

GENETIC STUDY OF SOME ALASKAN CHINOOK
POPULATIONS AND THE POTENTIAL FOR USE OF
THIS INFORMATION ON STOCK SEPARATION PROBLEMS

By

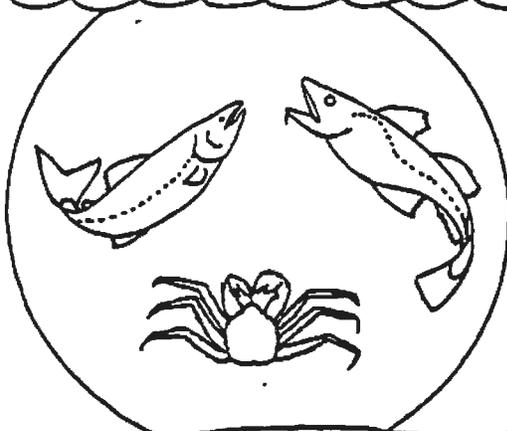
A. J. Gharrett
Associate Professor

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FINAL REPORT

to

Alaska Sea Grant College Program
Renewable Marine Resource
Project No. R/06-17



School of Fisheries
and Science

UNIVERSITY OF ALASKA, JUNEAU

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Introduction

The ownership and allocation of North American chinook salmon (Oncorhynchus tshawytscha) stocks are very much in question at the present time. Treaty renegotiations are currently taking place between the United States and Canada regarding the interception of the various species of Pacific salmon (Oncorhynchus sp.) by both U.S. and Canadian fishermen. There is also considerable controversy regarding the enumeration and subsequent allocation of fish originating in waters traditionally fished by native Americans. Because it is the most valuable species both in price per pound and price per fish, chinook salmon is a focal point for these problems.

The controversies are not limited to southeastern Alaska, British Columbia, and the northern Puget sound where chinook are primarily harvested by the troll fishery. Problems also occur in the western Alaska-Bering Sea area where chinook are incidentally caught by foreign trawlers and where many of the fish originate in the Canadian reaches of the Yukon River.

Many of the problems facing negotiators, allocators, and managers would be eliminated if it were possible to ascertain the origin of each fish caught or to accurately estimate the relative contribution to particular fisheries of stocks originating from different regions. Several techniques have been used in attempts to obtain this information: coded microwire tagging of juveniles,

marking of adults, scale pattern analysis, and biochemical genetic studies.

Coded micro-wire tags have been employed to mark hatchery releases and wild fish captured while migrating to sea. When such a fish is caught in the fishery there is no doubt as to its origin; however, such tagging is expensive and it is not possible to mark most of the wild fish in this manner.

Hoffman (1982) tagged adult pink salmon (O. gorbuscha) in salt water in an effort to determine their migration routes and timing. Similar work is currently being conducted on sockeye salmon (O. nerka) as a part of the U.S.-Canada treaty in order to determine which stocks are being exploited and by each nation. These species are particularly amenable to this kind of study because relatively large numbers can be captured for marking. This is not possible for chinook salmon which are less abundant and do not tend to school.

Scale-pattern analysis makes use of the fact that scale deposition reflects the environment to which a fish has been exposed (Krasnowski and Bethe 1979). Fish sharing the same environment would have similar scale patterns. Studies are presently being conducted on chinook salmon by the Alaska Department of Fish and Game (ADF&G).

The other technique that is applied to stock identification and separation problems is starch gel electrophoresis which genetically characterizes populations with respect to a number of

biochemically detectable genetic loci. The success of this technique depends on the extent of divergence among reproductively isolated populations. Divergence results from random changes that occur as a result of small (finite) population sizes or from directional influences such as selection. This technique is presently being used in the management of Canadian chum (O. keta) salmon stocks (Beacham et al. 1985). Milner and Utter (eg. 1981) have characterized the genetic compositions of Columbia River chinook salmon populations and successfully used these data to determine the destination of fish caught near the river mouth. These studies have been extended to coastal chinook populations (Milner et al. 1983).

Genetic compositions of stocks of fish can also be used to obtain information pertaining to diversity and number of populations of fish in a region, for brood stock selection and management in hatcheries (eg. Ryman and Staahl 1980), and for inferring relationships among populations (eg. Utter et al. 1980). Characterization of the genetic compositions of chinook populations has revealed differences among populations related to their watershed and to the timing of spawning runs (Utter et al. 1980). Studies in British Columbia indicate allozyme differences related to juvenile life history (Carl and Healey 1984).

This project was initiated to procure baseline data on the genetic compositions of Alaskan chinook populations and to examine the data to determine whether sufficient diversity exists

among the different populations within a region or between populations of different regions to be useful for stock separation purposes.

Ideally the baseline would reflect as many spawning populations as possible. Unfortunately, very little is known about Alaskan chinook populations. Many of the river systems that support chinook salmon are remote and glacial or turbid. Accurate population estimates for population size are rare; and for many larger systems such as the Yukon River, the locations of spawning grounds are not known. The largest problem encountered in this project has been the logistical problem of obtaining fresh or fresh-frozen tissue samples from all the major spawning populations in Alaska. It is not possible to sample a stock whose existence is unknown; in addition, transport of high-quality samples from remote sites to the laboratory is often impossible. In order to perform the most complete survey possible, we have requested and obtained the cooperation of state and federal agencies in procurement of tissue samples. We have often had to compromise and use samples of fish caught on their spawning runs, rearing in the stream, or migrating as smolts to salt water rather than samples from spawners on the spawning grounds. These samples should be representative of the general area from which they were taken if not of discrete breeding populations.

The genetic relationships among the populations of Alaskan chinook for which we were able to obtain data are reported in the

Canadian Journal of Fisheries and Aquatic Sciences (Gharrett et al. 1987). The relationship of Alaskan chinook populations to some more southern non-Alaskan, wild populations previously described by Milner et al. (1983) was also discussed. In this report we will briefly review those results and examine the utility of the data for stock identification purposes.

Material and Methods

Samples

Thirty-seven collections of young chinook salmon or tissue samples from adults, representing more than 2500 individuals, were taken from or near thirteen major Alaskan river systems (Figures 1 and 2 and Table 1). Tissues sampled from adults were heart, eyes, liver, and skeletal muscle. Juveniles were collected whole. Samples were kept on ice until they were frozen and stored at -20 C or -80 C. In most river systems, collections were made at more than one location and/or in more than one year.

Electrophoresis

Sample preparation and electrophoresis followed methods of Utter et al. (1974). The enzymatic activities were stained using methods described by Harris and Hopkinson (1976) and modified for use in our laboratory (McGregor 1982; Lane 1984). Genetic loci, the tissues in which they were observed, and the buffers in which they were resolved are presented in Table 2. Table 3 details the buffer systems used.

Figure 1. Sites of collection of Alaskan chinook salmon from western Alaska. Numbers and letters correspond to collections listed in Table 1.

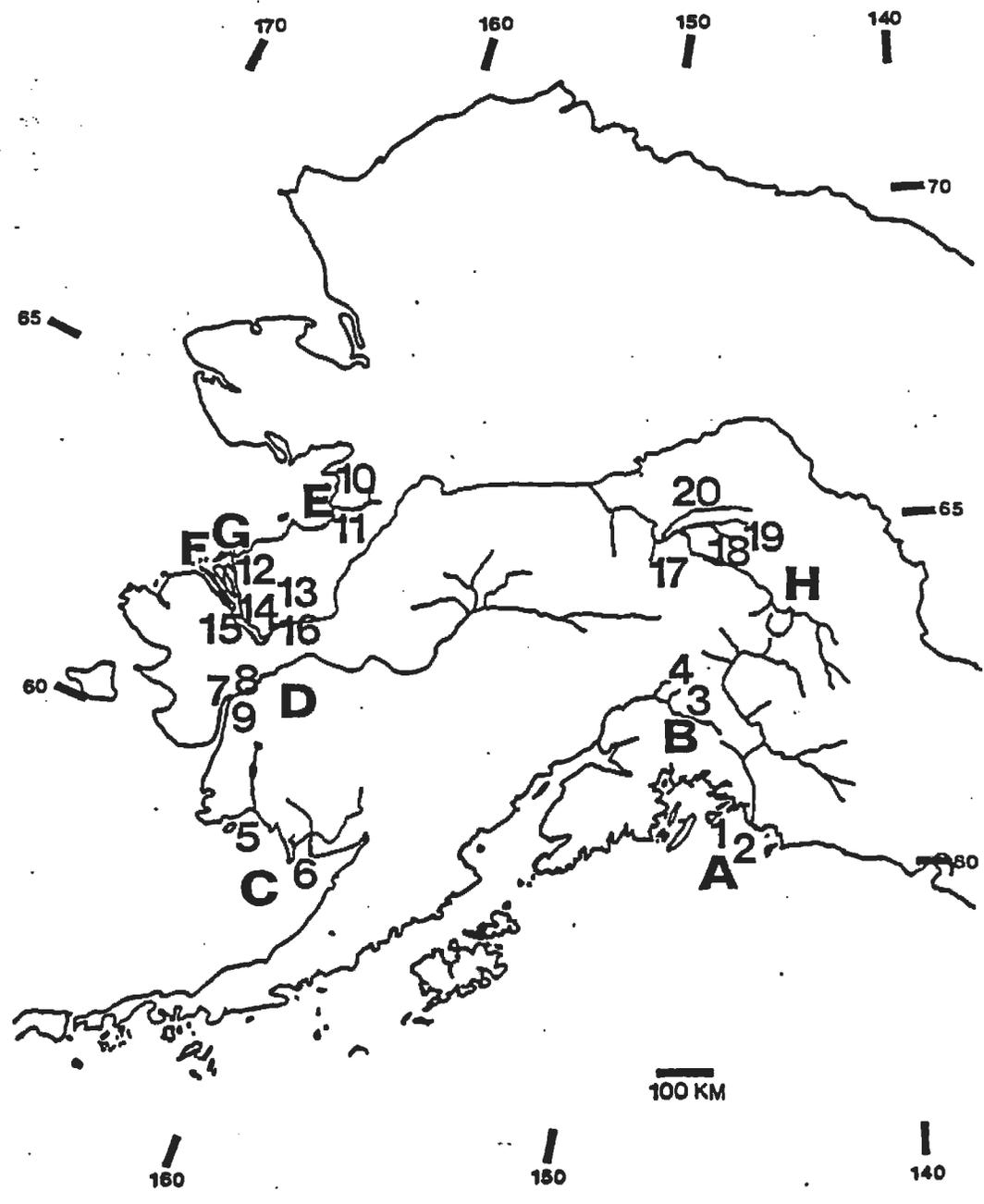


Figure 2. Sites of collection of Alaskan chinook salmon from southeastern Alaska. Numbers and letters correspond to collections listed in Table 1.

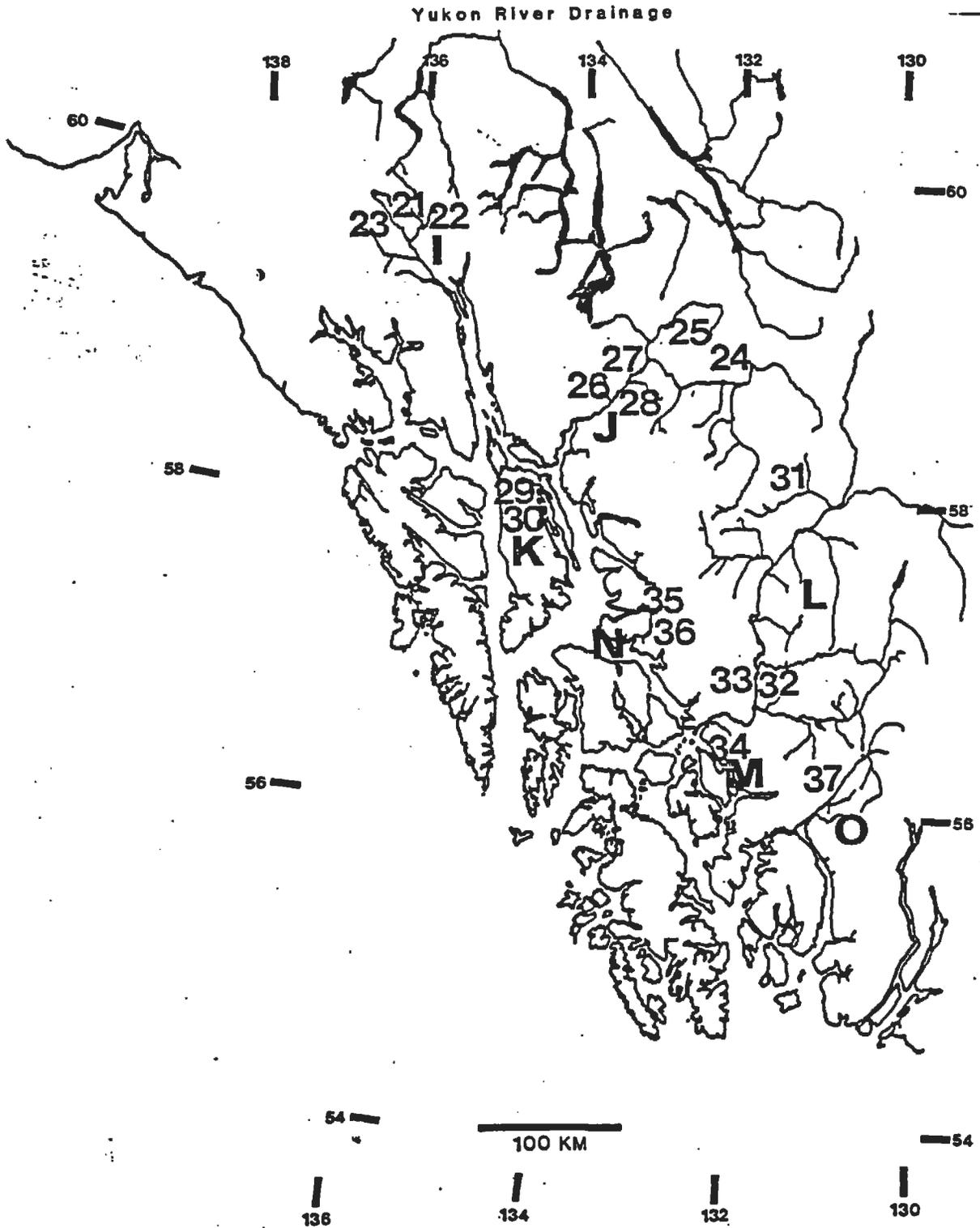


Table 1. Site, year, type of collections of chinook salmon reported in this study and agency making collection. Collections are numbered as in Figure 1, and grouped under designations for drainages and subdivisions of drainages. Agencies are ADF&G FRED Division (FRED), ADF&G Commercial Fish Division (CF), ADF&G Sport Fish Division (SF), Susitna Hydro Project (SH), Alaska Cooperative Fishery Research Unit (ACRFU), and ourselves (UAJ).

