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Biochemical Genetic Variation of Chinook Salmon Stocks of the Mid-Columbia River

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BIOCHEMICAL GENETIC VARIATION OF CHINOOK SALMON STOCKS OF THE MID-COLUMBIA RIVER

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ABSTRACT

Chinook salmon (Oncorhynchus tshawytscha) from 19 locations within the Mid-Columbia River drainage area, from Priest Rapids through the Methow River, were tested for biochemical genetic variation using starch gel electrophoresis. Genetic polymorphism was detected at 30 of 40 loci examined. Considerable genetic heterogeneity was observed. Two distinct groupings were apparent by cluster analysis based on genetic distances, and by plottings of allele frequencies for two highly polymorphic loci (Pgk-2 and Sod-1). Although no clear geographic patterns were apparent, all of the samples could be related to hatchery stocks known to contribute to runs of this area. One group was suggested to consist of summer- and fall-run fish supplemented by the production of Wells and Priest Rapids hatcheries, respectively. The other group was suggested to consist of spring-run fish supplemented by the adult chinook salmon returning to Leavenworth and Winthrop hatcheries. A cautious interpretation and application of these results was stressed because of the preliminary nature of the data, and the likelihood of additional genetic isolation among some populations that were not distinguished electrophoretically. Continuation of this study was recommended to examine year-class variation among sampled populations and relationships of unsampled populations, and to seek additional genetic markers for further distinction of genetically isolated groups.

PREFACE

This report fulfills a subcontract issued as a portion of a primary contract between Don Chapman Consultants, Inc., 3180 Airport Way, Boise, Idaho, and the University of Washington School of Fisheries, Seattle. This contract was based on a proposal from William K. Hershberger to Don Chapman Consultants, Inc. dated 16 September 1986 titled "Genetic identification of salmon and steelhead stocks in the mid-Columbia River." Under the terms of this proposal, up to 3,300 chinook salmon (Oncorhynchus tshawytscha), sockeye salmon (O. nerka), and steelhead trout (Salmo gairdneri) collected in tributaries of the mid-Columbia River would be analyzed using electrophoresis by personnel of the College of Fisheries and National Marine Fisheries Service (NMFS). These analyses were to be completed by 30 June 1987. The subcontract to NMFS concerned the analyses of the chinook salmon samples from 19 locations.

CONTENTS

INTRODUCTION

The genetic structure of most economically important fish populations still remains virtually unknown; however, in the last two decades the advent of electrophoretic methodology has provided a powerful tool for genetic studies of populations. Since stock identification of chinook salmon populations of the Columbia River has eluded biologists for years, the present study resorted to use of electrophoresis to identify the genetic structure of mid-Columbia River populations. Tissue samples from 1,214 chinook salmon were analyzed for electrophoretic variability at 40 protein-coding gene loci. The genetic variation observed among the 19 collection locations is discussed on the basis of geographic, seasonal, and historical variables. These findings provide a foundation for additional sampling and analysis of chinook salmon populations of the mid-Columbia River.

MATERIALS AND METHODS

Gel electrophoresis followed methods outlined in Aebersold et al. (in press). Three buffer systems were used: (1) 1:4 dilution of electrode solution, electrode, Tris (0.18M), boric acid (0.01 M), EDTA (0.004 M), pH 8.5 (Boyer et al. 1963); (2) 1:20 dilution of electrode solution, electrode, citric acid (0.04 M), adjusted to pH 7.0 with N-(3-aminopropyl)-morpholine (Clayton and Tretiak 1972) with EDTA (0.01 M); and (3) Tris (0.03 M), citric acid (0.005 M), 1% (final conc.) electrode buffer, pH 8.4, lithium hydroxide (0.06 M), boric acid (0.3 M), EDTA (0.01 M), pH 8.0 (modified from Ridgway et al. 1970). Procedures used to visualize enzyme activity on gel surfaces are described in Aebersold et al. (in press). Enzyme systems and loci detected are listed in Table 1. Nomenclature for loci and alleles was modified from a convention outlined in Allendorf and Utter (1979).

Chinook salmon were collected by the primary contractor and forwarded to NMFS from the School of Fisheries. Sampling data are listed in Table 2. Data for precise locations of some samples were not available. The area of sampling (except for Priest Rapids) is illustrated in Figure 1.

Data were entered into computer data files using a EDEP program (Milner et al. 1986). Analyses were carried out by the computer programs in BIOSYS (Swofford and Selander 1981) for listing of allele frequencies, testing of Hardy-Weinberg equilibrium, construction of a UPGM dendrogram (Sneath and Sokal 1973) from a matrix of genetic distances (Nei 1978), and calculation of fixation indices (Wright 1965).

RESULTS

Data were collected from a total of 40 genetic loci (see Table 1). Genetic variation was detected at 30 loci. Allele frequencies are tabulated for each of the 19 samples in the Appendix.

A total of 276 tests for Hardy-Weinberg equilibrium were made. These tests included 28 of the 30 polymorphic loci; Gpi-2 and Gpi-H were excluded because heterozygous phenotypes were not detected. Tests were made where two or more alleles were detectable at one of these loci among individuals of a particular sample. Twelve of these tests were significant at the 5% level. Since this number of deviations would be expected to occur by chance; it was concluded that forces having an influence on Hardy-Weinberg equilibrium (e.g., selection, immigration, population reduction) were not strongly affecting these loci at the times and locations of collection.

Table 1.--Background information for protein coding loci. Sequence of variant alleles corresponds to listing in Appendix. Tissues tested included eye (E), heart (H), liver (L), and muscle (M).

