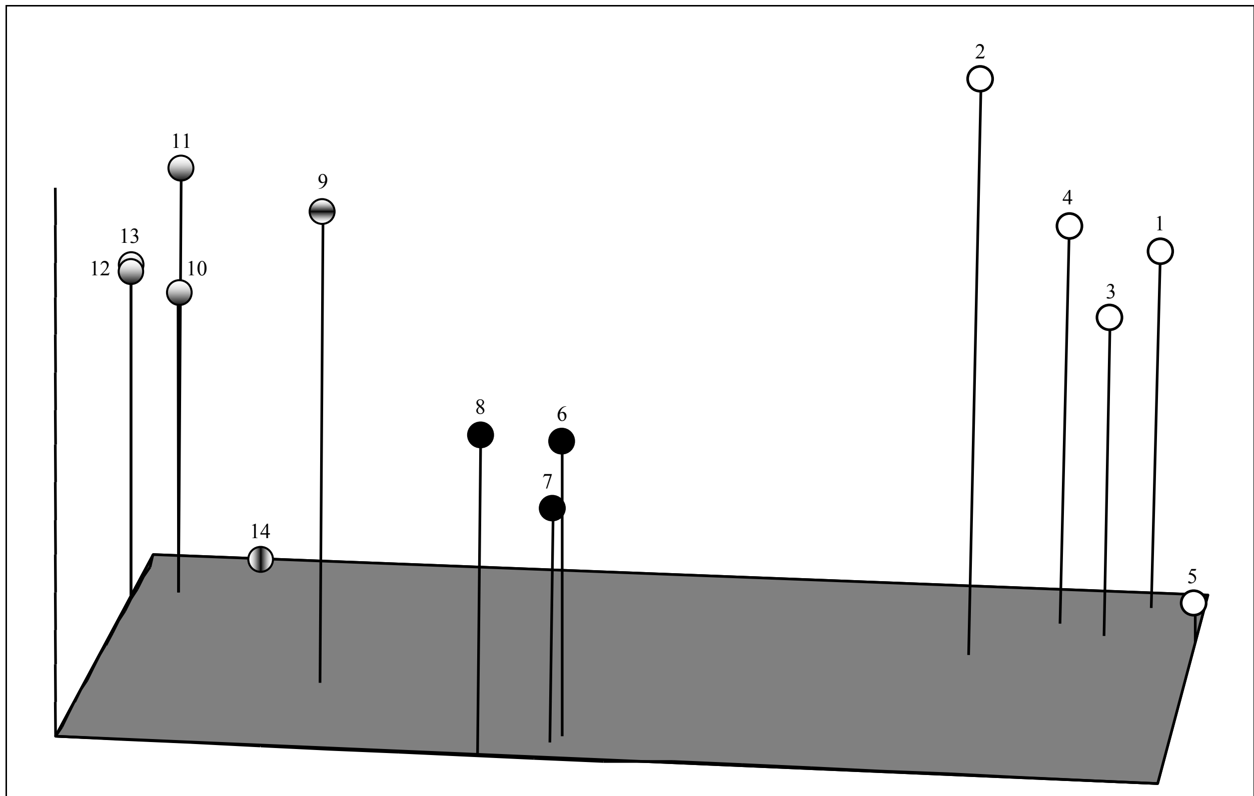

Figure 2.–Multidimensional scaling plot based on genetic distances calculated from 45 SNP loci.

Note: Population numbers and patterns correspond to Table 1 and Figure 1 respectively.

