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Timing and origin of Chinook salmon stocks in the Copper River and adjacent ocean fisheries using DNA markers

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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

FISHERY DATA SERIES NO. 09-58

TIMING AND ORIGIN OF CHINOOK SALMON STOCKS IN THE COPPER RIVER AND ADJACENT OCEAN FISHERIES USING DNA MARKERS

by
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ABSTRACT

The objectives of this project were to delineate major geographic and temporal stocks of Chinook salmon *Oncorhynchus tshawytscha* within the Copper River, investigate run timing within the Copper River, and characterize the timing and relative magnitude of Copper River stocks in the fisheries of the Copper River District. 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 drainages. Within the Lower Copper River group, 2 single collections were particularly divergent, Tebay River from the Chitina River drainage and Mendeltna Creek from the Tazlina River drainage. The inriver collections from Baird Canyon and collections from the marine fisheries consistently showed that the Upper Copper River stocks contributed early followed by the Gulkana River and Lower Copper River populations. Similar results were observed for the marine collections. The results also indicate that the marine fisheries are, to a great extent, targeting Chinook salmon bound for the Copper River.

Key words: Chinook, *Oncorhynchus tshawytscha,* Copper River, DNA, DNA markers, genetic divergence

INTRODUCTION

The Copper River drains a large inland valley in Southcentral Alaska bounded by the Alaska, Wrangell-St. Elias, and Chugach mountain ranges (Figure 1). As the river flows south through the Chugach Mountains to the Gulf of Alaska, several other rivers draining mountain valleys join the mainstem Copper River. Along its path, it drains an area of 70,000 km^2, making it the third largest river system in Alaska.

Chinook salmon *Oncorhynchus tshawytscha* from the Copper River provide opportunities for commercial, subsistence, personal use, and sport harvests. These salmon have been harvested commercially since the late 1800s (Moser 1899). 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 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; the remaining 32.8% (21,604) from 2005 was the 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 (Wuttig and Evenson 2001; Savereide 2005). In these studies, returning adult Chinook salmon were radiotagged near Baird Canyon and tracked to upriver destinations using ground-based receiving stations and aerial tracking techniques. Chinook salmon were tracked to 6 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.

Despite the large catches and escapements in the Copper River region, the effect of commercial fishing on the long-term abundance of salmon stocks spawning in these drainages is uncertain (Simeone and Valentine 2007), and more detailed stock-specific information is desirable to ensure future sustainability. 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 of the system 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 stocks.

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 (Gharrett et al. 1987; Utter et al. 1989; Crane et al. 1996; Waples et al. 2004; Templin et al. 2004, 2005) 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 (Seeb et al. 2007; Beacham et al. 2008). 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 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 using putative adaptive marker loci (Smith et al. 2005b; 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.

The objectives of this study were to use the techniques of molecular genetics to describe diversity within the Copper River and then apply GSI analyses to monitor inriver migration and run timing. The data from the Copper River were then combined with an existing standardized database composed of Chinook salmon stocks from northern Southeast Alaska to California (Seeb et al. 2007). The combined database was used to estimate the stock composition of the commercial fishery harvests in the marine waters of the Copper River district during 2005 on a weekly basis. These estimates provide one year of information on stock-specific run timing of Copper River spawners in the commercial fishery and demonstrate the potential use of GSI for estimating the proportion of migrating Chinook salmon from outside the drainage.

OBJECTIVES

Specific objectives associated with the multi-year contract between the United States Forest Service and the Alaska Department of Fish and Game 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 radiotagged and fish wheel 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; and
4. Standardize and contribute the Copper River data to a coastwide DNA database.

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 techniques 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 (guides) on the Klutina and Gulkana rivers and from minnow traps on the Tonsina River.

In addition to the spawning collections, tissue samples from radiotagged Chinook salmon were collected by NVE and ADF&G as part of 2 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, sampled, tagged with a radio transmitter, released, and relocated 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.

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 2006) sampled Chinook salmon over a 2 month period (May 12–July 14, 2005; statistical weeks 20–29) to provide a comprehensive set of samples to evaluate stock-specific run timing. Samples were divided into approximately weekly sets for analysis (Table 1). Statistical weeks 26–29 were combined to achieve a sufficient sample size.

Chinook salmon intercepted in the Copper River District fishery were sampled from landings at fish processing plants in Cordova during 2005. Samples were collected by an ADF&G sampling crew during the usual age, sex, and length sampling of the harvest. Individuals were selected without regard to size, sex, position in the tote, or the presence of an adipose fin. Axillary processes were collected and preserved in ethanol. Target sample sizes for each period were set at 200 individuals, reflecting the balance between logistic constraints and desired levels of accuracy and precision. Under worst-case scenarios with the assumption of perfect

identifiability, the estimates from samples of 200 should be +/- 7% of the true value 90% of the time (Thompson 1987).

LABORATORY ANALYSIS

Genomic DNA was extracted from individual Chinook salmon sampled from spawning populations, juveniles sampled from traps, individuals caught by the Baird Canyon fish wheel, and individuals caught by the sport and commercial fisheries of the Copper River drainage and Copper River District. DNA was extracted using DNAeasy 96 Tissue kits[1] (QIAGEN, Valencia, CA). Both single nucleotide polymorphism (SNP) and microsatellite genotyping was done for all samples except those from the commercial fishery. Only microsatellite genotyping was done for commercial fishery samples because only microsatellite loci have been included in the coastwide baseline available for statistical analyses.

SNP genotyping

SNP genotyping was conducted in 384-well reaction plates following the protocols outlined in Seeb et al. (*In press*); four wells in each plate served as negative (no-template) controls. Each reaction was conducted in a 5µL volume consisting of 0.10µL template DNA in 1X TaqMan Universal Buffer (Applied Biosystems, Foster City, CA), 900nM each polymerase chain reaction (PCR) primer, and 200nM each probe. Thermal cycling was performed on a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems) as follows: an initial denaturation of 10 min at 95°C followed by 50 cycles of: 92°C for 15 sec and annealing/extension temperature for 1 or 1.5 min. Cycling was conducted at a ramp speed of 1°C per second. The plates were read on an Applied Biosystems PRISM 7900HT Sequence Detection System after amplification and scored using Sequence Detection Software 2.2 (Applied Biosystems) to generate scatterplots that graphically depicted the amount of each allele-specific probe that bound to the PCR product of each individual. Baseline collections were genotyped for 51 SNPs in nuclear DNA and 1 SNP in mitochondrial DNA (*Ots_C3N3*; Table 2).

Microsatellite genotyping

Microsatellite genotyping was conducted in 384-well reaction plates in 5µl reaction volumes (10mM Tris-HCl, 50mM KCl, 0.2 mM each dNTP, 0.25 units *Taq* DNA polymerase (Applied Biosystems) using Dual 384-Well GeneAmp Thermal Cycler. PCR primer concentrations, MgCl concentrations and the corresponding annealing temperature for each locus are available upon request. PCR Fragment analysis was done on an Applied Biosystems 3730 capillary DNA sequencer. The 0.5µl PCR product was loaded into a 96 well reaction plate along with 0.5µl of GS500LIZ internal lane size standard and 9.0µl of Hi-Di (Applied Biosystems). Electropherograms were visualized and separated into bin sets using AB GeneMapper software v3.5. Data were collected for the 13 microsatellite loci currently included in the Chinook Technical Committee (CTC) standardized database (Table 2; Seeb et al. 2007). The microsatellite data were standardized following the procedures outlined in Seeb et al. (2007). Genotype data were stored as GeneMapper (*.fsa) files on a network drive that was backed up nightly.

[1] Product names used in this report are included for scientific completeness, but do not constitute a product endorsement.

Quality control methods

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. Discrepancies were 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 blanked.

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. 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 population structure analyses or the baseline for genetic stock identification (GSI). Samples from radiotagged individuals were evaluated for inclusion in the analyses based on criteria which included both the number and distribution of the spawning ground samples, statistical tests of the non-spawning samples to determine their conformance to Hardy-Weinberg equilibrium (HWE), and the relationship to spawning populations from the same drainage.

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

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 heterozygosities were calculated for each population and each locus using FSTAT v2.9.3.2 (Goudet 1995; Goudet 2001). Allelic richness, a measure of the number of alleles that is independent of sample size, was calculated using FSTAT for all loci for each population to compare levels of genetic diversity within populations.

Population structure

Genetic diversity as measured by F_{ST} was calculated 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 locations, pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between all sites using PHYLIP for each marker-type dataset (Felsenstein 2004). Pairwise tests of population differentiation based on *G*-statistics were

calculated using FSTAT. Significance levels for comparisons between population pairs were set at 0.05%, and standard Bonferroni corrections for multiple tests were applied. Correspondence between patterns of population structure based on the 2 marker types (SNPs and microsatellites) was measured as the correlation between matrices of pairwise population chord distances using the Mantel test (Sokal and Rohlf 1995). Genetic chord distances were then used to construct a neighbor-joining tree (N-J) of sample populations with bootstrap replicates over 1,000 iterations through PHYLIP. A consensus tree was drawn with the program TREEVIEW (Page 1996).

Allelic richness and F_{ST} were calculated across populations and loci and then averaged over samples and loci for regional groups using FSTAT. A permutation test was performed to test significance with 1,000 permutations. We evaluated 3 regional groups (Figure 1) based on the larger drainage systems: Upper Copper River, Gulkana River, and Lower Copper River (Klutina River, Tazlina River including Mendeltna Creek, Tonsina River, and Chitina River).

Partitioning of variance within and among collections for each marker type was calculated using an analysis of molecular variance (AMOVA) (Excoffier et al. 2005). Only nuclear (diploid) markers were used.

Spatial analysis

We 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).

In order to identify stream sections associated with potential restrictions (or lack of restriction) to gene flow, we used the program StreamTree (Kalinowski et al. 2008; http://www.montana.edu/kalinowski/Software/StreamTree.htm Accessed November 11, 2009) to map Cavalli-Sforza and Edwards (1967) chord distances onto the sections of streams that connect populations within a drainage. The sum of the resulting distances for the sections connecting any 2 populations became the "fitted" genetic distance and was approximately equal to the observed genetic distance between these populations. A separate analysis was done for each marker type.

Detection of loci under selection

We used the method of Beaumont and Nichols (1996) to identify "outlier loci" from a plot of heterozygosity versus F_{ST} (Cockerham and Weir 1993) using the program FDIST2 (M. A. Beaumont, University of Reading, UK; http://www.rubic.rdg.ac.uk/~mab/software.html Accessed November 11, 2009) by generating a distribution of F_{ST} based on 20,000 replicates of the SNP and microsatellite data and then plotting the 0.025 and 0.975 quantiles (between which 95% of the data points are expected to lie). Loci lying above or below these quantiles may be under directional or balancing selection, respectively. The null distribution was generated using an infinite-allele model for SNPs and a stepwise mutation model for microsatellites. The 2 models have similar distributions of F_{ST} until heterozygosities reach approximately 0.8 at which time the infinite allele model predicts a sharper decline in F_{ST} with heterozygosity than the stepwise model (FDIST2; M. A. Beaumont, University of Reading, UK).

INRIVER ANALYSES

Baseline evaluation

Simulations were conducted to evaluate the statistical power of the microsatellites and SNPs to proportionally assign unknown mixtures taken within the Copper River to regional groups in order to evaluate the composition of the run through time. Populations were assigned into 5 reporting groups based on geographic and population structure for genetic stock identification (GSI) analyses. 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 3 groups: 3) Mendeltna Creek, 4) Tonsina, Klutina, and Tazlina lakes (collectively referred to as "Lakes"), and 5) Chitina River.

The identifiability of these reporting groups was evaluated using 100% simulations in which each reporting group comprised 100% of the sample being tested. Simulated mixtures were first constructed with SPAM version 3.7b using parametric bootstrapping with replacement (PB-R) (Debevec et al. 2000). 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 (SPAM version 3.7b, Debevec et al. 2000; Reynolds 2001). 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 unbiased cross-validation over gene copies (CV-GC) 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. This bias may be significant when populations are closely related 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 and yields unbiased estimates of GSI accuracy. We conducted the simulation through the program ONCOR (S. Kalinowski, Montana State University, http://www.montana.edu/kalinowski Accessed November 11, 2009) 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.

Mixed stock analysis

Estimates of stock composition and their 90% credibility intervals for the Baird Canyon collections were generated using the Bayesian analysis implemented in the program BAYES (Pella and Masuda 2001). 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. A uniform prior was used in which the Dirichlet prior distribution parameters for all reporting group proportions were equal (1/N, where N = the number of reporting groups). Within each reporting group populations received the same proportional representation.

MARINE ANALYSES

Baseline evaluation

To evaluate the contribution of Copper River stocks to fisheries in the Copper River District, the microsatellite data collected in this study were combined with a coastwide data set containing populations ranging from Yakutat to California. These data were from an update (Version 2.1) of the database described in Seeb et al. (2007). Considerable care was exercised to insure data consistency and proper pooling of alleles following the procedures of Seeb et al. (2007).

For genetic stock identification in marine waters, populations in the Copper River drainage were assigned to the original 3 reporting groups (Upper Copper River, Gulkana River, and Lower Copper River) based on geographic structure (e.g. watersheds), number of populations in a group, and management needs. These 3 broader groupings were used instead of the 5 reporting groups used for the inriver analyses, because single-population groups (Mendeltna Creek and Chitina River) may not adequately represent the entire set of spawning populations that they are purported to characterize in samples from a large-scale, highly-mixed fishery. The Mendeltna Creek and Chitina River populations were included in the Lower Copper River group because they were genetically similar and located in the lower portion of the Copper River. Following recommendations by Wood et al. (1987), populations were estimated separately and then summed within reporting groups to provide reporting group estimates. The potential use of these 3 reporting groups for GSI applications was first assessed with 100% PB-R simulations as previously described for inriver analyses.

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 using BAYES (Pella and Masuda 2001) to further examine the utility of the baseline. 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. 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. A uniform prior for the Bayesian estimation was used as described for inriver analyses.

Mixed stock analysis

Stock composition proportions were estimated from samples taken from the commercial fishery harvest. Stock compositions for all mixture samples were estimated using BAYES (Pella and Masuda 2001) with the uniform prior to allow for the most conservative estimate of stock proportions. Estimates and their 90% credibility intervals were derived from the posterior distribution as previously described.

RESULTS

SAMPLE COLLECTION

Extensive efforts were made during the 2004–2006 summer field seasons to sample spawning populations of Chinook salmon from throughout the drainage. Our sampling efforts were more successful than anticipated in the upper Copper River drainage, but we had difficulty obtaining samples in the lower river (Chitina and Tonsina river drainages). 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 sampling during multiple years can improve the representative nature of 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 sportfishing guides operating on the mainstem Gulkana and Klutina rivers, juvenile samples from the Little Tonsina River, and individuals radiotagged at Baird Canyon and tracked to river system (Savereide 2005). After review of the collection information and preliminary genetic data, the river guide and juvenile samples were excluded from use in the baseline as the uncertainty associated with mixed stock nature of the samples was high. Radiotagged individuals returning to the mainstem Tonsina River were included, however, because of the reported relatively high abundance of mainstem spawners (Savereide 2005), lack of significant departure from HWE (indicating that these individuals may have come from a single spawning population), and the very low collecting success from traditional on-the-ground sampling. Radiotagged 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 Chitina River drainage. This set of individuals could not be considered to represent a single population without further corroboration.

LABORATORY ANALYSIS

Genomic DNA was extracted from 4,907 Chinook salmon (Table 1) including 1,644 individuals from spawning populations, 1,665 individuals sampled from the Baird Canyon fish wheel (including radiotagged individuals), and 1,598 individuals sampled from the Copper River District commercial fisheries.

STATISTICAL ANALYSES

Diversity within populations

Four SNP loci, *Ots_arf-188*, *Ots_HGFA-446*, *Ots_PSMB1-197,* and *Ots_LEI-292*, with known polymorphisms in Chinook salmon were found to be monomorphic in the Copper River drainage (Appendix A) 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 variant allele frequencies <0.02 (Table 2; Appendix B; *Ots_Ikaros-250*, *Ots_Ots2, Ots_RFC2-558, Ots_TAPBP,* and *Ots_u211-85*). Private alleles were

observed in Chistochina River at *Ots_RFC2-558* (relative frequency = 0.004), Gulkana River 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 H_E across all populations for each SNP locus 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 (Appendix A), 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 2 (*Ots9)* to 47 (*Omm1080*). The mean H_E across all populations for each microsatellite locus 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; 3 tests were significant after adjustment for multiple tests (α = 176/0.05 = 0.0003). When a test of the alternative hypothesis of heterozygote deficiency was performed using ML-Null, 3 loci showed no heterozygote deficiency and 9 loci showed potential heterozygote deficiency in from 1 to 4 collections. However, 12 of the 14 tests at *Ssa408* were significant prior to adjustments for multiple tests; 6 were significant at $P<0.05$ and the remaining 6 were significant at $P<0.01$. This suggests the presence of null alleles at *Ssa408.*

Genotypic disequilibrium was not detected at any microsatellite loci, but the 2 SNPs at *Ots_FGF6* (*Ots_FGF6A* and *Ots_FGF6B)* were significantly linked to each other, as were the 2 *Ots_HSP90B* SNPs (*Ots_HSP90B-100* and *Ots_HSP90B-385*). Based on these results, both *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

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 based on SNPs and microsatellites showed positive correlation, suggesting broadly concordant patterns between marker classes (R=0.883; Figure 2). The consensus N-J trees (Figures 3 and 4) based on these chord distances provide a graphical representation of the relative similarities of populations based on the 2 marker sets. The consensus trees from the 2 marker sets were highly concordant and showed 4 distinct clusters: 1) Upper Copper River, 2) Gulkana River, 3) Mendeltna Creek, and 4) Lower Copper River (Tonsina and Klutina lakes, Kaina Creek, and the Chitina River). These clusters were strongly corroborated by appearing in 75% or more of the bootstrap replicates. The placement of Mendeltna Creek differed between the 2 marker sets. SNPs placed Mendeltna Creek on the branch leading to the Lower Copper River while microsatellites placed Mendeltna Creek on the branch leading to the Gulkana River collections.

Pairwise tests for significant differences between populations based on SNPs detected no significant difference ($P>0.000549$ for 91 tests) between Indian Creek and Chistochina River in

the Upper Copper River; among the 3 collections in the Gulkana River; or among the populations in the Lakes reporting group (Kaina Creek, Manker Creek, Greyling Creek, Tonsina River, or Tonsina River Radio Tags). All other tests were significant. For microsatellites, all pairwise tests were significant (P>0.000549 for 91 tests) with the exception of tests between Gulkana River Middle Fork and Gulkana River Mainstem and between 2 Tonsina River populations (Greyling Creek and Little Tonsina River).

We also tested for patterns in genetic diversity. Tests for significant differences in allelic richness were conducted across all populations and then among the 3 regional groups: Upper Copper River, Gulkana River, and Lower Copper River. Average richness among groups was significantly different for both SNPs (P<0.01) and microsatellites (P<0.02). Average allelic richness values in order by region for SNPs were 1.60, 1.68, and 1.74, and for microsatellites 9.51, 11.65, and 13.27. For both marker sets, allelic richness was lowest in the Upper Copper River and increased for Gulkana River and Lower Copper River populations (Table 3, Figure 5). Again moving from upriver downstream, regional F_{ST} values among populations within regions for SNPs were 0.026, 0.003, and 0.026, and for microsatellites 0.027, 0.003, and 0.030.

The AMOVA based on all collections indicated that the mean percentage (standard error) of variation among collections was 6.98% (1.20%) for SNPs and 4.81% (1.99%) for microsatellites.

Spatial analyses

Isolation by distance between Chinook salmon populations in the Copper River drainage based on pairwise values of genetic and geographic distance among the 14 populations showed (P<0.01) positive correlation between genetic and geographic distance for both markers, SNPs (R=0.602) and microsatellites (R=0.594) (Figure 6). As expected from the correlation between genetic distance values for the 2 markers (Figure 2), isolation by distance relationships were highly concordant between the marker sets.

When genetic distances were "fitted" to the sections of the Copper River connecting Chinook salmon populations, the resulting stream tree based on SNP data (Figure 7) showed a strong concordance with the geographic structure (R=0.932). Fitted distances calculated from microsatellite data (Figure 8) were also concordant with geographic structure, but with lower correlation (R=0.811). Only 3 sections of the Copper River mainstem were associated with large changes in allele frequencies, the remaining sections showed no obstruction to gene flow. These 3 sections are the river sections separating the Upper Copper River, Gulkana River, Lakes/Mendeltna Creek, and Chitina River reporting groups. The Tazlina River was also identified as having no association with inter-population genetic distances. The variation was entirely associated with the separation between Kaina and Mendeltna creeks across Tazlina Lake. Most of the remaining genetic distances were associated with tributary sections connecting collection sites with the mainstem Copper River.

