OREGON COOPERATIVE FISHERY RESEARCH UNIT

Research Highlights - 1976/1977

Title of Project and Highlights — Page

Bob,

Just some things I thought might interest you.

Regards,

Carl

BIOCHEMICAL GENETIC VARIATION IN ROGUE RIVER CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA)

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Water development projects in the Rogue River drainage will cause changes in existing patterns of water flow and temperature in the river. Associated changes in the characteristics, distribution, and abundance of fish stocks that reside in the drainage for all or part of their life-cycles cannot be predicted. An understanding of the variation extant in present stocks in the Rogue system is required to detect changes in these populations. This report describes the results of a study of genetic variation in chinook salmon (Oncorhynchus tshawytscha) in the Rogue River Basin prior to the closure of the newly constructed Lost Creek Dam. This study was carried out by the Oregon Cooperative Fishery Research Unit in cooperation with the Research section of the Oregon Department of Fish and Wildlife during the period between 1 February 1976 and 30 June 1977. It is an updated and completion report for the chinook salmon phase of the work reported in the Annual Report (part 2) of the Rogue River Evaluation Program, May 1976 (Oregon Cooperative Fishery Research Unit [1976]).

Classical taxonomic characters do not permit discrimininination between stocks of fish of the same species in the same drainage. Biochemical genetic traits, however, have utility in partitioning variable, although closely related, stocks or populations.

Methods

General.--In recent years, the technology to observe and describe the frequencies of variant alleles at many loci in populations of organisms has become available. The methodology consists of electrophoretic separation of allozymes, addition of substrates to produce specific staining reactions, and cataloging the frequencies of variant forms. Allozyme variability often can be explained by a genetic hypothesis which subsequently must be verified by breeding experiments. The gene and genotype frequencies for groups of organisms then can be determined and used as a basis for characterizing specific groups and assessing the similarity between groups.

Genetic sampling.--Juvenile chinook salmon from nine sites in the Rogue River drainage were collected during 1976 and analyzed for their genetic variability with respect to seven different enzyme systems. The enzyme systems and tissue sources that were used in this study are listed in Table 1. In addition, a general protein stain was used routinely to confirm the species of each fish. The electrophoretic methods used were modified from those described by Shaw and Prasad (1970), Ridgway et al. (1970), Clayton and Tretiak (1972), Markert and Faulhaber (1965), and Kristiansson (1975). Enzymes found to be of little value in an earlier phase of this study were not included in the analyses.

The banding patterns observed for each of the systems studied are illustrated in Figures 1a and 1b. For chinook salmon, the inheritance patterns of MDH, TO, PGM, and LDH have been confirmed. PHI, SDH, and G6PDH have not been confirmed. In cases where inheritance patterns have not been identified, tentative designation according to the observed phenotype was used.

One enzyme system that had been used previously--isocitrate dehydrogenase (IDH)--was excluded from consideration this year because its level of resolution was too unreliable.

The locations of each sample, date of collection, and other pertinent information are summarized in Table 2. Each sample was assigned a code number for future reference. The locations from which samples were obtained in the Rogue River drainage are further identified in Figure 2.

Results

The observed genotype (or phenotype) proportions for each enzyme system by sample location are shown in Tables 3-8. The proportion of the common phenotype (or allele for enzymes in which the genetic basis has been confirmed) and corresponding confidence interval ($p = .05$) for each sample are represented graphically in Figures 3-8 for each enzyme system. One enzyme system, PGM, was fixed at the common type; hence, it is not represented graphically.

Each sample was compared to all other samples to identify significant differences in the proportion of the common phenotype or allele in each enzyme system (Fig. 9). In cases where no data were available, the samples to be compared were assumed to be identical; i.e., no significant difference. Of the 72 between sample comparisons, 81% had one or more significant differences while 48% had two or more differences. Much of the variation between samples can be attributed to differences for the G6PDH enzyme system; 55% of all significant differences were related to G6PDH. Experiments conducted in our laboratory (Solazzi, unpublished) suggest that the G6PDH phenotypes of steelhead trout (Salmo gairdneri) are labile and dependent upon

sampling methodology. By inference, we suspect that G6PDH in chinook salmon may be subject to artifactual bias.

The samples from Big Butte Creek and Quosatana Creek had the greatest number of significant differences (excluding G6PDH) associated with them. Most of these differences are related to variation in the TO enzyme system (Fig. 5). Big Butte Creek fish had the highest frequency of the E allele of TO, and Quosatana had the lowest for all samples taken during 1975.

