INFORMATIONAL LEAFLET NO. 213

A BIOCHEMICAL GENETIC ANALYSIS OF PINK SALMON (Oncorhynchus gorbuscha) FROM SELECTED STREAMS IN NORTHERN SOUTHEAST ALASKA

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March 1983

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March 1983

[1] Accepted by the University of Alaska, Juneau in partial fulfillment of the requirements for the author's Master of Science degree in December 1982

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ABSTRACT

This study was undertaken to determine the genetic structure of pink salmon, *Oncorhynchus gorbuscha*, stocks near the city of Juneau, in northern Southeast Alaska. It was accepted by the University of Alaska, Juneau in partial fulfillment of the requirements for the author's Master of Science degree in December 1982. Pink salmon were collected in 1978 and 1979 from twelve streams located within 64 km (40 mi) of Juneau. Intertidal and upstream areas of many streams were sampled on several different dates to allow both within stream (different spawning locations and times) and among stream comparisons to be made. Pink salmon from four streams in southern Southeast Alaska were compared with four systems in the Bering Sea region of Alaska. Tissue samples were electrophoretically analyzed at 25 loci. Analysis of breeding studies verified the genetic basis of the observed electrophoretic variation.

A high level of genetic variation was shown to exist in Alaskan pink salmon. Eighteen loci were polymorphic, with a variant allele frequency greater than 0.01, in at least one collection. No significant allele frequency differences appeared among different segments of runs returning to selected streams. Intertidal and upstream spawners, as well as early and late-run spawners, appeared to form a single spawning group in each stream. Heterogeneity among streams in the Juneau area was significant for the even-year class, but actually represented only a minor portion of the total genetic variation present. Genetic differences among regions were greater than within regions, and reflected the geographic distance between regions. The greatest differences in allele frequencies occurred between year classes.

INTRODUCTION

Pink salmon, *Oncorhynchus gorbuscha*, are the most abundant and economically the most valuable species of salmon in Southeast Alaska. Commercial catches of pink salmon in Southeast have exceeded those of each of the other four species of Pacific salmon every year since 1893 (INPFC 1979). Catches are currently depressed, however, relative to historic levels (INPFC 1979).

Hundreds of streams in which pink salmon spawn are scattered along the intricate network of channels and straits in Southeast Alaska. Few streams are major producers. Several migration routes are followed by pink salmon returning to the inside waters of Southeast Alaska (Nakatani et al. 1975; Hoffman 1982). Mixed stocks are present in many of the traditional fishing areas located along these migration corridors. A difficult problem faced by management biologists is to allow the largest catches possible, while insuring adequate escapement to each stream. To regulate fishing on separate stocks, individual breeding groups must be identifiable in mixed-stock fisheries.

A variety of techniques have been used to identify the stream of origin of pink salmon taken in commercial fisheries. Run timing differences have been noted for pink salmon destined for many Southeast Alaskan streams (Sheridan 1962; Hoffman 1982). A broad overlap in time of return exists in most pink salmon populations of this region, however, reducing the effectiveness of stock separation by run timing. Scale pattern analysis have proven successful in identifying even- and odd-year runs of pink salmon in British Columbia and Alaska (Bilton 1971). But Robertson (1979) found the usefulness of scale pattern analysis in identifying the home stream of pink salmon caught in Southeast Alaskan commercial fisheries to be limited, due to the absence of a freshwater growth zone and the overall similarity of marine growth characteristics expressed on the scales. Nickerson (1979) unsuccessfully attempted to distinguish pink salmon populations in the Prince William Sound region using both electrophoretic and size data. Electrophoretic differences were found among streams from Kodiak Island (Johnson 1979), but differences were not large enough to be of practical use in fisheries management.

Johnson (1979), and Allendorf and Utter (1979), suggested that genetic marking of pink salmon populations could be a valuable method for separating stocks in mixed fisheries. By enhancing the frequency of a relatively rare protein variant, a population could be genetically marked and made identifiable in a mixture of several spawning groups. Unlike marking techniques such as fin clipping and coded wire tagging, which must be performed annually to yield useful information over an extended period, a genetic mark will persist from generation to generation. Before genetically marking a population, however, the genetic composition of nearby populations must be known. With this information in hand a suitable protein variant may be chosen for use as a mark.

A full-scale genetic marking program in a hatchery population of late-run Auke Creek pink salmon was initiated in 1978. Auke Creek is located approximately 16 km (ten mi) north of Juneau, Alaska (Figure 1). The National Marine Fisheries Service, in conjunction with the local Territorial Sportsmen organization, operate a hatchery on Auke Creek.

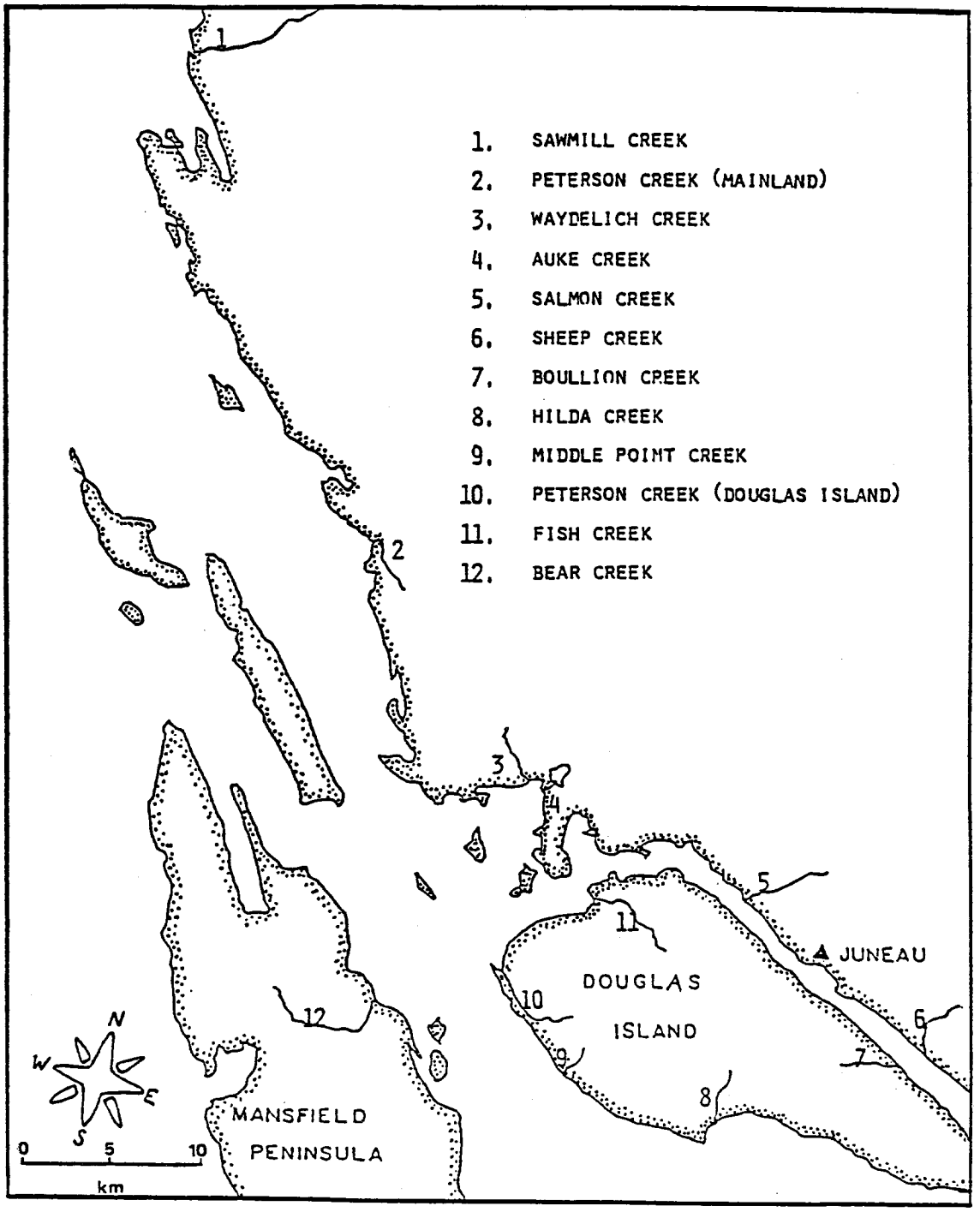

Figure 1. Map showing location of streams sampled in the Juneau area.

The primary objective of this study is to provide baseline genetic information on the pink salmon populations from streams in the area adjacent to Auke Creek prior to the implementation of a genetic mark in the Auke Creek population. Specific questions to be addressed include:

1) Do allele frequency differences exist among different life stages of pink salmon sampled from the same stream?

2) Are pink salmon populations in the Juneau area electrophoretically distinguishable?

3) Do genetic differences exist among spawning groups from the same stream, and if so, how do these differences compare with variation among streams in the Juneau area?

4) Are Juneau-area pink salmon electrophoretically distinguishable from pink salmon from other Alaskan regions, and if so what does this indicate about the overall genetic structure of Alaskan pink salmon populations?

MATERIALS AND METHODS

Sampling Procedure

Pink salmon were collected in 1978 and 1979 from streams close to Juneau that were easily accessible by car or small skiff (Figure 1). Spawned-out adults were dipnetted from streams. Eye, heart, liver, and muscle samples were taken from each fish. In most cases at least 75 adults per stream were sampled. Intertidal and upstream areas of many streams were sampled on several different dates to allow both within-stream and between-stream comparisons to be made.

Multiple collections made within a one-week period from the same stream were pooled and considered to compose one sample for all streams except Auke Creek and Waydelich Creek. Returns of fin-clipped releases from the Auke Creek hatchery allow the hatchery late run of pink salmon to be identified (Taylor 1980). The date of the first appearance of large numbers of marked late-run pink salmon to Auke Creek was used to separate samples collected from Auke Creek into early and late groups. The times of return of spawning groups to Waydelich Creek, which is adjacent to Auke Creek and which flows into the same saltwater bay, resemble those of Auke Creek spawning groups. Sample collections from Waydelich Creek were therefore pooled into early and late groups using the same criterion.

Small, portable fyke-nets (Appendix 1) were used to collect outmigrating pink salmon fry. These nets had small inlets, 20 cm in diameter, to ensure only modest catches avoiding overcrowding of fry in the live boxes. Mortality of fry due to netting was negligible. This was especially important because only a fraction of the fish collected in the nets were actually kept for electrophoretic analysis.

Fry were taken periodically throughout the period of emigration to ensure representation of offspring from as many spawning pairs as possible. Both adults

and fry were collected from three streams to allow a comparison between the two life stages. In addition, alevin samples from Auke Creek were collected by fry pumping, courtesy of the National Marine Fisheries Service Auke Bay Laboratory.

Samples from other geographic regions of Alaska were collected in 1980 with the aid of personnel from the Alaska Department of Fish and Game and the National Marine Fisheries Service (Figures 2 and 3). A complete list of all sample collections made for this study is given in Table 1.

Samples were kept on ice or frozen and transported to Juneau, where they were frozen (-20°C) until later analyzed by electrophoresis. Because some enzymatic activities deteriorate during storage of the tissues, samples were processed as soon as possible.

Electrophoresis

Portions (1-2 grams) of each tissue from adult pink salmon were placed in separate test tubes. Several drops of distilled water were added to all tubes except those containing eye samples. The samples were then refrozen. Fry and alevins were placed whole in separate tubes, along with several drops of distilled water. These samples had to be mascerated with a glass rod before refreezing to ensure detectable activity of all enzymes on the gels. All samples were centrifuged for approximately 5 minutes before electrophoresis, in order to thaw them and to remove cellular debris. One small piece (3 mm x 8 mm) of Schleicher and Schuell No. 470 chromatographic paper, called a wick, was immersed in the supernatant of each tube, and then inserted into a vertical cut in a starch gel. Forty to fifty of these wicks were placed on a gel.

Standard starch gel electrophoresis techniques (Utter et al. 1974) were used. Gels were made of 14% starch (Sigma Chemical Co., St. Louis, Mo.). Electrophoresis was accomplished in a commercial refrigerator at 5° C for 3 to 5 hours, using a maximum of 300 V at 55 mA/Gel. Four buffer systems were used and are listed in Table 2. Enzyme systems initially screened in this study are listed in Table 3. Histochemical staining solutions for the enzymes routinely examined were adapted from Shaw and Prasad (1970) and Harris and Hopkinson (1976), and are described in Appendix 2.

Nomenclature

Nomenclature of protein loci is based on revised guidelines proposed by B. May (1980). Abbreviations for enzymes examined in this study are listed in Table 3. When the abbreviations consist of one capitalized letter followed by one or more small letters, they represent specific loci that code for these enzymes. Multiple locus systems are designated by a hyphenated numeral following the abbreviation. Loci specific for the same enzyme are numbered sequentially, beginning with the locus with the least anodal migration. Alleles are assigned numerical values, relative to a common allele designated as 100, based on their electrophoretic mobilities.

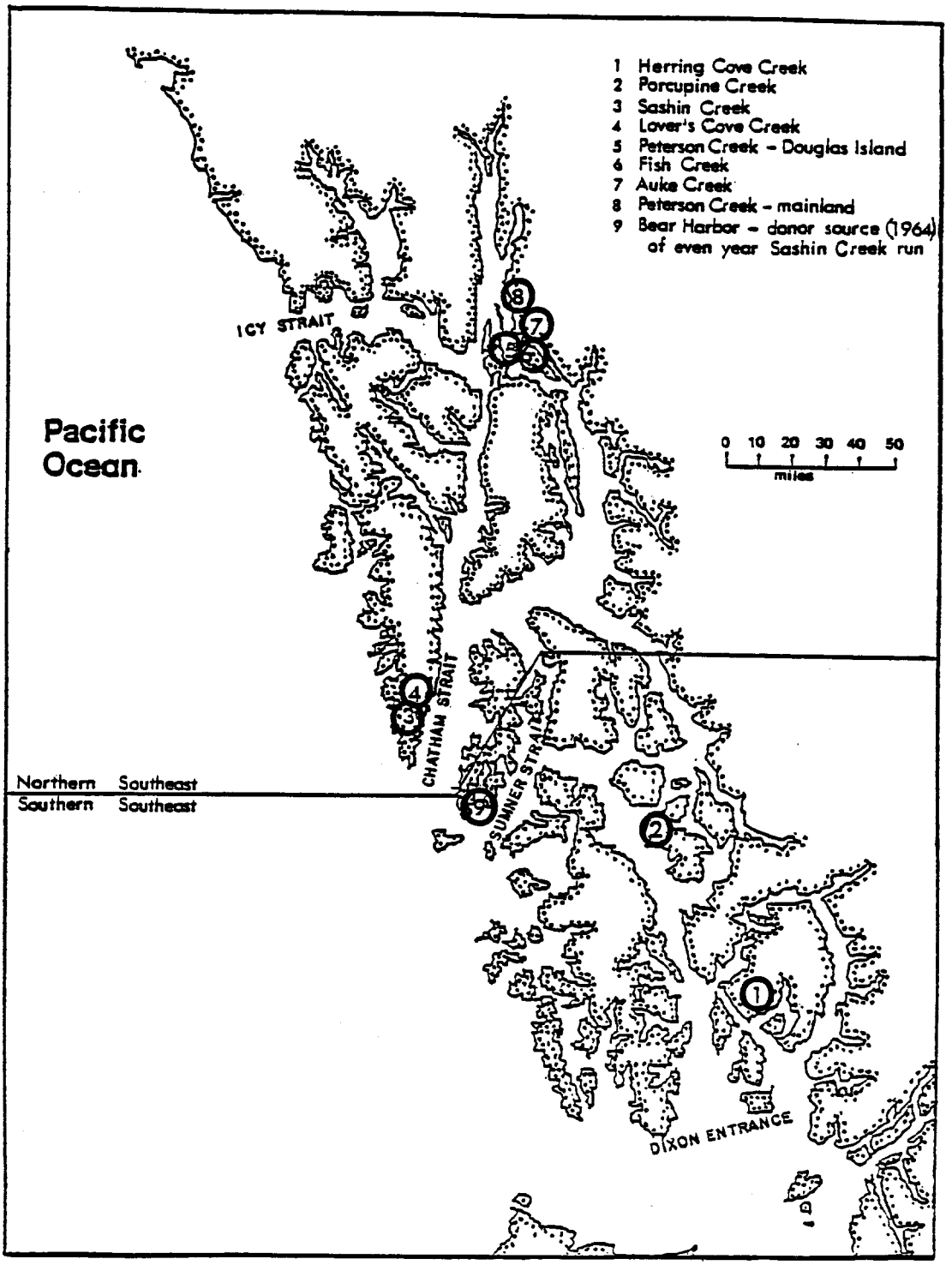

Figure 2. Map showing location of streams sampled for the even-year class in Southeast Alaska.

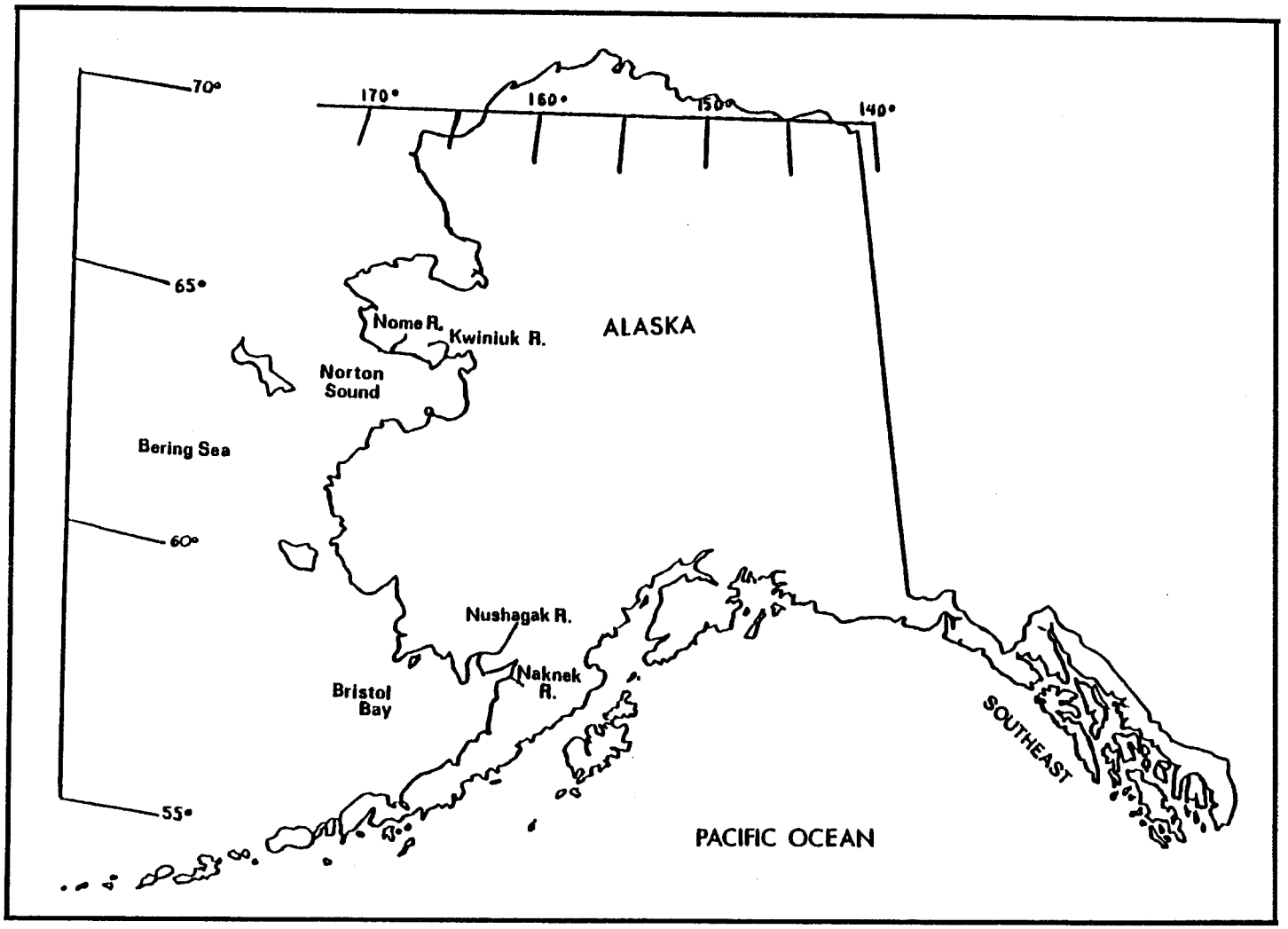

Figure 3. Map showing location of streams sampled for the even-year class in the Bering Sea region.

