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Title: BIOCHEMICAL GENETIC VARIATION AMONG SELECTED POPULATIONS OF CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) IN OREGON AND WASHINGTON

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John D. McIntyre

Biochemical variation in 12 different chinook populations, sampled from 10 hatcheries along the Columbia River and the Oregon coast, was studied with starch gel electrophoresis. An index was used to describe the genetic differences between pairs of populations. Differences were observed between spring and fall chinook and between Columbia River and Oregon coastal populations. Variation in inbreeding coefficients, calculated at four polymorphic loci, indicated that natural selection may alter the frequency of certain phenotypes.

Biochemical Genetic Variation among Selected Populations of Chinook Salmon (*Oncorhynchus tshawytscha*) in Oregon and Washington

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INTRODUCTION

Variation in life-cycle characteristics, behavior, and morphology among populations of chinook salmon (Oncorhynchus tschawytscha) has been described (Ricker, 1972). Within a single stream system, distinct runs of chinook salmon, identified by relatively specific spawning times, may be present. In addition, salmonid populations tend to be reproductively isolated because they usually return to their native streams to spawn. Selective responses to environmental pressures acting on isolated populations would be expected to produce different gene frequencies among these groups.

Characterization of the genetic variation among chinook salmon populations is possible through electrophoretic separation of proteins and subsequent histochemical staining (de Ligny, 1969; Utter et al., 1973; Ayala et al., 1974). The technical simplicity of electrophoretic analysis readily permits the scanning of large numbers of organisms and the subsequent comparison of different populations based on the isozyme variation among codominantly expressed alleles. Electrophoretic studies with other salmonid populations have revealed the existence of polymorphic genetic systems in steelhead trout (Salmo

gairdneri) (Allendorf, 1973), pink salmon (O. gorbuscha) (Aspinwall, 1974), sockeye salmon (O. nerka) and coho salmon (O. kisutch) (Utter et al., 1973). The objective of this study was to describe and compare the biochemical genetic variation for populations of chinook salmon from the Columbia River and Oregon coastal streams.