Comparison of differences between hatchery-reared fish and wild stock is hampered by the lack of samples which can be definitely identified as being of wild origin. Samples collected from the Rogue River mainstem are likely to contain at least a small percentage of hatchery-reared fish. Only the fish from Quosatana Creek can be confidently considered to be of wild origin. Quosatana Creek chinook salmon differed significantly from Cole Rivers Hatchery fish for the TO, PHI, and G6PDH enzyme systems. Because three of the seven enzyme systems we observed exhibited significant differences between the hatchery and wild stocks, one might conclude that a high degree of genetic variability exists between these stocks. However, during 1975 these stocks differed only in the G6PDH system.

Cole Rivers Hatchery and Big Butte Creek produce only spring-run chinook salmon. Quosatana Creek has only fall-run chinook. Samples from other locations may have had a mixture of both spring and fall chinook salmon. Fall chinook from Quosatana Creek differed consistently from spring chinook from Cole Rivers Hatchery and Big Butte Creek only for the TO enzyme (Fig. 5). This distinction was not apparent in samples taken during 1975.

Comparisons were made between samples taken in 1975 and 1976 from the same location. Little variability was apparent between years for the enzyme

systems other than G6PDH. Big Butte Creek and Quosatana Creek had significant differences between years for TO. Samples of chinook salmon from the estuary section of the Rogue River had different frequencies of the common phenotype for SDH between years.

Discussion

The purpose of this study was to provide a genetic description of the Rogue River chinook salmon prior to the closure of Lost Creek Dam. These data were collected to serve as a basis for assessing any genetic changes in these fish associated with environmental changes produced by the water management program in the Rogue River Basin or with the artificial propagation of fish at Cole Rivers Hatchery. Detection of any change in the gene frequencies that were calculated in this study will be restricted to changes at the relatively few loci. Some of these systems have been implicated in temperature adaptation. Johnson (1971) demonstrated a consistent correlation of LDH frequencies with temperature in the crested blenny, Anaplarchus purpurescens. Alterations of water temperatures in the Rogue River might be expected to produce associated changes in the frequencies of LDH and other systems that were studied.

It is important to keep in mind that few loci were examined relative to the total genome of the fish. One would expect that information at more loci would have permitted identification of discrete breeding units within fall and spring chinook salmon. Alternatively, the apparent homogeneity between most groups may have resulted from the straying of adult fish from their natal streams for spawning, producing a single breeding unit within each of these groups.

References

Clayton, J. W., and D. N. Tretiak. 1972. Amino-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Can. 29: 1169-1172.

Johnson, M. S. 1971. Adaptive lactate dehydrogenase variation in the crested blenny, Anoplarchus. Heredity 27(2):205-226.

Kristiansson, A. C. 1975. Biochemical genetic variation among selected population of chinook salmon (Oncorhynchus tshawytscha) in Oregon and Washington. MS Thesis, Oregon State University. 29 pp.

Markert, C. L., and I. Faulhaber. 1965. Lactate dehydrogenase isozyme patterns of fish. J. Wxp. Zool. 159:319-332.

Oregon Cooperative Fishery Research Unit. 1976. Biochemical genetic variation of Rogue River chinook salmon and steelhead trout. Annual Report (Part 2), Rogue Basin Evaluation Program, Research Section, Oregon Dept. Fish and Wildlife. Corvallis. 61 pp.

Table 1. Enzyme and tissue source of the enzyme in chinook salmon used in electrophoretic studies. Abbreviations are included in parentheses.

Enzyme	Tissue
Lactate dehydrogenase (LDH)	Liver
Tetrazolium oxidase (TO)	Liver
Malate dehydrogenase (MDH)	White muscle
Glucose-6-phosphate dehydrogenase (G6PDH)	Liver
Phosphoglucomutase (PGM)	White muscle
Phosphohexoisomerase (PHI)	White muscle
Sorbitol dehydrogenase (SDH)	Liver

Table 2. Codes and information for samples of chinook salmon from the Rogue River drainage taken for biochemical genetic studies. Sp = spring, F = fall, H = hatchery origin, RM = river mile.

Code	Location	Collection Date	Age class	Parental return season
R2-B	Cole Rivers Hatchery (h)	X - 76	0+	Sp
R3-B	Big Butte Creek	VII - 76	0+	Sp
R4-B	Table Rock	VIII - 76	0+	Sp?
R6-B	Lower Applegate	VIII - 76	0+	F
R8-B	Agness (RM 27)	IX - 76	0+	?
R9-B	Illinois (RM 27)	IX - 76	0+	?
R10-B	Quosatana	IX,X - 76	0+	F
R15	John's Hole (RM 4)	IX - 76	0+	?
R16	Station 2 (RM .5)	IX - 76	0+	?

Table 3. Summary of genotype proportions for lactate dehydrogenase in chinook salmon from the Rogue River drainage.