Group	Drainage	Collection No.	Site	Year	Type	Agency
A	Copper River	1.	gillnet fishery	1983	adults	CF
		2.	gillnet fishery	1984	adults	CF/UAJ
B	Susitna River	3.	Indian R.	1983	juveniles	SH
		4.	Indian R.	1984	juveniles	SH
C	Bristol Bay	5.	Togiak gillnet fishery	1983	adults	UAJ
		6.	Dillingham gillnet fishery	1983	adults	UAJ
D	Kuskokwim	7.	gillnet fishery	1982	adults	CF
		8.	gillnet fishery	1983	adults	UAJ
		9.	gillnet fishery	1984	adults	UAJ
E	Unalakleet	10.	gillnet fishery	1982	adults	CF
		11.	gillnet fishery	1983	adults	CF
F	Lower Yukon I	12.	Emmonak gillnet fishery	1982	adults	CF
		13.	Big Eddy test fishery	1982	adults	CF
		14.	Big Eddy test fishery	1983	adults	CF
G	Lower Yukon II	15.	Emmonak gillnet fishery	1983	adults	UAJ
		16.	St. Mary's gillnet fishery	1983	adults	UAJ
H	Tanana River	17.	Lower Chena R.	1982	juveniles	ACRFU
		18.	Upper Chena R.	1982	juveniles	ACRFU
		19.	Lower Chena R.	1983	juveniles	ACRFU
		20.	Chatinika R.	1984	juveniles	ACRFU
I	Chilkat River	21.	Tahini R. egg-take	1983	adults	FRED
		22.	Tahini R. egg-take	1984	adults	FRED
		23.	Tahini R.	1984	juveniles	SF
J	Taku River	24.	Nahlin R.	1982	juveniles	SF
		25.	Lower Nakina	1982	juveniles	SF
		26.	Mainstem	1981	juveniles	SF
		27.	Mainstem	1982	juveniles	SF
		28.	King Salmon Cr.	1982	juveniles	SF
		29.	Admiralty Island egg-take	1983	adults	FRED
K	King Salmon R.	30.	Admiralty Island egg-take	1984	adults	FRED
		31.	Little Tahltan	1981	juveniles	CF
L	Upper Stikine	32.	Mainstem	1981	juveniles	CF
		33.	Mainstem	1982	juveniles	SF
		34.	Andrew Cr. egg-take	1982	adults	FRED
M	Lower Stikine	34.	Andrew Cr. egg-take	1982	adults	FRED
N	Farragut River	35.	egg-take	1983	adults	FRED
		36.	egg-take	1984	adults	FRED
O	Unuk River	37.	mainstem	1982	juveniles	SF

Table 2. Protein coding loci, their Enzyme Commission (E.C.) numbers (International Union of Biochemistry 1984) and designations (May 1980), and the tissues and buffers in which they were resolved. The peptidase loci are designated according to their substrate specificity. Buffers used for electrophoresis were I (Ridgway et al. 1970), II (Markert and Faulhaber 1965), III (Clayton and Tretiak 1972), and IV a pH 7.0 tris-(hydroxymethyl)aminomethane buffer described by Shaw and Prasad (1970). The enzymatic activities were stained using methods described by Harris and Hopkinson (1976) and Shaw and Prasad (1970) modified for use in our laboratory (McGregor 1982; Lane 1984).

Enzyme	E.C. Number	designation	tissue	buffer
Alcohol dehydrogenase	1.1.1.1	<u>Adh</u>	liver	II&III
Aspartate aminotransferase	2.6.1.1	<u>Aat-3</u>	eye	I
Creatine kinase	2.7.3.2	<u>Ck-1</u>	muscle	I
		<u>Ck-2</u>	muscle	I
Glucose-6-phosphate isomerase	5.3.1.9	<u>Gpi-3</u>	muscle	I
Glycerol-3-phosphate dehydrogenase	1.1.1.8	<u>G3p-1</u>	heart	III
		<u>G3p-2</u>	heart	III
		<u>G3p-3</u>	heart	III
Isocitrate dehydrogenase	1.1.1.42	<u>Idh-3,4</u>	liver, eye	IV
Lactate dehydrogenase	1.1.1.27	<u>Idh-1</u>	muscle	I
		<u>Idh-3</u>	heart	I
		<u>Idh-4</u>	liver	I
		<u>Idh-5</u>	eye	I
Mannose-6-phosphate isomerase	5.3.1.8	<u>Mpi</u>	heart, eye	II
Malate dehydrogenase	1.1.1.37	<u>Mdh-1,2</u>	liver, muscle	III
		<u>Mdh-3,4</u>	muscle	III
Peptidases:	3.4.11/13			
Leucyl-glycyl-glycine activity		<u>Pep(Lgg)</u>	muscle	I
Phenylalanyl-proline activity		<u>Pep(Pp-1)</u>	heart, muscle	II
		<u>Pep(Pp-2)</u>	heart, muscle	II
glycyl-leucine activity		<u>Pep(Gl-1)</u>	muscle	II
		<u>Pep(Gl-2)</u>	eye	II
Phosphoglucomutase	5.4.2.2	<u>Pgm-2</u>	muscle	I
Phosphogluconate dehydrogenase	1.1.1.44	<u>Pgd</u>	liver	IV
Superoxide dismutase	1.15.1.1	<u>Sod-1</u>	liver	II
		<u>Sod-2</u>	liver	II

Table 3. Buffer systems used in this study. The designation used for each buffer is in parentheses.

1. Ridgway *et al.* (1970) (I)

gel buffer pH 8.5
0.03 M tris(hydroxymethyl)amino methane
0.005 M citric acid

electrode buffer pH 8.1

0.06 M lithium hydroxide
0.3 M boric acid

Gels are made using 99% gel buffer and 1% electrode buffer.

2. Markert and Faulhaber (1965) (II)

stock solution pH 8.7

0.9 M tris(hydroxymethyl)amino methane
0.5 M boric acid
0.02 M disodium ethylenediamine tetraacetate (EDTA)

Gels are made from a 1:20 dilution of stock solution
Electrode buffer is made from a 1:5 dilution of stock solution.

3. Clayton and Tretiak (1972) (III)

electrode buffer pH 6.1

0.04 M citric acid
adjusted to pH 6.1 with N-(3-aminopropyl)-morpholine

Gels are made from a 1:20 dilution of electrode buffer.

4. Shaw and Prasad (1970) (IV)

electrode buffer pH 7.0

0.155 M tris(hydroxymethyl)amino methane
0.043 M citric acid

Gels are made from a 1:20 dilution of electrode buffer.

Analysis

Departure of the genotypic frequencies of collections from Hardy-Weinberg equilibrium expectations was examined using Chi-square goodness-of-fit tests with a continuity correction of 0.25 (Emigh 1980). Pooling of genotypic frequencies was done where necessary (Sokal and Rohlf 1981, ch. 16).

Homogeneity of allelic frequencies among collections within a drainage, among drainages within a region, and among regions was examined using log-likelihood ratios (G-test) (Sokal and Rohlf 1981). Levels of heterogeneity were compared using F-distributions: $F(v_1, v_2) = (G_1/v_1)/(G_2/v_2)$.

The pooled data were analyzed using a number of techniques. The intent of applying these various analyses was to quantify the divergence among the collections and infer relationships among them. These data were compared with those of chinook populations from other geographical locations. The extent of divergence determines the extent to which biochemical genetic data may be used for stock identification.

Relationships among the collections were examined by principal component analysis and genetic distances. In principal component analysis, data from several loci are used to graphically portray the variability that exists among the collections by combining the variability that occurs at multiple loci into a smaller number of components, the "principal components". The data for each population are expressed as a

vector and the multi-dimensional space in which all the vectors lie is rotated to find planes which show the maximum variation among the populations (vectors).

Genetic distances are used to measure the extent of divergence in biochemical genetic compositions among populations (Nei 1972, Rogers 1972). From genetic distances, relationships among populations can be visualized with a dendrogram. As in principal component analysis, data for the loci used must exist in all collections to estimate genetic distance. For the data presented here, this means that fewer loci and more collections may be compared, or more loci and fewer collections. Several different combinations of loci and collections and several different clustering algorithms were used to form dendrograms; all depicted similar relationships.

Another way to visualize the relationships among the collections is with a tree. Such trees were obtained with Felsenstein's CONTML program (1973 and 1984). This unrooted tree (network) is constructed from a chord measure of genetic distances (Cavalli-Sforza and Edwards 1967) and by computing the topology with the maximum likelihood for the genetic distances between all pairs included in the data set. The program was run as recommended by the author.

Gene diversity analysis (Nei 1973; Chakraborty 1980) was used to decompose the genetic variability (heterozygosity) into hierarchical levels: $H_T = H_D + D_{DR} + D_{RT}$, where H_T is the

total heterozygosity observed, H_D the average heterozygosity within drainages (or major subdivisions of drainages) within regions, $D_{DR} = H_R - H_D$ the variability attributable to diversity among drainages within regions, and $D_{RT} = H_T - H_R$ that among regions. Coefficients of gene differentiation (Nei 1973) were computed to estimate the proportion of total gene diversity due to each level of hierarchy.

Simulations

The ability of the baseline data to discriminate among stocks was examined for several situations by computer modelling. Simulations were performed (see Figure 3) by initially defining the composition of a mixture of stocks and constructing the mixture with a computer. A mixture was produced by drawing individuals from each contributing stock according to the predefined stock composition of the mixture. Individuals were represented by their genotypes across all electrophoretic loci used in the simulation, and the genotypes of individuals drawn from a stock were determined from baseline data for that stock. The random element in the "sampling" genotypes from stocks means that mixtures constructed independently by this method are expected to vary in composition.