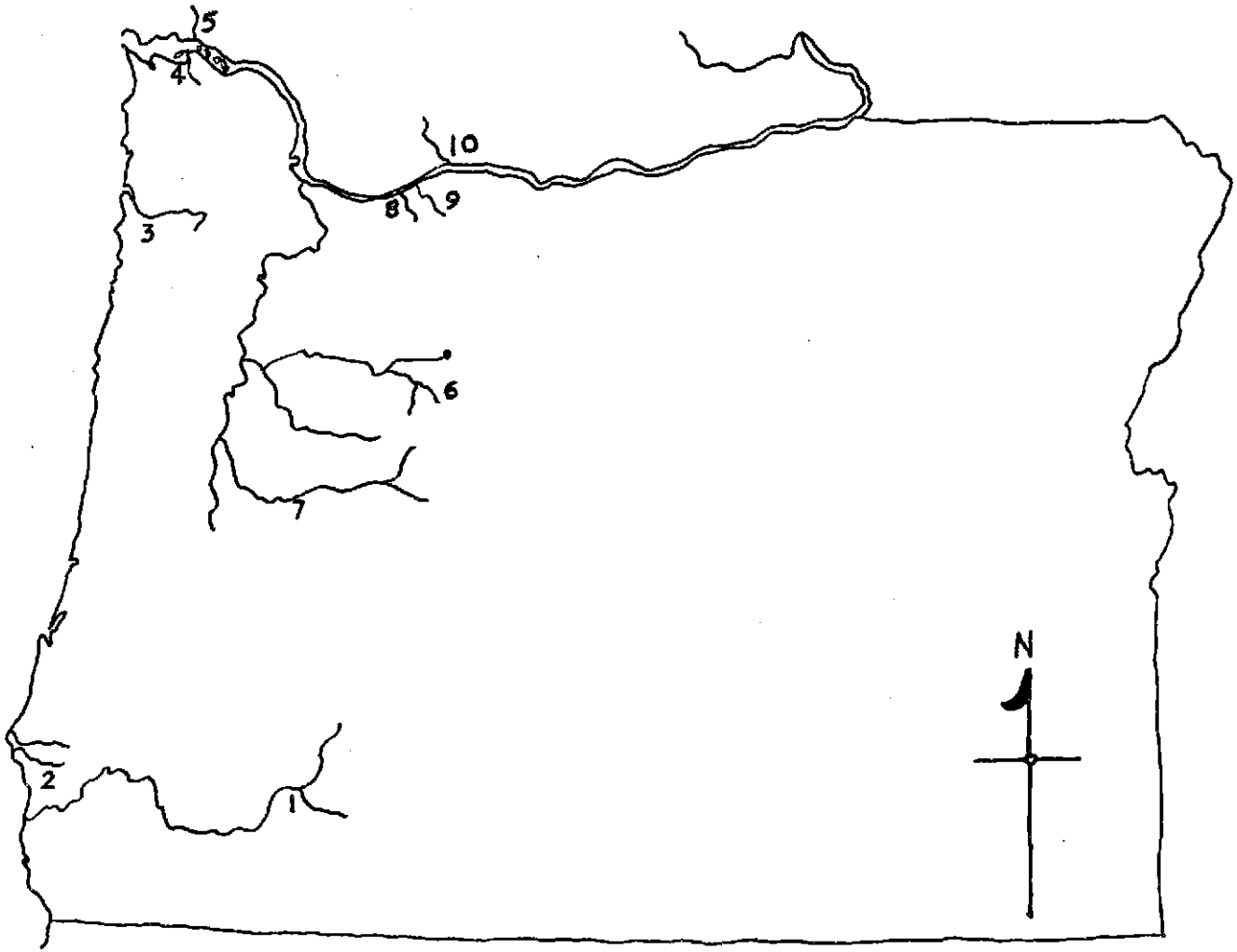
MATERIALS AND METHODS

Samples of juvenile chinook salmon were collected from 10 hatcheries along the Columbia River and the Oregon coast in 1974 (Fig. 1). Samples from both spring and fall races of chinook were obtained from the Trask River and Little White Salmon hatcheries. Tissue samples from adult fall chinook salmon were obtained in 1973 and 1974 from the Abernathy Salmon Cultural Development Center.

Preparation of samples and buffers for starch gel electrophoresis was as described by Utter et al. (1974). Initial analyses were performed on blood plasma, liver, white muscle, eye, and heart tissues. Heparnized hematocrit tubes were used to collect blood from the caudal artery. Samples were immediately centrifuged until the plasma separated from the red blood cells. Tissue extracts were ground in equal parts of distilled water just prior to electrophoresis. All samples were stored on ice in the field and then stored at -12 C. Tissue samples and whole juvenile fish could be frozen up to 6 mo with no significant loss of resolution for most enzyme systems; however, the clearest banding patterns were always obtained with fresh samples.

Three combinations of gel and electrode buffers were used in this study: A) electrode buffer--0.06 M LiOH, 0.3 M H_3BO_4, pH 8.3. Gel buffer--0.03 M tris, 0.005 M citric acid, 10 ml/l total solution

Fig. 1. Hatcheries from which chinook salmon were sampled for starch gel electrophoretic analyses. 1. Cole Rivers hatchery, Rogue River; 2. Elk River hatchery, Elk River; 3. Trask River hatchery, Trask River; 4. Big Creek hatchery, Big Creek; 5. Abernathy Salmon Cultural Development Center, Abernathy Creek; 6. Marion Forks hatchery, North Santiam River; 7. McKenzie River hatchery, McKenzie River; 8, Bonneville hatchery, Tanner Creek; 9. Oxbow hatchery, Herman Creek; 10. Little White Salmon hatchery, Little White Salmon River.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electrode buffer, pH 8.0 (Ridgway et al., 1970); B) electrode buffer--0.1 M Na_2HPO_4 with pH adjusted to 6.5 with 0.1 M NaH_2PO_4. Gel buffer--1:10 dilution of electrode buffer, pH 6.5 (Wolf et al., as cited by Allendorf, 1973); C) electrode buffer--0.04 M citric acid adjusted to pH 6.1 with N-(3-Aminopropyl)-morpholine. Gel buffer--1:20 dilution of electrode buffer, pH 6.1 (Clayton and Tretiak, 1972).