Table 1. List of sample collections.

Stream	Brood Year	Year Collected	Life Stage	Dates	Location/ Timing	Number Collected
Juneau-area streams						
Auke	1976	1978	adult	8/22-9/3	U/E	133
	1976	1978	adult	9/16-10/9	I/L	65
	1976	1978	adult	9/5-10/9	U/L	245
	1978	1979	alevin	3/27		62
	1978	1979	fry	3/31-5/17		180
	1977	1979	adult	8/27-8/30	U/E	81
	1977	1979	adult	8/15-8/27	I/E	91
	1977	1979	adult	9/17-9/27	U/L	98
	1977	1979	adult	9/19-9/27	I/L	100
Bear	1977	1979	adult	8/25	I+U	80
Boullion	1977	1979	adult	8/16-8/22	I	63
Fish	1976	1978	adult	8/28-8/31	U	79
	1976	1978	adult	8/28-8/31	I	195
	1978	1979	fry	3/24-5/30		200
	1977	1979	adult	8/13	U/E	87
	1977	1979	adult	8/7-8/13	I/E	84
	1977	1979	adult	9/18-9/21	I/L	89
Hilda	1977	1979	adult	8/19	I/E	33
	1977	1979	adult	9/7	I/L	58
Middle Pt.	1977	1979	adult	8/19	I/E	54
	1977	1979	adult	9/7	I/L	79
Peterson (mainland)	1976	1978	adult	8/25	U/E	47
	1976	1978	adult	9/13	U/L	46
	1978	1979	fry	4/7-6/5		200
	1977	1979	adult	8/14	U	49
Peterson (Douglas Is.)	1978	1979	fry	4/10-5/4		174
	1977	1979	adult	8/20	I/E	41
	1977	1979	adult	8/29	U/E	42
	1977	1979	adult	9/14-9/18	I/L	52
Salmon	1977	1979	adult	8/20	I+U	46
Sawmill	1977	1979	adult	8/12	I	101
Sheep	1977	1979	adult	8/20-8/27	I	105
Waydelich	1977	1979	adult	8/14-8/29	I+U/E	43
	1977	1979	adult	9/12	I+U/L	32
Other Southeast Alaskan streams						
Herring Cove	1978	1980	adult	9/29	I+U	114
Lover's Cove	1978	1980	adult	9/19	I	100
Porcupine	1978	1980	adult	9/27	I+U	113
Sashin	1978	1980	adult	9/10	U	100
Northern Alaskan streams						
Kwiniuk	1978	1980	adult	7/26		102
Nome	1978	1980	adult	7/23		100
Naknek	1978	1980	adult	summer		42
Nushagak	1978	1980	adult	7/27-7/30		104

I-intertidal U-upstream E-early run L-late run

Table 2. Buffer systems used in this study.

1. Ridgway et al. (1970)

   gel buffer (pH 8.5)

   Tris (0.03 M)
   Citric acid (0.005 M)

   electrode buffer (pH 8.1)

   Lithium hydroxide (0.06 M)
   Boric acid (0.3 M)

   Gels made using 99% gel buffer and 1% electrode buffer. Undiluted for electrode buffer.

2. Clayton and Tretiak (1972) (pH 6.1)

   Citric acid (0.04 M) adjusted to pH 6.1 with N-(3-Aminopropyl)-morphiline

   1:20 dilution used for gels. Undiluted for electrode buffer.

3. Markert and Faulhaber (1965) (pH 8.7)

   Tris (0.9 M)
   Boric acid (0.5 M)
   NaEDTA(disodium ethylenediamide tetraacetate) (0.02 M)

   1:20 dilution used for gels. 1:5 dilution used for electrode buffer.

4. Shaw and Prasad (1970) (pH 7.0)

   Tris (.155 M)
   Citric acid (.043 M)

   1:20 dilution used for gels. Undiluted for electrode buffer.

Table 3. List of enzymes initially screened in this study, and the tissues and buffers used for each. Abbreviations and Enzyme Commission numbers are listed after each enzyme. Loci analyzed for most samples are designated by a *.

Enzyme	Abbreviation	E.C.#	Locus Designation	Tissue	Buffer
Aspartate aminotransferase	AAT	2.6.1.1	Aat-1,2	muscle	2
			Aat-3*	eye	1
Aconitase	ACON	4.2.1.3	Acon-1,2	heart	4
			Acon-3*,4*	heart,muscle	4
Acid phosphatase	ACP	3.1.3.2	Acp	muscle	2
Adenosine deaminase	ADA	3.5.4.4	Ada-1	liver,heart	2
			Ada-2*	muscle,heart	2
Alcohol dehydrogenase	ADH	1.1.1.1	Adh	liver	1
Alpha-glycerol-3-phosphate dehydrogenase	AGP	1.1.1.8	Agp*	muscle	3
Adenylate kinase	AK	2.7.4.3	Ak	muscle,heart	1
Aldolase	ALD	4.1.2.13	Ald	muscle	2
B-glucuronidase	B-GUS	3.2.1.31	B-gus	liver	1,3
Creatine kinase	CK	2.7.3.2	Ck-1*,2*	muscle	1
			Ck-3	eye	1
Esterase	ES	3.1.1.1	Es	liver	1
Glyceraldehyde-phosphate dehydrogenase	GAPDH	1.2.1.12	Gapdh	heart	2
Glucose dehydrogenase	GDH	1.1.1.47	Gdh	liver	3
Glucose 6-phosphate dehydrogenase	G6PDH	1.1.1.49	G6pdh	liver	2
Glutamate-pyruvate transaminase	GPT	2.6.1.2	Gpt-1,2	liver	3
Isocitrate dehydrogenase	IDH	1.1.1.42	Idh-1,2	muscle	4
			Idh-3,4	liver	4
Lactate dehydrogenase	LDH	1.1.1.27	Ldh-1*,2	muscle	1
			Ldh-3	muscle	1
			Ldh-4*	liver,muscle	1
			Ldh-5*	eye	1
Malate dehydrogenase	MDH	1.1.1.37	Mdh-1*,2*	liver,heart	2
			Mdh-3*,4*	muscle,heart	2
Malic enzyme	ME	1.1.1.40	Me-1*	muscle	2
			Me-2	muscle,liver	2
Peptidase	PEP	3.4.13.9	Gl-1	muscle	3,4
			Gl-2	eye	3,4
			Lgg-1	muscle	3
			Ll-1*	muscle	4
			Ll-2	muscle	4
			Pp-1*	heart,muscle	3
			Pp-2*	heart,muscle	3
Phosphoglucomutase	PGM	2.7.5.1	Pgm*	muscle,heart	1
6-phosphogluconate dehydrogenase	6PG	1.1.1.44	6pg*	liver,heart, muscle	2,4
Phosphohexose isomerase	PHI	5.3.1.9	Phi-1*,2*	muscle	1
			Phi-3*	muscle	1
Phosphomannose isomerase	PMI	5.3.1.8	Pmi*	heart,eye	3,4
Sorbitol dehydrogenase	SDH	1.1.1.14	Sordh	liver	1
Superoxide dismutase	SOD	1.15.1.1	Sod-1*	liver	1
			Sod-2	heart	1

Interpretation of Electrophoretic Variations

Variation in electrophoretic gel patterns can be caused by a variety of factors (Allendorf and Utter 1979). It is necessary to be able to determine whether observed electrophoretic variation actually represents an underlying genetic difference in the organism being studied. Data from breeding experiments, in which phenotypes of progeny from selected matings are compared with known phenotypes of their parents, provide the strongest evidence for genetic control of electrophoretic variation.

In cases where no breeding experiment data existed for a specific locus examined in this study, other criteria were used to infer genetic control of electromorphs. Banding patterns had to reflect patterns expected from simple genetic models, and had to be reproducible upon repeated electrophoretic separations of tissue from the same individual. In addition, when particular loci were expressed in more than one tissue, the banding patterns observed had to be consistent among tissues. Electrophoretic variation observed that did not satisfy these criteria was not used.

Statistical Analysis

Genotypic frequencies were obtained by gene counting of phenotypes expressed on the gels. Allele (gene) frequencies were then calculated from genotypic frequencies. Several duplicated loci, including Mdh-1,2, Mdh-3,4, and Phi-1,2, were examined. Each of the duplicated systems expressed low levels of polymorphism. It could not be determined conclusively whether each locus of a duplicated pair shared the same variant alleles. To facilitate the calculation of allele frequencies it was assumed that only one locus of each of these duplicated systems was polymorphic.

Chi-square goodness-of-fit tests (Strickberger 1968) were used to test for Hardy-Weinberg equilibrium in each group of samples. Genotypic classes with expected total frequencies of less than four were pooled with the next largest class. The number of degrees of freedom for each test equaled the number of genotypes minus the number of alleles, after pooling.

A log-likelihood ratio analysis, or G-test (Sokal and Rohlf 1969), was used to test for heterogeneity of allele frequencies within and among streams as well as between even- and odd-year classes. Using this method, within- and between-stream components of variation can be partitioned in a manner similar to an analysis of variance. Classes with expected frequencies $2NP_i$, where P_i is equal to the frequency of the least common allele) less than four were pooled with other classes prior to testing. Only polymorphic loci for which two or more such classes existed for all groups of samples involved in a comparison were tested using the G-test. The G-statistic is approximately distributed as the chi-square with (alleles-1) (collections -1) degrees of freedom. Because numerous parallel tests were conducted at each locus the significance levels of the G-tests were adjusted to control the overall probability of type I error (Cooper 1968). The null hypothesis of no heterogeneity was accepted or rejected using the appropriate critical value for the desired level of significance from a chi-square table.

Unbiased estimates of the average heterozygosity per locus and their standard errors were calculated according to methods described by Nei (1978). Nei's gene diversity analysis (Nei 1973, 1977) was used as another method of analyzing the genetic differentiation among samples. This analysis is similar to the F-statistics developed by Wright (1943, 1951), but is designed to be applied to a large number of loci each of which may possess any number of alleles. Gene diversity analysis partitions the gene diversity, or heterozygosity, of a total population (H_T) into within (H_S) and among (D_{ST}) population components. G_{ST} is the coefficient of gene differentiation and is equal to D_{ST}/H_T. G_{ST} assumes values from 0 to 1, values which represent the extreme cases of a species with absolutely no population substructure to a species for which the entire genome of individual populations are fixed for different alleles.

Nei's measure of standard genetic distance (Nei 1972, 1974) was calculated for all possible pairs of streams. Fish that spawned in the same stream in alternate years were considered as separate groups. A computer program was obtained from Dr. Nei to perform these laborious computations. The program, which was written by A.K. Roychoudhury and later modified by Y. Tateno, computes the unbiased estimates of the minimum and standard genetic distances between each pair of streams, as well as the standard errors of these estimates. A detailed discussion of the calculations involved has been published by Nei (1978). The minimum genetic distance estimates revealed trends identical to the standard distance estimates. Only the standard distance estimates will be discussed. A matrix of D-values was obtained and a dendrogram was constructed to provide a visual representation of the results.

RESULTS

Protein Variation

Twenty-five loci were routinely analyzed for most samples collected. Eighteen of these loci were polymorphic at the 1 percent level in at least one of the sample groups.

The genetic basis for the variation that occurs at a number of these loci has been confirmed through breeding experiments. Aspinwall (1973, 1974a) and Johnson (1979) demonstrated the genetic nature of Agp and Mdh-3,4 variation in pink salmon. Johnson (1979) confirmed the genetic basis of Me-1 and Pgm variation in pink salmon. Variation occurred in low frequencies at a number of other loci examined in samples collected in this study, including Ldh-1, Ldh-4, Ck-1, Phi-3, and Pmi. Breeding experiments have confirmed the genetic basis of variation at each of these loci in at least one other salmonid species (May 1980). Variation observed at these loci was therefore considered to be genetic in nature, and allele frequency data was routinely collected for each.

In concurrence with the present study, breeding crosses were performed on pink salmon to determine the genetic basis for seven other enzyme systems. Results from breeding crosses are contained in Appendix 3. Loci routinely examined in this study that have not been previously reported for pink salmon, and those loci which have been described but for which no inheritance data has previously

existed are briefly described in the following section. Joint segregation statistics have been calculated for all applicable crosses, but only those crosses that showed nonrandom segregation are discussed. Electrophoretic patterns of protein variants of pink salmon observed at loci that have not been previously described are diagrammed in Appendix 4.

AAT:

Aspartate aminotransferase is a dimeric enzyme encoded by two loci expressed in muscle tissue (Aat-1,2) and one locus expressed in the eye (Aat-3) (May 1975). Breeding crosses confirmed that variation observed in the eye is encoded by a single locus with two codominant alleles.

Acon:

Aconitase is expressed in muscle and heart tissues. Fresh heart samples contain four zones of activity, the two least anodal of which are not visible in white muscle extracts. Due to the rapid loss of activity of these two zones with tissue storage they were not included in population analysis in this study. The most anodal bands, designated Acon-3 and Acon-4, displayed variability that was consistent between muscle and heart tissues. The variability observed in white muscle suggests a model of a monomeric enzyme encoded by two loci, each of which possess allelic variants.

Phenotypic ratios of progeny from a single cross of variation at the Acon-4 locus did not differ from those expected by this inheritance model. A single chi-square test of joint segregation between Acon-4 and 6pg was significant ($p < .005$). Nothing conclusive can be interpreted, however, from results of a single mating. The nonrandom segregation observed in this cross could result from linkage or pseudolinkage[1] of these two loci, since the male parent was a double heterozygote. Aberrant segregation ratios in a single family could be due to many other factors (May et al. 1979), necessitating the need for further crosses to document the relationship between Acon-4 and 6pg in pink salmon.

ADA:

Two zones of activity for adenosine deaminase are expressed in pink salmon. The more anodal zone appears best in muscle and heart tissues, whereas the least anodal zone appears best in the liver. Liver extracts often showed additional banding in the least anodal zone, designated Ada-1, but it was not possible to fit a simple Mendelian model to all of the variation observed. Variability observed at the most anodal zone, designated Ada-2, was consistent between tissues and appears consistent with a model of a monomeric enzyme, encoded by a single locus with three codominant alleles. Results of breeding crosses are consistent with this model.

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[1] Pseudolinkage is nonrandom segregation among the progeny of crosses in which the male is the informative parent (Morrison 1979; Davisson et al. 1973; Wright et al. 1975; May et al. 1979).

Peptidase:

A variety of substrate specific peptidases are expressed in pink salmon. Loci specific for glycyl-leucine, leucyl-leucine, leucyl-glycyl-glycine, and phenylalanyl-proline were examined. Of these, only leucyl-leucine and phenylalanyl-proline specific loci demonstrated variation that was clearly resolvable.

Ll:

Two zones of activity were observed for leucyl-leucine peptidase. Only the least anodal zone, designated Ll-1, could be reliably interpreted. Variants observed at this zone displayed banding patterns characteristic of a monomeric enzyme encoded by a single locus. Results of breeding crosses of variation at this zone are consistent with a model of a single locus possessing 3 codominant alleles.

Pp:

Two zones of activity were observed for phenylalanyl-proline peptidase. The least anodal band, designated Pp-1, was monomorphic in all the samples examined in this study. Variants observed at the more anodal zone, designated Pp-2, displayed banding patterns characteristic of a dimeric enzyme encoded by a single locus. Results of breeding crosses of variation at this zone are consistent with a model of a single locus possessing 3 codominant alleles.

6PG:

6-phosphogluconate dehydrogenase has previously been reported to be a dimeric enzyme, encoded by a single locus possessing 2 codominant alleles (May 1975). A third allele, designated 95, was identified in populations examined in this study. Only 2 alleles, 90 and 100, were consistently resolved, however, for all samples.

Phi:

Phosphohexose isomerase has been previously reported to be a dimeric enzyme encoded by three loci (May 1975). The common alleles that occur at Phi-1 and Phi-2 have identical mobilities (Johnson 1979).

Segregation ratios of phenotypes in the progeny from each of four crosses involving variability at Phi-1,2 were not significantly different from those expected (Appendix 3.1H). Nonrandom joint segregation between Phi-1,2 and Pp-2 was observed in two crosses (Appendix 3.2). Each mating was a double backcross which involved a doubly heterozygous male (different males were involved in each cross) possessing one dose of allele 33 at Phi-1,2. A complete absence of two expected progeny types was observed in each of these crosses. Random joint segregation was noted, however, between Phi-1,2 and Pp-2 in a double backcross which involved a doubly heterozygous male which possessed a different Phi-1,2 allele, the 200 allele.

At least two possible genetic models explain the aberrant segregation observed in these crosses. Alleles 33 and 200 could be variants of different Phi loci. If the locus at which allele 33 occurs is tightly linked with the Pp-2 locus and

the locus at which allele 200 occurs is not, the observed segregation ratios could result. The nonrandom segregation could also result from pseudolinkage. Pseudolinkage is observed only in males and may not be apparent in all males (May et al. 1979). Unfortunately only doubly heterozygous males were used in this study, since they were the only doubly heterozygous individuals found among the fish screened for use as parents. Further matings which involve doubly heterozygous females should be made to determine whether nonrandom segregation also occurs in females.

Frequency of variation at Phi-1,2 was low (< 1%) for pink salmon examined in this study. For this reason potential bias due to non-independence (caused by linkage) of Phi-1,2 and Pp-2 genotypes was thought to be of no significance in statistical analysis of the genetic structure of pink salmon stocks sampled in this study.

