

Genetic Implications of Steelhead Management

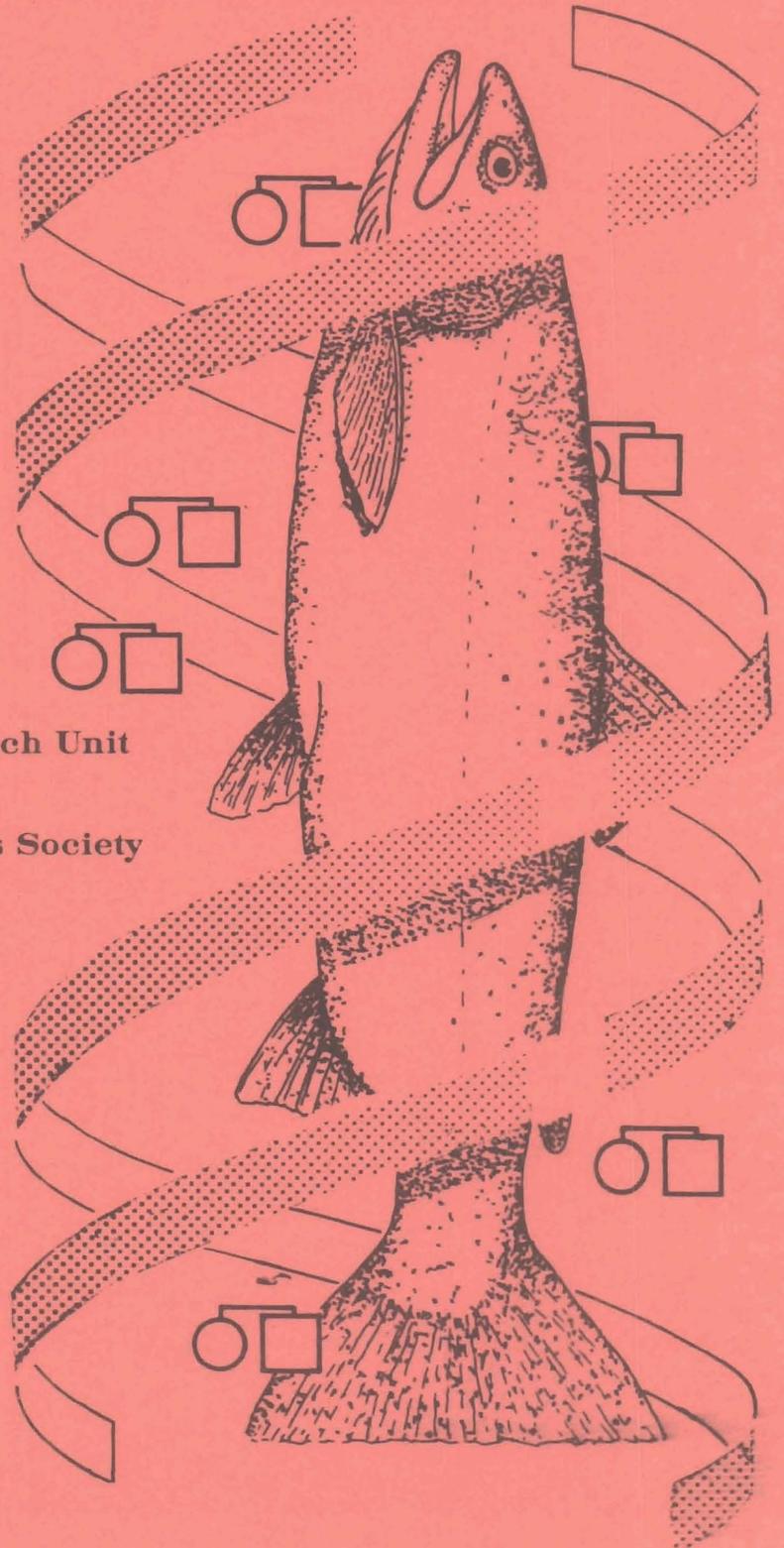
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DETERMINATION OF THE BREEDING STRUCTURE OF STEELHEAD POPULATIONS THROUGH GENE FREQUENCY ANALYSIS

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There is widespread agreement among people involved in the management of steelhead populations about the need for a better understanding of the breeding structure of these populations and the relationships among them. This attitude differs markedly from earlier management techniques where a tendency toward "typological" thinking resulted in treating salmonid populations of diverse ancestry as genetic equivalents; a practice which devastated some uniquely adapted gene pools (Behnke 1972). Such efforts often wasted time, money, and resources through ill-conceived plantings resulting in negligible survival (Finney and Kral 1965; Buchanan 1975; Utter, Allendorf, and May 1976).