Protein name and Enzyme Commission number	Locus	Mobility of Variant alleles 2	3	4	5	Tissue	Buffer system
Aconitate hydratase (4.2.1.3)	Ah-4	86	116	-	-	L	2
Adenosine deaminase (3.5.4.4)	Ada-1	83	-	-	-	E,H,M	1
Alcohol dehydrogenase (1.1.1.1)	Adh	-170	-52	-	-	L	1,2
Aspartate aminotransferase (2.6.1.1)	Aat-1	-	-	-	-	H,M	1
	Aat-2	85	105	-	-	H,M	1
	Aat-3	90	-	-	-	E	1
	Aat-4	130	63	-	-	L	1
Dipeptidase (3.4.13.11)	Gl-1	90	76	-	-	E,H,M	1,3
Fructose-biphosphate aldolase (4.1.2.13)	Ald-3	-	-	-	-	E	2
	Ald-4	110	-	-	-	E	2
Glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12)	Gap-1	-	-	-	-	M	2
	Gap-2	112	-	-	-	M	2
	Gap-5	-	-	-	-	E	2
	Gap-6	-	-	-	-	E	2
Glucose-6-phosphate isomerase (5.3.1.9)	Gpi-1	-	-	-	-	M	3
	Gpi-2	60	-	-	-	M	3
	Gpi-3	105	-	-	-	M	3
	Gpi-H	*	-	-	-	M	3
Glutathione reductase (16.4.2)	Gr	85	-	-	-	L,H	1
Hydroxyacylglutathione hydrolase (3.1.2.6)	Hagh	143	-	-	-	L	1
Isocitrate dehydroge-[a]	Idh-2	-	-	-	-	E,M	2
nase (1.1.1.42)	Idh-3	-	74	-	-	E,L	2
	Idh-4	127	-	-	50	E,L	2
Lactate dehydrogenase (1.1.1.27)	Ldh-4	112	134	-	-	E,L,M	3
	Ldh-5	90	-	-	-	E	3
Leucyl-tyrosine peptidase (3.4.11.-)	Lt	110	-	-	-	E,M	1
Malate dehydrogenase[b]	Mdh-1	-	-	-	-	L,H,M	2
	Mdh-2	120	-	-	-	L,H,M	2
(1.1.1.37)	Mdh-3	-	-	-	-	L,H,M	2
	Mdh-4	121	70	-	-	L,H,M	2
Mannose-6-phosphate isomerase (5.3.1.8)	Mpi	109	95	-	-	E,L	1
Phosphogluconate dehydrogenase (1.1.1.44)	Pgd	-	-	-	-	E,L	2

Table 1.--Continued.

Protein name and Enzyme Commission number	Locus	Mobility of Variant alleles 2	3	4	5	Tissue	Buffer system
Phosphoglycerate kinase (2.7.2.3)	Pgk-2	90	-	-	-	E,L,M	2
Phenylalanyl-proline peptidase (3.4.11.-)	Php-2	107	-	-	-	E,M	1
Superoxide dismutase (1.15.1.1)	Sod-1	-260	580	-	-	L,M	1
Triosephosphate isomerase (5.3.1.1)	Tpi-1	-	-	-	-	E,M	1
	Tpi-2	-	-	-	-	E,M	1
	Tpi-3	104	96	-	-	E,M	1
Tripeptide aminopeptidase (3.4.11.4)	Tpep	130	-	-	-	E,M	3

* Allele detected by absence of Gpi 1,3 heterodimer.

[a] Loci separated on the basis of differential mobilities of allelic proteins according to criteria of Shaklee and Phelps (Wash. Dep. Fisheries, personal communication, Feb. 1987).

[b] All variations of each isolocus assumed to reside in Mdh-2 and Mdh-4, respectively.

Table 2.--Collection data for samples. Data for run time are reliable only for adult fish, and were estimated by sampler when given on juvenile fish.

Sample no.	Location	Number of fish	Collection date(s)	Run time	Wild or hatchery	Adult or juvenile
1	Nason Creek, upper	53	11-10	Spring	Wild	Juvenile
2	Nason Creek, lower	47	11-10	Spring	Wild	Juvenile
3	Wenatchee R., upper	57	10-20 10-23	Summer	Wild	Adult
4	Wenatchee R., middle	58	10-17 10-20	Summer	Wild	Adult
5	Wenatchee R., Tumwater	65	10-1	-	Wild	Juvenile
6	Wenatchee R., Leavenworth	100	8-13 to 8-27	Spring	Hatchery	Adult
7	Wenatchee R., lower	50	10-29 10-31	Summer	Wild	Adult
8	Wenatchee R.	102	9-24	-	Wild	Juvenile
9	Wenatchee R.	70	10-1	-	Wild	Juvenile
10	Wenatchee R.	60	10-5	-	Wild	Juvenile
11	Priest Rapids	50	10-27	Fall	Hatchery	Adult
12	Entiat R.	94	10-16	Spring	Wild	Juvenile
13	Wells	61	10-16 10-22	Summer	Hatchery	Adult
14	Methow R.(Carlton)	45	11-3	Summer	Wild	Juvenile
15	Twisp R.	50	11-6	Spring	Wild	Juvenile
16	Winthrop	100	9-2 9-9	Spring	Hatchery	Adult
17	Chewack R.	58	11-6	Spring	Wild	Juvenile
18	Early Winters Cr.	56	11-7	Spring	Wild	Juvenile
19	Lost R.	50	11-7	Spring	Wild	Juvenile