Two types of hydrolyzed starch were used in gel preparation. Electrostarch (Electrostarch Co., Box 1294, Madison, Wisconsin 53701) was used for most enzyme assays. Connaught starch (Fisher Scientific Co.) produced optimal resolution for tetrazolium oxidase. A 12.1% (w/v) solution of Connaught starch or a 10.4% (w/v) solution of Electrostarch in a gel buffer was heated with vigorous swirling just to the boiling point in a side-arm flask. The starch solution was degassed with an aspirator and poured into an open-faced 22.5 cm x 15 cm plexiglass mold. The total volume of starch solution used was approximately 380 ml per gel. After 10 min, the gel was covered with plastic wrap and allowed to thoroughly cool.

Methods described by Ayala et al. (1972) were used for electrophoretic separation of proteins. Electrostarch gels were sliced into four or five slices with a taut 0.2 mm wire using plexiglass strips as guides. The Connaught starch gels were sliced into thirds. The top slice on all gels was discarded because it was found to distort the banding patterns.

Fourteen proteins were assayed using methods described by Shaw and Prasad (1970) and Johnson et al. (1972) with minor modifications. Electrophoresis proceeded at 50-75 ma with a maximum voltage of 300 V for 3-5 hrs. A dye marker, inserted adjacent to the tissue extracts, indicated when migration was complete at 8-10 cm from the origin. When required, phenazine methosulfate (PMS) was added just prior to application of the stain to the gel.

Proteins assayed, buffers, tissue sources, and stains used were:

Alcohol dehydrogenase (ADH): Buffer A. Liver tissue. Stain: 50 mg nicotinamide adenine dinucleotide (NAD), 30 mg nitro blue tetrazolium (NBT), 3 ml 95% ethanol, 5 ml 0.1 M KCN, 2 mg PMS in 100 ml 0.05 M tris-HCl (pH 7.1).

Aspartate aminotransferase (AAT): Buffer A. White muscle. Substrate: 0.8 g α-ketoglutaric acid, 2.7 g DL-aspartic acid, 28.4 g NaH_2PO_4, 10.0 g polyvinyl pyrrolidone, 1.0 g NaEDTA. Stain: 500 mg fast garnet GBC salt in 100 ml substrate solution.

Esterase (EST): Buffer A. Liver or serum. Stain: 50 mg α-napthyl acetate, 50 mg β-napthyl acetate dissolved in 3 ml acetone. Add: 100 mg fast blue RR salt in 100 ml 0.05 M tris-HCl (pH 7.1).

Glucose phosphate isomerase (GPI): Buffer A. White muscle. Stain: 10 mg nicotinamide adenine dinucleotide phosphate (NADP), 80 mg fructose 6-phosphate, 15 mg MTT tetrazolium, 1 mg PMS in

100 ml 0.05 M tris-HCl (pH 7.1). Adjust pH to 8.5 with 40% NaOH.

Hexokinase (HK): Buffer A. Liver tissue. Stain: 90 mg glucose, 21 mg $MgCl_2$, 25 mg ATP, 25 mg NADP, 20 mg NBT, 80 units glucose-6-phosphate dehydrogenase (G6PDH), 3 mg PMS in 100 ml 0.05 M tris-HCl (pH 7.1).

Isocitrate dehydrogenase (IDH): Buffer B. White muscle. Stain: 20 mg NBT, 10 mg NADP, 60 mg DL-Na-isocitrate, 50 mg $MgCl_2$, 5 mg PMS in 100 ml 0.05 M tris-HCl (pH 7.1). Adjust pH to 8.5 with 40% NaOH.

Lactate dehydrogenase (LDH): Buffer A. Liver or eye tissue. Stain: 30 mg NAD, 20 mg NBT, 1 ml lactic acid, 5 ml 0.1 M KCN, 2 mg PMS in 0.05 M tris-HCl (pH 7.1). Adjust pH to 7.5 with 40% NaOH.

Malate dehydrogenase (MDH): Buffer C. White muscle tissue. Stain: 20 mg MTT tetrazolium, 30 mg NAD, 0.70 g malic acid, 5 ml 0.1 M KCN, 2 mg PMS in 100 ml 0.05 M tris-HCl (pH 7.1). Adjust pH to 7.1 with 40% NaOH.

Malic enzyme (ME): Buffer B. Liver and white muscle. Stain: 10 mg NBT, 5 mg NADP, 200 mg malic acid, 50 mg $MgCl_2$, 10 mg PMS in 100 ml 0.05 M tris-HCl (pH 7.1). Adjust pH to 8.5 with 40% NaOH.