An EM algorithm (Dempster et al. 1977) described fully by Pella and Milner (1987) was used to estimate the composition of the mixture constructed as described. If the contributing stocks

Stock Separation Capability

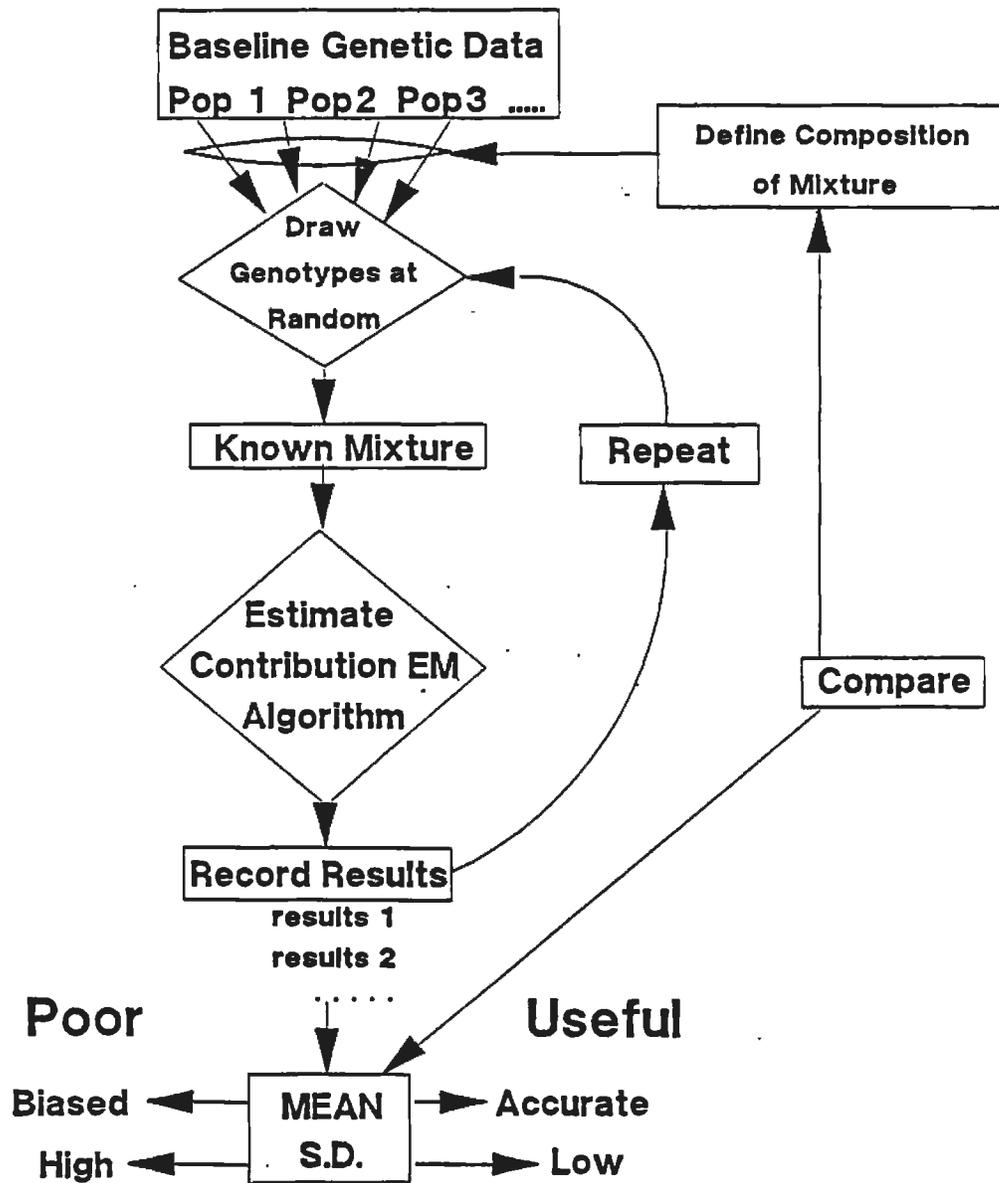


Figure 3. Flow diagram for simulations to determine stock separation capability of baseline data.

are sufficiently distinct, it is expected that the estimates of contribution would be quite close to the actual composition. If contributing stocks are not as distinct, estimates would not as closely reflect the actual composition.

The ability of an algorithm to estimate compositions can be examined by repeating the sampling and estimating procedure a number of times and examining the means and standard deviations of the estimates of contribution. Over a large number of iterations of the "sampling" and estimating process, the average contribution of a stock should be close to the actual composition. In addition, the smaller the deviation of estimates between iterations, the more accurately the estimation process is performing. Of course, the ability of the estimation process depends on the degree of differentiation among the stocks as reflected in the baseline.

Results

Electrophoresis

Approximately forty loci were routinely stained, but data are presented for only the twenty-eight loci we consider reliable. It was not possible to collect data for all loci for all collections because of the low activity of some enzymes (or small amounts of tissue) in fry or loss of activity during storage and shipping. Among the loci not presented were

phosphoglucose isomerase (Pgi-1,2), adenosine deaminase loci (Kobiashi et al. 1984), and aconitase (Aco-2), all of which displayed variability but could not be scored reliably.

Of the twenty-eight biochemical genetic loci presented (Appendix I), twelve were monomorphic and identical in all collections; six loci which had low levels of polymorphism (frequency of the common allele > 0.95 in all collections) were Gpi-3, Ldh-4, Pep(Gl-2), Pep(Pp-2), and isoloci (Allendorf and Thorgaard 1984) Mdh-1,2. Loci displaying more polymorphism (the frequency of the most common allele < 0.9 in at least one collection) were Mpi, Sod-1 and -2, Pep(Gl-1), Pep(Lgg), Aat-3, and isoloci Mdh-3,4 and Idh-3,4. No deviations from Hardy-Weinberg equilibrium expectations were observed ($P > 0.05$); however, only large deviations would be detectable given the collection sizes.

Neither spatial nor temporal variability was observed in the systems other than the Stikine and Yukon ($P < 0.001$) (Table 4). Heterogeneity within the Stikine River was attributable to the collection taken from Andrew Creek, near the mouth of the Stikine River suggesting that divergence has occurred between upstream and downstream chinook populations.

Heterogeneity within the Yukon could be partitioned into three groups within which no significant heterogeneity existed, two groups of lower Yukon gillnet and test fishery collections (Lower Yukon I and II) and the collections from the Tanana River

Table 4. Heterogeneity of Alaskan chinook salmon. Log-likelihood ratio analysis (Sokal and Rohlf 1981) of allelic frequencies. Group designations refers to drainages or subdivisions within drainages (see Table 1). No test indicates presence of a single collection.

Group Designation	Region/Drainage	Heterogeneity Within Drainages		Heterogeneity in Drainage Subdivisions		Heterogeneity within Regions	
		G	df	G	df	G	df
South Central Alaska						85.94	6 *
A	Copper River	3.17	4				
B	Susitna River	5.37	1				
Western Alaska						305.14	30 *
C	Bristol Bay	3.87	4				
D	Kuskokwim River	3.68	5				
E	Unalakleet River	0.82	1				
	Yukon River	106.14	17*				
F	lower river I			12.27	6		
G	lower river II			5.13	4		
H	Tanana River			18.90	9		
Southeastern Alaska						375.21	36 *
I	Chilkat River	13.28	8				
J	Taku River	25.56	12				
K	Admiralty Island	5.09	2				
	Stikine River	93.21	15*				
L	upper river			18.42	8		
M	lower river			<u>no test</u>			
N	Farragut River	0.86	2				
O	Unuk River	<u>no test</u>					
Heterogeneity among regions						956.66	14 *

(* P < 0.001; all others > 0.05)

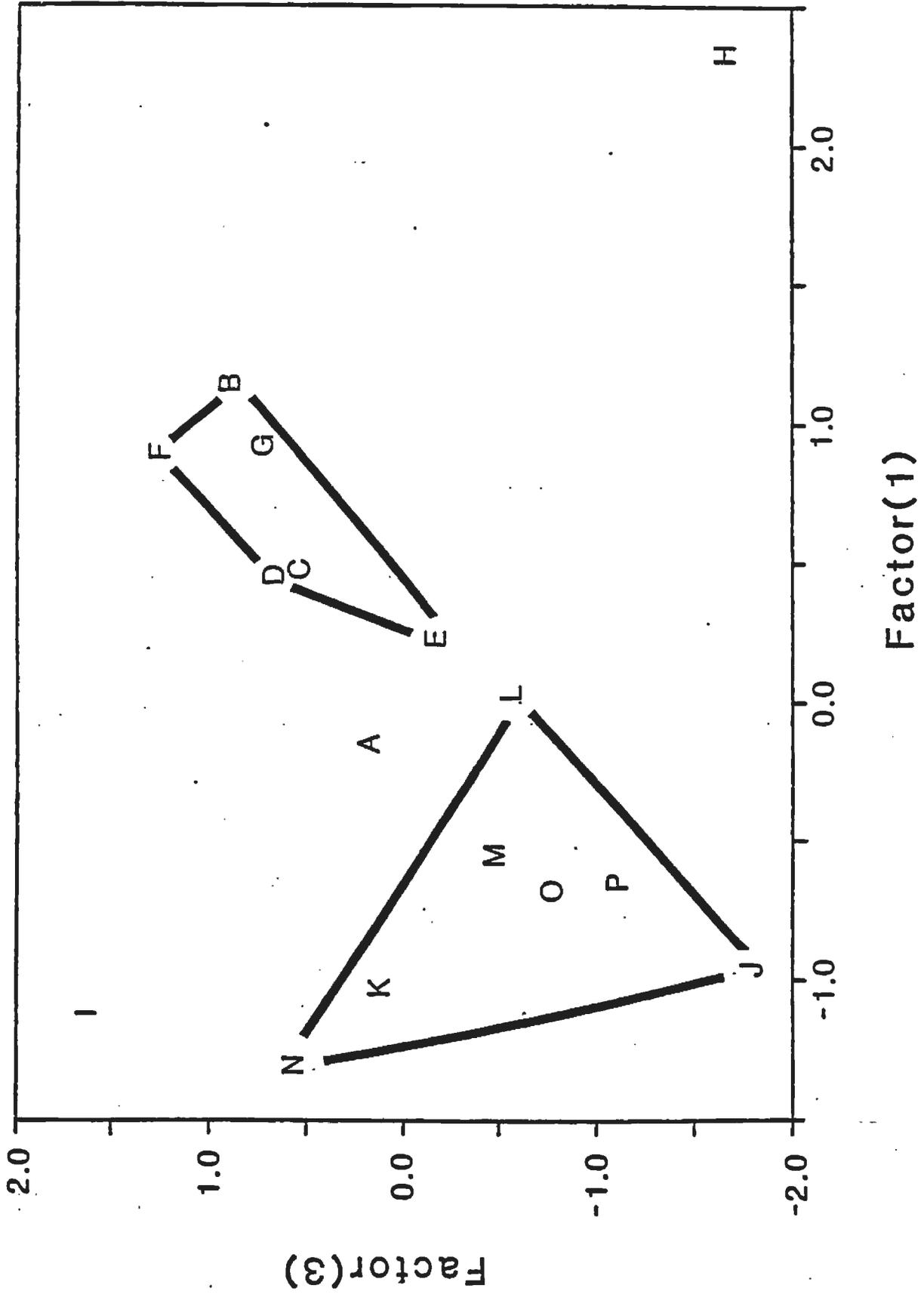
tributaries (Table 1). The Tanana collections were juveniles taken near their natal grounds and probably reflect one or a very few populations. In contrast the lower Yukon collections were taken from the particular mixture of populations passing through the fishery at the time of capture. All subsequent analyses were performed using data pooled from a drainage or from groups of collections minimizing heterogeneity within a drainage (Table 4).

A preliminary look at relationships among Alaskan chinook populations was done using principal component analysis (Figure 4). This analysis shows clearly that western and southeastern Alaskan populations cluster separately. Exceptions are Tanana River (upper Yukon) collections and Chilkat River collections. Copper River samples could have been assigned to either cluster.

The chinook collections within each of the three major geographic regions, western, south-central, and southeastern Alaska, were examined using log-likelihood ratio analysis to determine the extent of divergence within and among regions (Table 4). Significant heterogeneity ($P < 0.001$) was observed at both levels of hierarchy; however, relatively more ($P < 0.01$) was observed among regions than within regions.

Another way to compare divergence of populations is gene diversity analysis. This technique was used to partition the variation observed at twenty-eight genetic loci into components attributable to regional differences, to differences among significant subdivisions within regions, and to the average

Figure 4. Resolution of western Alaskan chinook salmon from southeastern Alaskan populations using principal component analysis of biochemical genetic data. Drainages and pooled subdivisions of drainages are designated according to Figures 1 and 2 and Table 1. The plot of the first principal component (Factor 1) and the third principal component (Factor 3) was typical of plots of pairs of the first five principal components.



observed within drainages (or their subdivisions). The Unuk River collection was not included in this analysis because data were not available for two of the loci. Most of the variability observed was expressed within drainages (94.09%). Divergence within regions accounted for 3.32% of the variability and differences among regions for 2.59% (Table 5).

Genetic relationships among western Alaskan populations (Figure 5A) and among southeastern Alaskan populations (Figure 5B) are depicted from dendrograms constructed by UMPGA (Sneath and Sokal 1973) from Nei's (1973, 1978) standard genetic distances. As with principal component analysis, they show that Tanana River collections and Chilkat River collections are distinct from other stocks in their regions. In addition, one of the lower Yukon composites appears to somewhat resemble the Tanana collections. When a single dendrogram (Figure 6) was constructed from all the collections, the similarity among western Alaskan collections remained, but the similarities among the southeastern Alaskan collections less obvious.

Because information is lost in the process of constructing the dendrogram, another method was used to illustrate genetic relationships of Alaskan collections and some wild southern stocks or composites of stocks, an unrooted maximum-likelihood tree (Felsenstein 1973 and 1984). Such a tree was constructed using data from allelic frequencies at twenty-three loci common to our data and to four groups of southern populations previously

Table 5. Gene diversity analysis of Alaskan chinook salmon using 28 loci.

Source	Gene diversity	Coefficient of gene differentiation
Average within drainages and subdivisions	$H_D = 0.03267$	$H_D/H_T = 0.9409$
Average among drainages within regions	$D_{DR} = 0.00115$	$G_{DR} = 0.0332$
Among regions	$D_{RT} = 0.00090$	$G_{RT} = 0.0259$
Total gene diversity	$H_T = 0.03472$	

Figure 5. Phenetic relationships of salmon from western Alaskan (A) and southeastern Alaskan drainages. Designations are as in Table 1 and Figures 1 and 2. Asterisks denote junctions for which heterogeneity exists between the two branches ($* P < 0.001$).

