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IDENTIFICATION OF KODIAK ISLAND PINK SALMON POPULATIONS
BASED ON BIOCHEMICAL GENETIC AND SCALE CHARACTER VARIATION

by

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BIOCHEMICAL GENETIC ANALYSES OF 1976 AND 1977 PINK SALMON

Five species of Pacific salmon are major contributors to the economy of the State of Alaska. Pink salmon (Oncorhynchus gorbuscha), on the average, provide a larger portion of the total annual catch of salmon than any other salmonid species (Neave et al. 1967) and are therefore very important to the commercial fishery.

Kodiak Island is one of the foremost areas of pink salmon production, contributing about 40% of the total pack in 1976 (Carson and Frohne 1977). Indeed, pink salmon is by far the most abundant and economically important species of salmon on this Island.

Adult salmon returning to Kodiak Island usually spawn during the period from late July to early October in intertidal and upstream portions of many water courses. Consequently, each stream may have two (or more) spawning populations. After the eggs hatch in late autumn the alevins remain in the gravel until sometime between March and early May, and then migrate into adjacent estuaries. Estuarine residence is approximately 6 months prior to leaving for the open sea. They remain in the open ocean for nearly 11 months before returning to spawn. Two exceptions to this are Karluk and Red rivers that have no estuary of any consequence. At present, the area of residence for these juveniles is unknown.

Intermingling adult pink salmon populations are subjected to the commercial fishery before reaching their natal streams in the Kodiak District. The primary means of capture is the purse seine; stationary gillnets and beach seines are used somewhat, but to a lesser extent. The management of pink salmon is based primarily upon the attainment of escapement goals, and once these salmon enter the purse seine fishery, it is virtually impossible to determine their stream of origin. This uncertainty presents a major problem in management because of the need to harvest each stock in a manner that will allow adequate escapement to each spawning ground and permit the maximum allowable catch.

In recent years, a new biochemical technique, starch gel electrophoresis, has been developed with the potential to distinguish spawning stocks by detecting genetic variations of certain proteins (enzymes). Frequencies of these genetic variants in a population of fish can sometimes be used to distinguish it from other populations. Utter et al. (1970) applied this technique to coho salmon of Washington and Oregon. They found a significant difference in the frequency of (variant) transferrin alleles and could thus distinguish Columbia River and Fraser River spawning stocks from coastal populations. Other researchers using this technique (Allendorf 1975; Utter et al. 1973; May 1975; Utter et al. 1976; Allendorf and Utter 1978; Utter et al. 1979) also detected major divisions of natural salmonid population units. Statistical differences in the frequencies of variant proteins may also exist between individual spawning stocks within natural population units.

The Fisheries Research Institute (FRI) in conjunction with the Alaska Department of Fish and Game (ADF&G) felt Kodiak Island would be a good location to examine the capabilities of this method for stock separation of pink salmon. The Fisheries Research Institute has done considerable biological work on both the juvenile and adult life stages of Kodiak pink salmon in cooperation with ADF&G, and these ongoing studies were easily modified for the collection of genetic data. Work was begun on this project in earnest during the summer of 1976. This is the fourth and final report concerning this work and includes a synthesis of all of the other reports, as well as more recently collected data. Our objective in this study was to ascertain if the spawning stocks of pink salmon on Kodiak Island could be distinguished on the basis of electrophoretically detectable protein variations.

Gene Frequency Determination for Stock Separation

Migratory habits of Kodiak Island pink salmon are highly variable within the Kodiak management area. Bevan (1959) showed that adults tagged on the northeastern side of Afognak Island were recovered from many locations adjacent to Afognak Island, Kodiak Island, and the mainland. This observation indicates that fish captured in one location may not be destined for that same area. Thus, to assure that individual populations are harvested in proportion to their production, a method is needed to identify individual stocks within a mixed fishery. The accuracy of management decisions by ADF&G would be greatly enhanced by a rapid, inexpensive method of distinguishing stocks.

Presently, many methods are available to provide data that will potentially distinguish individual stocks, or populations, but only two yield fairly definitive results in a relatively short analytical time. One is scale character analysis using various discriminant functions to separate populations (Cook and Lord 1978; Cook 1979). The other is biochemical determination and analysis of population genetic differences.

A previous electrophoretic study of pink salmon (Aspinwall 1974b) showed few differences between spawning populations within a year class based on the analysis of only two proteins. In this study we have looked at additional polymorphic enzymes which we hoped would enable us to better distinguish the individual spawning stocks.

Comparison of Year Classes and Life Stages

There is almost no genetic exchange between odd-year and even-year classes of pink salmon because of their 2-year life cycle. Aspinwall (1974b) found major gene frequency differences between odd-year and

even-year classes, presumably reflecting an absence of gene flow. Therefore, we examined both even-year (1976) and odd-year (1977) populations.

In addition, three different life stages of the pink salmon were studied to get a measure of gene frequency stability. Samples of adult spawners from their natal streams were analyzed for both 1976 and 1977. Samples of the offspring from these spawners were taken from fry digs in March and April of 1977 and 1978. During June of 1978 an additional sample of smolts (progeny of the 1977 year-class spawners) was captured by surface trawl (toward) in Alitak Bay. Although these samples were not necessarily from the same breeding populations, these data provided an assessment of generalized differences between life stages.

Breeding Experiments

Several enzyme systems (notably malic enzyme and glucose-6-phosphate dehydrogenase) possess what appears to be genetically determined variation; however, the results could not be interpreted with any known Mendelian model (Utter, personal communication). Therefore, genetic breeding experiments were conducted at Kitoi Bay hatchery with the purpose of determining whether inheritance of these allozymes followed Mendelian patterns.

MATERIALS AND METHODS

Horizontal starch gel electrophoresis is a method by which genetic differences among proteins of individual fish can be analyzed. In this procedure, mixtures of proteins are placed in a starch gel matrix and made to migrate by applying an electrical current. Since proteins have an electrical charge inherent in the components of their structure, each type has a characteristic migration distance. Thus, a change in the "typical" migration distance of an enzyme can be recognized by electrophoretic analysis and reflects a change in the gene that codes for that enzyme. Enzymes exhibiting genetically different forms are classified as allozymes or isozymes.

In order to detect where these isozymes are localized in the starch gel after migration, it is necessary to stain them. This is accomplished either by use of staining techniques which use the specific biochemical activity of individual enzymes or by non-specific staining which identifies all proteins present at concentrations above a threshold level. Thus, by combining the separation of isozymes and the specific staining characteristics of these molecules, we are able to measure genetic variability among individual fish in a population.

The quantity of the variable genes (gene frequency) is characteristic of a given population and will remain stable over generations provided the following three conditions are met: (1) consistently

large population size; (2) random mating; and, (3) no selection, mutation, or migration. While we cannot be completely assured that all of these conditions are met, work on other salmonid species (May 1975) and our previous work on the pink salmon populations of Kodiak Island indicate no serious discrepancies from expectations.

The importance of this stability to fish management is that we can obtain data on a basic biological characteristic of component populations of a fishery with a relatively easy and inexpensive method. Also, because the gene frequencies are characteristic of a population and tend to remain stable over time, they provide data that can separate stocks reliably.

One limitation is that relatively few genes can be analyzed compared to the total number of genes in an individual because techniques have been developed for only a limited number of proteins (20-30). This number varies with the developmental state of the animal and condition of tissue sample. In addition, not all enzymes we analyze show sufficient genetic variability or genetic divergence to be useful. For instance, in this study 14 enzymes were routinely analyzed, but only five demonstrated useful polymorphism (genetically determined multiple forms): alpha-glycerophosphate dehydrogenase (AGP), aspartate amino transferase (AAT), malate dehydrogenase (MDH), phosphoglucomutase (PGM), and malic enzyme (ME). Thus, theoretically, the number of populations that could be separated was that which showed distinctive variation in one or more of these five enzyme systems. For the purposes of this report the common form of the allele is referred to as "A" while all variants are designated "B," "C," and "D" in descending order of occurrence, (unless otherwise specified).

Isozymes were analyzed in adult tissue samples collected from 29 streams for the even-year and 22 streams for the odd-year cycles (Table 1, Fig. 1). The streams were located on Afognak and Kodiak Islands and were chosen for the magnitude of their contribution to the fishery. Approximately 50 fish were collected from each stream by personnel of the Kodiak office of the ADF&G under the direction of Larry Malloy, fishery biologist. The samples were frozen as soon as possible after collection, and remained frozen (-20°C) until processing for electrophoresis. Small portions (approximately 1 to 2 g) of liver and muscle tissue, and a few drops of vitreous eye fluid from each fish were placed in three separate test tubes. Since the liver and muscle tissue did not contain sufficient liquid to enable subsequent analysis, a few drops of distilled water were added to the test tubes containing liver and muscle tissue; the samples were homogenized and then centrifuged to remove cellular debris. A small amount of the supernatant from the test tubes was absorbed into a piece of filter paper, termed a wick. The wicks were then placed into previously prepared starch gels (May 1975). Each gel contained only wicks with one type of tissue sample. All starch gels were subjected to electrophoresis for periods ranging from 2 to 4 hours. At the termination of electrophoresis the

Table 1. Streams sampled for this study (for actual sites refer to numbered locations on Fig. 1).

Stream	Stream number	Life stage	Brood year	Year of collection	Number collected
Malina Cr.	251-105	Fry	1976	1977	17
Portage Cr.	251-825	Adult	1974	1976	45
		Fry	1976	1977	32
Seal Bay Cr.	251-901	Adult	1974	1976	44
Kitoi Cr.	252-314	Fry	1976	1977	50
		Adult	1975	1977	72
Danger Cr.	252-332	Fry	1976	1977	14
		Adults	1975	1977	50
Marka Cr.	252-334	Adult	1974	1976	44
		Adults	1975	1977	49
Afognak Cr.	252-342	Adults	1974	1976	50
		Fry	1976	1977	27
		Adults	1975	1977	50
Sharatin R.	252-371	Adults	1974	1976	45
		Adults	1975	1977	45
Uganik R.	253-122	Adults	1974	1976	50
		Fry	1976	1977	15
		Adults	1975	1977	50
Terror R.	253-331	Adults	1974	1976	50
		Fry	1976	1977	11
		Adults	1975	1977	50
		Fry	1977	1978	6
Baumann's Cr.	253-332	Adults	1974	1976	49
		Fry	1977	1978	2
Uyak R.	254-202	Adults	1974	1976	50
		Fry	1976	1977	12
		Fry	1977	1978	22
Brown's Lagoon	254-204	Adults	1974	1976	49
		Fry	1976	1977	29
Zacher R.	254-301	Adults	1974	1976	50
		Fry	1977	1978	12

Table 1. Streams sampled for this study (for actual sites refer to numbered locations on Fig. 1)-continued.

Stream	Stream number	Life stage	Brood year	Year of collection	Number collected
Karluk R.	255-101	Adults	1974	1976	45
		Fry	1976	1977	20
		Adults	1975	1977	49
Karluk Lagoon	255-101	Adults	1974	1976	50
		Adults	1976	1978	50
Red R.	256-201	Adults	1974	1976	28
		Fry	1976	1977	46
Red Lake	256-201	Adults	1974	1976	50
Akalura Lagoon	257-302	Adults	1975	1977	49
Upper Station Cr.	257-304	Adults	1974	1976	49
		Adults	1975	1977	47
Narrows Cr.	257-401	Adults	1974	1976	28
		Fry	1976	1977	19
		Adults	1975	1977	41
		Fry	1977	1978	8
Horse Marine Cr.	257-402	Adults	1974	1976	47
Dog Salmon R.	257-403	Adults	1974	1976	47
		Fry	1976	1977	61
		Adults	1975	1977	32
Deadman R.	257-502	Adults	1974	1976	48
		Fry	1976	1977	36
		Adults	1975	1977	33
		Fry	1977	1978	26
Humpy R.	257-701	Adults	1974	1976	39
		Fry	1976	1977	44
		Fry	1977	1978	42
Kiliuda Bay	258-206	Adults	1974	1976	50
Kiliuda Bay	258-207	Adults	1974	1976	50
		Fry	1976	1977	23

Table 1. Streams sampled for this study (for actual sites refer to numbered locations on Fig. 1)-continued.