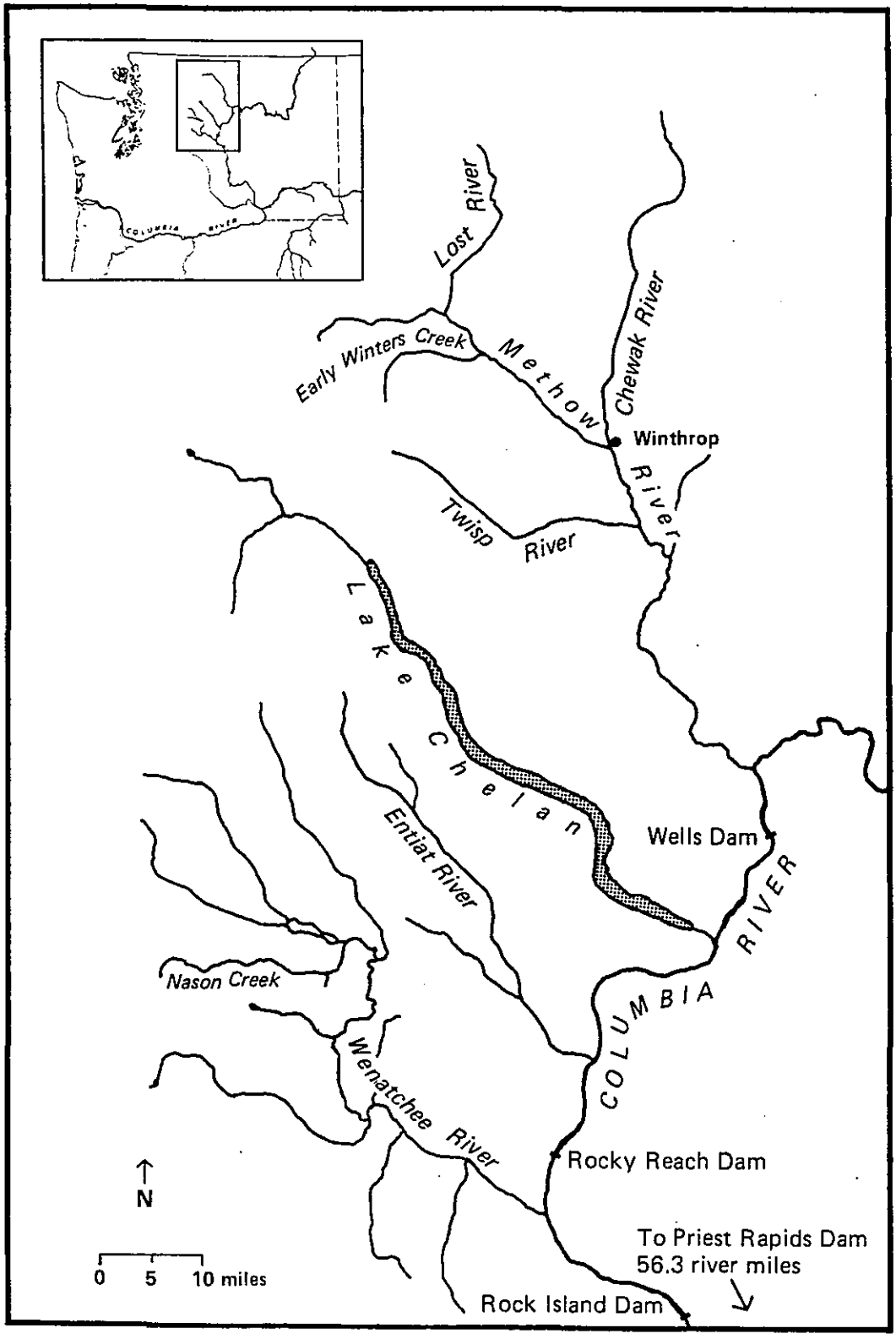

Figure 1.--Area of study for chinook salmon in the Columbia River Basin.

Nine of the 30 polymorphic loci had frequencies of the common allele less than 0.95 in one or more samples. Data for these loci are summarized over all samples in Table 3. The range of allelic variation was substantial for each locus, extending from a minimum of 0.80-1.00 for Hagh to a maximum of 0.02-0.97 for Pgk-2. The heterogeneity of the polymorphism of these nine loci was confirmed by contingency tests where significance levels exceeded 0.001 for each locus. The heterogeneity of the allelic distribution for these loci among the 19 samples was further demonstrated by their fixation indices which measure departures from a single panmictic population (i.e., Fst=0). The Pgk-2 locus, which had the greatest allele frequency range, also had the highest Fst value of 0.306.

Average heterozygosity ($\bar{H}$) values for each sample (listed roughly in a linear manner from the upper Wenatchee drainage downstream to the Columbia River, then upstream into the Methow drainage) were:

	$\bar{H}$		$\bar{H}$
(1) Upper Nason	0.043	(11) Priest Rapids	0.058
(2) Lower Nason	0.053	(12) Entiat	0.058
(3) Wenatchee, upper	0.080	(13) Wells	0.076
(4) Wenatchee, mid.	0.087	(14) Methow, Carlton	0.044
(5) Wenatchee, Tum.	0.047	(15) Twisp	0.046
(6) Leavenworth	0.041	(16) Winthrop	0.045
(7) Wenatchee, lower	0.086	(17) Chewack	0.057
(8) Wenatchee, 9-24	0.080	(18) Early Winter	0.055
(9) Wenatchee, 10-1	0.066	(19) Lost River	0.057
(10) Wenatchee, 10-5	0.058		

Mean heterozygosity for all 19 samples was 0.060. All populations of the Methow River had $\bar{H}$ values below the mean.

