AN ABSTRACT OF THE THESIS OF

Noah Swayambhu Adams for the degree of Master of Science in Fisheries Science presented on February 4, 1994.

Title: Variation in Mitochondrial DNA and Allozymes Discriminates Early and Late Forms of Chinook Salmon (Oncorhynchus tshawytscha) in the Kenai and Kasilof Rivers, Alaska.

Abstract approved: Redacted for Privacy
Dr. Carl B. Schreck

Redacted for Privacy
Dr. Hiram W. Li

Genetic differences between early and late forms of Alaskan chinook salmon (Oncorhynchus tshawytscha) were identified using two genetic approaches: mitochondrial DNA (mtDNA) analysis and protein electrophoresis. The study populations consisted of early- and late-run chinook salmon in each of the Kenai and Kasilof rivers in Alaska, and a single population from the Minam River, Oregon, that provided a relative scale for the differences among the Alaskan populations. Two segments of mtDNA were amplified separately using the polymerase chain reaction (PCR) and then digested with 14 to 16 restriction enzymes. Results showed that the two early runs were genetically similar to each other but different from either of the late runs. The late runs were different from each other based on the

frequency of the common haplotypes. The Minam River stock shared two haplotypes with the Alaskan stocks and displayed one unique haplotype. The frequency difference in the shared haplotypes together with the presence of a unique haplotype allowed us to separate the Oregon population from those in Alaska. In the protein analysis, each of the five populations was examined at 30 allozyme loci to determine variation within and between the runs. Based on 14 polymorphic loci, Minam River chinook salmon were genetically distinct from the Alaskan populations. Within the Alaskan populations, the two early runs were most similar to each other but different from the two late runs; the two late runs were also genetically most similar to each other. Based on all loci, protein electrophoresis proved to be a useful technique to separate stocks of chinook salmon. On a locus by locus basis, however, mtDNA was more powerful. Both mtDNA and allozyme analysis suggest that chinook salmon may segregate into genetically different early and late forms within a drainage.

Variation in Mitochondrial DNA and Allozymes Discriminates Early and Late Forms of Chinook Salmon (*Oncorhynchus tshawytscha*) in the Kenai and Kasilof Rivers, Alaska

by

Noah Swayambhu Adams

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed February 4, 1994

Commencement June 1995

APPROVED:

Redacted for Privacy

Co-Professor of Fisheries and Wildlife in charge of major

Redacted for Privacy

Co-Professor of Fisheries and Wildlife in charge of major

Redacted for Privacy

Head of Department of Fisheries and Wildlife

Redacted for Privacy

Dean of Graduate School

Date thesis is presented February 4, 1994

Typed by researcher for Noah Swayambhu Adams

ACKNOWLEDGEMENTS

I would like to acknowledge my major professors, Carl B. Schreck and Hiram W. Li, and Carl V. Burger, Matt A. Cronin and Kenneth P. Currens for constant guidance and advice throughout my thesis program. I thank Orvie Danzuka, Dan Farnsworth, Cam Sharpe, Ken Denning, Phil Harris, and Ken Currens for laboratory assistance and data analysis, and Michele L. Adams and Leslie Bartles for comments on the manuscript. Tissue samples could not have been obtained without the assistance of the Alaska Department of Fish and Game, the Oregon Department of Fish and Wildlife, the U.S. Fish and Wildlife Service, numerous fishing guides on the Kenai River, and Michele L. Adams. I am also thankful for the sacrifices made by and the special support of my wife, Michele.

TABLE OF CONTENTS

INTRODUCTION	1
METHODS	6
Collection of Samples	6
Tissue Analysis	8
DATA ANALYSIS	12
Among-Population Differences	12
Estimates of Within-Population Diversity	13
Estimates of Between-Population Diversity	13
RESULTS	15
Among-Population Difference	21
Estimates of Within-Population Diversity	23
Estimates of Between-Population Diversity	25
DISCUSSION	29
Ecology	33
Colonization and Evolution	36
SUMMARY	38
LITERATURE CITED	40

LIST OF FIGURES

Figure		Page
1.	Collection sites of chinook salmon samples in Southcentral Alaska and Central Oregon, 1990 through 1992.	3
2.	Phenograms showing genetic relationships among five populations of chinook salmon from the Kenai, Kasilof, and Minam rivers based on mtDNA haplotype and allozyme frequency data.	26

LIST OF TABLES

Table		Page
1.	International Union of Biochemistry (I.U.B.) enzyme names (1984), Enzyme Commission (E.C.) numbers, loci, tissues, and buffers used in this study.	10
2.	MtDNA segments with restriction site polymorphisms and the restriction fragment lengths.	16
3.	Composite haplotype definitions for chinook salmon.	17
4.	Distribution of chinook salmon mtDNA haplotypes among locations in the Kenai, Kasilof, and Minam rivers and haplotype and nucleotide diversity within each population as well as a matrix of nucleotide diversity and divergence among populations.	18
5.	Allelic frequencies at 14 polymorphic loci for chinook salmon sampled from the Kenai, Kasilof, and Minam rivers from 1990 to 1992.	19
6.	Results of hierarchial tests-of-homogeneity using mtDNA (haplotypes D1,D2,D3,D4) and allozyme (14 polymorphic loci) data among and within the chinook salmon populations in the Kenai, Kasilof, and Minam rivers, and tests-of-homogeneity between the two early and two late runs in the Kenai and Kasilof rivers.	22
7.	Means and SE (in parentheses) for the 12 morphological characteristics of the Kenai River early- and late-run chinook salmon.	24

CONTRIBUTION OF AUTHORS

William J. Spearman. Provided laboratory and statistical analysis of mitochondrial DNA samples and provided editorial comments on the manuscript..

Carl V. Burger. Was instrumental in designing the project, planning and assisting in the collection of tissues samples, and providing editorial comments on the manuscript.

Kenneth P. Currens. Trained and assisted in laboratory and statistical analysis of the allozyme data, and provided editorial comments on the manuscript.

Carl B. Schreck. Provided guidance in the design, implementation and completion of the project and provided editorial comments on the manuscript.

Hiram W. Li. Provided guidance in the design, implementation and completion of the project and provided editorial comments on the manuscript.

Variation in Mitochondrial DNA and Allozymes Discriminates Early and Late Forms of Chinook Salmon (*Oncorhynchus tshawytscha*) in the Kenai and Kasilof Rivers, Alaska

INTRODUCTION

The tendency of chinook salmon (*Oncorhynchus tshawytscha*) to return and spawn in their river of origin (Quinn 1982; McIsaac and Quinn 1988) results in separate breeding populations that may be biochemically, morphologically and ecologically different. Because conserving genetic diversity among populations of fish is critical to species survival (Meffe 1986; Nelson and Soule' 1987; Allendorf et al. 1987), a first step towards genetic conservation is to identify those groups that constitute separate breeding populations (Larkin 1981). Although it is often difficult to determine whether morphological and ecological differences among chinook salmon populations reflect separate breeding groups, the consequences of ignoring such differences may be a loss of genetic diversity for the whole species.