Stream	Stream number	Life stage	Brood year	Year of collection	Number collected
Barling R.	258-522	Adults	1974	1976	50
		Fry	1976	1977	11
		Adults	1975	1977	16
Kaiugnak R.	258-542	Adults	1974	1976	50
		Fry	1976	1977	42
		Adults	1975	1977	29
		Fry	1977	1978	40
Seven Rivers	258-701	Fry	1976	1977	28
		Adults	1975	1977	44
		Fry	1977	1978	18
Pillar Cr.	259-102	Adults	1974	1976	40
		Adults	1975	1977	48
Buskin R.	259-211	Adults	1974	1976	49
		Fry	1976	1977	15
		Adults	1975	1977	47
		Fry	1977	1978	22
American R.	259-231	Adults	1975	1977	50
Sid Olds R.	259-242	Adults	1974	1976	50
		Fry	1976	1977	25
Miam Cr.	259-412	Fry	1976	1977	13
		Adults	1975	1977	10
Hurst Cr.	259-414	Adults	1974	1976	50
		Fry	1976	1977	20
		Adults	1975	1977	65
Saltery Cr.	259-415	Adults	1975	1977	46
Kinak Cr.	262-451	Fry	1976	1977	16
Geographic Cr.	262-501	Fry	1976	1977	15

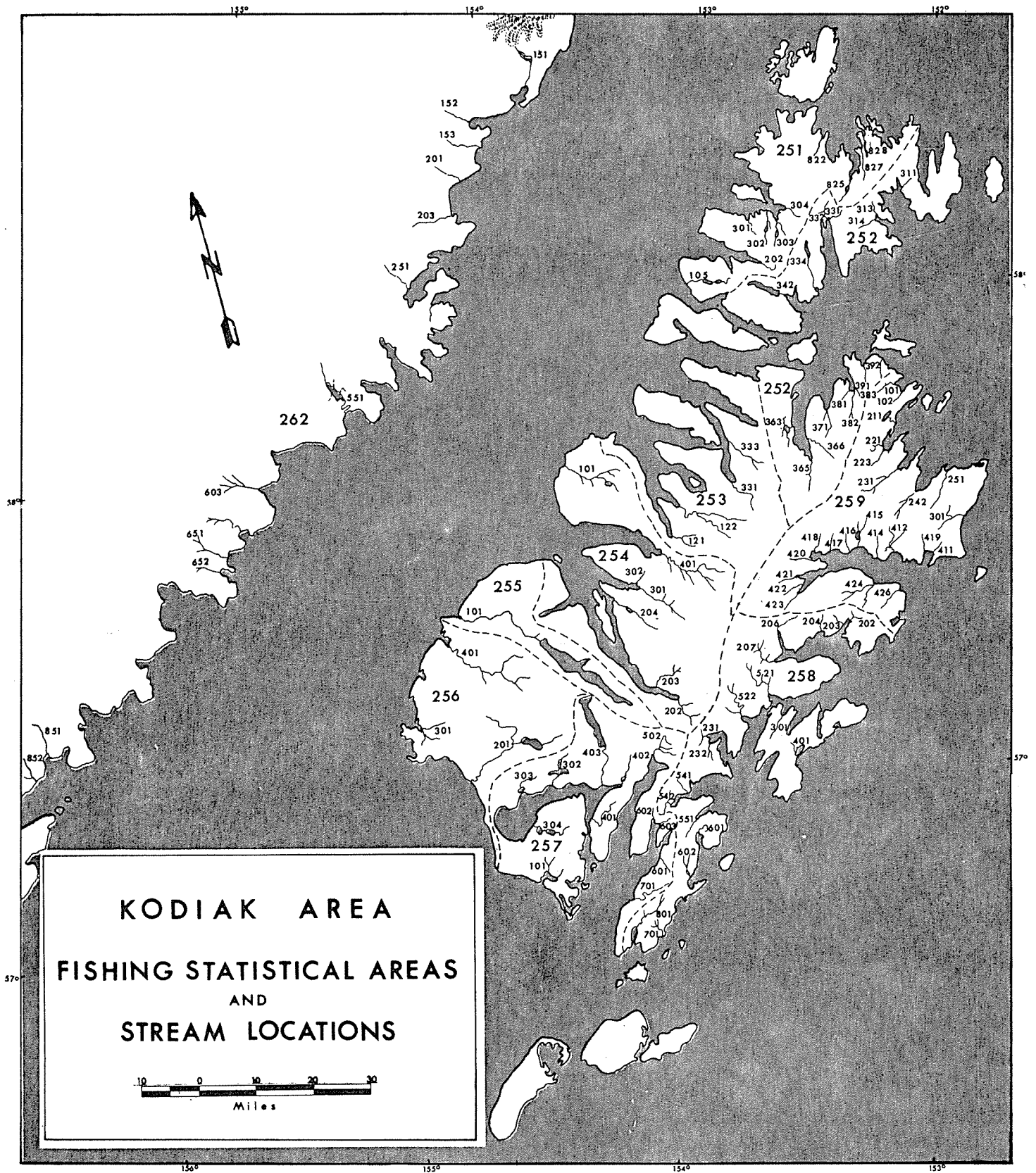


Fig. 1. Map of Kodiak Island and surrounding area showing statistical areas and stream designations used by Alaska Department of Fish and Game.

starch gels were sliced into several layers (usually 5) and each layer was stained for a different enzyme. The staining solutions used were those detailed by Shaw and Prasad (1970). For additional detail on this technique see May (1975). After staining, the phenotype of each fish was recorded for every enzyme system analyzed. Phenotypes were coded onto computer cards and the data were analyzed using existing computer facilities.

RESULTS AND DISCUSSION

The Genetic Basis of Protein Variation

Certain criteria must be met before we could assume that an observed protein variation was an actual reflection of genetic variation. A genetic basis was regarded as confirmed if progeny of parents having known isozyme variations conformed to models of simple Mendelian inheritance (Utter et al. 1974; Allendorf and Utter 1978).

Aspinwall (1973, 1974a) confirmed the genetic nature of AGP and MDH variations found in pink salmon. AAT-3 (numbers refer to the specific locus involved) variation was determined to be genetic in chum salmon by May (1975) and is presumed to be the same in pink salmon. Inheritance studies for PGM in sockeye (Utter and Hodgins 1970) have also demonstrated simple Mendelian segregation for this enzyme. Many other enzyme variations of salmonids seen on starch gels have been confirmed to be actual genetic variations (May 1975).

The genetic nature of the variation observed in three enzymes in Kodiak pink salmon populations (ME, IDH, and G₆PDH) had not yet been confirmed by breeding data. Therefore, specific crosses involving these enzyme variations were made. If they were found to have a genetic basis, we could then use them to enhance our capability for stock separation.

Another purpose for our genetic crosses was to determine if the genes which code for these enzymes are located on the same chromosome (i.e., linked). Linkage could lead to bias in the statistical analysis of their gene frequencies since independence of genes is assumed. Knowledge of the interrelationships of genes is also important for designing and evaluating any future selective breeding programs.

Genetic crosses were made at the Kitoi Bay hatchery during the summer of 1977 on the basis of variations found in the following enzymes: AGP, AAT, MDH, PGM, ME, IDH, G₆PDH, and esterase (EST). The progeny were reared on location until their yolk sacs were absorbed. They were then frozen, and sent to FRI for analysis. Unfortunately, only the enzymes expressed in the muscle tissue of the fry could be analyzed (AGP, MDH, PGM, and ME) because we were unable to rear them large enough to obtain sufficient liver and eye tissue.

The results of our specific crosses are listed in Appendix A. Malic enzyme (ME-1) variation showed simple Mendelian inheritance with what appears to be a one-locus, two-allele system. We therefore concluded that the observed variation of ME is indeed genetic and have included gene frequency data for this enzyme in our analysis.

We also confirmed the genetic nature of the enzymes AGP, MDH, and PGM in pink salmon. We were unable to detect any chromosomal linkage between any of the genes coding for AGP-1, MDH-3, PGM-1, and ME-1. The observation is not too surprising considering that pink salmon have 26 chromosome pairs and therefore the probability of these genes being located on the same chromosome is small.

Exploratory Staining

Another aspect of the project was to screen enzyme systems that were not reported previously for pink salmon, with the specific purpose of finding additional isozymes that might be useful in stock separation. Juvenile samples (1977) were used for this screening because they were in better condition than the 1976 adults. Table 2 shows all of the enzymes stained.

Specific Protein Staining

Alpha-Glycerophosphate Dehydrogenase (AGP)

This protein is expressed phenotypically in pink salmon as a single locus (Appendix C), having two alleles with the variant allele faster migrating than the common form. It is one of the easiest enzymes to read and interpret.

All populations (except Uganik River, for the 1974 brood year) gave quantifiable results. Chi-square analysis (Table 3) showed no significant difference between year classes for this protein. Gene frequency estimates of the common allele ranged from 0.77 to 1.00 (Appendix B).

Aspartate Aminotransferase (AAT)

Aspartate aminotransferase is a dimeric enzyme encoded by two loci in muscle tissue and one locus in the eye (May 1975). The two loci expressed in muscle tissue of pink salmon (AAT-1, 2) showed no variation. The locus expressed in the eye (AAT-3) had a slow migrating variant allele. The banding pattern displayed is characteristic of a dimeric enzyme encoded by a single locus having two codominant alleles (Appendix C).

The only polymorphic locus (AAT-3) was best expressed in the eye vitreous fluid. In contrast to the results obtained for the 1976 adults, most 1977 populations (17 out of 22) gave readable results.

Table 2. Protein enzymes screened for genetic variation.

Enzyme	Abbreviation	Results
Alpha glycerophosphate dehydrogenase	(AGP)	Variation
Aspartate aminotransferase - eye	(AAT)	Variation
Malate dehydrogenase	(MDH-A & B)	Variation
Lactate dehydrogenase	(LDH)	Variation
Phosphoglucomutase	(PGM)	Variation
Malic enzyme	(ME)	Variation
Creatin kinase	(CK)	Variation
Phosphoglucose isomerase	(PGI)	Variation
Phosphomannose isomerase	(PMI)	Variation
6-Phosphogluconate dehydrogenase	(6-PGHD)	Variation
Isocitrate dehydrogenase - liver	(IDH)	Variation
Glucose-6-phosphate dehydrogenase	(G ₆ PDH)	Genetic crosses will be necessary to interpret the results
β -hydroxybuterate dehydrogenase	(HBDH)	Monomorphic
Sorbitol dehydrogenase	(SDH)	Monomorphic
Peptidase	(PEP)	Monomorphic
Triose phosphate isomerase	(TPI)	Monomorphic
Glyceraldehyde-3-phosphate dehydrogenase	(G ₃ PDH)	Monomorphic
Acid phosphatase		Monomorphic
Aldolase		Monomorphic
Esterase	(EST)	Monomorphic
Alcohol dehydrogenase	(ADH)	Unreadable
Leucine aminopeptidase	(LAP)	Unreadable
Octanol dehydrogenase	(ODH)	Unreadable
Xanthine dehydrogenase	(XDH)	Unreadable
Adenylate kinase	(AK)	Unreadable
Hexokinase	(HK)	Unreadable
β -Glucuronidase		Unreadable
L-Alanine amino transferase		Unreadable

Table 3. Chi-square comparison of even-year and odd-year spawning pink salmon gene frequencies. "N" designates the sample size, "A" the common allele, "V" the variant allele(s).

Enzyme	Year of spawning	N	A	V _{fast}	V _{slow}	χ^2 comparison
AGP	even-1976 adults	1254	.888	.112	-	$\chi^2 = 0.18$ df=1
	odd- 1977 adults	967	.884	.116	-	
AAT-3	even-1978 Karluk Lagoon	50	.760	-	.240	$\chi^2 = 0.06$ df=1
	odd- 1977 adults	580	.769	-	.231	
MDH-B	even-1977 fry	640	.984	.015	.001	$\chi^2 = 43.9$ df=2
	odd- 1977 adults	990	.964	.016	.020	
PGM	even-1976 adults	1281	.975	.025	-	$\chi^2 = 17.3$ df=1
	odd- 1977 adults	969	.952	.048	-	
ME	even-1976 adults	503	.730	.270	-	$\chi^2 = 200$ df=1
	odd- 1977 adults	832	.931	.069	-	

Estimates of gene frequency for the common allele ranged from .68 to .90 (Appendix B). There was no detected difference between the 1977 frequencies and the frequency of the limited 1978 sample.

Malate Dehydrogenase (MDH)

MDH is a four-locus system that is expressed in two sets of duplicated loci (Appendix C); one group is designated MDH-A, the other MDH-B (Bailey et al. 1970). Significant differences in gene frequencies were noted between year classes for MDH-B (Table 3). Indeed, the odd-year class samples possessed a very slow MDH-B variant allele not found in the even-year sampling. This allele appears to have been classified as a fast MDH-A variant by Aspinwall (1974a). We had considerable difficulty obtaining clear results for the 1976 adults; therefore, only 8 of 19 populations could be scored (Appendix B). However, the progeny from that year class and all the odd-year samples gave excellent data; hence, the comparison of year classes.

We found the fast-migrating form of MDH-A to be rare in the 1974 brood year, occurring in samples from only three populations (Kaiugnak River, Seven River (lower fork), and Kitoi Creek) although this variant did occur in several populations of the 1975 brood year (Appendix B). In contrast, the slower migrating MDH-A variant allele was found in approximately one-half of the 1976 streams sampled, while only minor amounts of variation in samples from five streams of the 1975 brood year were detected (Appendix B).

Patterns for the MDH-B system were similar to MDH-A. The slow variant (as opposed to the very slow variant, Appendix C) was infrequent in both spawning years. The fast allele was expressed in most of the samples from 1976 and 1977 (19 for 1976 and all but 4 for 1977). The very slow allele was found at low frequency for most populations in the odd-year class, but was virtually absent in the even-year class (Appendix B).

Lactate Dehydrogenase (LDH)

Isozymes determined by five loci (numbered 1 through 5) were stained, but only two of the five loci (LDH-1 and LDH-4) showed any variation (Appendix C). Infrequent variation was noted for both loci in each year class. LDH-1 variants were slightly more frequent than LDH-4 variants. Due to the small number of samples that showed variation, this protein was not used in the year-class comparisons. Sample gene frequencies ranged from .07 to .00 for the LDH-1 variant (slower migrating than the common form). One note of interest, Karluk River and Geographic Creek, had sample gene frequencies of .07 (variant allele), considerably more variation than any other even-year streams (Appendix B).

LDH-4 variation in 1976 was only found in the Seven River (upper fork) samples (.04 for a fast-migrating form) while just four populations sampled demonstrated variation (.02 to .00) in 1977 (Appendix B).