The dendrogram constructed from pairwise measures of genetic distances (Fig. 2) forms two clusters at a genetic distance greater than 0.01. Cluster B contains Wenatchee River samples plus those of Priest Rapids and Wells. Cluster A contains all Methow River samples plus some samples from the Wenatchee River. A plotting of samples based on frequencies of common alleles of the Sod

Table 3.--Range of frequency of common (100) allele and fixation index (Fst) for nine polymorphic loci having frequencies less than 0.95 for 100 allele in one or more populations.

Locus	Range of 100 allele	Fst
Ah-4	0.74 - 1.00	0.106
Hagh	0.80 - 1.00	0.070
Idh-3	0.67 - 1.00	0.122
Idh-4	0.57 - 1.00	0.171
Mpi	0.58 - 0.95	0.101
Lt	0.56 - 1.00	0.167
Pgk-2	0.02 - 0.64	0.306
Sod-1	0.44 - 0.84	0.083
Tpep	0.70 - 0.99	0.073

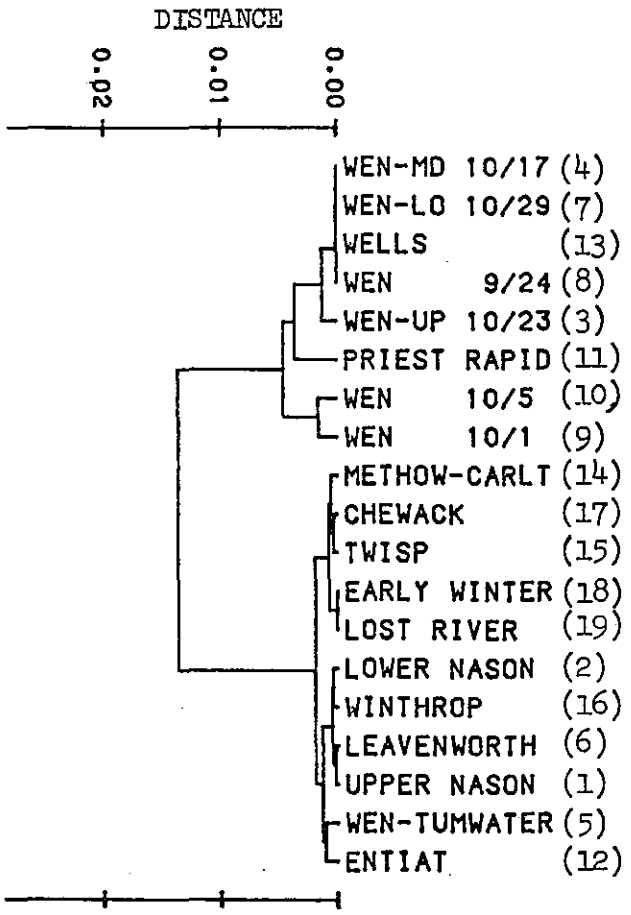

Figure 2.--UPGM dendrogram based on pairwise measurements of unbiased genetic distance (Nei 1978). Two major clusters (A and B) are indicated beyond a genetic distance of 0.01.

and the Pgk-2 loci (Fig. 3) indicates that these two loci are major contributors to the clustering of the dendrogram; three distinct groups are formed that are identical to the three major clusters of the dendrogram.

DISCUSSION

These data identify considerable genetic heterogeneity among chinook salmon populations of the mid-Columbia River. A comparison of allele frequencies with those from a much broader sampling of chinook salmon from British Columbia through California (Utter et al. in prep.) indicates a similar range for five polymorphic loci common to both studies (Ah-4, Mpi, Pgk-2, Sod-1, Tpep). The mean range for these loci was 0.371 in the present study and 0.614 for the more extended sampling. A dendrogram of the extended data set identified eight clusters diverging at a genetic distance greater than 0.01 contrasted with the two clusters within the present sampling area. It appears that much of the total genetic variation of this species in the Pacific Northwest is represented within this mid-Columbia sampling area.

One or more possibilities might be used to explain these observed allelic distributions. These possibilities include:

(1) The allele frequencies reflect ancestral runs of these areas prior to the construction of dams and intensive non-Indian fisheries.

(2) The distributions are a transitional array of genotypes fluctuating widely over time and distance as a reflection of widespread and continuing transplantation of stocks from many areas and resultant strayings.

(3) The distributions represent finely tuned combinations of protein-coding genes; each genotype is highly sensitive to the rigors of specific environments and thus highly transitory temporally and spatially.