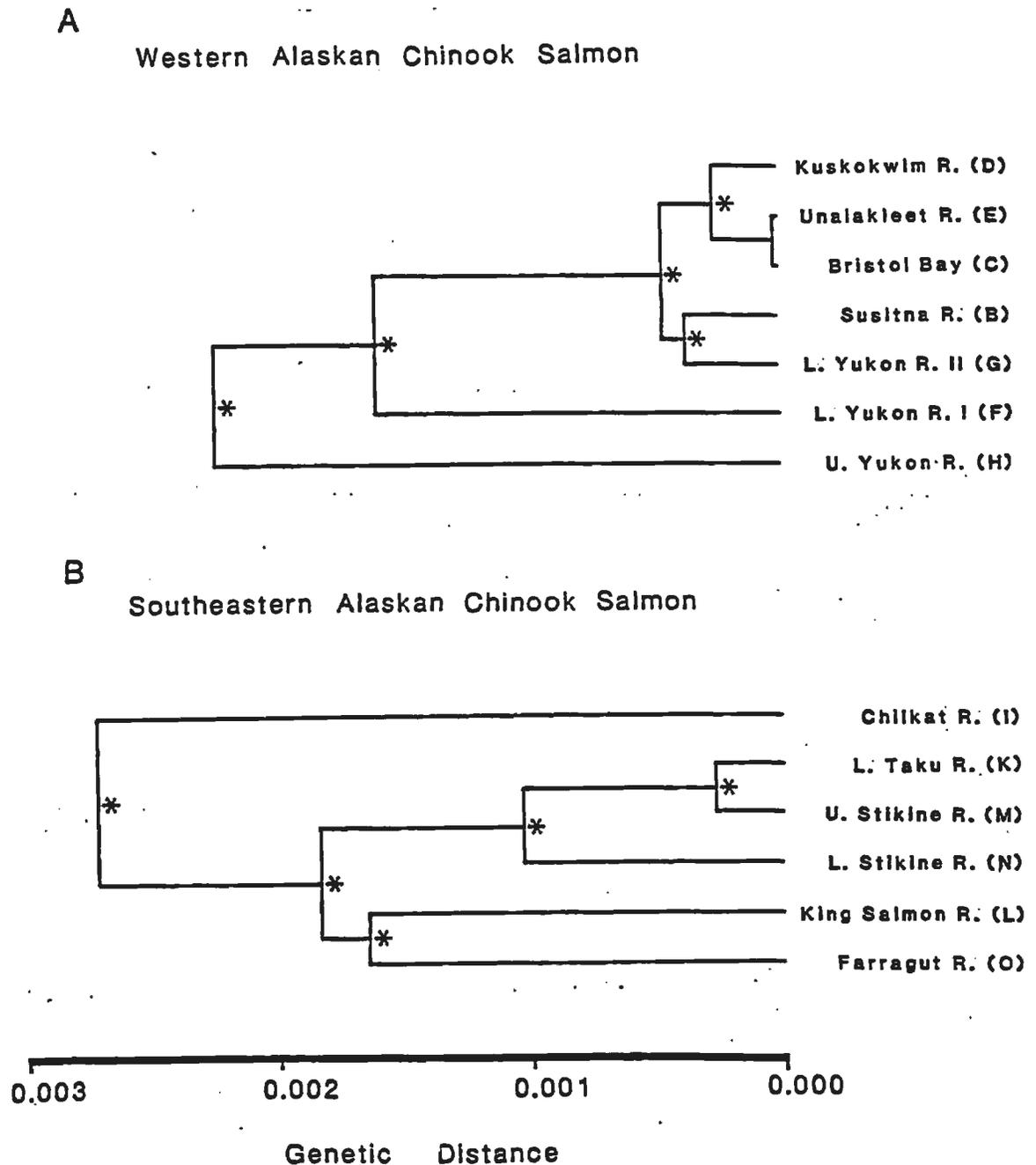
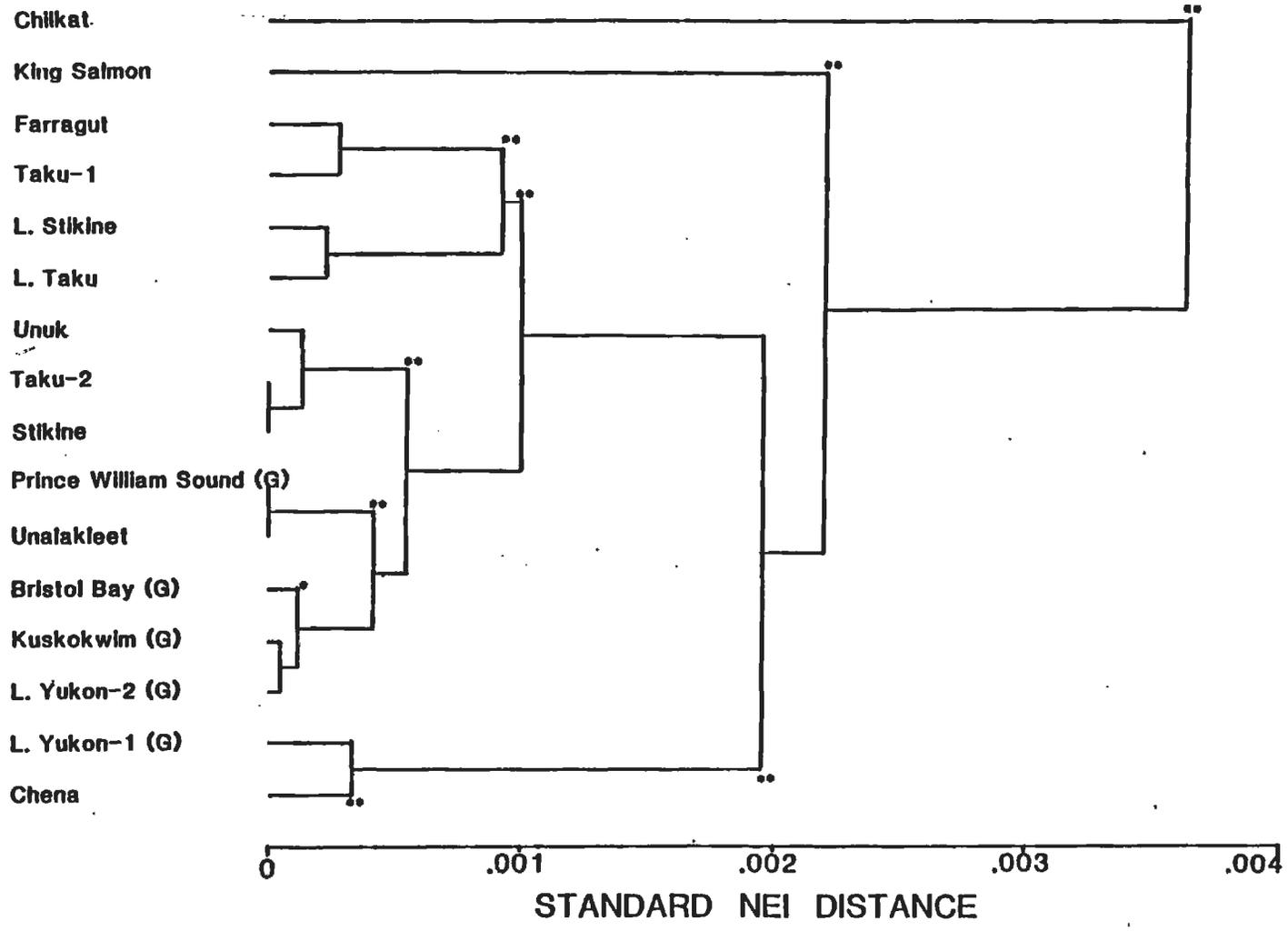


Figure 6. Dendrogram depicting relationships among Alaskan chinook populations.

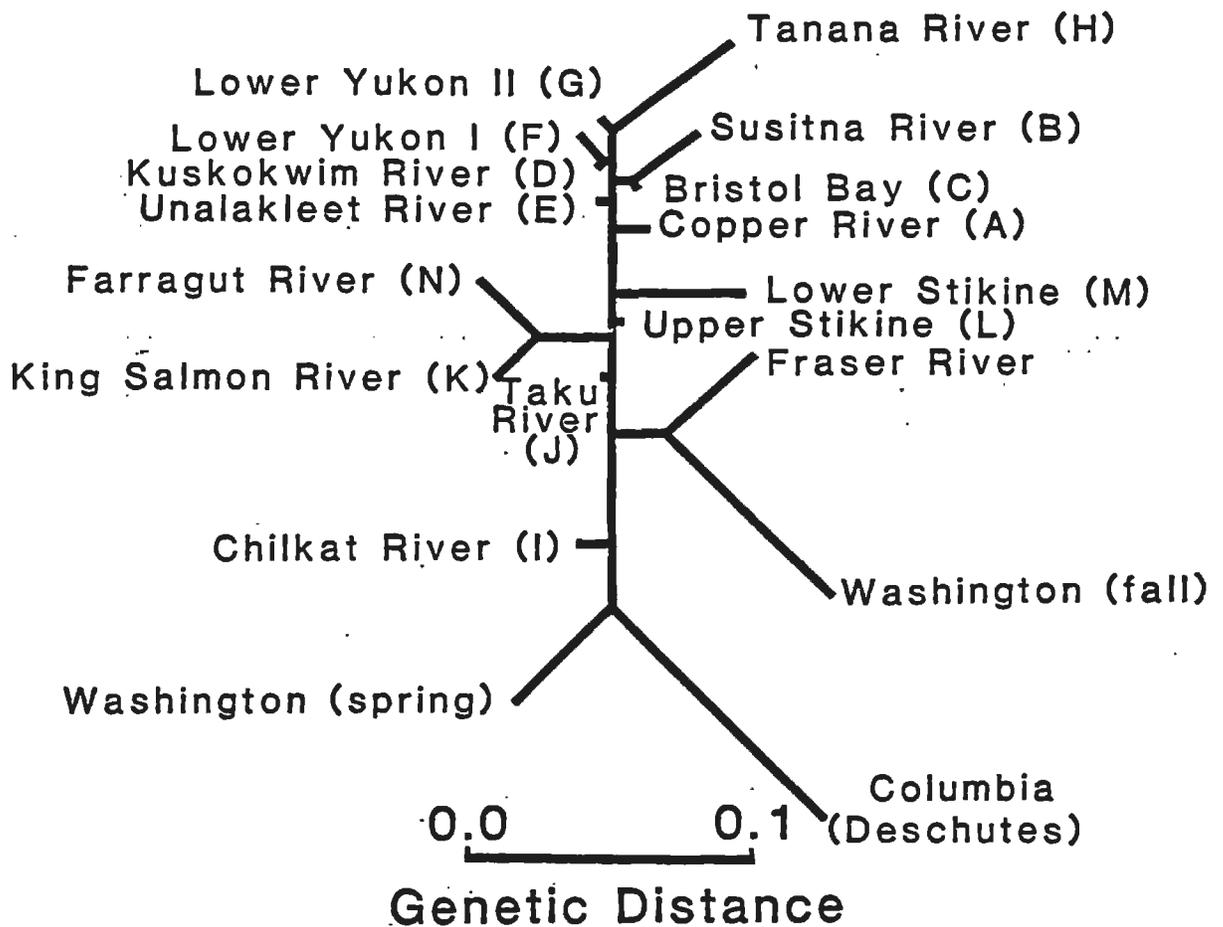


reported by Milner et al. (1983). The unused loci (Pep(Pp-1), Pep(Pp-2), Ldh-1, Adh, and G3p-3) had little or no polymorphism. Southern collections used included spring-run and fall-run wild populations chosen as representative of two life history types. Unweighted means of the allelic frequencies of chinook from Nooksack and Soleduc Rivers in Washington are referred to as Washington (spring). Similarly, allelic frequencies of chinook from Hoh and Queets Rivers in Washington were combined as Washington (fall). Data from three Fraser River collections pooled in the same way and included because of their proximity to southeastern Alaska. Spring-run chinook from Warmsprings on the Deschutes River, a Columbia River tributary, were chosen because of their geographical distance from all other collections.

Western Alaskan and southeastern Alaskan chinook are clearly separated from each other (Figure 7). Copper River chinook were genetically intermediate between western and southeastern Alaskan populations. Other trees generated from the CONTML program were similar to the maximum-likelihood tree presented. Only details of the relationships within the southeastern Alaskan region or within the western Alaskan region varied among the less likely trees produced.