Phosphoglucomutase (PGM)

This protein stained reliably only in the adult samples of both years, and was used in year class comparisons of adult populations where significant differences were found (Table 3). The common allele was slower migrating than the variant (Appendix C) with sample frequencies of .79 to 1.0 for 1976 and .81 to 1.0 for 1977 (Appendix B).

Malic Enzyme (ME)

Malic enzyme was not included in the analysis of the 1974 year class (Donnelly et al. 1977) because we were unsure of the genetic interpretation even though we tabulated the data (Appendices B & C). Subsequent breeding studies (discussed above) confirmed a Mendelian inheritance for this protein and these data were later included. We found a substantial difference between the year classes with average gene frequency estimates of .73 and .93 for the 1974 and 1975 brood years, respectively (Table 3).

Additional Isozymes

Creatine kinase (CK), phosphoglucose isomerase (PGI), phosphomannose isomerase (PMI), 6-phosphogluconate dehydrogenase (6-PGDH) and isocitrate dehydrogenase (IDH) all showed some variation in samples collected during 1976 (Donnelly et al. 1977). These proteins were not included in the analysis for separation capabilities or comparison of year classes due mainly to their low variability and unclear results for a large number of samples.

Three proteins (acid phosphatase, esterase (EST), and peptidase (PEP)) showed no variation for either year class. A fourth protein (glucose-6-phosphate dehydrogenase) showed variation, but the genetic interpretation is unclear.

Juveniles and Smolts

The juvenile pink salmon were not large enough to permit analysis of specific tissues. Therefore, a homogenate of the whole organism was made. Unfortunately, this homogenate lacked sufficient quantities of some enzymes. Indeed, no enzymes associated with liver (e.g., IDA) or eye tissue stained well enough to interpret. ME-1, PGM, or PEP, enzymes normally strongly expressed in muscle tissue, also did not stain satisfactorily in juveniles.

Of 39 different stream collections analyzed for the 1974 year class, 17 of these had both adult and juvenile progeny samplings (Table 1). Significant differences between adult and juvenile gene frequencies were found in only one population (Terror River); however, because of the small sample sizes of juveniles taken from these 17 streams, their estimated gene frequencies had correspondingly large statistical variances. Since these juvenile gene frequency estimates were sufficiently homogeneous, they were pooled to increase the statistical power of their comparison with the adult gene frequencies. The pooled average gene frequency of the AGP variant for the juveniles was significantly different from that of the adults (Table 4). The average frequency for the adults was .117, the juveniles .165.

Table 4. Comparison of pooled adults and juvenile AGP gene frequencies.

	<u>Adult</u>				<u>Juvenile</u>		
	<u>N</u>	<u>A</u>	<u>B</u>		<u>N</u>	<u>A</u>	<u>B</u>
	767	.883	.117		467	.835	.165

$$X^2 = 11.43 \quad DF = 1$$

There are a number of possible reasons that could be used to explain these results, e.g., sampling error, selection, sub-populations within the runs, etc.; however, the most logical reason relates to the actual sampling. There are indications that gene frequencies can vary both spatially and temporally within a given run, i.e., reflect heterogeneous gene pools (Utter, personal communication). The adult samples used in this study were taken from a limited area within most streams and from essentially one point in time. The juvenile samples may have the same shortcoming as the adult samples except the temporal problem is probably reduced when the manner of sampling and emergence conditions are considered (on sight observations). The egg digs were done in such a manner that samples were taken with the full spectrum of development, thereby eliminating some of the timing problems; however, adult sampling was not done in a systematic manner throughout the spawning grounds. Thus, we may not have obtained representative samples of the entire population.

Four samples of pink salmon smolts were taken from Alitak Bay in June of 1978. Gene frequencies of these four samples (Appendix B-5)

did not vary significantly from each other (Table 5), nor were they significantly different from the 1977 adult sample gene frequencies.

Because of the small samples of 1978 juveniles only the pooled average frequencies could be used, and the streams sampled for 1978 juveniles were not in all cases the same as those sampled for 1977 adults (Table 1). No definite conclusions can therefore be made concerning the causes of the differences between these life stages. However, we can conclude that when comparing gene frequencies from different geographical regions, it is best to sample fish at the same life stage using the same sampling method.

Population Structure

There are several methods of measuring the relationships between different populations based on their gene frequencies (Sangvhi 1953; Nei 1972; Rogers 1972; and others). Most are designed to show taxonomic relationships that may reflect genetic distances. The assumptions underlying these measurements are:

- 1) That the actual (not estimated) gene frequencies are known.
- 2) That a random sample of genome has been examined.
- 3) That all different forms (alleles) of a gene can be detected.

As with most purely theoretical models the actual application of a technique tends to use less than the theoretically best data. Usually, conscientiously designed sampling plans and adequate sample sizes result in close approximations of these assumptions. However, in our particular study we found that many sample sizes and detectable loci were far too few, resulting in a gross violation of assumptions. Therefore, we felt another technique, chi-square homogeneity test, would give better results. Table 5 lists the results of homogeneity tests of gene frequencies for all the streams sampled. The 1976 adult spawners, the 1977 (1976 brood year) juveniles, and the 1977 adult spawners showed significant heterogeneity of gene frequencies among their composite streams. These three groups were further analyzed to determine which streams or groups of streams gave distinctive gene frequencies.

The gene frequencies of all pairs of streams were compared and corresponding chi-square probabilities were calculated. The two populations whose gene frequencies were most similar were grouped and their gene frequencies averaged. Comparisons were again made, and again the two most similar populations (or groups of populations) were grouped and the process continued. The results were graphed as dendrograms from this cluster analysis, and are shown as Figs. 2, 3, and 4.

The dendrogram for the 1976 adults (Fig. 2) was based on AGP and PGM gene frequencies. These were the only two polymorphic enzymes we

Table 5. Chi-square homogeneity tests of gene frequencies.

Population	No. of subsamples	Enzyme	χ^2	d.f.	P
1976 adults	28 streams	AGP	75.7	27	< .001
	25 streams	PGM	225.8	24	< .001
		Total	301.5	51	< .001
1977 fry (1976 brood year)	25 streams	AGP	13.8	24	> .05
	6 streams	MDH-B	14.4	5	< .05
		Total	28.2	29	> .05
1977 adults	22 streams	AGP	24.4	21	> .05
	16 streams	AAT-3	15.4	15	> .05
	20 streams	MDH-B	55.8	38	< .01
	20 streams	PGM	44.9	19	< .01
	18 streams	ME	17.4	17	> .05
		Total	157.9	110	< .01
1978 fry (1977 brood year)	7 streams	AGP	4.2	6	> .05
	6 streams	MDH-B	3.1	5	> .05
	3 streams	PGM	1.0	2	> .05
		Total	8.3	13	> .05
1978 smolts (1977 brood year)	4 tows	AGP	0.6	3	> .05
	4 tows	AAT-3	1.0	3	> .05
	2 tows	MDH-B	0.3	1	> .05
	4 tows	PGM	4.9	3	> .05
	4 tows	ME	4.7	3	> .05
		Total	11.5	13	> .05

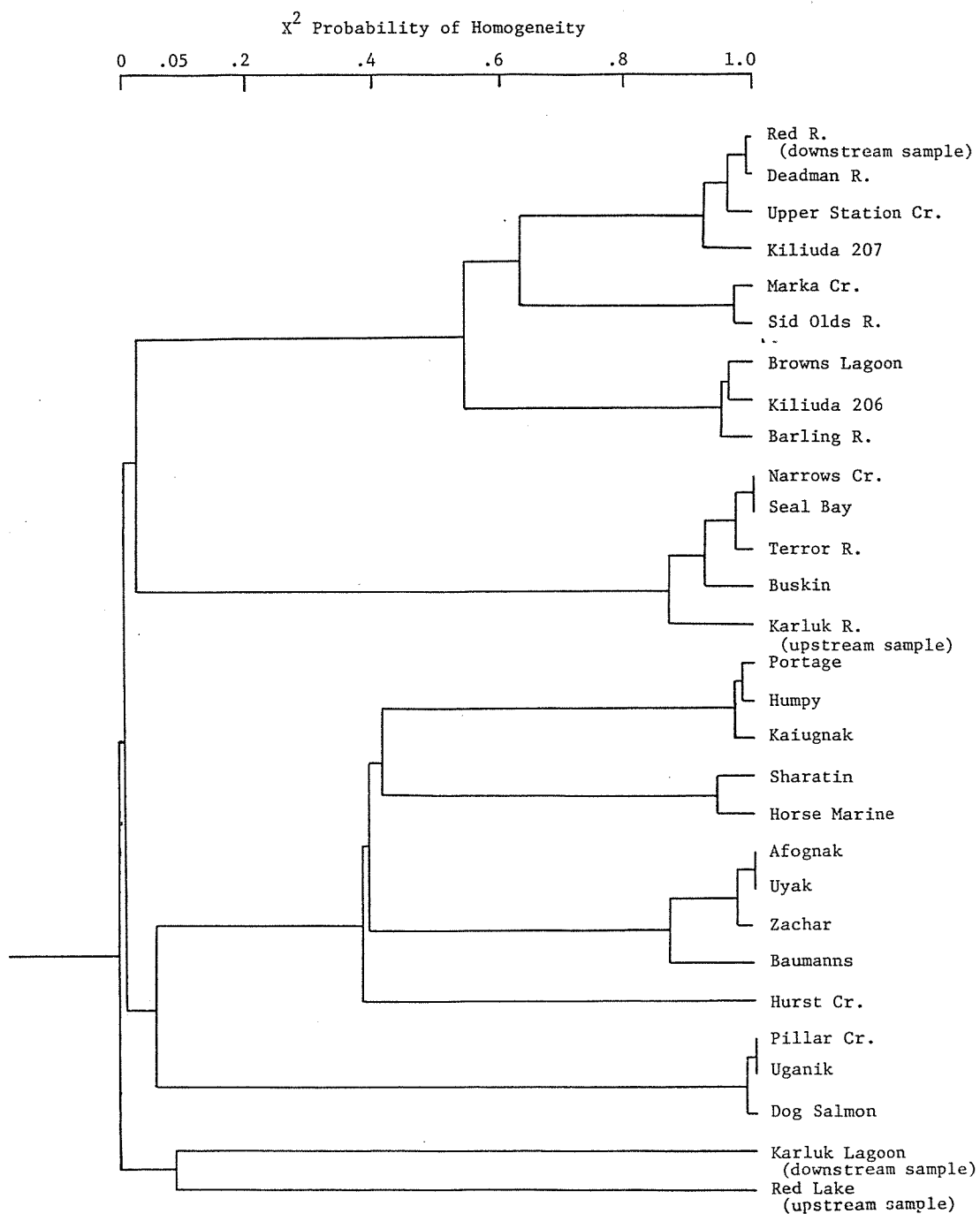


Fig. 2. Dendrogram of gene frequency clusters for 1976 adult samples, utilizing ACP and PGM gene frequencies.

χ^2 Probability of Homogeneity

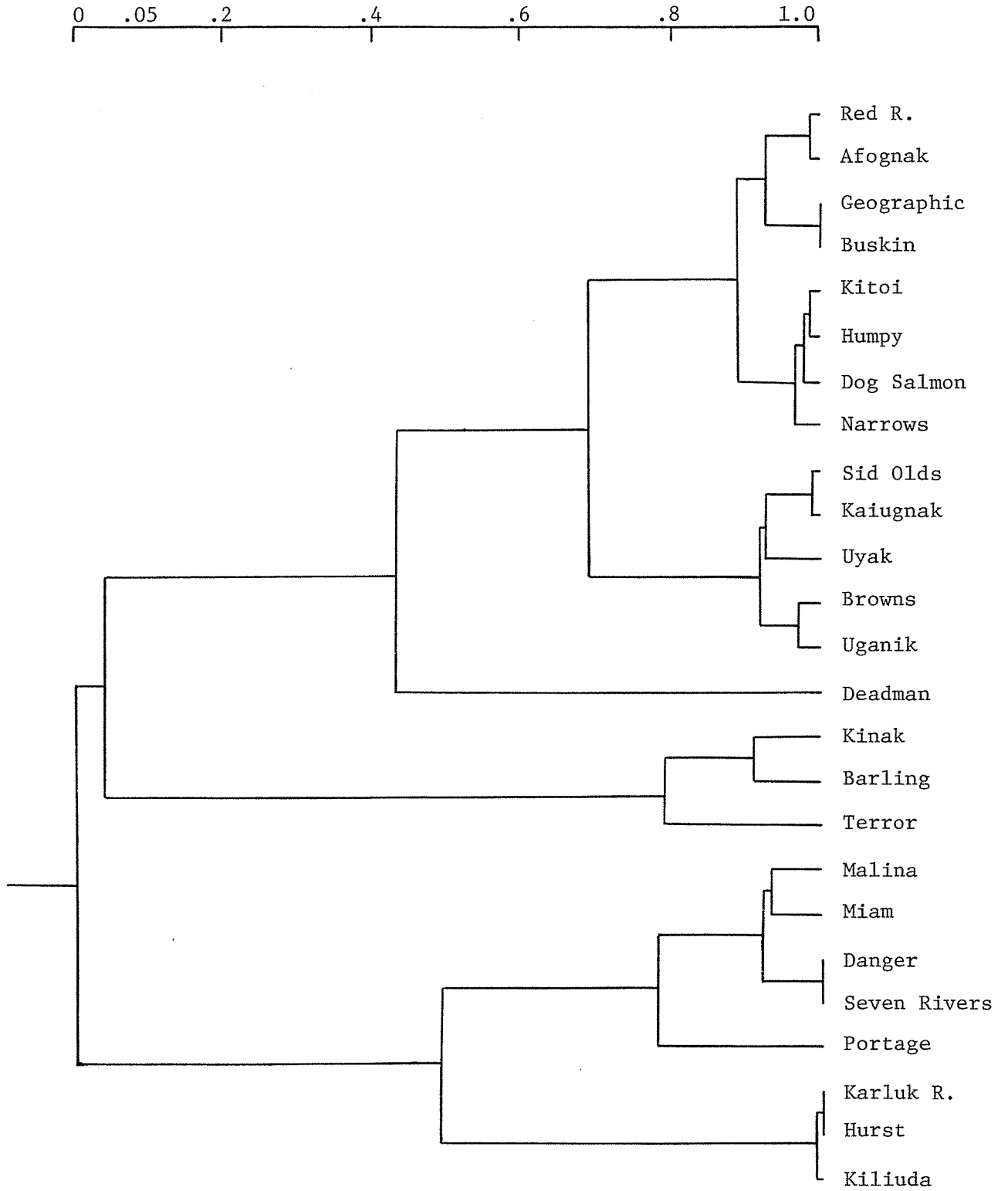


Fig. 3. Dendrogram of gene frequency clusters for 1976 brood year juveniles, using AGP and MDH-B gene frequencies.

