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STATE OF WASHINGTON
DEPARTMENT OF FISH AND WILDLIFE
FISH PROGRAM - SCIENCE DIVISION

31 May 2000

TO: Distribution list

FROM: Anne Marshall, Genetics

SUBJECT: Genetic analyses of 1999 Hood Canal area chinook samples

Chinook salmon were sampled in 1999 from several populations in the Hood Canal area to evaluate genetic characteristics, assess inter-population relationships, and continue the study of Skokomish basin chinook. The locations or populations sampled were George Adams Hatchery, Hoodsport Hatchery, Hamma Hamma River, Union River, South Fork Skokomish River, North Fork Skokomish River, and mainstem Skokomish River. Sample sizes are shown in Table 1, except for Union River, which had a sample size of five. A total of 108 chinook were sampled from the Skokomish Basin in 1999. Adult spawners comprised all samples.

As in last year's study of Skokomish River chinook samples, we assayed genetic variation at 57 allozyme loci in all 1999 samples using our standard baseline electrophoretic protocol. For samples of 50 or more fish, allele frequencies for each locus were calculated, and variable loci were tested for conformance to genotypic proportions expected in random samples of a population (Hardy-Weinberg equilibrium tests). Genetic comparisons were made among the 1999 samples with sample sizes (n) of 50 or greater and previous years' samples from the Hood Canal area, and among these and selected Puget Sound populations (Table 1).

I used pair-wise *G*-tests (log-likelihood ratio tests) to test homogeneity of allele frequencies between samples of a population from different years (temporal tests), and between different populations or locations. I computed genetic distances (Cavalli-Sforza and Edwards chord distance) among different sets of population samples as described below. Distances were then used in cluster analyses (unweighted pair-group method), and in multi-dimensional scaling analyses to produce dendrograms and three-dimensional diagrams, respectively, for displays of relationships among samples.

Hardy-Weinberg (H-W) tests for George Adams and Hoodsport hatcheries samples showed no overall significant deviations from expected genotypic proportions. In the mainstem Skokomish sample two loci out of 14 (14%) showed significant deviations from H-W equilibrium, an overall level higher than that expected by chance (5%). One locus had a deficiency of heterozygotes and the other, an excess. This could be due to a relatively small sample size (n=64), or possibly that the sample was not a random sample of the entire breeding population. In the Hamma Hamma sample 15% of the H-W tests showed significant deviations due to heterozygote deficiencies. Small sample size, or non-random sampling may

also explain this result, although heterozygote deficiencies may occur when fish from genetically different populations are present in a sample.

In temporal tests, I found no significant differences (probability, $p, > 0.05$) in allele frequencies between the 1999 and 1988 samples from Hoodsport Hatchery. Although spawners were sampled in the South Fork, North Fork and mainstem Skokomish with the intention of site-specific testing, none of the locations had sample sizes large enough for meaningful statistical comparisons between years. Thus, I pooled the samples of adult spawners from all three sites in each year to try a temporal comparison. Allele frequencies of adults sampled throughout the Skokomish system in 1998 ($n=61$) and 1999 ($n=108$) showed no significant differences ($p > 0.05$).

I compared each 1998 and 1999 Skokomish total adult sample to the 1999 hatchery samples and found no significant allele frequency differences ($p > 0.05$) between either group of natural spawners and Hoodsport Hatchery or George Adams Hatchery samples.

To examine genetic differentiation among spawner aggregations in the South Fork, North Fork and mainstem Skokomish, I combined 1998 and 1999 samples from each site and compared the pooled samples. The 1998 samples included adults and juveniles. Total samples sizes were: South Fork, $n=84$; North Fork, $n=66$; and mainstem, $n=79$. In each pair-wise *G*-test among the three samples, significant differences in allele frequencies occurred, but at a minor level ($0.01 < p < 0.05$). This differentiation was somewhat unexpected given that 1998 and 1999 total adult samples, which contained unequal numbers of fish from the three sub-basins, did not show significant differences. This result may be due to contributions from juveniles sampled from the South and North Forks. It is possible that the sub-basin samples do not represent random samples of a population, but are subsamples of the entire spawning population and unequal sampling between years produced the variation in allele frequencies. Nonetheless, the three sub-basin samples were used for some among-population comparisons to explore relationships further.