Code	Location	BB	BB'	BB'''	BB''	Sample size
R2-B	Cole Rivers Hatchery	1.00	-	-	-	59
R3-B	Big Butte Creek	.95	-	.04	.01	80
R4-B	Table Rock	.98	-	.02	-	84
R6-B	Lower Applegate	.99	-	.01	-	92
R8-B	Agness	.96	-	.03	.01	100
R9-B	Illinois	.96	-	.04	-	85
R10-B	Quosatana	.98	-	.02	-	42
R15	John's Hole	.98	-	.02	-	96
R16	Station 2	1.00	-	-	-	15

Table 4. Summary of genotype proportions for malate dehydrogenase in chinook salmon from the Rogue River.

Code	Location	BB	BB'	B'B'	BB''	Sample size
R2-B	Cole Rivers Hatchery	.95	.03	-	.02	59
R3-B	Big Butte Creek	1.00	-	-	-	94
R4-B	Table Rock	.96	-	.01	.03	92
R6-B	Lower Applegate	.95	.04	.01	-	92
R8-B	Agness	.96	.04	-	-	100
R9-B	Illinois	.92	.08	-	-	85
R10-B	Quosatana	.83	.17	-	-	42
R15	John's Hole	.93	.06	-	.01	96
R16	Station 2	.93	.07	-	-	15

Table 5. Summary of genotype proportions for tetrazolium oxidase in chinook salmon from the Rogue River drainage.

Code	Location	EE	EF	FF	Sample size
R2-B	Cole Rivers Hatchery	.83	.14	.03	59
R3-B	Big Butte Creek	.95	.05	-	61
R4-B	Table Rock	.80	.14	.06	70
R6-B	Lower Applegate	.64	.32	.04	92
R8-B	Agness	.69	.25	.06	100
R9-B	Illinois	.74	.20	.06	85
R10-B	Quosatana	.41	.45	.14	42
R15	John's Hole	.67	.31	.02	96
R16	Station 2	.60	.27	.13	15

Table 6. Summary of phenotype proportions for glucose phosphohexoisomerase in chinook salmon from the Rogue River drainage.

Code	Location	Common	Variant 1	Sample size
R2-B	Cole Rivers Hatchery	1.00	-	59
R3-B	Big Butte Creek	.95	.05	84
R4-B	Table Rock	.93	.07	91
R6-B	Lower Applegate	.96	.04	92
R8-B	Agness	.96	.04	95
R9-B	Illinois	.93	.07	85
R10-B	Quosatana	.88	.12	42
R15	John's Hole	.96	.04	96
R16	Station 2	1.00	-	15

Table 7. Summary of phenotype proportions for sorbitol dehydrogenase in chinook salmon from the Rogue River.

Code	Location	Common	Variant 1	Sample size
R2-B	Cole Rivers Hatchery	.83	.17	59
R3-B	Big Butte Creek	.95	.05	37
R4-B	Table Rock	-	-	-
R6-B	Lower Applegate	.91	.09	92
R8-B	Agness	.90	.10	100
R9-B	Illinois	.95	.05	85
R10-B	Quosatana	.88	.12	42
R15	John's Hole	.85	.15	96
R16	Station 2	1.00	-	15

Table 8. Summary of phenotype proportions for glucose-6-phosphate dehydrogenase in chinook salmon from the Rogue River.

Code	Location	Common	Variant 1	Variant 2	Sample size
R2-B	Cole Rivers Hatchery	.80	.05	.15	59
R3-B	Big Butte Creek	.17	.42	.41	81
R4-B	Table Rock	.41	.32	.27	85
R6-B	Lower Applegate	.03	.83	.14	92
R8-B	Agness	-	.57	.43	100
R9-B	Illinois	-	.67	.33	85
R10-B	Quosatana	.19	.64	.17	42
R15	John's Hole	.35	.39	.26	96
R16	Station 2	-	.60	.30	15