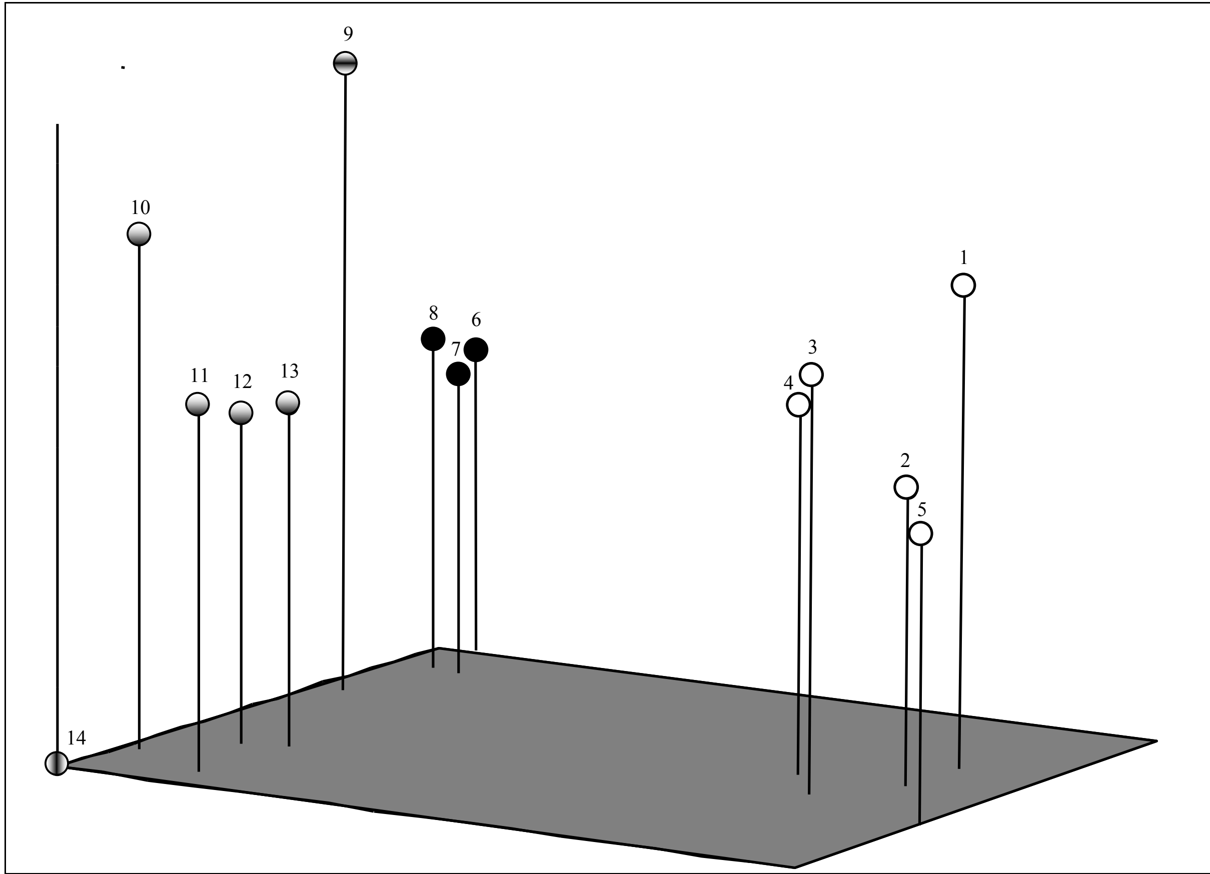

Figure 3.–Multi-dimensional scaling plot based on genetic distances calculated from 13 microsatellite loci.

Note: Population numbers and patterns correspond to Table 1 and Figure 1 respectively.