Results of *G*-tests among the three Skokomish sites and George Adams and Hoodsport Hatchery samples were as follows. No significant differences overall ($p > 0.05$) in allele frequencies were found between Skokomish mainstem sample and George Adams Hatchery, and 1999 and 1988 Hoodsport Hatchery samples. Allele frequencies for South Fork Skokomish were not significantly different from those of either Hoodsport Hatchery sample, but showed minor differences ($0.01 < p < 0.05$) with those for George Adams Hatchery. Allele frequencies for North Fork Skokomish were significantly different from those of all three hatchery samples ($p < 0.01$).

Allele frequencies of the Hamma Hamma sample were not significantly different ($p > 0.05$) from those of George Adams Hatchery, 1999 Hoodsport Hatchery, and 1998 and 1999 total Skokomish adults samples. The Hamma Hamma sample did show significant differentiation ($p < 0.01$) from the 1988 Hoodsport Hatchery sample.

To evaluate genetic distances among Skokomish natural spawners and other Hood Canal and Puget Sound chinook populations I made two sets of comparisons. For the first, I used the three sub-basin samples, South Fork, North Fork and mainstem Skokomish, each containing fish from 1998 and 1999, and secondly I used 1998 and 1999 Skokomish samples combined due to close similarities between years. I pooled the 1988 and 1999 Hoodsport Hatchery samples for a characterization of that population. For both comparisons, I computed genetic distances between all possible pairs of the Skokomish samples and the other population samples.

The results of the cluster analysis involving the three Skokomish sub-basin samples appear in the dendrogram of Figure 1. Hoodsport and George Adams hatcheries and South Fork and mainstem Skokomish samples formed a distinct cluster relative to a cluster of south Puget Sound population samples, and the North Fork Skokomish and Hamma Hamma samples appeared as outliers to these two groupings. Allele frequencies of these two samples were actually more similar to other 1999 Hood Canal area samples than those of south Puget Sound samples, but the step-wise clustering process can create distortions in relationships especially when genetic distances among samples overall are relatively small. Clustering of the two Snohomish basin samples (Skykomish and Snoqualmie) showed that Hood Canal area populations were most closely aligned with south Puget Sound than north Sound populations (Figure 1), which is consistent with previous results. A multi-dimensional scaling diagram of genetic distance relationships among the same samples (Figure 2) showed a similar overall pattern of clustering, although South Fork Skokomish appeared somewhat more divergent from Hoodsport, George Adams, and mainstem Skokomish than it did in the dendrogram.

In the second analysis using combined 1998 and 1999 samples to represent the total Skokomish population, the clustering of Hood Canal-area populations (Figure 3) was similar to results seen in the first dendrogram (Figure 1). The Hamma Hamma sample was an outlier again rather than clustering with the Hood Canal group as would be expected from allele frequency similarities. Note that I included in this cluster analysis two Nisqually basin hatchery populations (Kalama and Clear Creek hatcheries) not used in the previous analysis. Clustering of populations in three-dimensional space (Figure 4) reflected relationships shown in the dendrogram (Figure 3).

Thom Johnson compiled data on recoveries of chinook with coded-wire-tags (CWTs) in the Skokomish Basin, and data for fish with CWTs in the 1999 genetic samples are presented in Table 2. Based on scale age data from John Sneva, WDFW, eleven mainstem Skokomish chinook had hatchery scale patterns (release as yearlings) and five of these had CWTs. Within the 1999 mainstem Skokomish sample 15 of 64 fish (23%) were known hatchery-origin, based on scale pattern and/or CWT information. Five of 38 fish (13%) in the South Fork Skokomish, and two of six fish (33%) in the North Fork Skokomish samples were known hatchery-origin.

To test for genetic differentiation between Skokomish Basin natural spawners without a known hatchery origin and the hatchery population samples, I compiled a subsample of 1998 and 1999 Skokomish adults by excluding fish with CWTs and/or with the yearling release scale pattern. This would not be a complete exclusion of hatchery-released fish because fish released as subyearlings cannot be distinguished from wild fish (which normally outmigrate as sub-yearlings), and hatchery releases are not 100% marked with CWTs. I also excluded untagged fish without scale age data. Allele frequencies of Skokomish adults without a known hatchery origin were not significantly different from those of Hoodsport or George Adams hatchery samples. Additionally, no significant differentiation was found between Skokomish adults without a known hatchery origin and the Skokomish sub-basin and combined total samples. This latter result is not surprising given that individuals were shared between the potentially wild-origin subsample and other Skokomish samples.