Detection of loci under selection

The test for outlier loci that may be under natural selection identified the SNP locus, *Ots_MHC-2*, as above the 95% quantile given the F_{ST} value, 0.068 (Figure 9). For microsatellites, only 3 loci were within the distribution (Figure 10). The majority of the loci with high heterozygosity were outliers either above or below the 95% quantile. However, one locus, *Ots9*, was well above the 95% quantile at moderate levels of heterozygosity, indicative of natural selection.

INRIVER ANALYSES

Baseline evaluation

Results of the PB-R method for the 100% simulations for the 5 reporting groups (Upper Copper River, Gulkana River, Mendeltna Creek, Lakes, and Chitina River) indicated these groups were highly identifiable in mixtures. Mean values of 1,000 bootstrap iterations ranged from 0.931 to 0.994 for SNPs and 0.945 to 0.991 for microsatellites (Table 4). Chitina River, 1 of the 2 reporting groups with only a single population and characterized by only 68 individuals, was the lowest performer for both marker types, but still had correct classification above the 90% level commonly used in fishery analyses (Seeb et al. 2007).

Simulations performed using the unbiased CV-GC method with the SNP data provided correct proportional assignments of individual population mixtures to population (Table 5) ranging from a low of 0.115 for Gulkana River Mainstem to a high of 0.998 to Sinona Creek. Results based on microsatellites were very similar and varied from 0.254 for Gulkana River Mainstem to 0.995 for Sinona Creek. The resulting correct proportional assignment to region using the CV-GC method 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.

Mixed stock analysis

Estimates of stock composition of Chinook salmon passing the Baird Canyon fish wheel were made for approximately weekly periods based on statistical weeks from mid-May to mid-July, 2005 (Table 6, Figure 11). Sample sizes for each estimate varied from 65 to 274 depending on the availability of the fish during the period. Stock spawning in the upper portions of the drainage, represented by the Upper Copper River and Gulkana River reporting groups, comprised over 80% of samples during the period from May 12 through May 28 (weeks 20-22). The week of May 29–June 4 (week 23) was a transition period during which the contribution of the Upper Copper River and Gulkana River groups dropped to about 59% of the sample. Thereafter, during June 5–July 14, stocks from 3 lower river groups predominated samples, increasing from about 64% to 96% of samples. Migratory timing profiles for each of the reporting groups showed that 50% of the cumulative proportional contribution of the Upper Copper River and Gulkana River groups was reached by week 22 (Figure 12). The Mendeltna Creek, Lakes and Chitina River groups did not achieve this proportion until week 24.

MARINE ANALYSES

Baseline evaluation

Simulations were conducted to evaluate the accuracy and precision of the composite baseline to provide compositional estimates of mixtures of Chinook salmon sampled in marine waters of the Copper River District. These simulations were used to help assess whether the baseline of allele frequencies at the 13 microsatellite markers would provide sufficient information to identify individual stocks or groups of stocks (reporting groups) from the Copper River drainage in mixtures. Populations were combined into the 3 larger reporting groups: 1) Upper Copper River, 2) Gulkana River, and 3) Lower Copper River, and simulations indicated that these 3 reporting groups had mean correct allocations of 97.9%, 95.0%, and 96.8% in the context of the larger CTC baseline which extends from the Copper River to California, well above the 90% threshold for identifiability (Table 7).

When fish sampled from the sport fisheries in the Gulkana and Klutina rivers were used as mixtures, the Bayesian method demonstrated an ability to correctly allocate Chinook salmon to Copper River reporting groups within the larger coastwide microsatellite baseline (99% correct allocation) (Table 7).

Mixed stock analysis

A total of 1,612 individuals were sampled from the commercial harvest landed at processors in Cordova, Alaska in 2005 (Table 8). These samples were taken during 9 of 10 fishing periods across the entire fishery from May 16 through June 14. Only Period 6 (June 1–2) was not sampled due to other obligations by the sampling crew. Target sample sizes of 200 individuals were generally met except for periods where the harvest was small or logistics did not allow complete sampling.

Estimates of stock composition in the commercial harvest in the Copper River District indicate that Chinook salmon of Copper River origin contributed more than 94% of the harvest during 9 of 10 commercial fishing periods in 2005 (Table 9; Figure 13). The estimated relative proportions of the 3 reporting groups in the Copper River component varied across the season. Each of the 3 groups contributed similar proportions during the first period (25% - 37%), but the Upper Copper River contribution quickly dropped to less than 2% by the middle of the season (Period 5). The proportional contribution from the Gulkana River group remained relatively constant (35% - 40%) during the first 4 periods before it also decreased to 3% of the harvest sample during the last period. Contributions from populations from the Lower Copper River group increased over the season, rising from 25% during the first period to 92% by the last period. Contributions from stock groups outside the Copper River drainage ranged from 1% to 5% throughout the season.

DISCUSSION

The goals of this project were to develop genetic markers to delineate major geographic and temporal stocks of Chinook salmon spawning in the Copper River and then to use the markers to investigate run timing both within the Copper River and in commercial ocean fisheries in the Copper River District. Collections analyzed during this project spanned the entire drainage and represented most of the known spawning areas of the river. Chinook salmon in the Copper River exhibit significant genetic divergence both within and among populations in the major tributary drainages. Samples collected from Baird Canyon fish wheels and from fisheries in the Copper River District consistently showed that Upper Copper River populations had earlier run timing than populations from the Gulkana River and Lower Copper River.

GENETIC DIVERSITY

Considerable genetic divergence among Chinook salmon populations was detected across the major drainages of the Copper River with substantial allele frequency differences between populations for both SNPs (mean F_{ST}=0.068) and microsatellites (mean F_{ST}=0.054). These differences were generally organized by region as demonstrated by the consistency of the population groups on the N-J trees. Varying amounts of genetic divergence were found within the 3 regional groupings of populations, which may be related to the amount of habitat diversity present within and among these regions.

The Upper Copper River region is an area of non-glaciated lakes and upland highlands with small Chinook salmon populations. These populations exhibit significant allele frequency differences for both microsatellites and SNPs as supported by significant pairwise tests between every pair of populations for both markers and regional F_{ST} values of 0.026 and 0.027 for microsatellites and SNPs, respectively. These populations also exhibit the lowest range of allelic richness (Figure 5, Table 3); indicative of smaller, more isolated populations. This area clearly exhibits a high level of diversity with multiple genetically-diverse populations. Barriers to gene flow exist, as demonstrated by the StreamTree results, where relatively large values of "fitted" genetic distances were derived for each tributary (Figures 7 and 8).

The Gulkana River drainage flows through an area of rolling hills and upland highlands. Collections from the Gulkana River drainage were obtained from 3 geographically close reaches and genetic differences among collections from this region were low (F_{ST}=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, these collections were taken in relatively close proximity and it is possible that additional divergence could exist in populations from areas not yet sampled within this drainage.

Significant genetic diversity was found between Chinook salmon spawning in 2 tributaries to Tazlina Lake. From the north, clear-water Mendeltna Creek drains upland highlands and on the south shore, glacier-fed Kaina Creek descends from mountain slopes. Of the 2, the Mendeltna Creek population is particularly divergent, while the Kaina Creek population clusters with other populations from the glacial mountain valleys containing Klutina and Tonsina lakes (Figures 3 and 4). Mendeltna Creek shows some genetic similarity to other upland populations of the Gulkana River and Upper Copper River drainages. There were barriers indicated in the StreamTree results and marked differences in allele frequency indicative of restricted gene flow. For example, the estimated allele frequency for *Ots_MHC2*1* was 0.25 for Mendeltna Creek, but 0.60 for Kaina Creek. Also of note, a variant of *Ots_C3N3*, the mitochondrial DNA SNP, was observed in Mendeltna Creek at a frequency of 0.076, but was not observed in any other collection in the Copper River drainage. The only other *Ots_C3N3* variants found in Alaska appear in Southeast Alaska (Bill Templin, Fisheries Scientist, ADF&G, Anchorage; personal communication; Narum et al. 2008).

The drainages of the Klutina and Tonsina lakes are glacial systems with high-gradient, cold tributaries. Chinook salmon populations spawning in these drainages share similar genetic profiles as demonstrated by the close cluster visible on the N-J trees with both marker sets. Further, no significant differences in pairwise tests were found between these populations using SNPs. However, run timing differences may exist between mainstem and tributary spawners within these lakes and such segregation could promote divergence of populations within the lakes that was not detected in this study. For example, radiotelemetry studies (Savereide 2005) have suggested that there are early- (tributary) and late- (mainstem) components to the Klutina and Tonsina river systems. While more comprehensive sampling would be needed to evaluate genetic diversity associated with the timing and location (tributary vs. mainstem) of spawning, obtaining samples will be difficult. During our study the Tonsina River drainage was particularly challenging to sample. Only 16 spawners were obtained from the upper portion of the drainage at Greyling Creek and 61 were collected from the Little Tonsina River. Despite the difficulty in collecting genetic samples, radiotelemetry information indicates that the Tonsina River system

can produce a significant portion (estimated 27% in 2002, Savereide 2005) of the Copper River escapement.

Chinook salmon populations within the Chitina River drainage may also be more diverse than our study can show. Based on escapement and radiotelemetry results, Chitina River contributed from 22% to 34% of the spawners over the period 2002-2004 (Savereide 2005), and spawners were distributed both in the mainstem and the tributaries. Tebay River, at 29 km from the mouth of the Chitina River, was the only location where sufficient individuals (N=68) were sampled for inclusion in this study. Float trips on the mainstem Chitina River were unsuccessful. Additional diversity among Chinook salmon spawning within the Chitina River system is possible and should be investigated.

SPATIAL ANALYSIS

Spatial or landscape analyses have become an increasingly valuable tool used to place 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). Isolation-by-distance tests are commonly applied to explore the relationship between genetic and geographic distances. For both marker types, we found significant correlation between genetic and geographic distances, suggesting a rate of gene flow proportional to the geographic distance between populations. However, isolation by distance analysis does not account for other variables or barriers that shape population structure at different spatial scales. For example, low-level divergence between populations spawning in the Klutina and Tonsina river drainages may reflect the lack of ecological barriers to migration. Alternately, Mendeltna and Kaina creeks, both tributaries to Tazlina Lake and separated by a short distance, exhibit significant differences potentially due to large differences in spawning and rearing conditions.

A newly developed spatial analysis, StreamTree (Kalinowski et al. 2008), is useful for identifying migratory corridors and in-stream barriers. Sections of streams (corridors) through which fish migrate are first identified. Then genetic distances are mapped onto the drainage with a distance assigned to each section. The sum of the genetic distances across all sections of the stream between any 2 populations is equal to the observed genetic distance. Unlike the N-J tree, which is solely based on genetic divergence, the StreamTree retains geographic relationships. The results from StreamTree were highly concordant between marker types, supporting the ability of both markers to identify population structure within the drainage. The results indicate that the barriers to gene flow are the tributaries themselves with only certain sections of the mainstem Copper River acting as barriers.

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 Upper Copper River populations are the smallest and/or the most isolated within the drainage. Both SNPs and microsatellites indicated comparable levels of divergence among populations within regions as described by the AMOVA analysis. Similar to the results of this study, Smith et al. (2007) found that both marker types showed similar estimates of population divergence among collections of Chinook salmon from North America and Russia, with SNP values higher than those measured from microsatellites.

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 (Smith et al. 2005c; Narum et al. 2008). Currently, investigators working with a variety of organisms are developing baselines 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; Seeb et al. 2007; Morin et al. 2009).

Several recent studies have compared the ability of SNPs and microsatellites to reveal population structure (Ryynanen et al. 2007; Smith et al. 2007; Narum et al. 2008; Morin et al. 2009). Narum et al. (2008) evaluated the utility of the 2 marker sets for information content and population structure analysis for Chinook salmon using 37 of the 51 SNPs and the same set of 13 microsatellites used in our study. 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 that genetic differentiation measured with G'_{ST} (Hedrick 2005) ranked SNPs at the top. Similar to our study, the topologies of the N-J trees were very similar, and 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. (2009) found that approximately 30 SNPs were sufficient to detect moderate (F_{ST}=0.01) levels of differentiation, but that at least 80 SNPs would be needed to detect demographic independence (e.g. F_{ST}<0.005). These simulations results are consistent with our findings, since significant pairwise differences were not detected among Gulkana River drainage populations (F_{ST}=0.003) with SNPs. Cumulatively, results of these studies suggest that additional SNPs would improve fine-scale resolution and individual assignments of Chinook salmon in the Copper River drainage.

Outlier analyses can be particularly useful for identifying those loci potentially under selection. This type of analysis showed that the *Ots_MHC-2* SNP was an outlier with an F_{ST} value above the 95% quantile, which is indicative of directional selection. This same locus was previously identified as an outlier by Smith et al. (2007) in a range-wide survey of Chinook salmon, although Narum et al. (2008) did not report elevated F_{ST} for this locus. The major histocompatibility complex (MHC) is a multigene family that contains genes for the processing and presentation of antigens to cells of the immune system (Klein 1986). High levels of genetic variation have been observed at functional MHC genes, and this has been attributed to a number of forces including pathogen-mediated selection, kin selection, mate choice, and maternal/fetal interactions (Aguilar and Garza 2007).

We also found that the microsatellite locus, *Ots9*, was an outlier among the microsatellites. This locus exhibited the fewest alleles (N=2) of the 13 microsatellites surveyed in our study. This locus also expressed the fewest alleles in a survey of Chinook salmon populations from California to northern Southeast Alaska (N=9; Seeb et al. 2007), which suggests that there are constraints on the mutation of this locus. This locus was not surveyed by Smith et al. (2007) and was not identified as an outlier by Narum et al. (2008).

We identified departures from HWE in microsatellites, but not with 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 null alleles at this locus. The locus was ascertained from Atlantic salmon *Salmo salar* (Cairney et al. 2000) and found to be useful and within HWE for Chinook salmon in the Snake River which is in the southern portion of their geographic range (Narum et al. 2007; Neville et al. 2007). However, there appears to be a null allele in the northern portion of the species' range leading to ascertainment bias in diversity estimates based on this locus (see Smith et al. 2007).

INRIVER MIGRATION

Simulations using PB-R indicated that 5 reporting groups could be accurately identified in the Copper River drainage. Two of these groups were represented by single populations. In these cases, the underlying assumption was that unsampled populations within an area represented by a single population are more similar to the represented population than to populations in any other reporting group. We think that this assumption is likely valid for Mendeltna Creek, a localized area without much spawning habitat for Chinook salmon. This assumption may not be valid for Tebay River in the Chitina River drainage, a large drainage.

Chinook salmon samples from the Baird Canyon fish wheel show stock-specific differences in entry patterns. Populations from the Upper Copper River and Gulkana River regions enter first followed by populations from the Mendeltna, Lakes, and Chitina regions. These results confirm observations from radiotelemetry studies. However, the proportional contributions from the Chitina River reporting group were consistently lower in our study (1.4% to 8.1%) than those obtained from the radiotelemetry study in which estimates as large as 34% were reported (Savereide 2005). This difference may be due to annual differences in run abundance or over-estimation of the Chitina River escapement component by the radiotelemetry data. However, we suspect that the limited baseline samples obtained from Tebay River did not capture the actual diversity and genetic characteristics of Chitina River drainage populations. The Chitina River drainage is a large and potentially diverse system with many tributaries in which Chinook salmon are reported to spawn (Savereide 2005). More complete representation of the Chitina River in the genetic baseline would be required to evaluate the discrepancies between the studies.

Radiotelemetry results suggest that Chinook salmon populations with early- and late-run timing may be present in the Tonsina and Klutina drainages. Assuming the current genetic baseline adequately characterizes both runtimes, there was no clear signal of early- and late-run components in the analysis of cumulative proportions (Figure 12). However, the Lakes reporting group did represent 3 drainages (Tazlina, Klutina, and Tonsina lakes), which confounds our ability to identify population components of this group with different run timing. The genetic data do provide evidence that Mendeltna Creek has an earlier runtime than other populations returning to the Lakes reporting group (Figure 12). Since Kaina Creek was pooled in the Lakes reporting group, a specific comparison of run timing between the 2 Tazlina Lake tributaries was not possible with the current dataset. To better evaluate genetic diversity associated with run timing, more comprehensive baseline sampling, designed to characterize early- and late-run components within drainages, would be needed.

FISHERY ANALYSIS

We analyzed samples from the commercial fishery harvests in the Copper River District to investigate the migratory run timing of Copper River Chinook salmon stocks in marine waters. Patterns of migratory timing in the commercial fishery were similar to patterns observed with both radiotelemetry (Savereide 2005) and genetic stock identification at the Baird Canyon fish wheel. In general, populations from the Upper Copper River and Gulkana River reporting groups comprised the largest proportion of commercial harvest samples taken during the first 3 periods and then declined to minor contributors as the contribution from the Lower Copper River reporting group increased.

Commercial harvesting occurs in the marine waters of the Gulf of Alaska off the mouth of the Copper River and genetic stock identification detected low proportional contributions (less than 5%) of Chinook salmon from outside the Copper River drainage in samples from the commercial fishery harvests. Coded-wire tagged (CWT) recoveries from the harvest also indicate that migrating Chinook salmon from outside the Copper River have been intercepted periodically in the fishery. As an example, in 2002 CWT samples from the commercial harvest included a total of 23 Chinook salmon with tags from hatcheries outside Alaska (British Columbia, Washington, and Oregon) and 16 individuals with tags from hatcheries in Cook Inlet and Southeast Alaska (ADF&G, http://tagotoweb.adfg.state.ak.us/CWT/reports/ Accessed November 11, 2009). Since coded-wire tags are typically only used with hatchery-origin Chinook salmon, contributions from wild stocks must be estimated by extrapolating from recoveries of adjacent hatchery stocks (Hankin et al. 2005). Genetic data provide an accurate method for estimating the interception of both wild and non-coded wire tagged stocks.

Through the use of the standardized markers and methods by laboratories contributing to the Coastwide Chinook salmon database (Seeb et al. 2007), the Copper River microsatellite data collected during our project are being contributed to the 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 Accessed November 11, 2009). SNP markers are standardized by definition (Smith et al. 2007) and will be contributed to a growing coastwide Chinook salmon SNP database (e.g. Narum et al. 2008). In future years, we hope to further extend these databases west by including Chinook salmon samples from Cook Inlet, Kodiak Island, and Alaska Peninsula populations. Once the database is more complete, it will be a valuable resource for management agencies wishing to make accurate stock-contribution estimates for mixed stock fisheries.

CONCLUSIONS

This study provides the first comprehensive analysis of genetic diversity within Chinook salmon populations in the Copper River drainage, a highly valued and productive system. Significant genetic divergence was found both within and among Chinook salmon spawning in 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 used in our study 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 drainages. Within the Lower Copper River group, 2 single collections were particularly divergent, Tebay River from the Chitina River drainage and Mendeltna Creek from the Tazlina River drainage.

Results for both marker sets, SNPs and microsatellites, were very consistent and provided similar estimates of 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 diversity within the Tazlina River system with large allele frequency differences between Kaina and Mendeltna creeks.

Mixed stock collections from both within the Copper River at Baird Canyon and the marine waters of the Copper River District consistently showed the Upper Copper River stocks contributing their largest proportion early in the season followed by populations from the Middle (Gulkana River) and Lower Copper River groups. The results also indicate that the commercial fishery was, to a great extent, targeting Chinook salmon bound for the Copper River. While only small percentages of out-of-basin individuals were detected, use of these data for GSI on Copper River commercial fishery harvests is not recommended prior to the inclusion of populations from the western Gulf of Alaska.

RECOMMENDATIONS

1. Resource agencies should incorporate knowledge of the significant diversity of Chinook salmon within the Copper River into their management regimes and decisions. Conserving this diversity will help ensure that these populations remain productive and sustainable.
2. Use knowledge of Chinook salmon diversity to evaluate conservation strategies since populations within the Upper Copper River are diverse and likely isolated.
3. Expand sampling and analysis of additional populations within the Copper River since additional fine-scale differentiation likely exists within individual drainages beyond that revealed by this study. Improving the resolution and documentation of genetic diversity is particularly needed for the following Lower River group populations:
    a. Klutina and Tonsina rivers to evaluate genetic differences between early- and late-timing as well as mainstem and tributary spawning locations; and
    b. Chitina River system to evaluate the escapement to the system and differences between radiotelemetry and genetic inriver estimates.
4. Expand the number of SNPs used for Copper River Chinook salmon to at least the Coastwide standard of 75 to better allow the Copper River to be incorporated into Pacific Rim baselines for migration and bycatch studies. Increasing to 96 SNP loci is now economical with dynamic array analyses (Seeb et al. *In press*).
5. Continue monitoring the commercial fishery with genetic stock identification to allow comparisons to be made across multiple years and track the relative contribution of the major population groups within the Copper River. These analyses can be based on SNP markers in the near future as the SNP baseline expands.