The refining of electrophoretic techniques, together with breeding crosses which demonstrated the genetic nature of newly observed electrophoretic variation, greatly increased the number of loci that could be examined in this study compared to previous electrophoretic studies of pink salmon. A brief description of sample collections and the electrophoretic data collected for each year class and region follows.

Even-Year Sampling in the Juneau Area

Sampling of adult pink salmon in the Juneau area in 1978 was limited to three streams: Auke Creek, Fish Creek, and Peterson Creek (mainland) (Figure 1). Segregation of sampling dates and/or location of sampling efforts on each stream allowed comparisons to be made between sets of samples taken within each stream, as well as between streams.

New laboratory techniques were being developed during analysis of these fish. As a result, no data were collected for the Aat-3 and Ldh-5 loci from 1978 adults. Sample sizes for several other loci, including Acon-3, Acon-4, and Ll-1 were limited because enzyme activity had greatly decreased by the time a number of these samples were processed.

Four streams were repeatedly sampled by fyke-netting over periods of a month or more. At least 170 fry were taken from each of Auke Creek, Fish Creek, Peterson Creek (mainland) and Peterson Creek (Douglas Island). Due to the large sample sizes involved and the random nature of the sampling effort, fry data from these streams were included in data analysis. In addition, 62 alevins were taken from Auke Creek.

Allele frequencies of pink salmon samples from the even-year class are given in Table 4.

Odd-Year Sampling in the Juneau Area

Twelve streams in the Juneau area were sampled for adults in 1979: Auke, Bear, Boullion, Fish, Hilda, Middle Point, Peterson (mainland), Peterson (Douglas Island), Salmon, Sawmill, Sheep, and Waydelich Creeks (Figure 1). The sampling of six of these streams again was structured to permit within-stream comparisons

Table 4. Allele frequencies of even-year class pink salmon sampled from Juneau area streams. Adults were collected in the fall of 1978. Alevin and fry were collected in the winter and spring of 1979. The frequency of the common allele, designated "100", is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N", "U", and "I" denote sample size, upstream samples, and intertidal samples. Dashes (---) indicate that no data was taken.

Stream	Life Stage	Location/ Timing	N	Aat-3	N	Acon-3	N	Acon-4		N	Ada-2		
				(100)		(100)		(100)	(85)		(100)	(87)	(113)
Auke	adult	U. early	---	---	64	1.0000	64	.9375	.0625	60	.8750	.1250	.0000
	adult	I. early	---	---	15	1.0000	15	.8333	.1667	36	.9444	.0417	.0139
	adult	U. late	---	---	33	1.0000	33	.9394	.0606	29	.8966	.0862	.0172
	alevin		13	.8462	62	1.0000	62	.9919	.0081	60	.8500	.1000	.0500
	fry		123	.6829	59	1.0000	59	.9407	.0593	58	.9052	.0948	.0000
Fish	adult	I.	---	---	120	1.0000	120	.9542	.0458	120	.8292	.1333	.0375
	adult	U.	---	---	51	1.0000	51	.9608	.0392	47	.8830	.0851	.0319
	fry		179	.7989	---	---	---	---	---	---	---	---	---
Peterson (mainland)	adult	U. early	---	---	---	---	---	---	---	---	---	---	---
	adult	U.late	---	---	---	---	---	---	---	---	---	---	---
	fry		179	.7710	99	1.0000	99	.9293	.0707	99	.9192	.0303	.0505
Peterson (Douglas Is.)	fry		173	.7370	48	1.0000	48	.9896	.0104	50	.9500	.0300	.0200

Stream	Life Stage	Location/ Timing	N	Agp			N	Ck-1		N	Ck-2	N	Ldh-1	N	Ldh-4
				(100)	(200)	(175)		(100)	(80)		(100)		(100)		(100)
Auke	adult	U. early	132	.7841	.2159	.0000	77	1.0000	.0000	77	1.0000	132	.9962	133	.9962
	adult	I. late	64	.8281	.1719	.0000	58	1.0000	.0000	58	1.0000	64	1.0000	64	1.0000
	adult	U. late	244	.8135	.1865	.0000	30	1.0000	.0000	30	1.0000	224	1.0000	224	1.0000
	alevin		60	.7583	.2417	.0000	53	1.0000	.0000	53	1.0000	62	1.0000	62	1.0000
	fry		174	.7874	.2123	.0000	159	1.0000	.0000	159	1.0000	180	1.0000	180	1.0000
Fish	adult	I.	195	.7692	.2308	.0000	150	.9967	.0033	150	1.0000	188	1.0000	188	1.0000
	adult	U.	79	.7468	.2532	.0000	51	1.0000	.0000	51	1.0000	79	1.0000	79	1.0000
	fry		200	.7950	.2050	.0000	200	.9975	.0025	200	1.0000	200	1.0000	200	1.0000
Peterson (mainland)	adult	U. early	47	.7979	.2021	.0000	47	1.0000	.0000	47	1.0000	46	1.0000	47	.9894
	adult	U. late	46	.8261	.1739	.0000	23	1.0000	.0000	23	1.0000	46	1.0000	45	.9889
	fry		200	.8375	.1625	.0000	198	1.0000	.0000	198	1.0000	200	1.0000	200	.9825
Peterson (Douglas Is.)	fry		174	.8391	.1609	.0000	174	1.0000	.0000	174	1.0000	174	1.0000	174	1.0000

-Continued-

Table 4. Allele frequencies of even-year class pink salmon sampled from Juneau area streams. Adults were collected in the fall of 1978. Alevin and fry were collected in the winter and spring of 1979. The frequency of the common allele, designated "100", is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N", "U", and "I" denote sample size, upstream samples, and intertidal samples. Dashes (---) indicate that no data was taken (continued).

Stream	Life Stage	Location/ Timing	N	Ldh-5	N	Ll-1			N	Mdh-1			N	Mdh-2
				(100)		(100)	(85)	(115)		(100)	(70)	(13)		(100)
Auke	adult	U. early	---	---	---	---	---	---	133	.9850	.0113	.0037	133	1.0000
	adult	I. late	---	---	---	---	---	---	64	.9609	.0391	.0000	64	1.0000
	adult	U. late	---	---	---	---	---	---	235	.9936	.0064	.0000	235	1.0000
	alevin		62	1.000	20	.7000	.0000	.3000	62	.9758	.0242	.0000	62	1.0000
	fry		139	1.000	45	.9111	.0111	.0778	180	.9944	.0056	.0000	180	1.0000
Fish	adult	I.	---	---	54	.8148	.1296	.0556	194	.9948	.0052	.0000	194	1.0000
	adult	U.	---	---	20	.7250	.1500	.1250	79	.9937	.0063	.0000	79	1.0000
	fry		200	1.000	---	---	---	---	200	.9975	.0175	.0050	200	1.0000
Peterson (mainland)	adult	U. early	---	---	---	---	---	---	47	1.0000	.0000	.0000	47	1.0000
	adult	U. late	---	---	---	---	---	---	46	1.0000	.0000	.0000	46	1.0000
	fry		200	1.000	95	.8263	.0526	.1211	200	.9850	.0150	.0000	200	1.0000
Peterson (Douglas Is.)	fry		174	1.000	44	.8410	.0795	.0795	174	.9425	.0460	.0115	174	1.0000

Stream	Life Stage	Location/ Timing	N	Mdh-3			N	Mdh-4	N	Me-1			N	Pgm
				(100)	(130)	(70)		(100)		(100)	(130)	(70)		(100)
Auke	adult	U. early	133	.9774	.0188	.0038	133	1.0000	123	.7805	.2195	.0000	131	.9924
	adult	I. late	64	.9609	.0391	.0000	64	1.0000	61	.7131	.2869	.0000	64	.9766
	adult	U. late	235	.9894	.0085	.0021	235	1.0000	236	.7436	.2564	.0000	220	.9932
	alevin		62	.9838	.0162	.0000	62	1.0000	62	.7661	.2339	.0000	62	.9839
	fry		180	.9944	.0056	.0000	180	1.0000	129	.7946	.2054	.0000	160	.9844
Fish	adult	I.	194	.9948	.0052	.0000	194	1.0000	162	.7994	.1975	.0031	187	1.0000
	adult	U.	79	.9873	.0127	.0000	79	1.0000	62	.8467	.1452	.0081	76	.9934
	fry		200	.9850	.0125	.0025	200	1.0000	168	.7411	.2589	.0000	200	.9975
Peterson (mainland)	adult	U. early	47	.9894	.0106	.0000	47	1.0000	43	.8140	.1744	.0116	47	1.0000
	adult	U. late	46	1.0000	.0000	.0000	46	1.0000	38	.7895	.2105	.0000	45	1.0000
	fry		200	.9850	.0125	.0025	200	1.0000	168	.7411	.2589	.0000	200	1.0000
Peterson (Douglas Is.)	fry		174	.9828	.0057	.0115	174	1.0000	171	.8041	.1959	.0000	174	.9971

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Table 4. Allele frequencies of even-year class pink salmon sampled from Juneau area streams. Adults were collected in the fall of 1978. Alevin and fry were collected in the winter and spring of 1979. The frequency of the common allele, designated "100", is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N", "U", and "I" denote sample size, upstream samples, and intertidal samples. Dashes (---) indicate that no data was taken (continued).

Stream	Life Stage	Location/ Timing	N	Phi-1					N	Phi-2	N	Phi-3		
				(100)	(33)	(130)	(200)	(-33)		(100)		(100)	(90)	(110)
Auke	adult	U. early	80	1.0000	.0000	.0000	.0000	.0000	80	1.0000	78	.9808	.0000	.0192
	adult	I. late	58	.9741	.0259	.0000	.0000	.0000	58	1.0000	58	1.0000	.0000	.0000
	adult	U. late	30	1.0000	.0000	.0000	.0000	.0000	30	1.0000	30	1.0000	.0000	.0000
	alevin		62	.9354	.0646	.0000	.0000	.0000	62	1.0000	62	1.0000	.0000	.0000
	fry		180	.9722	.0083	.0111	.0083	.0000	180	1.0000	180	1.0000	.0000	.0000
Fish	adult	I.	155	.9968	.0000	.0032	.0000	.0000	155	1.0000	155	.9968	.0000	.0032
	adult	U.	53	1.0000	.0000	.0000	.0000	.0000	53	1.0000	53	1.0000	.0000	.0000
	fry		200	.9975	.0000	.0025	.0000	.0000	200	1.0000	200	1.0000	.0000	.0000
Peterson (mainland)	adult	U. early	47	1.0000	.0000	.0000	.0000	.0000	47	1.0000	47	1.0000	.0000	.0000
	adult	U. late	23	.9565	.0000	.0000	.0435	.0000	23	1.0000	23	1.0000	.0000	.0000
	fry		200	.9975	.0000	.0000	.0025	.0000	200	1.0000	200	1.0000	.0000	.0000
Peterson (Douglas Is.)	fry		174	.9885	.0029	.0029	.0029	.0057	174	1.0000	174	1.0000	.0000	.0000

Stream	Life Stage	Location/ Timing	N	Pmi			N	Pp-1	N	Pp-2			N	6pg		N	Sod-1
				(100)	(85)	(115)		(100)		(100)	(109)	(93)		(100)	(90)		(100)
Auke	adult	U. early	2	1.0000	.0000	.0000	74	1.0000	74	.5000	.2162	.2838	128	.9414	.0586	133	1.0000
	adult	I. late	28	1.0000	.0000	.0000	58	1.0000	58	.5086	.1638	.3276	63	.9206	.0794	63	1.0000
	adult	U. late	—	———	———	———	31	1.0000	31	.5161	.2258	.2581	133	.9624	.0376	202	1.0000
	alevin		62	1.0000	.0000	.0000	58	1.0000	58	.5517	.0862	.3621	62	.9839	.0161	180	1.0000
	fry		99	1.0000	.0000	.0000	93	1.0000	93	.5870	.1522	.2608	159	.9465	.0535	180	1.0000
Fish	adult	I.	—	———	———	———	153	1.0000	154	.5844	.2305	.1851	183	.9098	.0902	195	1.0000
	adult	U.	—	———	———	———	56	1.0000	57	.5526	.1930	.2544	78	.9359	.0641	79	1.0000
	fry		—	———	———	———	126	1.0000	124	.5645	.2702	.1653	199	.9347	.0653	200	1.0000
Peterson (mainland)	adult	U. early	32	.9844	.0156	.0000	36	1.0000	36	.5417	.2361	.2222	43	.9302	.0698	47	1.0000
	adult	U. late	—	———	———	———	37	1.0000	37	.5676	.2297	.2027	45	.9556	.0444	46	1.0000
	fry		99	1.0000	.0000	.0000	197	1.0000	197	.6472	.2005	.1522	200	.9425	.0575	200	1.0000
Peterson (Douglas Is.)	fry		50	1.0000	.0000	.0000	174	1.0000	174	.6437	.2241	.1322	174	.9138	.0862	174	1.0000

to be made. Samples were promptly processed in the laboratory and provided the most complete data of this study. Allele frequencies for all loci examined in the 1979 adults are given in Table 5.

Even-Year Sampling in Other Alaskan Regions

Eight additional Alaskan streams were sampled in 1980: Herring Cove, Porcupine, Sashin, and Lover's Cove Creeks are all located south of Juneau in Southeast Alaska, the Naknek and Nushagak Rivers are located in the Bristol Bay region, and the Kwiniuk and Nome Rivers are located in the Norton Sound region. Allele frequencies of pink salmon sampled from each of these systems are given in Table 6.

Single-Locus Tests for Hardy-Weinberg Equilibrium

Genotypic frequencies of all groups of pink salmon were tested to determine if they differed significantly from frequencies expected under Hardy-Weinberg equilibrium conditions. The Hardy-Weinberg Law is based on a number of assumptions; large population size, random mating, and the absence of mutation, migration, and selection. These assumptions are probably never completely fulfilled for any population in nature. The Hardy-Weinberg Law is not very sensitive, however, to minor violations in the assumptions (Hartl 1980). The most important ramification of this principle is that, under Hardy-Weinberg conditions, a population in Hardy-Weinberg equilibrium will exhibit stable gene and genotypic frequencies between generations.

Of the 99 chi-square goodness-of-fit tests conducted on single locus genotypic frequencies, only three deviated significantly ($p < .05$) from those expected under the Hardy-Weinberg Law. It is impossible to attribute these instances of non-equilibrium to a specific cause, but at least four of the 99 test would be expected to yield significant deviations by chance alone.

Stability of Allele Frequencies Over Generations

Allele frequency data for two loci was reported for Fish Creek runs during 1969-1971 (Aspinswall, 1974b) and 1978-1979 (this study). Within each year class allele frequencies have remained stable over this ten-year period (Table 7). If allele frequencies fluctuated wildly in each generation, the utility of electrophoresis for studying population structure would be minimal. Johnson (1979) and Utter et al. (1979) similarly found allele frequency stability over several generations of pink salmon from streams at Kodiak Island and Washington state.

Allele Frequency Comparisons Among Life Stages

Several electrophoretic studies have been conducted in which more than one life stage of fish have been examined (Kristianson and McIntryre 1976; Utter 1971; Johnson 1979). If Hardy-Weinberg equilibirum conditions exist among the populations being studied and if sampling is random, allele frequencies of different life stages of a population should be the same. Sampling techniques are seldom completely random, however. A variety of sampling methods must often be used to collect individuals from different life stages. Johnson (1979) found allele differences at the Agp locus between alevin and adult life stages of even-year

Table 5. Allele frequencies of odd-year class pink salmon sampled from Juneau area streams. All samples wer adults collected in the fall of 1979. The frequency of the common allele, designated "100", is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N", "U", and "I" denote sample size, upstream samples, and intertidal samples.

Stream	Location/ Timing	N	Aat-3 (100)	N	Acon-3 (100)	N	Acon-4 (100)	(85)	N	Ada-2 (100)	(87)	(113)
Auke	U. early	80	.8250	80	.9688	80	1.0000	.0000	81	.9198	.0802	.0000
	I. early	91	.7637	90	.9889	90	.9833	.0167	90	.9333	.0667	.0000
	U. late	96	.7813	98	.9898	98	1.0000	.0000	98	.8827	.1173	.0000
	I. late	97	.7629	100	1.0000	100	1.0000	.0000	100	.9250	.0750	.0000
Bear	I.+U.	80	.8375	80	.9750	80	.9875	.0125	79	.9304	.0696	.0000
Boullion	I	63	.7857	63	.9921	63	.9921	.0079	63	.9603	.0397	.0000
Fish	U. early	87	.6609	87	.9943	87	.9943	.0057	66	.9621	.0379	.0000
	I. early	83	.7651	83	.9699	83	.9940	.0060	83	.9398	.0602	.0000
	I. late	88	.7102	89	1.0000	89	.9944	.0056	89	.9045	.0955	.0000
Hilda	I. early	30	.6833	33	1.0000	33	.9848	.0152	32	.9375	.0625	.0000
	I. late	58	.7931	44	.9886	44	.9886	.0114	44	.8750	.1250	.0000
Middle Pt.	I. early	47	.7447	54	.9815	54	.9907	.0093	54	.7447	.2553	.0000
	I. late	76	.7961	76	.9934	76	.9934	.0066	75	.8933	.1067	.0000
Peterson (mainland)	U.	49	.8163	48	1.0000	48	1.0000	.0000	48	.9688	.0312	.0000
Peterson (Douglas Is.)	U. early	41	.7439	42	.9881	42	1.0000	.0000	42	.9286	.0714	.0000
	I. early	41	.7561	40	1.0000	40	1.0000	.0000	40	.9250	.0750	.0000
	I. late	52	.7212	51	.9902	51	1.0000	.0000	51	.9216	.0784	.0000
Salmon	I.+U.	46	.7500	45	.9778	45	1.0000	.0000	45	.9778	.0222	.0000
Sawmill	I.	98	.7908	101	.9901	101	1.0000	.0000	101	.9505	.0495	.0000
Sheep	I.	102	.7451	43	.9651	43	1.0000	.0000	43	.9419	.0581	.0000
Waydelich	I.+U. early	43	.7326	43	.9767	43	1.0000	.0000	43	.9186	.0814	.0000
	I.+U. late	31	.6935	30	1.0000	30	1.0000	.0000	31	.8710	.1290	.0000

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Table 5. Allele frequencies of odd-year class pink salmon sampled from Juneau area streams. All samples were adults collected in the fall of 1979. The frequency of the common allele, designated "100", is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N", "U", and "I" denote sample size, upstream samples, and intertidal samples (continued).