As we saw with the analysis of 1998 Skokomish samples, there appeared to be little genetic differentiation between natural spawners and local hatchery populations. The combined North Fork Skokomish sample showed more divergence from hatchery populations than the other two sub-basin samples. Sampling error, due to small sample sizes (only six adults sampled in 1999), could be the simplest explanation for this result. Also, if North Fork spawners are only a segment of the total population, sampling error (non-random sampling) may explain the apparent variation. For both sample years, 32% of spawners from the North Fork were hatchery-origin. This level of straying suggests a potential for gene flow that would tend to homogenize allele frequencies rather than promote genetic divergence. Other biological and demographic information may be more helpful in determining whether population segregation or structuring occurs within the Skokomish Basin.

This was our first evaluation of genetic characteristics of chinook spawners in the Hamma Hamma River. Although we found little differentiation of allele frequencies between this population sample and others in the Hood Canal area, it would be useful to increase sample size by sampling spawners in 2000. Also, if Hamma Hamma chinook are a small population, samples from more than one year may better characterize the population.

It did appear that Hood Canal area populations formed a group differentiated from south Puget Sound populations, although at a relatively low level. This is noteworthy given the history of stock transfers between the two areas. Presumably, this divergence would be maintained under the conditions of no further transfers of South Sound chinook. Although allozyme allele frequency divergence is not evidence of differences in local adaptations, it often signifies reproductive isolation. Local adaptations are more likely to be maintained in populations that are significantly reproductively isolated.

This is only a brief summary of results and interpretation. I will be glad to provide other details or clarifications as needed. WDFW Genetics staff contributing to this analysis were Bruce Baker, Cherril Bowman, and Norm Switzler. Field sampling was carried out by WDFW Genetics staff (Hoodsport Hatchery, George Adams Hatchery, North Fork Skokomish

samples), WDFW Hood Canal District Fish Program staff (mainstem and South Fork Skokomish samples), and volunteers with the Hood Canal Salmon Enhancement Group (Hamma Hamma and Union rivers samples).

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Skokomish Tribe- Dave Herrera
Hood Canal Salmon Enhancement Group- Al Adams
Northwest Indian Fisheries Commission- Ken Currens

Table 1. Hood Canal and Puget Sound chinook population samples used in this analysis. All samples are fall-run chinook unless indicated otherwise.

Name	Collection Year(s)	Number sampled
S.F. Skokomish River	99	38
S.F. Skokomish River (adults & juveniles)	98	46
N.F. Skokomish River	99	6
N.F. Skokomish River (adults & juveniles)	98	60
Skokomish River, mainstem	99	64
Skokomish River, mainstem	98	15
Hoodsport Hatchery	99	100
Hoodsport Hatchery	88	150
George Adams Hatchery	99	100
Hamma Hamma River	99	55
Nisqually River	98, 99	53
Clear Cr. (Nisqually) Hatchery	99	100
Kalama Hatchery (Nisqually)	99	100
Puyallup Hatchery	92-93	150
South Prairie Creek	92-93	86
Green River Hatchery	87, 88, 90, 98	399
Newaukum Creek	92-93	144
Cedar River	93-94	107
Issaquah Hatchery	92	99
Issaquah Creek	99	100
Bear/Cottage Lake Creek (Sammamish R.)	98-99	178
Skykomish River summer-run	89, 93, 96	178
Snoqualmie River	88	101

Table 2. Information on coded-wire tagged (CWT) chinook within genetic samples of 1999 Skokomish Basin natural spawners. F=female, M=male.