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REFERENCES CITED

ADF&G (Alaska Department of Fish and Game). 2007. News release 10/31/2007. Downloaded from: http://www.cf.adfg.state.ak.us/region2/finfish/salmon/pws/pwspos07.pdf (Accessed November 5, 2008).

Aguilar, A., and J. Garza. 2007. Patterns of Historical Balancing Selection on the Salmonid Major Histocompatibility Complex Class II β Gene. Journal of Molecular Evolution 65(1):34-43.

Anderson, E. C., R. S. Waples, and S. T. Kalinowski. 2008. An improved method for predicting the accuracy of genetic stock identification. Canadian Journal of Fisheries and Aquatic Sciences 65:1475–1486.

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in Chinook salmon (*Oncorhynchus tshawytscha*). Journal of Heredity 90:281-288.

Beacham, T. D., M. Wetklo, C. Wallace, J. B. Olsen, B. G. Flannery, J. K. Wenburg, W. D. Templin, A. Antonovich, and L. W. Seeb. 2008. The application of microsatellites for stock identification of Yukon River Chinook salmon. North American Journal of Fisheries Management 28(1):283-295.

Beaumont, M. A., and R. A. Nichols. 1996. Evaluating loci for use in the genetic analysis of population structure. Proceedings of the Royal Society of London B 263:1619-1626.

Brumfield, R. T., P. Beerli, D. A. Nickerson, and S. V. Edwards. 2003. The utility of Single Nucleotide Polymorphisms in inferences of population history. Trends in Ecology & Evolution 18(5):249-256.

Cairney, M., J. B. Taggart, and B. HØyheim. 2000. Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar L*) and cross-species amplification in other salmonids. Molecular Ecology 9(12):2175-2178.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21:550-570.

Crane, P. A., W. D. Templin, and L. W. Seeb. 1996. Genetic stock identification of Alaska Chinook salmon. Final report of the Alaska Department of Fish and Game pursuant to National Oceanic and Atmospheric Administration Awards No. NA46FD0356, Regional Information Report 5J96-17, Juneau.

Cockerham, C. C., and B. S. Weir. 1993. Estimation of gene flow from F-statistics. Evolution 47(3):855-863.

Debevec, E. M., R. B. Gates, M. Masuda, J. Pella, J. Reynolds, and L. W. Seeb. 2000. SPAM (Version 3.2): Statistics program for analyzing mixtures. Journal of Heredity 91:509-511.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Felsenstein, J. 2004. PHYLIP (Phylogeny Inference Package) Version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Gharrett, A. J., S. M. Shirley, and G. R. Tromble. 1987. Genetic relationships among populations of Alaskan Chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 44(4):765-774.

REFERENCES CITED (Continued)

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86:485-486.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www2.unil.ch/popgen/softwares/fstat.htm Updated from Goudet (1995).

Greig, C., and M. A. Banks. 1999. Five multiplexed microsatellite loci for rapid response run identification of California's endangered winter Chinook salmon. Animal Genetics 30(4):318-320.

Greig, C., D. P. Jacobson, and M. A. Banks. 2003. New tetranucleotide microsatellites for fine-scale discrimination among endangered Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology Notes 3(3):376-379.

Hankin, D. G., D. G. Hankin, J. H. Clark, R. B. Deriso, J. C. Garza, G. S. Morishima, B. E. Riddell, C. Schwarz, and J. B. Scott.. 2005. Report of the expert panel on the future of the coded wire tag recovery program for Pacific salmon. Pacific Salmon Commission Technical Report 18, Vancouver, British Columbia. Available at: www.psc.org/pubs/CWT/EPfinalreport.pdf

Hedrick, P. W. 2005. A standardized genetic differentiation measure. Evolution 59:1633-1638.

Hilborn, R., Quinn, T. P., Schindler, D. E. and D. E. Rogers. 2003. Biocomplexity and fisheries sustainability. Proceedings of the National Academy of Sciences 100:6564-6568.

Hollowell, G., B. Lewis, R. Merizon, and S. Moffitt. 2007. 2005 Prince William Sound Area finfish management report. Alaska Department of Fish and Game, Fishery Management Report No. 07-33, Anchorage. http://www.sf.adfg.state.ak.us/FedAidPDFs/fmr07-33.pdf

Kalinowski S. T., and M. L. Taper. 2006. Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. Conservation Genetics 7:991-995.

Kalinowski, S. T., M. H. Meeuwig, S. R. Narum, and M. L. Taper. 2008. Stream trees: a statistical method for mapping genetic differences between populations of freshwater organisms to the sections of streams that connect them. Canadian Journal of Fisheries and Aquatic Sciences 65:2752-2760.

Klein, J. 1986. Natural history of the major histocompatibility complex. John Wiley, New York.

Manel, S., M. K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology and Evolution 18(4):189-197.

Morin, P. A., G. Luikart, R. K. Wayne, and SNP Workshop Group. 2004. SNPs in Ecology, Evolution and Conservation. Trends in Ecology & Evolution 19(4):208-216.

Morin, P. A., K. K. Martien, and B. L. Taylor. 2009. Assessing statistical power of SNPs for population structure and conservation studies. Molecular Ecology Resources 9(1):66-73.

Moser, J. F. 1899. The salmon and salmon fisheries of Alaska. Report of the operations of the United States Fish Commission steamer Albatross for the year ending June 30, 1898. Washington, Government Printing Office.

NRCC (National Research Council Committee on Protection and Management of Pacific Northwest Anadromous Salmonids). 1996. Upstream: Salmon and Society in the Pacific Northwest. National Academy Press, Washington, DC, 452 p.

Narum, S. R., M. Banks, T. D. Beacham, M. R. Bellinger, M. R. Campbell, J. Dekoning, A. Elz, C. M. Guthriw III, C. Kozfkay, K. M. Miller, P. Moran, R. Phillips, L. W. Seeb, C. T. Smith, K. Warheit, S. F. Young, and J. C. Garza. 2008. Differentiating salmon populations at broad and fine geographical scales with microsatellites and single nucleotide polymorphisms. Molecular Ecology 17(15):3464-3477.

Narum, S. R., J. J. Stephenson, and M. R. Campbell. 2007. Genetic variation and structure of Chinook salmon life history types in the Snake River. Transactions of the American Fisheries Society 136(5):1252-1262.

Neville, H., D. Isaak, R. Thurow, J. Dunham, and B. Rieman. 2007. Microsatellite variation reveals weak genetic structure and retention of genetic variability in threatened Chinook salmon (*Oncorhynchus tshawytscha*) within a Snake River watershed. Conservation Genetics 8(1):133-147.

REFERENCES CITED (Continued)

Olsen, J. B., P. Bentzen, and J. E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7:1087-1089.

Page, R. D. M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12:357-358.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Pella, J., and M. Masuda. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fishery Bulletin 99(1):151-167. ftp://ftp.afsc.noaa.gov/sida/mixture-analysis/Bayes/

Rexroad, C. E. I., R. L. Coleman, A. M. Martin, W. K. Hershberger, and J. Killefer. 2001. Thirty-five polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). Animal Genetics 32(5):317-319.

Reynolds, J. H. 2001. SPAM Version 3.5: User's Guide Addendum. Addendum to Special Publication No. 15, Alaska Department of Fish and Game, Division of Commercial Fisheries, Gene Conservation Laboratory, 333 Raspberry Road, Anchorage, AK 99518. http://www.cf.adfg.state.ak.us/geninfo/pubs/special/sp15/spam_v35may02.pdf

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rousset, F. 2007. Inferences from spatial population genetics. Pages 945-979 [*in*]: D. J. Balding, M. Bishop, and C. Cannings, editors. Handbook of Statistical Genetics, 3rd edition, Wiley, Chichester, UK.

Rousset, F. 2008. GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8(1):103-106.

Ryynanen, H. J., A. Tonteri, A. Vasemagi, and C. R. Primmer. 2007. A comparison of biallelic markers and microsatellites for the estimation of population and conservation genetic parameters in Atlantic salmon (*Salmo salar*). Journal of Heredity 98(7):692-704.

Savereide, J. W. 2005. Inriver abundance, spawning distribution and run timing of Copper River Chinook salmon, 2002-2004. Alaska Department of Fish and Game, Fishery Data Series No. 05-50, Anchorage. http://www.sf.adfg.state.ak.us/FedAidPDFs/fds05-50.pdf

Scribner, K. T., J. A. Blanchong, D. J. Bruggeman, B. K. Epperson, C. Lee, Y. Pan, R. I. Shorey, H. H. Prince, S. R. Winterstein, and D. R. Luukkonen. 2005. Geographical genetics: Conceptual foundations and empirical applications of spatial genetic data in wildlife management. Journal of Wildlife Management 69(4):1434-1453.

Seeb, L. W., A. Antonovich, M. A. Banks, T. D. Beacham, M. R. Bellinger, S. M. Blankenship, M. R. Campbell, N. A. Decovich, J. C. Garza, C. M. Guthrie, T. A. Lundrigan, P. Morgan, S. R. Narum, J. J. Stephenson, K. J. Supernault, D. J. Teel, W. D. Templin, J. K. Wenburg, S. F. Young and C. T. Smith. 2007. Development of a standardized DNA database for Chinook salmon. Fisheries 32(11):540-552.

Seeb, J. E., C. E. Pascal, R. Ramakrishnan, and L. W. Seeb. *In press*. SNP genotyping by the 5'-nuclease reaction: advances in high throughput genotyping with non-model organisms. A. Komar, editor Methods in Molecular Biology, Single Nucleotide Polymorphisms, 2d Edition. Humana Press.

Simeone, W. E., and E. M. Valentine. 2007. Ahtna knowledge of long-term changes in salmon runs in the Upper Copper River drainage, Alaska. Alaska Department of Fish and Game, Division of Subsistence Technical Paper No. 324, Juneau.

Smith, C. T., A. Antonovich, W. D. Templin, C. M. Elfstrom, S. R. Narum, and L. W. Seeb. 2007. Impacts of marker class bias relative to locus-specific variability on population inferences in Chinook salmon: a comparison of single-nucleotide polymorphisms with short tandem repeats and allozymes. Transactions of the American Fisheries Society 136(6):1674-1687.

Smith, J. J. and K. M. van den Broek. 2006. Estimating Chinook salmon escapement on the Copper River, 2005 annual report. U.S. Fish and Wildlife Service, Office of Subsistence Management, Fisheries Resource Monitoring Program (Study No. 04-503), Anchorage, Alaska.

REFERENCES CITED (Continued)

Smith C. T., Elfstrom C. M., Seeb J. E., Seeb L. W. 2005a. Use of sequence data from rainbow trout and Atlantic salmon for SNP detection in Pacific salmon. Molecular Ecology 14:4193-4203.

Smith C. T., J. E. Seeb, P. Schwenke, and L. W. Seeb. 2005b. Use of the 5'-nuclease reaction for SNP genotyping in Chinook salmon. Transactions of the American Fisheries Society 134:207-217

Smith, C. T., W. D. Templin, J. E. Seeb, and L. W. Seeb. 2005c. Single nucleotide polymorphisms (SNPs) provide rapid and accurate estimates of the proportions of U.S. and Canadian Chinook salmon caught in Yukon River fisheries. North American Journal of Fisheries Management 25(3):944-953.

Smith, J. J. 2004. Feasibility of using fish wheels for long-term monitoring of Chinook salmon escapement on the Copper River, 2003 annual report. U.S. Fish and Wildlife Service, Office of Subsistence Management, Fisheries Resource Monitoring Program, Annual Report No. FIS01-020, Anchorage.

Smouse, P. E., and J. C. Long. 1992. Matrix correlation-analysis in anthropology and genetics. Yearbook of Physical Anthropology 35:187-213.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd edition. W. H. Freeman and Co.: New York.

Templin, W. D., C. T. Smith, D. Molyneaux, J. Wenburg and L. W. Seeb. 2004. Genetic diversity of Chinook salmon from the Kuskokwim River. USFWS Office of Subsistence Management, Fisheries Resource Monitoring Program, Final Report No. 01-070, Anchorage, Alaska.

Templin, W. D., R. L. Wilmot, C. M. I. Guthrie, and L. W. Seeb. 2005. United States and Canadian Chinook salmon populations in the Yukon River can be segregated based on genetic characteristics. Alaska Fisheries Research Bulletin 11:44-60.

Thompson, S. 1987. Sample sizes for estimating multinomial proportions. American Statistician 41:42-46.

Utter, F., G. Milner, G. Stahl, and D. Teel. 1989. Genetic population structure of Chinook salmon, *Oncorhynchus tshawytscha*, in the Pacific Northwest. Fishery Bulletin 87:239-264.

Utter, F., D. Teel, G. Milner, and D. McIsaac. 1987. Genetic estimates of stock compositions of 1983 Chinook salmon, *Oncorhynchus tshawytscha*, harvests off the Washington coast and the Columbia River. Fishery Bulletin 85:13-23.

Waples, R. S. 1990. Temporal changes of allele frequency in Pacific salmon - Implications for mixed stock fishery analysis. Canadian Journal of Fisheries and Aquatic Sciences 47(5):968-976.

Waples, R. S., D. J. Teel, J. M. Myers, and A. R. Marshall. 2004. Life history divergence in Chinook salmon: Historic contingency and parallel evolution. Evolution 58:386-403.

Williamson, K. S., J. F. Cordes, and B. May. 2002. Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Molecular Ecology Notes 2(1):17-19.

Wood, C. C., S. McKinnell, T. J. Mulligan, and D. A. Fournier. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Canadian Journal of Fisheries and Aquatic Sciences 44(4):866-881.

Workman, W. 1976. Ahtna archaeology: a preliminary statement. Paper presented at the 9th annual conference of the University of Calgary Archeology Association. Calgary, Alberta, Canada.

Wuttig, K. G., and M. J. Evenson. 2001. Inriver abundance, spawning distribution, and migratory timing of Copper River Chinook salmon in 2000. Alaska Department of Fish and Game, Fishery Data Series No. 01-22, Anchorage. http://www.sf.adfg.state.ak.us/FedAidPDFs/fds01-22.pdf

TABLES AND FIGURES

Table 1.–Chinook salmon collections for genetic analysis sampled from the Copper River drainage and fisheries.

Map #	Drainage Location	Sample Size	Year	Total Genotyped
	Baseline			
	Upper Copper River			
1	Bone Creek	70, 8	2004, 2005	78
2	Slana River, Ahtell Creek[a]	20, 1	2004, 2005	21
3	Otter Creek	128	2005	128
4	Indian Creek	43, 7	2004, 2005	50
5	East Fork Chistochina River	145	2004	145
6	Sinona Creek	7, 152	2004, 2005	159
	Gulkana River			
7	Mainstem	46	2004	46
8	Middle Fork	79	2004	79
9	Paxson Fork	88	2004	88
	Gulkana River (sport fishing guides) [a]	130	2004	130
	Tazlina River			
10	Mendeltna Creek	144	2004	144
11	Kaina Creek	75	2004	75
	Klutina River			
12	Manker Creek	41, 21	2004, 2005	62
	Klutina River (sport fishing guides) [a]	168	2004	168
	Tonsina River			
	Little Tonsina River (juveniles) [a]	20	2004	20
13	Little Tonsina River	31, 1, 29	2004, 2005, 2006	61
14	Greyling Creek	16	2004	16
15	Tonsina Radio Tags	106	2003, 2004	106
	Chitina River			
16	Tebay River – lake outlet	27, 34, 7	2004, 2005, 2006	68
	Total			1,644
	Radiotelemetry			
	Baird Canyon	477	2003	156
	Baird Canyon	496	2004	127
	Total			283
	Baird Canyon Fish wheel			
	May 12 – July 14, 2005	1,382	2005	1,382
	Commercial Fishery (Cordova processors)			
	May 16 – June 14, 2005	1,612	2005	1,598

Note: Map numbers reference the collection locations shown in Figure 1.

[a] Not included in analysis of baseline.

Table 2.–Genetic markers assayed in Copper River Chinook salmon and the locus-specific observed number of alleles, range of frequencies of the most common allele, mean expected heterozygosity (H_E), mean observed heterozygosity (H_O), and genetic diversity (F_{ST}) values are given.

Locus	Reference	Observed Allele Number	Range of Most Common 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
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

-continued-

Table 2.–Page 2 of 2.

Locus	Reference	Observed Allele Number	Range of Most Common Allele			H_E	H_O	F_{ST}
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
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	0.68	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	Greig et al. 2003	23	0.007	-	0.296	0.898	0.878	0.026
Ots208b	Greig et al. 2003	34	0.008	-	0.25	0.915	0.919	0.023
Ots211	Greig et al. 2003	32	0.011	-	0.628	0.831	0.832	0.086
Ots212	Greig et al. 2003	22	0.256	-	0.522	0.803	0.811	0.031
Ots213	Greig et al. 2003	30	0.052	-	0.275	0.891	0.907	0.024
Ots3M	Greig and Banks 1999	5	0.317	-	0.599	0.612	0.598	0.03
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	0.34	0.336	0.05
Ssa408	Cairney et al. 2000	23	0.063	-	0.38	0.838	0.759	0.03
						Average		0.054

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

	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 River Mainstem	46	1.69	1.69	0.22	0.21	46	11.69	11.55	0.74	0.74
Gulkana River Middle Fork	76	1.69	1.67	0.20	0.20	77	13.69	12.06	0.73	0.74
Gulkana River 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

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	
Reporting Group	Mean	90% CI	Mean	90% CI
Upper Copper River	0.994	(0.982–1.000)	0.988	(0.976–0.998)
Gulkana River	0.955	(0.919–0.989)	0.973	(0.955–0.990)
Mendeltna Creek	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 River	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 (Debevec et al. 2000).

Table 5.–Mean correct allocations of simulated mixtures to individual population and to reporting region using the baseline of 45 SNPs and 13 microsatellite markers from Copper River Chinook salmon.

Collection	Population				Region			
	SNPs		Microsatellites		SNPs		Microsatellites	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Bone Creek	0.957	0.023	0.903	0.019	1.000	0.000	1.000	0.001
Otter Creek	0.952	0.024	0.982	0.010	0.997	0.004	1.000	0.001
Indian Creek	0.434	0.062	0.573	0.035	1.000	0.001	0.998	0.002
Chistochina River	0.891	0.042	0.929	0.019	0.999	0.001	0.999	0.002
Sinona Creek	0.998	0.004	0.995	0.005	1.000	0.001	1.000	0.000
Gulkana River Mainstem	0.115	0.047	0.254	0.030	0.967	0.017	0.997	0.003
Gulkana River Middle Fork	0.637	0.064	0.560	0.034	0.995	0.006	0.985	0.008
Gulkana River Paxson Fork	0.797	0.051	0.764	0.029	0.996	0.005	0.996	0.004
Mendeltna Creek	0.979	0.013	0.992	0.006	0.979	0.013	0.992	0.006
Kaina Creek	0.543	0.060	0.803	0.025	0.976	0.015	0.995	0.004
Manker Creek	0.491	0.053	0.528	0.031	0.974	0.016	0.997	0.003
Little Tonsina/Greyling	0.208	0.064	0.505	0.031	0.997	0.004	0.995	0.004
Tonsina Radio Tags	0.459	0.080	0.645	0.033	0.994	0.007	0.989	0.006
Tebay River	0.977	0.014	0.959	0.013	0.977	0.014	0.959	0.013

Note: Each set of mixtures (N=400) was created from a single population based on estimated allelic frequencies for the population. 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. Standard deviations of the mean estimates to population and region are also provided.

Table 6.–Relative proportion and 90% credibility intervals (CI) of reporting groups in collections of Chinook salmon sampled approximately weekly from the Baird Canyon fish wheel, Copper River, 2005.

			Region									
			Upper Copper River		Gulkana		Mendeltna		Lakes		Chitina	
Statistical Week	Dates	Sample size	Est	90% CI	Est	90% CI	Est	90% CI	Est	90% CI	Est	90% CI
20	(5/12 - 5/14)	65	0.404	(0.287-0.525)	0.430	(0.288-0.581)	0.018	(0.000-0.088)	0.095	(0.015-0.199)	0.054	(0.000-0.149)
21	(5/15 - 5/21)	243	0.359	(0.288-0.438)	0.485	(0.373-0.582)	0.035	(0.000-0.091)	0.106	(0.057-0.166)	0.015	(0.000-0.053)
22	(5/22 - 5/28)	265	0.293	(0.236-0.352)	0.571	(0.501-0.641)	0.007	(0.000-0.032)	0.115	(0.072-0.163)	0.014	(0.000-0.043)
23	(5/29 - 6/04)	274	0.147	(0.106-0.191)	0.440	(0.370-0.511)	0.018	(0.000-0.068)	0.375	(0.304-0.445)	0.020	(0.000-0.053)
24	(6/05 - 6/11)	234	0.073	(0.041-0.109)	0.287	(0.217-0.359)	0.038	(0.000-0.091)	0.545	(0.465-0.624)	0.058	(0.015-0.109)
25	(6/12 - 6/18)	193	0.006	(0.000-0.027)	0.215	(0.154-0.279)	0.044	(0.000-0.108)	0.655	(0.572-0.733)	0.080	(0.033-0.138)
26-29	(6/19 - 7/14)	107	0.003	(0.000-0.016)	0.033	(0.000-0.081)	0.014	(0.000-0.054)	0.869	(0.776-0.951)	0.081	(0.006-0.167)

Note: Analysis is based on 45 SNP loci using BAYES (Pella and Masuda 2001).