Phosphoglucomutase (PGM): Buffer A. White muscle tissue. Stain: 600 mg Na_2 glucose-1-phosphate·$4H_2O$, 200 mg $MgCl_2$, 10 mg

NADP, 80 units G6PDH, 20 mg NBT, 1 mg PMS in 100 ml 0.05 M tris-HCl (pH 7.1).

Sorbitol dehydrogenase (SDH): Buffer A. Liver tissue. Stain: 10 mg NAD, 25 mg MTT tetrazolium, 0.5 g sorbitol, 2 mg PMS in 0.05 M tris-HCl (pH 7.1). Adjust pH to 8.5 with 40% NaOH.

Tetrazolium oxidase (TO): Buffer C. Liver tissue. Stain: 35 mg MTT tetrazolium, 5 mg PMS in 100 ml 0.05 M tris-HCl (pH 7.1). Adjust pH to 8.5 with 40% NaOH.

Transferrin (TFN): Buffer A. Serum. Stain: 1 mg buffalo black in 100 ml destain solution (10% acetic acid:40% methanol:50% distilled water).

Xanthine dehydrogenase (XDH): Buffer A. Liver and white muscle tissues. Stain: 0.41 g hypoxanthine in 2.4 ml H_2O and 0.6 ml KOH. Add: 50 mg NAD, 30 mg NBT, 2 mg PMS in 0.05 M tris-HCl (pH 7.1). Adjust pH to 7.1 with 1 N HCl.

Following electrophoresis, most gels were held in the dark at 37 C until bands appeared. TO was developed under a fluorescent light. TFN was left in the staining solution for 15 min and then destained. EST and AAT were both held in the staining solution until clear resolution of the bands was attained.

All gels were destained and preserved in a 1:4:5 solution of acetic acid:methanol:distilled water. Photographs of the gels were made for future reference.

All breeding experiments were performed with fall chinook salmon at the Abernathy Salmon Cultural Development Center (Longview, Washington) in conjunction with an experiment currently in progress at the Center. Adults were sampled at the time of spawning. Liver and white muscle tissue samples were obtained from adults used as parents of the 1973 and 1974 brood. Samples of heart tissue also were collected from the parents of the 1973 brood. Offspring from each mating made for the Center's study were marked with family-specific coded-wire tags. The analysis of tissues from the juveniles permitted comparisons of isozyme frequencies in offspring from specific matings with expected frequencies based on the parental types.

RESULTS

Many of the enzyme systems assayed were monomorphic in the populations studied. Polymorphism was observed for six enzyme systems: TO, MDH, LDH, PGM, SDH, and GPI. Banding patterns on gels stained for SDH and GPI presented interpretive problems but variant types were noted.

Three anodally migrating forms of TO were observed. Utter (1971) described TO as a dimeric molecule with two segregating alleles producing three phonotypes. This model was corroborated in this study. Frequencies of isozymic phenotypes for the juveniles were compared with expected frequencies predicted from parental phenotypes (Table 1). The nomenclature proposed by Utter et al. (1973) was used, with the E-allele representing the slow-migrating form.

MDH polymorphism in salmonids was first described by Bailey et al. (1970). According to their model, MDH is a dimeric protein coded by two duplicated loci. Polymorphism in chinook has been only detected at the high-mobility B-locus. The variant, high-mobility allele, B', was present in most populations studied. Intensity differences were used to distinguish the variant homo- and hetero-dimeric forms. Results of breeding studies conformed to the Bailey et al. (1970) model (Table 2). A previously undescribed slow-mobility variant was detected in one sample from the Rogue River population.

Table 1. TO phenotypes of parents and offspring in breeding experiments. Values in parentheses represent expected numbers assuming mendelian inheritance.

Phenotypes of Parents		Progeny Phenotypes		
Male	Female	EE	EF	FF
EF	FF	0 (0)	7 (9.5)	11 (9.5)
EF	EE	18 (13.5)	9 (13.5)	0 (0)
EF	EF	2 (2.75)	7 (5.5)	2 (2.75)
EE	EE	15 (16)	1 (0)	0 (0)
FF	FF	0 (0)	0 (0)	20 (20)

Table 2. MDH phenotypes of parents and offspring in breeding experiments. Values in parentheses represent expected numbers assuming mendelian inheritance.

