

POPULATION GENETIC STRUCTURE OF ARCTIC CHAR
(SALVELINUS ALPINUS) **FROM RIVERS OF THE**
NORTH SLOPE OF ALASKA

by

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SUMMARY

Potential problems with oil and gas development in the Beaufort Sea area include the effects of offshore construction of causeways and other structures on anadromous species such as Arctic char (Salvelinus alpinus). By studying the amount and pattern of genetic variation in the populations while they are associated with their natal drainages, we can make inferences about the evolutionary history of northern Arctic char, and predict their ability to respond to changing environmental conditions.

Electrophoretic detection of protein variation makes it possible to discriminate among stocks using quantifiable characters having a genetic basis. This proven method requires a relatively small sample of fish from different populations for baseline data. Further, electrophoretically distinguishable characters have generally proven to be stable characteristics of fish stocks that have been studied. If the species of concern has a suitable stock structure, biochemical genetics methods can be used to estimate the percent composition of various stocks represented in samples from mixed aggregations.

The objectives of this project are to 1) characterize the amount and pattern of genetic variation in populations of anadromous Arctic char from major drainages of the North Slope of Alaska, 2) determine whether the population structure of North Slope char is such that genetic stock identification of mixed populations collected from offshore waters would be possible, and 3) describe how a sampling program would be designed to use genetic stock identification to determine which stocks would be affected by specific development projects.

Samples from fifteen populations of juvenile Arctic char were collected from ten tributaries to the Beaufort Sea. We used horizontal starch-gel electrophoresis to identify protein products of forty-one loci coding for twenty enzymes in three tissues. We measured the amount of variation, the pattern of variation (genotypic distribution) within population samples, the similarity between populations, their heterogeneity, and the degree of gene diversity among groups.

Northern Alaska Arctic char have more genetic variation than might be expected given the relatively narrow range of waters they inhabit and the harsh environmental conditions. With an average heterozygosity per locus of 5.1%, they are typical of fish species in general; at the upper end of the range observed in other salmonid fishes; and higher than most other Arctic char populations that have been studied.

The genetic identities (Nei 1972) among North Slope Arctic char populations are high ($> .987$), indicating fairly recent common ancestry. High similarity values do not imply lack of significant differences between populations. Heterogeneity tests indicate the distinctness of the populations and the complexity of the relationships between them. Almost all North Slope Arctic char populations are significantly genetically distinct from each other. Thus, fish from different drainages are not freely interbreeding, and are most likely true to their spawning streams. There is no simple correlation between genetic relationships and geographical proximity.

It is not possible to determine the underlying cause of the observed relationships among North Slope Arctic char populations from protein studies. Selection, migration, mutation, behavioral isolation, founder effects, random genetic drift (chance changes due to small populations size) and combinations of these and other forces may all contribute.

North Slope Arctic char do not have the magnitude of difference between groups exhibited by the non-migratory char of northern Europe. They do, however, compare with the population structure of anadromous Pacific salmon. This is relevant because genetic stock identification methods have been successfully applied to these salmonids, and can apparently be applied to North Slope Arctic char.

To do genetic stock identification there must be sufficient detectable genetic variation between populations of different major drainages, combined with a low within-group variability. Our data indicate that North Slope char have a relatively large amount of genetic variation; there are significant differences among populations; and the observed variation is partitioned such that there is as much difference between char from different drainages as there is among populations of sockeye and chum salmon where genetic stock identification has been used successfully. As such, we can anticipate successful application of this technique to the identification of char at specific offshore sites.

Management Implications

We have determined that North Slope char have a relatively large amount of genetic variation, and that populations are genetically distinct from each other. From this we know that different stocks are currently reproductively isolated from each other. Since they do mix to some unknown degree in feeding areas, the differences that have been established between stocks are maintained by homing behavior. Populations of each drainage are probably discrete, locally adapted units. It is not clear at this time how non-migratory forms are related to anadromous

stocks.

It is unlikely that loss of any one stock would be mitigated by substitution of another. While the actual loci we have studied may be selectively neutral, underlying variation that is marked by these loci may be highly selected for in different environments, corresponding generally to different drainages. As such, Arctic char stocks of the North Slope should be managed as individual, unique gene pools.

Further work is needed to understand the relationships among populations. To get a complete picture of the resource, we should consider deliberately sampling resident populations. It is important that we identify and sample additional populations making major contributions to the Beaufort Sea admixture, as it is an important assumption of the GSI model that all major contributors to a mixed stock be represented in the baseline. It is also important to understand that genetic stock identification estimates the percent composition at only one point in space and time.

Distribution of offshore stocks of fish is related to environmental conditions which are highly variable from year to year. Also, Arctic char are highly mobile in offshore areas, so estimates should be made of stock composition at several times during the short summer feeding season. It must be realized that there will be considerable variation, regardless of study method used, between data from different years and different areas and at different times during the season. This means that stock identification must be done on a site-specific basis, with repeated sampling during the summer, and that data from more than one year will be required to establish the pattern of use by the fish.

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INTRODUCTION

Most current environmental concerns on the North Slope of Alaska are related to the impacts of oil exploration and development on fish and populations. Potential problems include the effects of offshore causeways and other structures on anadromous species such as Arctic char (Salvelinus alpinus) and Arctic cisco (Coregonus autumnalis). In offshore areas, these species are in mixed aggregations, which makes it difficult to determine specifically which stocks could be at risk.