The current awareness of genetic diversity among steelhead populations coincides with the maturation of a biochemical genetic methodology that permits - for the first time - the purely genetic definition of populations through the enumeration of variation at many loci. This methodology of electrophoretic separation of proteins coupled with histochemical staining procedures has revolutionized the understanding of genetic diversity in nature during the past decade (Ayala 1975; Lewontin 1974). These techniques also have great potential for use in applied biological problems but have received little attention in this regard until very recently (Utter, Hodgins, and Allendorf 1974; Allendorf and Utter, in press; Hedgecock *et al.* 1976; Moav *et al.* 1976).

Fisheries scientists of the Pacific Northwest have pioneered the use of gene frequency data as tools of applied biology. These applications have been particularly effective in defining populations (Allendorf 1975; May 1975; Utter, Allendorf, and May 1976) and in studying the interaction of hatchery and native populations (Utter, Allendorf, and May 1976; Reisenbichler, and McIntyre 1977).

This paper extends the range of the initial observations of Allendorf, which focused on the structure of steelhead populations of Washington

State and the upper Columbia River drainage (Allendorf 1975; Allendorf and Utter, in press). The data were collected from many sources (identified below) including much unpublished information that has been shared with us by other investigators. The paper (1) outlines the methodology and its application to studying the structure of steelhead populations, (2) presents an overview of the current knowledge of the structure of steelhead populations, and (3) discusses possible directions for future investigations of this nature. It is directed toward a general audience having a varied background with regard to the use of the methodology and the interpretation of the data. Some points of discussion that provide pertinent background information but that are peripheral to the purposes of the paper are therefore introduced as footnotes in order to give greater continuity to the text.

METHODOLOGY

The significant progress that has resulted from the development and application of electrophoretic methodology is a reflection of the useful attributes of these techniques for the collection of genetic data. Appropriately selected protein variants are a direct indication of single gene differences, and a tremendous amount of data can be collected with little effort relative to other methods.

It is very important to select only those protein variants that reflect simple genetic differences. A number of criteria for distinguishing such variation from artifactual expression have been detailed elsewhere (Utter, Hodgins, and Allendorf 1974; Allendorf and Utter, in press). The strongest test for simple inheritance of a particular protein variation is breeding data. However, other criteria can usually be reliably used in the absence of breeding data (e.g., repeatability of expression and conformance of the variation to simple genetic models).

Superoxide dismutase (SOD), one of the variant protein systems that was included in this survey, will be used to illustrate how electrophoretic data are translated into genetic data. This protein has been called tetrazoliumoxidase (TO) in most previous publications by our group and by many other investigators. The three patterns of SOD activity shown in Figure 1 are a direct reflection of two different genes (or more precisely, alleles) at the SOD locus.¹ Each individual carries two SOD genes which correspond to the paired chromosome complement, and therefore, genes of higher animals including steelhead and man. Therefore, three different genetic types are possible from these two alleles; the common homozygous type (No. 5 of Figure 1), the heterozygous type (No. 2 of Figure 1), and the alternate homozygous type (No. 1 of Figure 1).

The frequency of the two SOD alleles can be measured in a particular collection by direct counts from the electrophoretic expression. The number of SOD genes in a particular collection is always twice the number of individuals sampled because two doses of SOD are expressed in each individual.

A homozygous individual expresses two doses of the particular allele reflected by its phenotype and a heterozygous individual one dose of each allele. The allelic frequencies for the 17 individuals of Figure 1 are calculated as follows:

Frequency of phenotype	Frequency of allele	
	100	152
100/100 7	14	0
100/152 8	8	8
152/152 2	0	4
Total allelic frequency	22	12

Proportion of SOD (100) allele = $22/34 = .647$

Proportion of SOD (152) allele = $12/34 = .353$

Phenotype and allelic frequencies like those reflected by the SOD variation are the basic data of electrophoretic analyses. All comparisons of genetic variation within and between populations are based on this kind of data. Data at a single locus (e.g., SOD) can yield useful information about population structure because significant differences in gene frequencies between two populations are positive evidence for genetic differences. Reliable estimates of different allelic frequencies of two populations at a single locus can be straightforwardly used to estimate the proportion of each population where the two groups intermingle. This estimate is determined by solving the direct proportionality between the known allelic frequencies of the two contributing populations and the allelic frequencies determined in the mixed fishery—i.e., $P_1 = \frac{F_m - F_2}{F_1 - F_2}$, where P_1 is the proportion of population I in the mixed fishery, F_1 and F_2 the frequency of allele in populations I and II respectively, and F_m the frequency of allele in mixed fishery.

The simultaneous use of data reflecting many loci significantly magnifies the discriminatory power of electrophoretic data. More subtle divisions become apparent as more data are used as will be demonstrated later in this paper.