Stream	Location/ Timing	N	Agp				N	Ck-1		N	Ck-2	N	Ldh-1	N	Ldh-4
			(100)	(200)	(175)	(65)		(100)	(80)		(100)		(100)		(100)
Auke	U. early	81	.9259	.0741	.0000	.0000	81	.9815	.0185	81	1.0000	81	1.0000	81	1.0000
	I. early	90	.8944	.1056	.0000	.0000	90	.9889	.0111	90	1.0000	90	1.0000	90	1.0000
	U. late	98	.8827	.1173	.0000	.0000	98	1.0000	.0000	98	1.0000	98	1.0000	97	1.0000
	I. late	100	.9050	.0850	.0000	.0000	100	.9950	.0050	100	1.0000	100	1.0000	100	1.0000
Bear	I.+U.	80	.9063	.0937	.0000	.0000	80	1.0000	.0000	80	1.0000	80	1.0000	80	.9938
Boullion	I.	63	.8810	.1032	.0158	.0000	63	.9921	.0079	63	1.0000	63	1.0000	63	1.0000
Fish	U. early	87	.9253	.0747	.0000	.0000	67	.9851	.0149	67	1.0000	83	.9940	87	1.0000
	I. early	83	.9277	.0723	.0000	.0000	83	.9880	.0120	83	1.0000	83	1.0000	83	1.0000
	I. late	89	.8652	.1348	.0000	.0000	89	1.0000	.0000	89	1.0000	89	1.0000	89	1.0000
Hilda	I. early	33	.8939	.1061	.0000	.0000	33	1.0000	.0000	33	1.0000	33	.9967	33	1.0000
	I. late	44	.8977	.0909	.0000	.0114	44	.9886	.0114	44	1.0000	44	1.0000	58	.9914
Middle Pt.	I. early	54	.8704	.1296	.0000	.0000	54	1.0000	.0000	54	1.0000	54	1.0000	54	1.0000
	I. late	76	.8684	.1250	.0066	.0000	76	.9868	.0132	76	1.0000	76	1.0000	76	1.0000
Peterson (mainland)	U.	47	.9362	.0638	.0000	.0000	48	1.0000	.0000	48	1.0000	48	1.0000	48	1.0000
Peterson (Douglas Is.)	U. early	42	.8214	.1786	.0000	.0000	42	.9881	.0119	42	1.0000	42	1.0000	42	1.0000
	I. early	40	.8750	.1250	.0000	.0000	40	1.0000	.0000	40	1.0000	40	1.0000	40	.9875
	I. late	51	.9118	.0882	.0000	.0000	51	1.0000	.0000	51	1.0000	51	1.0000	51	1.0000
Salmon	I.+U.	45	.8333	.1667	.0000	.0000	45	1.0000	.0000	45	1.0000	45	1.0000	46	.9783
Sawmill	I.	101	.8762	.1238	.0000	.0000	101	.9951	.0049	101	1.0000	101	1.0000	101	.9901
Sheep	I.	43	.9070	.0930	.0000	.0000	43	.9884	.0116	43	1.0000	43	1.0000	101	.9951
Waydelich	I.+U. early	43	.8837	.1163	.0000	.0000	43	1.0000	.0000	43	1.0000	43	1.0000	43	1.0000
	I.+U. late	31	.9355	.0645	.0000	.0000	31	1.0000	.0000	31	1.0000	31	1.0000	31	1.0000

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Table 5. Allele frequencies of odd-year class pink salmon sampled from Juneau area streams. All samples were adults collected in the fall of 1979. The frequency of the common allele, designated "100", is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N", "U", and "I" denote sample size, upstream samples, and intertidal samples (continued).

Stream	Location/ Timing	N	Ldh-5	N	Ll-1			N	Mdh-1			N	Mdh-2
			(100)		(100)	(115)	(85)		(100)	(70)	(130)		(100)
Auke	U. early	81	1.0000	81	.7778	.2222	.0000	81	1.0000	.0000	.0000	81	1.0000
	I. early	90	1.0000	90	.7556	.2444	.0000	90	1.0000	.0000	.0000	90	1.0000
	U. late	97	1.0000	98	.7653	.2347	.0000	98	1.0000	.0000	.0000	98	1.0000
	I. late	99	1.0000	100	.7900	.2100	.0000	100	1.0000	.0000	.0000	100	1.0000
Bear	I.+U.	80	1.0000	80	.7313	.2687	.0000	80	1.0000	.0000	.0000	100	1.0000
Boullion	I.	63	1.0000	63	.7857	.2143	.0000	63	1.0000	.0000	.0000	63	1.0000
Fish	U. early	87	1.0000	63	.8492	.1508	.0000	87	.9885	.0115	.0000	87	1.0000
	I. early	83	1.0000	83	.8012	.1988	.0000	83	.9940	.0060	.0000	83	1.0000
	I. late	89	1.0000	89	.7640	.2360	.0000	89	1.0000	.0000	.0000	89	1.0000
Hilda	I. early	33	1.0000	33	.8485	.1515	.0000	33	.9848	.0152	.0000	33	1.0000
	I. late	58	1.0000	43	.8140	.1860	.0000	44	1.0000	.0000	.0000	44	1.0000
Middle Pt.	I. early	54	1.0000	53	.8113	.1887	.0000	54	1.0000	.0000	.0000	54	1.0000
	I. late	76	1.0000	75	.7200	.2800	.0000	76	1.0000	.0000	.0000	76	1.0000
Peterson (mainland)	U.	48	1.0000	48	.8438	.1562	.0000	48	1.0000	.0000	.0000	48	1.0000
Peterson (Douglas Is.)	U. early	42	1.0000	42	.7143	.2857	.0000	42	1.0000	.0000	.0000	42	1.0000
	I. early	40	1.0000	40	.8125	.1875	.0000	40	.9875	.0125	.0000	40	1.0000
	I. late	51	1.0000	51	.7549	.2451	.0000	51	1.0000	.0000	.0000	51	1.0000
Salmon	I.+U.	46	1.0000	45	.8333	.1667	.0000	45	1.0000	.0000	.0000	45	1.0000
Sawmill	I.	101	1.0000	101	.8564	.1436	.0000	101	1.0000	.0000	.0000	101	1.0000
Sheep	I.	101	1.0000	43	.7326	.2674	.0000	43	1.0000	.0000	.0000	43	1.0000
Waydelich	I.+U. early	43	1.0000	42	.8333	.1667	.0000	43	1.0000	.0000	.0000	43	1.0000
	I.+U. late	31	1.0000	31	.7097	.2903	.0000	31	1.0000	.0000	.0000	31	1.0000

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Table 5. Allele frequencies of odd-year class pink salmon sampled from Juneau area streams. All samples were adults collected in the fall of 1979. The frequency of the common allele, designated "100", is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N", "U", and "I" denote sample size, upstream samples, and intertidal samples (continued).

Stream	Location/ Timing	N	Mdh-3			N	Mdh-4	N	Me-1			N	Pgm
			(100)	(130)	(70)		(100)		(100)	(130)	(70)		(100)
Auke	U. early	81	.8766	.0864	.0370	81	1.0000	81	.9383	.0494	.0123	81	.9316
	I. early	90	.9167	.0222	.0611	90	1.0000	90	.9556	.0389	.0555	90	.9500
	U. late	98	.9031	.0459	.0510	98	1.0000	98	.9439	.0510	.0051	98	.9643
	I. late	100	.8600	.0300	.1100	100	1.0000	100	.9700	.0300	.0000	100	.9450
Bear	I.+U.	80	.9188	.0125	.0687	80	1.0000	80	.9688	.0250	.0062	80	.9375
Boullion	I.	63	.8889	.0397	.0714	63	1.0000	63	.9444	.0556	.0000	63	.9127
Fish	U. early	87	.9196	.0517	.0287	87	1.0000	86	.9477	.0523	.0000	87	.9368
	I. early	83	.8735	.0663	.0602	83	1.0000	83	.9639	.0361	.0000	83	.9458
	I. late	89	.8989	.0225	.0786	89	1.0000	89	.9719	.0225	.0056	89	.9607
Hilda	I. early	33	.8788	.0758	.0454	33	1.0000	33	.9697	.0303	.0000	33	.9394
	I. late	44	.8636	.1023	.0341	44	1.0000	44	.9773	.0227	.0000	44	.9659
Middle Pt.	I. early	54	.9260	.0370	.0370	54	1.0000	54	.9445	.0370	.0185	54	.9444
	I. late	76	.9276	.0132	.0592	76	1.0000	76	.9803	.0197	.0000	76	.9211
Peterson (mainland)	U.	48	.9271	.0312	.0417	48	1.0000	48	.9792	.0208	.0000	48	.9583
Peterson (Douglas Is.)	U. early	42	.8810	.0833	.0357	42	1.0000	42	.9405	.0595	.0000	42	.9167
	I. early	40	.9250	.0375	.0375	40	1.0000	40	.9375	.0375	.0250	40	.9000
	I. late	51	.9118	.0196	.0686	51	1.0000	51	.9706	.0294	.0000	51	.9902
Salmon	I.+U.	45	.8778	.0778	.0444	45	1.0000	45	.9444	.0556	.0000	45	.9445
Sawmill	I.	101	.9109	.0544	.0347	101	1.0000	100	.9650	.0300	.0050	101	.9406
Sheep	I.	43	.9302	.0000	.0697	43	1.0000	43	.9419	.0581	.0000	43	.9302
Waydelich	I.+U. early	43	.8023	.1047	.0930	43	1.0000	43	.9651	.0349	.0000	43	.9535
	I.+U. late	31	.8871	.0323	.0806	31	1.0000	31	.9678	.0161	.0161	31	.9355

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Table 5. Allele frequencies of odd-year class pink salmon sampled from Juneau area streams. All samples were adults collected in the fall of 1979. The frequency of the common allele, designated "100", is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N", "U", and "I" denote sample size, upstream samples, and intertidal samples (continued).

Stream	Location/ Timing	N	Phi-1					N	Phi-2	N	Phi-3		
			(100)	(33)	(130)	(200)	(-33)		(100)		(100)	(90)	(110)
Auke	U. early	81	.9938	.0062	.0000	.0000	.0000	81	1.0000	81	1.0000	.0000	.0000
	I. early	90	1.0000	.0000	.0000	.0000	.0000	90	1.0000	90	1.0000	.0000	.0000
	U. late	98	1.0000	.0000	.0000	.0000	.0000	98	1.0000	98	.9949	.0051	.0000
	I. late	100	1.0000	.0000	.0000	.0000	.0000	100	1.0000	100	.9900	.0100	.0000
Bear	I.+U.	80	1.0000	.0000	.0000	.0000	.0000	80	1.0000	80	1.0000	.0000	.0000
Boullion	I.	63	.9842	.0079	.0000	.0079	.0000	63	1.0000	63	1.0000	.0000	.0000
Fish	U. early	87	1.0000	.0000	.0000	.0000	.0000	87	1.0000	87	1.0000	.0000	.0000
	I. early	83	1.0000	.0000	.0000	.0000	.0000	83	1.0000	83	1.0000	.0000	.0000
	I. late	89	1.0000	.0000	.0000	.0000	.0000	89	1.0000	89	1.0000	.0000	.0000
Hilda	I. early	33	1.0000	.0000	.0000	.0000	.0000	33	1.0000	33	1.0000	.0000	.0000
	I. late	44	.9773	.0227	.0000	.0000	.0000	44	1.0000	44	1.0000	.0000	.0000
Middle Pt.	I. early	54	.9815	.0000	.0000	.0000	.0185	54	1.0000	54	1.0000	.0000	.0000
	I. late	76	.9934	.0066	.0000	.0000	.0000	76	1.0000	76	.9868	.0066	.0066
Peterson (mainland)	U.	48	1.0000	.0000	.0000	.0000	.0000	48	1.0000	48	.9896	.0000	.0104
Peterson (Douglas Is.)	U. early	42	1.0000	.0000	.0000	.0000	.0000	42	1.0000	42	.9881	.0119	.0000
	I. early	40	1.0000	.0000	.0000	.0000	.0000	40	1.0000	40	1.0000	.0000	.0000
	I. late	51	1.0000	.0000	.0000	.0000	.0000	51	1.0000	51	1.0000	.0000	.0000
Salmon	I.+U.	45	.9889	.0000	.0111	.0000	.0000	45	1.0000	45	1.0000	.0000	.0000
Sawmill	I.	101	1.0000	.0000	.0000	.0000	.0000	101	1.0000	101	.9851	.0149	.0000
Sheep	I.	43	.9884	.0000	.0000	.0000	.0116	43	1.0000	43	1.0000	.0000	.0000
Waydelich	I.+U. early	43	1.0000	.0000	.0000	.0000	.0000	43	1.0000	43	1.0000	.0000	.0000
	I.+U. late	31	.9839	.0000	.0000	.0000	.0161	31	1.0000	31	1.0000	.0000	.0000

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Table 5. Allele frequencies of odd-year class pink salmon sampled from Juneau area streams. All samples were adults collected in the fall of 1979. The frequency of the common allele, designated "100", is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N", "U", and "I" denote sample size, upstream samples, and intertidal samples (continued).

Stream	Location/ Timing	N	Pmi (100)	(85)	(115)	N	Pp-1 (100)	N	Pp-2 (100)	(109)	(93)	N	6pg (100)	(90)	N	Sod-1 (100)
Auke	U. early	81	.9878	.0062	.0062	81	1.0000	81	.6605	.1975	.1420	80	.9875	.0125	81	1.0000
	I. early	90	.9889	.0000	.0111	88	1.0000	88	.6761	.2102	.1136	89	.9944	.0056	90	1.0000
	U. late	98	1.0000	.0000	.0000	98	1.0000	97	.6598	.2113	.1289	98	.9949	.0051	98	1.0000
	I. late	100	.9900	.0100	.0000	100	1.0000	100	.6900	.2050	.1050	100	.9850	.0150	100	1.0000
Bear	I.+U.	80	1.0000	.0000	.0000	80	1.0000	79	.6835	.1899	.1266	80	.9875	.0125	80	1.0000
Boullion	I.	63	.9762	.0000	.0238	63	1.0000	63	.6746	.1825	.1429	63	.9762	.0238	63	1.0000
Fish	U. early	64	1.0000	.0000	.0000	62	1.0000	62	.7177	.1371	.1452	87	.9655	.0345	87	1.0000
	I. early	83	1.0000	.0000	.0000	83	1.0000	82	.7683	.1646	.0671	83	.9458	.0542	83	1.0000
	I. late	89	.9944	.0056	.0000	89	1.0000	89	.6910	.2022	.1068	89	.9831	.0169	89	1.0000
Hilda	I. early	33	1.0000	.0000	.0000	33	1.0000	33	.6667	.2424	.0909	33	.9848	.0152	33	1.0000
	I. late	44	1.0000	.0000	.0000	44	1.0000	44	.7386	.1364	.1250	44	1.0000	.0000	44	1.0000
Middle Pt.	I. early	54	.9815	.0000	.0185	54	1.0000	54	.7315	.2037	.0648	54	.9815	.0185	54	1.0000
	I. late	76	.9934	.0066	.0000	76	1.0000	76	.7303	.1579	.1118	67	1.0000	.0000	76	1.0000
Peterson (mainland)	U.	48	.9792	.0104	.0104	48	1.0000	48	.7292	.1667	.1042	48	.9375	.0625	48	1.0000
Peterson (Douglas Is.)	U. early	42	1.0000	.0000	.0000	42	1.0000	42	.7262	.1548	.1190	42	.9643	.0357	42	1.0000
	I. early	40	1.0000	.0000	.0000	40	1.0000	40	.6750	.2000	.1250	40	.9750	.0250	40	1.0000
	I. late	51	.9804	.0196	.0000	51	1.0000	51	.6274	.1765	.1961	51	.9804	.0196	51	1.0000
Salmon	I.+U.	45	1.0000	.0000	.0000	45	1.0000	45	.7111	.2667	.0222	45	.9889	.0111	45	1.0000
Sawmill	I.	101	1.0000	.0000	.0000	101	1.0000	101	.6485	.2079	.1436	100	.9850	.0150	101	1.0000
Sheep	I.	43	1.0000	.0000	.0000	43	1.0000	43	.6512	.2209	.1279	43	.9884	.0116	43	1.0000
Waydelich	I.+U. early	43	1.0000	.0000	.0000	43	1.0000	43	.7326	.1744	.0930	43	.9767	.0233	43	1.0000
	I.+U. late	31	1.0000	.0000	.0000	31	1.0000	31	.7097	.1935	.0968	31	1.0000	.0000	31	1.0000

Table 6. Allele frequencies of even-year class pink salmon sampled from streams in 1980 in southern Southeast Alaska, Norton Sound, and Bristol Bay. The frequency of the common allele, "100", is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N" denotes sample size.

Stream	N	Aat-3	N	Acon-3	N	Acon-4			N	Ada-2		
		(100)		(100)		(100)	(85)	(115)		(100)	(87)	(113)
Southern S.E.AK												
Herring Cove Cr.	114	.6842	114	.9868	114	.9079	.0921	.0000	114	.9342	.0614	.0044
Lover's Cove Cr.	99	.7475	99	1.0000	99	.9040	.0960	.0000	100	.9250	.0450	.0300
Porcupine Cr.	112	.7946	113	.9912	113	.9425	.0575	.0000	113	.9513	.0398	.0089
Sashin Cr.	97	.7371	99	.9950	99	.9040	.0960	.0000	100	.9000	.0900	.0100
Norton Sound												
Kwiniuk R.	101	.8663	102	1.0000	102	.9853	.0098	.0049	102	.9461	.0098	.0441
Nome R.	98	.8776	99	1.0000	99	.9747	.0202	.0051	99	.8990	.0303	.0707
Bristol Bay												
Naknek R.	35	.9429	42	1.0000	42	.9524	.0357	.0119	42	.8690	.0952	.0357
Nushagak R.	104	.8942	104	1.0000	104	.9663	.0288	.0048	104	.9471	.0144	.0385

	N	Agp				N	Ck-1			N	Ck-2	N	Ldh-1
		(100)	(200)	(175)	(65)		(100)	(80)	(120)		(100)		(100)
Southern S.E.AK													
Herring Cove Cr.	114	.8114	.1842	.0044	.0000	114	1.0000	.0000	.0000	114	1.0000	114	1.0000
Lover's Cove Cr.	100	.8300	.1700	.0000	.0000	100	1.0000	.0000	.0000	100	1.0000	100	.9950
Porcupine Cr.	113	.8319	.1637	.0044	.0000	113	.9956	.0044	.0000	113	1.0000	113	1.0000
Sashin Cr.	100	.8400	.1600	.0000	.0000	100	1.0000	.0000	.0000	100	1.0000	100	.9950
Norton Sound													
Kwiniuk R.	102	.9118	.0882	.0000	.0000	102	1.0000	.0000	.0000	102	1.0000	102	1.0000
Nome R.	99	.9293	.0707	.0000	.0000	99	1.0000	.0000	.0000	99	1.0000	99	1.0000
Bristol Bay													
Naknek R.	42	.8690	.1310	.0000	.0000	42	1.0000	.0000	.0000	42	1.0000	42	1.0000
Nushagak R.	104	.8414	.1538	.0048	.0000	104	.9904	.0096	.0000	104	1.0000	104	1.0000

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Table 6. Allele frequencies of even-year class pink salmon sampled from streams in 1980 in southern Southeast Alaska, Norton Sound, and Bristol Bay. The frequency of the common allele, "100" is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N" denotes sample size (continued).