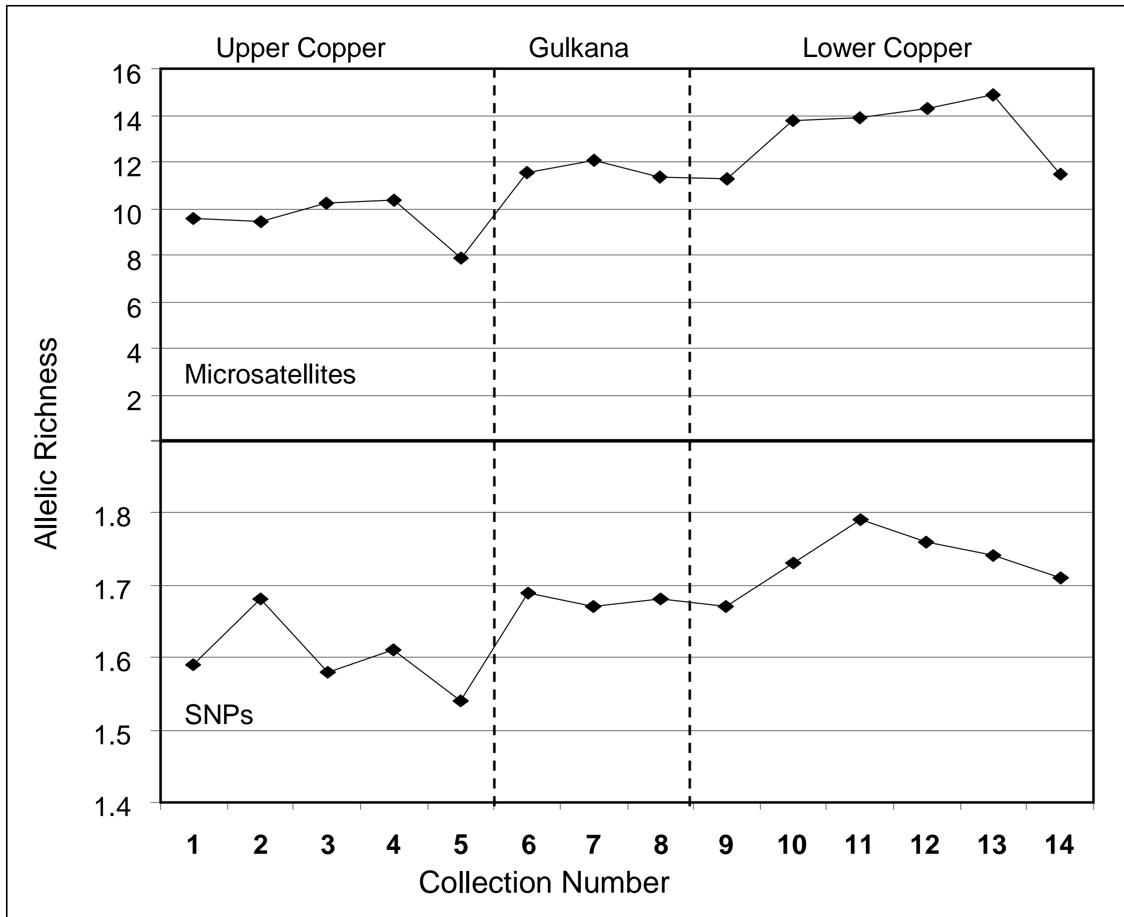

Figure 4.–Allelic richness for Copper River Chinook salmon populations measured with microsatellite and SNP loci.

Note: Collection numbers are found in Table 1.