The use of data from many loci has proven to be a powerful tool for estimating relationships among species within a family (Avisé 1974). Such data have also been useful for examining relationships among populations at the sub-species level (Allendorf 1975; Allendorf and Utter, in press). The use of data from multiple loci extends the power for analyses of mixed fisheries to complex situations involving many contributing populations through automatic data processing methods and more sophisticated statistical analyses (see Milner, proceedings of this symposium).

GENETIC VARIATION OF POPULATIONS

Genetic data from both anadromous and non-anadromous rainbow trout populations have been collected over a broad geographic range, and

1. It is beyond the scope of this paper to review the details relating the sequence of nucleotides of the genetic material - DNA - and the sequences of amino acids of the protein, but it has been very well established that a colinearity exists and therefore that protein differences of this kind directly reflect genetic differences. See Watson, 1975, for a thorough and highly readable account of this relationship.

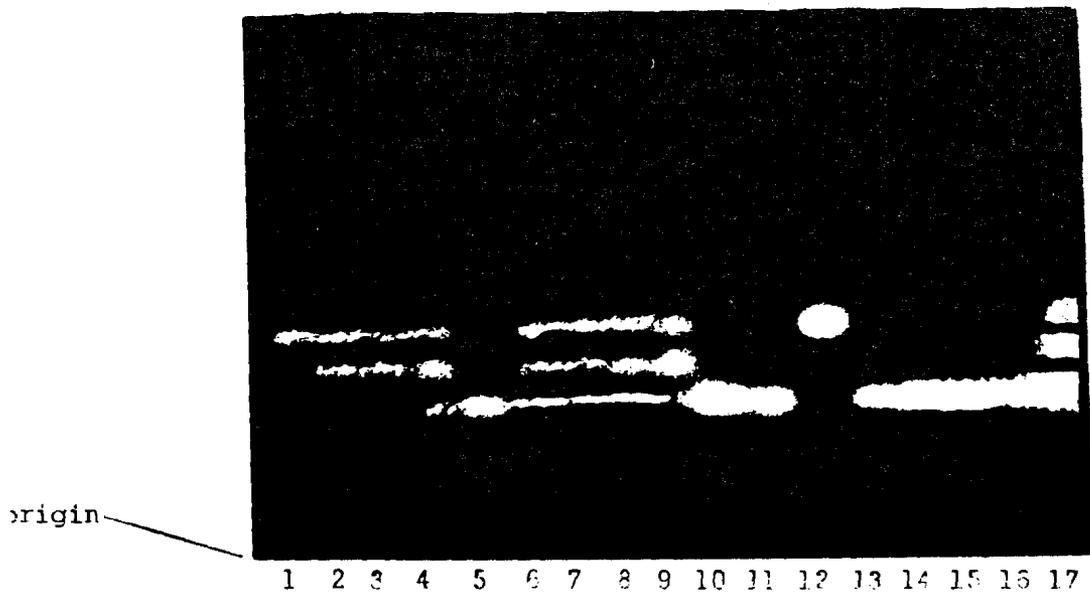


FIGURE 1. Electrophoretic expression of genetic variation of SOD in rainbow trout reflecting two alleles and three phenotypes. SOD (100)/SOD (100) 5, 10, 11, 13-16; SOD (100)/SOD (152) 2-4, 6-9, 17; SOD (152)/SOD (152) 1, 12.