Phenotypes of Parents		Progeny Phenotypes		
Male	Female	BB	BB'	B'B'
BB	B'B'	0 (0)	50 (50)	0 (0)
BB	BB'	27 (25)	23 (25)	0 (0)
BB'	BB'	16 (12.5)	24 (25)	10 (12.5)
BB	BB	49 (49)	0 (0)	0 (0)

Due to its extremely low frequency, this variant was omitted from the analysis.

LDH has been described as a multiple locus tetrameric molecule in other salmonids (Wright and Atherton, 1970; Utter et al., 1973). The banding patterns observed in this study conformed to Wright and Atherton's (1970) model with variability limited to the B_1 locus (Fig. 2). The common homotetrameric B_1 allele corresponded in mobility to the B_1 allele in steelhead trout. Three previously undescribed heterotetrameric forms were found among southern coastal populations and in a single Columbia River population. They were designated B_1B_1', B_1B_1'', B_1B_1''' (Fig. 2) as suggested by Wright (personal communication). No variant homotetrameric forms were found due to the low frequency of each variant allele. Because variant alleles were observed in such low frequencies ($\leq .03$), polymorphism was recorded as being present or absent. Although adults with variant phenotypes were not available for breeding, experiments with LDH isozymes in other salmonids (Wright and Atherton, 1970; Allendorf, 1973) and adherence of the observed patterns to Hardy-Weinberg equilibrium (Table 3) supported the hypothesized model.

PGM polymorphism has been described in rainbow trout and sockeye salmon (Roberts et al., 1969; Utter and Hodgins, 1970). Of the two common bands, C and D, observed in chinook, the C band was present in all samples (Fig. 3). Two variant alleles have been

Figure 2. Diagrammatic representation of the LDH variability in chinook salmon. a, common homozygote B_1B_1; b, B_1B_1'; c, B_1B_1''; d, B_1B_1'''.

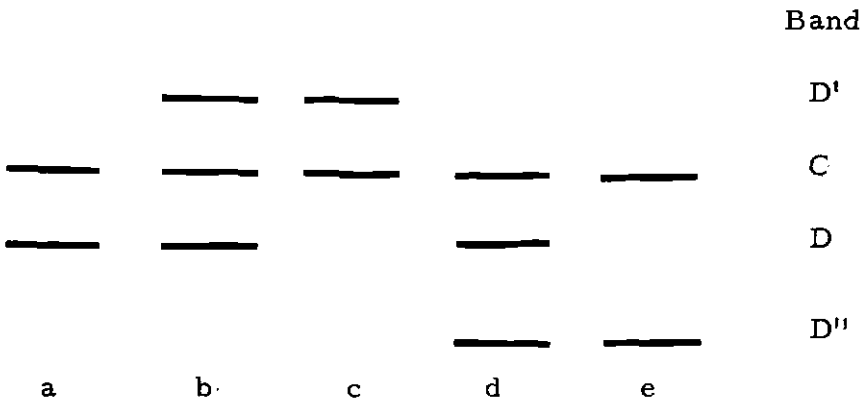

Figure 3. Diagrammatic representation of the PGM variability in chinook salmon. a, common homozygote DD; b, DD'; c, D'D'; d, DD''; e, D''D''.

Table 3. Observed and expected (in parentheses) values based on Hardy-Weinberg distribution of LDH phenotypes in polymorphic populations of chinook salmon.

Sample Source	LDH Phenotypes			
	BB	BB'	BB''	BB'''
Rogue River	112 (112)	2 (2)	0 (0)	3 (3)
Elk River	113 (113)	0 (0)	0 (0)	2 (2)
Little White Salmon (spring run)	124 (124)	0 (0)	3 (3)	0 (0)

designated D' and D''. The low mobility D'' allele was limited to spring chinook from the Trask River. Because polymorphic adults were not available for breeding, it was assumed that the PGM system in chinook salmon corresponded to the monomeric system described for sockeye salmon by Utter and Hodgins (1970). Deviations from equilibrium were generally associated with small sample sizes.

Utter et al. (1973) described SDH in chinook salmon as a two-locus tetrameric molecule with variability at the slow locus. Difficulty in obtaining satisfactory resolution in the present study prevented substantiation of their findings. Due to this difficulty in interpretation, SDH variability was recorded as present or absent.