Analysis of genetic variation has proven to be a useful technique in identifying separate breeding populations. Genotypic data inferred through protein electrophoresis has been successful in discriminating among broad geographical groups of chinook salmon (Allendorf and Phelps 1981; Kristiansson and McIntyre

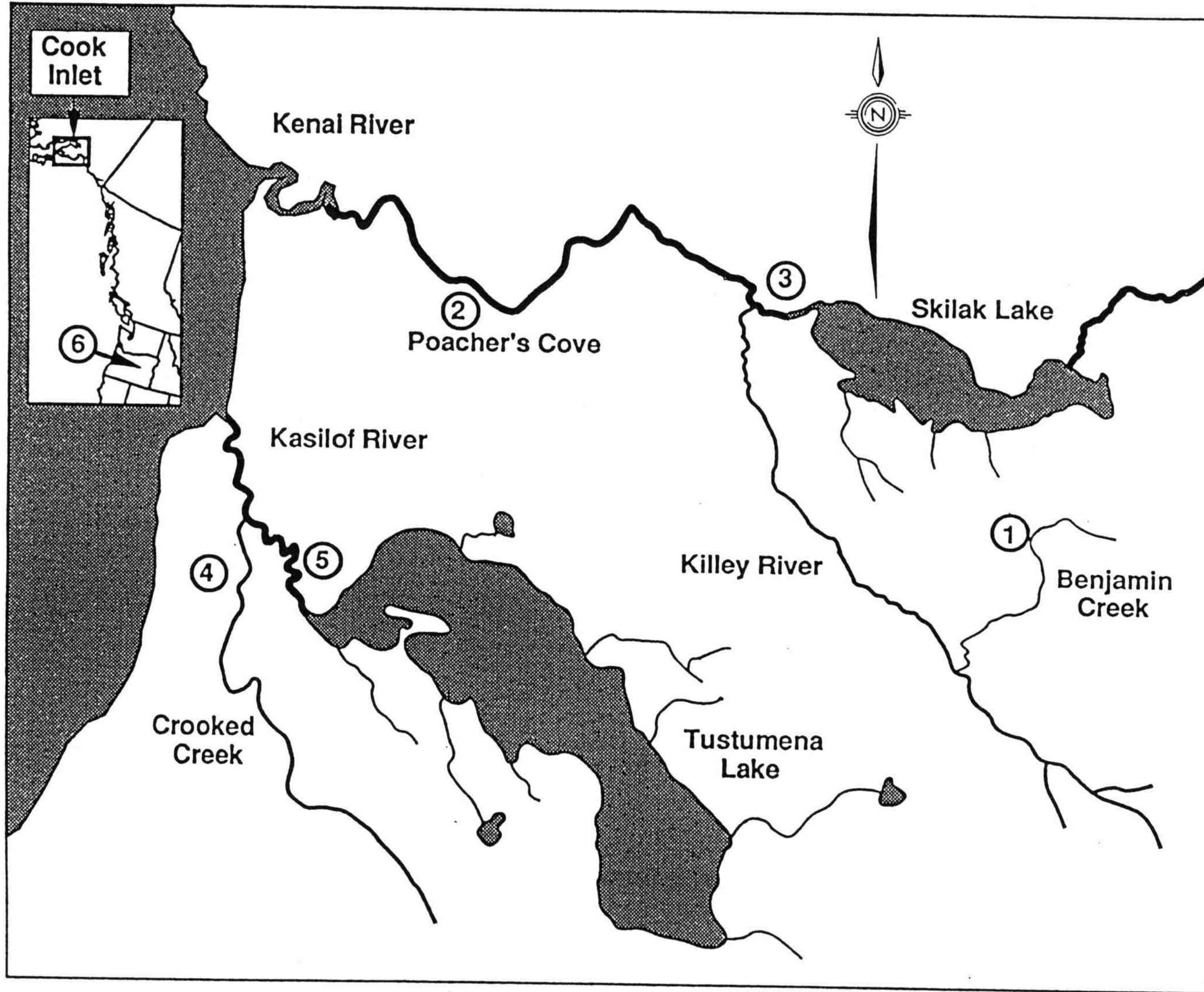
1976; Winans 1989; Utter et al. 1973, 1989; Gharrett et al. 1987).

Analysis of mitochondrial DNA (mtDNA) is an alternative way of examining genetic diversity among groups. Detecting variation within species and organizing individuals into matriarchal phylogenes is facilitated by the maternal inheritance of mtDNA (Hutchison et al. 1974; Giles et al. 1980; Gyllensten et al. 1985), its individual homogeneity (Avise et al. 1979), and its apparently high rate of sequence evolution (Brown et al. 1979). The fast rate of evolution, which appears to be approximately four times faster than that of nuclear genes (Birky et al. 1983; Wilson et al. 1985), offers a magnified view of the divergence between closely related populations (Gyllensten and Wilson 1987) and aids tremendously in the analysis of interspecific relationships. MtDNA analysis in fish has been successful in detecting differences both between and within species (Thomas et al. 1986; Cronin et al. 1993). However, few studies have examined mtDNA variation of morphologically and ecologically differentiated chinook salmon.

Our objective was to determine if genetic differences existed among chinook salmon that exhibit different ecological spawning characteristics. Chinook salmon populations from the Kasilof and Kenai rivers, Alaska, (Fig. 1) were chosen for two reasons. First, both rivers

Figure 1. Collection sites of chinook salmon samples in Southcentral Alaska and Central Oregon, 1990 through 1992: 1, Kenai River early-run; 2, Kenai River early- and late-run; 3, Kenai River late-run; 4, Kasilof River early-run; 5, Kasilof River late-run; 6, Minam River (Insert, shows general sampling sites in Alaska and Oregon).

Figure 1.



support early- and late-run salmon. Cold winter water temperatures and a short summer growing season in Alaska presumably cause the runs to be concentrated over a 3-month period (Burger et al. 1985). In the Kasilof River, the early run enters the river during June and the late run returns from late July through early September (Faurot and Jones 1990). Similarly, Kenai River early-run chinook enter the river through June whereas the late run returns during July and early August (Burger et al. 1985). Late run fish in both rivers spawn in the mainstem downstream of large lakes whereas early run fish spawn in tributaries that are not influenced by lakes (Burger et al. 1985; Faurot and Jones 1990).

Geographical proximity was a second factor in choosing the Alaska study populations. If genetic differences existed among geographically close yet ecologically different populations, detectable genetic differences might also exist among other chinook salmon populations in Alaska. In that case, a baseline could be developed to determine the genetic origins of the stocks harvested in various commercial and sport fisheries.

METHODS

Collection of Samples

Skeletal muscle, liver, heart, eye and caudal fin tissues were collected from chinook salmon during the summers of 1990, 1991, and 1992 from the Kenai and Kasilof rivers and the Minam River, Oregon (Fig. 1). Tissues were stored at -80°C until analyzed.

In the Kenai River, post-spawning early-run chinook salmon were collected on the spawning grounds in a tributary (Fig. 1) by dipnetting. Late-run fish were collected with drift nets on the spawning grounds downstream of Skilak Lake (Fig. 1). Additional samples of early and late-run salmon were collected from angled fish at Poacher's Cove each week from June 4 through July 27, 1991 (Fig. 1). Based on previous studies of seasonal entry into the river (Hammarstrom 1981) and geographical spawning distribution (Burger et al. 1985), fish collected before July 1 were considered the early-run form whereas fish collected after July 1 were considered to be late-run salmon.

During field sampling at Poacher's Cove and prior to obtaining tissues for genetic analysis, 12 morphological characteristics were measured on each fish to determine if morphological differences existed between the early and

late runs of chinook salmon in the Kenai River. These morphological features are a collection of those used by other investigators (Riddell and Leggett 1981; Beacham 1984; Taylor and McPhail 1985; Hénault and Fortin 1989). Some of these characters have been successfully used to separate stocks within a basin (Riddell and Leggett 1981; Beacham 1984). Measurements were: 1) distance from mid-eye to fork of tail; 2) distance from snout to fork of tail; 3) weight; 4) girth (circumference of fish at anterior insertion of dorsal fin); 5) circumference of caudal peduncle; 6) length of base of dorsal fin; 7) length of longest dorsal fin ray; 8) length of base of anal fin; 9) length of longest anal fin ray; 10) length of longest pelvic fin ray; 11) length of longest pectoral fin ray; and 12) length of adipose fin from anterior end of base to tip.