Stream	N	Ldh-4	N	Ldh-5	N	Ll-1			N	Mdh-1		
		(100)		(100)		(100)	(85)	(115)		(100)	(87)	(113)
Southern S.E.AK												
Herring Cove Cr.	114	.9956	114	1.0000	114	.8114	.0526	.1360	114	.9868	.0088	.0044
Lover's Cove Cr.	100	1.0000	100	1.0000	100	.8150	.0800	.1050	100	.9800	.0200	.0000
Porcupine Cr.	113	.9867	113	1.0000	113	.7876	.0310	.1814	113	.9867	.0089	.0044
Sashin Cr.	100	1.0000	100	1.0000	100	.7650	.0550	.1800	100	.9850	.0150	.0000
Norton Sound												
Kwiniuk R.	102	1.0000	102	1.0000	102	.9755	.0098	.0147	102	.9608	.0392	.0000
Nome R.	98	1.0000	97	1.0000	95	.9263	.0474	.0263	99	.9798	.0152	.0050
Bristol Bay												
Naknek R.	42	1.0000	42	1.0000	37	.9865	.0000	.0136	42	.9762	.0233	.0000
Nushagak R.	104	1.0000	104	1.0000	104	.9856	.0096	.0048	104	.9615	.0385	.0000

	N	Mdh-2	N	Mdh-3			N	Mdh-4	N	Me-1		
		(100)		(100)	(130)	(70)		(100)		(100)	(130)	(70)
Southern S.E.AK												
Herring Cove Cr.	114	1.0000	114	.9956	.0000	.0044	114	1.0000	114	.8289	.1711	.0000
Lover's Cove Cr.	100	1.0000	100	.9800	.0150	.0050	100	1.0000	100	.8100	.1900	.0000
Porcupine Cr.	113	1.0000	113	.9690	.0265	.0045	113	1.0000	113	.8894	.1106	.0000
Sashin Cr.	100	1.0000	100	.9650	.0350	.0000	100	1.0000	100	.7900	.2100	.0000
Norton Sound												
Kwiniuk R.	102	1.0000	102	.9901	.0049	.0049	102	1.0000	100	.7400	.2600	.0000
Nome R.	99	1.0000	99	.9646	.0202	.0152	99	1.0000	99	.8081	.1919	.0000
Bristol Bay												
Naknek R.	42	1.0000	42	1.0000	.0000	.0000	42	1.0000	42	.6905	.2976	.0119
Nushagak R.	104	1.0000	104	.9952	.0048	.0000	104	1.0000	104	.6827	.3173	.0000

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Table 6. Allele frequencies of even-year class pink salmon sampled from streams in 1980 in southern Southeast Alaska, Norton Sound, and Bristol Bay. The frequency of the common allele, "100" is given for each locus. Where more than one variant allele occurs at a locus, frequencies for each allele are listed. "N" denotes sample size (continued).

Stream	N	Pgm	N	Phi-1			N	Phi-2	N	Phi-3		
		(100)		(100)	(33)	(130)		(100)		(100)	(110)	(90)
Southern S.E.AK												
Herring Cove Cr.	114	.9956	114	.9824	.0088	.0088	114	1.0000	114	1.0000	.0000	.0000
Lover's Cove Cr.	100	1.0000	100	.9900	.0050	.0050	100	1.0000	100	1.0000	.0000	.0000
Porcupine Cr.	113	.9956	113	.9912	.0044	.0044	113	1.0000	113	1.0000	.0000	.0000
Sashin Cr.	100	1.0000	100	1.0000	.0000	.0000	100	1.0000	100	1.0000	.0000	.0000
Norton Sound												
Kwiniuk R.	102	1.0000	102	1.0000	.0000	.0000	102	1.0000	102	1.0000	.0000	.0000
Nome R.	99	1.0000	99	1.0000	.0000	.0000	99	1.0000	99	1.0000	.0000	.0000
Bristol Bay												
Naknek R.	42	1.0000	42	1.0000	.0000	.0000	42	1.0000	42	1.0000	.0000	.0000
Nushagak R.	104	1.0000	104	1.0000	.0000	.0000	104	1.0000	104	.9952	.0000	.0048

Stream	N	Pmi			N	Pp-1	N	Pp-2			N	6pg		N	Sod-1
		(100)	(85)	(115)		(100)		(100)	(93)	(109)		(100)	(90)		(100)
Southern S.E.AK															
Herring Cove Cr.	114	1.0000	.0000	.0000	114	1.0000	114	.5877	.1711	.2412	114	.9430	.0570	114	1.0000
Lover's Cove Cr.	100	.9950	.0000	.0050	100	1.0000	100	.5200	.2100	.2700	100	.9050	.0950	100	1.0000
Porcupine Cr.	113	.9956	.0044	.0000	113	1.0000	113	.5664	.1947	.2389	112	.9063	.0937	100	1.0000
Sashin Cr.	100	1.0000	.0000	.0000	100	1.0000	100	.5450	.1800	.2750	99	.8788	.1212	100	1.0000
Norton Sound															
Kwiniuk R.	102	.9951	.0049	.0000	102	1.0000	102	.5980	.2304	.1716	99	.9848	.0152	102	1.0000
Nome R.	90	1.0000	.0000	.0000	97	1.0000	97	.5412	.2835	.1753	86	.9884	.0116	99	1.0000
Bristol Bay															
Naknek R.	42	1.0000	.0000	.0000	42	1.0000	42	.5122	.3171	.1707	42	.9881	.0119	42	1.0000
Nushagak R.	104	.9952	.0048	.0000	104	1.0000	104	.4712	.3654	.1635	104	.9904	.0096	104	1.0000

Table 7. Allele frequencies from even- and odd-year runs of pink salmon in Fish Creek, 1969-1979.

Year \ Locus	N	Agp (100)	Agp (150)	N	Mdh-3 (100)	Mdh-3 (130)	Mdh-3 (70)
			Odd-year				
1969*	76	.9079	.0921	75	.8933	.0467	.0600
1971*	64	.8984	.1016	64	.8437	.0313	.1250
1979	259	.9054	.0946	259	.8978	.0463	.0559
			Even-year				
1970*	81	.7099	.2901	81	.9877	.0123	.0000
1978	474	.7764	.2236	473	.9894	.0095	.0011

* Data taken from Aspinwall (1974b). Mdh-3 locus allele frequencies have been recalculated for this comparison from Aspinwall's phenotypic data.

class Kodiak Island pink salmon stocks. Although allele frequency differences between life stages could be caused by variations in the fitness of specific genotypes, Johnson suggested a more likely cause was the nonrandom nature of the collection of alevin samples. Due to the extremely small sample sizes of alevins he examined from most of the Kodiak Island streams, meaningful comparisons (of allele frequencies with small statistical variances) could be made only between pooled totals for each life stage. Since these alevins and adult totals were pooled over a different set of streams, this comparison was not as rigorous as one might hope. A more detailed comparison of allele frequencies of different life stages within each of three Juneau-area streams was made in this study. Three life stages of pink salmon were sampled using three methods of capture. Adults were sampled on the spawning grounds in Auke, Fish, and Peterson (mainland) Creeks. Fry were captured in fyke nets in each stream on many occasions over a large portion of the outmigration period (see Table 1). Alevins were taken from Auke Creek by fry-pumping, a method of pre-emergent sampling used to estimate egg survival.

No significant allele frequency differences were found among the three life stages sampled from Auke Creek (Table 8), or among the fry and adults from both Fish Creek and Peterson Creek (mainland) (see Table 9). Since alevins were collected only from Auke Creek, alevin data was not included in Auke Creek totals used in subsequent analyses.

Despite the lack of allele frequency differences between life stages of pink salmon in Juneau area streams, sampling techniques used in future electrophoretic studies of this species should be carefully examined. It is likely that limited pre-emergent sampling of alevins will not provide a genetically random sample. To ensure a collection of alevins from many families a large number of egg digs should be made in each stream. In this study allele frequencies of fry and adults collected from the same stream were similar, but it must be stressed that in each case fry were sampled over a large portion of the outmigration period. It is likely that fry collected by single, overnight fyke-net settings would not comprise random samples of outmigrating fry in small streams.

Heterogeneity of Even-Year Class Pink Salmon Sampled in the Juneau Area

Heterogeneity of allele frequencies within and among streams was examined using a log-likelihood ratio technique. Results of the log-likelihood ratio analysis of even-year-class pink salmon sampled from Juneau area streams in 1978 and 1979 are shown in Tables 9 and 10. This analysis is based on allele frequencies obtained from adult and fry samples collected in each of Auke Creek, Fish Creek, and Peterson Creek (mainland) as well as fry samples from Peterson Creek (Douglas Island).

Within-stream heterogeneity was partitioned so that heterogeneity among life stages and among adult spawning groups could be examined for each stream. None of these tests were significant, so adult and fry data were pooled for each stream and tested for heterogeneity among streams.

Tests for heterogeneity among streams were highly significant ($p < .01$; Table 9) at two loci; Pp-2 and Mdh-1. Samples from Peterson Creek (Douglas Island) had a significantly higher frequency of the Mdh-1 (70) allele than samples from the other three streams, and Auke Creek samples had a significantly higher frequency

Table 8. Comparison of 3 life stages of even-year class pink salmon from Auke Creek. Adults were collected in the autumn of 1978, alevins were collected in the winter of 1979, and fry were collected in the spring of 1979. "N" denotes sample size. Numbers enclosed in parenthesis represent each allele's mobility.

Locus	Life Stage	N	Allelic Frequencies			G	df
			(100)	(87+113)			
Ada-2	adult	125	.9000	.1000		2.327	2
	alevin	60	.8500	.1500			
	fry	58	.9052	.0948			
			(100)	(200)			
Agp	adult	440	.8068	.1932		1.786	2
	alevin	60	.7583	.2417			
	fry	174	.7874	.2126			
			(100)	(70+130)			
Me-1	adult	420	.7500	.2500		2.228	2
	alevin	62	.7661	.2339			
	fry	129	.7946	.2054			
			(100)	(109)	(93)		
Pp-2	adult	163	.5061	.1994	.2945	11.533	4
	alevin	58	.5517	.0862	.3621		
	fry	92	.5869	.1522	.2609		

Table 9. Log-likelihood ratio analysis of variation at 10 polymorphic loci of even-year class pink salmon from 4 streams in the Juneau area.

Source of Variation	df	Ada-2	df	Agp	df	Me-1	df	6pg	df	Pp-2	df	Acon-4	df	Ll-1	df	Aat-3	df	Mdh-1	df	Mdh-3
Among Streams	3	13.311*	3	9.812	3	9.666	3	6.471	6	37.754**	3	9.066	3	6.515	3	11.482	3	26.712**	3	0.935
Within Streams	4	4.224	7	4.490	7	8.264	7	5.574	14	15.108	3	0.449	1	1.366	—	———	—	———	3	0.001
within adult groups	3	4.200	4	1.928	4	3.733	4	4.615	8	4.673	1	0.076	1	1.366	—	———	—	———	—	———
Auke Cr.	2	2.639	2	1.376	2	2.215	2	3.059	4	1.989	—	———	—	———	—	———	—	———	—	———
Fish Cr.	1	1.561	1	0.309	1	1.366	1	1.027	2	2.568	1	0.076	1	1.366	—	———	—	———	—	———
Peterson Cr.(M.)	-	———	1	0.243	1	0.152	1	0.529	2	0.116	—	———	—	———	—	———	—	———	—	———
within life stages	1	0.024	3	2.562	3	4.531	3	0.959	6	10.435	2	0.373	—	———	—	———	—	———	—	———
Auke Cr.	1	0.024	1	0.587	1	2.206	1	0.001	2	3.370	1	0.334	—	———	—	———	—	———	—	———
Fish Cr.	—	———	1	1.392	1	0.005	1	0.957	2	2.874	—	———	—	———	—	———	—	———	—	———
Peterson Cr.(M.)	—	———	1	0.583	1	2.320	1	0.001	2	4.191	1	0.039	—	———	—	———	—	———	—	———
Total	7	17.535	10	14.302	10	17.930	10	12.045	20	52.862**	6	9.515	4	7.881	3	11.482	3	26.712**	4	0.936

* $.01 < p < .05$

** $p < .01$

Table 10. Totals of log-likelihood ratio analysis of variation, pooled over all loci, for even-year class pink salmon from the Juneau area.

Source	df	G	F-ratio
Among streams	33	131.724**	4.565(33,44df)**
Within streams	44	39.476	
Adult spawning groups	25	20.591	
Auke Creek	12	11.278	
Fish Creek	8	8.273	
Peterson Cr.(mainland)	5	1.040	
Life stages	19	18.885	
Auke Creek	7	6.522	
Fish Creek	6	5.229	
Peterson Cr.(mainland)	6	7.134	
Total	77	171.200**	

** $p \leq .01$

of the Pp-2 (93) allele than did samples from the other streams (Table 11). Among streams heterogeneity was also significant ($p < .05$) at the Ada-2 locus.

The total sums of the likelihood tests over all loci (Table 10) reveal the major characteristics of the genetic structure of even-year class pink salmon in the Juneau area. No significant heterogeneity existed within streams, either among adult spawning groups or among different life stages. Highly significant heterogeneity exists ($p < .01$) among streams. A ratio of the among to within stream variation was calculated by dividing the standardized among-stream measure (sum over all loci/df) by the standardized within stream measure, and has a sampling distribution approximated by an F-distribution (Winer, 1971). The F-ratio was highly significant ($p < .01$), which indicates that the heterogeneity in the even-year class was due primarily to allele frequency differences among streams.

Heterogeneity of Odd-Year Class Pink Salmon Sampled in the Juneau Area

Results of the log-likelihood ratio analysis of odd-year class pink salmon sampled from the Juneau area streams in 1979 are shown in Tables 12 and 13. Since only mature adults were sampled for this year class, comparisons of life stages within streams was not possible.

Tests for heterogeneity both within and among streams were not significant at any particular loci (Table 12). The pooled totals reveal that there was not significant heterogeneity either among streams or within streams. An F-ratio of among to within stream variation was not significant, which indicates that unlike the even-year class, the magnitude of the differences among streams was not significantly greater than the magnitude of the differences within streams.

Comparison of Even-Year and Odd-Year Class

Pink salmon almost invariably mature and die in their second year of life. As a result of this rigid two year life cycle, very little or no genetic exchange occurs between even- and odd-year class pink salmon on the west coast of North America. An electrophoretic comparison of even- and odd-year class pink salmon that spawned in the same streams (Aspinwall 1974) revealed significant differences in allele frequencies for AGP and MDH enzymes between year classes. Johnson (1979) found significant differences between even- and odd-year classes spawning in Kodiak Island streams for AGP, MDH, and PGM loci. Numerous loci examined in pink salmon from the Juneau area have not been previously studied, so a more detailed comparison of the two year classes from this region could be made. Samples collected from Auke, Fish, Peterson (mainland) and Peterson (Douglas Island) Creeks were pooled by year class in this comparison.

Table 14 lists allele frequencies of samples pooled for each year class and the G-statistic calculated for each locus. Differences at nine of the twelve loci compared were highly significant ($p < .01$), and significant ($p < .05$) at one additional locus. This evidence supports previous findings of genetic distinctness among year classes of pink salmon, and suggests that the differences are perhaps greater than previously realized. Three alleles, Ada-2 (113), LI-1 (83), and Mdh-1 (130), were present in only the even-year class in Juneau area streams.

Table 11. Selected allele frequencies at the Mdh-1 and Pp-2 loci for even-year class pink salmon from 4 streams in the Juneau area.

Stream	Locus (allele) N	Mdh-1(70)	95%C.I.	N	Pp-2(93)	95%C.I.
Auke	612	.0106	(.0055-.0173)	255	.2824	(.2434-.3231)
Fish	473	.0106	(.0050-.0183)	335	.1896	(.1603-.2208)
Peterson(mainland)	293	.0102	(.0036-.0202)	270	.1685	(.1375-.2019)
Peterson(Douglas Is.)	174	.0460	(.0262-.0710)	174	.1322	(.0981-.1706)

Table 12. Log-likelihood ratio analysis of variation at 9 polymorphic loci of odd-year class pink salmon collected from 12 streams in the Juneau area.

Source of Variation	df	Aat-3	df	Ada-2	df	Agp	df	Ll-1	df	Mdh-3	df	Pp-2	df	Me-1	df	Pgm	df	6pg
Among Streams	11	20.207	11	17.149	11	10.509	11	19.341	11	13.336	22	27.127	—	———	—	———	—	———
Within Streams	10	11.068	10	15.079	10	11.490	10	12.719	10	8.755	20	17.115	5	3.956	8	15.457	2	3.698
Auke Cr.	3	2.584	3	3.530	3	2.049	3	0.727	3	3.765	6	1.451	3	2.557	3	4.320	—	———
Fish Cr.	2	4.519	2	4.290	2	4.912	2	3.423	2	1.959	4	6.873	2	1.399	3	1.064	2	3.698
Hilda Cr.	1	2.517	1	1.707	1	0.006	1	0.317	1	0.077	2	2.984	—	———	—	———	—	———
Middle Pt.Cr.	1	0.873	1	4.634	1	0.002	1	2.879	1	0.003	2	2.317	—	———	1	0.548	—	———
Peterson Cr.(Doug.Is.)	2	0.306	2	0.033	2	3.348	2	2.217	2	0.978	4	3.387	—	———	2	9.525	—	———
Waydelich	1	0.269	1	0.885	1	1.171	1	3.156	1	1.973	2	0.103	—	———	—	———	—	———
Total	21	31.275	21	32.228	21	21.999	21	32.060	21	22.091	42	44.242	5	3.956	8	15.457	2	3.698

Table 13. Totals of log-likelihood ratio analysis of variation, pooled over all loci, for odd-year class pink salmon from the Juneau area.

Source	df	G	F-ratio
Among streams	77	107.669	1.196(77,85 df)
Within streams	85	99.337	
Auke Creek	26	20.983	
Fish Creek	21	32.137	
Hilda Creek	7	7.608	
Middle Pt.Creek	8	11.256	
Peterson Cr.(Dougl.Is.)	16	19.794	
Waydelich Creek	7	7.559	
Total	162	207.006	

* .01 < .05
** p < .01

Table 14. Comparison of even- and odd-year class samples from Auke, Fish, Peterson (mainland), and Peterson (Douglas Island) Creeks. "N" denotes sample size. "p", "q", and "r" are relative allele frequencies for all fish sampled from each individual year class.