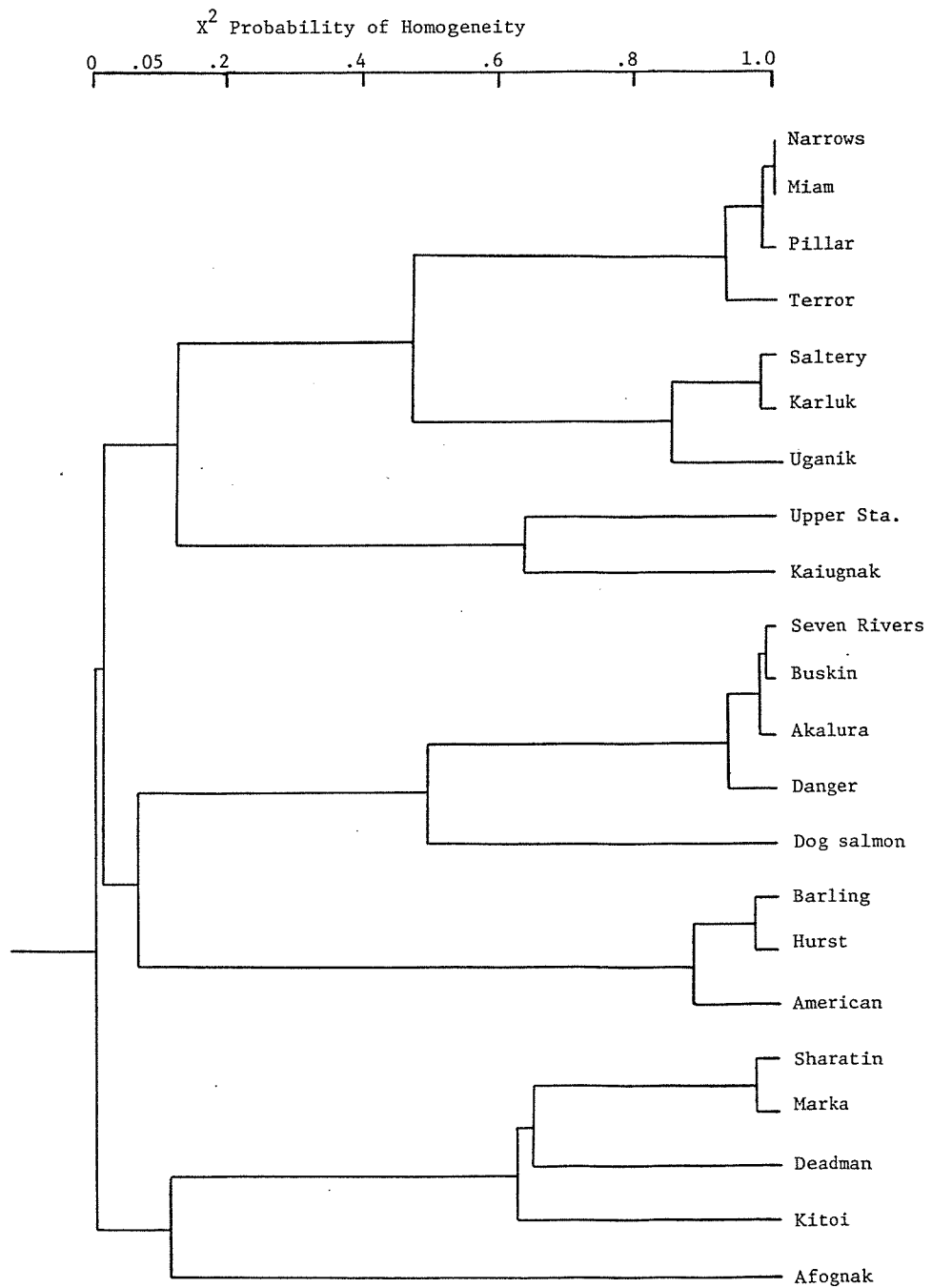


Fig. 4. Dendrogram of gene frequency clusters for 1977 adult samples, using ACP, AAT-3, MDH-B, PGM-1, and ME-1 gene frequencies.

could reliably score for all the populations (due to the poor condition of some tissue samples). The clustering of the streams based on these gene frequencies does not reflect any geographical structure. Some streams from widely separated areas are clustered together and some streams from the same geographical area are found in separate clusters.

The juveniles of the 1976 brood year were also subjected to cluster analysis of their gene frequencies (Fig. 3). Again, there is no apparent geographic structure to the populations based on gene frequencies for AGP and MDH-B. The populations of juveniles clustered differently from the adults. This may be due to the fact that the adults' dendrogram is based on AGP and PGM gene frequencies whereas the juveniles' dendrogram is based on AGP and MDH-B gene frequencies. Also, the streams sampled for juveniles were not in all cases the same as those sampled for adults.

The 1977 adult spawners provided the best data in this study. A large number of tissue samples in good conditions was collected and analyzed from each of the 22 streams studied. Cluster analysis was based on the gene frequencies of five polymorphic enzymes, AGP, AAT-3, MDH, PGM, and ME-1 (Fig. 4). The increased number of enzymes appears to make the structure somewhat more realistic; however, the situation is still not good, as evidenced by Deadman River being grouped with Sharatin, Marka, Kitoi, and Afognak rivers (all the latter are on Afognak Island, while the former is located at the southern end of Kodiak Island).

The two largest even-year spawning streams on Kodiak Island are Karluk River and Red River, with recorded spawning escapements sometimes in excess of one million pink salmon. Gene frequencies differed significantly for AGP and PGM between upstream and downstream spawners in both rivers. In addition, the LDH-1 frequencies were different in Karluk River for upstream and downstream (Karluk Lagoon) populations. Karluk Lagoon and Red Lake samples were genetically similar (except for LDH-1) but different from all other samples, including mainstream Karluk and Red River (Fig. 2). Based on this evidence, there seem to be at least two, and possibly more, subpopulations within each of the two river systems.

Tissue samples were collected in Karluk Lagoon from the 1978 spawners to determine if the distinctive gene frequencies for this area were consistent from spawning year to spawning year. Table 6 lists the gene frequencies of the 1978 Karluk Lagoon spawners and compares them with the gene frequencies obtained from the 1976 Karluk Lagoon spawners. The 1978 Karluk Lagoon sample did not have the distinctive gene frequencies of the 1976 sample. This implies that there may be several subpopulations within the lagoon spawning at different locations or times, a not unreasonable assumption considering that returning salmon are more likely to spawn near the location where they hatched. If sampling is done in just one small area, progeny from only

Table 6. Gene frequencies and 95% confidence intervals (C.I.) of the 50 adults sampled in 1976 and 1978 from Karluk Lagoon; "A" designates the common allele and "V" the variant allele(s).

Enzyme	A	95% C.I.	V _{fast}	V _{slow}
<u>1976</u>				
AGP	1.00	.97 - 1.00	0	--
LDH-1, 2, 3, 4	1.00	.97 - 1.00		
PGM	.89	.81 - .94	.11	--
CK	1.00	.97 - 1.00		
<u>1978</u>				
AGP	.85	.78 - .92	.15	--
AAT-3	.76	.67 - .85	--	.24
MDH-A	.98	.94 - .99	.005	.015
MDH-B	.97	.93 - .99	.025	.005
LDH-1	.99	.95 - 1.00	--	.01
LDH-2, 3, 4, 5	1.00	.97 - 1.00		
PGM	.99	.95 - 1.00	.01	--
ME-1	.69	.60 - .78	.31	--
CK	1.00	.97 - 1.00		
PGI-1, 2, 3	1.00	.97 - 1.00		
PMI	1.00	.97 - 1.00		

a few spawning pairs might be collected and these may not be representative of the total stock. The observation of more than one subpopulation of pink salmon inhabiting the same stream is not unique to Kodiak Island. The same phenomena was observed in Prince William Sound pink salmon populations (Seeb and Wishard 1977). The lack of geographic structuring and the possibility that several distinct subpopulations inhabit the same stream further complicates any attempt at separating stocks of pink salmon.

The small samples of 1978 juveniles exhibited gene frequencies not significantly different from random samples taken from one large homogeneous population (Table 5). The four samples of smolts also showed no significant heterogeneity. Therefore, no further statistical analysis was done on these two groups.

An analysis of Kodiak pink salmon gene frequencies suggests a rather complex population structure. Within a year class, gene frequencies among individual stream systems are quite similar, possibly reflecting a large degree of interbreeding or straying. Yet there are some streams and even some samples from the same stream that have distinct gene frequencies. Severe population constrictions or limited sampling could account for gene frequency differences if the fish sampled were, by chance, not representative of the total population. Thus, random drift could account for the few observed instances of gene frequency differences, while the large degree of straying or migration would tend to diminish these differences (Utter et al. 1979).

Because of the pink salmon's tendency to stray and interbreed with other populations (Merrell 1962; Vernon 1962), spawning populations (at least within the Kodiak area) do not have gene frequencies sufficiently distinct to enable separation. However, if particular rare protein variants were bred into a population, it could then be distinguished from other populations in a mixed fishery.

Although pink salmon populations on Kodiak show few differences in gene frequencies between streams within a year class, there are definite differences between the even-year and odd-year classes (Table 3). Even though the same streams were not always sampled for both year classes (because some streams only support one year class of any consequence), we feel these results are valid. The apparent straying of pink salmon spawners probably reduces the heterogeneity within each year class; therefore, we feel we were (for the most part) comparing two panmictic populations. Of the five isozyme frequencies compared, MDH-B, PGM, and ME-1 showed significant gene frequency differences. Aspinwall (1974b) also found significant differences in gene frequencies between the year classes of pink salmon.

These results support the view of two genetically distinct groups of pink salmon (even-year and odd-year classes). This difference has management implications because we would not expect the two year

classes to respond in the same manner to either natural or man-made environments. For example, Ricker et al. (1978) suggests that each year class has different heritabilities for size of adult fish. Therefore, each year class should be managed as a separate entity.

One note of caution should also be mentioned in regard to the management of individual spawning populations. Although the gene frequencies we examined differed little among these populations, this does not mean that these populations are genetically the same. Electrophoresis cannot detect all genetic differences. The enzymes we examined are all basic to general metabolism. Other genes controlling less basic functions may be more likely to differ among spawning populations because of differential selection pressures presented by the individual stream environments. Indeed, Bams (1976) found evidence of locally-adapted genes affecting the homing ability of pink salmon.

Although the results of this investigation indicated that naturally-reproducing pink salmon stocks could not be reliably separated on a geographical (home-stream) basis, there is a potential for using biochemical genetic analyses to aid fishery management. With the availability of pink salmon hatcheries and their control of reproduction, artificial manipulation might be employed to increase genetic variation among stocks. By marking populations with genetic tags, their contribution to the fishery could be determined and some indication obtained on the importance of these genetic differences to the fish populations. In addition, the relative cost, and stress on the marked fish, are reduced to the maximum possible. The data thus gleaned should provide a better picture of the dynamics of pink salmon stocks and, therefore, a more productive, reliable commercial fishery.

ANALYSIS OF PINK SALMON SCALES OF THE 1980 RETURN
FROM SELECTED RIVER SYSTEMS

The biochemical genetic part of this study demonstrated that within each year class the spawning populations of pink salmon on Kodiak Island were not genetically distinguishable. This varied somewhat between the odd- and even-year classes, with some distinguishing characters apparent in several of the populations in even-years. Generally, however, the starch gel technique was not able to provide the separation desirable for effective identification. Thus, it was decided to investigate the use of scale pattern analysis which has been shown to be a useful technique for separating North American and Asian sockeye salmon (Marshall et al. 1978). In fact, Cook (1979) employed discriminant function analysis of scale measurements to classify Bristol Bay sockeye salmon to river system of origin.