In the Kasilof River, tissues from adult early-run chinook salmon and their progeny were obtained from fish at the Crooked Creek Hatchery (Fig. 1). The hatchery has propagated early-run chinook salmon since 1974 from native chinook salmon that spawned in Crooked Creek (Robert Och, Alaska Department of Fish and Game, personal communication). Tissues were obtained from adult late-run chinook salmon collected with drift nets on the spawning grounds downstream of Tustumena Lake (Fig. 1). Because fertilized ova from Kasilof late-run salmon were incubated

(Alaska Department of Fish and Game) for fry releases in other drainages, tissues were obtained from hatchery-reared progeny of the late-run stock.

Tissues from juvenile chinook salmon from the Minam River, Oregon, were used to examine regional differences among chinook salmon populations and to provide a relative scale for interpreting differences among Alaskan populations. The Minam River is a tributary to the Snake River. The distant geographical isolation of Minam River chinook salmon suggested a high probability of genetic difference between this stock and Alaskan chinook salmon.

Tissue Analysis

Procedures described by Cronin et al. (1993) were used to extract, amplify, and digest mtDNA segments (NADH dehydrogenase subunit 1 [ND-1] and the control region) and to visualize restriction fragment patterns. Restriction enzymes used in the analysis of the ND-1 and control region segments were: Ase I, Ava II, Bgl I, Bgl II, BstU I, Dde I, EcoR I, Hae II, Hae III, Hinc II, Hind III, Msp I, Rsa I, and Xba I. Additionally, BsaJ I and BstN I were used only for the control region segment. The sizes of the restriction fragments were estimated by comparison with size standards (PhiX174 Am3cs70 virus DNA digested with Hae III or lambda phage DNA digested with Hind III).

Restriction fragment patterns produced by each of the mtDNA segment-restriction enzyme combinations were used to define composite haplotypes (Lansman et al. 1981).

Twenty fish from each of the Kasilof River chinook salmon runs were used to determine the repeatability of the mtDNA analysis techniques applied in this study. DNA was extracted and analyzed from muscle at the Oregon Cooperative Fisheries Research Unit, Oregon State University, laboratory. Liver samples from the same fish were analyzed at the Alaska Fish and Wildlife Research Center laboratory in Anchorage, Alaska. Different tissues (muscle and liver) were used to determine if identical results could be achieved regardless of tissue type. The haplotype of each individual was identified at both labs and the results were examined for inconsistencies. Procedures for detecting genetic variation at 30 allozyme loci (Table 1) followed those of Aebersold et al. (1987).

Table 1. International Union of Biochemistry (I.U.B.) enzyme names (1984), Enzyme Commission (E.C.) numbers, loci, tissues, and buffers used in this study. Tissues: M, muscle; L, liver; E, eye; H, heart. Buffers: TBE--a Tris-borate-EDTA-gel and tray buffer pH 8.5; CAME--a citric acid-EDTA gel and tray buffer pH 6.8; CAMEN--a citric acid-EDTA-NAD^+ gel and tray buffer pH 6.8; TC-4--a tris-citric acid gel and tray buffer pH 5.8; and KG--a tris-glycine gel and tray buffer pH 8.4 (Wilmot et al. 1992).

I.U.B. Enzyme Name	E.C. Number	Locus	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1	mAAT-1*	H	CAMEN 6.8
		sAAT-1,2*	H,M	CAME 6.8
Adenosine deaminase	3.5.4.4	ADA-1*	H,M	KG,TC-4
		ADA-2*	H,M	KG,TC-4
Aconitate hydratase	4.2.1.3	sAH-1*	L	CAME 6.8, TC-4
Alanine aminotransferase	2.6.1.2	ALAT*	H,M	KG
Creatine kinase	2.7.3.2	CK-B*	M,E	KG
Glucose-6-phosphate isomerase	5.3.1.9	GPI-B1*	M	KG,TBE
		GPI-B2*	M	KG,TBE
		GPI-A*	M	KG,TBE
Glutathione reductase	1.6.4.2	GR*	H,M	TC-4
			E	CAME 6.8
Isocitrate dehydrogenase (NADP+)	1.1.1.42	sIDHP-1*	H,M,L	TC-4
			M,E,L	CAME 6.8
		sIDHP-2*	H,M,L	TC-4
			M,E,L	CAME 6.8

Table 1. (Continued)

I.U.B. Enzyme Name	E.C. Number	Locus	Tissue	Buffer
L-lactate dehydrogenase	1.1.1.27	LDH-B1*	E,L	KG,CAME 6.8
		LDH-B2*	E,L	KG,CAME 6.8
		LDH-C*	E	KG
Malate dehydrogenase	1.1.1.37	mMDH-1*	H	CAMEN 6.8
		sMDH-A1,2*	H,M,E,L	CAME 6.8
		sMDH-B1,2*	H,M,E,L	CAME 6.8
Malic enzyme (NADP+)	1.1.1.40	sMEP-1*	H,M	CAME 6.8
			L	TC-4
		sMEP-2*	H,M	CAME 6.8
			L	TC-4
Dipeptidase	3.4.-.-	PEPA*	E	KG, CAME 6.8
Tripeptide aminopeptidase	3.4.-.-	PEPB-1*	H,M	KG,TC-4
Proline dipeptidase	3.4.13.9	PEPD2*	H	CAME 6.8
			M,L	TC-4
Phosphoglucomutase	5.4.2.2	PGM-1*	H,M	KG
			L	TC-4
		PGM-2*	H,M	KG
			L	TC-4
Superoxide dismutase	1.15.1.1	sSOD-1*	L	TC-4
			H,M	KG
Triose-phosphate isomerase	5.3.1.1	TPI-1*	H,M,E	KG
		TPI-2*	H,M,E	KG
		TPI-3*	H,M,E	KG

DATA ANALYSIS

Based on seasonal entry into the rivers (Hammarstrom 1981; Faurot and Jones 1990) and geographical spawning distribution (Burger et al. 1985), data were partitioned into five groups: Kenai River early-run, Kenai River late-run, Kasilof River early-run, Kasilof River late-run, and Minam River. Tests-of-homogeneity using the log likelihood ratio statistic (G; Sokal and Rohlf 1981) were used to determine if haplotype frequency data as well as allele frequency data could be pooled for different years and maturity classes.

Among-Population Differences

Relationships among groups were defined by hierarchial tests of homogeneity, using the log-likelihood-ratio statistic (G; Nei 1973, 1975; Sokal and Rohlf 1981), for both haplotype and allele frequencies. The level of significance for each comparison within the hierarchy was calculated following the procedures of Sokal and Rohlf (1981).

Morphological differences between early and late run chinook salmon in the Kenai River were examined by analysis of variance (ANOVA). A random sample of 20 fish from each population was used in the analysis. A principal component analysis (PCA) was conducted to

examine the effect overall fish size had on the 12 morphological characteristics measured. Removal of the first component of the PCA, which represents fish size, allowed us to determine if there was a difference in body shape between the early and late runs of chinook salmon in the Kenai River.

Estimates of Within-Population Diversity

Genetic diversity within each population was estimated using both mtDNA and allozyme data. MtDNA haplotype and nucleotide diversity was estimated according to Nei (1987) and Nei and Tajima (1981) using haplotype frequencies. Within-population diversity based on allozyme data was estimated from mean heterozygosities and the percentage of polymorphic loci. Goodness-of-fit tests using Pearson's X^2 were used to determine departure from Hardy-Weinberg equilibrium.