Locus	Year Class	N	p	q [1]	r	G	df
Aat-3	Even	654	.753	.247		0.003	1
	Odd	805	.754	.246			
Acon-4[1]	Even	521	.943	.057		78.315***	1
	Odd	808	.996	.004			
Ada-2[1]	Even	499	.891	.109		8.759**	1
	Odd	788	.925	.0759			
Agp[1]	Even	1555	.803	.197		77.535***	1
	Odd	808	.900	.100			
Ll-1[1]	Even	258	.833	.167		6.296*	1
	Odd	785	.783	.217			
Mdh-1[1]	Even	1552	.983	.017		23.561***	1
	Odd	809	.997	.003			
Mdh-3	Even	1552	.988	.009	.003	218.459***	2
	Odd	808	.895	.048	.056		
Me-1	Even	1391	.782	.217	.001	295.470***	2
	Odd	808	.956	.040	.004		
Pgm	Even	1504	.995	.005		108.617***	1
	Odd	809	.947	.053			
Phi-3[1]	Even	1198	.998	.002		0.818	1
	Odd	809	.997	.003			
Pp-2	Even	1034	.588	.215	.197	51.588***	2
	Odd	780	.692	.188	.120		
6pg	Even	1405	.935	.0651		41.817***	1
	Odd	807	.977	.0238			

* .01 < .05
** .001 < p < .01
*** p < .001

[1] "q" represents pooled frequencies of more than 1 allele

Comparison of Juneau Area Streams with Other Southeast Alaskan Streams

The Alaska Department of Fish and Game, for management purposes, partitions Southeast Alaska into northern and southern regions (Figure 2). Tagging studies have revealed that pink salmon stocks migrate into Southeast Alaskan waters through several major routes (Nakatani et al. 1975; Hoffman 1982). Pink salmon returning to streams in northern Southeast Alaska generally enter through Icy Strait or southern Chatham Strait, whereas those bound for streams in southern Southeast generally enter through Dixon Entrance or Sumner Strait. Differences in migration routes followed by Southeast Alaskan pink salmon could act to isolate one region's stocks from those of the other. Once isolated, stocks from the two regions could become genetically distinct and electrophoretically identifiable.

Four Southeast Alaskan streams located far south of Juneau were sampled in 1980 to permit a comparison between even-year pink salmon populations from northern and southern Southeast Alaska. Two streams located near the regional boundary, Sashin Creek and Lover's Cove Creek, were sampled as well as two streams, Porcupine Creek and Herring Cove Creek, located in southern Southeast Alaska (Figure 2). The Sashin Creek run was reestablished in 1964, following a pink salmon eradication project that had all but eliminated the native run (McNeil et al. 1969). Live pink salmon adults were captured in Bear Harbor and transported to Sashin Creek, where they were released to spawn. A healthy even-year run has subsequently developed in this creek. Due to the proximity of Sashin Creek and Lover's Cove Creek to the division boundary, as well as the fact that the donor source of the Sashin Creek run is located in southern Southeast, these samples were grouped with those from Porcupine Creek and Herring Cove Creek for comparisons with samples from the Juneau area. The results of the log-likelihood ratio analysis on these samples are displayed in Tables 15 and 16.

Samples from streams from the southern group were homogeneous ($p > .05$). As discussed previously, even-year class streams within the Juneau area did exhibit significant heterogeneity. Differences between the regions were significant ($p < .05$) at the Ada-2, Me-1, and Pp-2 loci. The ranges of allele frequencies for the two regions overlapped at each of these loci, so allele frequency data at any one of these particular loci alone was not sufficient to accurately make a regional classification for the streams.

The total sums of the likelihood tests over all loci (Table 16) reveal that there was highly significant heterogeneity between regions. An F-ratio of the among to within region variation was highly significant ($p < .005$), indicating differences among major geographic regions are greater than within these regions.

The relationship between migration route differences and increased genetic heterogeneity was not as definite. Herring Cove, Porcupine, Sashin, and Lover's Cove populations appear to form a homogeneous group, even though several of these populations almost surely follow different migration routes when returning to their natal streams. Sashin Creek and Lover's Cove Creek populations appear to enter inland Southeast Alaskan waters via lower Chatham Strait (Hoffman 1982). Preliminary analysis of tagging studies performed by the Alaska Department of Fish and Game in 1981 indicate that pink salmon destined for Porcupine Creek migrate through Sumner Strait (Steve Hoffman, Alaska Department of

Table 15. Log likelihood ratio analysis of variation at 12 polymorphic loci of even-year class pink salmon collected from 4 streams in the Juneau area and 4 streams south of Juneau.

Source of Variation	df	Aat-3	df	Acon-4	df	Ada-2	df	Agp	df	Ldh-4	df	Ll-1
Among regions	1	0.430	1	5.704	1	8.006*	1	2.686	1	0.333	1	3.090
Within regions	6	18.741	9	12.700	10	21.865	13	14.968	—	———	7	9.911
within Juneau area	3	11.482	6	9.515	7	17.535	10	14.302	—	———	4	7.881
within southern area	3	7.259	3	3.185	3	4.330	3	0.666	—	———	3	2.030
Total	7	19.171*	10	18.404	11	29.871*	14	17.654	1	0.333	8	13.001

Source of Variation	df	Mdh-1	df	Mdh-3	df	Me-1	df	Phi-1	df	Pp-2	df	6pg
Among regions	1	0.105	1	4.297	1	10.264*	1	0.002	2	12.249*	1	6.098
Within regions	3	26.712**	7	7.937	13	26.905	—	———	26	55.692**	13	17.727
within Juneau area	3	26.712**	4	0.936	10	17.930	—	———	20	52.862**	10	12.045
within southern area	—	———	3	7.001	3	8.975	—	———	6	2.830	3	5.682
Total	4	26.817**	8	12.234	14	37.160**	1	0.002	28	67.941**	14	23.825

Table 16. Totals of log-likelihood ratio analysis of variation, pooled over all loci, for even-year class pink salmon from 4 streams in the Juneau area and 4 streams south of Juneau.

Source of variation	df	G	F-ratio
Among regions	13	53.264**	2.057(13,107 df)*
Within regions	107	213.158**	
within Juneau area	77	171.200**	
within southern area	30	42.958	
Total	120	266.422**	

* .01 < p < .05
** p < .01

Fish and Game, personal communication). Previous tagging studies indicate that fish bound for streams farther south along the inland coast of Southeast Alaska (i.e., Herring Cove Creek) primarily enter through Dixon Entrance.

Average Heterozygosities and Gene Diversity Analysis

Data from all Alaskan regions sampled were included in this analysis. Allele frequency data for all samples from a stream within a particular year class were pooled. Only loci for which data were collected from all streams were used. No data was collected for the Pmi locus from the even-year run from Fish Creek, so average heterozygosity estimates as well as the gene diversity analysis were based on the remaining 24 loci examined.

Unweighted averages of allele frequencies at all loci were computed for streams within a region. Average expected heterozygosities (H_T) were then calculated for each region. H_T values varied from 0.0713 ± .0285 for pink salmon from the Norton Sound region to 0.1032 ± .0329 for southern Southeast pink salmon (Table 17). The heterozygosity of odd-year pink salmon from northern Southeast Alaska (0.0820) was slightly lower than that of the even-year class (0.0990) from this region. Within the even-year class a cline of heterozygosities appears to exist. A general trend of decreasing heterozygosity northward along the Alaskan coastline from southern Southeast Alaska was apparent, although standard errors of H_T overlapped for all regions.

Interpopulational gene diversity ($\overline{D}_{ST}$) and gene differentiation coefficient ($\overline{G}_{ST}$) values within regions were uniformly small (Table 17). Measures of two standard errors for each value overlapped zero for all regions except the even-year class from northern Southeast Alaska. A very small portion of the total genetic diversity within each region was therefore due to differences among streams.

Gene diversity analysis was extended two more levels. Unweighted averages of regional H_S and H_T values were calculated for the even-year class, and were designated $\overline{H}_S$ and $\overline{H}_T$. A value designated $\overline{H}_r$, the average expected heterozygosity of all regions pooled and considered as one, was calculated from the unweighted averages of mean regional allele frequencies at all 24 loci. $\overline{D}_{tr}$ and $\overline{G}_{tr}$ values, analogous to $\overline{D}_{st}$ and $\overline{G}_{st}$ except that they measure genetic diversity among instead of within regions, were derived. The same procedure was followed to calculate $\overline{D}_{ry}$ and $\overline{G}_{ry}$, which represent the average inter-year class gene diversity and the coefficient of gene differentiation among year classes. Results are displayed in Tables 18 and 19.

$\overline{\overline{D}}_{st}$ and $\overline{D}_{tr}$ values, which represent the average within and among region gene diversities of the even-year class, were 0.000205 and 0.003300 (Table 18). Diversity among regions was more than ten times greater than within regions. The estimate of $\overline{G}_{tr}$, the coefficient of gene differentiation among regions, was only 0.03636, however. This means that less than four percent of the total genetic diversity within the even-year class was attributable to differences among regions, and that greater than 96 percent exists within the stream themselves.

By far the most conspicuous grouping of pink salmon was by year class. The coefficient of gene differentiation among year classes was 0.14920 (Table 19).

Table 17. Gene diversity analysis of even- and odd-year class pink salmon within different Alaskan regions.

Year Class	Region	$\bar{H}s$	Ht	(s.e.)	$\bar{D}st$	(s.e.)	$\bar{G}st$	(s.e.)
Even								
	Norton Sound	.071196	.071285	.028484	.000089	.000074	.001245	.000986
	Bristol Bay	.076386	.076318	.031732	-.000068	.000145	-.000891	.001738
	North.S.E.AK	.098359	.099037	.032224	.000678	.000251	.006845	.001214
	South.S.E.AK	.103040	.103167	.032858	.000128	.000119	.001238	.001164
Odd								
	North.S.E.AK	.081905	.082029	.027080	.000125	.000075	.001522	.000765

Table 18. Gene diversity among regions sampled for the even-year class.

Value	Description
$\bar{\bar{H}}s$ = .087245	average gene diversity within populations within a region
$\bar{H}t$ = .087450	average total regional gene diversity
$\bar{H}r$ = .090750	total gene diversity of the even-year class
$\bar{\bar{D}}st$ = .000205	average gene diversity among populations within a region
$\bar{D}tr$ = .003300	gene diversity between regions
$\bar{G}tr$ = .036360	coefficient of gene differentiation between regions

Table 19. Gene diversity among year classes.

Value	Description
$\bar{\bar{H}}r$ = .086390	average total gene diversity within a year class
$\bar{H}y$ = .101540	total gene diversity
$\bar{D}ry$ = .015150	gene diversity between year classes
$\bar{G}ry$ = .149200	coefficient of gene differentiation between year classes

Nei (1975) lists comparable estimates for a variety of other organisms; 0.07 among the three major races of man, 0.072 among four populations of horseshoe crabs and 0.119 among five populations of fruit flies.

Genetic Distance

Genetic distance measures permit grouping populations by the magnitude of genetic differences. Many genetic distance measures have been developed but few are actually based on biological principles. Nei's method of calculating genetic distance was chosen for use because it estimates a biological parameter; the number of electrophoretically detectable codon differences per locus between two populations (Nei 1972; Nei and Roychoudhury 1974).

Heterogeneity within streams was not significant; allele frequency data was pooled by stream within each year class for this analysis. A matrix of genetic distances values was generated (Table 20). Unbiased estimates of genetic distances were calculated, which made it possible for genetically similar populations to be related by a negative value. This was indeed the case between a number of streams within regions. Nei (1978) suggests that these values be considered to be zero.

The genetic distance ± 2 standard errors overlapped zero for all but two of the 80 pairwise comparisons between streams located in the same region. Values between even-year samples from Fish Creek and Peterson Creek (Douglas Island) as well as between odd-year samples from Sheep Creek and Boullion Creek were slightly higher than the range of two standard errors of the genetic distance. All other statistical analyses performed in this study suggest that the differences within regions sampled for a particular year class for each region. Unweighted averages of allele frequencies for all populations within a region from the same year class were calculated for each locus. Unweighted arithmetic average clustering techniques (UPGMA; Sneath and Sokal 1973) were then used to construct dendrogram of Nei's genetic distances (Figure 4).

The dendrogram reveals clusters that correspond perfectly to results from the other statistical analyses. Even- and odd-year classes form the most distinct clusters. The genetic distance estimate between year classes (D = 0.00424) roughly compares with estimates between races of *Drosophila*, *Astyanax mexicanus* (cave fish) and *A. carolinensis* (lizard), and is slightly lower than estimates between races of man (.011-.019) (Nei 1975).

Within a year class fish from regions which are geographically closer formed clusters. The genetic distance between Southeast Alaskan pink salmon and those from the Bering Sea region is much higher, however, confirming that gene flow between widely separated geographic areas is much lower than that between populations from the same region.

DISCUSSION

Electrophoretic analysis of 25 loci revealed that a high level of genetic variation exists in Alaskan populations of pink salmon. Eighteen loci were polymorphic (> 1%) in at least one collection.

Table 20. Matrix of unbiased estimates of the standard genetic distance between populations. All values are less than 1, and are preceded by a decimal point.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
2	0054																							
3	0013	0034																						
4	-0001	0026	-0022																					
5	0537	0502	0511	0361																				
6	0540	0468	0473	0361	-0015																			
7	0331	0234	0316	0201	0118	0060																		
8	0344	0310	0354	0245	0080	0044	0019																	
9	0037	0193	0085	0048	0383	0389	0313	0275																
10	0116	0114	0099	0060	0366	0305	0210	0279	0167															
11	0071	0103	0119	0072	0354	0370	0185	0167	0141	0090														
12	0051	0093	0061	0036	0287	0269	0164	0145	0103	0073	0024													
13	0388	0324	0480	0394	0831	0759	0360	0513	0588	0303	0276	0395												
14	0478	0358	0552	0460	0904	0810	0404	0572	0729	0344	0353	0460	-0002											
15	0327	0260	0426	0331	0766	0723	0327	0445	0521	0284	0219	0335	-0022	-0019										
16	0346	0348	0484	0412	0963	0916	0463	0571	0557	0370	0244	0400	0026	0072	0004									
17	0357	0315	0478	0401	0861	0777	0367	0498	0538	0329	0260	0386	0000	0050	-0008	-0006								
18	0409	0365	0542	0457	0952	0876	0465	0595	0649	0331	0293	0422	0001	-0007	-0018	0016	0009							
19	0397	0270	0487	0397	0797	0715	0306	0406	0604	0363	0229	0369	0033	0031	-0009	0029	0009	0031						
20	0311	0292	0420	0342	0893	0848	0426	0559	0514	0273	0237	0369	-0003	0018	-0027	0002	0004	-0008	0050					
21	0286	0206	0400	0289	0644	0598	0243	0360	0437	0253	0193	0300	0022	0052	-0018	0044	-0005	0039	-0001	0025				
22	0363	0329	0530	0415	0727	0740	0376	0469	0551	0318	0190	0335	0048	0100	0012	0033	0012	0033	0050	0046	0011			
23	0339	0318	0452	0357	0845	0817	0389	0538	0562	0293	0242	0375	-0024	-0026	-0044	0004	0026	-0022	0047	-0037	0032	0041		
24	0429	0420	0557	0494	1049	0979	0510	0656	0643	0409	0323	0479	0002	0055	0002	-0018	-0030	0007	0056	-0006	0055	0030	-0001	

1-Herring Cove(even);2-Porcupine(even);3-Sashin(even);4-Lover's Cove(even);5-Nushagak(even);6-Naknek(even);7-Nome(even);8-Kwiniuk(even);
9-Auke(even);10-Fish(even);11-Peterson(Douglas Island)(even);12-Peterson(mainland)(even);13-Auke(odd);14-Bear(odd);15-Boullion(odd);
16-Fish(odd);17-Hilda(odd);18-Middle Pt.(odd);19-Peterson(mainland)(odd);20-Peterson(Douglas Island)(odd);21-Sawmill(odd);22-Salmon(odd);
23-Sheep(odd);24-Waydelich(odd)

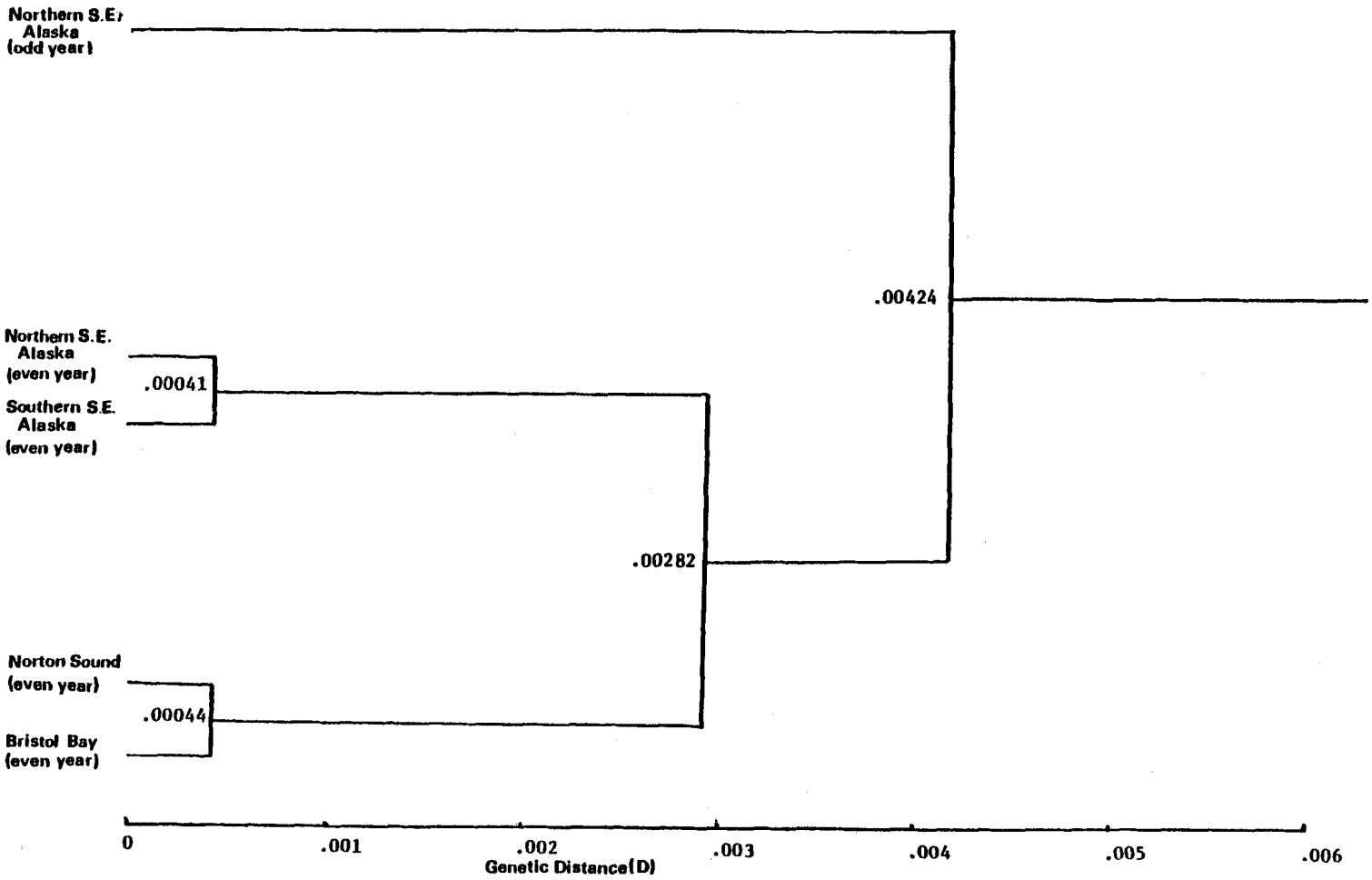

Figure 4. Dendrogram of standard genetic distances based on 24 loci of pink salmon stocks from various North American regions.