With the exception of the Tanana River collections, the the overall genetic differentiation among the different populations of chinook salmon from western Alaskan drainages was small. Southeastern Alaskan collections (Figure 7) showed relatively

Figure 7. Phenetic relationships among Alaskan chinook and other more southerly groups of chinook. The maximum-likelihood method of Felsenstein (1973 and 1984) was employed to estimate this unrooted tree using data from 23 loci. The tree is oriented to approximate the geographical relationships among the collections. The distances between nodes and collections are chord measure genetic distances (Cavalli-Sforza and Edwards 1967). Angles are arbitrary. Data for non-Alaskan collections are from Milner et al. (1983).



more differentiation among drainages.

The genetic compositions of most of the southeastern Alaska chinook populations were intermediate between those of western Alaskan chinook and of Washington fall-run (Queets and Hoh) and Fraser River chinook (Figure 6). An exception is Chilkat River chinook which differ from other southeastern Alaskan collections and which branch between the branch for the Fraser River and Washington fall-run (Queets and Hoh) and the branch for Washington spring-run (Soleduc and Nooksack) chinook.

Note the similarity of southeastern Alaskan populations shown both by principal component analysis (Figure 4) and by the maximum-likelihood tree (Figure 7), is lost in the dendrogram (Figure 6).

Simulations

Simulations were done to determine the utility of the preliminary baseline data for stock separation problems. In particular, differences observed within the Yukon River were examined as were differences within southeastern Alaska and between southeastern Alaskan stocks and stocks from further south.

The only substantial differences observed among western Alaskan populations occurred in the Yukon River. Genetic relationships indicate that Tanana River collections are distinct from all other western Alaskan collections. In addition, two

groupings of lower Yukon River collections differ; one resembled other western Alaskan collections but the other appeared to share some of the upper Yukon character. Differences in genetic compositions of the collections probably reflect differences in run timing of different stocks within the Yukon. A simplistic hypothesis is that the second lower Yukon grouping may be a mixture of up-river fish (eg. Tanana) and fish spawning in the lower river (eg. the other lower grouping).

Too little data exists for the Yukon River stocks to test this hypothesis or to describe Yukon River stock structure. However, to test the utility of the observed differences for addressing such questions, simulations were run to determine the resolution capabilities of the two available distinct types. Subsequently, assuming that the second grouping of lower river collections was a mixture of the two types, an estimate was made of the composition.

Efficacy of the preliminary baseline data for a two component estimate was examined by constructing mixtures that varied from 5 to 95% of Tanana River fish and the remainder of presumed reference lower river types. Mixtures of 100 fish were constructed and 1000 iterations performed to estimate means and standard deviations (Figure 8). For this relatively small mixture size, surprisingly good resolution was achieved for mixtures between 25 and 75%. Even more precise estimates were possible

using larger mixture sizes, 200 or 500 (Figure 9). However, estimates of contributions less than 10% are still not reliable.

The stock separation capabilities of the preliminary baseline obtained from southeastern Alaska were also examined with this technique. Separations of simulated mixtures of southeastern Alaskan stocks or combinations of "stocks" and key southern stocks were examined. The basis for selection of these "stocks" was their genetic distinctness (see Figures 5B and 7). The southeastern Alaskan stocks included in the simulations were Chilkat River, Farragut River, King Salmon River (on Admiralty Island), and a combination of Stikine and Taku stocks.

Southern stocks and combination of stocks included were chosen from a list provided by Dr. J. Helle (NMFS Auke Bay Laboratory) which were deemed to be important components of the most pressing stock separation problems involving Alaskan and southern stocks. The availability of data (Milner et al. 1983) determined which problems could be addressed. Three combinations of stocks were used: data from three Fraser River tributaries (Chilko, Clearwater, and Stuart), data from upper Columbia stocks (Deschutes, Priest Rapids, and Ice Harbor), and from a composite of B.C. stocks (Puntledge, Robertson Creek, and Quinsam). All composites were made using unweighted averages of the allelic frequencies reported for the contributors.

Four different sets of simulations were run. Three involved all four southeastern Alaskan "stocks" and one of the southern

Figure 8. Simulation results showing error of estimation of 2 component Yukon River model. Different mixtures comprising samples of 100 were used.

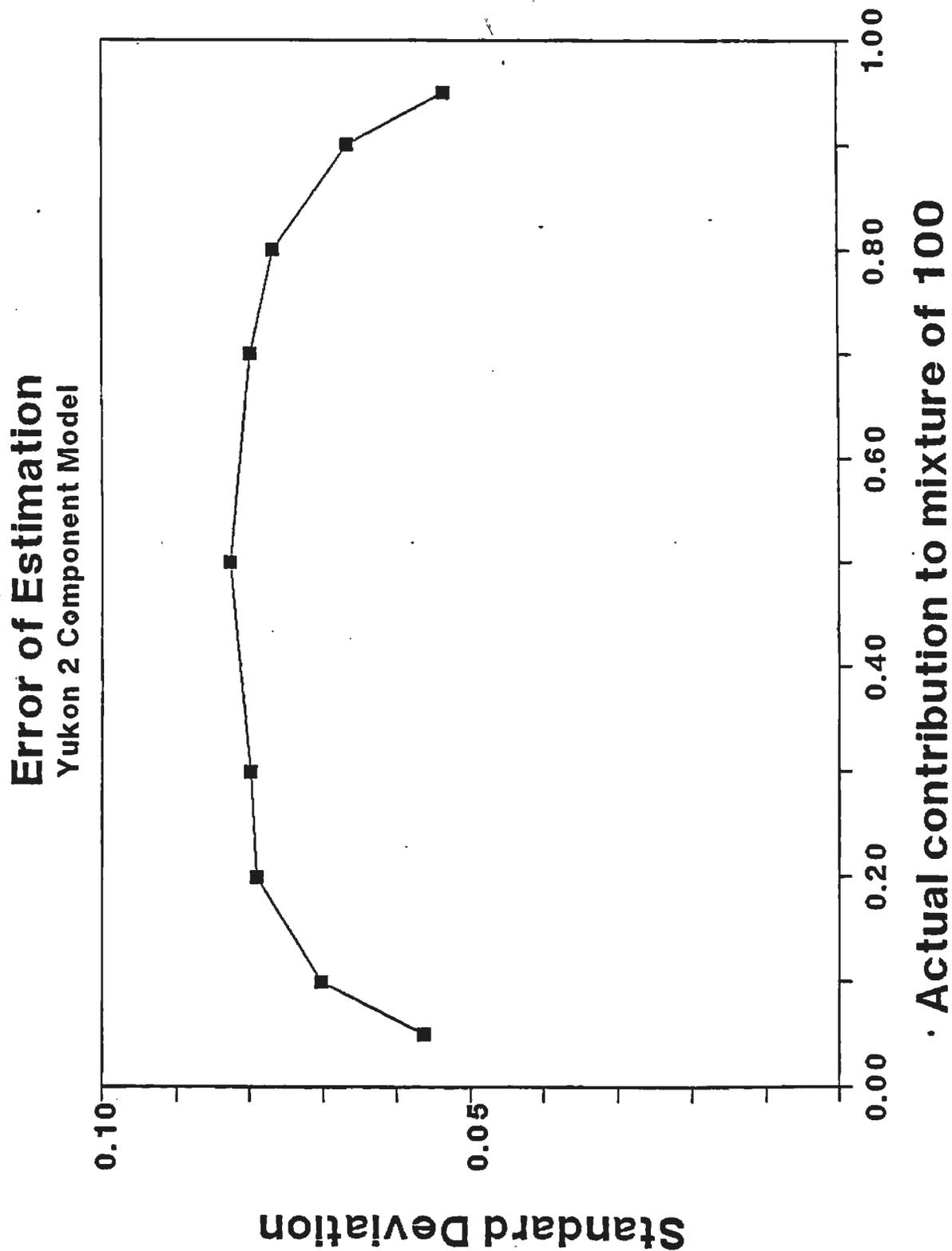
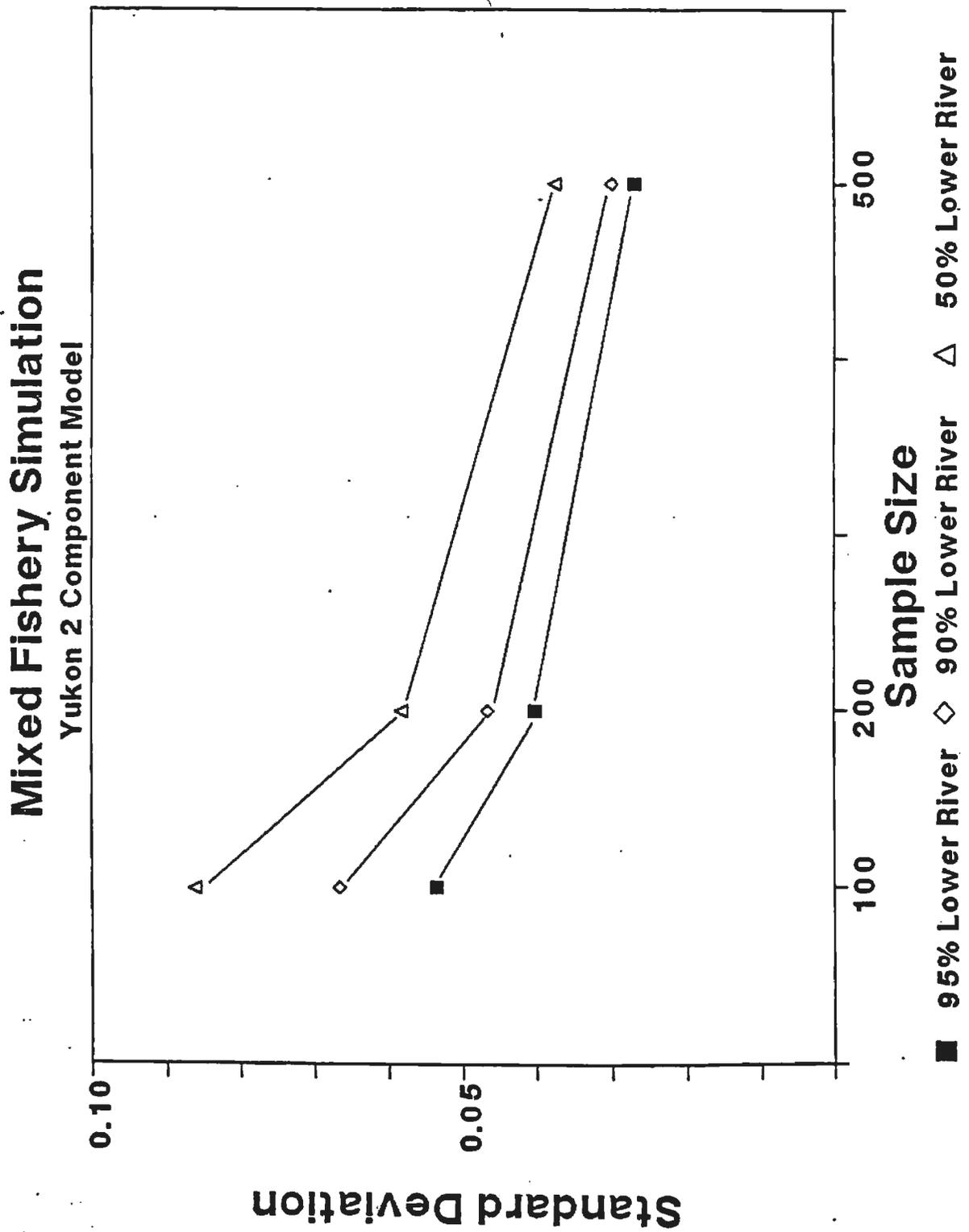


Figure 9. Simulation results showing error of estimation of 2 component Yukon River model. Different mixture sizes were examined for 3 different compositions.



"stocks". The fourth set of simulations included all three southern "stocks" and Chilkat River and the Taku/Stikine combination from southeastern Alaska. Each component of the mixture contributed equally (20%). Three repetitions of 100 iterations was performed for each set of conditions. Means were always within 1% of the actual (predefined) contributions, but the standard deviations of the estimates of contribution varied. Presented are the means and ranges of standard deviations for each set of simulations (Figures 10-13).

For all four sets of simulations, mixtures as small as one hundred were too small for accurate estimates of the mixture composition; between 200 and 500 fish appear to be necessary for accurate determination of those particular mixtures. The larger standard deviations for the estimates of contributions of some stocks results from their similarity to other stocks in the mixture. For example, note that King Salmon River (Admiralty), Taku/Stikine, and Farragut stocks have larger standard deviations (Figures 10-12). These are the most similar contributors and the variation in estimates results from classification of genotypes from one stock to a second similar stock. In contrast the accuracy of estimates of contributions of the more southern stocks was higher, both when examined singly with southeastern Alaskan stocks (Figures 10-12) and together (Figure 13). Lower variation is an indication of greater genetic divergence among the stocks.

Figure 10. Simulation results showing error of estimation of 5 component southeastern Alaskan model: Farragut, Chilkat, King Salmon, and Taku/Stikine drainages and upper Columbia drainages were included, each at 20%.