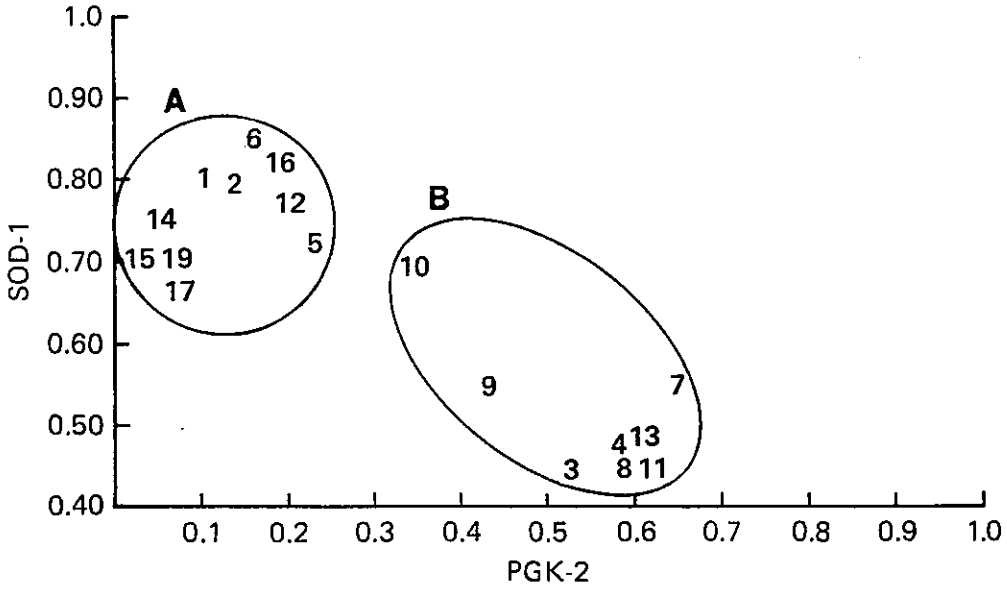

Figure 3. --Plot of frequencies of common (100) alleles for Pgk-2 and Sod-1 loci for mid-Columbia River chinook salmon.

(4) The distributions reflect exaggerated genetic drift resulting from sharply reduced populations sizes (i.e., "bottlenecks") and limited gene flow.

Each of these possibilities or a combination could be operating with reasonable intensity. Sorting out these interactions would be a hopeless chore on the basis of the available data. However, the effects of two of these possibilities can be considered to be minimal. Strong selection of the electrophoretically detected allelic variation (possiblity 3) appears unlikely based on a primarily geographic basis for allelic distributions in chinook salmon (Utter et al. in prep., Gharrett et al. 1987); a general case for minimal selection of electrophoretic variants in anadromous salmonids is presented in Utter et al. (1980). Dramatic population reductions of chinook salmon runs in the mid-Columbia River is indisputable. Nevertheless, the effects of possiblity 4 are assumed (for the moment at least) to be minimal because of the counteracting effects of transplantation and straying (possibility 2).

Attempts to explain the observed allelic distribution will therefore assume that this variation reflects ancestral native genotypes with some influences from introduced populations. First, we will consider that genetic structure of these populations is based solely on ancestral relationships. Genetic divergence among populations could result either from geographic or temporal isolation (it has been postulated above that geographic isolation is the primary basis for genetic isolation of chinook salmon over broad geographic ranges). The observed variation does not cleanly conform to either geographic or temporal lines. Geographic conformation is suggested by the absence of Methow River samples in Cluster B. However, samples from both the Wenatchee and Methow rivers fall into Cluster A. Time of return could be determined only for the seven adult samples; both summer and fall run times were represented in Cluster B.

In the absence of gene flow through transplantation, two ancestral lineages could be postulated for Clusters A and B. Under this scenario, Cluster B could represent ancestral fall- and summer-run populations. Natural populations of this major group are supplemented by hatchery rearing at Priest Rapids (for the fall-run component) and at Wells (for the summer-run component); gene flow between these two hatchery sources is discussed in Utter et al. (in prep.). Cluster A would represent spring-run populations isolated from those of Cluster B by different spawning times and, possibly, areas.

The above model is at least partially invalidated since transplantations of fish from exogenous sources have been made within the study area. Specifically, both the Leavenworth and Winthrop hatcheries have currently (as well as in the past) released fish predominantly of Carson Hatchery origins (Howell et al. 1985). The Carson stock, in turn, originated in the 1950's from downstream interceptions of spring-run fish destined for different areas in the Columbia River drainage upstream. The persistence of the Carson gene pool in the Leavenworth and Winthrop stocks is confirmed by (1) similarities in this study, (2) similar allele frequencies recorded for the Leavenworth stock in this study and in Utter et al. (in prep.), and (3) by the statistically indistiguishable allele frequencies of the Carson and Leavenworth samples in Utter et al. (in prep.). The influence of these two hatchery stocks on the remaining populations of Cluster A is uncertain. There is a distinct possibility that wild stocks of this group may have originated or been supplemented by gene flow from hatchery stocks of Carson lineage (a potential exception is the juvenile Methow-Carlton sample which was estimated to be of summer-run origin). The same reasoning extends to populations of Cluster B, with the difference being that the origins of hatchery stocks were primarily of native fish of the study area.

This report ends with words of caution. The suggested possibility of two major population groups within the study area that may have originated (and are perhaps being maintained) through hatchery operations is speculation and is not intended as a justification for intensifying hatchery transplantations within the area. First, the data are too preliminary to exclude the existence of additional distinct populations that have neither been sampled, nor detected with the present array of polymorphic loci.

Second, each population (be it hatchery or wild) that is perpetuated by fish returning to a particular area is a valuable resource. Such populations are maintained by fish that have successfully completed the requirements for survival, migration, and reproduction at a particular location and time. Genes affecting biological fitness of these populations are not usually represented among those expressed electrophoretically (which are much more useful for tracing ancestries). Thus, two populations that are electrophoretically very similar or indistinguishable may differ markedly with regard to genes that are strongly related to fitness (e.g., Idaho steelhead and "redband" trout, Wishard et al. 1984). The identification of three major groups summarizes the positive data of this study; each group should be treated as a genetically distinct entity. However, it would be highly inappropriate to consider populations within a group (e.g., the Lower Nason in the Wenatchee drainage and the Winthrop in the Methow drainage) as genetically identical (and thus subject to management as a single unit) solely on the basis of similar frequencies of protein-coding genes detected electrophoretically.