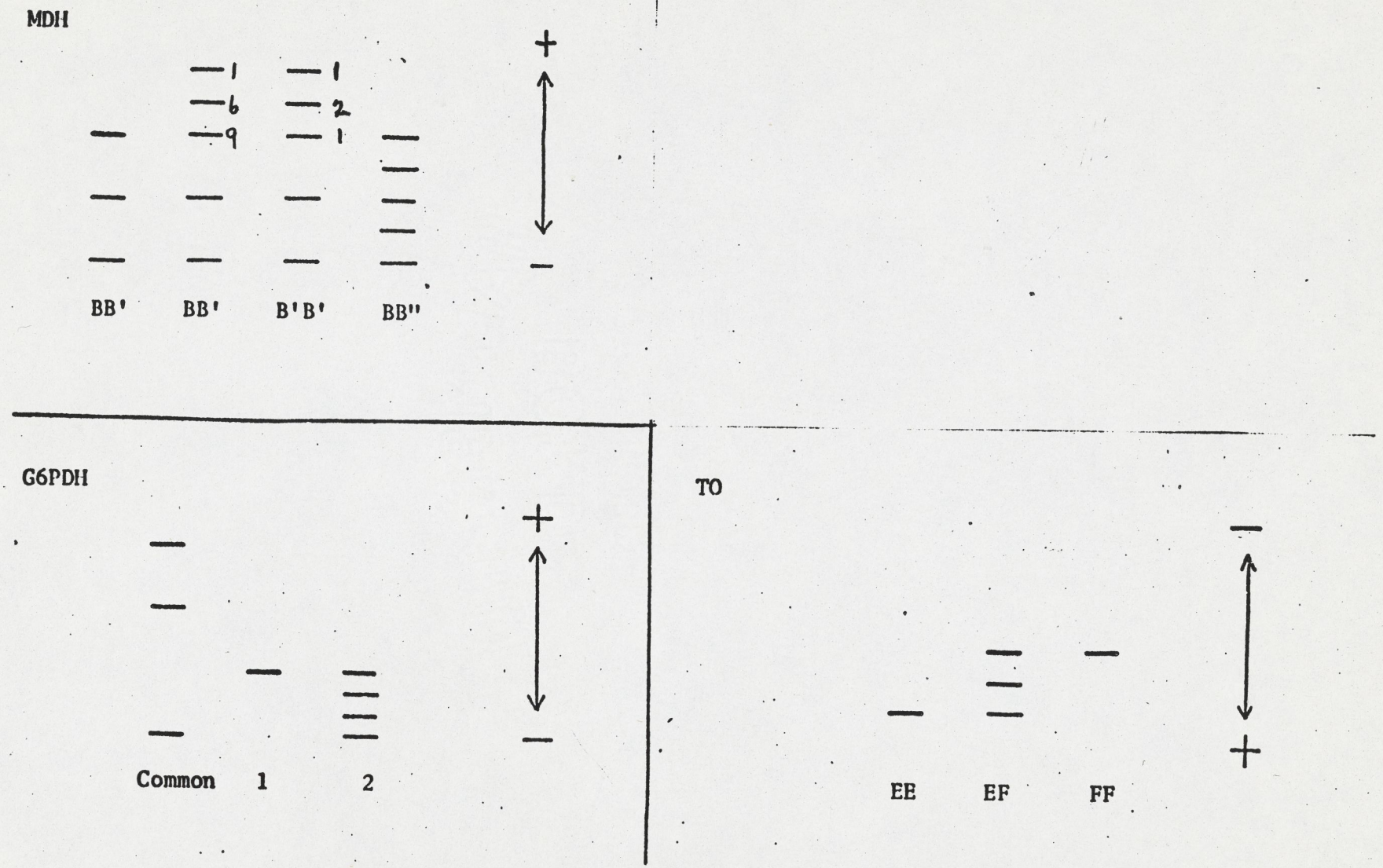

Fig. 1a. Electrophoretic patterns observed for MDH, G6PDH, and TO in tissues of chinook salmon. Proposed genotypes (or phenotypes) are indicated beneath each pattern. Numberals adjacent to bands in MDH denote relative doses of subunits in patterns with equal numbers of bands.