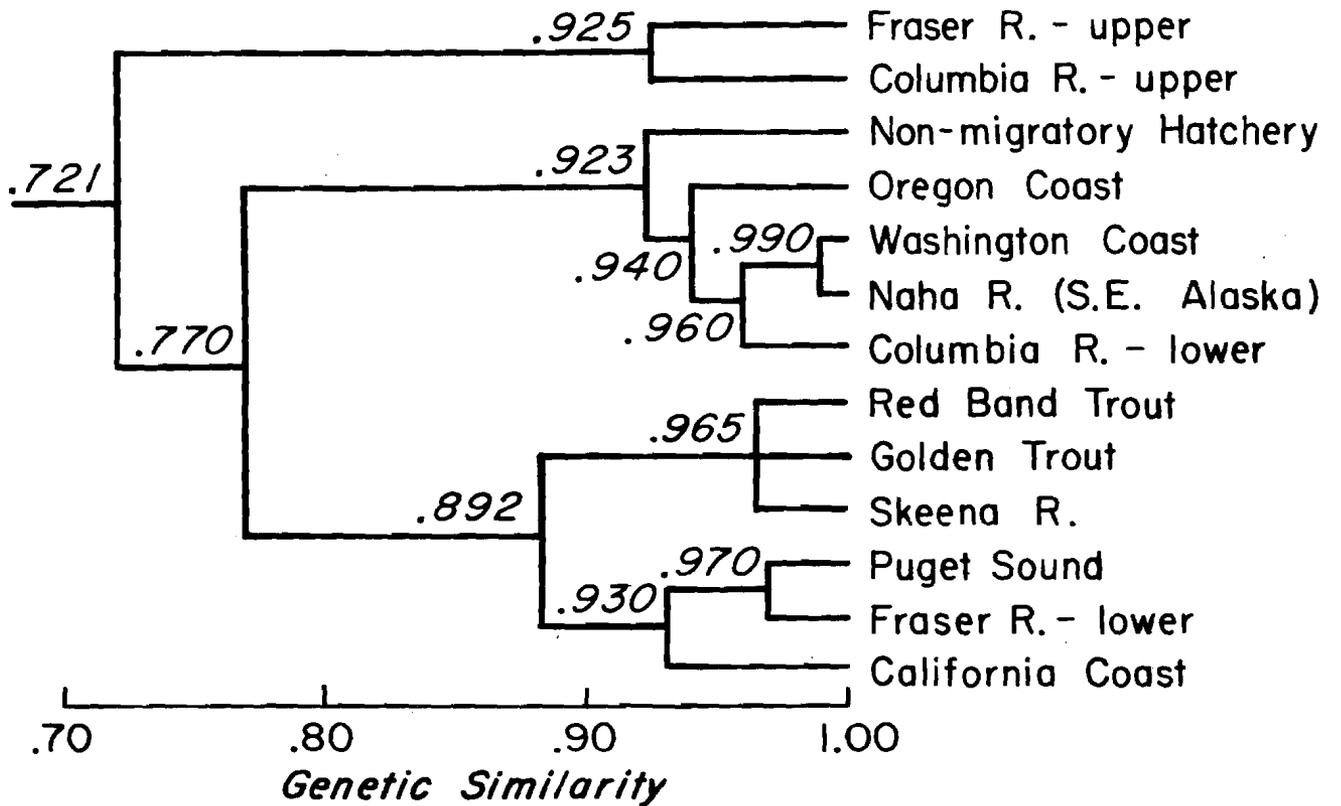


FIGURE 2. Dendrogram based on genetic similarity matrix (Table 2) using the unweighted average linkage method.

these data examined for distributional patterns that suggest relationships among populations. Only two polymorphic loci have been used in this survey - the lactic dehydrogenase locus expressed in the liver *LDH-4* and *SOD* - because data for these loci were most universally available from various sources and because Allendorf (1975) found these two loci particularly useful for identifying major population groups of rainbow trout of Washington State. We currently examine approximately 40 loci in our analysis of genetic variation in steelhead populations. Results here, based on only two loci, are reported for illustrative purposes rather than as a precise definition of the known genetic structure of steelhead populations.

Data for the frequency of the common alleles of the *LDH-4* and *SOD* loci from a substantial number of collections and a variety of sources have been pooled on the basis of geographic distribution into 13 groups (Table 1)¹. Data were pooled in a particular drainage where a consistency of gene frequency was observed (e.g., Babine and Zymoet tributaries of the Skeena River) but were separated where abrupt transitions in allelic distribution occurred (e.g., upper and lower Fraser and Columbia Rivers). The red band and golden trouts have been included because of their close kinship to rainbow trout (Behnke 1965; Allendorf and Utter 1974; Gold, in Press) and because of the possible relationship of these populations and rainbow trout populations of the upper Columbia and Fraser River drainages (Gold, In Press).

A number of methods exist for comparing genetic differences or similarities between two species or populations on the basis of electrophoretic data. Matrices of such pairwise comparisons over many groups of related organisms are useful for the construction of dendrograms which depict the genetic similarity among these groups on the basis of the

electrophoretic characters used. A simple and effective method for deriving pairwise genetic indices has been described by Rogers (1972). This method averages the allelic similarities between two groups over all loci studied on a scale of 0 to 1 where 0 indicates no electrophoretic similarity and 1 complete electrophoretic similarity. A similarity matrix (Table 2) was computed using the data of Table 1 and Rogers' method, and a dendrogram (Figure 2) constructed from this matrix using the unweighted average linkage method (Sneath and Sokal 1973).

The relationships suggested by Figure 2 should be conservatively interpreted because of the small number of loci used and the limited number of individuals and populations included in some groupings. The major feature of the dendrogram - the separation of populations from the upper Fraser and Columbia River drainages from all other groups - is regarded as a valid reflection of different evolutionary lineages because of the consistent differences observed over a broad geographic (and political) area, and the fact that a similar separation over a more restricted geographic area but including over 30 loci has previously been described (Allendorf 1975). Allendorf proposed that the major taxonomic distinction of these rainbow trout populations be based on a major group of populations from the upper Fraser and Columbia River drainages contrasted with another major group of coastal populations from drainages of the west slope of the Cascade Mountains. It was presumed that the inland group descended from a common ancestral stock which migrated to a large inland basin impounded during the last glacial period (ending approximately 10,000 years ago) while the coastal group represented descendents of Asiatic or American coastal populations which existed beyond the range of the glacial mass.