Arctic char are of special interest because of their relatively low abundance, limited range in a narrow band of coastal waters, and their importance in subsistence fisheries. Studies (e.g. Furniss 1975; Craig and McCart 1975) characterizing the marine and freshwater phases of Arctic char life history emphasize the importance of the coastal area of the Beaufort Sea. Populations of Arctic char migrate from freshwater, springfed spawning and overwintering areas to nearshore marine feeding grounds. Migration studies using mark and recapture techniques indicate that movements are generally limited to an area adjacent to the river of origin (Furniss 1975). However, examples of extended migration and overwintering in drainages other than those used for spawning have been documented (Craig and McCart 1976; Glova and McCart 1974).

In order to determine which stocks could be affected by development , and possibly to predict how they would be affected, we need to understand the stock structure of both the inland and offshore stages of the Arctic char life history. By studying the amount and pattern of genetic variation in the populations while they are associated with their natal drainages, we can make inferences about the evolutionary history of northern Arctic char, and predict their ability to respond to changing environmental conditions. Also, with preliminary data we can determine the applicability of genetic stock identification methods to management problems involving the mixed stocks at specific offshore sites.

Most methods that have been used in the past to study the relationships among salmonid populations require intensive annual field sampling because the distinguishing characteristics are usually growth rings of scales and/or otoliths. The marks on these structures are determined by environmental differences reflected in patterns of fish growth, and are not static population characteristics. Acquisition of adequate sample sizes of salmon of known and unknown origin is essential to the success of stock identification using these characteristics. Large sample sizes are needed to develop the "standards" required for tests with specimens of unknown origin. In order to ensure that regional stocks are represented in the analysis, the sampling

strategies must account for stock and species-specific characteristics relative to age and size of fish smelting and the intermingling of migrating stocks in coastal waters.

Electrophoretic detection of protein variation makes it possible to discriminate among stocks using quantifiable characters having a genetic basis. This proven method requires a relatively small sample of fish from different populations to establish baseline data. Further, electrophoretically distinguishable characters have generally proven to be stable characteristics of fish stocks that have been studied (see Utter et al. 1981), although there are exceptions (Wilmot, unpublished data) .

If the species of concern has a suitable stock structure, biochemical genetics methods can be used to estimate the percent composition of various stocks represented in mixed aggregations sampled from offshore areas. The amount of effort required to study mixed stocks is relatively small using these techniques compared to methods requiring extensive extrinsically applied marks (Ihssen et al. 1981). This type of information provides site-specific information on stocks at risk from habitat alteration, and biological data at migratory stages of the life cycle of these natural populations.

Objectives

The objectives of this portion of the Arctic Fish Habitats and Sensitivities project are to: 1) characterize the amount and pattern of genetic variation in populations of **anadromous** Arctic char from major drainages of the North Slope of Alaska, 2) determine whether the population structure of North Slope char is such that genetic stock identification of mixed populations collected from offshore waters would be possible, and 3) describe how a sampling program would be designed to use genetic stock identification to determine which stocks would be affected by specific development projects.

METHODS

Samples from fifteen populations of Arctic char were collected from the North Slope of Alaska by U.S. Fish and Wildlife Service (USFWS) biologists of the Fairbanks Fisheries Assistance Office. Figure 1 shows the study area and Table 1 the sampling location, number of fish used for electrophoresis, and the date of collection. Minnow traps and electrofishing gear were used to catch juvenile char which were shipped to the Alaska Fish and Wildlife Research Center (AFWRC) laboratory in Anchorage either alive or frozen whole. Skeletal muscle, liver, and eye tissues were dissected from the samples in the laboratory and used for protein electrophoresis.

ELECTROPHORETIC METHODS

We used horizontal starch-gel electrophoresis to identify protein products of gene loci following the methods described by Utter et al. (1974). Buffers and staining procedures were after Allendorf et al. (1977), and isozyme nomenclature was that of Allendorf et al. (1983). Gel buffers included: AC (Clayton and Tretiak 1972) pH 6.3 - 6.8; AC+ (AC plus 30 mg NAD and one drop of mercaptoethanol); RW (Ridgway et al. 1970) PH 8.2; MF (Markert and Faulhaber 1965) PH 8.7.