Scale growth of pink salmon is somewhat different from sockeye salmon since the fry migrate to salt water shortly after emergence, and before scale formation is much advanced. Thus, there is little or no differentiation possible among populations based on their freshwater experience, whereas this provides a large part of scale differences in salmon with an extensive freshwater residence (e.g., sockeye). However, there may be differences in the early life history experiences among populations in that some rivers on Kodiak Island have extensive estuaries for early rearing and others have basically none. Therefore the possibility exists that populations are differentiable if the scale characters reflect environmental variation among the habitats in which the young fish grow. Our objective in this part of the study was thus to ask the question: Are there analyzable scale pattern differences among pink salmon populations that may experience different rearing conditions?

MATERIALS AND METHODS

Scales were obtained by Mr. Larry Malloy of the Alaska Department of Fish and Game from approximately 50 fish from each of the following river systems: Zachar, Uyak, Karluk, Red, Upper Station, and Dog Salmon. Two of these rivers, Karluk and Red, have virtually no estuaries; the other four have relatively large estuaries. After collection, ADF&G personnel made plastic impressions of the scales and sent the impressions to us for analysis.

Data for analysis of the scales consisted of measurements of the distance from the focus to the outer edge of selected circuli to a point on the outer edge of the annulus. In many cases the entire series of measurements was not possible because of resorption of the outer part of the scale. Therefore, a scale was only digitized (measured) if at least 15 circuli were present.

The measurements were grouped so that five categories of data were collected from each scale. These categories were: 1) the distance from the focus to the outer edge of the third circulus; 2) the distance from the focus to the outer edge of the sixth circulus; 3) the distance from the focus to the outer edge of the ninth circulus; 4) the distance from the focus to the outer edge of the twelfth circulus; and 5) the distance from the focus to the outer edge of the fifteenth circulus.

Analysis of these values was performed using a BMDP discriminate analysis program (Dixon 1979).

RESULTS AND DISCUSSION

A summary of the data collected on the scales measured is shown in Table 7. These data show that the scales from Dog Salmon River fish

Table 7. Summary of scale measurement data.

Variable	Upper station	Zachar river	Karluk river	Red river	Dog Salmon river	Uyak river
Sample size	5	53	34	52	52	64
Character 1	74.2	71.1	63.6	60.3	54.7	66.5
Character 2	110.0	117.1	105.0	100.9	88.1	113.2
Character 3	145.2	165.1	148.1	142.7	126.2	156.4
Character 4	189.2	211.5	191.8	186.4	169.2	199.4
Character 5	230.4	259.6	235.4	230.9	215.9	243.2

have consistently smaller measurements and indicate that some discrimination from other populations is possible. Discriminate function analysis did, in fact, correctly classify 40 of 52 samples from this river (Table 8). This result suggests some potential for management since

Table 8. Number of correct and incorrect classifications for each river system.

	Upper river	Zachar river	Karluk river	Red river	Dog Salmon river	Uyak river
Correct	2	20	6	10	40	28
Incorrect	3	33	28	42	12	33
Total	5	53	34	52	52	64

since the Dog Salmon fish are an early returning population of pink salmon. However, the same analysis on samples from the other five rivers showed very poor classification. Figure 5 shows these results graphically, and vividly demonstrates that there is no separation between rivers with and without estuaries. Thus, similar to the biochemical genetic technique, the scale pattern method seems to show limited capability to separate Kodiak Island pink salmon populations.

Two explanations for these results can be forwarded. First, the juvenile pink salmon from the Red and Karluk rivers may migrate into neighboring estuaries after leaving freshwater, and therefore the scale patterns would be similar to other populations in close geographic proximity that use the same saltwater growth areas. Second, there may be a sufficiently strong genetic component determining scale patterns so that straying, during the spawning migration, leads to a similarity that is relatively unaffected by differences in environmental circumstances. However, a more thorough investigation should be conducted with larger sample sizes and more populations before definite conclusions are stated.

SUMMARY

- 1) Breeding studies showed that malic enzyme (ME) variation in pink salmon can be explained by codominant autosomal inheritance.
- 2) No chromosomal linkage was detected among AGP, MDH-B, PGM and ME-1 loci.
- 3) Differences of gene frequencies between adults and juveniles were detected but may be due to the different sampling methods employed.
- 4) Gene frequency differences between samples suggest that the Karluk River and Red River (as well as other river systems) may each contain more than one spawning population.
- 5) No apparent geographical patterns of gene frequencies were observed.
- 6) Although pink salmon populations on Kodiak Island exhibited some heterogeneity of gene frequencies, the differences were not of sufficient magnitude to be used in management-related stock separation.
- 7) Significant differences of three isozyme frequencies (MDH-B, PGM, and ME-1) were found between the even-year and odd-year classes.
- 8) Genetic heterogeneity among the populations sampled appears (for the most part) to be a reflection of the unique life history of the pink salmon rather than a reflection of geographic heterogeneity.
- 9) Scale pattern analysis of pink salmon from selected river systems, based primarily on the presence or absence of estuarine growth areas, yielded little discrimination of the populations on the basis of home river.
- 10) Scales in one population, Dog Salmon River pink salmon, were classified correctly for 77% of the samples apparently because of smaller scale measurements.
- 11) Although the results of this study do not show a great amount of population separation on the basis of scale patterns, further study is needed with larger sample sizes and more populations to adequately assess the technique.

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APPENDICES

Appendix Table A. Results of genetic crosses performed on pink salmon at Kitoi Bay hatchery.

(Legend: Presumed genotypes (refer to Appendix C))

A = Common allele

B,C,D = Variant alleles)

Cross No. 1:	Parent	AGP	MDH-3,4	ME-1
	Male	AB	AAAC	AB
	Female	AB	AAAA	AA

Progeny phenotypes--single-locus segregation:

	AGP			MDH-3,4		ME-1	
	AA	AB	BB	AAAA	AAAC	AA	AB
Observed	33	59	27	50	69	61	58
Expected	29.75	59.5	29.75	59.5	59.5	59.5	59.5
	$X^2=0.61$ df=2			$X^2=3.03$ df=1		$X^2=0.08$ df=1	

Progeny phenotypes--joint segregation:

ME-1/MDH-3,4	observed	AGP/MDH-3,4	observed	AGP/ME-1	observed
AA/AAAA	30	AA/AAAA	14	AA/AA	17
AA/AAAC	31	AA/AAAC	19	AA/AB	16
AB/AAAA	20	BB/AAAA	15	BB/AA	15
AB/AAAC	38	BB/AAAC	12	BB/AB	12
	$X^2 = \frac{(30+38-31-20)^2}{119} = 2.43$		$X^2 = \frac{(14+12-19-15)^2}{60} = 1.07$		$X^2 = \frac{(17+12-16-15)^2}{60} = 0.07$
	df=1		df=1		df=1

Cross No. 2:	Parent	AGP	MDH-3,4	ME-1
	Male	AB	AAAC	AB
	Female	AA	AAAA	AA

Progeny phenotypes--single-locus segregation:

	AGP			MDH-3,4		ME-1	
	AA	AB	BB	AAAA	AAAC	AA	AB
Observed	86	63	27	82	67	56	44
Expected	74.5	74.5	29.75	74.5	74.5	50	50
	$X^2=3.55$ df=1			$X^2=1.51$ df=1		$X^2=1.44$ df=1	

Progeny phenotypes--joint segregation:

AGP/MDH-3,4	observed	MDH-3,4/ME-1	observed	AGP/ME-1	observed
AA/AAAA	50	AAAA/AA	30	AA/AA	36
AA/AAAC	35	AAAA/AB	23	AA/AB	22
AB/AAAA	32	AAAC/AA	26	AB/AA	20
AB/AAAC	31	AAAC/AB	20	AB/AB	21
	$X^2 = \frac{(50+31-35-32)^2}{148} = 1.32$		$X^2 = \frac{(30+20-23-26)^2}{99} = 0.01$		$X^2 = \frac{(36+21-22-20)^2}{99} = 2.27$
	df=1		df=1		df=1

Appendix Table A. Results of genetic crosses performed on pink salmon at Kitoi Bay hatchery - continued.

(Legend: Presumed genotypes (refer to Appendix C)

A = Common allele

B,C,D = Variant alleles)

Cross No. 3:	<u>Parent</u>	<u>AGP</u>	<u>MDH-3,4</u>	<u>ME-1</u>
	Male	BB	AAAB	AA
	Female	AB	AAAA	AA

Progeny phenotypes--single-locus segregation:

	<u>AGP</u>		<u>MDH-3,4</u>		<u>ME-1</u>
	BB	AB	AAAA	AAAB	AA
Observed	56	59	59	60	120
Expected	57.5	57.5	59.5	59.5	120
	$X^2=0.08$ df=1		$X^2=0.01$ df=1		

Cross No. 4:	<u>Parent</u>	<u>AGP</u>	<u>MDH-3,4</u>	<u>ME-1</u>
	Male	AB	AAAA	AA
	Female	AA	AAAB	AA

Progeny phenotypes--single-locus segregation:

	<u>AGP</u>		<u>MDH-3,4</u>		<u>ME-1</u>
	AA	AB	AAAA	AAAB	AA
Observed	42	58	41	59	100
Expected	50	50	50	50	100
	$X^2=2.56$ df=1		$X^2=3.24$ df=1		

Cross No. 5:	<u>Parent</u>	<u>AGP</u>	<u>MDH-3,4</u>	<u>ME-1</u>	<u>PGM-1</u>
	Male	AA	AAAA	AA	AA
	Female	AB	AAAA	AA	AB

Progeny phenotypes--single-locus segregation:

	<u>AGP</u>		<u>MDH-3,4</u>	<u>ME-1</u>	<u>PGM-1</u>	
	AA	AB	AAAA	AA	AA	AB
Observed	55	45	100	100	35	26
Expected	50	50	100	100	30.5	30.5
	$X^2=1.0$ df=1				$X^2=1.33$ df=1	

Progeny phenotypes--joint segregation:

<u>AGP/PGM-1</u>	observed	
AA/AA	19	
AA/AB	13	
AB/AA	16	
AB/AB	13	
		$X^2 = \frac{(19+13-13-16)^2}{61} = 0.15$
		df=1

Appendix Table A. Results of genetic crosses performed on pink salmon at Kitoi Bay hatchery - continued.

(Legend: Presumed genotypes (refer to Appendix C)

A - Common allele
B,C,D - Variant alleles)

Cross No. 6: Parent AGP MDH-3,4 ME-1
 Male AA AAAA AB
 Female AB AAAB AA

Progeny phenotypes--single locus segregation:

	<u>AGP</u>		<u>MDH-3,4</u>		<u>ME-1</u>	
	AA	AB	AAAA	AAAB	AA	AB
Observed	47	53	56	44	56	46
Expected	50	50	50	50	50	50
	$\chi^2 = 0.36 \text{ df}=1$		$\chi^2 = 1.44 \text{ df}=1$		$\chi^2 = 0.64 \text{ df}=1$	

Progeny phenotypes--joint segregation:

<u>AGP/MDH-3,4</u>	observed	
AA/AAAA	22	$\chi^2 = \frac{(22+20-24-33)^2}{99} = 2.27$ df=1
AA/AAAB	24	
AB/AAAA	33	
AB/AAAB	20	

Cross No. 7: Parent AGP MDH-3,4 ME-1
 Male AA AAAC AA
 Female AA AAAB AA

Progeny phenotypes--single-locus segregation:

	<u>AGP</u>	<u>MDH-3,4</u>			<u>ME-1</u>	
	AA	AAAA	AAAB	AAAC	AABC	AA
Observed	145	31	42	30	42	145
Expected	145	36.25	36.25	36.25	36.25	145
		$\chi^2 = 3.66 \text{ df}=3$				

Appendix Table B-1. Gene frequencies and 95% confidence intervals (C.I.) of 1976 adults for LDH-1 and LDH-4; "n" designates sample size. Continued.

District number	Stream name	Stream number	LDH-1		LDH-4		
			n	A 95% C.I.	n	A 95% C.I.	
251	Seal Bay Cr.	901	44	1.00 (.97-1.00)	44	1.0 (.98-1.0)	0.0
	Portage Cr.	825	50	1.00 (.97-1.00)	50	1.0 (.98-1.0)	0.0
252	Afognak R.	342	50	.98 (.93-.99)	50	1.0 (.98-1.0)	0.0
	Sharatin Bay	371	50	.99 (.95-1.00)	50	1.0 (.98-1.0)	0.0
	Marka R.	334	44	1.00 (.97-1.00)	44	1.0 (.98-1.0)	0.0
253	Terror R.	331	50	.97 (.92-.99)	50	1.0 (.98-1.0)	0.0
	Iganik R.	122	50	.98 (.93-.99)	50	1.0 (.98-1.0)	0.0
	Baumann's Cr.	332	50	.99 (.95-1.00)	50	1.0 (.98-1.0)	0.0
254	Brown's Lagoon	204	49	.99 (.94-1.00)	49	1.0 (.98-1.0)	0.0
	Iyak R.	202	50	.99 (.95-1.00)	50	1.0 (.98-1.0)	0.0
	Zachar R.	301	50	1.00 (.97-1.00)	50	1.0 (.98-1.0)	0.0
255	Karluk R.	101	47	.98 (.93-.99)	47	1.0 (.98-1.0)	0.0
	Karluk Lagoon	101	50	1.00 (.97-1.00)	50	1.0 (.98-1.0)	0.0
256	Red R.	201	-	-	-	-	-
	Red Lake	201	50	1.00 (.97-1.00)	50	1.0 (.98-1.0)	0.0
257	Narrows Cr.	401	29	.98 (.91-1.00)	29	1.0 (.97-1.0)	0.0
	Deadman R.	502	48	1.00 (.97-1.00)	48	1.0 (.98-1.0)	0.0
	Dog Salmon R.	403	47	.99 (.94-1.00)	47	1.0 (.98-1.0)	0.0
	Humpy R.	701	39	1.00 (.96-1.00)	39	1.0 (.98-1.0)	0.0
	Horse Marine Cr.	402	47	1.00 (.97-1.00)	47	1.0 (.98-1.0)	0.0
258	Upper Station Cr.	304	49	1.00 (.97-1.00)	49	1.0 (.98-1.0)	0.0
	Kaliuda Bay	206	-	-	-	-	-
	Kaliuda Bay	207	-	-	-	-	-
	Barling R.	522	50	1.00 (.97-1.00)	50	1.0 (.98-1.0)	0.0
	Katugnak R.	542	50	.99 (.95-1.00)	50	1.0 (.98-1.0)	0.0
259	Hurst Cr.	414	50	.99 (.95-1.00)	50	1.0 (.98-1.0)	0.0
	Sid Olds R.	242	50	1.00 (.97-1.00)	50	1.0 (.98-1.0)	0.0
	Buskin E.	211	49	.96 (.90-.98)	49	1.0 (.98-1.0)	0.0
	Pillar Cr.	102	40	.98 (.91-.99)	40	1.0 (.98-1.0)	0.0
	KODIAK Total		1231	.992 (.988-.996)	1231	1.0 (.99-1.0)	0.0

Appendix Table B-1. Gene frequencies and 95% confidence intervals (C.I.) of 1976 adultst for ME; "n" designates sample size - continued.