Estimates of Between-Population Diversity

Genetic diversity between populations was also estimated using both mtDNA and allozyme data. MtDNA nucleotide divergence was estimated according to Nei (1987). Nei's genetic identity values (Nei 1972, 1978) were used to estimate allozyme diversity between populations. Phenograms based on both haplotype and

allele frequencies were examined for mtDNA and allozyme similarities among populations. Phenograms were constructed from matrices of genetic identity values (Nei 1972, 1978) using the unweighted pair-group method with arithmetic averages (UPGMA) algorithm (Sneath and Sokal 1973). Cluster analysis of the mtDNA data was also conducted using nucleotide divergence values and compared with the phenogram based on haplotype frequencies.

RESULTS

Results of the replicate testing were 100% repeatable between the two labs. The haplotype of each individual fish was consistently identified at both labs regardless of the type of tissue (liver or muscle) used in the analysis.

Within each population, both haplotype and allele frequency data from different years and maturity classes showed no statistically significant differences, allowing us to pool the data for each population. The mtDNA analysis revealed variable fragment patterns when the ND-1 segment was digested with Dde I and Rsa I and the control region segment was digested with Rsa I (Table 2). The four different composite haplotypes (D1,D2,D3,D4) are described in Table 3 and their distribution among populations is shown in Table 4. Of the 30 allozyme loci examined, 16 were monomorphic for all populations. Of the remaining 14, 5 loci had low levels of polymorphism (frequency of the common allele > 0.95); these were AH-1*, ALAT*, LDH-B2*, PEPD2*, and PEPB-1*. Loci which displayed higher levels of polymorphism (frequency of the most common allele < 0.95 in at least one population) were ADA-1*, sIDHP-1*, sIDHP-2*, sMDH-B1,2*, sMEP-1*, sMEP-2*, PEPA*, sSOD-1*, and TPI-4* (Table 5). No deviations from Hardy-Weinberg equilibrium were observed (P>0.05).

Table 2. MtDNA segments with restriction site polymorphisms and the restriction fragment lengths. Fragment length is presented as number of base pairs.

MtDNA Segment	Restriction Site	Fragment Length	Pattern	
Control Region	_Rsa_ I	1153	-	B
		887	A	-
		441	A	-
		292	-	B
ND-I	_Dde_ I	475	A	-
		422	-	B
		349	A	B
		324	A	B
		279	A	B
		258	A	B
		193	A	B
		183	-	B
	Rsa I	663	A	C
		542	-	C
		381	A	-
		361	A	C
		327	A	C
		305	A	-

Table 3. Composite haplotype definitions for chinook salmon. Letters refer to the banding patterns seen in individual samples. The banding patterns are shown in Table 2.

		Haplotype Definition			
MtDNA Segment	Restriction Enzyme	D1	D2	D3	D4
Control Region	*Rsa* I	A	A	A	B
ND-I	*Dde* I	A	A	B	A
	Rsa I	A	C	C	C

Table 4. Distribution of chinook salmon mtDNA haplotypes among locations in the Kenai, Kasilof, and Minam rivers and haplotype and nucleotide diversity within each population as well as a matrix of nucleotide diversity and divergence among populations. Numbers of locations (L) correspond to those in Figure 1. M, Maturity: A, Adult; J, Juvenile: Y, Year(s) samples were collected.

L	Sample Group	Y	M	Composite Haplotype D1	D2	D3	D4	Haplotype Diversity	Nucleotide Diversity
1,2	Kenai Early Run	1991	A	4	70	-	3	0.1716	0.0019
2,3	Kenai Late Run	1990,91	A	52	76	-	-	0.4806	0.0044
4	Kasilof Early Run	1990,91	A	4	21	-	-	0.3094	0.0030
		1991,92	J	4	29	-	-		
5	Kasilof Late Run	1990,91,92	A	66	16	-	-	0.2809	0.0026
		1991,92	J	26	9	-	-		
6	Minam River	1990	J	1	21	3	-	0.2900	0.0028

Nucleotide Diversity (above diagonal) and Divergence (below diagonal) Among Populations

	Kenai Early Run	Kenai Late Run	Kasilof Early Run	Kasilof Late Run	Minam River
Kenai Early Run	_	0.0042	0.0025	0.0079	0.0024
Kenai Late Run	0.0010	-	0.0043	0.0052	0.0048
Kasilof Early Run	0.0001	0.0006	_	0.0073	0.0029
Kasilof Late Run	0.0056	0.0018	0.0046	_	0.0085
Minam River	0.0001	0.0012	0.0001	0.0059	_

Table 5. Allelic frequencies at 14 polymorphic loci for chinook salmon sampled from the Kenai, Kasilof, and Minam rivers from 1990 to 1992. The most common allele is designated as 100, and other alleles are assigned numbers according to their mobility relative to the 100 allele. N is 100 for all five populations. Allele mobility numbers separated with a slash indicate that the data for those two alleles have been pooled.

	Locus					
	ADA-1		AH-1		ALAT	
Population	100	83	100	86	100	94
Kenai Early	0.965	0.035	0.990	0.010	0.995	0.005
Kenai Late	0.980	0.020	0.980	0.020	0.990	0.010
Kasilof Early	0.940	0.060	1.000	0.000	0.985	0.015
Kasilof Late	0.995	0.005	0.995	0.005	0.995	0.005
Minam River	0.970	0.030	1.000	0.000	1.000	0.000

	Locus					
	LDH-B2		sMDH-B1,2		sMEP-1	
Population	100	56	100	121/126	100	92/86
Kenai Early	1.000	0.000	0.985	0.015	0.090	0.910
Kenai Late	1.000	0.000	0.990	0.010	0.045	0.955
Kasilof Early	1.000	0.000	1.000	0.000	0.105	0.895
Kasilof Late	1.000	0.000	0.990	0.010	0.025	0.975
Minam River	0.990	0.010	0.895	0.105	0.060	0.940

	Locus					
	sMEP-2		PEPA		PEPD2	
Population	100	78	100	90	100	83
Kenai Early	0.615	0.385	0.990	0.010	1.000	0.000
Kenai Late	0.615	0.385	0.910	0.090	0.995	0.005
Kasilof Early	0.590	0.410	0.995	0.005	0.980	0.020
Kasilof Late	0.640	0.360	0.980	0.020	1.000	0.000
Minam River	1.000	0.000	0.990	0.010	1.000	0.000

Table 5. (Continued)

Population	PEPB-1 100	PEPB-1 130	sSOD-1 100	sSOD-1 -260	TPI-1 100	TPI-1 104
Kenai Early	0.975	0.025	0.990	0.010	0.930	0.070
Kenai Late	0.990	0.010	0.900	0.100	0.960	0.040
Kasilof Early	0.980	0.020	0.990	0.010	0.855	0.145
Kasilof Late	0.980	0.020	0.940	0.060	1.000	0.000
Minam River	0.990	0.010	0.880	0.120	0.915	0.085

Population	sIDHP-1 100	sIDHP-1 74	sIDHP-1 136	sIDHP-2 100	sIDHP-2 127	sIDHP-2 50
Kenai Early	0.995	0.000	0.005	0.995	0.000	0.005
Kenai Late	1.000	0.000	0.000	0.990	0.000	0.010
Kasilof Early	1.000	0.000	0.000	1.000	0.000	0.000
Kasilof Late	1.000	0.000	0.000	0.945	0.000	0.055
Minam River	0.900	0.100	0.000	0.965	0.030	0.005

Population	Summary: Mean Heterozygosity	Summary: Percentage of Polymorphic Loci
Kenai Early	0.034	40.0
Kenai Late	0.037	40.0
Kasilof Early	0.040	30.0
Kasilof Late	0.029	33.3
Minam River	0.036	33.3

Among-Population Differences

Tests of homogeneity identified similar patterns of genetic differentiation for both mtDNA and allozyme data (Table 6). The mtDNA data indicated that the early runs in the Kenai and Kasilof rivers were genetically similar to each other but different from either of the late runs; the late runs were different from each other based on the frequency of the common haplotypes (haplotypes D1 and D2). Although all populations shared the D1 and D2 mtDNA haplotypes, the frequency difference of the shared haplotypes together with a unique haplotype (D3) in the Minam River stock allowed us to separate the Oregon population from those in Alaska. Since, the Minam River population shared haplotypes D1 and D2 with the Alaska populations it was genetically most similar to the two early runs in this respect. There was also a unique haplotype (D4) found at a low frequency in the Kenai River early-run population.