Building on the work of Andersson et al. (1983), Hindar et

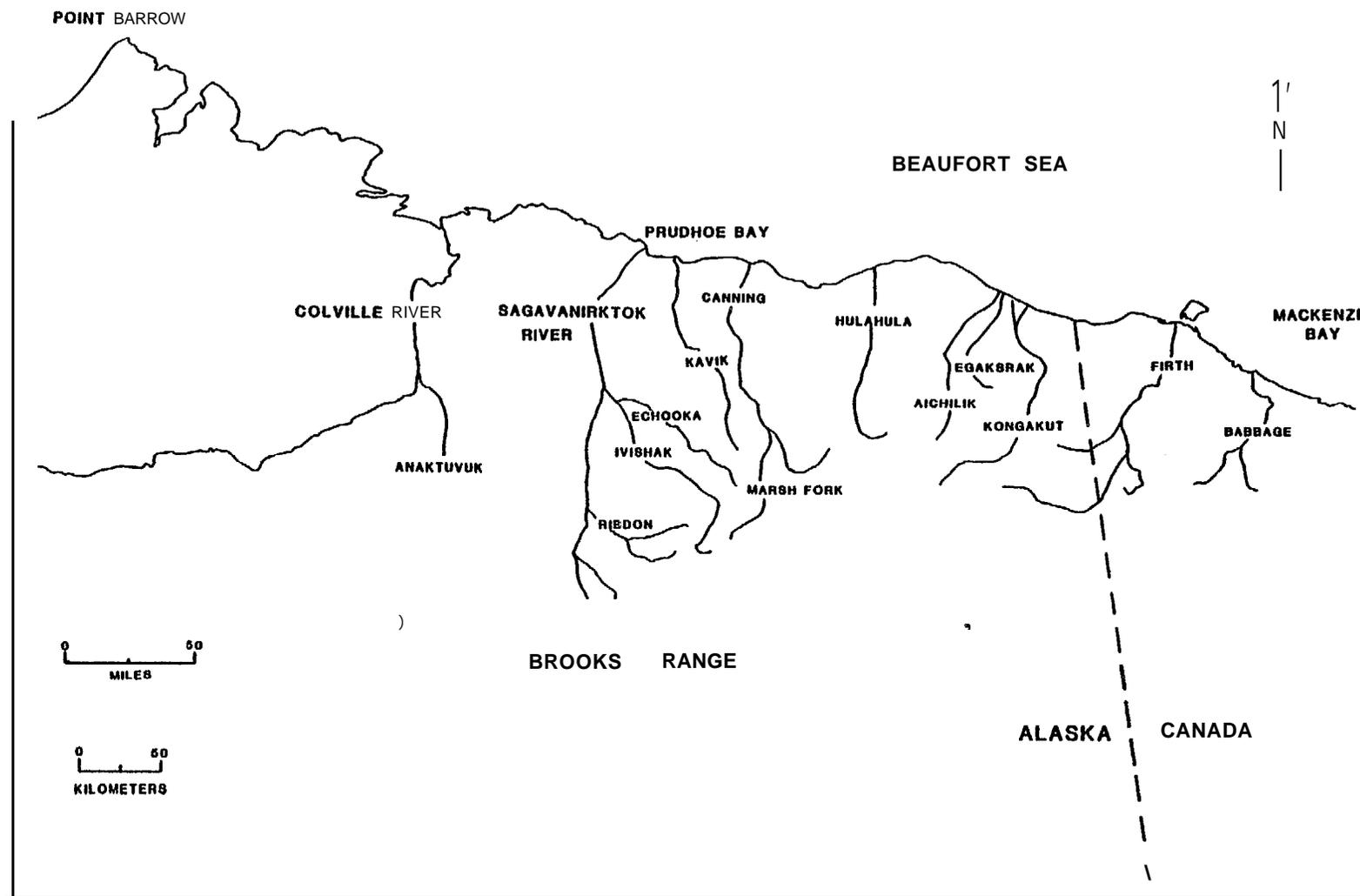


Figure 1. North Slope of Alaska study area for Arctic char genetic research.

Table 1. population, location (latitude and longitude), number of char sampled, and date of collection.

Population	Location	Number	Date
Aichilik	69°22'N, 143°05'W	40	9 / 8 6
Anaktuvuk	68°42'N, 151°13'W	40	5/86
Babbage (Canoe River)	68°37'N, 138°42'W	35	9/86
Canning	69°54'N, 145°45'W	27	5/86
Canning Marsh Fork	69°05'N, 146°00'W	29	5/86
Echooka	69°15'N, 147°18'W	24	4/86
Egaksrak River	69°24'N, 142°35'W	41	5/86
Firth (Joe Creek)	68°57'N, 140°58'W	40	9/86
Hula Hula Site #1	69°45'N, 144°15'W	15	10/85
Hula Hula Site #2	69°28'N, 144°20'W	37	10/85
Hula Hula Site #3	69°18'N, 144°33'W	59	10/85
Ivishak	68°58'N, 148°08'W	50	5/86
Kavik	69°24'N, 146°34'W	40	4/86
Kongakut	69°31'N, 141°42'W	40	9/86
Ribdon	68°38'N, 148°12'W	40	5/86
Total Sampled		557	

al. (1986), Johnson (1984), Kartavtsev et al. (1983). Kornfield et al. (1981), and Robb Leary (University of Montana, personal communication), more than 25 enzymes were tested for activity and resolution on various buffers and tissues. Our statistical results are based on successful resolution of forty-one loci coding for twenty enzymes in three tissues (Table 2). Other tissues were tested but added little or no additional information. The loci we used are those with nearly complete data sets and consistent results, including good resolution and a repeatable pattern of expression.

Inferences were made regarding enzyme expression based on 1) assumptions of parallel expression with that of other salmonids with experimentally determined patterns of inheritance (especially Johnson 1984), 2) comparisons based on different tissue expression, and 3) on the known molecular subunit structure of the enzymes. Mobilities of enzymes were measured relative to the common electrophoretic phenotype observed in samples of Anaktuvuk River Arctic char.