Banding patterns for GPI were well-defined; however, the number of bands indicated a complex system of inheritance. The absence of variant types among the Abernathy fish prevented a breeding study. A duplicated, multi-locus system, similar to that proposed by Schmidkte et al. (1975) in salmonid and cyprinid fishes, may explain

the observed banding pattern. Variability was recorded only as present or absent.

Variance for a binomial distribution with two alleles is defined by Kempthorne (1969) as $\sigma^2 = \frac{pq}{2N}$ where p is the frequency of one allele, q or (1-p) is the frequency of the other allele, and N is the sample size. The 95% confidence intervals for the gene frequencies in each population (Table 4) were calculated as: the gene frequency $\pm$ 1.96 σ; where σ represents the standard deviation. In populations where more than two alleles were present at a single locus, the low frequency alleles were pooled as 1-p to calculate σ.

Higher frequencies of the TO E-allele were generally observed among spring populations than among fall populations (Table 4) with the exceptions of Elk River and Trask River fall chinook. Variability among populations at the MDH locus (Table 4) indicated that a cline may exist along the coast. A decrease in latitude along the coast corresponded to a decrease in variability at this locus. Among the coastal populations sampled, the southernmost sample site, the Rogue River, was the most polymorphic. This cline may continue north from the Columbia River as Utter (personal communication) has detected only negligible variation among chinook salmon from the Washington coast and Puget Sound.

Polymorphism at the PGM locus was restricted to coastal populations with the exception of the McKenzie River (Table 4).

Table 4. Allelic and phenotypic frequencies at TO, MDH, and PGM loci with associated 95% confidence intervals and qualitative assessment of variability at LDH, SDH, and GPI loci in 12 hatchery populations of chinook salmon.

Enzyme		Rogue River (Spring Run)	Elk River (Fall Run)	Trask River (Spring Run)	Trask River (Fall Run)	Big Creek (Fall Run)	Abernathy Creek (Fall Run)	Marion Forks (Spring Run)	McKenzie River (Spring Run)	Bonneville (Fall Run)	Oxbow (Fall Run)	Little White Salmon (Spring Run)	Little White Salmon (Fall Run)
TO	Sample Size	115	114	109	85	84	62	100	108	99	101	124	137
	EE	.57	.42	.75	.82	.30	.29	.72	.71	.25	.23	.60	.30
	EF	.33	.49	.23	.17	.48	.40	.24	.29	.57	.53	.34	.49
	FF	.10	.09	.02	.01	.23	.31	.04	.00	.18	.24	.06	.21
	E	.74	.67	.87	.90	.54	.49	.84	.86	.54	.50	.77	.54
	1.96 σ	.06	.06	.05	.04	.08	.09	.05	.05	.07	.07	.06	.06
MDH	Sample Size	171	116	113	87	102	63	98	106	102	95	127	161
	BB	.93	.90	.99	1.0	.75	.76	.72	.73	.80	.83	.88	.71
	BB'	.05	.09	.01	.00	.20	.14	.23	.18	.17	.16	.11	.26
	B'B'	.02	.01	.00	.00	.05	.10	.05	.09	.03	.01	.01	.02
	B	.96	.94	.99	1.0	.85	.83	.83	.82	.88	.91	.94	.85
	1.96 σ	.02	.03	.01	---	.05	.07	.05	.05	.06	.04	.03	.04
PGM	Sample Size	No Results	114	113	82	67	63	109	108	102	66	107	71
	DD		.81	.77	.44	1.0	1.0	1.0	.99	1.0	1.0	1.0	1.0
	DD'		.12	.16	.44	.00	.00	.00	.01	.00	.00	.00	.00
	D'D'		.07	.00	.12	.00	.00	.00	.00	.00	.00	.00	.00
	DD''		.00	.06	.00	.00	.00	.00	.00	.00	.00	.00	.00
	D''D''		.00	.01	.00	.00	.00	.00	.00	.00	.00	.00	.00
	D		.87	.88	.66	1.0	1.0	1.0	.99	1.0	1.0	1.0	1.0
	1.96 σ		.04	.04	.07	---	---	---	.01	---	---	---	---
LDH	Absent(-)	+	+	-	-	-	-	-	-	-	-	+	-
SDH	or	+	+	+	+	-	-	-	-	-	-	+	-
GPI	Present(+)	+	+	+	+	-	-	-	+	-	-	-	-

Within the coastal populations, there was some variability in gene frequencies. The D'' allele was only found among spring chinook from the Trask River.