The allozyme data revealed genetic differences between the populations that were very similar to those determined by mtDNA analysis (Table 6). Among the Alaska populations, the test of homogeneity indicated that the two early runs were genetically most similar to each other but different from both of the late runs. The two late

Table 6. Results of hierarchial tests-of-homogeneity using mtDNA (haplotypes D1,D2,D3,D4) and allozyme (14 polymorphic loci) data among and within the chinook salmon populations in the Kenai, Kasilof, and Minam rivers, and tests-of-homogeneity between the two early and two late runs in the Kenai and Kasilof rivers. (G=log-likelihood ratio statistic, df=degrees of freedom, p=the probability of rejecting the null hypothesis when it is true based on the observed G and df, los=level of significance for each test in the hierarchy where the overall los=0.05).

	Mitochondrial DNA				Allozyme			
Group	G	df	p	los	G	df	p	los
Total	293.33	15	<0.001	0.050	505.66	64	<0.05	0.050
Among Basins	181.85	12	<0.001	0.050	358.12	32	<0.05	0.050
Within Basins								
Kenai River	40.40	2	<0.001	0.013	44.90	16	<0.05	0.013
Kasilof River	71.04	1	<0.001	0.013	102.60	16	<0.05	0.013
Between Like Runs								
Early Runs	6.18	2	0.045	0.013	24.73	16	>0.05	0.013
Late Runs	37.71	1	<0.001	0.013	38.47	16	<0.05	0.013

runs were statistically different from each other and the Minam River chinook were statistically different from the Alaska populations.

There was a significant difference in size of the fish from the Kenai River early and late runs. Based on all 12 morphological characteristics, late-run fish were larger than early-run salmon (Hotelling-T; $p<0.0000$) (Table 7). The first component of the PCA accounted for 85.7% of the variation between groups while the second and third components accounted for 4.5% and 2.7% respectively. After accounting for the effect that fish size had on body shape by removing the first component, there was no statistically significant difference between early- and late-run salmon in the Kenai River. Consequently, we were unable to identify any differences in shape despite the clear difference in overall fish size.

Estimates of Within-Population Diversity

The most genetically diverse population was the Kenai River late run. Haplotype diversity within this population was 0.4806 and nucleotide diversity was 0.0044 (Table 4). Mean heterozygosity and the percentage of polymorphic loci were 0.037 and 40% (Table 5). Mean heterozygosity (0.034) and percentage of polymorphic loci (40%) in the Kenai early run were comparable but the early

Table 7. Means and SE (in parentheses) for the 12 morphological characteristics of the Kenai River early- and late-run chinook salmon. All measurements are in centimeters except for weight which is in kilograms. N=20 for each population.

Morphological Character	Early Run	Late Run
Distance from mid-eye to fork of tail	88.61 (1.92)	99.15 (1.16)
Distance from snout to fork of tail	97.28 (2.25)	110.92 (1.48)
Weight	10.56 (0.63)	15.61 (0.57)
Girth	57.65 (1.23)	67.21 (0.89)
Circumference of caudal peduncle	20.47 (0.46)	22.89 (1.36)
Length of base of dorsal fin	10.59 (0.26)	12.33 (0.26)
Length of longest dorsal fin ray	11.08 (0.29)	13.02 (1.02)
Length of base of anal fin	11.85 (0.26)	13.51 (0.27)
Length of longest anal fin ray	8.60 (0.25)	10.36 (0.20)
Length of longest pelvic fin ray	9.78 (0.23)	11.69 (0.18)
Length of longest pectoral fin ray	11.76 (0.26)	13.44 (0.22)
Length of adipose fin from anterior end of base to tip.	5.76 (0.28)	6.58 (0.32)

run had the lowest haplotype diversity (0.1716) as well as the lowest nucleotide diversity (0.0019) of all populations.

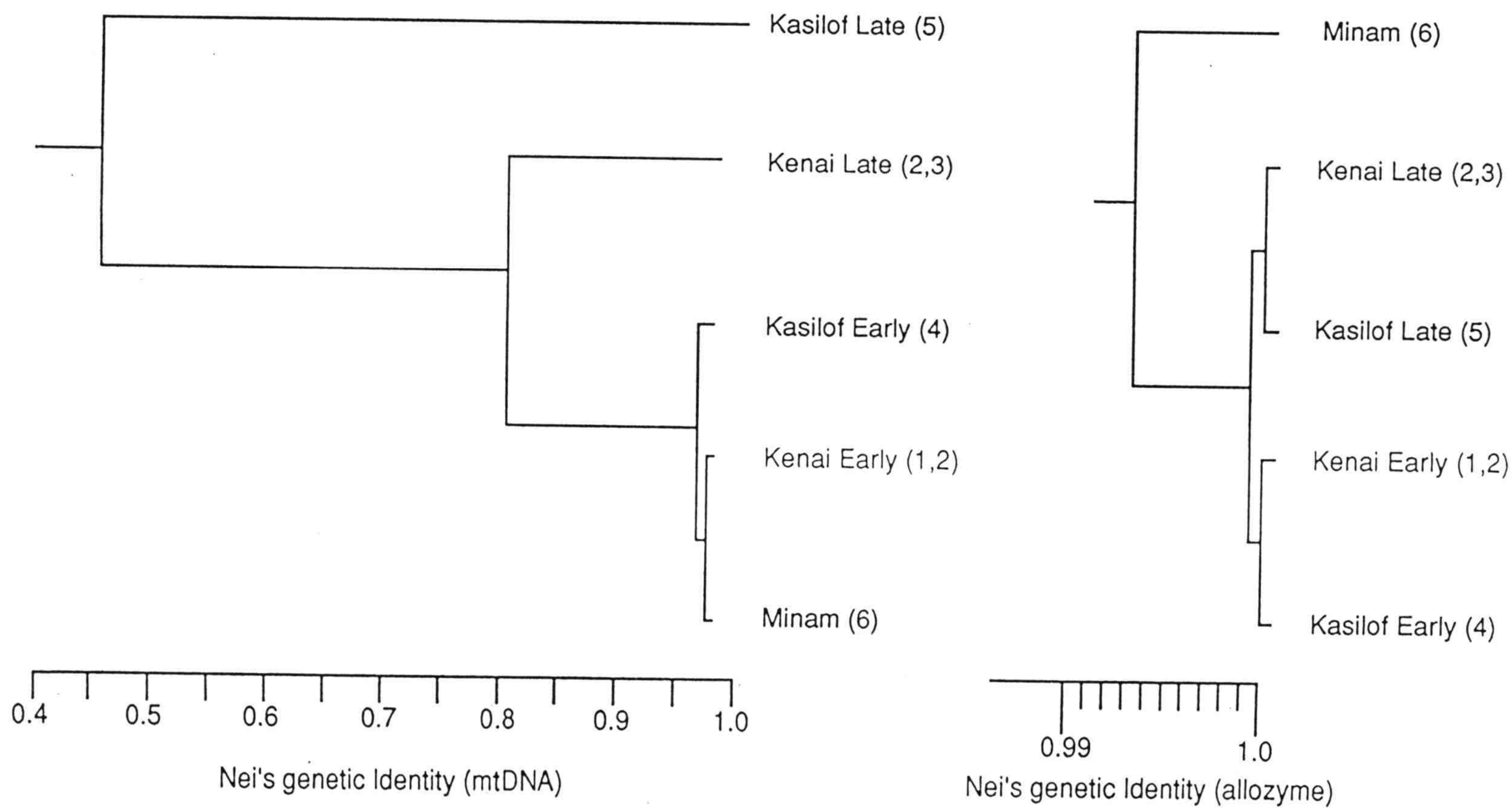
Estimates of Between-Population Diversity

Nucleotide divergence ranged from 0.0001 to 0.0096 and averaged 0.0021 (Table 4). Although these values are low, intraspecific mtDNA divergence values less than 0.01 have been reported for chinook salmon from Alaska and British Columbia (Wilson et al. 1987). The greatest divergence among populations occurred between the Kasilof River late run and the other four populations. The divergence of the Kasilof River late run was, on average, 2.5 times greater than the divergence among the other populations.