In many cases, expression of different gene loci is specific to a particular tissue or tissues. Often the pattern of expression of genes among tissues is a species-specific trait. We studied tissues rather than blood because blood is difficult to collect; special handling is required to maintain the quality of blood samples; enzymes are less stable in blood; and because

Table 2. Enzymes, Enzyme Commission (E. C.) numbers, and loci examined in samples of Arctic char from northern Alaska. Buffers include: AC (Clayton and Tretiak 1972) PH 6.3-6.8; AC+ (AC plus 30 mg **NAD** and one drop of **mercaptoethanol**); RW (Ridgway et al. 1970) pH 8.2; MF (Markert and **Faulhaber** 1965) PH 8.7. Tissues include muscle (M), liver (L), and eye (E). The pairs of loci listed in parentheses are **electrophoretically** indistinguishable (**isoloci**). For this analysis they were considered as individual loci.

Enzyme	E.C. #	Loci	Buffer	Tissue
Acetylglucosaminidase	3.2.1.30	Hex1	RW	L
Adenylate kinase	2.7.4.3	Adk1,2	AC	M
Alcohol dehydrogenase	1.1.1.1	Adh1	RW	L
Aconitate hydratase	4.2.1.3	Ac03	AC	L
Aspartate aminotransferase	2.6.1.1	Aat1,2 Aat(3,4)	RW, AC RW, AC	L, E M
Creatine kinase	2.7.3.2	Ck1,2 Ck3	RW RW	M E
Glucose phosphate isomerase	5.3.1.9	Gpi(1,2),3	RW	M
Glyceraldehyde-3-phosphate dehydrog.	1.2.1.12	Gap(3,4)	AC+	E
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3p1	AC, RW	L, M
Glycyl-leucine peptidase	3.4.11	G11	MF	E
Isocitrate dehydrogenase	1.1.1.42	Idh1,2 Idh(3,4)	AC AC	M L
Lactate dehydrogenase	1.1.1.27	Ldh1,2 Ldh4 Ldh3, 4,5	RW RW RW	M L E
Leucyl-glycyl-glycine peptidase	3.4.13	Lgg1	MF	E
Malate dehydrogenase	1.1.1.37	Mdh(1,2) Mdh(3,4)	AC AC	L M
Malate dehydrogenase (NADP-dependent)	1.1.1.40	Me1, 2, 3 Me4	AC AC	M L
Phosphoglucomutase	2.7.5.1	Pgm1, 2	AC, RW	L, M
6-Phosphogluconate dehydrogenase	1.1.1.44	6Pg1	AC	M
Sorbitol (iditol) dehydrogenase	1.1.1.14	Sdh1	RW	L
Superoxide dismutase	1.15.1.1	Sod1	RW, AC	L
Xanthine oxidase*	1.2.3.2	Xol	RW	L

* Observed phenotype probably represents diaphorase activity.

more information is available from tissues. Carmichael et al. (1986) found that of 64 loci they studied in largemouth bass, only 24 (37%) were adequately expressed in blood. Muscle tissue expressed 37 loci (57%) and liver, 39 (60%). A combination of liver and muscle expressed 80% of the loci tested. We have found that eye tissue adds at least four more loci to the combination of muscle- and liver-specific loci.

STATISTICAL METHODS

Sample size

Ideal sample size is evaluated based on the 95% probability of including variants in the sample, if those variants are present in some minimum frequency within the sample. For example, to be 95% sure of observing variants present in a sample in a frequency of at least 2%, N (the sample size) for a diploid organism would have to be approximately 40 ($.95^{2N} = .02$). We chose forty individuals as a reasonable sample size, though samples with less than forty were included in the analyses.

Amount of genetic variation

The amount of genetic variation is estimated by determining the percent of loci that are polymorphic (P), and the mean percent of heterozygous loci per individual (H). Expected

average heterozygosity for each locus is calculated using allele frequencies of observed genotypes in each population and expected random mating (Hardy-Weinberg) proportions:

$$H = 1 - \left(\sum_{j=1}^L \sum_{i=1}^{A_j} P_{ij}^2 \right) / L$$

where L is the number of loci, A_j is the number of alleles at the j^{th} locus, and P_{ij} is the frequency of the i^{th} allele at the j^{th} locus.

The standard criteria for polymorphism (P) is the percent of the loci examined in a population in which the frequency of the common allele is less than or equal to 0.99.

For this and subsequent analyses, **isoloci** (duplicated locus pairs with indistinguishable nobilities) were counted as two individual loci and all observed variation was attributed arbitrarily to only one locus of the pair.

Genotypic distribution

Observed genotypes in samples were tested for conformance to random mating (Hardy-Weinberg) proportions. A chi-square test was used to determine whether the frequency of genotypes for each locus equal those expected from calculations of probable

combinations of alleles (with the frequencies we observed) joining at random. For each population sampled, a multiple simultaneous chi-square test was done by summing the chi-square values over all the variable loci, summing the degrees of freedom, and comparing these values to the expected distribution.

Genetic similarity

The genetic similarity between the 15 char samples was determined using computer programs by Donald Campton of University of Washington (UW). The program calculates Nei's index of genetic identity (1972; 1978) using the probability of identity of gene pairs between populations averaged over all loci. The normalized identity of genes between two populations, X and Y, is defined as:

$$I = J_{XY} / \text{SQRT} (J_X J_Y)$$

where J_X , J_Y , and J_{XY} are the arithmetic means over all loci of the probabilities of identity between gene pairs among populations.