Table 7.–Mean correct assignment and 90% credibility intervals from 100% simulations (SPAM, Debevec et al. 2000) and estimated composition of 2 mixture samples from sport-caught Chinook salmon in the Copper River drainage (BAYES, Pella and Masuda, 2001).

	100% simulations		Klutina Guides		Gulkana Guides	
Region	Mean	90% CI	Estimate	90% CI	Estimate	90% CI
			N=169		N=130	
Upper Copper River	0.979	(0.964-0.991)	0.000	(0.000-0.001)	0.000	(0.000-0.000)
Gulkana River	0.950	(0.926-0.970)	0.000	(0.000-0.003)	0.991	(0.972-1.000)
Lower Copper	0.968	(0.951-0.983)	0.993	(0.978-1.000)	0.002	(0.000-0.011)
Other [a]			0.006	(0.000-0.020)	0.007	(0.000-0.021)

Note: These analyses were based on the 13 microsatellite loci and the combined Copper River and Coastwide Chinook salmon baselines.

[a] All non-Copper River populations combined.

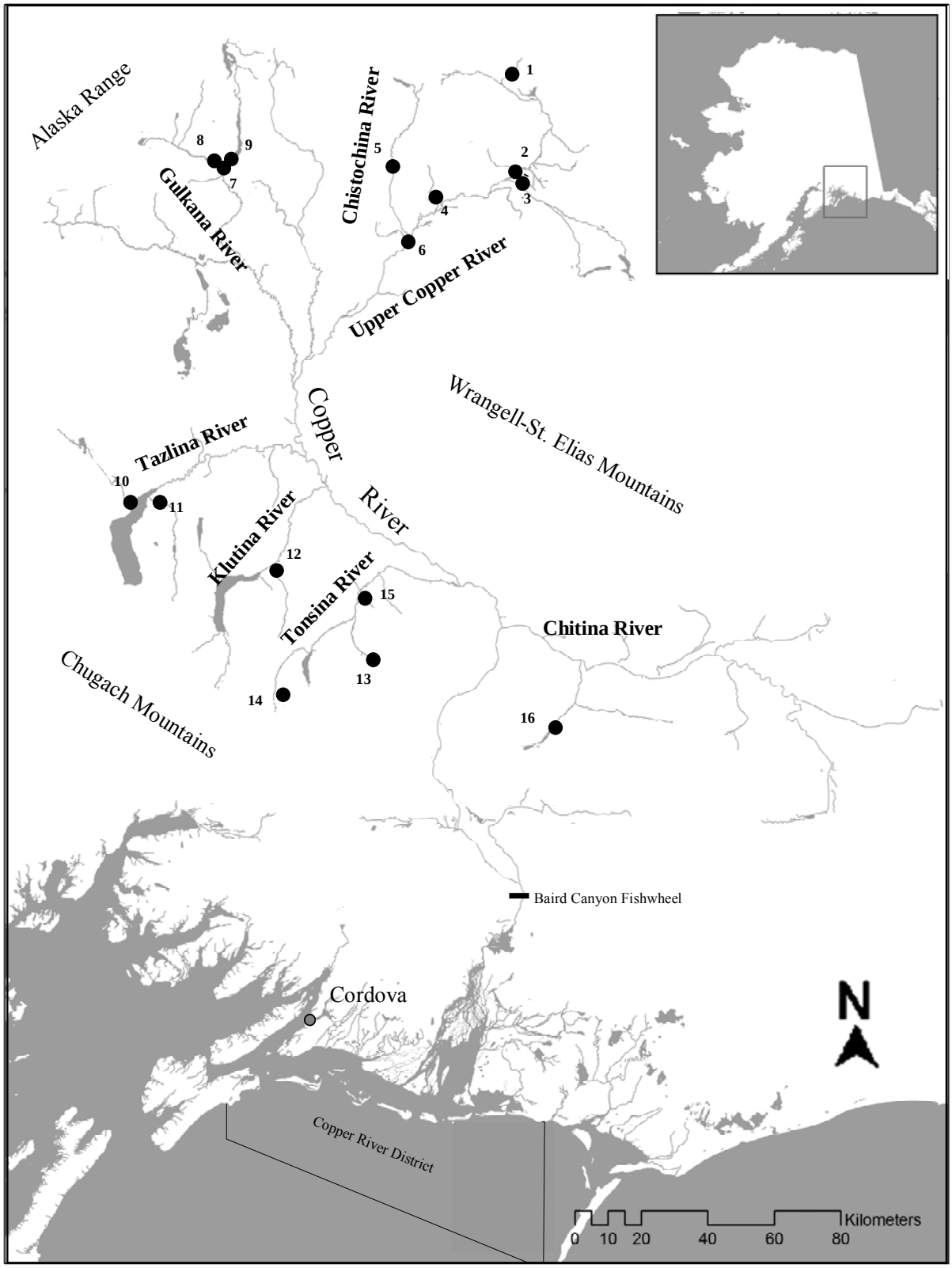
Table 8.–Collection dates and sizes of Chinook salmon sampled from the Copper River commercial harvest landed at processors in Cordova, Alaska, 2005.

Period	Dates	Sample size		Harvest
		Collected	Analyzed	
1	5/16-5/17	150	150	7,500
2	5/19-5/20	200	198	4,191
3	5/23-5/24	160	160	3,717
4	5/26-5/28	200	200	3,404
5	5/30-5/31	209	206	3,356
6	6/01-6/02	Not sampled	0	2,400
7	6/03-6/04	200	196	1,675
8	6/06-6/07	201	201	2,364
9	6/09-6/10	200	196	2,096
10	6/13-6/14	92	91	1,105
	Total	1,612	1,598	31,808

Table 9.–Estimated relative proportions (Est) and 90% credibility intervals (CI) of reporting regions for Chinook salmon sampled approximately weekly from the commercial gillnet fishery in the Copper River District, 2005.

		Region							
		Upper Copper River		Gulkana River		Lower Copper River		Other	
Period	Dates	Est	90% CI	Est	90% CI	Est	90% CI	Est	90% CI
1	5/16-5/17	0.371	(0.301-0.438)	0.356	(0.287-0.428)	0.253	(0.189-0.319)	0.020	(0.006-0.043)
2	5/19-5/20	0.286	(0.233-0.341)	0.407	(0.345-0.469)	0.289	(0.232-0.349)	0.019	(0.004-0.039)
3	5/23-5/24	0.220	(0.167-0.278)	0.389	(0.322-0.459)	0.353	(0.285-0.423)	0.038	(0.015-0.068)
4	5/26-5/28	0.117	(0.080-0.159)	0.414	(0.352-0.476)	0.455	(0.393-0.518)	0.015	(0.003-0.034)
5	5/30-5/31	0.013	(0.002-0.030)	0.203	(0.154-0.254)	0.740	(0.682-0.794)	0.045	(0.023-0.072)
6	6/01-6/02	Not sampled							
7	6/03-6/04	0.039	(0.018-0.066)	0.159	(0.116-0.208)	0.748	(0.692-0.801)	0.054	(0.030-0.084)
8	6/06-6/07	0.009	(0.000-0.026)	0.127	(0.085-0.174)	0.844	(0.794-0.890)	0.020	(0.007-0.038)
9	6/09-6/10	0.009	(0.000-0.025)	0.079	(0.047-0.118)	0.876	(0.830-0.916)	0.036	(0.017-0.061)
10	6/13-6/14	0.000	(0.000-0.000)	0.028	(0.002-0.066)	0.922	(0.866-0.965)	0.050	(0.018-0.094)

Note: Analysis is based on the 13 microsatellite loci and the combined Copper River and Coastwide Chinook salmon baselines using BAYES (Pella and Masuda 2001).



Note: Numbers on the map correspond to collection numbers in Table 1.

Figure 1.–Collection locations for genetic samples of Chinook salmon from the Copper River drainage, Alaska.

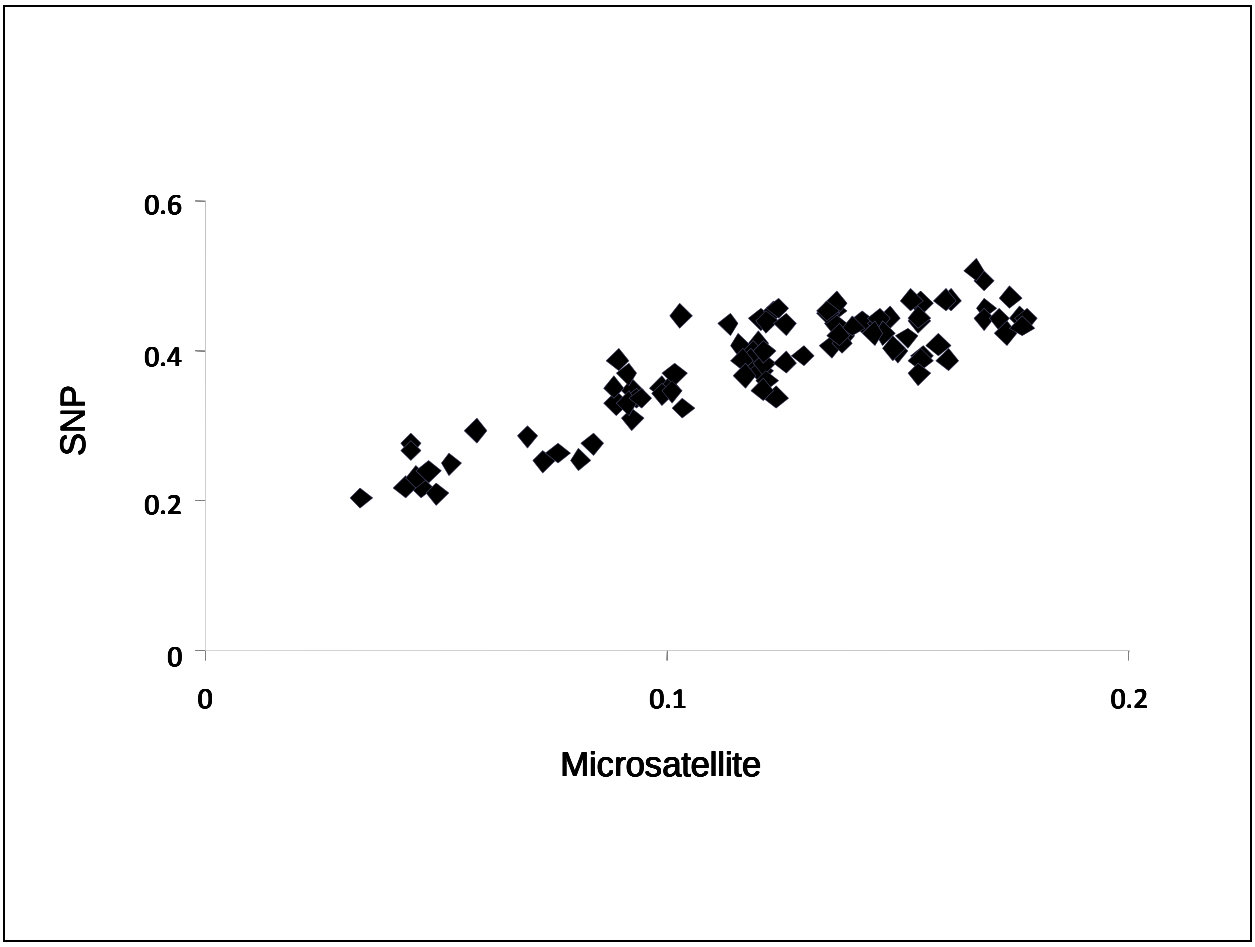
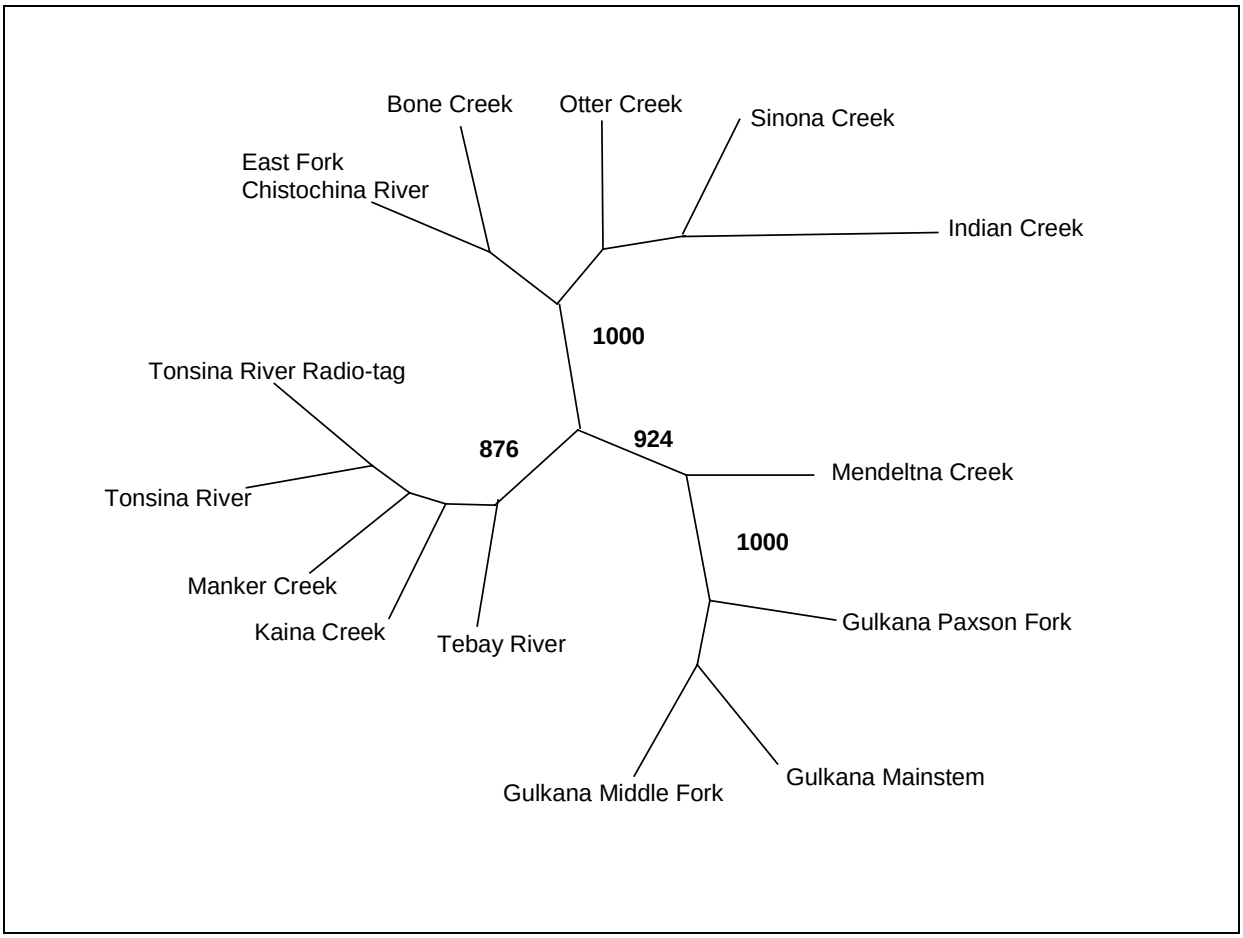
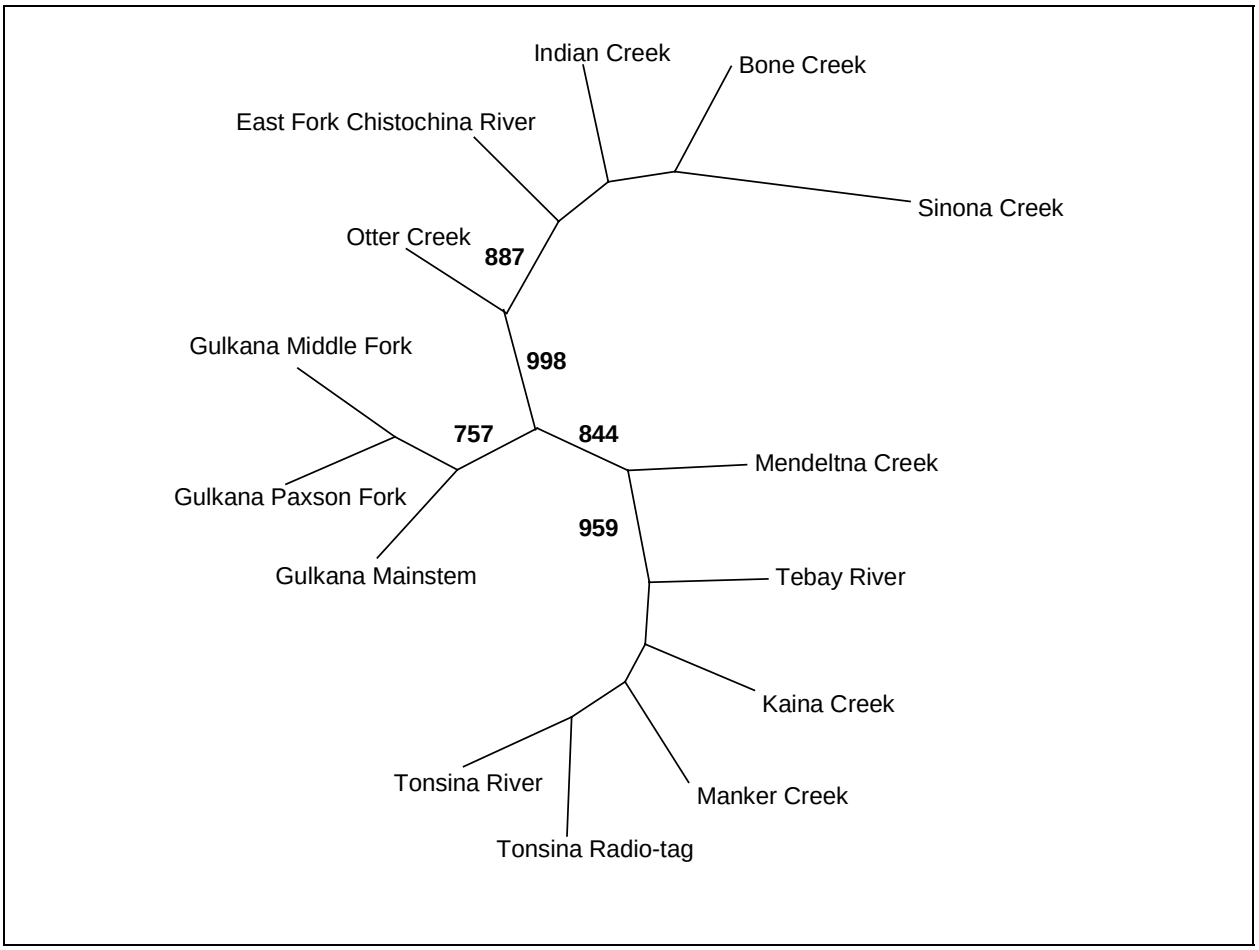


Figure 2.–Correlation between matrices of pairwise population chord distances based on allele frequencies at 45 SNPs and 13 microsatellites for Chinook salmon populations in the Copper River, Alaska.