The divergence of the Kasilof River late run is clearly depicted in the mtDNA phenogram shown in Figure 2. However, the phenogram depicting the allozyme similarities among the populations does not show a clear divergence of the Kasilof River late run. Instead, the populations appeared to cluster in accordance with their ecological spawning characteristics and geographic locations. The two early runs grouped separately from the two late runs, and a clear distinction exists between the Minam River and Alaska chinook salmon populations. The same groupings did

Figure 2. Phenograms showing genetic relationships among five populations of chinook salmon from the Kenai, Kasilof, and Minam rivers based on mtDNA haplotype and allozyme frequency data. Numbers in parentheses correspond to locations shown in Figure 1.

Figure 2.



not occur when cluster analysis was conducted using mtDNA data. In this case, the Minam River population was similar to the two Alaska early runs and the two late runs were different from each other. An identical mtDNA phenogram resulted when cluster analysis was conducted using the nucleotide divergence values.

DISCUSSION

Based on both mtDNA and allozyme analysis, we identified genetic differences among ecologically different forms of chinook salmon in the Kenai and Kasilof rivers. The largest genetic difference, based on the test of homogeneity using mtDNA data, occurred between the Kasilof River early and late runs. Genetic differences among salmon within a drainage have previously been reported based on allozyme analysis (Currens et al. 1990; Wilmot et al. 1992). However, we could not locate published accounts of mtDNA differences between populations in a drainage that are as temporally or spatially close as the early and late runs in the Kasilof River. The spawning times of early- and late-run chinook salmon in the Kasilof River differ by only two months and their spawning grounds are separated by only 19 river km. Because the Kasilof River early run has been propagated in a hatchery since 1974, a founder effect as well as genetic drift could account for the differences we identified in the Kasilof River populations. To evaluate the potential for founder effect in hatchery populations (Waples 1990), the number of fish used as brood stock since 1974 in the Crooked Creek hatchery was examined. Twelve females (estimated fecundity = 8000) and 19 males from the naturally spawning population in Crooked Creek were used

to start the hatchery population in 1974 (Robert Och, Alaska Department of Fish and Game, personal communication). Chinook salmon take 2 to 5 years to reach sexual maturity. As a result, adults from the naturally spawning population were used in the years following 1974 because the progeny of the initial hatchery population would not have returned to spawn for at least 2 to 5 years. In 1991 and 1992, an average of 68.4 females (estimated fecundity = 6700) and 36.2 males of the fish returning to the hatchery were used to propagate the early run. Although the effective population size (Ne; Hartl and Clark 1989) for the initial spawning was relatively low (N_e=29.42), continued use of wild spawning fish in the years immediately following 1974 and the presence of their haplotypes (D1 and D2) in similar frequencies in the Kenai River populations suggest that the hatchery influence does not account for all of the variability identified in the Kasilof River populations. The genetic difference between the early and late runs in the Kasilof River is further supported by the test of homogeneity (Table 6) and cluster analysis (Figure 2) based on the allozyme data.

Genetic differences were also identified between ecologically different forms in the Kenai River. Both the test of homogeneity and the cluster analysis revealed genetic differences between the early and late runs.

Among the populations that exhibit ecologically similar spawning characteristics, tests of homogeneity between the two early runs in the Kenai and Kasilof rivers showed no statistically significant difference. Conversely, there was a statistically significant difference between the two late runs. The sampling design for obtaining fish from the Kenai River late-run population could explain the difference between the two late runs. Because all but 7 of the 128 fish categorized as late-run salmon were obtained in the lower section of the Kenai River at Poacher's Cove (Figure 1), it is likely that we unintentionally included some early-run fish in the late-run category and artificially altered the haplotype frequencies within the Kenai River late-run group. Studies in previous years showed all fish collected at Poacher's Cove after July 1 to be late-run salmon (Burger et al, 1985; Hammarstrom 1981). During 1991 and 1992, however, early-run salmon may have migrated through the lower river to spawning tributaries after July 1. The only difference between the two late runs is the frequency of the shared haplotypes, D1 and D2. The D2 haplotype predominates in the early runs from both the Alaska rivers. The D1 haplotype is characteristic of late-run fish in both rivers. If Kenai River early-run fish (haplotype D2) were inadvertently included in the late-run group, the results would be an over abundance of

D2 haplotypes in the Kenai River late-run group. This could account for the haplotype frequency difference identified between the two late runs.

The sampling design in the Kenai River might also account for the relatively high values of within-population diversity in the Kenai River late-run. The likely inclusion of some early-run fish in the late-run category may have artificially increased estimates of genetic variability of Kenai River late-run salmon.

Although both mtDNA and allozyme analysis identified similar genetic differences between the populations, genetic identity values indicate that mtDNA analysis was more powerful in discriminating between the groups on a locus by locus basis. Nei's unbiased genetic identity based on haplotype data (1978) ranged from 0.454 to 0.998 in the mtDNA phenogram. Separation between populations according to allozyme data occurred between 0.994 and 0.999 (Fig 2). Put into perspective, all the groupings in the allozyme phenogram occur in the same amount of space as that between the Minam River and Kenai River early-run populations in the mtDNA phenogram.

Although the magnitude of the differences was greater using the mtDNA data, the two phenograms were different. The difference may be a consequence of the reduced effective population size of mtDNA (1/4 that of nuclear DNA) and the resulting increased susceptibility to genetic

drift and bottleneck effects. The difference may also be attributed to the number of loci used in constructing the phenograms. The phenogram depicting the allozyme differences uses 14 loci whereas the mtDNA phenogram is based on only one locus. Based on the single mtDNA locus, the degree of separation between the populations was greater than any one, or all, of the 14 polymorphic allozyme loci. The MEP-2* locus had the greatest frequency difference between populations (Table 5). Nevertheless, when cluster analysis was conducted using this locus no genetic difference was identified among the Alaska populations (Nei's Identity = 1). A distinction was made between the Alaska populations as a whole and Minam River chinook salmon (Nei's Identity = 0.849), however the magnitude of the separation was still less then those identified using mtDNA analysis.

Ecology

Natural selection for differences in spawning habitat may explain the genetic differences between the early and late runs in the Kenai and Kasilof rivers. Late-run salmon return to spawn in the main stem of their respective rivers downstream of glacial-fed lakes. The thermal capacity of lakes may maintain elevated temperatures downstream (Carmack et al. 1979) to enable

successful spawning late in the year. Late-run chinook salmon in the Kenai and Kasilof rivers are the latest known spawning populations in Southcentral Alaska. Spawning by late-run chinook salmon peaks in the Kenai River during late August (Burger et al. 1985) and through mid-September in the Kasilof River (Carl Burger, unpublished data). The warmer water temperatures maintained by the lakes may limit spawning late in the year to areas downstream of lakes (Burger et al. 1985). Thus, late-run fish could not successfully spawn in the same upstream areas as early runs due to suboptimal (colder) water temperatures late in the year.