Identity values are scaled from 0.0 to 1.0; 0.0 corresponds to complete allele substitution at all loci, and 1.0 to populations that are electrophoretically indistinguishable at all

loci studied. Genetic distance is calculated as the negative natural log of the identity value.

Genetic identity values were used in a clustering algorithm (UPGMA: Sneath and Sokal 1973) modified by Donald Campton (UW) to produce a dendrogram of relationships among populations. The average linkage method of clustering was used, and the analysis was weighted to reflect unequal sample sizes. The three Hula Drainage populations were combined because of the lack of heterogeneity among them.

We also used a multidimensional scaling procedure (Kruskal and Wish 1977) to show relationships among populations. This method uses Nei's indices of genetic similarity among populations and defines each population as a point in Euclidean space. The multidimensional construct is then reduced to a two-dimensional plot. As such, the relative distances among points on the diagram illustrate the relative genetic distance among populations .

Genetic heterogeneity

To test the heterogeneity between paired populations, we used multiple simultaneous G-tests (Sokal and Rohlf 1981). G-tests were performed for each locus, and G-values and degrees of freedom for each locus were summed over all loci in all pairs and

tested against a chi-square distribution. Because of the robustness of the test, only cells with expected values less than 1.0 were combined.

The large number of non-independent pairwise comparisons (78) makes it possible that a percentage of the comparisons could appear significantly different by chance. Consequently, the probability value required to demonstrate a significant difference was modified for this analysis according to Cooper (1968) to eliminate spurious correlations.

Gene diversity analysis

Gene diversity analysis determines the source of observed variation, i.e., what proportion of the observed variation is between individuals within populations, as opposed to differences among populations or groups of populations. Our analysis was done with a computer program by Donald Campton (UW) based on the work of Nei (1973) and Chakraborty (1980). Modifications include use of simple unweighed arithmetic averages of population samples within sites rather than weighting gene frequencies within sites by the number of samples.

Sample data were analyzed in levels: as individual subpopulations (sites) , as subpopulations of different drainages, and as a whole. The total amount of genetic variation of all

populations studied was partitioned into within- and between-subpopulation diversity components. The total gene diversity (H_T) over all subpopulations equals the average heterozygosity within the subpopulations (H_S) plus the average gene diversity between subpopulations (D_{S_T}). The diversity between subpopulations (D_{S_T}) can be broken down to differences between sites within a drainage (D_{B_S}) and differences between populations of different drainages (D_{B_D}). The relative magnitude of gene differentiation among populations (G_{S_T}) was estimated as D_{S_T} / H_T or $(D_{B_S} + D_{B_D}) / H_T$, and can be expressed as a percentage.

Figure 2 shows how the North Slope char data were combined for this analysis, excepting that in the actual analysis the combination of Ivishak and Echooka was not considered.

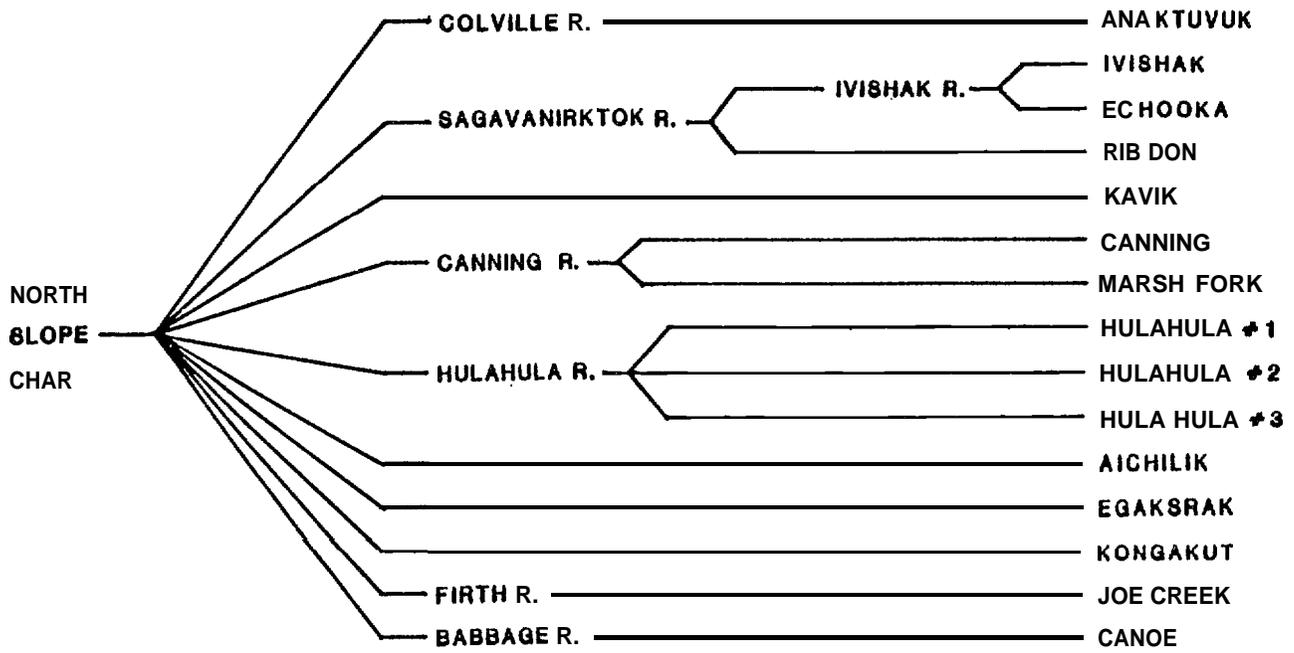


Figure 2. Hierarchical structuring used in the gene diversity analysis of 15 North Slope Arctic char populations.