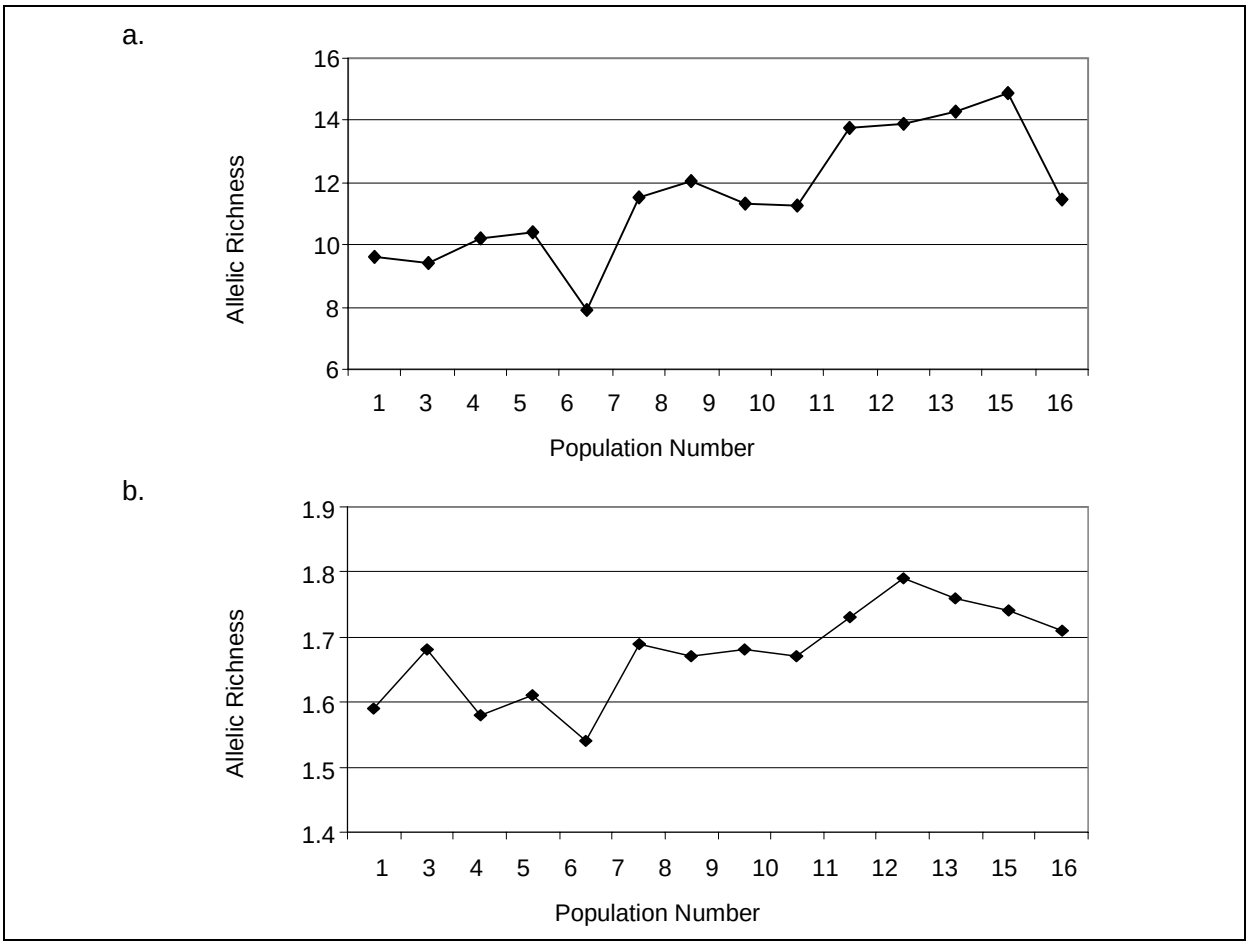
Note: The numbers at each major node indicate the number of times that all the populations on the branch below the node were grouped in each of 1000 bootstrap iterations of the tree.

Figure 3.–Neighbor-joining dendrogram based on genetic chord distances between Chinook salmon populations in the Copper River, Alaska, calculated from 13 microsatellite loci.



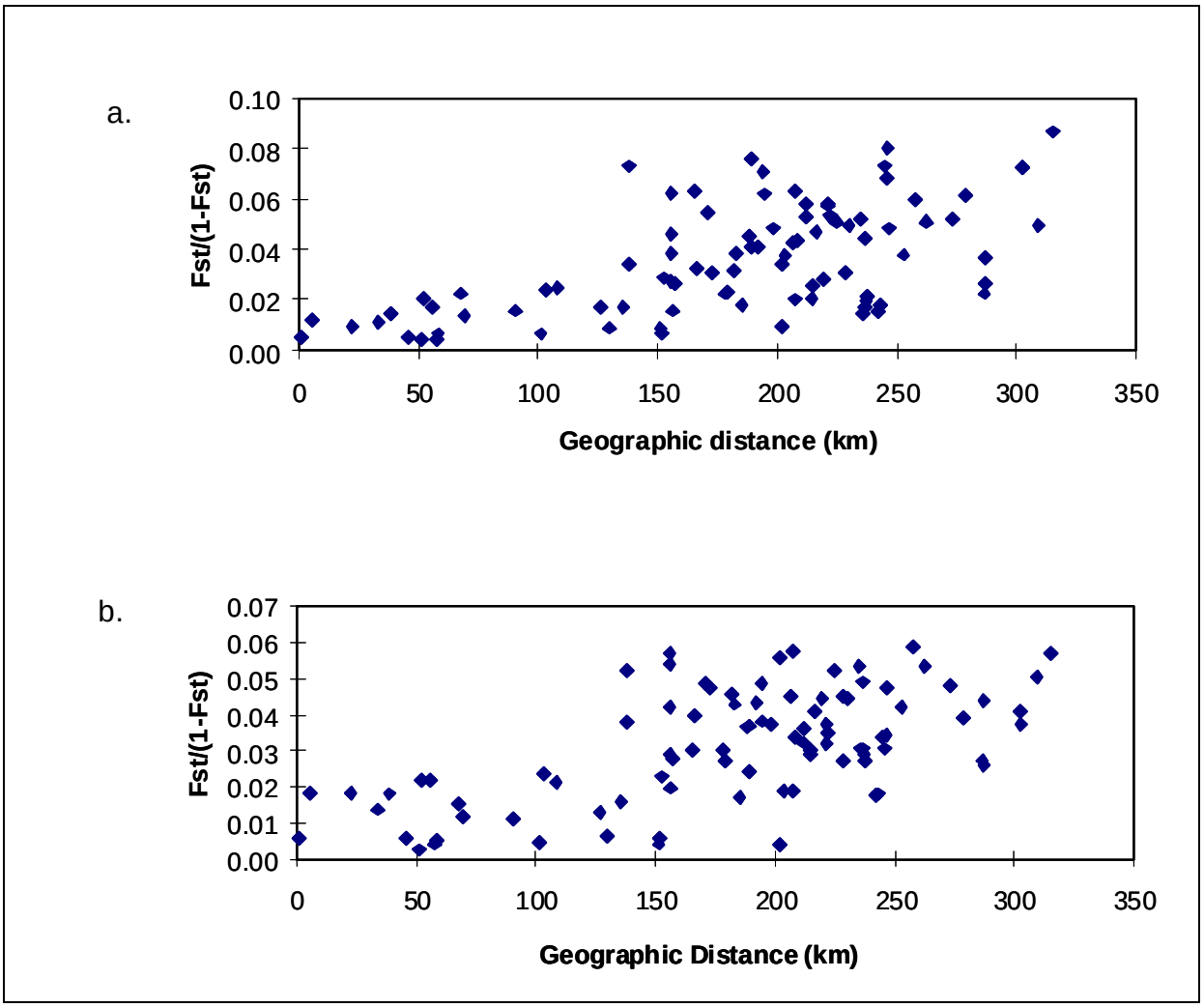
Note: The numbers at each major node indicate the number of times that all the populations on the branch below the node were grouped in each of 1000 bootstrap iterations of the tree.

Figure 4.–Neighbor-joining dendrogram based on genetic chord distances between Chinook salmon populations in the Copper River, Alaska, calculated from 45 SNP loci.



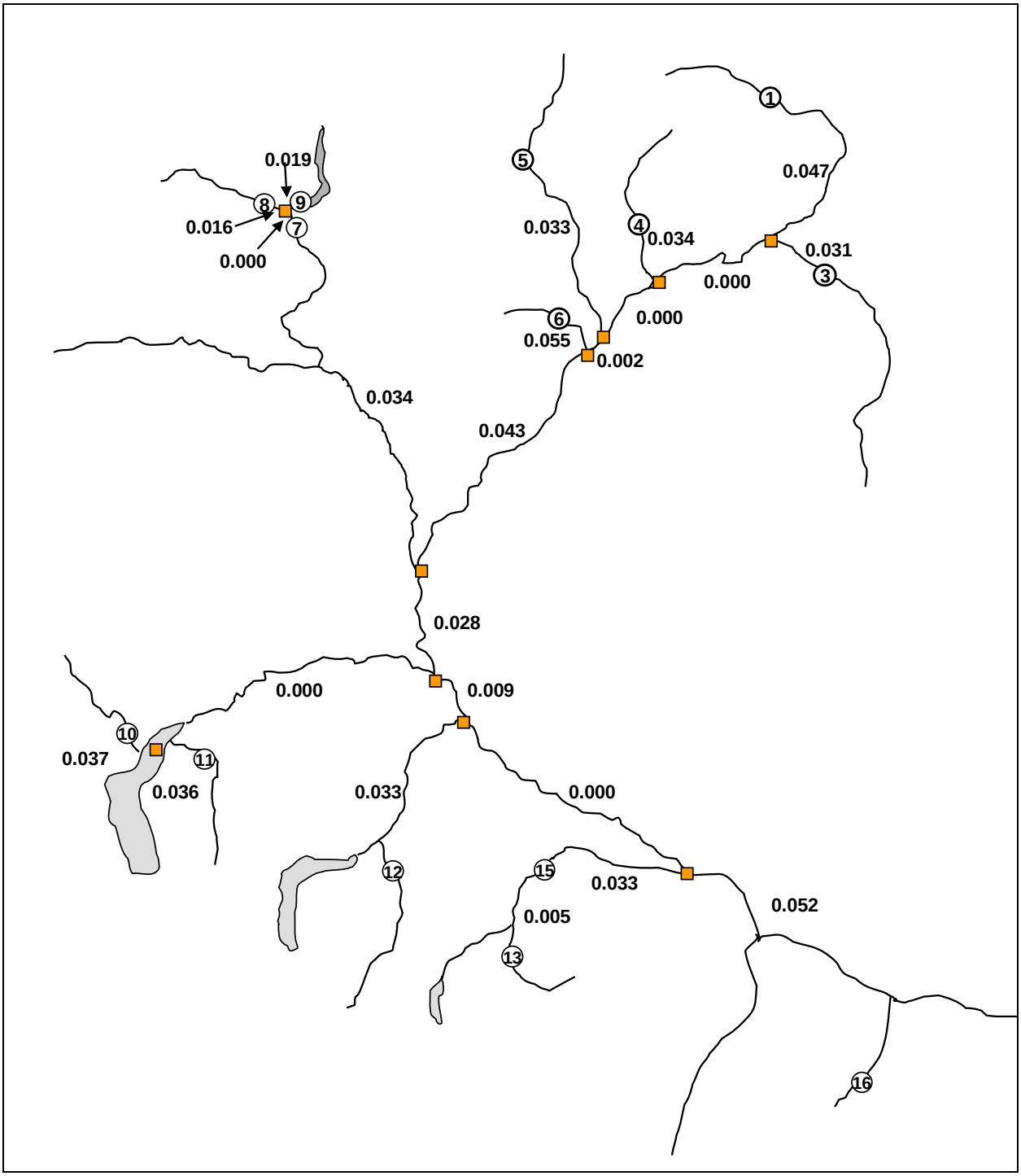
Note: Collection numbers correspond to the numbers in Table 1. Allelic richness values were calculated for a) 13 microsatellites and b) 45 SNPs.

Figure 5.–Allelic richness for Chinook salmon populations in the Copper River drainage.



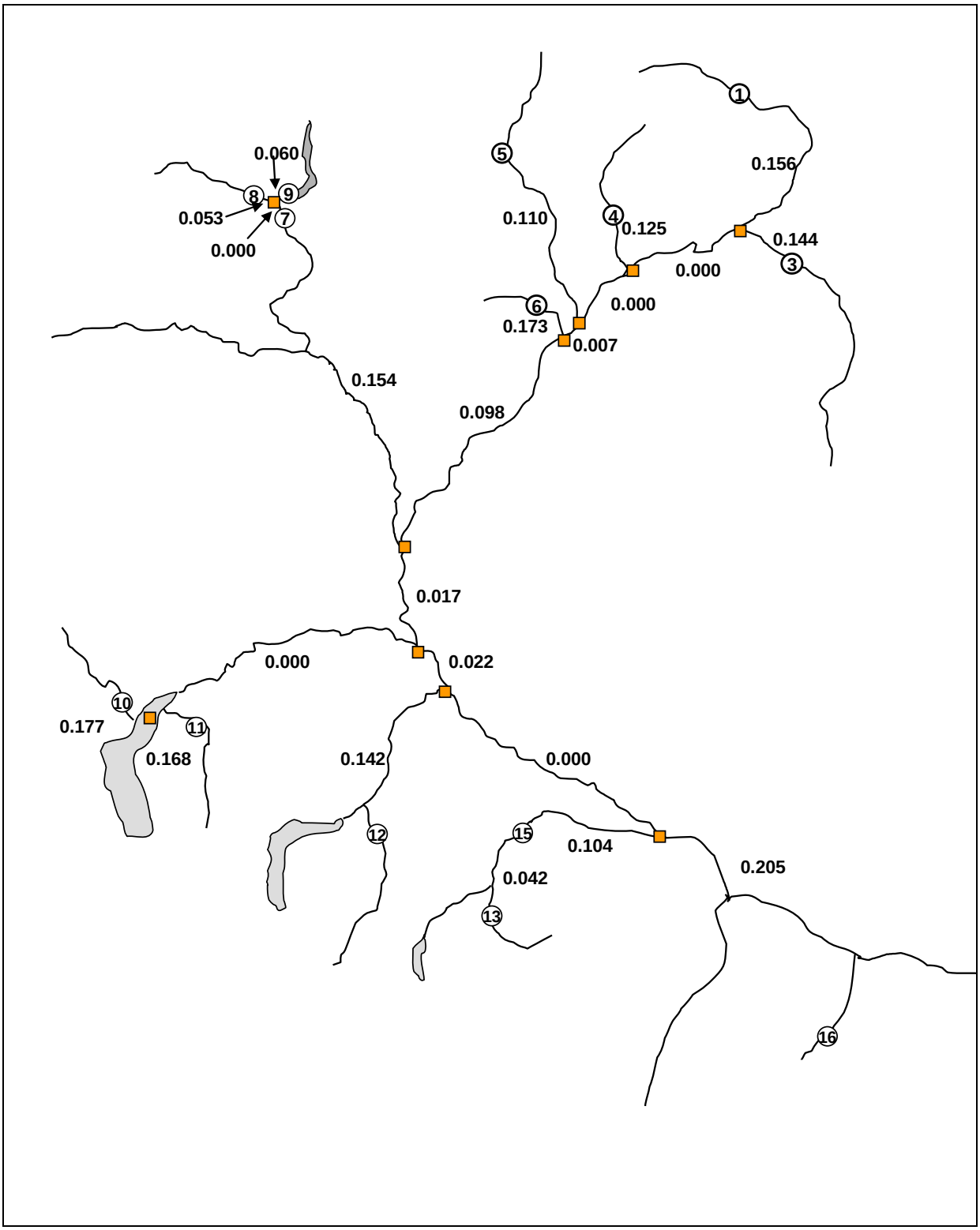
Note: Genetic distances for both microsatellites and SNPs show a significant (P<0.01) positive correlation with geographic distance.

Figure 6.–Isolation by distance relationships between populations of Chinook salmon in the Copper River, Alaska as measured using a) 13 microsatellites and b) 45 SNPs.



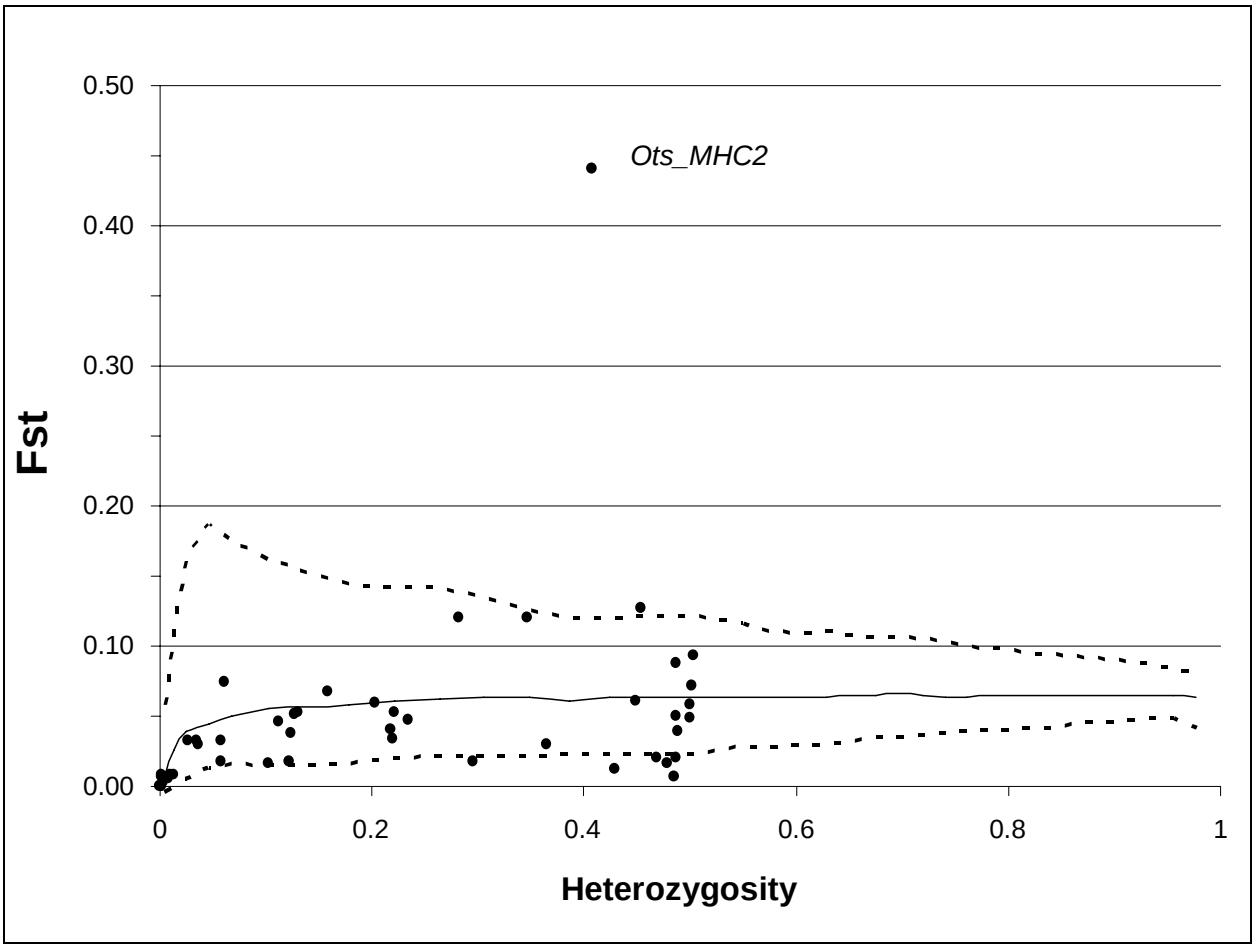
Note: Circles indicate sampling sites; squares are stream nodes; and numbers indicate the genetic distance associated with the section between nodes.

Figure 7.–Stream tree for Chinook salmon populations from the Copper River drainage, Alaska, based on genetic distances estimated from SNP markers.