It was concluded by Allendorf (1975), and

1. It is assumed throughout this discussion that the reported allelic frequencies are more a reflection of selectively neutral protein variants rather than of the action of natural selection. This assumption is supported by a considerable amount of empirical data for the *LDH-4* and *SOD* loci of rainbow trout which indicate (1) no differences in hatchery populations in allelic or genotypic frequencies between juvenile and adult fish of a given year class or between year classes (unpublished data from this laboratory), (2) no differential survival or growth of different genotypes placed in different environments (Reisenbichler and McIntyre 1977), and (3) a distribution of variation among populations over many loci which is indicative of random processes (Allendorf 1975). It is not surprising that the effects of selection are not being detected at these loci. It must be kept in mind the *LDH-4* and *SOD* each represent only a single genetic locus out of (conservatively) many thousands of loci that represent the total genetic expression of an individual fish, and that dozens or perhaps hundreds of loci interact to affect a measurable quality such as growth or survival. Thus, a different allelic form at a single locus (particularly a difference that occurs at moderate to high frequencies in a multitude of environments) would not be expected to have a large effect even if it were differentially involved in the expression of a measurable quality. Protein polymorphisms having large effects, such as sickle cell hemoglobin, do exist but they are very exceptional.

We, therefore, do not expect to see any measurable effect of a given allelic form of a protein on any specific quantitative trait for most of the polymorphic loci that we are detecting. However, we believe that it is important to look for different effects of allelic proteins to justify the assumption that allelic frequencies of natural populations reflect primarily random processes, and to exclude the use of inadaptable alleles in genetic marking programs.

Table 1. Frequencies of common alleles for LDH-4 and SOD loci in a rainbow trout (populations 1 through 11) and two closely related species; populations 1 through 10 are anadromous rainbow trout (i.e., steelhead). Data sources include: (a) Northwest and Alaska Fisheries Center; (b) Erick Parkinson, University of British Columbia, personal communication; (c) Huzyk and Tsuyuki (1974); (d) J. McIntyre, Oregon State University, personal communication; (e) G. A. E. Gall, University of California, Davis, personal communication; (f) Gall et al., (1976); (g) Allendorf (1975); (h) Utter and Hodgins (1972); (i) Wilmot (1974).

	Allelic Frequency		Number of Collections	Total Number of Individuals
	LDH-4 (100)	SOD (100)		
1. Naha River (Southeastern Alaska) ^a	.86	.64	1	7
2. Skeena River (Babine and Zymoet Rivers) ^b	.97	.96	2	37
3. Fraser River, upper ^{b,c}	.51	1.00	8	400
4. Fraser River, lower ^b	.91	.90	2	50
5. Puget Sound ^g	.93	.86	5	800
6. Washington Coast ^g	.88	.64	9	300
7. Columbia River, lower ^d	.84	.69	7	400
8. Columbia River, upper ^g	.43	.93	6	280
9. Oregon Coast ^d	.93	.71	11	1000
10. California Coast ^d	.98	.80	2	220
11. Non-migratory hatchery rainbow trout ^h	1.00	.64	3	150
12. Red band trout ^{e,i}	1.00	.93	3	131
13. Golden trout ^f	1.00	.93	2	102

supported by the additional data contained here, that neither the tendency toward anadromy, nor the timing of upstream migration of anadromous fish are characteristics of distinct evolutionary lines. Allelic frequencies of both resident and migratory populations of a particular region were invariably similar (e.g., Thompson River steelhead and Kamloops rainbow trout) while summer and winter run steelhead of a particular coastal stream tended to resemble one another more than they resembled populations of adjacent drainages (Thorgaard, proceedings of this symposium). Thus, the terms "steelhead," and "summer-run," and "winter-run" are useful for the description of life history patterns of a particular population but do not imply close evolutionary relationships among populations of different areas.

The upper Columbia and Fraser River populations are also distinctly separated from the red band and golden trout populations. This separation indicates different evolutionary lineages for these two groups that predate at least the last period of glaciation.

The occurrence of allelic frequencies typical of coastal populations in both upstream and downstream populations sampled from the Skeena River drainage indicates that the inland group does not extend to this drainage. This group may very well, then, be restricted to the regions of the upper Columbia and Fraser Rivers although fish in some unsampled drainages cannot be excluded, such as rainbow trout natively occurring east of the Continental Divide in the Athabasca River of Alberta (MacCrimmon 1971).

The previously mentioned restrictions limiting conclusions drawn from Figure 2 preclude further positive statements regarding the inference of additional subgroupings from these data. For instance, it would be inappropriate to suggest from these data that a closer relationship exists between the populations of the Skeena River and the red band and golden trouts than exists between fish of the Skeena River and the Naha River of southeastern Alaska.