District number	Stream name	Stream number	n	ME		95% C.I.	B
				A	B		
251	Seal Bay Cr.	901	37	.78	(.69-.87)	.22	-
	Portage Cr.	825	-	-	-	-	-
252	Afognak R.	342	-	-	-	-	-
	Sharatin Bay	371	29	.79	(.69-.89)	.21	.21
253	Marka R.	334	46	.72	(.63-.81)	.28	.28
	Terror R.	331	-	-	-	-	-
254	Uganik R.	122	-	-	-	-	-
	Baumann's Cr.	332	-	-	-	-	-
255	Brown's Lagoon	204	-	-	-	-	-
	Uyak R.	202	32	.81	(.71-.92)	.19	.19
256	Zachar R.	301	-	-	-	-	-
	Karluk R.	101	42	.67	(.57-.77)	.33	.33
257	Karluk Lagoon	101	-	-	-	-	-
	Red R.	201	27	.76	(.65-.87)	.24	.24
258	Red Lake	201	-	-	-	-	-
	Narrows Cr.	401	24	.79	(.67-.91)	.21	.21
259	Deadman R.	502	45	.65	(.55-.75)	.35	.35
	Dog Salmon R.	403	-	-	-	-	-
258	Humpy R.	701	-	-	-	-	-
	Horse Marine Cr.	402	-	-	-	-	-
258	Upper Station Cr.	304	-	-	-	-	-
	Kiliuda Bay	206	40	.69	(.59-.79)	.31	.31
259	Kiliuda Bay	207	42	.71	(.61-.81)	.29	.29
	Barling R.	522	47	.80	(.72-.88)	.20	.20
259	Kaiugnak R.	542	47	.71	(.62-.80)	.29	.29
	Hurst Cr.	414	45	.68	(.58-.78)	.32	.32
259	Sid Olds R.	242	-	-	-	-	-
	Buskin R.	211	-	-	-	-	-
Total	Pillar Cr.	102	-	-	-	-	-
			503	.73	(.69-.77)	.27	.27

Appendix Table B-2. Gene frequencies and 95% confidence intervals (C.I.) of the 1977 emergent fry (1976 brood year) for AGP, LDH-1 and LDH-4; "n" designates sample size.

District number	Stream name	Stream number	AGP			LDH-1			LDH-4		
			n	A	95% C.I.	n	A	95% C.I.	n	A	95% C.I.
251	Malina Cr.	105	17	.88	(.73-.95)	17	1.0	(.92-1.0)	17	1.0	(.92-1.0)
		825	32	.91	(.81-.96)	32	1.0	(.95-1.0)	32	1.0	(.95-1.0)
252	Kitoi Cr.	314	50	.84	(.76-.90)	50	.99	(.95-1.0)	50	1.0	(.97-1.0)
		332	14	.89	(.73-.96)	14	1.0	(.90-1.0)	14	1.0	(.90-1.0)
		342	27	.83	(.71-.91)	27	1.0	(.95-1.0)	27	1.0	(.95-1.0)
			122	15	.83	(.66-.93)	15	1.0	(.91-1.0)	15	1.0
253	Terror R.	331	11	.73	(.52-.87)	11	1.0	(.87-1.0)	11	1.0	(.87-1.0)
		202	12	.83	(.64-.93)	12	1.0	(.88-1.0)	12	1.0	(.88-1.0)
254	Brown's Lagoon	204	29	.81	(.69-.89)	29	1.0	(.95-1.0)	29	1.0	(.95-1.0)
		101	20	.85	(.71-.93)	20	.93	(.80-.97)	20	1.0	(.93-1.0)
255	Karluk R.	201	46	.85	(.76-.91)	39	.99	(.93-1.0)	39	1.0	(.96-1.0)
256	Narrows Cr.	401	19	.82	(.67-.91)	19	1.0	(.92-1.0)	19	1.0	(.92-1.0)
		403	58	.83	(.75-.89)	61	1.0	(.98-1.0)	61	1.0	(.98-1.0)
		502	36	.88	(.78-.93)	36	1.0	(.96-1.0)	36	1.0	(.96-1.0)
		701	44	.83	(.74-.89)	44	1.0	(.97-1.0)	44	1.0	(.97-1.0)
		207	23	.85	(.72-.93)	23	1.0	(.94-1.0)	23	1.0	(.94-1.0)
257	Dog Salmon R.	522	10	.75	(.53-.89)	11	1.0	(.87-1.0)	11	1.0	(.87-1.0)
		542	42	.82	(.73-.89)	42	1.0	(.97-1.0)	42	1.0	(.97-1.0)
258	Seven Rivers (lower fork)	701	1	.88	(.75-.95)	14	1.0	(.91-1.0)	14	1.0	(.91-1.0)
		701	14	.86	(.69-.94)	14	1.0	(.90-1.0)	14	.96	(.82-.99)
259	Seven Rivers (upper fork)	211	15	.83	(.66-.93)	15	1.0	(.91-1.0)	15	1.0	(.91-1.0)
		242	23	.80	(.67-.89)	25	1.0	(.94-1.0)	25	1.0	(.94-1.0)
		412	13	.92	(.76-.98)	13	1.0	(.89-1.0)	13	1.0	(.89-1.0)
		414	20	.83	(.68-.91)	20	1.0	(.93-1.0)	20	1.0	(.93-1.0)
		451	16	.72	(.55-.84)	16	.97	(.84-.99)	16	1.0	(.91-1.0)
262	Kinak Cr.	501	15	.87	(.70-.95)	15	.93	(.79-.98)	15	1.0	(.91-1.0)
		Total	637	.84	(.82-.86)	636	.99	(.99-1.0)	636	.99	(.99-1.0)

Appendix Table B-2. Gene frequencies and 95% confidence intervals (C.I.) of the 1977 emergent fry (1976 brood year) for PGI-1, PGI-3, and MDH-A; "n" designates sample size - continued.

District number	Stream name	Stream number	PGI-1			PGI-3			MDH-A					
			n	A	95% C.I.	B	n	A	95% C.I.	n	A	95% C.I.		
251	Malina Cr.	105	17	1.0	(.95-1.0)	0.0	17	1.0	(.95-1.0)	0.0	17	.97	(.95-.99)	.03
	Portage Cr.	825	32	1.0	(.98-1.0)	0.0	32	1.0	(.98-1.0)	0.0	31	1.0	(.98-1.0)	0.0
252	Kitroi Cr.	314	50	1.0	(.98-1.0)	0.0	50	1.0	(.98-1.0)	0.0	50	.98	(.93-.99)	.02
	Danger Cr.	332	14	1.0	(.95-1.0)	0.0	14	1.0	(.95-1.0)	0.0	14	1.0	(.95-1.0)	0.0
	Afognak R.	342	-	-	-	-	-	-	-	-	27	.97	(.89-.99)	.03
253	Uganik R.	122	-	-	-	-	-	-	-	-	15	1.0	(.95-1.0)	0.0
	Terror R.	331	11	1.0	(.93-1.0)	0.0	11	1.0	(.93-1.0)	0.0	11	1.0	(.93-1.0)	0.0
254	Uyak R.	202	12	1.0	(.94-1.0)	0.0	12	1.0	(.94-1.0)	0.0	12	.98	(.82-1.0)	.02
	Brown's Lagoon	204	29	1.0	(.97-1.0)	0.0	29	1.0	(.97-1.0)	0.0	29	.99	(.92-1.0)	.01
255	Karluk R.	101	20	1.0	(.96-1.0)	0.0	20	1.0	(.96-1.0)	0.0	20	1.0	(.96-1.0)	0.0
256	Red R.	201	39	1.0	(.98-1.0)	0.0	39	.99	(.94-1.0)	.01	47	.98	(.93-1.0)	.02
257	Narrows Cr.	401	19	1.0	(.96-1.0)	0.0	19	1.0	(.96-1.0)	0.0	19	1.0	(.96-1.0)	0.0
	Dog Salmon R.	403	61	.99	(.97-1.0)	0.0	61	1.0	(.99-1.0)	0.0	61	1.0	(.99-1.0)	0.0
	Deadman R.	502	36	1.0	(.98-1.0)	0.0	36	1.0	(.98-1.0)	0.0	36	.97	(.91-1.0)	.03
	Humpy R.	701	44	1.0	(.98-1.0)	0.0	44	1.0	(.98-1.0)	0.0	44	.98	(.97-1.0)	0.0
	Kiliuda Bay	207	23	1.0	(.96-1.0)	0.0	23	1.0	(.96-1.0)	0.0	23	.99	(.90-1.0)	.01
258	Barling R.	522	11	1.0	(.93-1.0)	0.0	11	1.0	(.93-1.0)	0.0	11	1.0	(.93-1.0)	0.0
	Kaiugnak R.	542	42	1.0	(.98-1.0)	0.0	42	1.0	(.98-1.0)	0.0	42	.99	(.94-1.0)	.01
	Seven Rivers (lower fork)	701	16	1.0	(.95-1.0)	0.0	16	1.0	(.95-1.0)	0.0	16	1.0	(.95-1.0)	0.0
259	Seven Rivers (upper fork)	701	14	1.0	(.95-1.0)	0.0	14	1.0	(.95-1.0)	0.0	14	.93	(.77-.98)	.07
	Buskin R.	211	15	1.0	(.95-1.0)	0.0	15	1.0	(.95-1.0)	0.0	15	.93	(.78-.98)	.07
262	Sid Olds R.	242	25	1.0	(.97-1.0)	0.0	25	1.0	(.97-1.0)	0.0	25	.99	(.91-1.0)	.01
	Miam R.	412	13	1.0	(.94-1.0)	0.0	13	1.0	(.94-1.0)	0.0	13	.98	(.84-1.0)	.02
	Hurst Cr.	414	20	1.0	(.96-1.0)	0.0	20	1.0	(.96-1.0)	0.0	20	1.0	(.96-1.0)	0.0
	Kinak Cr.	415	16	1.0	(.95-1.0)	0.0	16	1.0	(.95-1.0)	0.0	16	.98	(.87-1.0)	.02
Total	Geographic Cr.	501	15	1.0	(.95-1.0)	0.0	15	1.0	(.95-1.0)	0.0	15	1.0	(.95-1.0)	0.0
			594	.99	(.98-1.0)	.01	594	.99	(.98-1.0)	0.0	640	.99	(.98-1.0)	.01

Appendix Table B-2. Gene frequencies and 95% confidence intervals (C.I.) of the 1977 emergent fry (1976 brood year) for MDH-B; "n" designates sample size - continued.