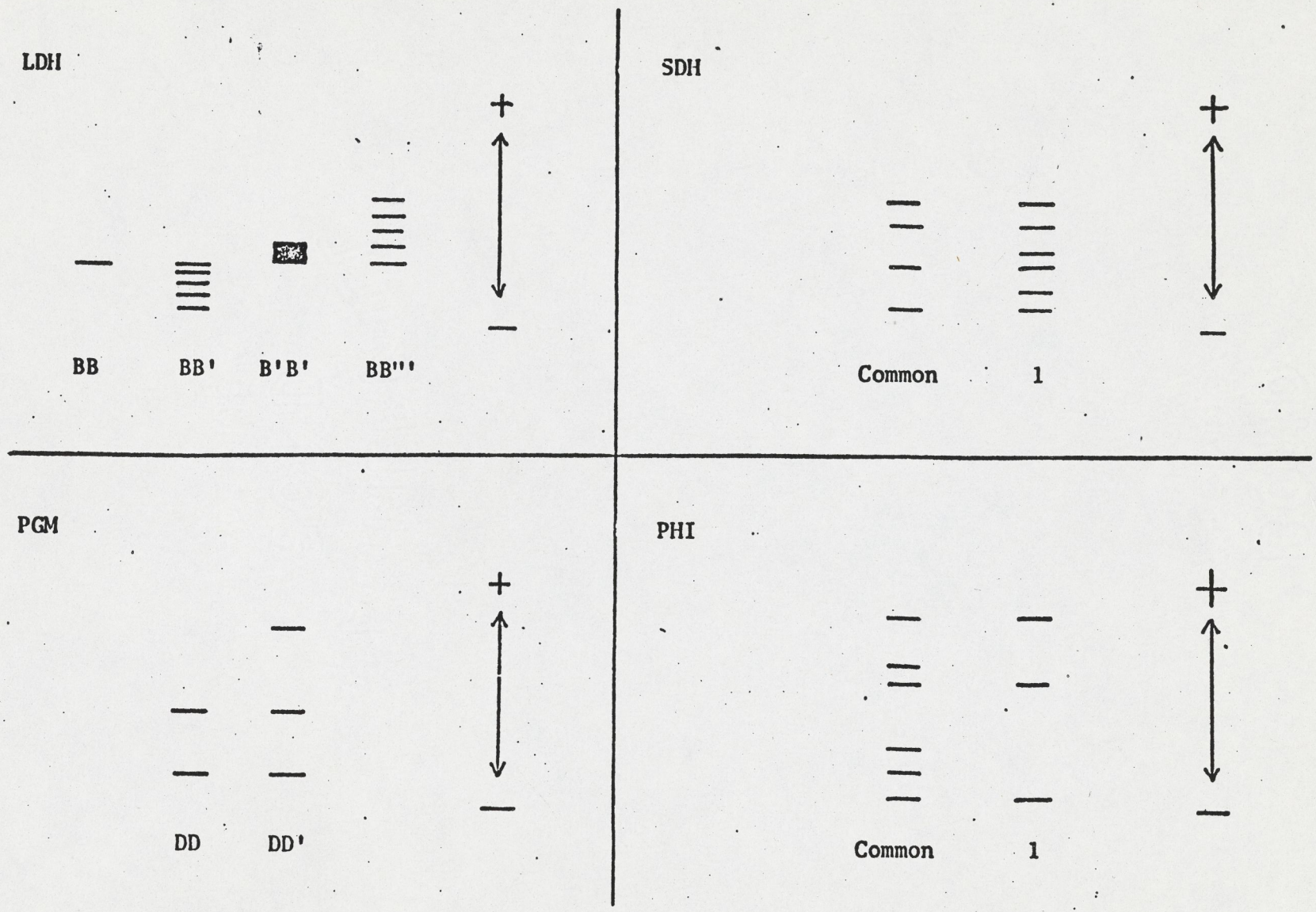

Fig. 1b. Electrophoretic patterns observed for LDH, SDH, PGM, and PHI in tissues of chinook salmon. Proposed genotypes (or phenotypes) are indicated beneath each pattern.

Fig. 2. Locations on the Rogue River from which chinook salmon were obtained for genetic studies during 1975 and 1976. One, 2 - Cole Rivers Hatchery; 3 - Big Butte Creek; 4 - Table Rock Canal; 5 - Savage Rapids; 6 - Lower Applegate; 7 - Winkle Bar; 8 - Agness; 9 - Illinois River; 10 - Quosatana Creek; 11 - Canfield Riffle; 12 - Station 7; 13 - Gold Beach; 15 - John's Hole; 16 - Station 2.

Code	Location
R2-B	Cole Rivers Hatchery
R3-B	Big Butte Creek
R4-B	Table Rock Canal
R6-B	Lower Applegate
R8-B	Agness
R9-B	Illinois
R10-B	Quosatana Creek
R15	John's Hole
R16	Station 2

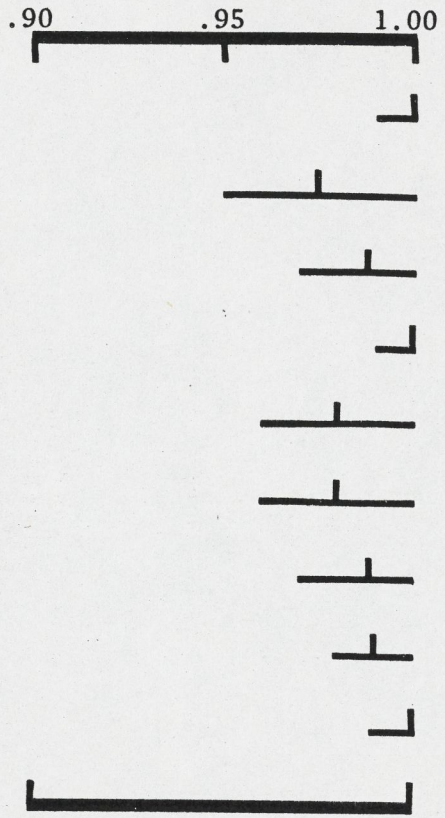

Fig. 3. Sample proportions and approximate 95% confidence intervals for LDH-B allele in chinook salmon from the Rogue River drainage.