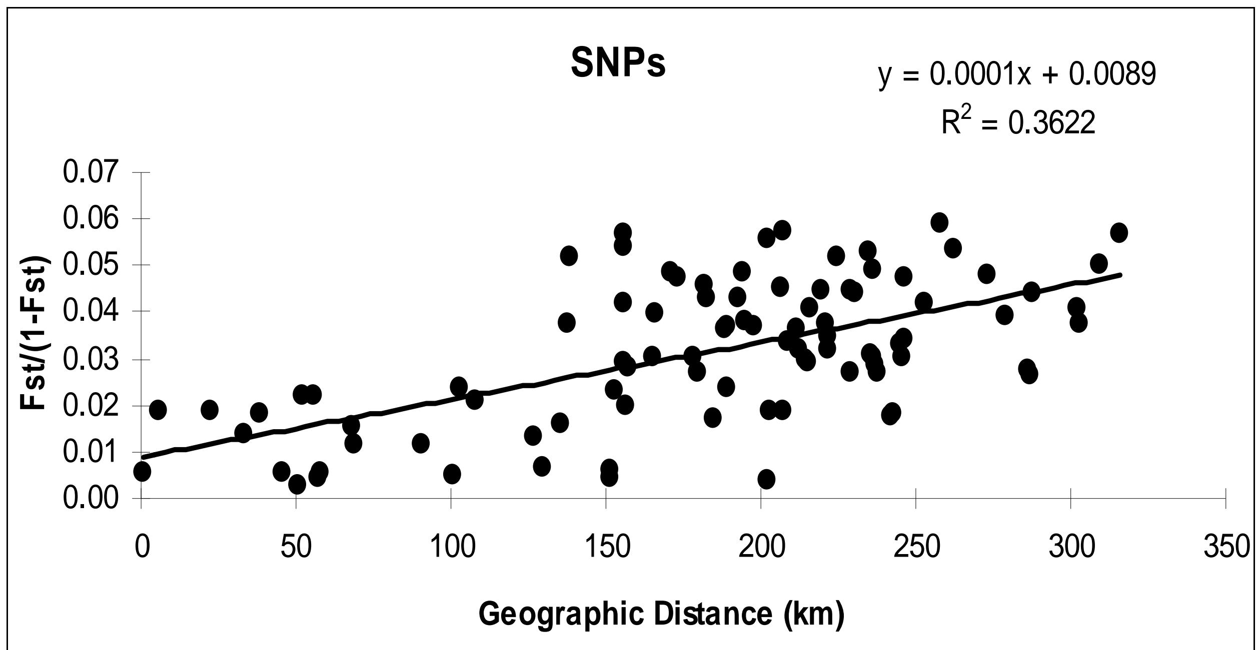

Figure 5.–Isolation by distance relationship based on pairwise comparison of genetic and geographic distances between Copper River Chinook salmon populations using 45 SNPs.