These findings justify continuation of this study. Replication for location and time are recommended to study year class variability. A search for additional genetic variation (through protein-coding loci or DNA markers) is

warranted to seek population differences among presently indistinguishable groups.

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APPENDIX

ALLELE FREQUENCIES IN POPULATIONS

LOCUS	POPULATION 1	16	12	11
AAT1				
(N)	48	92	94	50
1	1.000	1.000	1.000	1.000
AAT2				
(N)	48	92	94	50
1	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000
AAT3				
(N)	50	90	94	10
1	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000
AAT4				
(N)	47	55	74	1
1	0.968	0.964	0.939	1.000
2	0.000	0.000	0.000	0.000
3	0.032	0.036	0.061	0.000
ADA1				
(N)	50	80	71	40
1	0.980	0.981	0.986	0.950
2	0.020	0.019	0.014	0.050
ADA2				
(N)	50	78	72	40
1	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000
ADH				
(N)	50	61	92	50
1	0.970	1.000	0.995	1.000
2	0.010	0.000	0.005	0.000
3	0.020	0.000	0.000	0.000
AH4				
(N)	50	89	94	50
1	1.000	0.978	0.989	0.850
2	0.000	0.022	0.011	0.150
ALD3				
(N)	1	55	76	39
1	1.000	1.000	1.000	1.000
ALD4				
(N)	1	55	86	39
1	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000
GAP1				
(N)	50	45	94	40
1	1.000	1.000	1.000	1.000
GAP2				
(N)	50	36	93	20
1	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000
GAP5				
(N)	50	99	94	47
1	1.000	1.000	1.000	1.000
GAP6				
(N)	50	99	94	47
1	1.000	1.000	1.000	1.000
GL1				
(N)	50	95	94	50
1	1.000	1.000	0.995	0.990
2	0.000	0.000	0.005	0.010
3	0.000	0.000	0.000	0.000
4	0.000	0.000	0.000	0.000

LOCUS	POPULATION 1	16	12	11
GPI1				
(N)	50	97	91	50
1	1.000	1.000	1.000	1.000
GPI2				
(N)	50	59	91	50
1	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000
GPI3				
(N)	50	94	90	50
1	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000
GPIH				
(N)	50	58	90	50
1	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000
GR				
(N)	50	98	85	49
1	0.990	0.974	0.976	1.000
2	0.010	0.026	0.024	0.000
3	0.000	0.000	0.000	0.000
HAGH				
(N)	50	60	52	50
1	0.900	0.942	0.981	1.000
2	0.100	0.058	0.019	0.000
IDH2				
(N)	50	97	90	50
1	1.000	1.000	1.000	1.000
IDH3				
(N)	49	100	94	50
1	0.724	0.790	0.819	0.990
2	0.000	0.000	0.000	0.000
3	0.276	0.210	0.181	0.010
IDH4				
(N)	49	99	94	50
1	1.000	0.995	0.894	0.840
2	0.000	0.005	0.005	0.160
3	0.000	0.000	0.000	0.000
4	0.000	0.000	0.000	0.000
5	0.000	0.000	0.101	0.000
LDH4				
(N)	50	100	93	40
1	0.990	0.990	0.995	1.000
2	0.010	0.005	0.005	0.000
3	0.000	0.005	0.000	0.000
LDH5				
(N)	42	81	86	50
1	0.976	0.938	1.000	1.000
2	0.024	0.012	0.000	0.000
TPEP				
(N)	47	84	80	31
1	0.957	0.958	0.969	0.839
2	0.043	0.042	0.031	0.161
LT				
(N)	47	88	94	46
1	1.000	0.989	0.973	1.000
2	0.000	0.011	0.027	0.000
MDH1				
(N)	50	61	94	50
1	1.000	1.000	1.000	1.000

LOCUS	POPULATION 1	16	12	11
MDH2				
(N)	50	100	94	50
1	1.000	1.000	0.995	0.980
2	0.000	0.000	0.005	0.020
MDH3				
(N)	50	61	93	50
1	1.000	1.000	1.000	1.000
MDH4				
(N)	50	100	94	50
1	0.980	0.985	0.910	0.980
2	0.020	0.015	0.090	0.020
3	0.000	0.000	0.000	0.000
MPI				
(N)	49	99	93	37
1	0.918	0.838	0.769	0.838
2	0.082	0.136	0.231	0.162
3	0.000	0.025	0.000	0.000
PGD				
(N)	50	100	94	50
1	1.000	1.000	1.000	1.000
PGK2				
(N)	37	99	94	50
1	0.095	0.182	0.197	0.610
2	0.905	0.818	0.803	0.390
PHP2				
(N)	50	82	89	45
1	0.980	1.000	1.000	0.933
2	0.020	0.000	0.000	0.067
SOD1				
(N)	49	100	93	50
1	0.806	0.835	0.769	0.440
2	0.194	0.165	0.231	0.560
3	0.000	0.000	0.000	0.000
TPI1				
(N)	50	98	94	50
1	1.000	1.000	1.000	1.000
TPI2				
(N)	50	98	94	50
1	1.000	1.000	1.000	1.000
TPI3				
(N)	1	40	40	1
1	1.000	0.913	0.875	1.000
2	0.000	0.088	0.125	0.000
3	0.000	0.000	0.000	0.000