The importance of temperature in governing the spawning activity of rainbow trout (*Oncorhynchus mykiss*) was demonstrated by Morrison and Smith (1986). They successfully altered the spawning time by manipulating water temperatures. Temperature affects the development rate and viability of gametes prior to spawning and also influences the rate of embryonic development and subsequent emergence of the fry during optimal environmental conditions. Water temperature may be a factor in the reproductive isolation identified between early- and late-run chinook salmon in the Kenai and Kasilof rivers. A similar conclusion was reached in explaining the occurrence of a genetically unique late run

of sockeye salmon in the upper Kasilof River (Carl Burger, unpublished data).

Natural selection for differences in spawning habitat may explain the genetic isolation between populations of chinook salmon in the Kenai and Kasilof rivers. It may also account for the difference in body size observed between the early and late runs in the Kenai River. Late-run chinook salmon spawn almost exclusively in the main stem of the Kenai River (as opposed to the tributaries used by the early run) (Burger et al. 1985), and natural selection may have favored a larger body size. We theorize that larger body size may be an adaptation to the greater water velocities found in the main stem of the Kenai River. This idea is supported by studies on chum salmon (Oncorhynchus keta) (Beacham 1984), brown trout (Salmo trutta) (Yevsin 1977), and Atlantic salmon (Salmo salar) (Jones 1975; Schaffer and Elson 1975; Riddell and Leggett 1981). Breeding experiments conducted under controlled conditions have shown that these interpopulational differences in morphological characteristics are heritable and represent adaptations to natal rearing environments (Riddell and Leggett 1981). Further information is needed to verify that the same adaptations have occurred in the Kenai River populations. Nevertheless, difference in fish size between the two runs correlates with the genetic differences we identified

between populations that exhibit different ecological spawning characteristics.

Colonization and Evolution

Our results can be used to support either of two current theories concerning the colonization and evolution of the Alaska chinook salmon. One theory (Bartley and Gall 1990) suggests that colonization of glaciated areas in Alaska took place approximately 10,000 years ago by chinook salmon from the Columbia River and Bering Sea. The similarity in distribution of the D1 and D2 haplotypes among Minam River and the two early runs in the Kenai and Kasilof rivers supports this hypothesis. The frequency of the shared haplotypes suggests that an ancestral form from the Columbia River may have colonized the early runs in the Kenai and Kasilof rivers. Genetically different late runs in both rivers may have diverged later due to local adaptation and reproductive isolation. Evidence in favor of the divergence of the two late runs is shown by the cluster analysis based on allozyme data. The two early and two late runs were more similar to each other then to the late runs (Figure 2).

It is also possible that recolonization occurred out of a central Alaskan refugium (Cronin et al. 1993; Gharrett et al. 1987). If populations with similar mtDNA

lineages survived in each of the Alaskan and Columbia River refugia, then the shared haplotypes in the Minam and Alaskan populations could be explained. As previously mentioned, a more recent divergence of the two late runs in the Kenai and Kasilof rivers would explain the within-basin differences we identified.

Equally plausible is that allopatric divergence may account for the genetic differences we identified. The potential existence of an Alaskan and Columbian refugia during glaciation may have resulted in genetic differences due to reproductive isolation with the Alaskan fish utilizing lakes to facilitate successful spawning late in the year and Columbian fish spawning in the rivers early in the year. The Columbian fish could have subsequently spread north with the retreat of the glaciers and either displaced any river spawning populations in Alaska or simply colonized unused river spawning habitat.

SUMMARY

Conserving genetic diversity among populations of fish is critical to species survival (Nelson and Soule' 1987; Meffe 1986; Allendorf et al. 1987). The first step towards genetic conservation is to identify those groups that constitute separate breeding populations (Larkin 1981). Our results demonstrate the usefulness of mtDNA and protein electrophoresis techniques in identifying separate breeding populations that are temporally and spatially close. Other studies have used mtDNA techniques to identify differences between stocks of the same species, yet little published data exist that identify significant genetic differences between runs within the same drainage. Furthermore, our ability to consistently identify haplotypes of each individual at the two laboratories demonstrates the repeatability of the mtDNA techniques we used. The replicate tests also demonstrated that mtDNA from muscle or liver tissue can be used to achieve identical results.

Results of this study suggest that sufficient variation exists to develop a genetic baseline for stocks of chinook salmon originating in Cook Inlet in Alaska. This information would aid in the management of the species in Alaska and contribute to the coast-wide management of the species.

Our results also demonstrate the potential loss of genetic diversity that could occur by ignoring ecological and morphological differences that exist among chinook salmon within or between a drainage.

LITERATURE CITED

AEBERSOLD, P.B., G.A. WINANS, D.J. TEEL, G.B. MILNER, AND F.M. UTTER. 1987. Manual for starch gel electrophoresis: a method for the detection of genetic variation. NOAA Technical Report NMFS 61. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Springfield, Virginia.

ALLENDORF,F.W., AND S.R. PHELPS. 1981. Use of allelic frequencies to describe population structure. Can. J. Fish. Aquat. Sci. 38: 1507-1514.

ALLENDORF,F.W., N. RYMAN, AND F.M. UTTER. 1987. Genetics and fishery management: past, present, and future. Pages 1-19 in N. Ryman and F.M. Utter, eds. Population genetics and fishery management. University of Washington Press, Seattle, WA.

AVISE, J.C., C. GIBLIN-DAVIDSON, J. LAERM, J.C. PATTON, AND R. A. LANSMAN. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of pocket gophers, <u>Geomys pinetis</u>. Pro. Nat. Aca. Sci. U.S.A. 76: 6694-6698.

BARTLEY, D.M., AND G.A.E. GALL. 1990. Genetic structure and gene flow in chinook salmon populations in California. Trans. Am. Fish. Soc. 119: 55-71.

BEACHAM, T.D. 1984. Age and morphology of chum salmon in southern British Columbia. Trans. Am. Fish. Society 113: 727-736.

BIRKY, C.W., T. MARUYAMA, AND P. FUERST. 1983. An approach to population genetic and evolutionary genetic theory for genes in mitochondrial and chloroplasts, and some results. Genetics. 103: 513-527.

BROWN, W.M., M. GEORGE, Jr., AND A.C. WILSON. 1979. Rapid evolution of animal mitochondrial DNA. Pro. Nat. Aca. Sci. U.S.A. 76: 1967-1971.

BURGER, C.V., R.L. WILMOT, AND D.B. WANGAARD. 1985. Comparison of spawning areas and times for two runs of chinook salmon (<u>Oncorhynchus tshawytscha</u>) in the Kenai River, Alaska. Can. J. Fish. Aquat. Sci. 42: 693-700.

CARMACK, E.C., C.B.J. GRAY, C.H. PHARE, AND R.J. DALEY. 1979. Importance of lake-river interactions on seasonal patterns in the general circulation of Kamloops Lake, British Columbia. Limnol. Oceanogr. 24:634-644.

CRONIN, M.A., W.J. SPEARMAN, R.L. WILMOT, J.C.PATTON, AND J.W. BICKHAM. 1993. Mitochondrial DNA variation in chinook (Oncorhynchus tshawytscha) and chum salmon (O keta) detected by restriction enzyme analysis of polymerase chain reaction (PCR) products. Can. J. Fish. Aquat. Sci. 50: 708-715.