CWT No.	Skokomish Site	Sex/Age	Release Site
63-01-48	North Fork	M/3.1	Purdy Creek (George Adams Hatchery)
63-53-41	South Fork	M/4.2	Endicott Pond (Skokomish R.)
63-59-34	South Fork	M/4.2	Skokomish R.
63-01-48	Mainstem	M/4.1	Purdy Creek
63-01-48	Mainstem	F/3?	Purdy Creek
63-03-38	Mainstem	F/3.1	(origin- George Adams Hatchery)
63-53-41	Mainstem	F/4.2	Endicott Pond
63-53-41	Mainstem	F/4.2	Endicott Pond
63-53-41	Mainstem	M/4.2	Endicott Pond
63-53-41	Mainstem	F/4.2	Endicott Pond
63-53-41	Mainstem	M/4.2	Endicott Pond
Unreadable	Mainstem	M/3.1	unknown

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Figure 1. Dendrogram resulting from cluster analysis of pair-wise genetic distances among Hood Canal area and Puget Sound population samples. Populations are fall-run unless otherwise indicated. Skok.=Skokomish, H=Hatchery, R = River, CR=Creek, SU=summer-run.

29 loci 17 pops ° (N

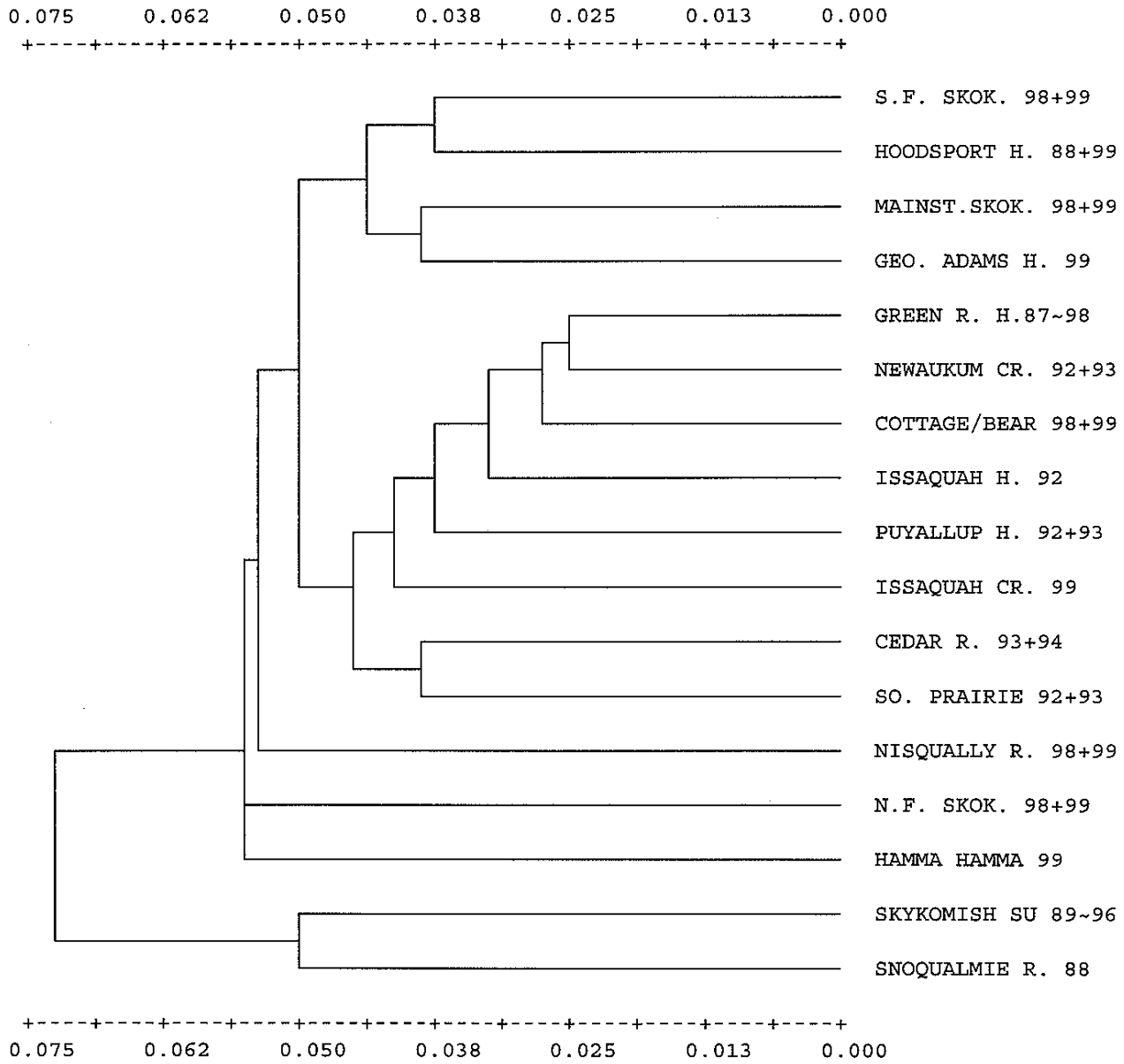

Figure 2. Multidimensional scaling diagram of genetic distances among 17 Hood Canal and Puget Sound chinook population samples. Skokomish chinook are represented by three sub-basin samples.

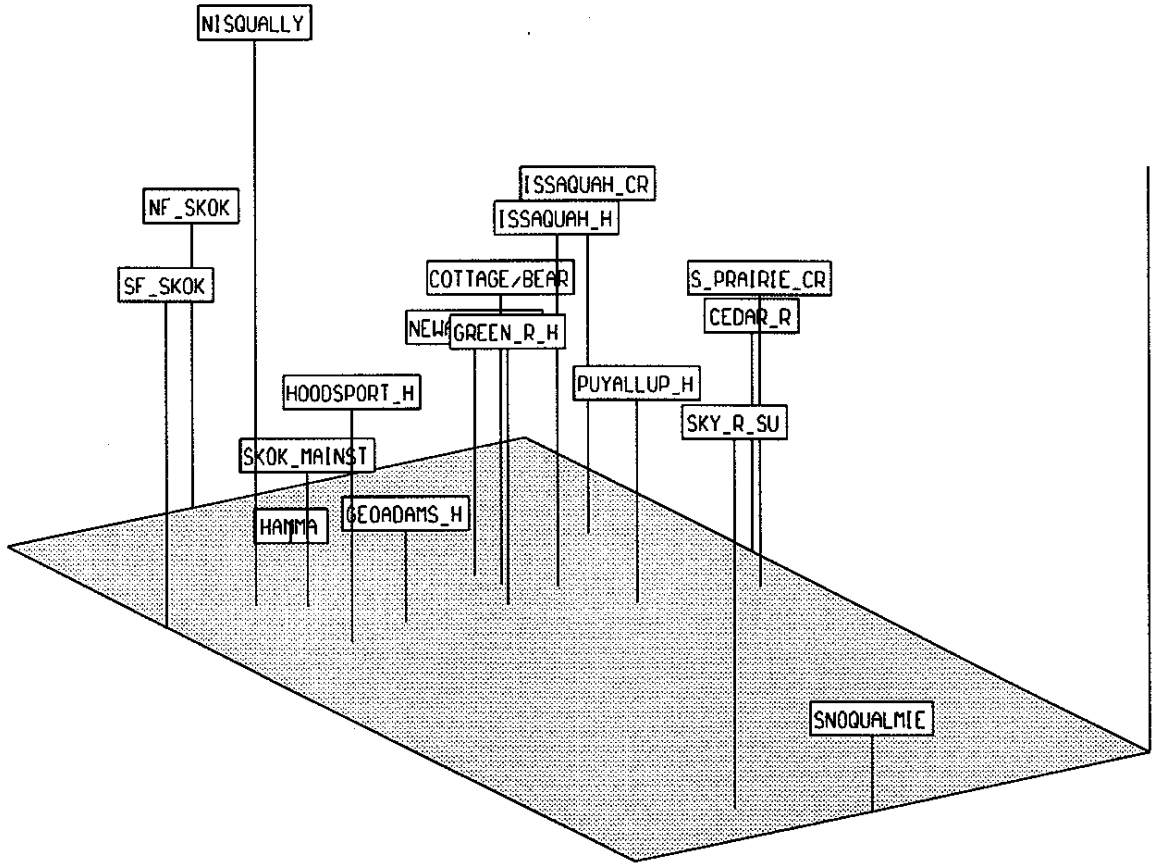

Figure 3. Dendrogram resulting from cluster analysis of pair-wise genetic distances among Hood Canal area and Puget Sound population samples. All Skokomish River basin samples from 1998 and 1999 were pooled. Populations are fall-run unless otherwise indicated. H=Hatchery, R=River, CR=Creek, SU=summer-run.