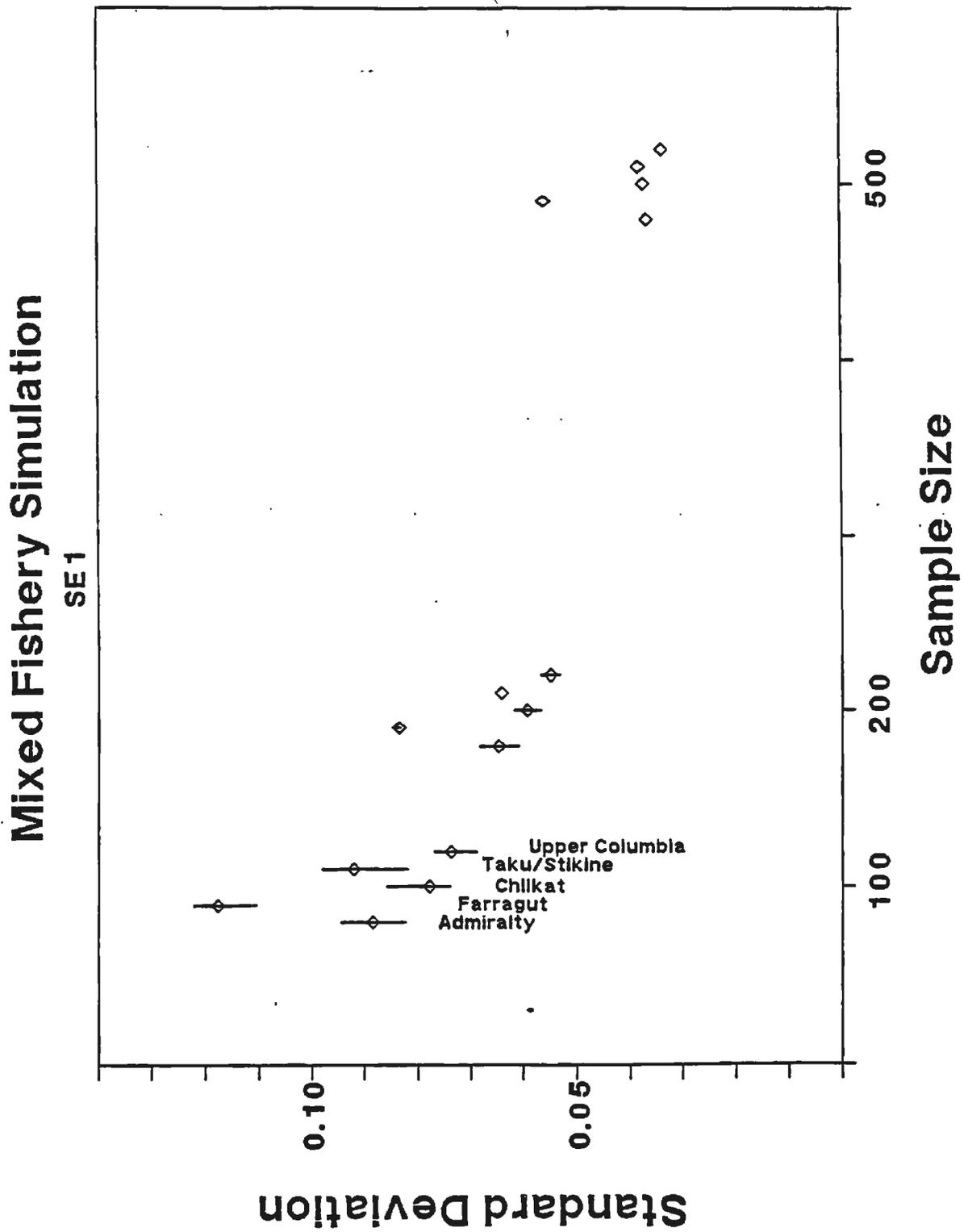


Figure 11. Simulation results showing error of estimation of 5 component southeastern Alaskan model: Farragut, Chilkat, King Salmon, and Taku/Stikine drainages and Fraser River drainages were included, each at 20%.

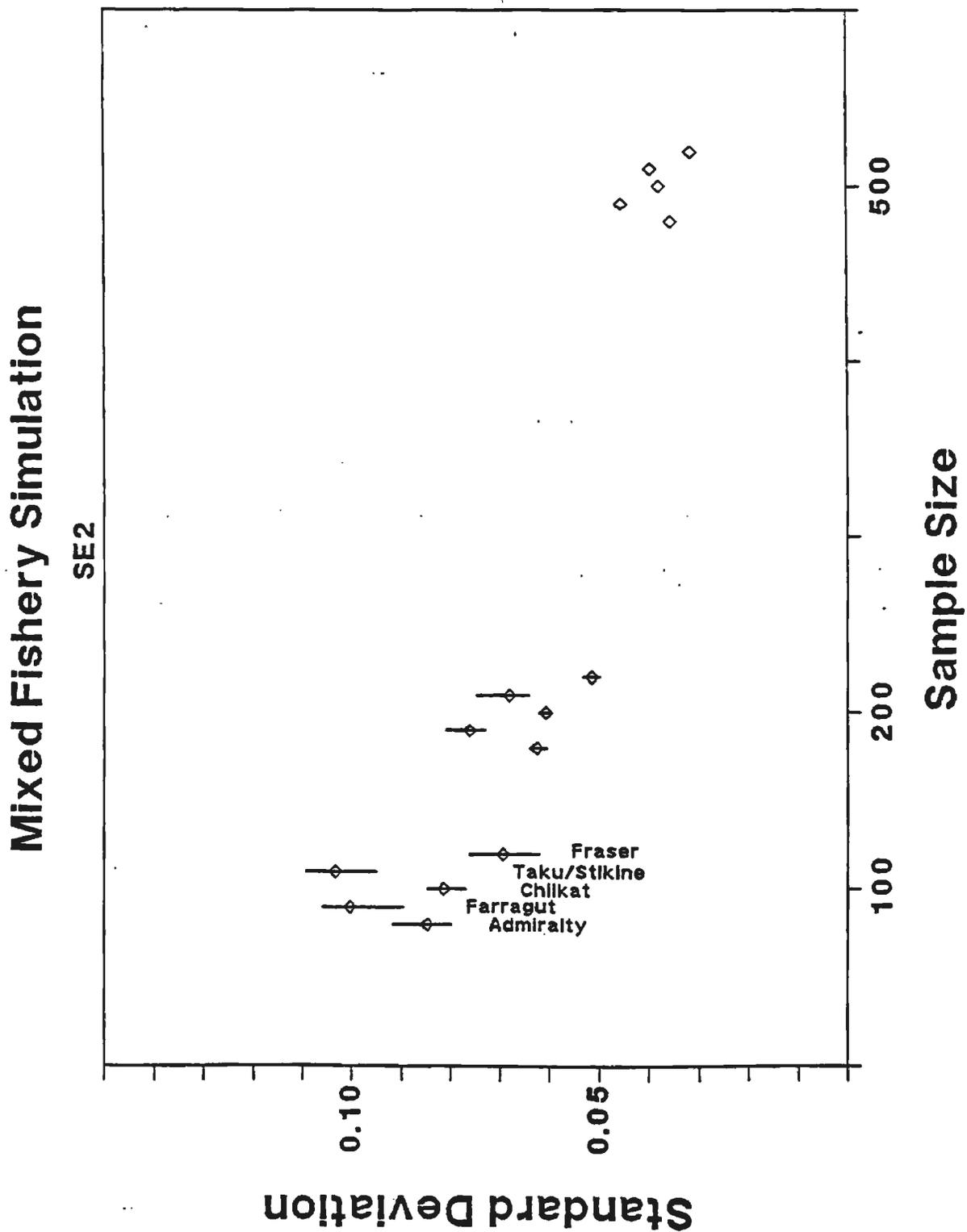


Figure 12. Simulation results showing error of estimation of 5 component southeastern Alaskan model: Farragut, Chilkat, King Salmon, and Taku/Stikine drainages and B.C. fall stock composite were included, each at 20%.

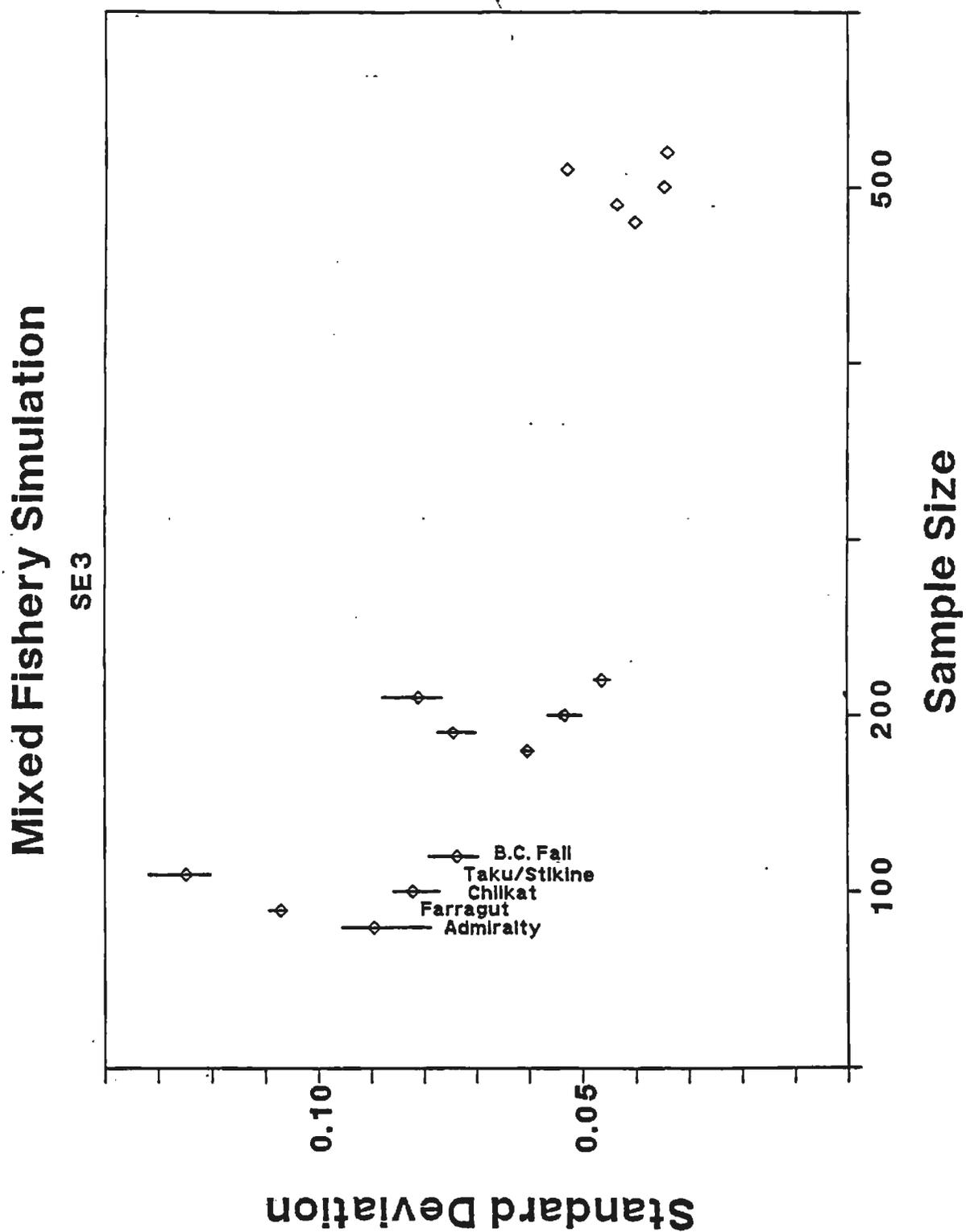
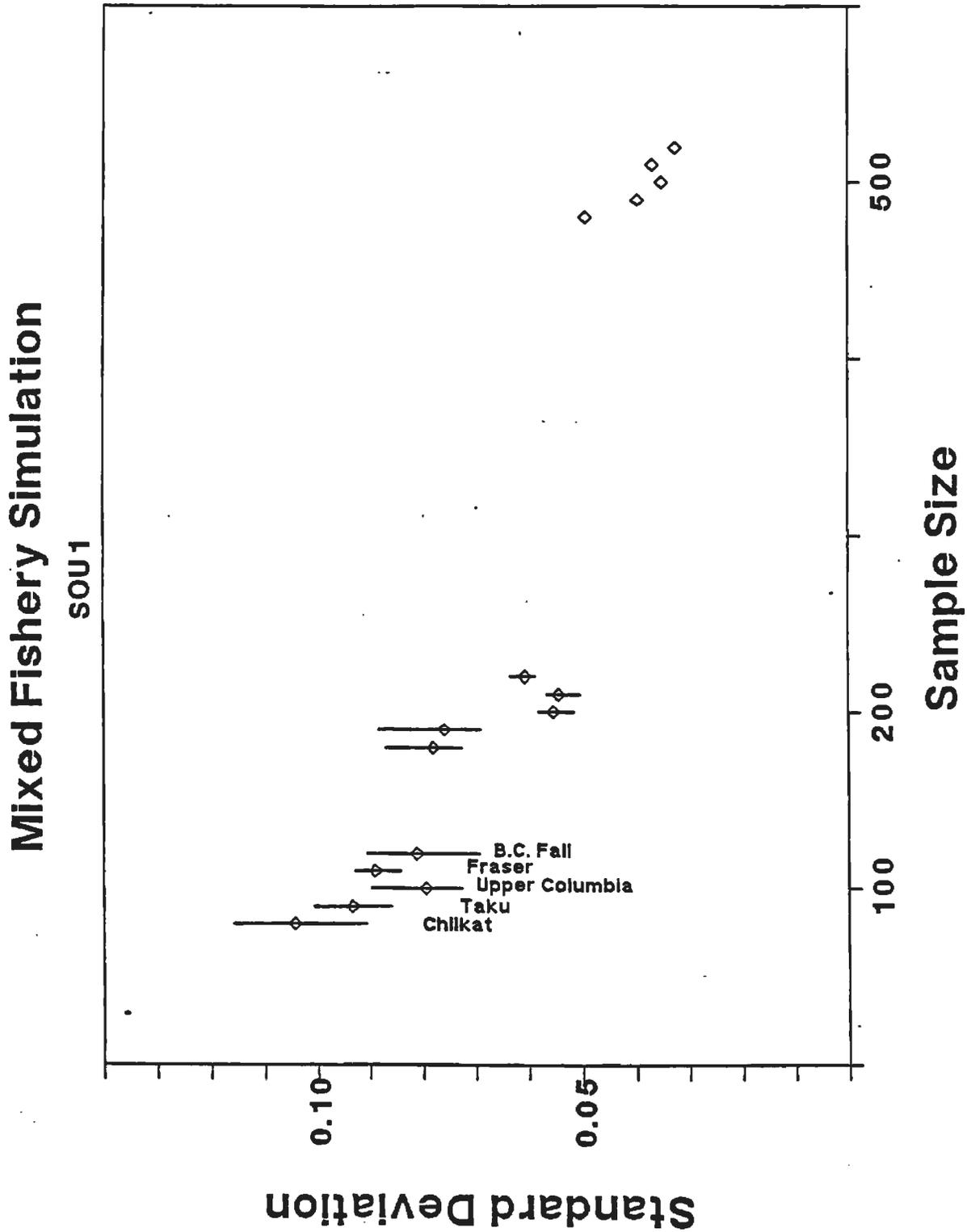