RESULTS

The genetic variation of populations can be quantified, making it possible to determine not only the amount of variation within populations, but also the pattern of variation among them. We have measured the amount of variation, the pattern of variation (genotypic distribution) within population samples, the similarity between populations, their heterogeneity, and the degree of gene diversity among groups, studied hierarchically.

Amount of genetic variation

Allele frequencies and relative mobility of variable loci are listed in Appendix 1. Percent of loci polymorphic and average heterozygosity for the 15 populations of Arctic char sampled are reported in Table 3. Over 40% of the gene loci studied are variable in at least one of the populations. The values of P range from 13.2 to 29.3%. Average heterozygosity ranges from 3.1 to 7.0% for the samples, and the weighted average over all 15 populations is 5.1%.

Genotypic distributions

Significant deviation from expected values can indicate non-random mating, unequal fertility among parents, unequal viability

Table 3. Average percent of fish heterozygous per locus (H), and percent of loci examined that were polymorphic (P) in 15 populations of Arctic char from the North Slope of Alaska.

Population	% H	% P
Babbage (Canoe River)	4.72	15.0
Firth (Joe Creek)	5.17	29.3
Kongakut	5.92	22.0
Egaksrak	6.47	26.8
Aichilik	7.02	24.4
Hula Hula Site #1	4.12	13.2
Hula Hula Site #2	5.41	26.3
Hula Hula Site #3	4.47	23.7
Canning	6.21	25.0
Canning Marsh Fork	6.16	29.3
Kavik	3.14	15.0
Echooka	4.19	22.5
Ivishak	4.26	25.6
Ribdon	6.06	25.0
Anaktuvuk	5.37	23.1
Average	5.25	23.08

among offspring (selection) , migration from other populations, or failure to collect a random sample from the population. In the 15 samples of Arctic char we studied, there is no evidence of departure from the expected genotypic distributions. The parental generations have apparently been mating at random (no more than one population was detected in any sample), and the collections appear to represent random samples of the populations .

Genetic similarity

No allele substitutions were observed at any locus. Genetic identities are high among North Slope char, all greater than or equal to 0.987 (corresponding to a genetic distance of 0.013). The values range up to complete identity, 1.000, and are reported in Table 4,

The dendrogram of Figure 3 illustrates the genetic relationships among Arctic char populations of tributaries of the Beaufort Sea. Three main groups are apparent at approximately the .9950 level. Figure 4 is another representation of the relationships among North Slope char populations, and illustrates relative Euclidean distances among subpopulations. Although loose clusters of points are evident, there is generally as much difference between points within a cluster as there is between points of different clusters.

Table 4. Matrix of Nei's (1978) gene identity values pairwise among 15 populations of Arctic char from the North Slope of Alaska.

1 Babbage	1.000																
2 Firth	.992	1.000															
3 Kongakut	.993	.998	1.000														
4 Egaksrak	.996	.992	.998	1.000													
5 Aichilik	.999	.990	.994	.998	1.000												
6 Hula #1	.991	.992	.997	.999	.993	1.000											
7 Hula #2	.992	.990	.996	.999	.994	1.000	1.000										
8 Hula #3	.991	.987	.994	.998	.991	1.000	1.000	1.000									
9 Canning	.991	.998	1.000	.997	.993	.997	.995	.993	1.000								
10 Marsh Fork	.997	.994	.996	.998	.999	.995	.992	.990	.997	1.000							
11 Kavik	.990	.997	.998	.996	.992	.998	.995	.993	.998	.996	1.000						
12 Echooka	.991	.998	.998	.994	.992	.997	.995	.992	.999	.996	1.000	1.000					
13 Ivishak	.991	.998	.998	.997	.993	.998	.995	.993	.999	.997	.999	.999	1.000				
14 Ribdon	.994	.998	1.000	.996	.993	.996	.995	.993	.998	.994	.997	.998	.998	1.000			
15 Anaktuvuk	.993	.999	.997	.995	.993	.993	.990	.987	.999	.998	.998	.998	.998	.996	1.000		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		