District number	Stream name	Stream number	n	MDH-B			C	95% C.I.	
				A	95% C.I.	B			
251	Malina Cr.	105	17	.94	(.81-.98)	.06	(0.0-.12)	0.0	(0.0-.04)
	Portage Cr.	825	31	.98	(.91-1.0)	.02	(0.0-.04)	0.0	(0.0-.01)
252	Kitoi Cr.	314	50	.98	(.93-.99)	.01	(0.0-.02)	.01	(0.0-.02)
	Danger Cr.	332	14	.96	(.82-.99)	.04	(0.0-.09)	0.0	(0.0-.05)
	Afognak R.	342	27	.97	(.89-.99)	.03	(0.0-.06)	0.0	(0.0-.03)
253	Uganik R.	122	15	1.0	(.95-1.0)	0.0	(0.0-.05)	0.0	(0.0-.05)
	Terror R.	331	11	.98	(.82-1.0)	.02	(0.0-.04)	0.0	(0.0-.07)
254	Uyak R.	202	12	.98	(.82-1.0)	.02	(0.0-.04)	0.0	(0.0-.06)
	Brown's Lagoon	204	29	.98	(.91-1.0)	.02	(0.0-.04)	0.0	(0.0-.05)
255	Karluk R.	101	20	.96	(.85-.99)	.04	(0.0-.08)	0.0	(0.0-.04)
256	Red R.	201	47	1.0	(.98-1.0)	0.0	(0.0-.02)	0.0	(0.0-.02)
257	Narrows Cr.	401	19	.97	(.87-1.0)	.03	(0.0-.07)	0.0	(0.0-.04)
	Dog Salmon R.	403	61	.99	(.95-1.0)	0.0	(0.0-.01)	.01	(0.0-.02)
	Deadman R.	502	36	1.0	(.98-1.0)	0.0	(0.0-.02)	0.0	(0.0-.02)
	Humpy R.	701	44	1.0	(.98-1.0)	0.0	(0.0-.02)	0.0	(0.0-.02)
	Kiliuda Bay	207	23	.98	(.89-1.0)	.02	(0.0-.04)	0.0	(0.0-.03)
258	Barling R.	522	11	.93	(.75-.98)	.07	(0.0-.15)	0.0	(0.0-.07)
	Kaiugnak R.	542	42	1.0	(.98-1.0)	0.0	(0.0-.02)	0.0	(0.0-.02)
	Seven Rivers (lower fork)	701	16	.93	(.80-.98)	.05	(0.0-.10)	.02	(0.0-.05)
	Buskin R.	211	15	.98	(.86-1.0)	.02	(0.0-.05)	0.0	(0.0-.05)
259	Sid Olds R.	242	25	.96	(.87-.99)	.03	(0.0-.06)	.01	(0.0-.03)
	Miam R.	412	13	1.0	(.94-1.0)	0.0	(0.0-.06)	0.0	(0.0-.06)
	Hurst Cr.	414	20	.98	(.87-1.0)	.02	(0.0-.04)	0.0	(0.0-.04)
262	Kinak Cr.	451	16	.97	(.84-.99)	.03	(0.0-.07)	0.0	(0.0-.05)
	Geographic Cr.	501	15	1.0	(.95-1.0)	0.0	(0.0-.05)	0.0	(0.0-.05)
Total			640	.984	(.979-.989)	.015	(-.010-.020)	.001	(0.00-.002)

Appendix Table B-3. Gene frequencies and 95% confidence intervals (C.I.) of the 1977 adults for AGP, PGM and AAT; "n" designates the sample size.

District number	Stream name	Stream number	AGP			PGM			AAT					
			A	n	95% C.I.	B	n	95% C.I.	A	n	95% C.I.			
252	Kitoi Cr.	314	.81	72	(.73-.86)	.19	72	.94	.06	(.80-.97)	.79	49	(.69-.86)	.21
"	Danger R.	332	.91	50	(.84-.95)	.09	50	.96	.04	(.90-.98)	.69	49	(.60-.78)	.31
"	Marka Cr.	334	.85	49	(.76-.91)	.15	49	.96	.04	(.90-.98)	.74	38	(.63-.82)	.26
"	Afognak R.	342	.94	50	(.88-.97)	.06	47	.96	.04	(.90-.98)	.74	44	(.64-.82)	.26
"	Sharatin R.	371	.89	45	(.81-.94)	.11	47	.94	.06	(.87-.97)	.74	44	(.64-.82)	.26
253	Uganik R.	122	.88	50	(.80-.93)	.12	50	.97	.03	(.92-.99)	.77	46	(.68-.85)	.23
"	Terror R.	331	.89	50	(.81-.94)	.11	46	.90	.10	(.82-.95)	.77	46	(.68-.85)	.23
255	Karluk R.	101	.86	45	(.77-.91)	.14	49	.97	.03	(.91-.99)	.77	13	(.58-.89)	.23
257	Akalura Lagoon	302	.91	48	(.83-.95)	.09	48	.98	.02	(.93-.99)	.81	36	(.70-.88)	.19
"	Upper Station Cr.	304	.84	47	(.75-.90)	.16	47	.95	.05	(.88-.98)	.81	36	(.70-.88)	.19
"	Narrows Cr.	401	.89	41	(.80-.94)	.11	40	.90	.10	(.81-.95)	.81	36	(.70-.88)	.19
"	Dog Salmon R.	403	.83	32	(.72-.90)	.17	30	1.0	0.0	(.95-1.0)	.81	36	(.70-.88)	.19
"	Deadman R.	502	.86	33	(.76-.93)	.14	33	.88	.12	(.78-.94)	.68	25	(.54-.79)	.32
258	Barling Cr.	522	.88	16	(.72-.95)	.12	16	1.0	0.0	(.91-1.0)	.90	15	(.74-.97)	.10
"	Kaiugnak R.	542	.91	29	(.81-.96)	.09	29	.86	.14	(.75-.93)	.80	28	(.68-.89)	.20
"	Seven Rivers	701	.91	44	(.83-.95)	.09	44	.98	.02	(.92-.99)	.77	42	(.67-.85)	.23
259	Pillar Cr.	102	.92	48	(.84-.96)	.08	49	.94	.06	(.87-.97)	.74	48	(.64-.82)	.26
"	Buskin R.	211	.87	47	(.79-.93)	.13	47	.97	.03	(.91-.99)	.81	44	(.71-.88)	.19
"	American R.	231	.93	50	(.86-.97)	.07	50	.97	.03	(.92-.99)	.85	50	(.77-.91)	.15
"	Miam Cr.	412	1.0	10	(.86-1.0)	0.0	10	1.0	0.0	(.86-1.0)	.75	10	(.53-.89)	.25
"	Hurst Cr.	414	.89	65	(.83-.93)	.11	65	1.0	0.0	(.98-1.0)	.75	10	(.53-.89)	.25
"	Saltery Cr.	415	.88	46	(.80-.93)	.12	46	.95	.05	(.88-.98)	.74	43	(.64-.82)	.26
Total			.884	967	(.869-.899)	.116	969	.952	.048	(.942-.962)	.769	580	(.744-.794)	.231

Appendix Table B-3. Gene frequencies and 95% confidence intervals (C.I.) of 1977 adults for MDH-B and ME; "n" designates the sample size - continued.

District number	Stream name	Stream number	MDH-B				ME						
			A	95% C.I.	B	95% C.I.	D	95% C.I.	Slow variant	n	A	95% C.I.	B
252	Kitoi Cr.	314	.986	(.951-.996)	.023	(.005-.041)	.014	(.004-.049)	0.00	40	.950	(.90-1.0)	.050
"	Danger R.	332	.980	(.930-.995)	.010	(.002-.055)	.010	(.002-.055)	0.00	50	.950	(.91-.99)	.050
"	Marka Cr.	334	.969	(.914-.990)	.026	(.008-.079)	.005	(.001-.047)	0.00	50	.890	(.83-.95)	.110
"	Afognak R.	342	.925	(.856-.962)	.045	(.919-.105)	.015	(.024-.004)	.015	50	.970	(.94-1.0)	.030
"	Sharatin R.	371	.966	(.905-.988)	.028	(.009-.087)	.006	(.001-.051)	0.00	47	.904	(.84-.96)	.096
253	Uganik R.	122	.950	(.888-.979)	.015	(.004-.062)	.025	(.008-.079)	.010	50	.960	(.92-1.0)	.040
"	Terror R.	331	.970	(.916-.990)	.010	(.002-.055)	.020	(.006-.070)	0.00	50	.950	(.91-.99)	.050
255	Karluk R.	101	.949	(.886-.978)	.015	(.004-.064)	.036	(.013-.093)	0.00	33	.955	(.90-1.0)	.045
257	Akalura R.	302	.974	(.920-.992)	.010	(.002-.057)	.016	(.004-.065)	0.00	47	.894	(.83-.95)	.106
"	Upper Station Cr.	304	.968	(.910-.989)	0.00	(.00-.031)	.032	(.011-.090)	0.00	37	.932	(.87-.99)	.068
"	Narrows Cr.	401	.945	(.873-.977)	.012	(.002-.066)	.043	(.016-.111)	0.00	30	.917	(.85-.99)	.083
"	Frazer Lk.	403	.975	(.923-.992)	.015	(.004-.062)	.010	(.002-.055)	0.00	30	.917	(.85-.99)	.083
"	Deadman R.	502	.932	(.844-.972)	.045	(.016-.125)	.023	(.005-.003)	0.00	11	1.00	(.93-1.0)	.00
258	Barling Cr.	522	.953	(.820-.989)	.016	(.002-.133)	.031	(.006-.157)	0.00	29	.880	(.80-.96)	.120
"	Kaiugnak R.	542	.957	(.871-.987)	.009	(.001-.077)	.026	(.006-.105)	.008	44	.909	(.85-.97)	.091
"	Seven Rivers	701	.955	(.889-.982)	.017	(.004-.071)	.023	(.006-.079)	.005	49	.939	(.89-.99)	.061
259	Pillar Cr.	102	.954	(.893-.981)	.015	(.004-.064)	.031	(.011-.086)	0.00	47	.894	(.83-.95)	.106
"	Buskin R.	211	.989	(.942-.998)	.011	(.002-.058)	0.00	(.00-.031)	0.00	50	.940	(.89-.99)	.060
"	American R.	231	.955	(.895-.981)	.010	(.002-.055)	.035	(.013-.092)	0.00	9	.944	(.83-1.0)	.056
"	Miam Cr.	412	10.975	(.800-.997)	0.00	(.00-.139)	.025	(.003-.200)	0.00	62	.944	(.90-.98)	.056
"	Hurst Cr.	414	65.977	(.934-.992)	0.00	(.00-.023)	.023	(.008-.066)	0.00	47	.936	(.89-.99)	.064
"	Saltery Cr.	415	46.973	(.916-.992)	.005	(.001-.050)	.022	(.006-.076)	0.00	832	.931	(.919-.943)	.069
	Total		990	.962	(.956-.968)	.016	(.012-.020)	.020	(.016-.024)	.002			

Appendix Table B-3. Gene frequencies and 95% confidence intervals (C.I.) of the 1977 adults for LDH-1, LDH-4 and MDH-A; "n" designates the sample size. -- continued.

District number	Stream name	Stream number	LDH-1			LDH-4			MDH-A					
			n	A	95% C.I.	B	n	A	95% C.I.	B	n	A	95% C.I.	B
252	Kitoi Cr.	314	72	1.0	(.98-1.0)	0.0	72	.98	(.94-.99)	.02	72	1.0	(.98-1.0)	0.0
"	Danger R.	332	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0
"	Marka Cr.	334	50	.99	(.95-1.0)	.01	50	1.0	(.97-1.0)	0.0	49	.97	(.91-.99)	.03
"	Afognak R.	342	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0	50	.99	(.95-1.0)	.01
"	Sharatin R.	371	47	.99	(.94-1.0)	.01	47	.99	(.94-1.0)	.01	47	1.0	(.97-1.0)	0.0
253	Uganik R.	122	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0	50	.99	(.95-1.0)	.01
"	Terror R.	331	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0
255	Karluk R.	101	49	.99	(.94-1.0)	.01	49	.99	(.94-1.0)	.01	49	.99	(.95-1.0)	.01
257	Akalura R.	302	48	1.0	(.97-1.0)	0.0	48	1.0	(.97-1.0)	0.0	48	1.0	(.97-1.0)	0.0
"	Upper Station Cr.	304	-	-	-	-	-	-	-	-	47	1.0	(.97-1.0)	0.0
"	Narrows Cr.	401	41	1.0	(.96-1.0)	0.0	41	.99	(.93-1.0)	.01	41	.99	(.87-.98)	.01
"	Frazer Lk.	403	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0
"	Deadman	502	33	.97	(.90-.99)	.03	33	1.0	(.96-1.0)	0.0	34	1.0	(.96-1.0)	0.0
258	Barling Cr.	522	16	1.0	(.91-1.0)	0.0	16	1.0	(.91-1.0)	0.0	16	1.0	(.91-1.0)	0.0
"	Kaiugnak R.	542	29	.91	(.81-.96)	.09	29	1.0	(.95-1.0)	0.0	29	1.0	(.95-1.0)	0.0
"	Seven Rivers	701	44	.99	(.94-1.0)	.01	44	1.0	(.97-1.0)	0.0	44	1.0	(.97-1.0)	0.0
259	Fillar Cr.	102	49	1.0	(.93-.99)	.02	49	1.0	(.97-1.0)	0.0	49	1.0	(.97-1.0)	0.0
"	Buskin R.	211	47	.98	(.93-.99)	.02	47	1.0	(.97-1.0)	0.0	47	1.0	(.97-1.0)	0.0
"	American R.	231	50	.91	(.84-.95)	0.9	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0
"	Miam Cr.	412	10	1.0	(.86-1.0)	0.0	10	1.0	(.86-1.0)	0.0	10	1.0	(.86-1.0)	0.0
"	Hurst Cr.	414	65	.99	(.97-1.0)	.01	65	1.0	(.98-1.0)	0.0	65	1.0	(.98-1.0)	0.0
"	Saltery Cr.	415	46	.99	(.94-1.0)	.01	46	1.0	(.97-1.0)	0.0	46	1.0	(.97-1.0)	0.0
Total			946	.987	(.982-.992)	.013	946	.997	(.994-.999)	.003	993	.997	(.995-.999)	.003

Appendix Table B-4. Gene frequencies and 95% confidence intervals (C.I.) of 1978 emergent fry (from the 1977 brood) for ACP, PGM and PMI; "n" designates sample size.