Nei (1972) developed a distance index, I_D, that ranges from an infinitely large value, for two totally dissimilar populations, to zero, for two identical populations. This index was defined as:

$$I_D = -\log_e \frac{\sum_{i=1}^{m} P_{i \cdot x} P_{i \cdot y}}{\left[\left(\sum_{i=1}^{m} P_{i \cdot x}^2 \right) \left(\sum_{i=1}^{m} P_{i \cdot y}^2 \right) \right]^{1/2}}$$

where $P_{i \cdot x}$ is the frequency of the i^{th} allele in population x, and m is the number of alleles. The nonlinear distribution of the Nei index emphasizes the differences between two populations where the most prevalent allele in one population has a low frequency in the other population. Relatively small I_D values were obtained in this study since comparisons are made between populations with relatively small frequency differences for the same allele.

Distance indices were calculated with the above equation for paired populations of chinook salmon (Tables 5 and 6). In calculating I_D values at the PGM locus, all monomorphic Columbia River populations were pooled. At the TO locus, greater differences existed between spring and fall chinook ($\bar{I}_D = .111 \pm .006$) than between populations of the same race ($\bar{I}_D = .052 \pm .007$) (Table 5). Fall chinook from the Elk and Trask rivers had a greater frequency of the

TABLE 5. Genetic distance, I_D, in comparisons between paired populations at the TO locus.

		SPRING CHINOOK				FALL CHINOOK						
		Marion Forks	McKenzie River	Little White Salmon	Trask River	Trask River	Elk River	Big Creek	Bonneville	Oxbow	Abernathy Creek	Little White Salmon
SPRING CHINOOK	Rogue	.011	.015	.065	.017	.028	.008	.071	.071	.108	.138	.065
	Marion Forks		.000	.006	.001	.004	.039	.145	.145	.197	.240	.136
	McKenzie			.008	.000	.002	.045	.158	.158	.212	.257	.148
	Little White Salmon				.010	.019	.014	.090	.090	.130	.164	.082
	Trask River					.001	.049	.166	.166	.128	.269	.157
FALL CHINOOK	Trask River						.066	.198	.198	.261	.312	.188
	Elk River							.031	.031	.057	.078	.027
	Big Creek								.000	.003	.010	.000
	Bonneville									.003	.010	.000
	Oxbow										.002	.005
	Abernathy Creek											.012

Table 6. Genetic distance, I_D, in comparisons between paired populations at the PGM locus with most Columbia River samples considered as a single pooled population (see text).

	Trask River (fall run)	Elk River	McKenzie River	Remaining Columbia River Populations
Trask River				
spring run	.078	.003	.004	.005
fall run		.054	.111	.119
Elk River			.009	.011

TO E-allele than other fall populations (Table 4). Elimination of these populations from this analysis indicated distinct differentiation between inter-racial ($\bar{I}_D$ = .148 ± .003) and intra-racial ($\bar{I}_D$ = .009 ± .001) comparisons. Distance measures at the PGM locus (Table 6) indicated that Trask River fall chinook exhibited the greatest differentiation ($\bar{I}_D$ = .090 ± .001) in comparisons with other populations. The low frequency of polymorphism among the remaining populations was reflected in their similar I_D values.

DISCUSSION

Genetic differences between coastal and Columbia River populations and between fall and spring chinook were observed. The TO E-allele in fall chinook from the Trask and Elk rivers was more frequent than in other fall populations. These coastal populations tended to approach TO frequencies observed in spring populations. In general, fall chinook are reared in hatcheries for approximately six months and spring chinook are reared for approximately one year prior to their release. Fall chinook at the Elk and Trask River hatcheries are reared on a schedule most characteristic of spring chinook programs. These extended rearing programs with fall populations may be favoring the survival of phenotypes typical of spring chinook. Samples of both spring and fall chinook were available from the Trask River and Little White Salmon hatcheries. As expected, the TO E-allele was prevalent among the spring chinook from Little White Salmon hatchery and was less frequent among fall fish. A clear distinction in TO gene frequencies was not evident between the two Trask River races. The method of broodstock selection at the Trask River may favor some interbreeding of the two races partially accounting for the observed results.