ALLELE FREQUENCIES IN POPULATIONS
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	POPULATION														
LOCUS	4	3	7	14	13	2	17	18	19	8	6	10	9	5	15
AAT1															
(N)	40	35	42	40	60	45	58	52	49	101	95	60	70	65	28
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT2															
(N)	40	35	46	40	60	45	58	52	48	101	95	60	70	65	28
1	1.000	1.000	1.000	0.988	1.000	0.956	0.991	1.000	0.979	1.000	1.000	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.013	0.000	0.044	0.009	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000
AAT3															
(N)	45	44	50	40	57	37	58	52	47	99	79	44	70	63	48
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.989	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000
AAT4															
(N)	35	1	20	1	28	32	37	39	39	89	82	26	57	51	36
1	1.000	1.000	1.000	1.000	1.000	0.969	0.932	0.962	0.936	1.000	0.976	0.962	0.974	0.980	0.958
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.031	0.068	0.038	0.064	0.000	0.000	0.038	0.026	0.020	0.042
ADA1															
(N)	58	57	50	40	60	44	58	52	48	102	88	59	62	61	50
1	0.991	0.991	1.000	1.000	1.000	0.920	0.940	0.952	0.948	0.990	0.966	1.000	0.992	0.984	0.940
2	0.009	0.009	0.000	0.000	0.000	0.080	0.060	0.048	0.052	0.010	0.034	0.000	0.008	0.016	0.060
ADA2															
(N)	58	57	50	40	61	40	40	40	49	95	90	50	67	63	50
1	1.000	1.000	1.000	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ADH															
(N)	1	1	1	1	1	45	58	52	49	100	95	60	70	62	50
1	1.000	1.000	1.000	1.000	1.000	0.900	0.983	0.990	1.000	0.990	1.000	0.975	0.979	0.944	0.980
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.025	0.007	0.008	0.000
3	0.000	0.000	0.000	0.000	0.000	0.100	0.017	0.010	0.000	0.005	0.000	0.000	0.014	0.048	0.020
AH4															
(N)	56	38	49	40	56	45	58	52	48	99	100	60	69	60	46
1	0.804	0.842	0.827	1.000	0.741	1.000	0.991	1.000	0.979	0.783	1.000	0.858	0.884	0.967	1.000
2	0.196	0.158	0.173	0.000	0.259	0.000	0.009	0.000	0.021	0.217	0.000	0.142	0.116	0.033	0.000
ALD3															
(N)	48	42	16	40	20	45	58	52	9	54	93	30	1	1	1
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ALD4															
(N)	58	42	17	40	20	45	58	52	9	58	93	30	1	1	1
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.991	1.000	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000

	POPULATION														
LOCUS	4	3	7	14	13	2	17	18	19	8	6	10	9	5	15
GAP1															
(N)	1	1	1	40	1	40	40	40	49	102	42	60	67	65	50
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAP2															
(N)	1	1	1	40	1	40	40	40	49	102	42	60	67	65	50
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.977	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000
GAP5															
(N)	58	57	50	40	21	45	58	52	49	99	96	53	70	65	22
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAP6															
(N)	58	57	50	40	21	45	58	52	49	99	96	53	70	65	22
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GL1															
(N)	49	51	49	40	59	40	58	52	49	100	89	59	70	63	41
1	0.949	0.980	0.969	1.000	1.000	0.963	0.957	0.952	1.000	0.990	0.989	0.983	0.986	0.960	0.951
2	0.041	0.020	0.031	0.000	0.000	0.025	0.043	0.000	0.000	0.010	0.011	0.017	0.014	0.040	0.049
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.010	0.000	0.000	0.000	0.000	0.013	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GPI1															
(N)	58	52	49	40	61	44	58	52	49	92	94	47	70	65	50
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI2															
(N)	58	52	49	40	61	44	58	52	49	92	94	50	70	65	44
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.989	1.000	1.000	1.000	0.985	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.015	0.000
GPI3															
(N)	58	52	49	40	61	44	58	52	49	92	94	50	70	65	50
1	0.983	0.962	0.990	1.000	0.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	0.017	0.038	0.010	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GPIH															
(N)	58	53	50	40	61	44	58	52	49	92	94	50	70	65	44
1	0.931	0.981	0.960	1.000	0.934	1.000	1.000	1.000	1.000	0.978	1.000	1.000	1.000	0.985	1.000
2	0.069	0.019	0.040	0.000	0.066	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.015	0.000
GR															
(N)	59	54	47	40	60	40	58	52	49	100	87	50	70	62	48
1	0.973	1.000	0.968	1.000	0.958	1.000	1.000	1.000	1.000	0.940	1.000	0.980	0.993	0.984	0.990
2	0.027	0.000	0.032	0.000	0.042	0.000	0.000	0.000	0.000	0.015	0.000	0.020	0.007	0.016	0.010
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000
HAGH															
(N)	58	52	50	40	39	37	58	51	45	56	92	58	68	61	36
1	1.000	1.000	1.000	0.938	1.000	0.919	0.888	0.804	0.822	0.991	0.935	1.000	0.912	0.992	0.972
2	0.000	0.000	0.000	0.063	0.000	0.081	0.112	0.196	0.178	0.009	0.065	0.000	0.088	0.008	0.028