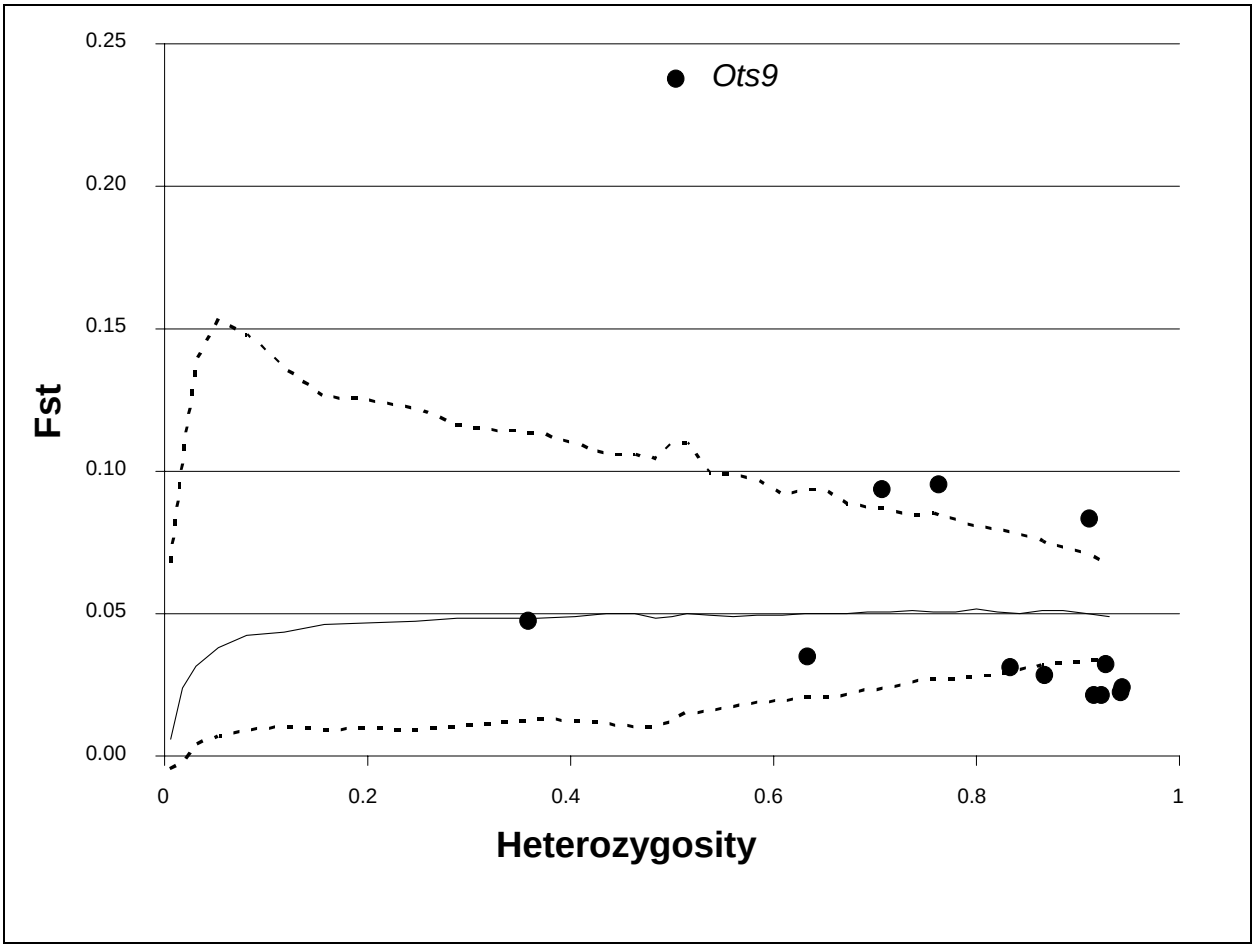
Note: Circles indicate sampling sites; squares are stream nodes; and numbers indicate the genetic distance associated with the section between nodes.

Figure 8.–Stream tree for Chinook salmon populations from the Copper River drainage, Alaska, based on genetic distances estimated from microsatellite markers.



Note: Dashed lines represent 0.025 and 0.975 quantiles.

Figure 9.–Locus-specific F_{ST} values estimated for 45 SNP loci plotted against heterozygosity for Chinook salmon from the Copper River, Alaska.



Note: Dashed lines represent 0.025 and 0.975 quantiles.

Figure 10.–Locus-specific F_{ST} values estimated for 13 microsatellite loci plotted against heterozygosity for Chinook salmon from the Copper River, Alaska.

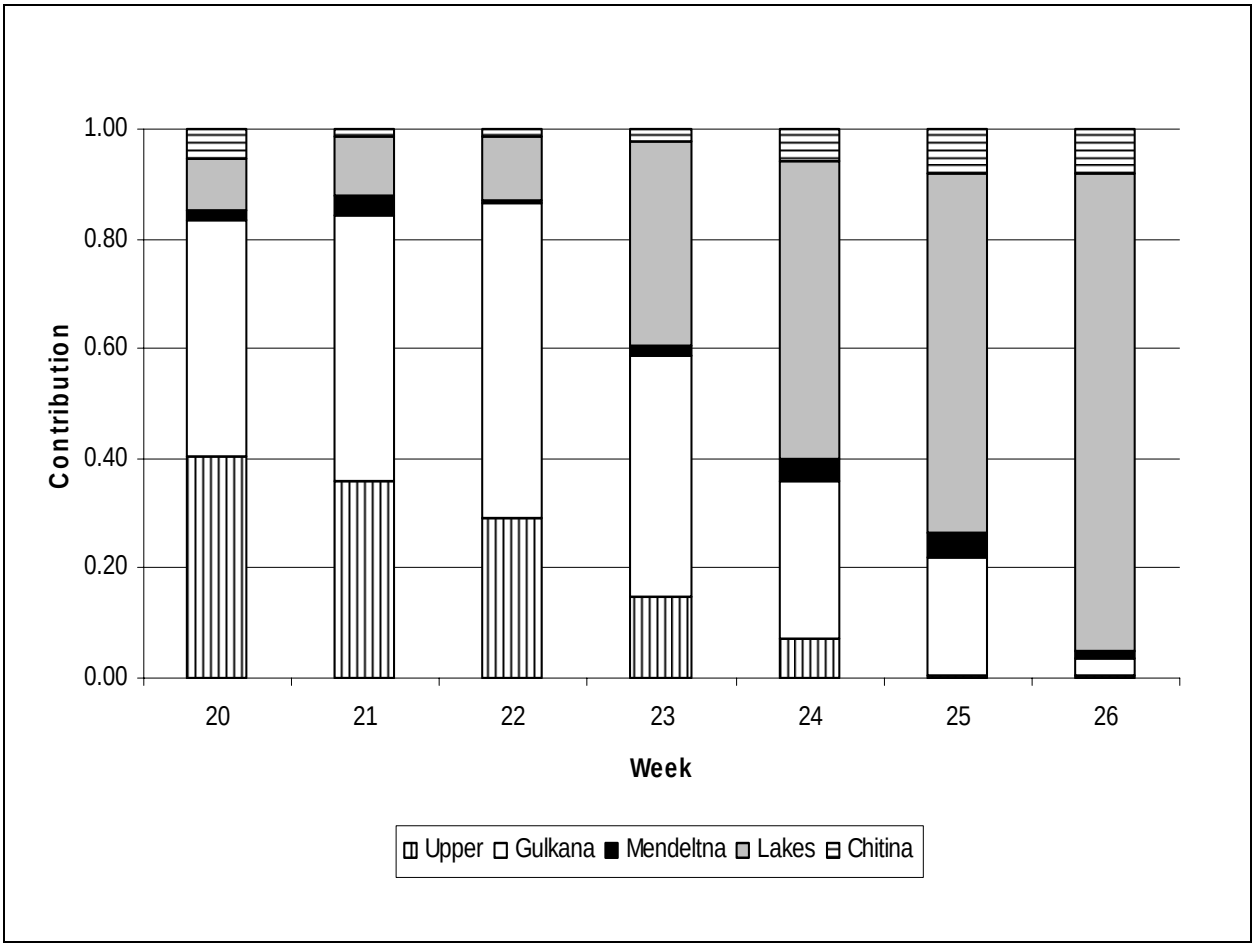


Figure 11.–Weekly stock composition of 5 regional groups of Chinook salmon in samples taken from fish wheels at Baird Canyon in the Copper River, Alaska, 2005.

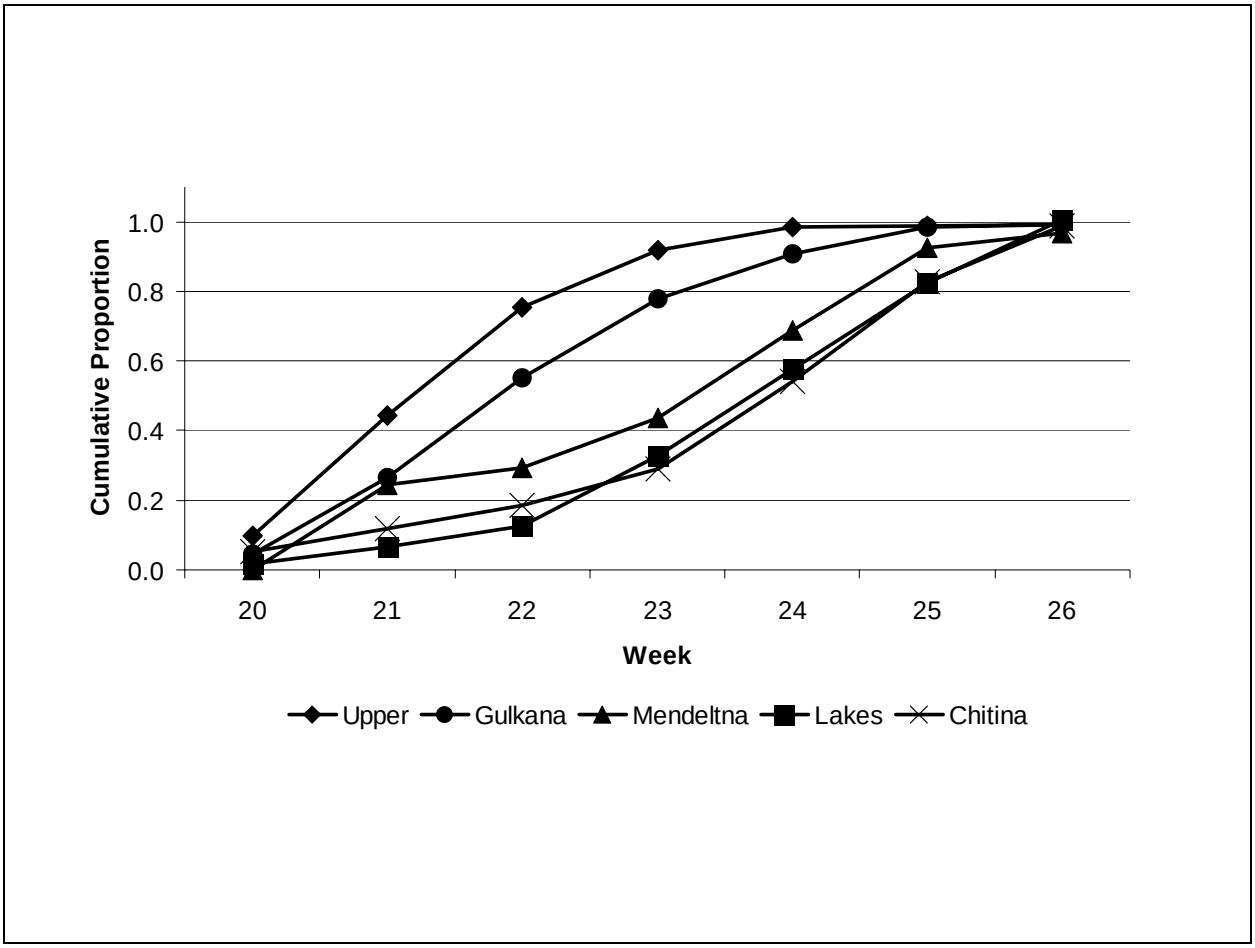


Figure 12.–Cumulative return for 5 regional groups of Chinook salmon estimated from samples taken at the fish wheels in Baird Canyon, Copper River, Alaska, 2005.

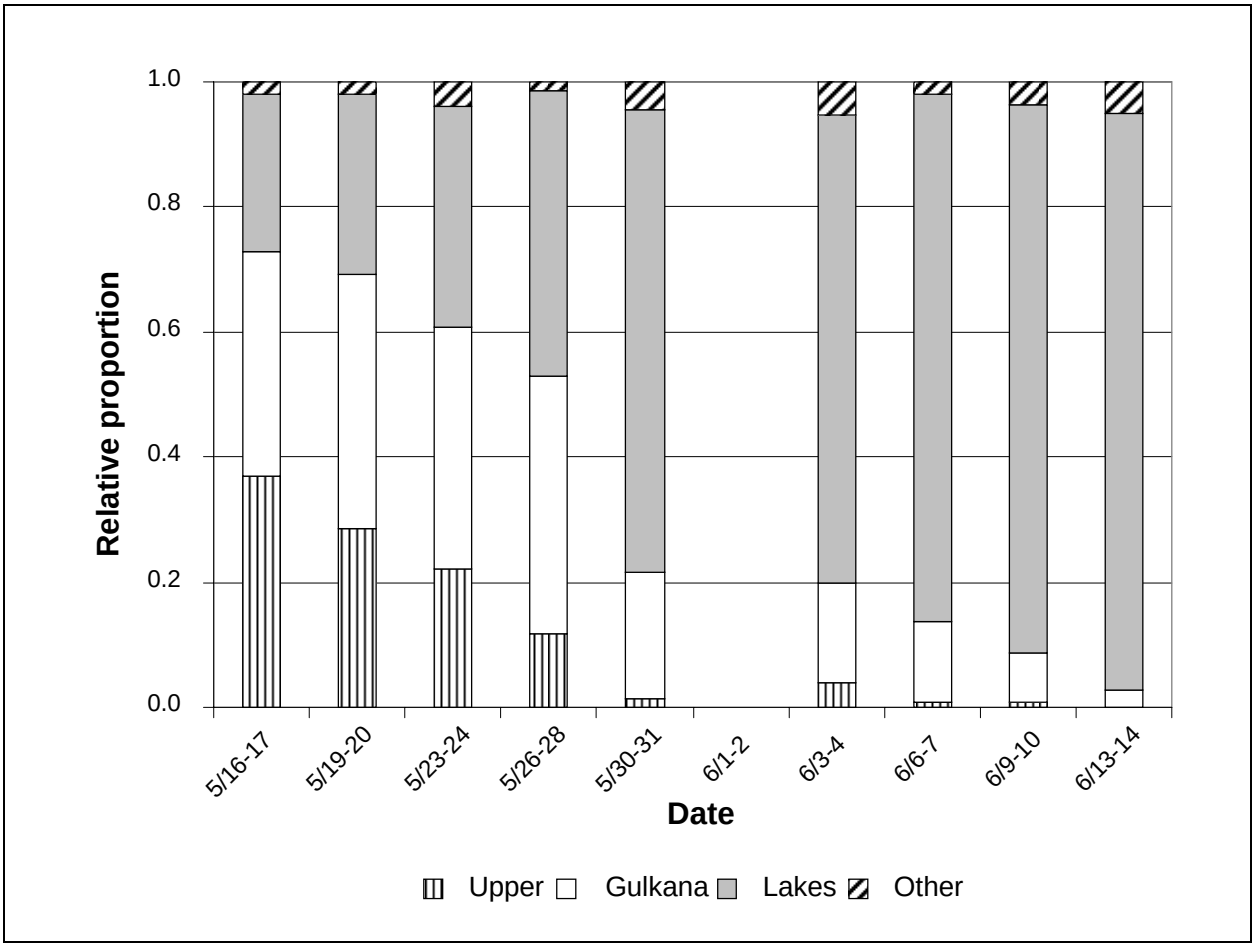


Figure 13.–Stock composition of bi-weekly samples of Chinook salmon from the commercial fishery in the Copper River District, Alaska, 2005.

APPENDICES

Appendix A.–Estimated relative allele frequencies for single nucleotide polymorphisms assayed in Chinook salmon populations in the Copper River drainage, Alaska.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots_arf-188	N	77	126	49	132	154	46	76	86	144	74	62	75	105	61
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ots_AsnRS-60	N	78	125	49	132	154	46	75	87	141	74	62	74	105	61
	1	0.481	0.260	0.327	0.265	0.377	0.413	0.487	0.425	0.525	0.649	0.492	0.628	0.629	0.689
	2	0.519	0.740	0.673	0.735	0.623	0.587	0.513	0.575	0.475	0.351	0.508	0.372	0.371	0.311
Ots_C3N3	N	78	126	49	132	155	46	75	88	144	74	61	75	106	60
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.924	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.076	0.000	0.000	0.000	0.000	0.000
Ots_ETIF1A	N	78	124	50	133	154	46	75	87	141	73	62	74	105	61
	1	0.872	0.802	0.770	0.846	0.896	0.739	0.647	0.753	0.592	0.795	0.758	0.716	0.748	0.721
	2	0.128	0.198	0.230	0.154	0.104	0.261	0.353	0.247	0.408	0.205	0.242	0.284	0.252	0.279
Ots_FARSLA-220	N	78	127	50	133	154	46	76	86	141	74	60	75	106	61
	1	0.077	0.256	0.120	0.117	0.250	0.098	0.086	0.064	0.082	0.128	0.158	0.133	0.160	0.025
	2	0.923	0.744	0.880	0.883	0.750	0.902	0.914	0.936	0.918	0.872	0.842	0.867	0.840	0.975
Ots_FGF6A	N	78	126	49	132	154	46	76	85	144	74	62	75	105	61
	1	0.000	0.028	0.122	0.080	0.019	0.261	0.237	0.229	0.267	0.439	0.323	0.320	0.314	0.434
	2	1.000	0.972	0.878	0.920	0.981	0.739	0.763	0.771	0.733	0.561	0.677	0.680	0.686	0.566
Ots_GH2	N	78	126	49	131	155	46	76	88	144	74	62	75	106	61
	1	0.712	0.667	0.541	0.611	0.548	0.707	0.671	0.705	0.441	0.655	0.605	0.620	0.585	0.697
	2	0.288	0.333	0.459	0.389	0.452	0.293	0.329	0.295	0.559	0.345	0.395	0.380	0.415	0.303
Ots_GnRH-271	N	78	125	49	129	151	46	75	87	144	72	60	75	100	60
	1	0.962	0.944	0.939	0.973	0.934	1.000	1.000	1.000	1.000	1.000	0.992	0.993	1.000	1.000
	2	0.038	0.056	0.061	0.027	0.066	0.000	0.000	0.000	0.000	0.000	0.008	0.007	0.000	0.000

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Appendix A.–Page 2 of 7.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots_GPDH-338	N	78	126	46	129	154	46	76	86	142	74	62	75	100	60
	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.016	0.027	0.015	0.025
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.986	0.984	0.973	0.985	0.975
Ots_GPH318	N	78	128	50	133	155	46	77	88	144	75	62	75	106	61
	1	0.577	0.723	0.670	0.680	0.681	0.935	0.916	0.972	0.885	0.920	0.919	0.940	0.892	0.934
	2	0.423	0.277	0.330	0.320	0.319	0.065	0.084	0.028	0.115	0.080	0.081	0.060	0.108	0.066
Ots_GST-207	N	78	128	50	133	155	46	77	88	144	75	61	74	106	61
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.955	0.927	0.959	0.959	0.953	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.073	0.041	0.041	0.047	0.000
Ots_GST-375	N	78	128	50	133	155	46	75	88	143	74	62	75	106	61
	1	1.000	1.000	1.000	1.000	1.000	1.000	0.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ots_GTH2B-550	N	78	124	49	133	147	45	74	87	144	75	62	74	106	61
	1	0.724	0.714	0.663	0.620	0.619	0.511	0.385	0.385	0.448	0.360	0.298	0.365	0.255	0.410
	2	0.276	0.286	0.337	0.380	0.381	0.489	0.615	0.615	0.552	0.640	0.702	0.635	0.745	0.590
Ots_HGFA-446	N	78	123	48	130	154	45	76	87	143	74	61	75	100	60
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ots_hnRNPL-533	N	78	128	50	133	155	46	77	88	143	75	62	75	106	61
	1	0.083	0.133	0.130	0.256	0.000	0.098	0.039	0.074	0.119	0.153	0.298	0.133	0.193	0.057
	2	0.917	0.867	0.870	0.744	1.000	0.902	0.961	0.926	0.881	0.847	0.702	0.867	0.807	0.943
Ots_HSP90B-100	N	78	128	50	133	155	46	75	88	144	75	62	75	106	60
	1	0.942	1.000	1.000	0.985	1.000	0.957	1.000	0.977	0.927	0.953	0.976	0.940	0.939	0.983
	2	0.058	0.000	0.000	0.015	0.000	0.043	0.000	0.023	0.073	0.047	0.024	0.060	0.061	0.017

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Appendix A.–Page 3 of 7.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots_IGF-I.1-76	N	78	125	49	132	154	46	75	87	144	74	62	75	105	61
	1	0.397	0.520	0.265	0.235	0.406	0.609	0.593	0.626	0.542	0.547	0.548	0.500	0.476	0.320
	2	0.603	0.480	0.735	0.765	0.594	0.391	0.407	0.374	0.458	0.453	0.452	0.500	0.524	0.680
Ots_Ikaros-250	N	78	126	49	132	155	46	76	88	143	74	60	75	105	61
	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.007	0.000	0.000
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.992	0.993	1.000	1.000
Ots_il-1racp-166	N	78	125	49	131	155	45	73	86	144	74	62	75	104	60
	1	0.250	0.284	0.245	0.244	0.142	0.422	0.425	0.436	0.573	0.514	0.589	0.467	0.514	0.617
	2	0.750	0.716	0.755	0.756	0.858	0.578	0.575	0.564	0.427	0.486	0.411	0.533	0.486	0.383
Ots_LEI-292	N	78	128	50	132	155	46	77	88	144	75	61	75	107	61
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ots_MetA	N	78	127	49	133	153	46	77	88	144	75	62	75	106	61
	1	0.000	0.008	0.000	0.000	0.007	0.065	0.084	0.102	0.003	0.020	0.032	0.027	0.052	0.016
	2	1.000	0.992	1.000	1.000	0.993	0.935	0.916	0.898	0.997	0.980	0.968	0.973	0.948	0.984
Ots_MHC1	N	77	125	48	132	155	46	76	88	144	74	62	74	106	61
	1	0.240	0.188	0.281	0.250	0.413	0.196	0.204	0.148	0.205	0.486	0.468	0.446	0.495	0.754
	2	0.760	0.812	0.719	0.750	0.587	0.804	0.796	0.852	0.795	0.514	0.532	0.554	0.505	0.246
Ots_MHC2	N	78	126	49	132	155	45	74	88	142	74	61	75	106	61
	1	0.000	0.036	0.000	0.004	0.000	0.078	0.081	0.131	0.250	0.601	0.648	0.767	0.722	0.459
	2	1.000	0.964	1.000	0.996	1.000	0.922	0.919	0.869	0.750	0.399	0.352	0.233	0.278	0.541