The origin of the geographical cline in MDH is difficult to assess. The populations may be responding to variable environmental

pressures associated with spatial variation of the different streams. The cline may also reflect the historical distribution of mutations from a common point due to adults straying from their native streams.

Variability at the PGM locus was extensive among coastal populations and virtually absent from Columbia River chinook. Environmental conditions in the Columbia River drainage may influence the survival of some phenotypes. For instance, Columbia River fall chinook were found to be more resistant to gas bubble disease than fall chinook from the Trask River (Cramer and McIntyre, 1975) and Columbia River populations of fall chinook were found to be more resistant to the parasite Ceratomyxa shasta than fall chinook from the Trask River (Johnson, 1975). High levels of gas supersaturation and Ceratomyxa shasta occur in the Columbia River and are nearly absent in coastal streams. Selection pressures resulting from these kinds of factors may be of the magnitude required to realize the complete elimination of a particular allele through natural selection. Alternatively, the variant PGM alleles found among coastal chinook may never have been present in the Columbia River fish. The variation in coastal populations may represent differentiation occurring after the divergence of the sampled populations from a common progenitor. The polymorphic sample from the McKenzie River may represent a random mutation.

Differences in both allelic frequencies and alleles present at the PGM locus were observed in spring and fall populations from the Trask River. An allele totally absent from all other sampled chinook salmon was found in this spring population. If selective pressures are influencing allelic expression at this locus, the D'' allele must represent a selective advantage either in the ocean life or in the extended river life of returning spring adults since both populations are otherwise raised under nearly identical conditions.

Historically, extensive transfers of fall chinook eggs and juveniles have occurred between many hatcheries. In recent years, these transfers have been largely restricted to Columbia River hatcheries. In addition, the sampled Columbia River hatcheries have generally been in operation longer than the sampled coastal hatcheries.

Lewontin and Krakauer (1973) devised a method of assessing the effect of selective pressures on specific loci. They defined an estimate of effective inbreeding, $\hat{F}_e$, as

$$\hat{F}_e = \frac{s_p^2}{\bar{p}(1-\bar{p})}$$

where s_p^2 is the "variance in the frequency of one of two alternate alleles" at a specific locus over all populations and $\bar{p}$ is the mean allele frequency over all populations.

Variance, $\sigma^2_{\hat{f}}$, about $\hat{F}_e$ was defined as:

$$\sigma^2_{\hat{f}} = \frac{2\bar{f}^2}{n-1}$$

where $\bar{f}$ is the average of all $\hat{F}_e$ and n is the number of populations characterized.

Inbreeding coefficients with associated s_p^2 values for all populations combined were calculated at four loci: TO, MDH, PGM, and LDH (Table 7). Values for $\hat{F}_e$ ranged from 0.0436 to 0.1448 with a mean of $\bar{f} = 0.1082$ and a corresponding variance of $\sigma^2_{\hat{f}} = 0.002$.

According to Lewontin (1974), great variability in $\hat{F}_e$ values would be expected if selective pressures were influencing gene frequency distributions among the loci studied. The $\hat{F}_e$ for MDH was smaller than those for TO, PGM, and LDH. This heterogeneity may reflect selective responses associated with the cline observed for MDH. The small number of populations sampled and the limited number of polymorphic loci assayed limits the interpretive value of $\hat{F}_e$ in this study. With increased knowledge of the genetics of salmonid species, calculation of $\hat{F}_e$ values may provide a useful method of assessing comparative selection pressures.

Table 7. Mean gene frequencies ($\bar{p}$) with associated variance (s_p^2) and inbreeding coefficients ($\hat{F}_e$) over all sampled populations.

Locus	$\bar{p}$	s_p^2	$\hat{F}_e$
TO	.685	.024	.113
MDH	.901	.004	.044
LDH	.996	.001	.131
PGM	.867	.017	.145

The biochemical genetic data obtained in this study showed differences between spring and fall runs of chinook and between Columbia River and coastal chinook populations. At the present time, the adaptive significance of this variation cannot properly be assessed. With an increased understanding of the genetic structure of fish populations, the kinds of information obtained in this study may become useful to fishery managers as tools to aid in the assessment of the long-term effects of major environmental changes on fish populations and in the manipulation of the genetic structure of managed populations.

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