Figure 13. Simulation results showing error of estimation of 5 component southeastern Alaskan model: Chilkat and Taku/Stikine drainages as well as upper Columbia and Fraser River drainages and B.C. fall stock composite were included, each at 20%.



Discussion

One of the objectives of this work was to examine the genetic variation within and between Alaskan chinook salmon populations and to use that information to study the relationships among them. A second objective was to determine whether the divergence among populations was sufficient for separating stocks within regions and for distinguishing Alaskan chinook from more southerly stocks.

Differences do exist between the genetic compositions of most Alaskan chinook populations and between Alaskan populations and those from British Columbia, Washington, and Oregon. In addition there are differences between western Alaskan chinook and southeastern Alaskan chinook. The Alaskan populations generally display lower biochemical genetic variability than those reported by Milner et al. (1981). This may be a result of separate origins in the founding of these populations after glacial recession following the Wisconsin Age. Alternatively, this may reflect divergence related to natural selection or loss of variability as a result of random drift.

Western Alaskan populations do show some genetic differentiation, but with exceptions detailed below, the extent of divergence appears inadequate for distinguishing among stocks originating from this area. The collections studied in southeastern Alaska display more divergence; but while some

populations are readily distinguishable, others are too similar to segregate.

Within drainages, collections are generally somewhat similar, but there is evidence of discrete, identifiable populations within the Yukon, Taku, and Stikine River drainages. Within the Yukon drainage, the Tanana River is very different from all other collections examined. Although it was not possible to extensively examine the Yukon drainage, it is evident that the Tanana River collections are very different from all other collections examined. A related question is the genetic composition of the Canadian Yukon populations. Upstream and downstream collections within the Taku and Stikine River systems also show differences. Since these differences may be related to the rearing habitat differences between upstream and downstream populations, existence of these differences should be taken into consideration during brood stock selection for hatcheries.

Having shown that genetic differences exist among Alaskan populations and between Alaskan and more southern populations, the next step is to determine the utility these differences have for addressing stock separation problems. Of the western Alaskan systems examined, only the Yukon system has potential for electrophoretic applications to stock separation. Simulations reported here provide an example of how applications may be made. However, further applications can not be made without a substantial improvement in the data base of genetic compositions

of Yukon chinook populations.

The questions involving stock separation problems in southeastern Alaska are: is it possible to separate stocks within southeast Alaska? and can southeastern Alaskan stocks be resolved from British Columbia, Oregon, Washington, and Idaho stocks with which they may intermingle? Of the systems examined, Chilkat River chinook are quite distinct from other southeastern Alaskan stocks. Other stocks, while more divergent than those in western Alaska, are still similar. This similarity suggests against using electrophoresis for distinguishing among most southeastern Alaskan populations but may serve as an advantage for separating southeastern Alaskan stocks as a whole from southern stocks. The similarity, coupled with distinctness of the southern stocks used (Figure 13) suggests that with the exception of Chilkat stocks, southeastern Alaskan chinook might be pooled into a regional stock for some applications.

Stock separation potential relies on the divergence among stocks. The potential can be increased by including more loci which reflect the divergence of stocks. Recently techniques have been developed to resolve additional electrophoretic loci. Data from such loci may improve ability to identify Alaskan stocks and to estimate relationships among them. In addition, techniques for looking directly at genetic variation at the level of the DNA base sequences, rather than from products of that information exist and will soon be applied to fisheries

management problems. With such additional and powerful tools, it is possible that many of our present identification/separation problems may be resolved.

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Appendix. Biochemical genetic variation in collections of chinook salmon from drainages in Alaska. Allelic frequencies and collection sizes (N) for biochemical genetic loci. Collections are numbered as in Figure 1, and grouped under designations for drainages and subdivisions of drainages. Alleles are designated by their mobility relative to the most common allele (100).

Group	Source of sample and year of collection	Loci						
		Mpi			Sod-1			
		N	100	110	95	N	-100	-260
A	Copper River gillnet fishery							
	1. 1983	45	0.93	0.03	0.03	43	0.91	0.09
	2. 1984	102	0.93	0.07	0.00	102	0.89	0.11
B	Susitna River juveniles							
	3. Indian R. 1983	73	1.00	0.00	0.00	20	1.00	0.00
	4. Indian R. 1984	98	0.995	0.005	0.00	100	0.97	0.03
C	Bristol Bay gillnet fishery							
	5. Togiak 1983	106	0.90	0.10	0.00	84	0.93	0.07
	6. Dillingham 1983	46	0.91	0.04	0.04	—	—	—
D	Kuskokwim gillnet fishery							
	7. 1982	28	0.84	0.16	0.00	66	0.95	0.05
	8. 1983	110	0.91	0.09	0.00	114	0.97	0.03
	9. 1984	119	0.90	0.10	0.00	119	0.96	0.04
E	Unalakleet gillnet fishery							
	10. 1982	38	0.92	0.08	0.00	38	0.86	0.14
	11. 1983	25	0.94	0.06	0.00	27	0.91	0.10
F	Lower Yukon gillnet fishery Subdivision I							
	12. Emmonak 1982	43	0.93	0.07	0.00	45	1.00	0.00
	13. Big Eddy 1982	122	0.92	0.08	0.00	116	0.97	0.03
	14. Big Eddy 1983	48	0.96	0.04	0.00	48	0.98	0.02
G	Subdivision II							
	15. Emmonak 1983	45	0.90	0.10	0.00	—	—	—
	16. St. Mary's 1983	96	0.94	0.06	0.005	80	0.99	0.01
H	Tanana River juveniles							
	17. L. Chena R. 1982	116	0.996	0.004	0.00	116	0.96	0.04
	18. U. Chena R. 1982	90	0.98	0.02	0.00	—	—	—
	19. L. Chena R. 1983	79	0.99	0.01	0.00	79	0.98	0.02
	20. Chatinika R. 1984	119	0.98	0.02	0.00	119	0.99	0.01
I	Chilkat River							
	21. Tahini R. 1983 egg-take	34	0.62	0.38	0.00	34	0.84	0.16
	22. Tahini R. 1984 egg take	34	0.63	0.37	0.00	34	0.84	0.16
	23. Tahini R. 1984 juveniles	81	0.67	0.33	0.00	72	0.83	0.17
J	Taku River juveniles							
	24. Nahlin R. 1982	42	0.81	0.19	0.00	42	0.83	0.17
	25. L. Nakina 1982	85	0.76	0.24	0.00	73	0.80	0.20
	26. Mainstem 1981	100	0.82	0.18	0.00	98	0.92	0.08
	27. Mainstem 1982	92	0.78	0.19	0.03	95	0.90	0.10
	28. King Salmon Cr. 1982	47	0.77	0.23	0.00	47	0.92	0.08
K	Admiralty Island egg-take							
	29. King Salmon R. 1983	64	0.94	0.06	0.00	21	0.95	0.05
	30. King Salmon R. 1984	64	0.95	0.05	0.00	64	0.96	0.04
L	Stikine River Upper Stikine juveniles							
	31. Little Tahitan 1981	170	0.85	0.15	0.00	110	0.89	0.11
	32. Mainstem 1981	108	0.90	0.10	0.00	68	0.92	0.08
	33. Mainstem 1982	152	0.84	0.16	0.00	204	0.95	0.05
M	Lower Stikine egg-take							
	34. Andrew Cr. 1982	125	0.73	0.27	0.00	116	0.94	0.06
N	Farragut River egg-take							
	35. 1983	26	0.88	0.12	0.00	26	0.75	0.25
	36. 1984	24	0.92	0.08	0.00	24	0.81	0.19
O	Unuk River juveniles							
	37. 1982	82	0.82	0.18	0.00	82	0.87	0.13

N	Mdh-3,4			N	Pep(Gl-1)		N	Pep(Lgg)		
	100	80	121		100	90		100	130	95
46	1.00	0.00	0.00	42	0.95	0.05	45	0.97	0.03	0.00
102	1.00	0.00	0.00	99	0.94	0.06	102	0.95	0.05	0.00
85	1.00	0.00	0.00	86	1.00	0.00	79	0.97	0.03	0.00
100	1.00	0.00	0.00	100	1.00	0.00	100	0.91	0.09	0.00
109	0.99	0.00	0.01	109	0.995	0.005	106	0.91	0.06	0.03
95	0.99	0.00	0.01	96	1.00	0.00	91	0.94	0.05	0.01
63	0.98	0.00	0.02	37	0.99	0.01	12	0.88	0.12	0.00
105	0.99	0.00	0.01	108	0.995	0.005	84	0.90	0.09	0.01
156	0.98	0.00	0.02	114	0.97	0.03	112	0.87	0.10	0.03
38	0.97	0.00	0.03	38	0.96	0.04	36	0.96	0.04	0.00
27	0.96	0.04	0.00	27	0.98	0.02	27	0.93	0.04	0.04
45	0.99	0.01	0.00	43	0.99	0.01	29	0.76	0.24	0.00
132	0.98	0.02	0.004	107	0.97	0.03	95	0.79	0.20	0.01
48	0.97	0.02	0.01	40	0.99	0.01	44	0.91	0.07	0.02
45	1.00	0.00	0.00	45	0.97	0.03	44	0.92	0.07	0.01
85	0.97	0.01	0.02	98	1.00	0.00	76	0.91	0.05	0.05
116	0.91	0.04	0.05	116	0.996	0.004	116	0.80	0.20	0.00
135	0.90	0.06	0.04	134	0.99	0.01	—	—	—	—
79	0.87	0.10	0.03	78	1.00	0.00	—	—	—	—
119	0.94	0.03	0.03	119	1.00	0.00	119	0.74	0.26	0.00
34	1.00	0.00	0.00	34	1.00	0.00	34	0.96	0.04	0.00
34	1.00	0.00	0.00	34	1.00	0.00	34	0.88	0.12	0.00
82	1.00	0.00	0.00	82	1.00	0.00	82	0.92	0.08	0.00
42	0.99	0.01	0.00	34	0.93	0.07	42	0.96	0.04	0.00
90	0.94	0.00	0.06	84	0.91	0.09	90	1.00	0.00	0.00
100	0.98	0.02	0.00	100	0.88	0.12	100	0.98	0.02	0.01
95	0.97	0.03	0.00	85	0.95	0.05	59	1.00	0.00	0.00
47	1.00	0.00	0.00	47	0.89	0.11	44	0.98	0.02	0.00
64	1.00	0.00	0.00	64	0.78	0.22	60	1.00	0.00	0.00
64	1.00	0.00	0.00	64	0.88	0.12	64	0.98	0.02	0.00
144	0.96	0.04	0.00	88	0.97	0.03	—	—	—	—
108	0.98	0.02	0.00	40	0.95	0.05	68	0.98	0.02	0.00
204	0.98	0.02	0.00	152	0.93	0.07	102	0.995	0.005	0.00
126	0.98	0.02	0.00	119	0.86	0.14	116	0.97	0.03	0.00
26	1.00	0.00	0.00	23	0.91	0.09	26	1.00	0.00	0.00
24	1.00	0.00	0.00	23	1.00	0.00	23	0.98	0.02	0.00
82	0.98	0.02	0.00	82	0.98	0.02	54	1.00	0.00	0.00