Code	Location
R2-B	Cole Rivers Hatchery
R3-B	Big Butte Creek
R4-B	Table Rock Canal
R6-B	Lower Applegate
R8-B	Agness
R9-B	Illinois
R10-B	Quosatana Creek
R15	John's Hole
R16	Station 2

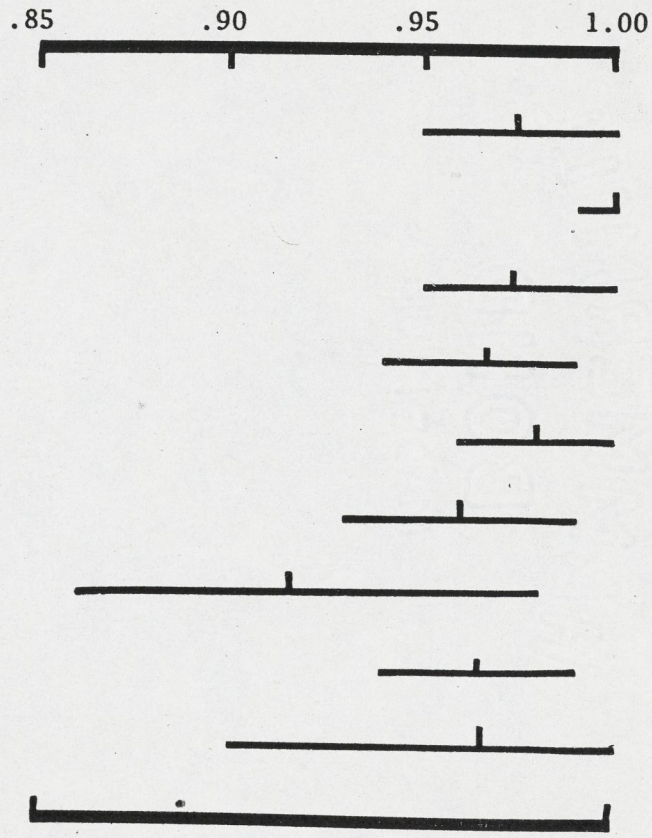

Fig. 4. Sample proportions and approximate 95% confidence intervals for MDH-B allele in chinook salmon from the Rogue River drainage.

Code	Location
R2-B	Cole Rivers Hatchery
R3-B	Big Butte Creek
R4-B	Table Rock Canal
R6-B	Lower Applegate
R8-B	Agness
R9-B	Illinois
R10-B	Quosatana Creek
R15	John's Hole
R16	Station 2

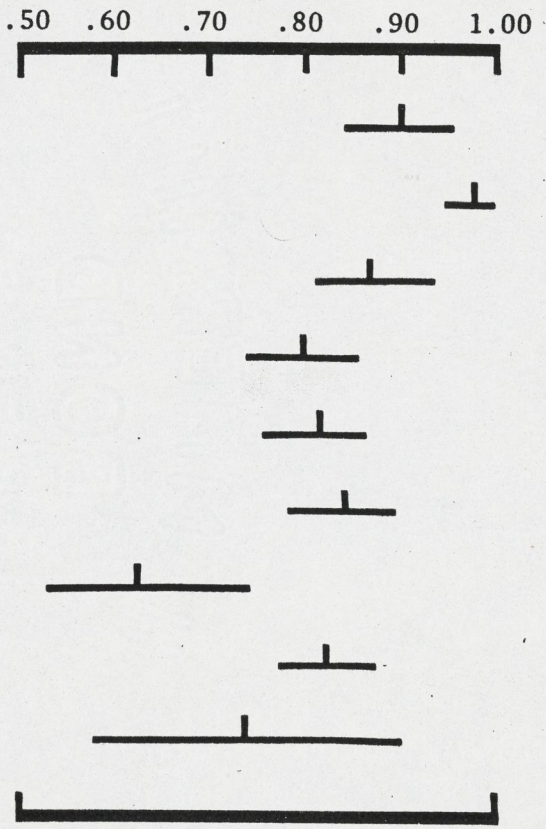

Fig. 5. Sample proportions and approximate 95% confidence intervals for TO-E allele in chinook salmon from the Rogue River drainage.