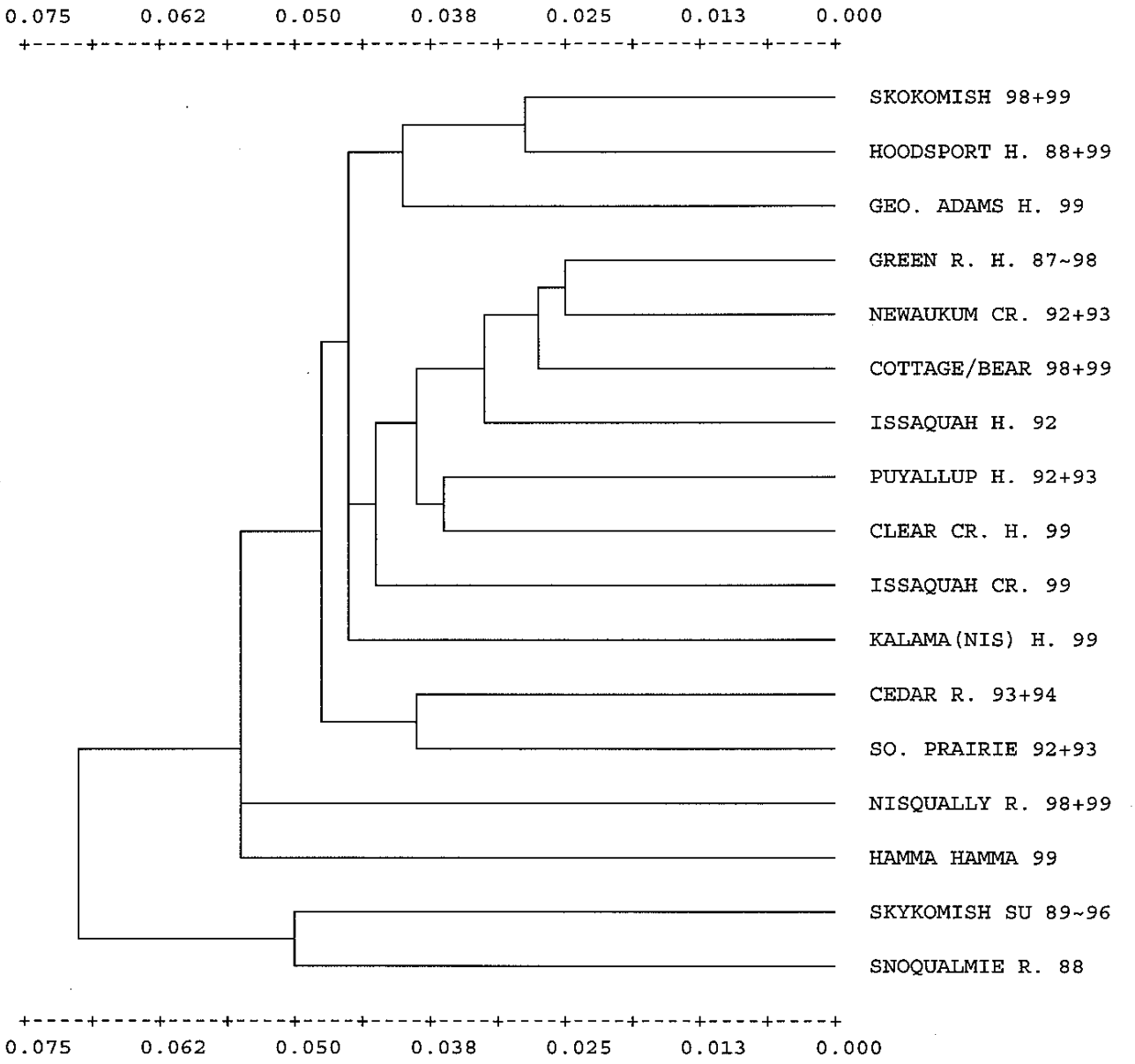

Figure 4. Multidimensional scaling diagram of genetic distances among Hood Canal and Puget Sound chinook population samples. All 1998 and 1999 Skokomish Basin samples combined.