District number	Stream name	Stream number	ACP			PGM			PMI					
			n	A	95% C.I.	B	n	A	95% C.I.	B	n	A	95% C.I.	B
253	Terror R. Baumann's Cr.	331	6	.92	(.76-1.0)	.08	6	1.0	(.78-1.0)	0.0	6	1.0	(.78-1.0)	0.0
		332	2	1.0	(.47-1.0)	0.0	2	1.0	(.47-1.0)	0.0	2	1.0	(.47-1.0)	0.0
254	Uyak R. Zachar R.	202	22	.80	(.68-.92)	.20	-	-	-	-	22	1.0	(.93-1.0)	0.0
		301	11	.95	(.87-1.0)	.05	11	.95	(.87-1.0)	.05	12	1.0	(.88-1.0)	0.0
257	Narrows Cr. Humpy R. Deadman R.	401	6	.75	(.51-1.0)	.25	7	.93	(.79-1.0)	.07	8	1.0	(.83-1.0)	0.0
		701	42	.81	(.73-.89)	.19	-	-	-	-	-	-	-	-
		502	26	.87	(.77-.96)	.13	-	-	-	-	26	1.0	(.94-1.0)	0.0
258	Kaugnak R. Seven Rivers	542	40	.85	(.77-.93)	.15	-	-	-	-	40	1.0	(.96-1.0)	0.0
		701	18	.86	(.75-.97)	.14	18	.92	(.83-1.0)	.08	18	1.0	(.92-1.0)	0.0
259	Lower Buskin R. Upper Buskin R.	211	14	.86	(.73-.99)	.14	10	1.0	(.86-1.0)	0.0	14	1.0	(.90-1.0)	0.0
		211	8	1.0	(.83-1.0)	0.0	8	.94	(.82-1.0)	.06	8	1.0	(.83-1.0)	0.0
	Unknown		28	.82	(.72-.92)	.18	28	.98	(.94-1.0)	.02	28	1.0	(.95-1.0)	0.0
	Total		217	.846	(.811-.881)	.154	124	.956	(.930-.982)	.044	184	1.0	(.99-1.0)	0.0

Appendix Table B-4. Gene frequencies and 95% confidence intervals (C.I.) of 1978 emergent fry (from the 1977 brood) for 6-PGDH and MDH-B; "n" designates sample size - continued.

District number	Stream name	Stream number	6-PGDH			MDH-B					
			n	A	95% C.I.	B	95% C.I.	D	95% C.I.		
253	Terror R.	331	6	.92	(.76-1.0)	.08	6	.916	(.806-1.00)	.042	(0.00-.122)
	Baumann's R.	332	2	1.0	(.47-1.0)	0.0	2	1.00	(.473-1.00)	0.00	(0.00-.527)
254	Uyak R.	202	-	-	-	-	22	.955	(.912-.998)	.034	(0.00-.072)
	Zacher R.	301	6	.92	(.76-1.0)	.08	12	.958	(.902-1.00)	.021	(0.00-.061)
257	Narrows Cr.	401	-	-	-	-	8	.969	(.909-1.00)	.031	(0.00-.092)
	Humpy R.	701	-	-	-	-	42	.970	(.944-.996)	.030	(.004-.056)
	Deadman R.	502	-	-	-	-	26	.961	(.924-.998)	.029	(0.00-.061)
258	Kaiugnak R.	542	-	-	-	-	40	.962	(.933-.991)	.025	(.001-.049)
	Seven Rivers	701	-	-	-	-	18	.972	(.934-1.00)	.028	(0.00-.066)
259	Lower Buskin R.211		11	.91	(.79-1.0)	.09	14	.929	(.861-.996)	0.00	(0.00-0.52)
	Upper Buskin R.211		8	1.0	(.83-1.0)	0.0	8	1.00	(.911-1.00)	0.00	(0.00-.089)
	Unknown		-	-	-	-	28	.982	(.957-1.00)	.009	(0.00-.027)
	Total		33	.939	(.880-.998)	.061	220	.969	(.957-.981)	.023	(.013-.033)

.008 (.002-.014)

Appendix Table B-4. Gene frequencies and 95% confidence intervals (C.I.) of 1978 emergent fry (from the 1977 brood) for PGI, LDH-1 and LDH-4; "n" designates sample size - continued.

District number	Stream name	Stream number	PGI			LDH-1			LDH-4			
			n	A	95% C.I.	n	A	95% C.I.	n	A	95% C.I.	
253	Terror R.	331	6	1.0	(.78-1.0)	6	1.0	(.78-1.0)	6	1.0	(.78-1.0)	
	Baumann's R.	332	2	1.0	(.47-1.0)	2	1.0	(.47-1.0)	2	1.0	(.47-1.0)	
254	Uyak R.	202	-	-	-	22	.98	(.93-1.0)	.02	22	1.0	(.93-1.0)
	Zacher R.	301	12	1.0	(.88-1.0)	12	1.0	(.88-1.0)	0.0	12	1.0	(.88-1.0)
257	Narrows Cr.	401	8	1.0	(.83-1.0)	8	1.0	(.83-1.0)	0.0	8	1.0	(.83-1.0)
	Humpy R.	201	-	-	-	42	.99	(.95-1.0)	.01	42	1.0	(.97-1.0)
	Deadman R.	502	-	-	-	26	1.0	(.94-1.0)	0.0	26	1.0	(.94-1.0)
258	Kaiugnak R.	542	-	-	-	40	1.0	(.96-1.0)	0.0	40	1.0	(.96-1.0)
	Seven Rivers	701	18	1.0	(.92-1.0)	18	1.0	(.92-1.0)	0.0	18	1.0	(.92-1.0)
259	Lower Buskin R.	211	-	-	-	14	1.0	(.90-1.0)	0.0	14	1.0	(.90-1.0)
	Upper Buskin R.	211	8	1.0	(.83-1.0)	8	1.0	(.83-1.0)	0.0	8	1.0	(.83-1.0)
	Unknown		28	1.0	(.95-1.0)	28	1.0	(.95-1.0)	0.0	28	1.0	(.95-1.0)
	Total		82	1.0	(.98-1.0)	226	.995	(.99-1.0)	.005	226	1.0	(.99-1.0)

Appendix Table B-5. Gene frequencies and 95% confidence intervals (C.I.) of AGP, PGM and AAT-3 for 1978 smolts; "n" designates the sample size. Tow number refers to which sequenced surface trawl was sampled.

Tow number	AGP			PGM			AAT-3		
	n	A	95% C.I.	n	A	95% C.I.	n	A	95% C.I.
6	23	.87	(.77-.97)	17	1.000	(.03-.23)	0.0	.82	(.70-.93)
13	48	.91	(.85-.96)	48	.90	(.83-.96)	.10	.76	(.67-.86)
29	22	.91	(.82-.99)	22	.93	(.86-1.0)	.07	.77	(.65-.90)
30	48	.88	(.82-.95)	48	.95	(.90-.99)	.05	.74	(.65-.83)
Total	141	.894	(.857-.930)	135	.933	(.903-.963)	.067	.766	(.713-.819)

Appendix Table B-5. Gene frequencies and 95% confidence intervals (C.I.) of ME, PMI and 6-PGDH for 1978 smolts; "n" designates the sample size. Tow number refers to which sequenced surface trawl was sampled - continued.

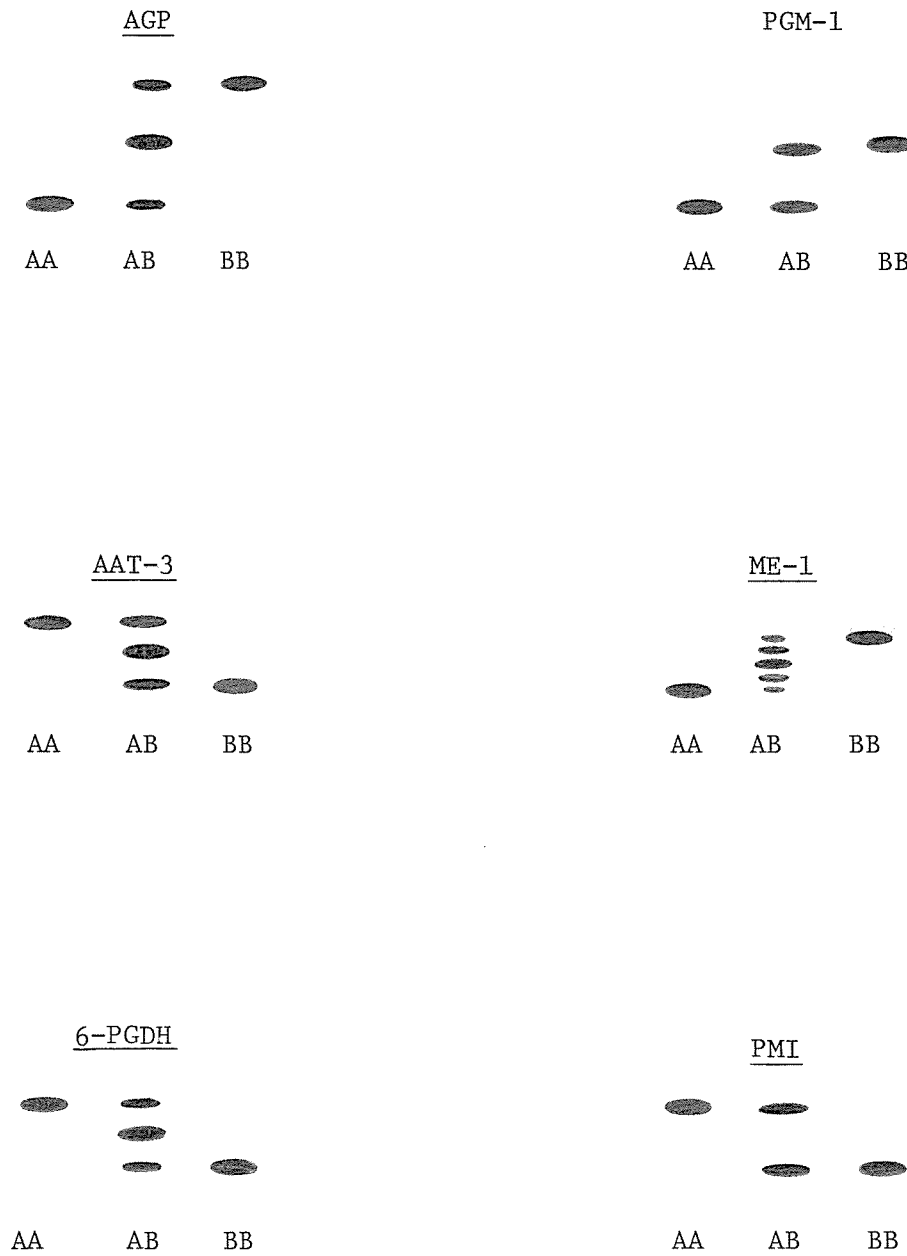
Tow number	ME			PMI			6-PGDH					
	n	A	95% C.I.	B	n	A	95% C.I.	B	n	A	95% C.I.	B
6	23	.89	(.80-.98)	.11	23	1.0	(.94-1.0)	0.0	23	1.0	(.94-1.0)	0.0
13	48	.94	(.89-.99)	.06	48	.99	(.97-1.0)	.01	47	.93	(.87-.98)	.07
29	22	1.0	(.93-1.0)	0.0	22	1.0	(.93-1.0)	0.0	22	.93	(.86-1.0)	.07
30	48	.94	(.89-.99)	.06	48	1.0	(.97-1.0)	0.0	48	.90	(.83-.96)	.10
Total	141	.940	(.912-.968)	.060	141	.996	(.99-1.0)	.004	140	.931	(.901-.961)	.069

Appendix Table B-5. Gene frequencies and 95% confidence intervals (C.I.) of MDH-A and MDH-B for 1978 smolts; "n" designates the sample size. Tow number refers to which sequenced surface trawl was sampled - continued.

Tow number	MDH-A			MDH-B									
	n	A	95% C.I.	B	n	A	95% C.I.	B	n	A	95% C.I.	D	95% C.I.
6	23	1.0	(.94-1.0)	0.0	23	.967	(.931-1.00)	.011			(0.00-.032)	.022	(0.00-.052)
13	48	.99	(.97-1.0)	.01	48	.969	(.945-.994)	.010			(0.00-.024)	.021	(.001-.041)
29	22	1.0	(.93-1.0)	0.0	22	.943	(.895-.991)	.023			(0.00-.054)	.034	(0.00-.072)
30	48	1.0	(.97-1.0)	0.0	48	.984	(.966-1.00)	0.00			(0.00-.016)	.016	(0.00-.031)
Total	141	.996	(.99-1.0)	.004	141	.970	(.956-.984)	.009			(.001-.017)	.021	(.009-.033)

Appendix Table B-5. Gene frequencies and 95% confidence intervals (C.I.) of PGI and LDH-4 for 1978 smolts; "n" designates the sample size. Tow number refers to which sequenced surface trawl was sampled - continued.

Tow number	PGI			LDH-4		
	n	A	95% C.I.	n	A	95% C.I.
6	23	1.0	(.94-1.0)	23	1.0	(.94-1.0)
11	48	1.0	(.97-1.0)	48	.99	(.97-1.0)
29	22	1.0	(.93-1.0)	22	1.0	(.93-1.0)
30	48	1.0	(.97-1.0)	48	1.0	(.97-1.0)
Total	141	1.00	(.994-1.00)	141	.996	(.99-1.0)
						.004



Appendix Fig. C-1. Observed electrophoretic patterns.

Legend: Presumed genotypes

A = common allele

B, C, D = variant alleles