Estimates of the average heterozygosity per locus for pink salmon from different Alaskan regions varied from 0.0713 ± 0.0285 to 0.1032 ± 0.0329, and were significantly higher than those reported by Utter and Allendorf (1980) for six populations of pink salmon (H = .039; range = 0.032 to 0.047).

A problem central to the interpretation of heterozygosity estimates is that in almost every electrophoretic study of a given species different assortments of loci have been examined. Heterozygosities vary greatly from locus to locus (Selander 1976) and a heavy reliance on a single group of functionally related enzymes may bias estimates. For example, glycolytic enzymes may be less variable than enzymes not involved in energy metabolism (Lewontin 1974). An estimate of the average heterozygosity per locus based primarily on data from glycolytic enzymes might be lower than one based on a different assortment of enzymes for a particular species. No simple remedy to this source of error is apparent.

A clinal trend of heterozygosities was evident among regions sampled for pink salmon. The range of pink salmon extends from northern California northward to the Arctic Ocean (Hart 1973). Southeast Alaska is centrally located within this range, while Norton Sound and Bristol Bay are located near the northern extremity. The average heterozygosity per locus was highest in pink salmon from southern Southeast Alaska. Heterozygosities steadily decreased northward from this region along the Alaskan coastline, although standard errors of these estimates overlapped for all regions. Mayr (1963) hypothesizes that populations that are centrally located within the range of a species should possess higher levels of genetic variation than populations located near the peripheries. Marginal environmental conditions and reduced gene flow among peripheral populations could reduce levels of genetic variation in these populations. Centrally located populations, occupying more congenial environments and experiencing gene flow from populations in two directions, would maintain higher levels of variation.

Mayr's hypothesis suggests that stocks from the southern periphery of the range of pink salmon might likewise have reduced heterozygosities. Unfortunately large gaps exist in the data collected in this study, since no samples were analyzed from regions south of Southeast Alaska or regions between northern Southeast Alaska and Bristol Bay. Collection of this data would permit a more conclusive judgment on the existence of a clinal trend of heterozygosities among stocks from the entire coast of western North America.

Given that a high degree of genetic variation exists in Alaskan pink salmon, what does analysis of this variation reveal about the population structure of fish from this area? Log-likelihood ratio analysis revealed no significant heterogeneity among different segments of runs returning to selected streams in the Juneau area. Intertidal and upstream spawners, as well as early and late run spawners, appeared to comprise a single spawning group in each stream. Electrophoretically distinct subpopulations of pink salmon inhabiting the same stream have been found, however, in several Kodiak Island and Prince William Sound streams (Johnson 1979; Seeb and Wishard 1979).

Although gene frequencies within Juneau area streams were similar, it must be emphasized that electrophoresis detects only approximately one-third of all nucleotide substitutions (Selander 1976). The twenty-five loci examined in

this study represent only a minute portion of the total genome of the pink salmon. It is possible that differences occur in other genes, such as those coding for structural and regulatory proteins. Indeed, Bams (1976) has reported evidence of a genetic influence on the homing behavior of pink salmon.

Genetic heterogeneity did occur among Juneau area streams for the even-year class. Allele frequency differences were not great, however. Within the even-year class, Auke Creek fish were characterized by a high frequency of the Pp-2 (93) allele, and Peterson Creek (Douglas Island) fish had a higher frequency of the Mdh-1 (70) allele than did fish from other streams. Heterogeneity was not significant among odd-year runs. Differences among streams were significantly greater than differences within streams ($F = 4.565$; $p < .01$) for the even-year class, though not for the odd-year class.

The allele frequency difference observed within each year class in Juneau-area streams were not closely correlated with geographic location. Johnson (1979) similarly found distinct allele frequencies among pink salmon that did not fit a simple pattern with respect to the geographic location of Kodiak Island streams. Because allele frequencies did not vary greatly among streams in the Juneau area, the electrophoretic discrimination of natural pink salmon populations from this limited geographic area in terminal mixed fisheries is not feasible.

Among-region differences were much larger than differences within regions. Johnson (1979) found that within a year class, allele frequency differences among regions were generally related to geographic distance: the farther apart two regions were, the greater were the differences between them. The same trend is seen in this study between populations from four Alaskan regions that were examined for many more loci.

Regional differences between northern and southern Southeast Alaskan pink salmon were analyzed using the log-likelihood ratio techniques. Significant heterogeneity existed among regions, but allele frequencies at individual loci were not large enough to allow a regional classification to be made for the streams. Fish returning to the streams sampled in Southeast Alaska are known to follow several different migration routes when returning to their natal streams. Although these migration routes could serve as at least minor isolating "barriers" between several of the populations examined, this relationship was not supported by the data.

By far the largest portion of the genetic variation present within a year class existed within the samples themselves. D_{st} and G_{st} values were extremely small for both even- and odd-year pink salmon from the Juneau area, indicating that variation among individuals is of a much greater magnitude than variation among streams. Similar results were obtained for populations sampled in southern Southeast Alaska, Bristol Bay, and Norton Sound.

Aspinwall (1974b) and Johnson (1979) noted that, for the limited number of loci examined in each of their studies, differences between year classes within a particular stream were greater than differences between streams from the same year class. Because selection pressures should be similar to populations occupying the same stream and ocean environments, both authors concluded that genetic differences between the year classes were primarily a result of neutral, rather

than selected, processes. A much more intensive analysis of both year lines of pink salmon from streams surrounding Juneau revealed significant differences between years at ten of the twelve loci compared. Five alleles were present in only one of the two year classes of Alaskan pink salmon. Three of these alleles were found in extremely low frequencies in samples from only one year class. Sampling error alone could account for the rare appearance of an allele in one year class and not another. However two alleles, Ada-2 (113) and Ll-1 (83), were present at over the 5 percent level in several streams in the even year, but were completely absent from the odd year streams. The presence of these two alleles at relatively high levels in only even-year pink salmon could be due to genetic differences in the founder populations of each year class, or to a loss of alleles in the odd-year class as a result of an extreme reduction in population size.

The overall genetic structure of pink salmon stocks sampled in this study is graphically represented in the dendrogram of Nei's genetic distances. Genetic distances between streams within major geographic regions were very small. Estimates of two standard errors overlapped zero in almost every case. Regional differences were much larger and were roughly proportional to the distance between regions. By far the most conspicuous clustering was by year class.

Johnson (1979) and Utter et al. (1979) have developed an elegant interpretation of the results of pink salmon electrophoretic studies performed by a number of investigators over the past ten years. Most pink salmon spawn close to salt water in small coastal streams. Environmental conditions in such streams are extremely dynamic, changing radically with fluctuations in rainfall, water temperature, and depth and numerous other destabilizing factors. Several reductions in population sizes, known as bottlenecks, consequently often occur in many streams, causing a limited degree of random genetic heterogeneity among populations within a region. They theorize that high levels of heterozygosity are maintained by straying between populations but that the rate of straying is not great enough to completely mask all differences that result from population constrictions. Populations located more distantly from one another experience reduced rates of genetic mixing and would be expected to maintain greater levels of genetic diversity.

The in-depth electrophoretic analysis of pink salmon from streams in Southeast Alaska and several other Alaskan regions performed in this study supports the population characteristics described by Johnson (1979) and Utter et al. (1979).

Important questions remain about the rate of straying between populations and the potential usefulness of electrophoresis in management of the pink salmon resource. How much straying takes place between pink salmon populations? Is gene flow actually great enough within and between streams in a limited geographic area to essentially homogenize all populations? If electrophoretic differences are relatively small and randomly distributed within region, of what use is this technique in separating mixed stocks in terminal fisheries?

An attempt to answer these and other questions is presently being undertaken as a follow-up to the collection of baseline information on the genetic structure of Juneau area pink salmon populations that was performed in this study.

A rare protein has been bred into both the even- and odd-year hatchery populations of pink salmon in Auke Creek. The proteins chosen for these "genetic marks" were demonstrated to be present in uniformly low frequencies in all streams near Auke Creek. By increasing the frequency of these rare proteins, the Auke Creek population suddenly becomes uniquely identifiable from other populations in this region. Evaluating the success of this experiment should provide some indication of migration rates between populations, as well as important information about the usefulness of this technique in separating populations of pink salmon in terminal fisheries.

SUMMARY

1. Results of breeding crosses confirmed that observed variation at the Aat-3, Ada-2, Ll-1, Pp-2, and 6pg loci is genetic in nature. Nonrandom segregation occurred between Phi-1,2 and Pp-2 in two crosses, as well as between Acon-4 and 6pg in another cross. Due to the nature of the crosses it was not possible to determine whether the aberrant segregation ratios were a result of linkage or pseudolinkage.

2. Estimates of the average heterozygosity per locus (± standard error) for pink salmon from four Alaskan regions varied from .0713 ± .0285 to .1032 ± .0329.

3. A clinal trend of heterozygosities was apparent, although standard errors of the estimates overlapped for all regions.

4. Within particular streams in the Juneau area no allele frequency differences were noted among intertidal and upstream spawners or early and late run spawners.

5. Heterogeneity among streams in the Juneau area was significant for the even-year class, but actually represented only a minor portion of the total genetic variation present and was not generally observable in stream by stream comparisons. Heterogeneity among Juneau-area streams for the odd-year class was not significant.

6. Gene frequency differences within this small geographic area were not large enough to be of potential use as a management tool for separating populations in mixed stock fisheries.

7. Genetic differences between different geographic regions were greater than within regions, and were roughly proportional to the geographic distance between regions.

8. The greatest differences in allel frequencies occurred between year classes. Even- and odd-year pink salmon differed at ten of the twelve loci compared. Five alleles were found among samples from only one of the two year classes.

9. Gene frequency data of pink salmon from streams in the Juneau area provides baseline data for a genetic marking project of a hatchery population of pink salmon from Auke Creek, Alaska.

ACKNOWLEDGMENTS

This work is a result of research sponsored by the Alaska Sea Grant Program, cooperatively supported by the National Oceanographic and Atmospheric Administration, Office of Sea Grant, Department of Commerce, under grant number M/79-01, and by the University of Alaska with funds appropriated by the State of Alaska.

I especially want to thank Dr. Anthony Gharrett for his enthusiasm and guidance throughout this project. I am grateful to the Alaska Sea Grant Program for funding the study. I appreciate and value the advice and support Scott Marshall offered toward completing my research. I would also like to acknowledge Dr. Jack Helle and Dr. Lew Haldorson for their helpful review of the manuscript. Fellow gel jockies Sandro Lane, Margie Thomason, and Scott McPherson were of great help in sample collection and processing. Thanks to Dr. John Clark, Bill Heard, Herb Jaenicke, and Jerry Taylor for assistance in obtaining many of the samples analyzed in this study. Finally, I thank Virginia Burton and June Grant for their meticulous typing.

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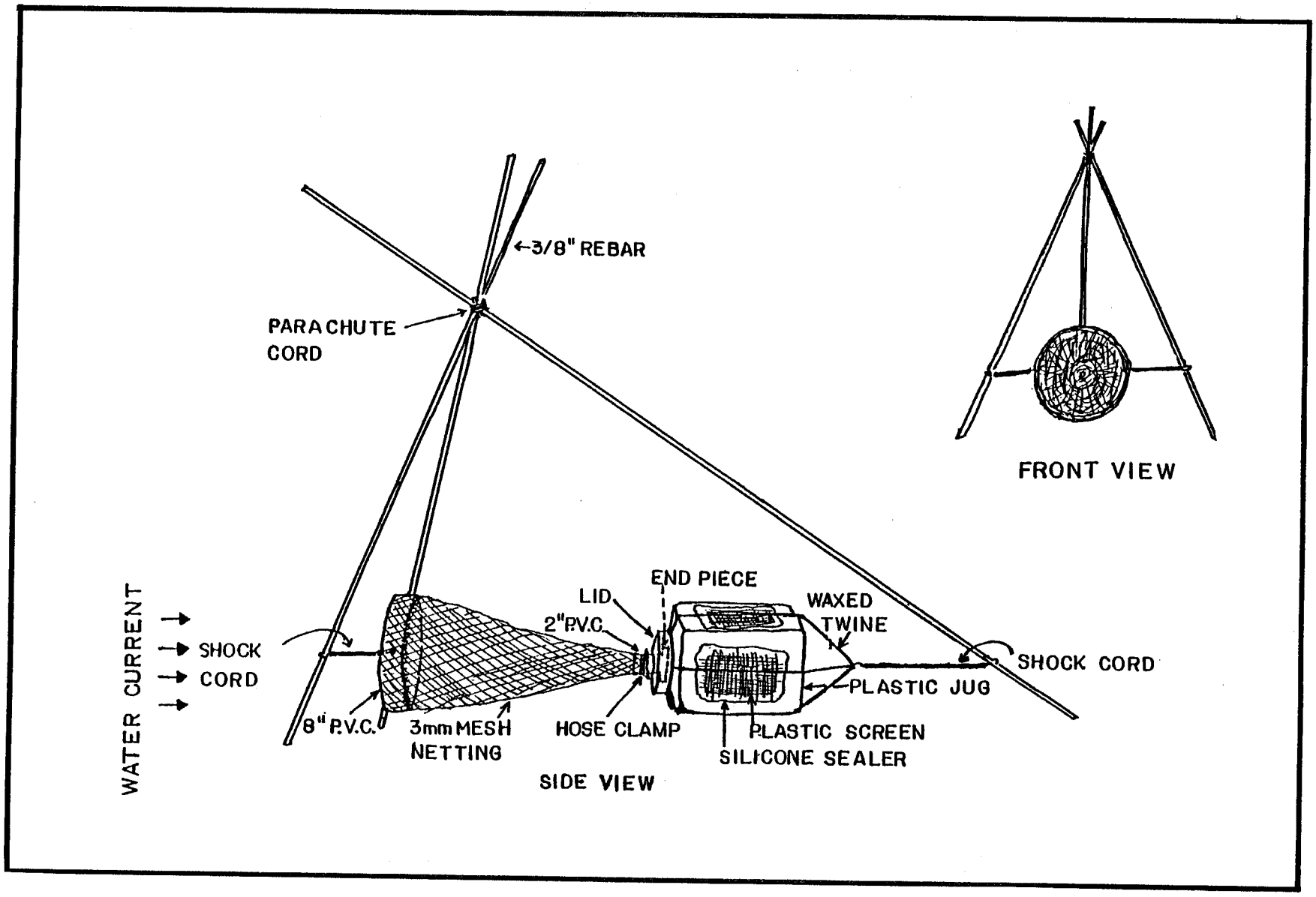
APPENDICES



Appendix 1. Design of fyke-net.

Appendix 2. Composition of staining solutions used for electrophoretic analysis of the enzymes routinely used in this study. Agar-overlay staining solutions, in which the stain ingredients were mixed with 10 ml of warm (60°C) 2% agar to increase resolution of banding patterns, are denoted by an "A".

Enzyme	MTT *PMS	Cofactor (5 mg)	Other Components
Aspartate aminotransferase			1.2 g tris, 200 mg L-aspartic acid, 110 mg ∝-ketoglutaric acid, 50 ml H_2O. Adjust to pH 8.0 with HCL 150 mg Fast Blue B salt
Aconitase	+	NADP	4 ml 1.0 M tris/HCL buffer(pH 8.0), 7 ml 0.2 M $MgCl_2$, 40 mg cis-aconitic acid, 3 units isocitric dehydrogenase, A
Adenosine deaminase	+		10 ml 0.05 M phosphate buffer(pH 7.5), 10 mg adenosine,.20 units nucleoside phosphorylase, .50 units xanthine oxidase, A
Alpha-glycerol-3-phosphate dehydrogenase	+	NAD	100 ml Ridgway gel buffer, 50 mg DL-alpha-glycerophosphate
Creatine kinase	+	NADP	1 ml 1.0 M tris/HCL buffer(pH 7.5),1 ml 0.2 M $MgCl_2$, 8 ml H_2O, 15 mg phosphocreatine, 30 mg adenosine diphosphate,35 mg ∝-D-glucose, 20 units G6PDH, 40 units hexokinase, A
Lactate dehydrogenase	+	NAD	100 ml Ridgway gel buffer,10 ml DL-Na-Lactate(pH7.0)
Malate dehydrogenase	+	NAD	100 ml Ridgway gel buffer,10 ml DL-Na-Malate(pH7.0)
Malic enzyme	+	NADP	100 ml Ridgway gel buffer,10 ml DL-Na-Malate(pH7.0),5 ml 0.2 M $MgCl_2$
Peptidase L-leucyl-L-leucine			.25 ml 1.0 M tris/HCL buffer(pH 8.0),.5 ml $MgCl_2$,9 ml H_2O,10 mg L-leucyl-L-leucine,2.5 mg snake venom L-amino acid oxidase,250 units peroxidase, 10 mg 3-amino-9-ethylcarbazole(in acetone) A
L-phenylalanyl-L-proline			.25 ml 1.0 M tris/HCL buffer(pH 7.5),.5 ml $MnCl_2$,9 ml H_2O,10 mg L-phenylalanyl-L-proline,2.5 mg snake venom L-amino acid oxidase, 200 units peroxidase,10 mg O-dianisidine DiHCL(in water), A
Phosphohexose isomerase	+	NADP	3 ml 0.1 M tris/HCL buffer(pH 8.0),1 ml 0.2 M $MgCl_2$,6 ml H_2O,10 mg D-fructose 6-phosphate, 20 units G6PDH, A
Phosphoglucomutase	+	NADP	100 ml Ridgway gel buffer, 5 ml 0.2 M $MgCl_2$,200 mg ∝-D-glucose-1-phosphate, 40 units G6PDH

-Continued-

Appendix 2. Composition of staining solutions used for electrophoretic analysis of the enzymes routinely used in this study. Agar-overlay staining solutions, in which the stain ingredients were mixed with 10 ml of warm (60°C) 2% agar to increase resolution of banding patterns, are denoted by an "A" (continued).

Enzyme	MTT *PMS	Cofactor (5 mg)	Other Components
6-Phosphogluconate dehydrogenase	+	NADP	100 ml Ridgway gel buffer, 5 ml 0.2 M $MgCl_2$,20 mg 6-phosphogluconic acid
Phosphomannose isomerase	+	NADP	2 ml 1 M tris/HCL buffer(pH 7.5),2 ml 0.2 M $MgCl_2$,6 ml H_2O, 10 mg D-mannose 6-phosphate, 50 units phosphohexose isomerase, 20 units G6PDH, A
Superoxide dismutase	+		100 ml Ridgway gel buffer

NAD β-diphosphopyridine nucleotide
NADP triphosphopyridine nucleotide
MTT [3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide]
PMS phenazine methosulfate
G6PDH glucose 6-phosphate dehydrogenase

* a "+" in this column indicates that both MTT(5 mg) and PMS(5 mg) were used in the staining solutions.