CURRENS, K.P., C.B. SCHRECK, AND H.W. LI. 1990. Allozyme and morphological divergence of rainbow trout (Oncorhynchus mykiss) above and below waterfalls in the Dechutes River, Oregon. Copeia. 3: 730-746.

FAUROT, D., AND R.N. JONES. 1990. Run timing and spawning distribution of coho and late-run chinook salmon in the Kasilof River watershed, Alaska, 1987. Alaska Fisheries Technical Report No. 9, U.S. Fish and Wildlife Service, Kenai, Alaska.

GHARRETT, A.J., S.M. SHIRLEY, AND G.R. TROMBLE. 1987. Genetic relationships among populations of Alaskan chinook salmon (Oncorhynchus tshawytscha). Can J. Fish. Aquat. Sci. 44: 765-774.

GILES, R.E., H. BLANC, H.M. CANN, AND D.C. WALLACE. 1980. Maternal inheritance of human mitochondrial DNA. Pro. Nat. Acad. Sci. U.S.A. 77: 6715-6719.

GYLLENSTEN, U., D. WHARTON, AND A.C. WILSON. 1985. Maternal inheritance of mitochondrial DNA during backcrossing of two species of mice. J. Heredity 76: 321-324.

GYLLENSTEN, U. AND A.C. WILSON. 1987. Mitochondrial DNA of salmonids: Inter- and intraspecific variability detected with restriction enzymes. Pages 301-317 in N. Ryman and F.M. Utter, eds. Population genetics and fishery management. University of Washington Press, Seattle, WA.

HAMMARSTROM, S.L. 1981. Evaluation of chinook salmon fisheries of the Kenai Peninsula. Alaska Dep. Fish Game, Annu. Rep., 1980-1981. Project F-9-13, 22(G-II-L): 39-66.

HARTL, D.L., AND A.G. CLARK. 1989. Principles of population genetics, second edition. Sinauer associates, inc., Sunderland, Massachusetts. 682 p.

HÉNAULT, M., AND R. FORTIN. 1989. Comparison of meristic and morphometric characters among spring- and fall-spawning ecotypes of cisco (Coregonus artedii) in southern Quebec, Canada. Can. J. Fish. Aquat. Sci. 46: 166-173.

HUTCHISON, C.A., J.E. NEWBOLD, S.S. POTTER, AND M.H. EDGELL. 1974. Maternal inheritance of mammalian mitochondrial DNA. Nature 251: 536-537.

INTERNATIONAL UNION OF BIOCHEMISTRY NOMENCLATURE COMMITTEE. 1984. Enzyme nomenclature, 1984. Academic Press, Orlando, Florida.

JONES, A.N. 1975. A preliminary study of fish segregation in salmon spawning streams. J. Fish Bio. 7: 95-104.

KRISTIANSSON, C., AND J. MCINTYRE. 1976. Genetic variation in chinook salmon (Oncorhynchus tshawytscha) from the Columbia River and three Oregon coastal rivers. Trans. Am. Fish. Soci. 105: 620-623.

LANSMAN, R.A., R.U. SHADE, J.F. SHAPIRA, AND A.C. AVISE. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. J. Mol. Evol. 17: 214-226.

LARKIN, P.A. 1981. A perspective on population genetics and salmon management. Can. J. Fish. Aquat. Sci. 38: 1469-1475.

MCISAAC, D.O., AND T.P. QUINN. 1988. Evidence for a hereditary component in homing behavior of chinook salmon (Oncorhynchus tshawytscha). Can. J. Fish. Aquat. Sci. 45: 2201-2205.

MEFFE, G.K. 1986. Conservation genetics and the management of endangered fishes. Fisheries 11: 14-22.

MORRISON, J.K., AND C.E. SMITH. 1986. Altering the spawning cycle of rainbow trout by manipulating water temperature. Pro. Fish. Cult. 48: 52-54.

NEI, M. 1972. Genetic distance between populations. Am. Nat. 106: 283-292.

------. 1973. Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. 70: 3321-3323.

------. 1975. Molecular population genetics and evolution. American Elsevier, New York, New York.

------. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

------. 1987. Molecular evolutionary genetics. Columbia University Press. New York.

NEI, M., AND F. TAJIMA. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97: 145-163.

NELSON, K., AND M. SOULE'. 1987. Genetical conservation of exploited fishes. Pages 345-368 in N. Ryman and F.M. Utter, eds. Population genetics and fishery management. University of Washington Press, Seattle, WA.

QUINN, T.P. 1982. Homing and straying in Pacific salmon. Pages 257-263 in J.D. McCleave, G.P. Arnold, J.J. Dodson, and W.H. Neill, editors. Mechanisms of migration in fish. Plenum Press, New York.

RIDDELL, B.E., AND W.C. LEGGETT. 1981. Evidence of an adaptive basis for geographic variation of body morphology, and time of downstream migration of juvenile Atlantic salmon (Salmo salar). Can. J. Fish. Aquat. Sci. 38: 308-320.

SCHAFFER, W.M., AND P.F. ELSON. 1975. The adaptive significance of variation in life history among local populations of Atlantic salmon in North America. Ecology 56: 577-590.

SNEATH, P.H.A., AND R.R. SOKAL. 1973. Numerical taxonomy: the principles and practice of numerical classification. W.H. Freeman & Co., San Francisco, California.

SOKAL, R.R., AND F.J. ROHLF. 1981. Biometry. Second edition. W.H. Freeman and Company, New York. 859 p.

TAYLOR, E.B., AND J.D. MCPHAIL. 1985. Variation in body morphology among British Columbia populations of coho salmon, Oncorhynchus kisutch. Can. J. Fish. Aquat. Sci. 42: 2020-2028.

THOMAS, W.K., R.E. WITHLER, AND A.T. BECKENBACH. 1986. Mitochondrial DNA analysis of Pacific salmonid evolution. Can J. Zool. 64: 1058-1064.

UTTER, F.M., F.W. ALLENDORF, AND H.O. HODGINS. 1973. Genetic variability and relationships in Pacific salmon and related trout based on protein variations. Syst. Zool. 22: 257-270.

UTTER, F., G. MILNER, G. STAHL, AND D. TEEL. 1989. Genetic population structure of chinook salmon, <u>Oncorhynchus tshawytscha</u>, in the Pacific Northwest. Fish. Bull. 87: 239-264.

WAPLES, R.S. 1990. Conservation Genetics of Pacific Salmon. II. Effective population size and the rate of loss of genetic variability. J. Heredity. 81: 267-276.

WILSON, G.M., W.K. THOMAS, AND A.T. BECKENBACH. 1985. Intra-and interspecific mitochondrial DNA sequence divergence in <u>Salmo</u>: rainbow, steelhead, and cutthroat trout. Can. J. Zool. 63: 2088-2094.

WILSON, G.M., W.K. THOMAS, AND A.T. BECKENBACH. 1987. Mitochondrial DNA analysis of Pacific Northwest populations of <u>Oncorhynchus</u> <u>tshawytscha</u>. Can. J. Fish. Aquat. Sci. 44: 1301-1305.

WILMOT, R.L., R. EVERETT, W.J. SPEARMAN, AND R. BACCUS. 1992. Genetic stock identification of Yukon River chum and chinook salmon 1987 to 1990. Alaska Fish and Wild. Res. Cen., U.S. Fish and Wild. Ser. Prog. Rep. 132 p.

WINANS, G.A. 1989. Genetic variability in chinook salmon stocks from the Columbia River basin. Nort. Am. J. Fish. Man. 9: 47-52.

YEVSIN, Y.N. 1977. Morphological characteristics and variability of the summer sea trout (<u>Salmo</u> <u>trutta</u>) from the Pulmg'a and Malaya Kumzhevaya River. J. Ichthyol. 17: 350-356.