Code	Location
R2-B	Cole Rivers Hatchery
R3-B	Big Butte Creek
R4-B	Table Rock Canal
R6-B	Lower Applegate
R8-B	Agness
R9-B	Illinois
R10-B	Quosatana Creek
R15	John's Hole
R16	Station 2

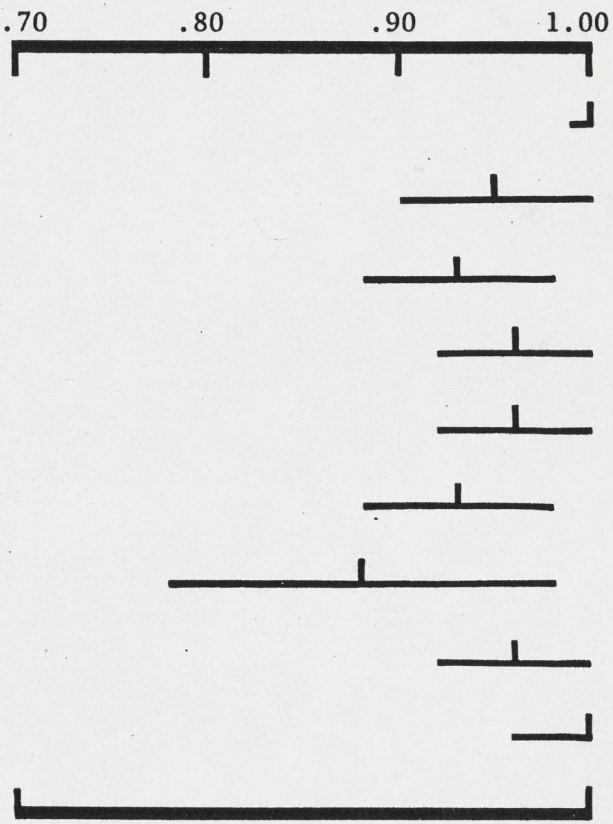

Fig. 6. Sample proportions and 95% confidence intervals for PHI-common phenotype in chinook salmon from the Rogue River drainage.

Code	Location
R2-B	Cole Rivers Hatchery
R3-B	Big Butte Creek
R4-B	Table Rock Canal
R6-B	Lower Applegate
R8-B	Agness
R9-B	Illinois
R10-B	Quosatana Creek
R15	John's Hole
R16	Station 2

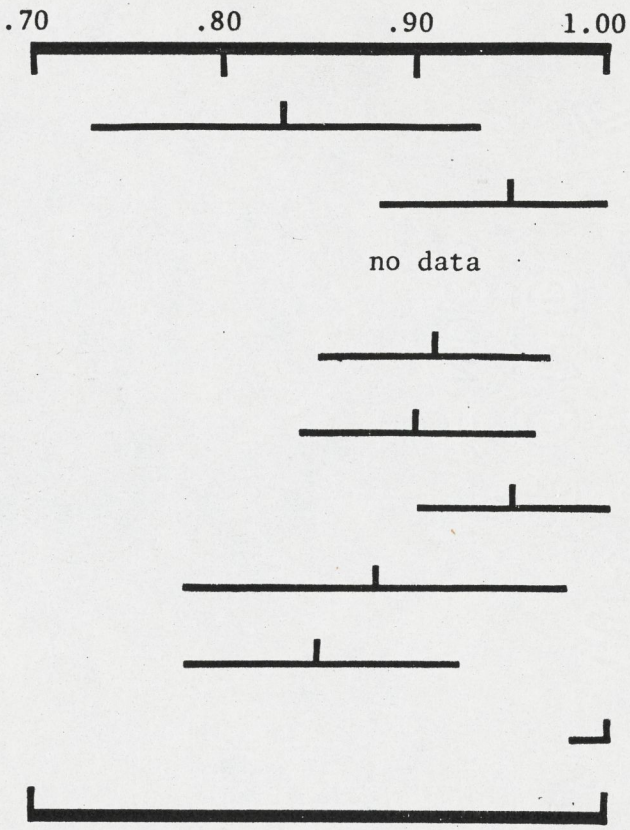

Fig. 7. Sample proportions and 95% confidence intervals for SDH-common phenotype in chinook salmon from the Rogue River drainage.