The two major population groups of rainbow trout distinguished by allelic frequency data are outlined in Figure 3. The coastal group has been tentatively extended through Kodiak Island as the result of reports of a sample of steelhead from Kodiak having *LDH-4* and *SOD* frequencies typical of the coastal group (Thorgaard, personal communication).

The *LDH-4* and *SOD* data of the steelhead populations from the Marion Forks hatchery of the North Santiam River in Oregon were sufficiently different from all other populations examined to warrant separate consideration and to preclude their

inclusion in either the coastal or the inland population groups. The frequencies of the common alleles based on a sampling of 50 individuals were:

LDH-4 (100) - .640
SOD (100) - .380

The *LDH-4* frequency, though higher than the average of either inland group, is much lower than any of the coastal frequencies. The *SOD* frequency is much lower than the average frequency of either the inland or the coastal group. These comparisons are graphically presented in Figure 4. (Similar allelic frequencies for this population have been independently observed by personnel of Oregon State University, who have also indicated that the hatchery run was derived from native fish of this area (McIntyre, personal communication). The intermediate frequency of the *LDH-4* alleles from the Marion Forks population coupled with the proximity of this population to the Deschutes River drainage, where allelic frequencies are typical of inland populations (Allendorf 1975), suggest an ancestral contribution from the Deschutes River drainage via possibly a formerly existing direct connection between the two drainages. However, such an explanation does not account for the anomalous *SOD* frequencies. Alternatively, the frequencies of both loci may be a reflection of one or more population "bottlenecks" in which allelic frequencies can become dramatically perturbed through a transiently extreme reduction of population size. A detailed examination of natural populations of this area should be extremely interesting in light of these data.

The population groups depicted in Figure 2 represent a valid, but hazy, first approximation of the breeding structure of these populations, analogous to examination of celestial areas with the unaided eye contrasted with various powers of telescopic magnification. A more detailed structure is certain to emerge as more populations are examined with a greater number of genetic variants. The cytogenetic data of Thorgaard (proceedings of this symposium) give a better focus to the sub-structure of the coastal group. Direct evidence for a sub-structure of the inland group was recently found in a comparison of three populations of the upper Columbia River drainage through differing frequencies of peptidase (PEP) variants (Milner, proceedings of this symposium). The pattern of variation (Figure 5) suggests a separation of populations from the Columbia River above its confluence with the Snake River and those of the Snake River drainage, although more data points are clearly needed before the generality of such a separation can be assumed.

Table 2. Similarity matrix of Rogers' measure of genetic similarity based on allelic frequencies of populations listed in Table 1.

	1	2	3	4	5	6	7	8	9	10	11	12
1. Naha River												
2. Skeena River	78											
3. Fraser River, upper	64	95										
4. Fraser River, lower	84	94	75									
5. Puget Sound	85	93	72	97								
6. Washington Coast	99	79	63	85	86							
7. Columbia River, upper	96	80	68	86	82	95						
8. Columbia River, lower	64	71	92	74	71	63	67					
9. Oregon Coast	93	85	64	88	92	94	94	64				
10. California Coast	86	91	66	91	94	87	87	66	93			
11. Hatchery rainbow trout	93	82	57	82	91	94	89	57	93	91		
12. Red band trout	75	96	75	90	89	76	76	68	82	81	82	
13. Golden trout	78	97	72	94	93	79	80	61	85	92	80	96