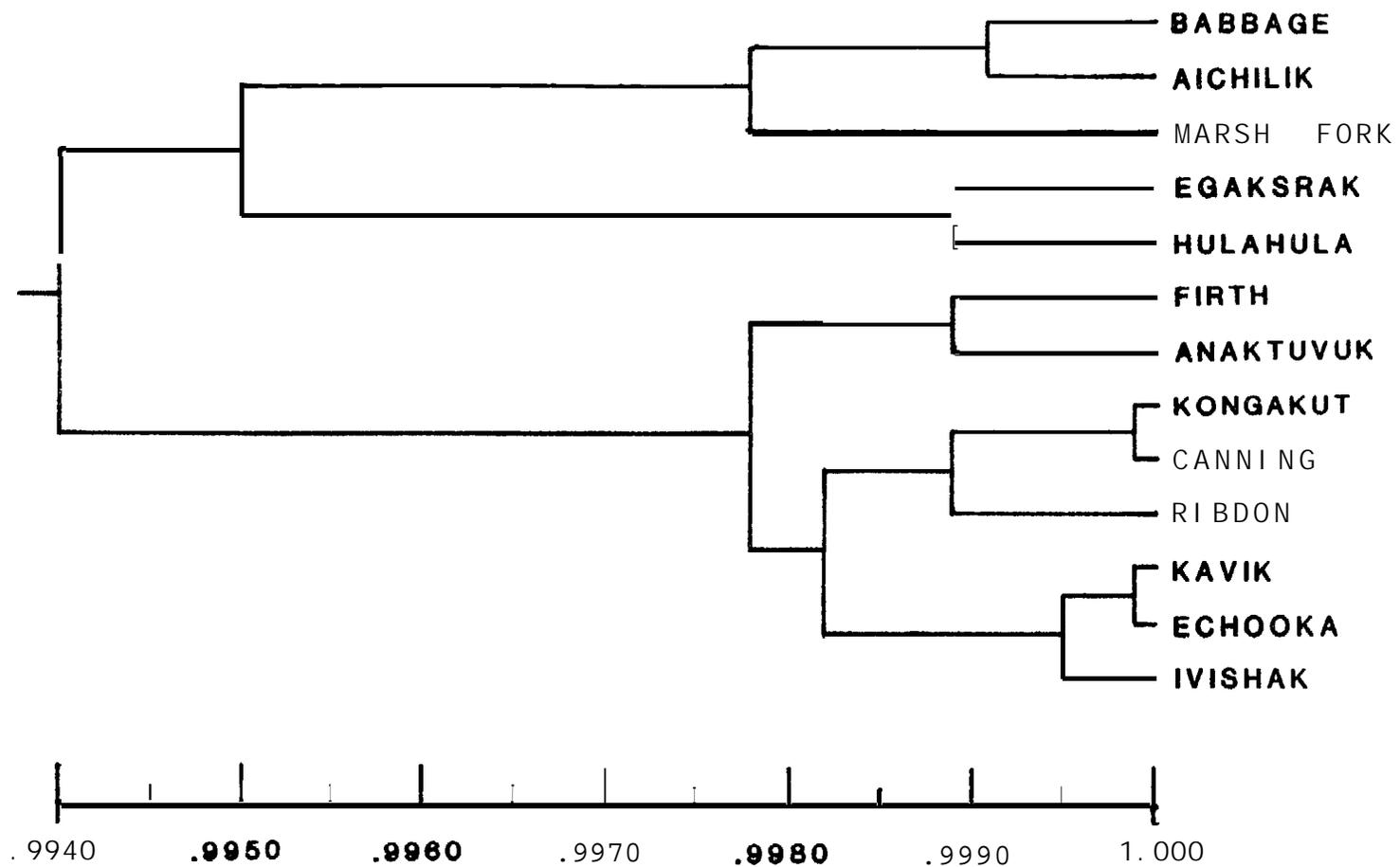


Figure 3. Dendrogram showing relationships of 15 populations of Arctic char from the North Slope of Alaska. The clustering program uses Nei's (1978) unbiased estimates of genetic identity.