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Appendix A.–Page 4 of 7.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots_NOD1	N	78	127	50	133	155	46	77	88	144	75	62	75	107	61
	1	0.372	0.173	0.320	0.222	0.313	0.359	0.377	0.381	0.340	0.300	0.282	0.287	0.248	0.369
	2	0.628	0.827	0.680	0.778	0.687	0.641	0.623	0.619	0.660	0.700	0.718	0.713	0.752	0.631
Ots_ZNF330-181	N	78	124	49	131	155	45	76	86	144	74	62	75	100	60
	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.984	1.000	1.000	1.000
Ots_LWSop-638	N	78	126	49	132	155	46	76	88	144	74	61	74	105	61
	1	0.000	0.036	0.000	0.004	0.000	0.163	0.171	0.142	0.069	0.081	0.066	0.095	0.124	0.025
	2	1.000	0.964	1.000	0.996	1.000	0.837	0.829	0.858	0.931	0.919	0.934	0.905	0.876	0.975
Ots_SWS1op-182	N	78	125	48	131	155	46	74	87	143	73	62	75	105	61
	1	0.590	0.672	0.656	0.687	0.581	0.565	0.547	0.609	0.514	0.555	0.516	0.560	0.533	0.648
	2	0.410	0.328	0.344	0.313	0.419	0.435	0.453	0.391	0.486	0.445	0.484	0.440	0.467	0.352
Ots_Ots2	N	77	126	49	132	155	39	69	75	144	74	60	75	104	58
	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.008	0.020	0.019	0.009
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.993	0.992	0.980	0.981	0.991
Ots_P450	N	78	126	49	131	154	46	76	87	144	74	62	75	100	59
	1	0.314	0.310	0.418	0.370	0.302	0.402	0.349	0.437	0.455	0.439	0.484	0.473	0.525	0.551
	2	0.686	0.690	0.582	0.630	0.698	0.598	0.651	0.563	0.545	0.561	0.516	0.527	0.475	0.449
Ots_P53	N	77	126	49	132	155	46	76	87	140	74	61	74	105	61
	1	0.565	0.397	0.469	0.390	0.252	0.522	0.513	0.661	0.379	0.338	0.311	0.392	0.357	0.262
	2	0.435	0.603	0.531	0.610	0.748	0.478	0.487	0.339	0.621	0.662	0.689	0.608	0.643	0.738
PGK54	N	78	128	49	131	155	46	77	88	144	75	62	75	107	61
	1	0.122	0.070	0.082	0.088	0.210	0.098	0.071	0.051	0.156	0.060	0.113	0.040	0.084	0.352
	2	0.878	0.930	0.918	0.912	0.790	0.902	0.929	0.949	0.844	0.940	0.887	0.960	0.916	0.648

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Appendix A.–Page 5 of 7.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots_Prl2	N	78	126	49	131	155	46	75	87	143	73	62	75	105	61
	1	0.160	0.183	0.153	0.183	0.277	0.446	0.500	0.443	0.458	0.370	0.371	0.400	0.424	0.361
	2	0.840	0.817	0.847	0.817	0.723	0.554	0.500	0.557	0.542	0.630	0.629	0.600	0.576	0.639
Ots_ins-115	N	78	125	49	132	155	46	76	88	144	74	62	75	105	61
	1	0.891	0.932	0.837	0.833	0.923	0.946	0.941	0.994	0.997	0.993	0.976	0.973	0.971	0.967
	2	0.109	0.068	0.163	0.167	0.077	0.054	0.059	0.006	0.003	0.007	0.024	0.027	0.029	0.033
Ots_PSMB1-197	N	78	128	50	133	155	46	76	87	140	74	61	75	106	58
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ots_RAG3	N	78	128	50	133	155	46	77	88	144	75	62	75	107	61
	1	0.000	0.125	0.000	0.000	0.000	0.141	0.143	0.102	0.170	0.040	0.081	0.053	0.056	0.033
	2	1.000	0.875	1.000	1.000	1.000	0.859	0.857	0.898	0.830	0.960	0.919	0.947	0.944	0.967
Ots_RFC2-558	N	77	124	49	128	155	46	74	87	142	69	60	75	99	60
	1	1.000	1.000	1.000	0.996	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ots_S7-1	N	78	127	50	132	155	46	74	88	142	74	61	75	106	61
	1	0.506	0.488	0.550	0.466	0.655	0.598	0.709	0.659	0.577	0.419	0.508	0.547	0.476	0.238
	2	0.494	0.512	0.450	0.534	0.345	0.402	0.291	0.341	0.423	0.581	0.492	0.453	0.524	0.762
Ots_SClkF2R2-135	N	78	126	49	131	155	46	76	88	143	74	61	75	104	60
	1	0.462	0.385	0.418	0.393	0.284	0.413	0.461	0.557	0.434	0.392	0.352	0.380	0.356	0.258
	2	0.538	0.615	0.582	0.607	0.716	0.587	0.539	0.443	0.566	0.608	0.648	0.620	0.644	0.742
Ots_SERPC1-209	N	78	128	50	133	155	45	77	88	141	75	62	75	107	61
	1	0.096	0.133	0.020	0.113	0.084	0.056	0.032	0.034	0.099	0.007	0.089	0.047	0.056	0.049
	2	0.904	0.867	0.980	0.887	0.916	0.944	0.968	0.966	0.901	0.993	0.911	0.953	0.944	0.951

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Appendix A.–Page 6 of 7.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots_SL	N	77	126	48	132	155	46	75	88	144	73	62	75	106	58
	1	0.532	0.484	0.490	0.557	0.374	0.489	0.547	0.608	0.684	0.685	0.613	0.733	0.717	0.655
	2	0.468	0.516	0.510	0.443	0.626	0.511	0.453	0.392	0.316	0.315	0.387	0.267	0.283	0.345
Ots_TAPBP	N	78	128	50	132	155	46	77	87	143	75	61	75	106	61
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.987	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
Ots_Tnsf	N	78	124	49	129	152	45	76	88	142	73	61	74	105	60
	1	0.147	0.198	0.143	0.198	0.122	0.167	0.105	0.222	0.254	0.233	0.246	0.189	0.233	0.058
	2	0.853	0.802	0.857	0.802	0.878	0.833	0.895	0.778	0.746	0.767	0.754	0.811	0.767	0.942
Ots_u202-161	N	78	125	48	132	154	46	75	86	144	74	61	75	105	61
	1	0.276	0.224	0.156	0.087	0.049	0.033	0.047	0.035	0.215	0.155	0.213	0.113	0.119	0.172
	2	0.724	0.776	0.844	0.913	0.951	0.967	0.953	0.965	0.785	0.845	0.787	0.887	0.881	0.828
Ots_u211-85	N	78	125	49	132	155	46	76	88	143	74	61	75	106	61
	1	0.994	0.992	1.000	1.000	1.000	1.000	1.000	0.989	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.006	0.008	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000
Ots_U212-158	N	76	125	49	130	155	46	75	87	144	74	61	75	101	60
	1	0.000	0.008	0.000	0.000	0.000	0.109	0.087	0.121	0.108	0.000	0.008	0.000	0.000	0.000
	2	1.000	0.992	1.000	1.000	1.000	0.891	0.913	0.879	0.892	1.000	0.992	1.000	1.000	1.000
Ots_u4-92	N	78	125	49	131	155	46	76	88	144	74	61	74	100	61
	1	0.013	0.104	0.061	0.027	0.026	0.141	0.059	0.063	0.021	0.061	0.033	0.068	0.050	0.033
	2	0.987	0.896	0.939	0.973	0.974	0.859	0.941	0.938	0.979	0.939	0.967	0.932	0.950	0.967

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Appendix A.–Page 7 of 7.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots_u6-75	N	77	125	48	132	155	46	75	88	144	74	61	75	105	61
	1	0.909	0.944	0.938	0.966	0.990	0.978	0.993	0.972	0.906	0.932	0.795	0.893	0.905	0.959
	2	0.091	0.056	0.063	0.034	0.010	0.022	0.007	0.028	0.094	0.068	0.205	0.107	0.095	0.041
Ots_E2-275	N	78	125	49	131	155	46	76	88	144	74	61	74	106	61
	1	0.962	0.828	0.939	0.905	0.997	0.880	0.908	0.875	0.854	0.784	0.770	0.770	0.858	0.943
	2	0.038	0.172	0.061	0.095	0.003	0.120	0.092	0.125	0.146	0.216	0.230	0.230	0.142	0.057
Ots_Zp3b-215	N	76	126	48	132	155	46	75	87	144	74	60	74	105	61
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.953	0.983	0.980	0.971	0.926
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.017	0.020	0.029	0.074

Note: N = number of individuals analyzed to estimate allele frequencies.

Appendix B.–Estimated relative allele frequencies for the GAPS microsatellite loci assayed in Chinook salmon populations in the Copper River drainage, Alaska.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ogo2	N	78	128	50	131	156	46	77	86	142	72	61	75	106	68
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.041	0.027	0.019	0.000
	214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
	216	0.506	0.641	0.560	0.649	0.455	0.152	0.240	0.244	0.197	0.250	0.254	0.400	0.415	0.699
	218	0.122	0.098	0.110	0.183	0.250	0.098	0.104	0.070	0.021	0.028	0.025	0.067	0.033	0.022
	220	0.205	0.125	0.210	0.095	0.135	0.554	0.468	0.523	0.401	0.375	0.336	0.260	0.288	0.096
	222	0.141	0.133	0.100	0.061	0.160	0.185	0.188	0.163	0.349	0.326	0.279	0.193	0.212	0.176
	224	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	226	0.026	0.004	0.020	0.011	0.000	0.000	0.000	0.000	0.004	0.007	0.057	0.013	0.009	0.007
	228	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.014	0.008	0.040	0.019	0.000
Ogo4	N	78	127	50	132	156	46	76	88	143	74	61	75	106	68
	136	0.103	0.461	0.390	0.273	0.471	0.152	0.151	0.068	0.273	0.196	0.410	0.300	0.245	0.566
	140	0.006	0.150	0.030	0.057	0.090	0.163	0.204	0.091	0.192	0.108	0.057	0.160	0.108	0.162
	144	0.000	0.000	0.000	0.000	0.000	0.033	0.007	0.017	0.000	0.000	0.008	0.007	0.014	0.000
	150	0.051	0.000	0.010	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	154	0.000	0.004	0.000	0.000	0.000	0.087	0.072	0.085	0.052	0.149	0.107	0.120	0.132	0.037
	156	0.038	0.004	0.080	0.034	0.006	0.011	0.007	0.017	0.000	0.000	0.016	0.007	0.014	0.000
	158	0.000	0.063	0.000	0.011	0.000	0.022	0.092	0.068	0.007	0.041	0.016	0.027	0.057	0.088
	160	0.545	0.157	0.200	0.261	0.157	0.511	0.428	0.642	0.476	0.480	0.377	0.347	0.387	0.118
	162	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.008	0.027	0.024	0.000
	164	0.256	0.161	0.280	0.352	0.276	0.022	0.039	0.011	0.000	0.007	0.000	0.007	0.014	0.029
	168	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000

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Appendix B.–Page 2 of 11.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Oki100	N	77	128	46	131	155	46	76	88	140	74	59	75	102	68
	208	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.006	0.000	0.000	0.000	0.007	0.000	0.000
	216	0.013	0.000	0.000	0.008	0.000	0.011	0.000	0.011	0.000	0.000	0.000	0.013	0.000	0.000
	220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.007	0.025	0.007	0.015	0.051
	224	0.045	0.000	0.000	0.008	0.000	0.000	0.013	0.000	0.004	0.014	0.000	0.000	0.005	0.000
	228	0.039	0.008	0.011	0.008	0.000	0.022	0.007	0.040	0.011	0.007	0.017	0.000	0.020	0.022
	232	0.078	0.082	0.109	0.038	0.068	0.109	0.099	0.068	0.104	0.000	0.042	0.020	0.034	0.007
	236	0.136	0.000	0.022	0.046	0.035	0.043	0.026	0.068	0.132	0.041	0.008	0.033	0.010	0.044
	240	0.000	0.059	0.054	0.034	0.087	0.054	0.053	0.023	0.021	0.223	0.102	0.067	0.044	0.015
	244	0.084	0.129	0.141	0.187	0.200	0.011	0.013	0.011	0.093	0.027	0.068	0.087	0.069	0.103
	248	0.084	0.121	0.098	0.092	0.019	0.141	0.066	0.125	0.111	0.101	0.186	0.060	0.137	0.162
	252	0.110	0.313	0.163	0.248	0.265	0.109	0.118	0.199	0.082	0.115	0.051	0.093	0.088	0.022
	256	0.214	0.043	0.076	0.084	0.087	0.065	0.092	0.102	0.286	0.088	0.076	0.120	0.118	0.059
	260	0.013	0.023	0.033	0.050	0.116	0.185	0.178	0.097	0.029	0.027	0.034	0.100	0.088	0.088
	264	0.071	0.039	0.120	0.103	0.013	0.033	0.046	0.034	0.007	0.027	0.076	0.033	0.059	0.066
	268	0.032	0.152	0.098	0.057	0.016	0.054	0.066	0.068	0.011	0.027	0.025	0.040	0.020	0.015
	272	0.019	0.008	0.033	0.015	0.032	0.011	0.013	0.028	0.014	0.007	0.017	0.047	0.049	0.125
	275	0.006	0.000	0.000	0.004	0.000	0.065	0.053	0.006	0.021	0.000	0.042	0.033	0.020	0.007
	279	0.013	0.000	0.022	0.015	0.000	0.033	0.039	0.063	0.025	0.061	0.017	0.013	0.034	0.022
	283	0.000	0.000	0.000	0.000	0.000	0.033	0.013	0.011	0.000	0.034	0.042	0.040	0.020	0.162
	287	0.000	0.008	0.000	0.000	0.000	0.000	0.007	0.000	0.021	0.047	0.008	0.073	0.025	0.022
	290	0.000	0.016	0.000	0.000	0.000	0.000	0.013	0.000	0.007	0.007	0.017	0.013	0.064	0.000
	294	0.006	0.000	0.000	0.000	0.010	0.000	0.007	0.000	0.004	0.068	0.085	0.053	0.034	0.000
	298	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.007	0.025	0.000
	302	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000
	305	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.020	0.025	0.013	0.005	0.007
	309	0.000	0.000	0.000	0.000	0.000	0.022	0.007	0.000	0.014	0.000	0.008	0.000	0.010	0.000
	313	0.032	0.000	0.022	0.004	0.052	0.000	0.039	0.017	0.000	0.047	0.000	0.000	0.005	0.000
	317	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.006	0.000	0.007	0.025	0.013	0.000	0.000
	321	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000

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Appendix B.–Page 3 of 11.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Omm1080	N	78	127	47	130	155	46	77	88	141	74	59	73	98	68
	186	0.051	0.000	0.000	0.035	0.000	0.011	0.026	0.000	0.000	0.000	0.034	0.014	0.005	0.000
	190	0.000	0.020	0.032	0.023	0.077	0.000	0.000	0.000	0.004	0.081	0.110	0.048	0.036	0.051
	194	0.122	0.185	0.149	0.146	0.100	0.217	0.227	0.295	0.298	0.196	0.051	0.212	0.133	0.132
	198	0.045	0.043	0.000	0.015	0.061	0.109	0.065	0.080	0.074	0.027	0.017	0.055	0.026	0.029
	202	0.045	0.008	0.000	0.038	0.000	0.000	0.006	0.000	0.004	0.020	0.000	0.014	0.020	0.007
	206	0.000	0.000	0.000	0.008	0.000	0.000	0.006	0.000	0.004	0.020	0.034	0.000	0.005	0.000
	210	0.173	0.091	0.096	0.115	0.039	0.033	0.026	0.051	0.025	0.020	0.051	0.048	0.041	0.088
	214	0.032	0.094	0.064	0.054	0.200	0.022	0.019	0.006	0.000	0.007	0.000	0.014	0.036	0.007
	218	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.054	0.017	0.034	0.046	0.132
	222	0.006	0.000	0.000	0.004	0.000	0.043	0.032	0.045	0.082	0.000	0.000	0.000	0.005	0.007
	226	0.000	0.000	0.000	0.000	0.003	0.011	0.039	0.068	0.011	0.000	0.000	0.000	0.026	0.000
	230	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.060	0.000	0.000	0.000	0.000	0.000
	234	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.011	0.014	0.000	0.000	0.000	0.000	0.000
	238	0.083	0.051	0.032	0.012	0.000	0.000	0.006	0.006	0.000	0.000	0.000	0.000	0.005	0.000
	242	0.006	0.000	0.000	0.000	0.068	0.000	0.000	0.000	0.000	0.014	0.000	0.007	0.020	0.022
	246	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.051	0.007	0.015	0.110
	250	0.000	0.016	0.021	0.004	0.000	0.011	0.000	0.011	0.000	0.020	0.008	0.000	0.005	0.051
	254	0.000	0.004	0.011	0.004	0.032	0.000	0.000	0.000	0.060	0.047	0.025	0.014	0.015	0.000
	258	0.147	0.032	0.106	0.027	0.042	0.054	0.039	0.028	0.046	0.007	0.085	0.068	0.031	0.015
	262	0.045	0.083	0.106	0.100	0.032	0.011	0.006	0.000	0.007	0.020	0.017	0.055	0.020	0.022
	266	0.077	0.094	0.064	0.073	0.023	0.011	0.000	0.000	0.000	0.020	0.000	0.014	0.041	0.000
	270	0.051	0.043	0.000	0.046	0.006	0.000	0.032	0.028	0.078	0.014	0.008	0.034	0.010	0.007
	274	0.045	0.016	0.074	0.023	0.081	0.076	0.071	0.023	0.004	0.061	0.076	0.027	0.036	0.066
	278	0.006	0.055	0.043	0.058	0.065	0.043	0.045	0.040	0.039	0.061	0.042	0.055	0.092	0.007
	282	0.000	0.012	0.032	0.050	0.052	0.043	0.026	0.017	0.021	0.068	0.025	0.034	0.036	0.007