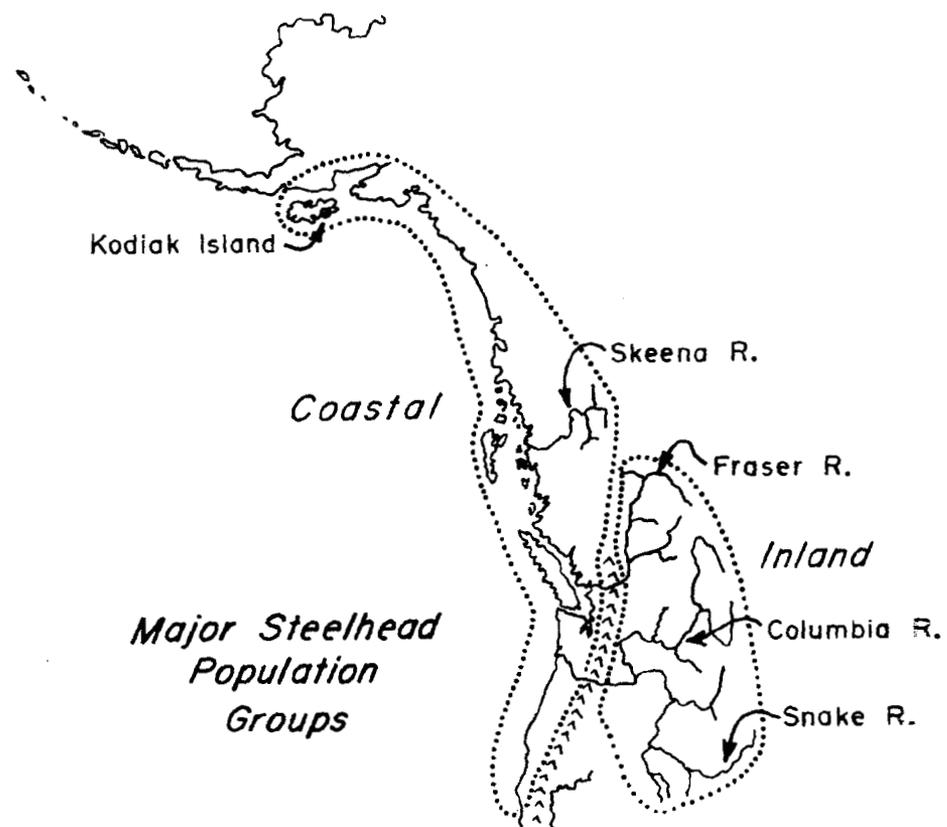


FIGURE 3. Major steelhead population groups based on LDH-4 and SOD data.

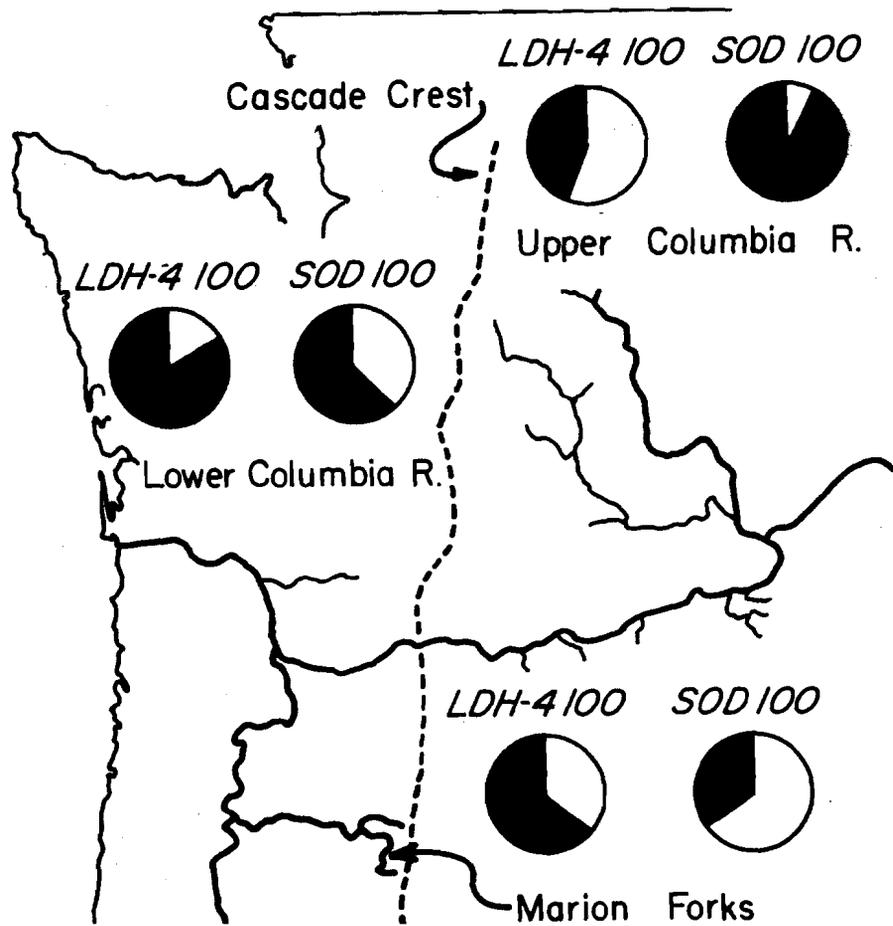


FIGURE 4. Frequencies of LDH-4 (100) and SOD (100) alleles in the upper and lower Columbia River and the Marion Forks hatchery.