<u>Gpi-3</u>				<u>Mdh-1,2</u>				<u>Ldh-4</u>		
N	100	105	93	N	100	120	82	N	100	117
46	1.00	0.00	0.00	46	1.00	0.00	0.00	45	1.00	0.00
102	0.995	0.005	0.00	102	1.00	0.00	0.00	80	1.00	0.00
86	1.00	0.00	0.00	86	1.00	0.00	0.00	86	1.00	0.00
100	1.00	0.00	0.00	100	1.00	0.00	0.00	100	1.00	0.00
109	1.00	0.00	0.00	193	1.00	0.00	0.00	109	1.00	0.00
55	1.00	0.00	0.00	95	1.00	0.00	0.00	40	1.00	0.00
50	1.00	0.00	0.00	63	1.00	0.00	0.00	68	1.00	0.00
104	1.00	0.00	0.00	105	1.00	0.00	0.00	105	0.995	0.005
119	0.996	0.004	0.00	119	1.00	0.00	0.00	119	0.996	0.004
38	1.00	0.00	0.00	38	1.00	0.00	0.00	38	1.00	0.00
27	1.00	0.00	0.00	27	1.00	0.00	0.00	27	1.00	0.00
—	—	—	—	45	1.00	0.00	0.00	45	0.98	0.02
81	1.00	0.00	0.00	133	1.00	0.00	0.00	131	0.99	0.01
46	1.00	0.00	0.00	48	1.00	0.00	0.00	41	1.00	0.00
45	1.00	0.00	0.00	45	1.00	0.00	0.00	45	1.00	0.00
97	1.00	0.00	0.00	85	1.00	0.00	0.00	98	1.00	0.00
34	1.00	0.00	0.00	116	1.00	0.00	0.00	116	1.00	0.00
45	1.00	0.00	0.00	135	1.00	0.00	0.00	135	1.00	0.00
79	1.00	0.00	0.00	79	0.98	0.00	0.02	79	1.00	0.00
119	1.00	0.00	0.00	119	1.00	0.00	0.00	119	1.00	0.00
34	0.97	0.03	0.00	34	1.00	0.00	0.00	34	1.00	0.00
34	0.98	0.02	0.00	34	1.00	0.00	0.00	34	1.00	0.00
82	0.96	0.04	0.00	82	1.00	0.00	0.00	82	1.00	0.00
42	0.96	0.04	0.00	42	1.00	0.00	0.00	42	1.00	0.00
50	0.99	0.01	0.00	90	1.00	0.00	0.00	90	1.00	0.00
100	0.96	0.04	0.00	—	—	—	—	—	—	—
95	0.99	0.01	0.00	95	1.00	0.00	0.00	95	1.00	0.00
47	1.00	0.00	0.00	47	1.00	0.00	0.00	47	1.00	0.00
64	1.00	0.00	0.00	64	1.00	0.00	0.00	64	1.00	0.00
64	1.00	0.00	0.00	64	1.00	0.00	0.00	64	1.00	0.00
140	1.00	0.00	0.00	—	—	—	—	—	—	—
108	0.99	0.01	0.00	—	—	—	—	—	—	—
152	0.97	0.03	0.00	204	1.00	0.00	0.00	152	1.00	0.00
93	0.97	0.03	0.00	126	1.00	0.00	0.00	126	0.996	0.004
26	1.00	0.00	0.00	26	1.00	0.00	0.00	26	1.00	0.00
24	0.98	0.02	0.00	24	1.00	0.00	0.00	24	1.00	0.00
40	0.99	0.01	0.00	82	1.00	0.00	0.00	82	1.00	0.00

	N	<u>Pep(G1-2)</u>			N	<u>Sod-2</u>		N	<u>Aat-3</u>		
		100	70	105		100	142		100	85	
A											
	1.	42	1.00	0.00	0.00	43	1.00	0.00	45	0.68	0.32
	2.	99	1.00	0.00	0.00	79	0.96	0.04	100	0.77	0.23
B											
	3.	86	1.00	0.00	0.00	—	—	—	75	0.86	0.14
	4.	100	1.00	0.00	0.00	92	0.92	0.08	—	—	—
C											
	5.	109	1.00	0.00	0.00	83	0.92	0.08	109	0.88	0.12
	6.	96	1.00	0.00	0.00	73	0.95	0.05	93	0.92	0.08
D											
	7.	37	1.00	0.00	0.00	16	0.91	0.09	—	—	—
	8.	103	1.00	0.00	0.00	112	0.95	0.05	105	0.86	0.14
	9.	119	1.00	0.00	0.00	156	0.93	0.07	105	0.83	0.17
E											
	10.	38	1.00	0.00	0.00	37	0.96	0.04	—	—	—
	11.	27	1.00	0.00	0.00	26	0.94	0.068	25	0.86	0.14
F											
	12.	45	1.00	0.00	0.00	35	0.93	0.07	—	—	—
	13.	133	1.00	0.00	0.00	109	0.95	0.05	—	—	—
	14.	41	1.00	0.00	0.00	41	0.90	0.10	48	0.92	0.08
G											
	15.	45	1.00	0.00	0.00	45	0.96	0.04	45	0.81	0.19
	16.	98	0.995	0.005	0.00	89	0.90	0.10	95	0.76	0.24
H											
	17.	116	1.00	0.00	0.00	36	0.92	0.08	—	—	—
	18.	134	1.00	0.00	0.00	—	—	—	—	—	—
	19.	78	1.00	0.00	0.00	11	1.00	0.00	—	—	—
	20.	119	1.00	0.00	0.00	—	—	—	117	0.70	0.30
I											
	21.	18	1.00	0.00	0.00	34	1.00	0.00	32	0.93	0.03
	22.	34	1.00	0.00	0.00	34	1.00	0.00	34	0.94	0.06
	23.	82	1.00	0.00	0.00	—	—	—	81	0.96	0.04
J											
	24.	39	0.97	0.00	0.03	42	1.00	0.00	—	—	—
	25.	89	1.00	0.00	0.00	—	—	—	—	—	—
	26.										
	27.	85	1.00	0.00	0.00	95	1.00	0.00	46	0.95	0.05
	28.	47	1.00	0.00	0.00	—	—	—	—	—	—
K											
	29.	63	1.00	0.00	0.00	21	1.00	0.00	51	1.00	0.00
	30.	64	1.00	0.00	0.00	64	1.00	0.00	44	0.98	0.02
L											
	31.	—	—	—	—	—	—	—	—	—	—
	32.	—	—	—	—	—	—	—	—	—	—
	33.	50	1.00	0.00	0.00	204	1.00	0.00	147	0.90	0.10
M											
	34.	126	1.00	0.00	0.00	69	1.00	0.00	33	0.83	0.17
N											
	35.	23	1.00	0.00	0.00	26	1.00	0.00	—	—	—
	36.	24	1.00	0.00	0.00	24	1.00	0.00	23	1.00	0.00
O											
	37.	82	1.00	0.00	0.00	—	—	—	40	0.95	0.05

N	100	<u>Idh-3,4</u>		127	50	N	<u>Pep(Pp-2)</u>		
		136	74				100	110	105
—	—	—	—	—	—	46	1.00	0.00	0.00
84	0.994	0.006	0.00	0.00	0.00	80	1.00	0.00	0.00
81	1.00	0.00	0.00	0.00	0.00	86	0.994	0.006	0.00
82	1.00	0.00	0.00	0.00	0.00	100	1.00	0.00	0.00
106	0.995	0.005	0.00	0.00	0.00	109	1.00	0.00	0.00
95	0.995	0.005	0.00	0.00	0.00	46	0.99	0.01	0.00
—	—	—	—	—	—	58	0.99	0.00	0.01
65	1.00	0.00	0.00	0.00	0.00	101	1.00	0.00	0.00
100	0.98	0.02	0.00	0.00	0.00	115	1.00	0.00	0.00
—	—	—	—	—	—	38	0.97	0.03	0.00
27	0.98	0.02	0.00	0.00	0.00	27	1.00	0.00	0.00
—	—	—	—	—	—	45	1.00	0.00	0.00
—	—	—	—	—	—	112	0.996	0.00	0.004
38	0.961	0.039	0.00	0.00	0.00	41	1.00	0.00	0.00
45	1.00	0.00	0.00	0.00	0.00	45	1.00	0.00	0.00
86	0.994	0.006	0.00	0.00	0.00	83	1.00	0.00	0.00
—	—	—	—	—	—	78	1.00	0.00	0.00
—	—	—	—	—	—	135	1.00	0.00	0.00
41	1.00	0.00	0.00	0.00	0.00	79	1.00	0.00	0.00
97	1.00	0.00	0.00	0.00	0.00	119	1.00	0.00	0.00
34	1.00	0.00	0.00	0.00	0.00	34	1.00	0.00	0.00
34	0.93	0.00	0.07	0.00	0.00	34	1.00	0.00	0.00
65	0.92	0.00	0.08	0.00	0.00	82	1.00	0.00	0.00
—	—	—	—	—	—	39	1.00	0.00	0.00
90	1.00	0.00	0.00	0.00	0.00	50	0.99	0.01	0.00
100	0.89	0.01	0.04	0.06	0.00	—	—	—	—
14	1.00	0.00	0.00	0.00	0.00	92	1.00	0.00	0.00
47	1.00	0.00	0.00	0.00	0.00	47	0.96	0.04	0.00
40	1.00	0.00	0.00	0.00	0.00	64	1.00	0.00	0.00
53	1.00	0.00	0.00	0.00	0.00	64	1.00	0.00	0.00
—	—	—	—	—	—	—	—	—	—
108	0.96	0.00	0.04	0.00	0.00	—	—	—	—
140	1.00	0.00	0.00	0.00	0.00	151	1.00	0.00	0.00
68	0.89	0.11	0.00	0.00	0.00	67	1.00	0.00	0.00
26	1.00	0.00	0.00	0.00	0.00	26	1.00	0.00	0.00
22	1.00	0.00	0.00	0.00	0.00	24	1.00	0.00	0.00
82	1.00	0.00	0.00	0.00	0.00	78	1.00	0.00	0.00

Sample Sizes of Monomorphic Loci											
<u>Ldh-3</u>	<u>Ck-1</u>	<u>Ck-2</u>	<u>Ldh-5</u>	<u>G3p-1</u>	<u>G3p-2</u>	<u>Pgm-2</u>	<u>Pod</u>	<u>G3p-3</u>	<u>Pep</u> <u>(Pp-1)</u>	<u>Ldh-1</u>	<u>Adh</u>
45	34	34	45	43	43	43	41	43	46	46	35
81	102	102	102	80	80	97	101	80	80	102	74
86	77	77	86	86	86	86	86	86	86	86	76
100	100	100	100	100	100	100	97	100	100	100	60
193	25	25	109	84	84	109	72	84	109	109	108
40	87	87	102	72	72	96	97	72	46	95	105
65	57	57	68	63	63	65	—	63	58	65	43
105	98	98	105	112	112	104	87	112	101	104	102
119	119	119	119	119	119	119	87	119	115	119	76
38	37	37	38	38	38	38	38	38	38	38	38
27	—	—	27	25	25	27	25	25	27	27	27
45	—	—	45	45	45	45	45	45	45	45	45
131	102	102	127	80	80	107	35	80	112	133	81
45	27	27	48	41	41	41	42	41	41	48	41
45	27	27	45	—	—	45	23	—	45	45	38
98	45	45	98	75	75	86	87	75	94	85	101
116	76	76	40	116	116	76	—	116	78	116	—
135	135	135	135	45	45	135	—	45	135	135	—
79	79	79	79	77	77	28	41	77	79	79	73
119	118	118	119	119	119	119	119	119	119	119	119
34	16	16	34	34	34	16	34	34	34	34	26
34	31	31	34	34	34	34	34	34	34	34	34
82	82	82	82	82	82	82	81	82	82	62	82
42	42	42	42	42	42	—	42	42	42	42	42
90	90	90	—	90	90	—	90	90	45	90	90
—	—	—	—	—	—	100	—	—	—	—	—
95	85	85	95	95	95	45	94	95	93	95	90
47	47	47	42	47	47	47	45	47	47	47	47
—	64	64	64	64	64	64	64	64	64	64	64
64	59	59	64	64	64	64	59	64	64	64	64
—	—	—	—	—	—	170	—	—	—	—	—
—	—	—	—	—	—	108	—	—	—	—	—
102	130	130	152	142	142	152	90	142	152	102	142
126	126	126	126	126	126	109	81	126	125	126	92
26	24	24	26	25	25	25	26	25	26	26	22
24	24	24	20	24	24	—	24	24	24	24	24
82	56	56	82	82	82	—	42	82	82	82	82