	POPULATION														
LOCUS	4	3	7	14	13	2	17	18	19	8	6	10	9	5	15
IDH2															
(N)	58	57	49	40	61	45	58	52	49	56	97	60	70	65	50
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IDH3															
(N)	39	40	49	40	61	45	58	52	49	61	92	59	70	63	46
1	1.000	1.000	1.000	0.600	1.000	0.833	0.569	0.750	0.673	0.975	0.761	0.856	0.914	0.857	0.674
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.400	0.000	0.167	0.431	0.250	0.327	0.025	0.239	0.144	0.086	0.143	0.326
IDH4															
(N)	39	40	49	40	61	44	58	52	48	61	92	59	70	65	46
1	0.833	0.825	0.806	0.988	0.844	1.000	1.000	1.000	1.000	0.852	1.000	0.949	0.893	0.985	1.000
2	0.167	0.175	0.194	0.013	0.156	0.000	0.000	0.000	0.000	0.148	0.000	0.051	0.107	0.015	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH4															
(N)	58	56	50	40	61	39	58	52	49	100	95	60	70	65	50
1	1.000	0.991	1.000	0.988	1.000	0.987	0.966	0.923	0.959	1.000	0.995	0.992	0.993	0.985	1.000
2	0.000	0.009	0.000	0.013	0.000	0.013	0.034	0.077	0.041	0.000	0.005	0.008	0.007	0.015	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH5															
(N)	56	55	50	38	60	37	58	52	49	102	99	60	70	65	48
1	0.991	0.991	0.960	1.000	0.975	1.000	1.000	1.000	1.000	0.946	0.995	1.000	0.986	1.000	1.000
2	0.009	0.009	0.040	0.000	0.025	0.000	0.000	0.000	0.000	0.054	0.005	0.000	0.014	0.000	0.000
TPEP															
(N)	55	54	49	38	59	40	58	52	49	97	93	60	67	57	34
1	0.773	0.759	0.704	0.961	0.703	0.988	0.905	0.904	0.908	0.753	0.919	0.925	0.858	0.868	0.941
2	0.227	0.241	0.296	0.039	0.297	0.013	0.095	0.096	0.092	0.247	0.081	0.075	0.142	0.132	0.059
LT															
(N)	56	51	40	40	55	40	58	52	40	101	95	27	61	65	48
1	0.759	0.559	0.788	0.975	0.818	0.938	0.966	0.981	0.988	0.762	0.911	0.833	1.000	1.000	1.000
2	0.241	0.441	0.213	0.025	0.182	0.063	0.034	0.019	0.013	0.238	0.089	0.167	0.000	0.000	0.000
MDH1															
(N)	58	57	50	40	61	44	58	52	49	98	100	59	70	65	45
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH2															
(N)	58	57	50	40	61	44	58	52	49	96	100	59	70	65	45
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.992	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000
MDH3															
(N)	58	57	50	40	61	44	56	52	48	102	100	59	69	65	50
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

	POPULATION														
LOCUS	4	3	7	14	13	2	17	18	19	8	6	10	9	5	15
MDH4															
(N)	58	57	50	40	61	44	56	52	48	100	100	59	69	65	50
1	0.940	0.912	0.910	0.913	0.910	0.977	0.911	0.923	0.969	0.940	0.975	0.941	0.920	0.908	0.990
2	0.034	0.044	0.060	0.088	0.033	0.023	0.089	0.077	0.031	0.040	0.025	0.059	0.051	0.092	0.010
3	0.026	0.044	0.030	0.000	0.057	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.029	0.000	0.000
MPI															
(N)	54	56	50	40	61	39	58	51	49	97	95	58	65	63	49
1	0.583	0.670	0.580	0.950	0.615	0.923	0.871	0.941	0.918	0.608	0.937	0.724	0.723	0.849	0.857
2	0.417	0.330	0.420	0.050	0.369	0.077	0.129	0.059	0.082	0.392	0.063	0.276	0.277	0.151	0.143
3	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGD															
(N)	58	57	48	40	61	45	58	52	49	62	100	60	70	65	50
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGK2															
(N)	57	50	46	40	60	44	58	52	48	99	77	55	61	63	50
1	0.579	0.530	0.641	0.063	0.583	0.148	0.069	0.019	0.052	0.591	0.156	0.336	0.426	0.230	0.030
2	0.421	0.470	0.359	0.938	0.417	0.852	0.931	0.981	0.948	0.409	0.844	0.664	0.574	0.770	0.970
PHP2															
(N)	58	56	50	40	61	40	58	52	49	60	98	59	61	65	39
1	0.974	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.990	1.000	1.000	1.000	0.987
2	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.013
SOD1															
(N)	57	47	50	40	59	39	58	51	49	98	93	56	68	60	49
1	0.482	0.447	0.560	0.763	0.475	0.808	0.672	0.686	0.694	0.454	0.839	0.714	0.551	0.717	0.684
2	0.518	0.553	0.440	0.237	0.525	0.192	0.328	0.314	0.306	0.546	0.161	0.286	0.434	0.283	0.316
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000
TPI1															
(N)	56	57	50	40	61	45	58	52	49	100	49	1	55	63	48
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI2															
(N)	56	57	50	40	61	45	58	52	49	100	49	1	55	63	48
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI3															
(N)	54	56	50	40	61	45	58	52	49	40	1	1	1	1	1
1	0.991	0.973	0.960	0.938	1.000	0.922	1.000	0.933	0.939	0.975	1.000	1.000	1.000	1.000	1.000
2	0.009	0.027	0.000	0.063	0.000	0.078	0.000	0.067	0.061	0.025	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000