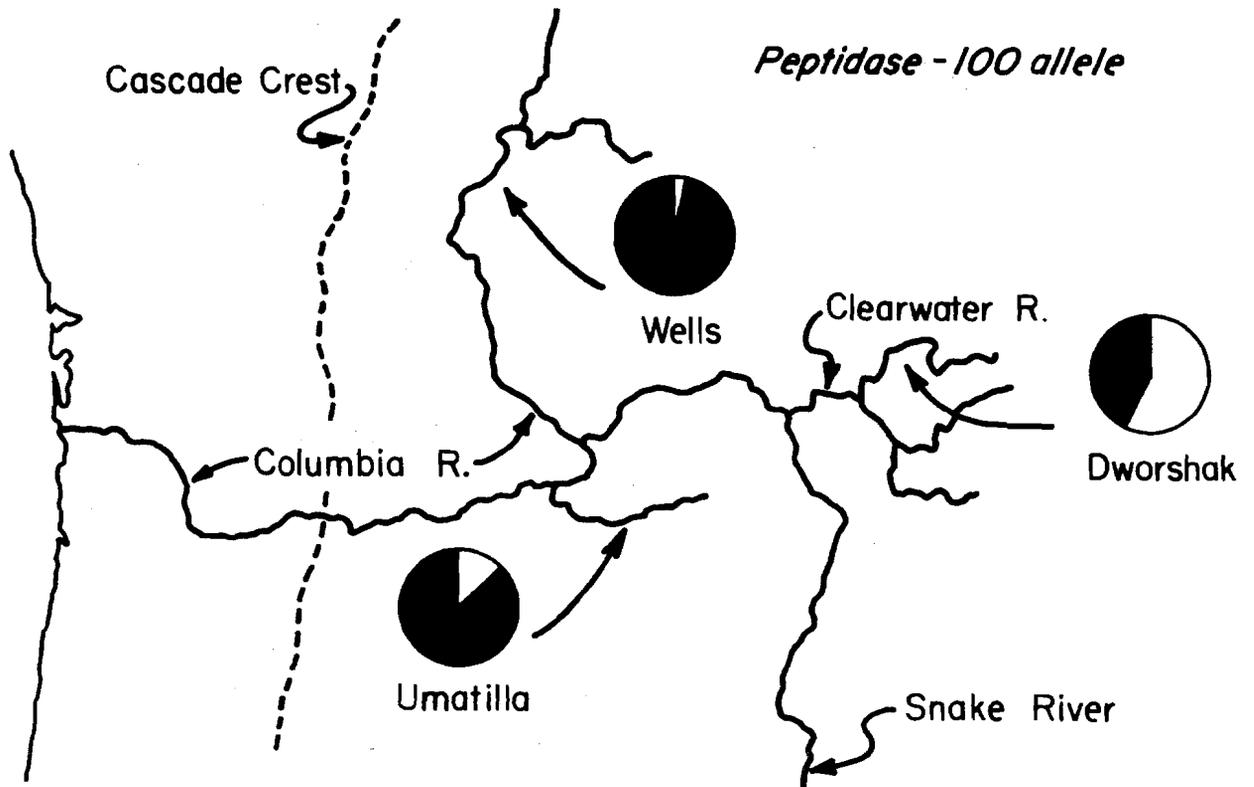


FIGURE 5. Frequencies of the PEP (100) allele from three areas of the upper Columbia River drainage.

The limit to which genetic differences can be used to define natural populations of rainbow trout varies according to the amount of natural or man-induced straying that has occurred between different populations, and the time that has elapsed since significant gene flow has occurred. The probability of defining two spawning populations occupying adjacent areas of a particular stream on the basis of naturally occurring genetic differences is remote unless geographical or physiological barriers exist precluding long term gene flow between these populations. It is therefore highly unlikely that a hatchery population derived from the natural population of an area can be distinguished from the parent population on the basis of naturally occurring genetic markers. Such differences can readily be created artificially, however, through the selection of uncommon genotypes in the establishment of the hatchery population (Allendorf and Utter, in press). A Breeding program of this kind is being successfully applied to the study of steelhead populations of the Kalama River in Washington (Utter *et. al.* 1976; Crawford, proceedings of this symposium). Proper implementation of this type of breeding program significantly broadens the capability for defining and managing steelhead populations through the use of genetic variation.

Studies of the genetic structure of populations of other salmonid species are in progress by persons associated with our group. These studies are designed to aid in the solution of a wide variety of management problems unique to each species (Utter, Allendorf, and May 1976; Seeb, and Wishard 1977; Grant 1977).

SUMMARY

Data were compiled from many sources to compare the genetic structure of steelhead populations on the basis of the frequencies of allelic variants at two loci, *LDH-4* and *SOD*. The data revealed two major population groups: an inland group comprising both anadromous and non-anadromous populations of the Fraser and Columbia River drainages east of the Cascade Crest, and a coastal group extending from Alaska to California. The inland populations were genetically distinct from the California golden and red band trouts. Limited additional data indicated that further structuring of both groups will inevitably be found as more loci and populations are studied. The ultimate resolving power of the method for distinguishing natural populations is dependent on the amount of gene flow that is occurring between populations and on the time that has elapsed since significant gene flow has occurred. The capability for distinguishing cultured populations is not limited by this restriction and may be significantly enhanced through the selection of specific genotypes in breeding programs.

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