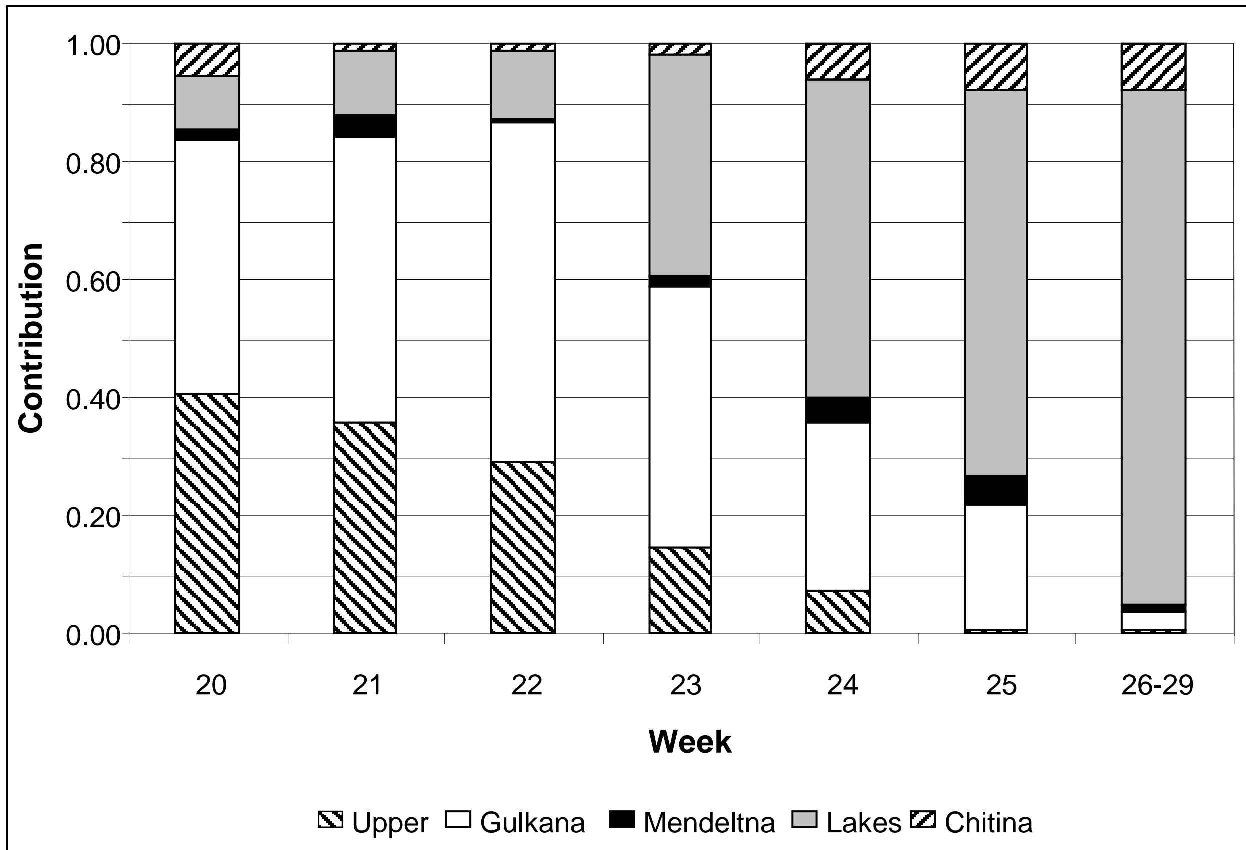

Figure 6.–Weekly stock composition estimates of Chinook salmon captured by the Baird Canyon fish wheel, 2005.

Note: Five regional groups are identified.

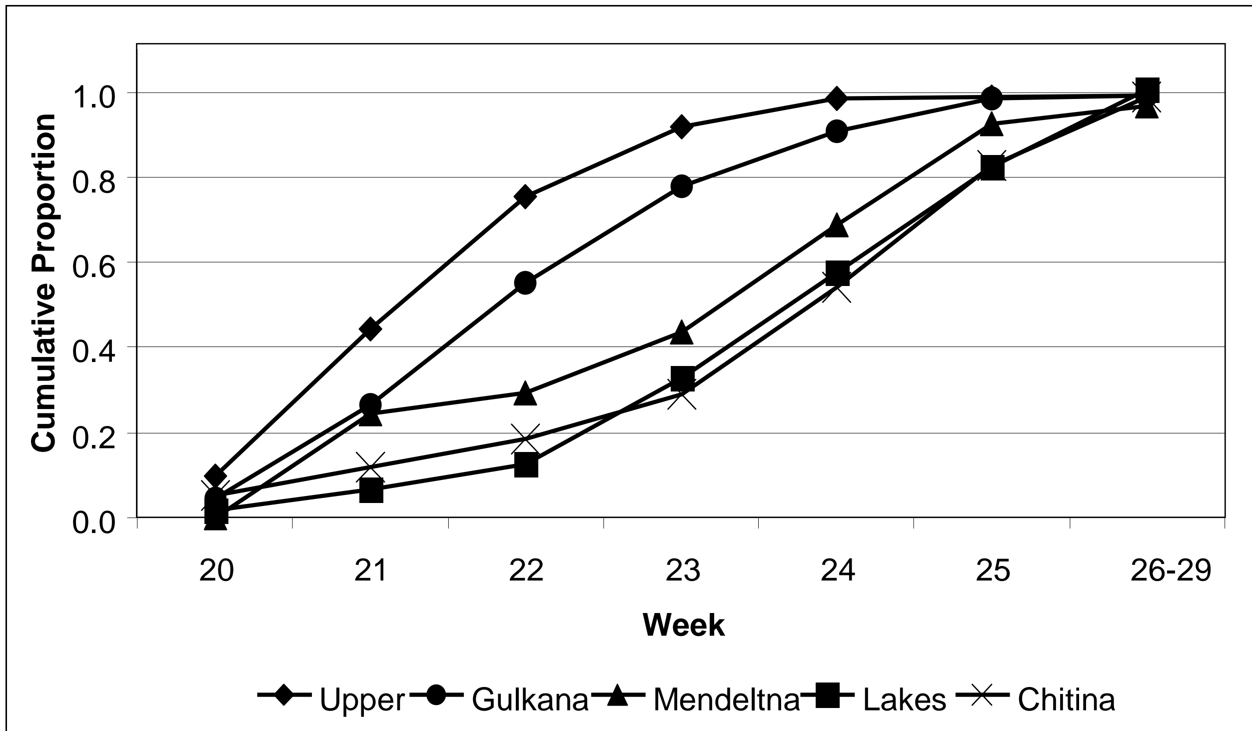

Figure 7.–Cumulative return for five regional groups of Chinook salmon in samples taken at the Baird Canyon fish wheel, 2005.