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Appendix B.–Page 4 of 11.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
	286	0.006	0.016	0.011	0.042	0.090	0.087	0.058	0.051	0.018	0.047	0.059	0.027	0.046	0.000
	290	0.019	0.000	0.000	0.015	0.023	0.141	0.091	0.091	0.064	0.027	0.000	0.048	0.015	0.059
	294	0.013	0.008	0.000	0.004	0.003	0.000	0.032	0.045	0.046	0.041	0.017	0.021	0.020	0.037
	298	0.013	0.043	0.064	0.050	0.003	0.011	0.006	0.011	0.007	0.000	0.025	0.007	0.031	0.007
	302	0.000	0.043	0.021	0.004	0.000	0.022	0.000	0.011	0.007	0.047	0.059	0.041	0.046	0.022
	306	0.000	0.024	0.043	0.027	0.000	0.011	0.045	0.006	0.004	0.000	0.000	0.014	0.010	0.000
	310	0.006	0.016	0.021	0.000	0.000	0.000	0.013	0.023	0.004	0.027	0.008	0.007	0.026	0.044
	314	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.017	0.021	0.015	0.044
	318	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.021	0.020	0.000
	322	0.000	0.004	0.011	0.000	0.000	0.000	0.000	0.006	0.000	0.007	0.051	0.014	0.015	0.015
	326	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.010	0.000
	330	0.006	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.011	0.000	0.017	0.000	0.010	0.000
	334	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.005	0.000
	338	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.008	0.014	0.020	0.000
	342	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.042	0.007	0.005	0.000
	346	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.007	0.000	0.000
	350	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.008	0.000	0.000	0.000
	354	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.028	0.000	0.000	0.000	0.000	0.000	0.007
	358	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.000	0.000	0.000	0.000	0.000	0.000
	362	0.000	0.000	0.000	0.000	0.000	0.011	0.026	0.011	0.000	0.000	0.000	0.000	0.000	0.000
	366	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	370	0.000	0.000	0.000	0.000	0.000	0.022	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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Appendix B.–Page 5 of 11.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots201b	N	71	124	45	125	155	46	76	85	137	74	60	66	103	67
	161	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.005	0.000
	165	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.015	0.014	0.000	0.000	0.000	0.000
	169	0.049	0.024	0.089	0.076	0.194	0.130	0.132	0.124	0.124	0.061	0.033	0.053	0.039	0.067
	173	0.162	0.149	0.167	0.152	0.187	0.098	0.105	0.124	0.091	0.182	0.133	0.136	0.160	0.127
	178	0.063	0.077	0.089	0.116	0.006	0.087	0.059	0.018	0.069	0.081	0.050	0.053	0.044	0.060
	182	0.035	0.020	0.011	0.060	0.000	0.011	0.026	0.041	0.047	0.128	0.092	0.106	0.097	0.052
	186	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.041	0.022	0.068	0.042	0.091	0.107	0.104
	190	0.000	0.000	0.000	0.016	0.000	0.000	0.007	0.018	0.000	0.068	0.000	0.038	0.019	0.045
	194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.007	0.050	0.023	0.034	0.007
	210	0.000	0.000	0.011	0.000	0.000	0.000	0.007	0.006	0.080	0.007	0.067	0.030	0.019	0.022
	214	0.028	0.012	0.033	0.048	0.006	0.011	0.000	0.029	0.004	0.020	0.008	0.008	0.019	0.015
	218	0.141	0.129	0.144	0.036	0.145	0.033	0.013	0.029	0.022	0.041	0.075	0.038	0.053	0.052
	222	0.106	0.056	0.100	0.096	0.097	0.076	0.125	0.118	0.026	0.014	0.083	0.076	0.083	0.075
	226	0.077	0.060	0.044	0.084	0.026	0.087	0.072	0.029	0.073	0.027	0.067	0.030	0.049	0.060
	230	0.042	0.008	0.056	0.104	0.000	0.054	0.039	0.035	0.037	0.054	0.067	0.106	0.092	0.142
	234	0.000	0.048	0.067	0.036	0.077	0.098	0.112	0.124	0.051	0.000	0.017	0.061	0.024	0.037
	238	0.169	0.169	0.100	0.088	0.035	0.239	0.211	0.188	0.296	0.135	0.100	0.068	0.078	0.007
	242	0.035	0.161	0.033	0.020	0.113	0.022	0.007	0.053	0.026	0.047	0.067	0.045	0.019	0.015
	246	0.007	0.004	0.033	0.016	0.000	0.043	0.026	0.018	0.018	0.027	0.033	0.038	0.034	0.090
	250	0.049	0.065	0.011	0.044	0.081	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.019	0.007
	254	0.000	0.016	0.011	0.000	0.032	0.000	0.000	0.000	0.000	0.014	0.008	0.000	0.005	0.015
	258	0.035	0.000	0.000	0.008	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ots208b	N	76	126	50	131	156	46	77	86	142	71	61	74	106	68
	150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.008	0.007	0.005	0.081
	154	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.004	0.007	0.016	0.000	0.014	0.022
	158	0.033	0.075	0.030	0.053	0.077	0.033	0.052	0.070	0.011	0.035	0.057	0.054	0.038	0.044
	162	0.118	0.020	0.060	0.073	0.109	0.022	0.032	0.006	0.063	0.106	0.033	0.047	0.052	0.132

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Appendix B.–Page 6 of 11.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
	166	0.099	0.075	0.030	0.038	0.083	0.228	0.214	0.250	0.162	0.014	0.008	0.061	0.009	0.022
	170	0.059	0.040	0.040	0.031	0.038	0.065	0.071	0.052	0.046	0.042	0.066	0.074	0.090	0.169
	174	0.066	0.139	0.110	0.050	0.051	0.054	0.032	0.047	0.092	0.070	0.148	0.128	0.151	0.110
	178	0.178	0.060	0.150	0.149	0.183	0.174	0.182	0.157	0.134	0.106	0.123	0.074	0.066	0.022
	182	0.072	0.190	0.070	0.111	0.048	0.065	0.026	0.105	0.028	0.070	0.066	0.061	0.080	0.074
	186	0.053	0.008	0.010	0.057	0.013	0.065	0.065	0.058	0.053	0.021	0.049	0.041	0.071	0.118
	190	0.033	0.012	0.030	0.015	0.006	0.033	0.039	0.070	0.032	0.049	0.016	0.034	0.033	0.007
	194	0.039	0.087	0.090	0.076	0.035	0.000	0.000	0.000	0.085	0.070	0.057	0.014	0.038	0.022
	198	0.020	0.020	0.010	0.023	0.032	0.033	0.045	0.006	0.028	0.021	0.025	0.034	0.028	0.029
	202	0.026	0.028	0.020	0.046	0.013	0.000	0.000	0.006	0.000	0.056	0.057	0.074	0.066	0.007
	206	0.007	0.000	0.000	0.000	0.000	0.011	0.013	0.000	0.000	0.070	0.016	0.020	0.028	0.000
	210	0.000	0.000	0.000	0.004	0.000	0.022	0.026	0.029	0.042	0.000	0.049	0.020	0.028	0.000
	214	0.033	0.012	0.000	0.031	0.000	0.011	0.032	0.006	0.021	0.042	0.025	0.027	0.028	0.015
	218	0.039	0.008	0.010	0.034	0.003	0.065	0.026	0.023	0.028	0.007	0.008	0.007	0.005	0.015
	222	0.007	0.000	0.020	0.019	0.029	0.033	0.058	0.058	0.028	0.000	0.008	0.000	0.000	0.000
	226	0.007	0.103	0.080	0.011	0.144	0.011	0.000	0.000	0.000	0.000	0.016	0.007	0.024	0.000
	230	0.007	0.024	0.000	0.008	0.000	0.000	0.006	0.000	0.000	0.021	0.008	0.000	0.005	0.015
	234	0.079	0.012	0.080	0.046	0.032	0.011	0.013	0.023	0.011	0.014	0.008	0.027	0.019	0.022
	238	0.013	0.016	0.070	0.061	0.074	0.000	0.000	0.000	0.004	0.007	0.008	0.027	0.019	0.000
	242	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.011	0.007	0.000	0.047	0.024	0.000
	246	0.000	0.000	0.020	0.000	0.000	0.011	0.000	0.006	0.014	0.021	0.016	0.041	0.000	0.037
	250	0.007	0.063	0.050	0.031	0.000	0.000	0.000	0.000	0.056	0.049	0.057	0.020	0.009	0.015
	254	0.000	0.008	0.020	0.023	0.000	0.000	0.006	0.000	0.032	0.021	0.000	0.027	0.024	0.007
	258	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.007	0.025	0.020	0.000	0.000
	262	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.012	0.000	0.014	0.000	0.007	0.009	0.000
	266	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.007	0.000	0.000	0.000	0.009	0.015
	270	0.000	0.000	0.000	0.000	0.000	0.033	0.013	0.000	0.000	0.007	0.000	0.000	0.014	0.000
	274	0.000	0.000	0.000	0.000	0.000	0.011	0.006	0.000	0.007	0.000	0.000	0.000	0.014	0.000
	278	0.007	0.000	0.000	0.004	0.029	0.000	0.019	0.012	0.004	0.014	0.016	0.000	0.000	0.000
	282	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000

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Appendix B.–Page 7 of 11.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots211	N	78	126	50	133	155	46	77	88	143	74	61	75	106	68
	212	0.000	0.012	0.020	0.000	0.000	0.000	0.000	0.000	0.007	0.007	0.016	0.013	0.028	0.000
	220	0.006	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.017	0.000	0.016	0.020	0.019	0.015
	224	0.019	0.016	0.100	0.056	0.126	0.022	0.058	0.028	0.255	0.081	0.107	0.100	0.075	0.015
	228	0.628	0.385	0.480	0.496	0.519	0.011	0.052	0.017	0.056	0.095	0.205	0.140	0.146	0.059
	232	0.006	0.000	0.010	0.083	0.003	0.054	0.045	0.063	0.063	0.108	0.066	0.173	0.146	0.110
	236	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.034	0.000	0.027	0.033	0.080	0.066	0.037
	240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.007	0.000	0.000
	244	0.000	0.000	0.000	0.000	0.000	0.076	0.026	0.045	0.000	0.020	0.000	0.007	0.000	0.000
	248	0.103	0.083	0.100	0.034	0.032	0.000	0.006	0.023	0.007	0.027	0.016	0.000	0.005	0.007
	252	0.032	0.052	0.040	0.068	0.013	0.033	0.052	0.040	0.042	0.101	0.000	0.013	0.014	0.000
	256	0.045	0.032	0.030	0.049	0.052	0.022	0.000	0.011	0.080	0.074	0.074	0.033	0.042	0.029
	260	0.058	0.000	0.000	0.019	0.000	0.022	0.052	0.102	0.028	0.041	0.041	0.053	0.057	0.368
	264	0.000	0.000	0.000	0.000	0.000	0.011	0.019	0.000	0.010	0.000	0.016	0.040	0.038	0.000
	268	0.000	0.000	0.000	0.000	0.000	0.043	0.084	0.057	0.000	0.027	0.057	0.027	0.033	0.037
	272	0.000	0.119	0.000	0.004	0.000	0.000	0.032	0.011	0.000	0.000	0.000	0.000	0.005	0.066
	276	0.000	0.000	0.000	0.000	0.000	0.087	0.052	0.034	0.091	0.007	0.000	0.007	0.005	0.000
	278	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
	280	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.020	0.005	0.000
	282	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000
	284	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.008	0.033	0.014	0.000
	286	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.000	0.000
	288	0.000	0.032	0.000	0.000	0.000	0.033	0.039	0.006	0.007	0.020	0.057	0.013	0.028	0.015
	292	0.000	0.000	0.000	0.008	0.000	0.065	0.065	0.074	0.042	0.047	0.033	0.047	0.033	0.029
	296	0.026	0.091	0.050	0.038	0.003	0.163	0.175	0.153	0.080	0.142	0.074	0.020	0.061	0.154
	300	0.013	0.008	0.040	0.053	0.000	0.043	0.045	0.097	0.091	0.095	0.041	0.060	0.047	0.037
	304	0.038	0.103	0.100	0.064	0.216	0.109	0.052	0.074	0.063	0.020	0.066	0.027	0.071	0.007
	308	0.026	0.067	0.030	0.026	0.035	0.130	0.097	0.119	0.045	0.034	0.041	0.007	0.042	0.000
	312	0.000	0.000	0.000	0.000	0.000	0.065	0.032	0.011	0.014	0.027	0.008	0.007	0.005	0.015
	316	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
	320	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000

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Appendix B.–Page 8 of 11.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots212	N	78	127	49	116	155	43	76	88	143	74	61	69	106	68
	131	0.000	0.000	0.000	0.000	0.000	0.023	0.033	0.028	0.031	0.007	0.000	0.000	0.000	0.000
	135	0.000	0.000	0.000	0.000	0.000	0.012	0.013	0.000	0.014	0.000	0.000	0.000	0.009	0.000
	139	0.051	0.020	0.041	0.022	0.058	0.023	0.053	0.045	0.080	0.061	0.025	0.087	0.080	0.051
	143	0.397	0.335	0.429	0.522	0.426	0.291	0.309	0.256	0.259	0.264	0.475	0.348	0.278	0.265
	147	0.032	0.063	0.051	0.047	0.113	0.326	0.257	0.227	0.133	0.155	0.139	0.109	0.127	0.015
	151	0.077	0.134	0.092	0.099	0.119	0.081	0.118	0.108	0.021	0.027	0.057	0.087	0.047	0.132
	155	0.032	0.051	0.082	0.017	0.006	0.047	0.046	0.051	0.066	0.027	0.098	0.036	0.085	0.110
	159	0.013	0.071	0.020	0.022	0.003	0.058	0.020	0.108	0.042	0.088	0.033	0.087	0.113	0.074
	163	0.276	0.181	0.153	0.172	0.090	0.070	0.059	0.091	0.045	0.034	0.041	0.058	0.061	0.103
	167	0.071	0.146	0.092	0.091	0.126	0.012	0.000	0.011	0.035	0.034	0.025	0.043	0.047	0.015
	169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000
	171	0.013	0.000	0.000	0.004	0.010	0.012	0.020	0.017	0.045	0.101	0.074	0.058	0.061	0.066
	175	0.038	0.000	0.020	0.004	0.048	0.023	0.039	0.017	0.000	0.074	0.033	0.051	0.052	0.074
	179	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	191	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.028	0.126	0.014	0.000	0.022	0.014	0.000
	195	0.000	0.000	0.000	0.000	0.000	0.023	0.026	0.011	0.000	0.014	0.000	0.000	0.009	0.000
	199	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.014	0.000	0.007	0.000	0.022
	203	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.066
	207	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.005	0.000
	211	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.049	0.061	0.000	0.000	0.000	0.007
	215	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.049	0.007	0.000	0.000	0.000	0.000
	219	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000
Ots213	N	77	128	48	130	153	44	77	86	141	74	59	72	98	68
	222	0.000	0.000	0.000	0.000	0.000	0.034	0.026	0.070	0.007	0.000	0.000	0.000	0.005	0.007
	226	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	230	0.000	0.043	0.021	0.042	0.003	0.023	0.006	0.023	0.007	0.000	0.000	0.000	0.000	0.000

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Appendix B.–Page 9 of 11.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
	234	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.015	0.000
	238	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.014	0.008	0.035	0.031	0.000
	242	0.000	0.016	0.010	0.012	0.000	0.023	0.006	0.000	0.000	0.041	0.025	0.014	0.020	0.000
	246	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.008	0.028	0.041	0.029
	250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.047	0.017	0.000	0.005	0.000
	254	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.017	0.014	0.026	0.007
	258	0.000	0.000	0.000	0.012	0.000	0.023	0.026	0.035	0.135	0.000	0.008	0.021	0.031	0.007
	262	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.008	0.007	0.010	0.000
	266	0.000	0.012	0.000	0.023	0.000	0.000	0.026	0.017	0.011	0.020	0.000	0.000	0.000	0.000
	270	0.000	0.004	0.010	0.008	0.013	0.000	0.006	0.017	0.039	0.027	0.017	0.021	0.031	0.000
	274	0.149	0.059	0.115	0.065	0.059	0.000	0.000	0.017	0.032	0.027	0.008	0.021	0.020	0.044
	278	0.000	0.000	0.000	0.000	0.003	0.080	0.039	0.105	0.007	0.007	0.051	0.076	0.010	0.044
	282	0.006	0.055	0.031	0.062	0.000	0.023	0.045	0.035	0.057	0.128	0.068	0.104	0.036	0.110
	286	0.149	0.117	0.240	0.119	0.275	0.080	0.078	0.052	0.092	0.061	0.093	0.132	0.122	0.103
	290	0.130	0.098	0.063	0.062	0.010	0.125	0.136	0.110	0.043	0.101	0.110	0.090	0.061	0.213
	294	0.078	0.078	0.021	0.077	0.075	0.080	0.123	0.041	0.043	0.027	0.034	0.056	0.102	0.051
	298	0.162	0.164	0.177	0.196	0.121	0.193	0.227	0.203	0.195	0.068	0.102	0.069	0.107	0.074
	302	0.078	0.059	0.135	0.165	0.225	0.068	0.084	0.087	0.160	0.135	0.144	0.097	0.153	0.081
	306	0.130	0.172	0.104	0.050	0.206	0.102	0.065	0.052	0.078	0.128	0.119	0.090	0.061	0.088
	310	0.110	0.117	0.052	0.081	0.010	0.011	0.065	0.064	0.018	0.041	0.059	0.069	0.056	0.059
	314	0.006	0.008	0.021	0.015	0.000	0.045	0.006	0.006	0.057	0.027	0.017	0.028	0.015	0.000
	318	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.059	0.014	0.005	0.000
	322	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.020	0.025	0.007	0.015	0.066
	338	0.000	0.000	0.000	0.012	0.000	0.011	0.013	0.006	0.000	0.000	0.000	0.000	0.000	0.000
	342	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
	346	0.000	0.000	0.000	0.000	0.000	0.068	0.013	0.041	0.000	0.000	0.000	0.007	0.015	0.015
	350	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.017	0.000	0.000	0.000	0.000	0.000	0.000

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Appendix B.–Page 10 of 11.

Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
Ots3M	N	78	120	50	133	154	46	77	88	144	75	61	75	106	61
	138	0.000	0.042	0.010	0.000	0.000	0.022	0.006	0.000	0.003	0.033	0.025	0.033	0.014	0.107
	144	0.231	0.258	0.270	0.301	0.221	0.130	0.078	0.148	0.135	0.060	0.066	0.073	0.085	0.041
	146	0.327	0.317	0.340	0.342	0.461	0.554	0.578	0.517	0.535	0.587	0.582	0.573	0.599	0.557
	148	0.423	0.308	0.380	0.353	0.318	0.293	0.312	0.330	0.323	0.293	0.311	0.280	0.259	0.295
	150	0.019	0.075	0.000	0.004	0.000	0.000	0.026	0.006	0.003	0.027	0.016	0.040	0.042	0.000
Ots9	N	78	128	49	133	140	46	77	88	144	75	60	75	105	68
	105	0.064	0.125	0.214	0.252	0.161	0.783	0.747	0.722	0.601	0.493	0.483	0.467	0.548	0.669
	107	0.936	0.875	0.786	0.748	0.839	0.217	0.253	0.278	0.399	0.507	0.517	0.533	0.452	0.331
OtsG474	N	78	128	50	133	155	46	77	88	143	74	61	75	106	68
	156	0.718	0.641	0.770	0.778	0.790	0.793	0.870	0.898	0.724	0.689	0.820	0.807	0.882	0.919
	160	0.192	0.273	0.110	0.158	0.113	0.207	0.117	0.102	0.059	0.041	0.016	0.013	0.028	0.000
	164	0.000	0.016	0.030	0.008	0.026	0.000	0.006	0.000	0.017	0.047	0.033	0.060	0.047	0.081
	168	0.019	0.008	0.060	0.038	0.000	0.000	0.006	0.000	0.000	0.014	0.025	0.013	0.005	0.000
	172	0.032	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.199	0.155	0.041	0.027	0.014	0.000
	176	0.038	0.055	0.030	0.019	0.071	0.000	0.000	0.000	0.000	0.054	0.066	0.080	0.024	0.000
Ssa408	N	71	122	44	122	155	46	75	85	136	72	59	64	103	67
	188	0.000	0.004	0.011	0.000	0.003	0.000	0.000	0.000	0.004	0.021	0.017	0.023	0.015	0.000
	192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.008	0.008	0.000	0.022
	196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.110	0.042	0.017	0.016	0.044	0.000
	200	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.041	0.022	0.007	0.000	0.031	0.019	0.000

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Locus	Allele	Bone	Otter	Indian	Chistochina	Sinona	Gulkana Mainstem	Gulkana Middle Fork	Gulkana Paxson Fork	Mendeltna	Kiana	Manker	Tonsina/ Greyling	Tonsina Radio	Tebay
	204	0.070	0.008	0.000	0.008	0.003	0.011	0.000	0.012	0.007	0.021	0.000	0.031	0.024	0.007
	208	0.148	0.279	0.159	0.201	0.103	0.185	0.187	0.235	0.136	0.153	0.127	0.117	0.180	0.269
	212	0.007	0.041	0.000	0.049	0.003	0.054	0.073	0.047	0.029	0.014	0.008	0.008	0.015	0.022
	216	0.000	0.000	0.000	0.008	0.000	0.054	0.047	0.047	0.055	0.181	0.144	0.227	0.126	0.075
	220	0.014	0.180	0.159	0.070	0.181	0.076	0.087	0.118	0.110	0.042	0.161	0.133	0.102	0.172
	224	0.380	0.160	0.352	0.336	0.265	0.217	0.247	0.159	0.235	0.118	0.144	0.063	0.170	0.104
	228	0.254	0.262	0.227	0.184	0.213	0.207	0.147	0.118	0.140	0.139	0.186	0.156	0.112	0.052
	230	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.007
	232	0.077	0.033	0.057	0.033	0.219	0.043	0.100	0.065	0.029	0.083	0.042	0.063	0.049	0.067
	234	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.005	0.022
	236	0.035	0.033	0.023	0.102	0.010	0.130	0.047	0.124	0.077	0.056	0.051	0.039	0.044	0.067
	240	0.014	0.000	0.011	0.008	0.000	0.022	0.013	0.029	0.007	0.014	0.017	0.016	0.024	0.000
	244	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.042	0.023	0.019	0.007
	248	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.017	0.008	0.015	0.000
	256	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.017	0.023	0.029	0.097
	260	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.008	0.005	0.007
	264	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.049	0.000	0.008	0.005	0.000
	268	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000

Note: N = number of individuals analyzed to estimate allele frequencies.