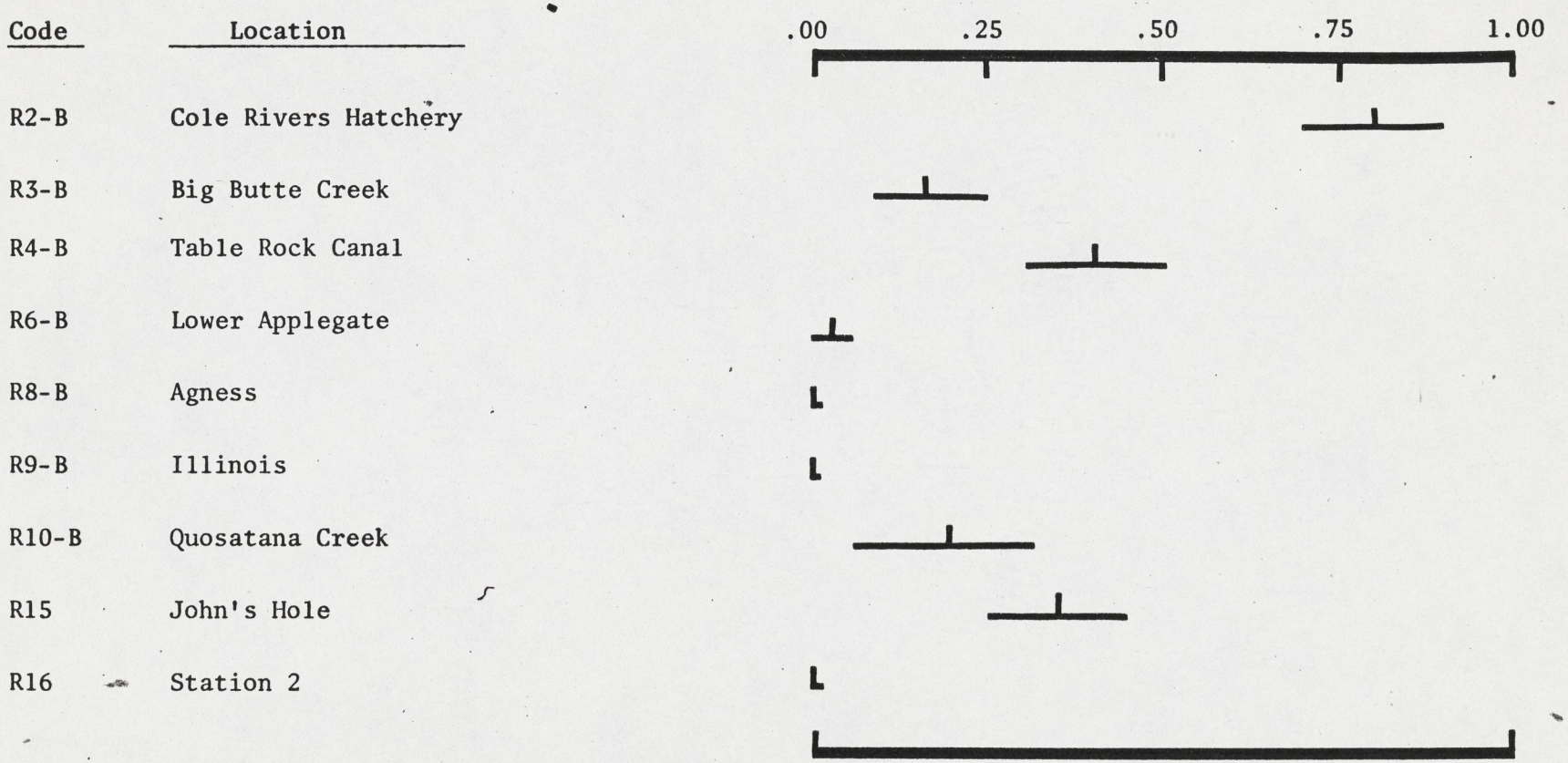

Fig. 8. Sample proportions and approximate 95% confidence intervals for G6PDH-common phenotype in chinook salmon from the Rogue River drainage.

Location	Code	Code: R2-B	R3-B	R4-B	R6-B	R8-B	R9-B	R10-B	R15	R16	Total no. of significant differences: with G6PDH	without G6PDH
Cole Rivers Hatchery	R2-B		•	⊡	•	•	⊡	⊡	•	⊡	13	5
Big Butte Creek	R3-B	•		⊡	⊡	⊡	⊡	⊡	⊡	⊡	14	8
Table Rock	R4-B	⊡	⊡		•	•	•	⊡		•	9	3
Lower Applegate	R6-B	•	⊡	•						⊡	6	2
Agness	R8-B	•	⊡	•				⊡	•	⊡	8	3
Illinois	R9-B	⊡	⊡	•				⊡	•		8	3
Quosatana Creek	R10-B	⊡	⊡	⊡		⊡	⊡		⊡	•	12	8
John's Hole	R15	•	⊡			•	•	⊡		⊡	7	3
Station 2	R16	⊡	⊡	•	⊡	⊡		•	⊡		11	5

Fig. 9. Comparison matrix of significantly different (p .05) sample proportions for the common phenotype or allele in all observed enzyme systems by location. Symbols on either side of the diagonal are redundant but are included to facilitate visualization. Each dot represents a significant difference for one or more enzyme system. A square around a dot denotes one or more differences in enzyme systems other than G6PDH.