APPENDIX 3

Pink salmon used in breeding experiments performed in this study were collected from Auke Creek[1]. Adults used as breeders were killed early in the morning and their gametes were removed and immediately refrigerated. Tissue samples were taken from the gamete donors and were electrophoretically screened. Appropriate crosses were made that same evening, and eggs from each cross were incubated in separate incubator trays. Fry from the crosses were examined once they had reached the button-up stage.

Chi-square statistics were used to test for random segregation among phenotypes at each locus for every cross. Chi-square statistics described by Mather (1951) were used in appropriate crosses to test for joint segregation between pairs of loci. In some of the crosses one of the parents was considered to be homozygous at a locus even though it was heterozygous for a third allele, since this third allele did not added the calculation of joint segregation statistics (May et al. 1979).

To test for linkage in double backcross matings (AABBxAA'BB') the following formulat was used (from Mather 1951):

$$X^2 = (a_1 - a_2 - a_3 + a_4)2/N \qquad (df=1)$$

Where a_1 = observed AABB progeny

a_2 = observed AABB" progeny

a_3 = observed AA"BB progeny

a_4 = observed AA"BB" progeny

N = total number of progeny

Single backcross matings, in which both parents were heterozygous at one of the loci (AA'BB' x AA'BB), were also tested for linkage relationships. Heterozygous progeny at the locus for which both parents were heterozygous were excluded from this test. The chi-square test for joint segregation of loci from such a cross was (from May et al. 1978):

---

[1] Lot numbers beginning with 0 were spawned in the fall of 1979. All other lots were spawned in the fall of 1980.

$$X^2 = (a_1 - a_2 - a_3 + a_4)2/N \qquad (df=2)$$

Where a_1 = observed AABB progeny

a_2 = observed AABB" progeny

a_3 = observed AA"BB progeny

a_4 = observed AA"BB" progeny

N (informative $N = a_1 + a_2 + a_3 + a_4$

Since the linkage phase of the loci pairs was unknown, it was assumed that the smallest progeny class $(a1 + a4)$ or $(a2 + a3)$ represented non-parental genotypes. The proportion of non-parental genotypes (r) was then calculated as follows:

$$r = \frac{a_1 + a_4}{N} \text{ or } \frac{a_2 + a_3}{N}$$

$$S.E. = \sqrt{r\ (1-r)/N} \qquad \text{(May et al. 1979)}$$

Appendix 3.1A. Segregation at the Aat-3 locus. Chi-square values are listed for each cross.

Lot Number	Parental Phenotypes		N	Progeny Phenotypes Observed (Expected)			χ^2	df
	Male	Female		100/100	100/85	85/85		
2	100/100	100/85	50	22(25)	28(25)		0.72	1
11	100/100	100/85	140	76(70)	64(70)		1.03	1
019	100/100	100/85	50	22(25)	28(25)		0.72	1
6	100/100	85/85	114		114(114)		——	-
5	100/85	100/100	94	47(47)	47(47)		0.00	1
024	100/85	100/100	50	31(25)	19(25)		2.88	1
3	100/85	100/85	90	23(22.5)	48(45)	19(22.5)	0.76	2
9	100/85	100/85	56	20(14)	28(28)	8(14)	5.14	2
02	100/85	100/85	45	16(11.25)	22(22.5)	7(11.25)	4.02	2
12	85/85	100/100	100		100(100)		——	-
022	85/85	100/85	50		24(25)	26(25)	0.08	1

Appendix 3.1B. Segregation at the Acon-4 locus. Chi-square values are listed for each cross.

Lot Number	Parental Phenotypes		N	Progeny Phenotypes Observed (Expected)		X^2	df
	Male	Female		100/100	100/85		
12	100/85	100/100	143	81(71.5)	62(71.5)	2.52	1

Appendix 3.1C. Segregation at the Ada-2 locus. Chi-square values are listed for each cross.

Lot Number	Parental Phenotypes		N	Progeny Phenotypes Observed (Expected)			X^2	df
	Male	Female		100/100	100/87	100/113		
3	100/100	100/87	91	50(45.5)	41(45.5)		0.89	1
5	100/100	100/113	88	40(44)		48(44)	0.73	1
018	100/100	100/87	48	27(24)	21(24)		0.75	1
2	100/87	100/100	50	29(25)	21(25)		1.28	1
024	100/87	100/100	50	29(25)	21(25)		1.28	1

Appendix 3.1D. Segregation at the Agp locus. Chi-square values are listed for each cross.

Lot Number	Parental Phenotypes		N	Progeny Phenotypes Observed (Expected)			X^2	df
	Male	Female		100/100	100/200	200/200		
12	200/200	100/200	98		43(49)	55(49)	1.47	1
02	100/100	100/200	49	24(24.5)	25(24.5)		0.02	1
018	100/100	100/200	50	25(25)	25(25)		0.00	1
023	100/200	100/100	43	22(21.5)	21(21.5)		0.02	1

Appendix 3.1E. Segregation at the L1-1 locus. Chi-square values are listed for each cross.

Lot Number	Parental Phenotypes		N	Progeny Phenotypes Observed (Expected)			X^2	df
	Male	Female		100/100	100/117	100/83		
6	100/117	100/100	112	49(56)	63(56)		1.75	1
11	100/83	100/83	140	39(35)	64(70)	37(35)	1.09	2
022	100/100	100/83	50	28(25)		22(25)	0.72	1
019	100/100	100/83	48	23(24)		25(24)	0.08	1

Appendix 3.1F. Segregation at the Me-1 locus. Chi-square values are listed for each cross.

Lot Number	Parental Phenotypes		N	Progeny Phenotypes Observed (Expected)			X^2	df
	Male	Female		100/100	100/130	130/130		
2	100/100	100/130	49	23(24.5)	26(24.5)		0.18	1
3	100/100	100/130	92	48(46)	44(46)		0.17	1
5	100/130	100/100	86	45(43)	41(43)		0.19	1
6	100/130	100/130	114	28(28.5)	60(57)	26(28.5)	0.39	2
019	100/100	100/100	50	50(50)			——	-
023	100/100	100/100	50	50(50)			——	-

Appendix 3.1G. Segregation at the Pgm locus. Chi-square values are listed for each cross.

Lot Number	Parental Phenotypes		N	Progeny Phenotypes Observed (Expected)		X^2	df
	Male	Female		100/100	100/150		
9	100/150	100/100	39	25(19.5)	14(19.5)	3.10	1
019	100/100	100/100	50	50(50)		——	-
015	100/150	100/100	80	47(40)	33(40)	2.45	1

Appendix 3.1H. Segregation at Phi-1,2 loci. Chi-square values are listed for each cross.

Lot Number	Parental Phenotypes		N	Progeny Phenotypes Observed (Expected)			X^2	df
	Male	Female		100/100 100/100	100/100 100/33	100/100 100/200		
6	100/100 100/33	100/100 100/100	351	171(175.5)	180(175.5)		0.23	1
11	100/100 100/33	100/100 100/100	369	192(184.5)	177(184.5)		0.61	1
5	100/100 100/200	100/100 100/100	94	47(47)		47(47)	0.00	1
8	100/100 100/200	100/100 100/100	24	15(12)		9(12)	1.50	1

Appendix 3.1I. Segregation at the Pp-2 locus. Chi-square values are listed for each cross.

Lot Number	Parental Phenotypes		N	Progeny Phenotypes Observed (Expected)						X^2	df
	Male	Female		100/100	100/109	109/109	100/93	109/93	93/93		
5	100/100	100/109	72	36(36)	36(36)					0.00	1
015	100/100	100/109	79	38(39.5)	41(39.5)					0.11	1
3	100/100	100/93	91	43(45.5)			48(45.5)			0.27	1
019	100/100	100/93	50	24(25)			26(25)			0.08	1
6	100/93	100/100	351	171(175.5)			180(175.5)			0.23	1
02	100/93	100/100	50	28(25)			22(25)			0.72	1
8	100/109	100/93	24	8(6)	6(6)		3(6)	7(6)		2.34	3
024	100/109	109/93	48		12(12)	8(12)	14(12)	14(12)		2.00	3
025	100/109	100/109	48	17(12)	24(24)	7(12)				4.17	2
11	109/93	100/109	369		106(92.25)	86(92.25)	93(92.25)	84(92.25)		0.59	3
12	109/93	100/93	96		20(24)		33(24)	24(24)	19(24)	5.08	3
023	109/109	109/93	49			22(24.5)		27(24.5)		0.51	1
2	93/93	100/100	50				50(50)			——	-

Appendix 3.1J. Segregation at the 6pg locus. Chi-square values are listed for each cross.

Lot Number	Parental Phenotypes		N	Progeny Phenotypes Observed (Expected)			X^2	df
	Male	Female		100/100	100/90	100/95		
12	100/90	100/100	185	89(92.5)	96(92.5)		0.26	1
018	100/90	100/100	50	23(25)	27(25)		0.32	1
022	100/95	100/100	50	25(25)		25(25)	0.00	1

Appendix 3.2. Joint segregation of various loci of pink salmon. Results of chi-square tests of joint segregation.

Lot Number	N	Female	Female	Male	Male	Number of phenotypes in progeny						x^2	r	2 S.E.
						AA BB	AA' BB	AA BB'	AA' BB'	A'A' BB	A'A' BB'			
12	136	Acon-4 100/100=AA	6pg 100/100=BB	Acon-4 100/115=AA'	6pg 100/90=BB'	47	31	19	39			9.53**	.37	.08
3	42	Aat-3 100/85=AA'	Ada-2 100/87=BB'	Aat-3 100/85=AA'	Ada-2 100/100=BB	11	22	12	26	13	6	1.52	.40	.15
024	50	Ada-2 100/100=AA	Aat-3 100/100=BB	Ada-2 100/87=AA'	Aat-3 100/85=BB'	20	12	11	7			0.32	.46	.14
3	91	Ada-2 100/87=AA'	Me-1 100/130=BB'	Ada-2 100/100=AA	Me-1 100/100=BB	22	25	28	16			2.47	.42	.10
3	89	Ada-2 100/87=AA'	Pp-2 100/93=BB'	Ada-2 100/100=AA	Pp-2 100/100=BB	25	17	23	24			0.91	.45	.11
5	70	Ada-2 100/87=AA'	Pp-2 100/109=BB'	Ada-2 100/100=AA	Pp-2 100/100=BB	15	20	15	20			0.00	.50	.12
018	48	Ada-2 100/87=AA'	Agp 100/200=BB'	Ada-2 100/100=AA	Agp 100/100=BB	15	12	9	12			0.75	.44	.14
12	39	Pp-2 100/93=AA'	Agp 200/100=BB'	Pp-2 109/93=AA'	Agp 200/200=BB	16	27	4	30	11	8	2.08	.38	.16
6	54	Me-1 100/130=AA'	Ll-1 100/100=BB	Me-1 100/130=AA'	Ll-1 100/83=BB'	17	22	11	36	10	16	2.67	.39	.13
6	112	Ll-1 100/100=AA	Pp-2 100/100=BB	Ll-1 100/83=AA'	Pp-2 100/93=BB'	18	30	31	33			0.89	.46	.09
11	76	Ll-1 100/117=AA'	Pp-2 100/109=BB	Ll-1 100/117=AA'	Pp-2 109/93=BB'	22	36	17	28	18	19	0.47	.46	.11
019	48	Ll-1 100/117=AA'	Pp-2 100/93=BB'	Ll-1 100/100=AA	Pp-2 100/100=BB	12	11	11	14			0.33	.46	.14

*.01<p<.05
**.001<p<.01

-Continued-

Appendix 3.2. Joint segregation of various loci of pink salmon. Results of chi-square tests of joint segregation (continued).

Lot Number	N	Female	Female	Male	Male	Number of phenotypes in progeny						x^2	r	2 S.E.
						AA BB	AA' BB	AA BB'	AA' BB'	A'A' BB	A'A' BB'			
3	91	Me-1 100/130=AA'	Pp-2 100/93=BB'	Me-1 100/100=AA	Pp-2 100/100=BB	21	22	26	22			0.27	.47	.10
6	54	Me-1 100/130=AA'	Pp-2 100/100=BB	Me-1 100/130=AA'	Pp-2 100/93=BB'	10	29	18	31	10	16	0.07	.48	.14
12	39	Pp-2 100/93=AA'	6pg 100/100=BB	Pp-2 109/93=AA'	6pg 100/90=BB'	9	26	11	31	8	11	0.03	.49	.16
11	76	Ll-1 100/117=AA'	Aat-3 100/85=BB'	Ll-1 100/117=AA'	Aat-3 100/100=BB	22	30	17	34	24	13	0.47	.46	.11
019	48	Aat-3 100/85=AA'	Ll-1 100/117=BB'	Aat-3 100/100=AA	Ll-1 100/100=BB	11	12	10	15			0.33	.46	.14
022	50	Aat-3 85/100=AA'	Ll-1 100/117=BB'	Aat-3 85/85=AA	Ll-1 100/100=BB	15	13	11	11			0.08	.48	.14
3	42	Aat-3 100/85=AA'	Me-1 100/130=BB'	Aat-3 100/85=AA'	Me-1 100/100=BB	16	21	7	27	10	9	1.51	.40	.15
5	86	Aat-3 100/100=AA	Me-1 100/100=BB	Aat-3 100/85=AA'	Me-1 100/130=BB'	24	21	19	22			0.42	.47	.11
3	41	Aat-3 100/85=AA'	Pp-2 100/93=BB'	Aat-3 100/85=AA'	Pp-2 100/100=BB	10	24	13	24	9	9	0.22	.46	.16
11	61	Pp-2 100/109=AA'	Aat-3 100/85=BB'	Pp-2 93/109=AA'	Aat-3 100/100=BB	16	50	12	30	11	22	3.69	.38	.12
019	50	Aat-3 100/85=AA'	Pp-2 100/93=BB'	Aat-3 100/100=AA	Pp-2 100/100=BB	14	10	8	18			3.92*	.36	.14

*.01<p<.05
**.001<p<.01

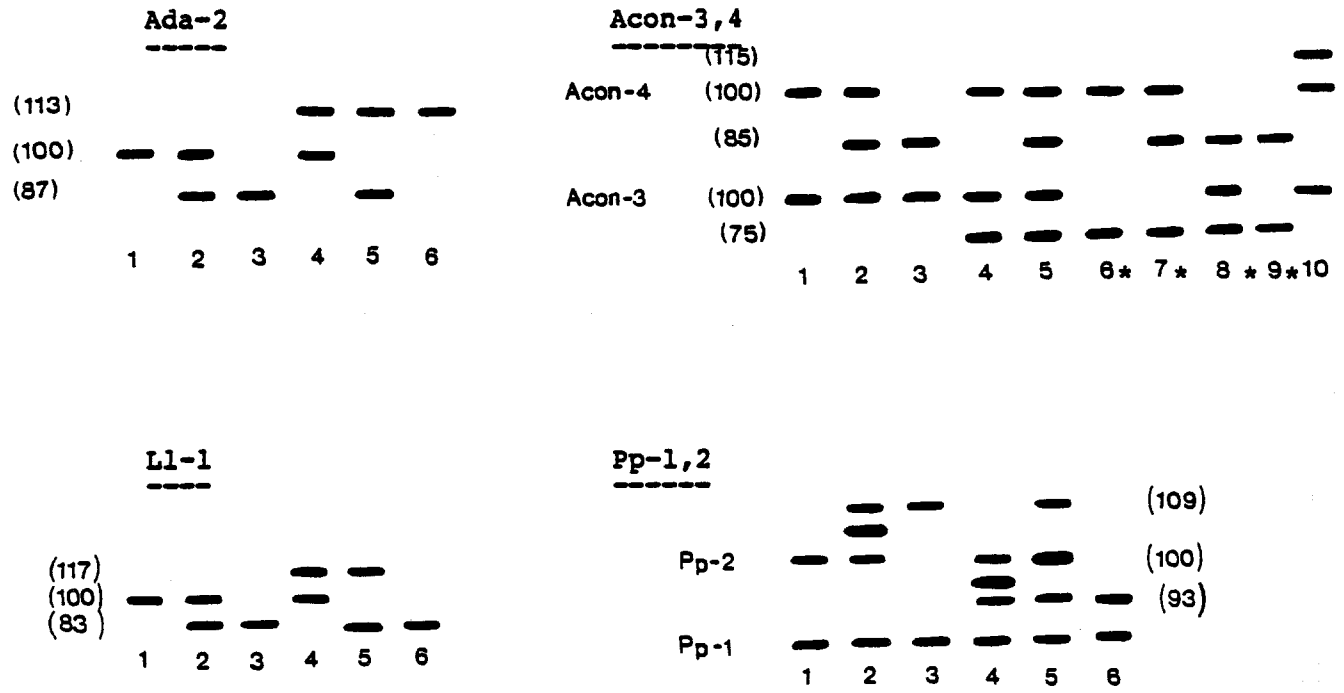
-Continued-

Appendix 3.2. Joint segregation of various loci of pink salmon. Results of chi-square tests of joint segregation (continued).

Lot Number	N	Female	Female	Male	Male	Number of phenotypes in progeny						X^2	r	2 S.E.
						AA AA BB	AA AA BB'	AA AA B'B'	AA AA' BB	AA AA' BB'	AA AA' B'B'			
5	94	Phi-1,2 100/100/100/100=AAAA	Aat-3 100/100=BB	Phi-1,2 100/100/100/200=AAAA'	Aat-3 100/85=BB'	25	22		22	25		0.38	.47	.10
6	112	Phi-1,2 100/100/100/100=AAAA	Ll-1 100/100=BB	Phi-1,2 100/100/100/33=AAAA'	Ll-2 100/83=BB'	18	30		31	33		0.89	.46	.09
11	76	Phi-1,2 100/100/100/100=AAAA	Ll-1 100/117=BB'	Phi-1,2 100/100/100/33=AAAA'	Ll-1 100/117=BB'	22	36	18	17	28	19	0.47	.46	.11
6	62	Phi-1,2 100/100/100/100=AAAA	Me-1 100/130=BB'	Phi-1,2 100/100/100/33=AAAA'	Me-1 100/130=BB'	9	18	9	29	31	15	3.16	.39	.12
6	351	Phi-1,2 100/100/100/100=AAAA	Pp-2 100/100=BB	Phi-1,2 100/100/100/33=AAAA'	Pp-2 100/93=BB'	171	0		0	180		351.00***	.00	.00
8	24	Phi-1,2 100/100/100/100=AAAA	Pp-2 100/93=BB	Phi-1,2 100/100/100/200=AAAA'	Pp-2 100/109=BB'	7	8		4	5		0.00	.50	.20
11	369	Phi-1,2 100/100/100/100=AAAA	Pp-2 100/109=BB	Phi-1,2 100/100/100/33=AAAA'	Pp-2 100/93=BB'	192	0		0	177		369.00***	.00	.00

*.01<p<.05
**.001<p<.01
***p<.001

Appendix 4. Electrophoretic patterns of protein variants at loci previously unreported for pink salmon.



*patterns not actually observed

Other previously unreported alleles

Locus	Allele
Agp	65,175
Ck-1	80,120
Me-1	70
Phi-1,2	-33,33,200
Phi-3	90
6pg	110