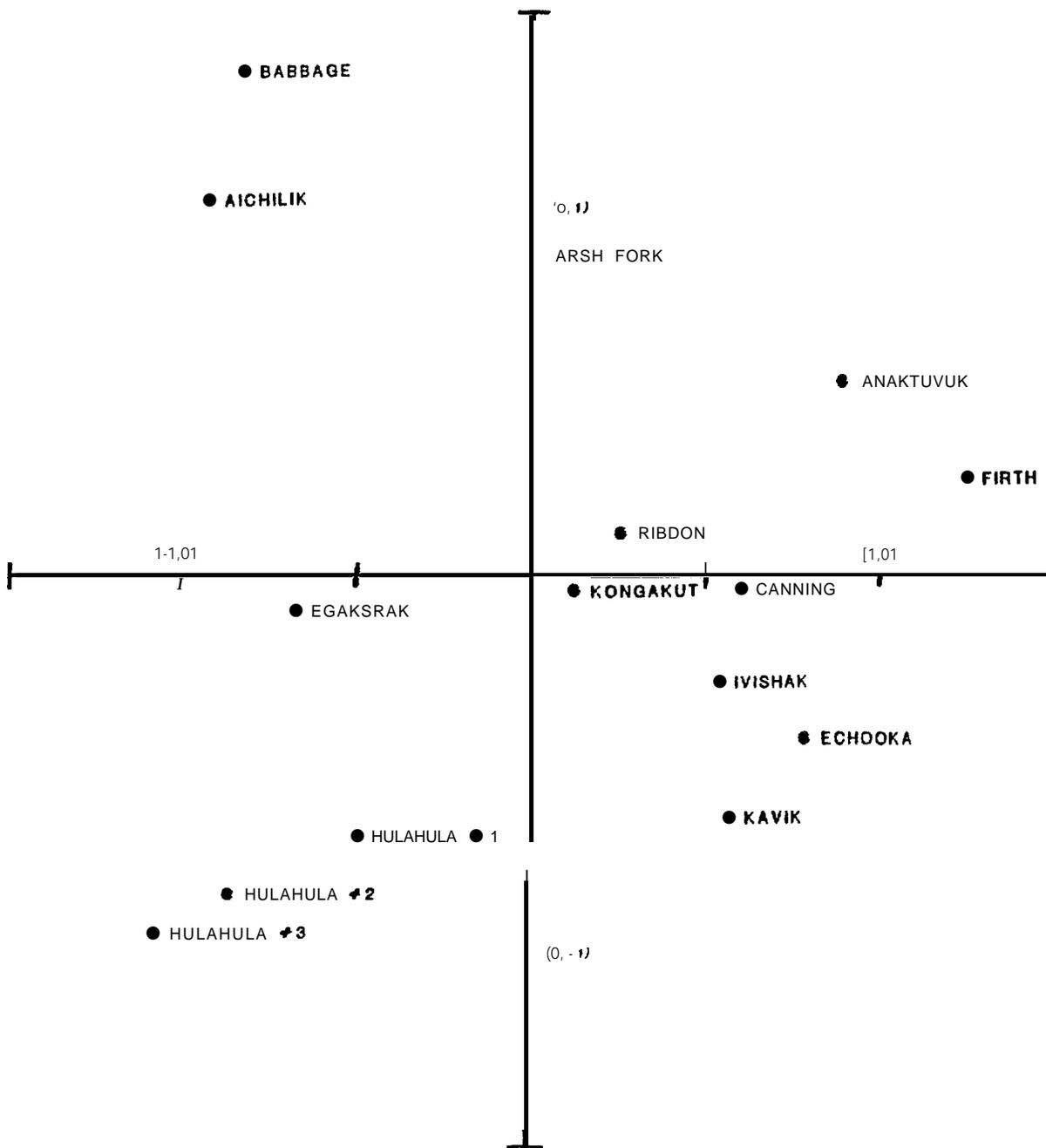


Figure 4. Plot of the relationships among North Slope Arctic char populations as determined using a multidimensional scaling procedure (Kruskal and Wish 1977). Scale of the diagram is standard Euclidean distance.

Genetic heterogeneity

Table 5 lists the results of the heterogeneity tests between North Slope Arctic char populations. The data for the three Hula Hula char samples were combined to simplify the table since they are not significantly different from each other. Sixty-nine out of 78 pairwise comparisons indicate significant genetic differences ($p < .05$) among North Slope char populations. A summary G-test, including all populations and all loci, shows that the Arctic char studied are highly different from each other ($G = 802.4$ with 127 degrees of freedom; $p \ll 0.001$).

Gene diversity analysis

Table 6 shows the absolute and relative magnitude of the diversity among **subpopulations**, analyzed hierarchically. Approximately 8% of the observed variation is due to differences among the populations of the ten drainages sampled. Less than 1% is due to differences among populations of different **sampling** sites within drainages. Variation among individuals within populations accounts for 91.5% of the total gene diversity.

Table 5. Matrix of genetic heterogeneity, tested pairwise among North Slope char populations. Data from the three Hula Hula populations were combined because they are not different from each other. The significance level was modified according to Cooper (1968) to reflect the number of pairwise tests (78).

1	Babbage													
2	Firth	*												
3	Kongakut	*	*											
4	Egaksrak	*	*	*										
5	Aichilik	*	*	*	NS									
6	Hula-all	*	*	*	*	*								
7	Canning	*	*	NS	*	*	*							
8	Marsh Fork	*	*	*	NS	NS	*	*	-					
9	Kavik	*	*	*	*	*	*	*	*	-				
10	Echooka	*	*	*	*	*	*	NS	*	NS	-			
11	Ivishak	*	*	*	*	*	*	NS	*	*	NS	-		
12	Ribdon	*	*	*	*	*	*	*	*	*	*	*	-	
13	Anaktuvuk	*	*	*	*	*	*	*	NS	*	*	*	*	-

* = $p < .05$.

NS = not significantly different.

Table 6. Gene diversity analysis among populations of Arctic char from the North Slope of Alaska. The average values represent data from all 15 sites from the ten drainages studied.

Drainage	# sites	Absolute gene diversity				Relative gene diversity (Percent)		
		Within sites	Between sites	Between drainages	Total	Within sites	Between sites	Between drainages
Hula Hula	3	.0456	.0008			98.29	1.71	
Canning	2	.0599	.0021			96.63	3.37	
Sagavanirktok	3	.0468	.0014			97.16	2.84	
Average	15	.0550	.0004	.0046	.0601	91.55	0.71	7.73

DISCUSSION

Genetic variation in populations is important because environmental alteration is inevitable, from both natural and man-caused conditions . Populations need to be responsive to change, and with less genetic variation there is a reduced potential to adapt to changing environments. While the immediate consequences of loss of variation are not known for Arctic char, in other species genetic variation is related to growth rate, developmental stability, survivorship, and the ability to compete (Frankel and Soule' 1981; Mitton and Grant 1984).

At the species level; genetic variation is present both within populations and distributed among populations. To determine how best to manage population to ensure their continued health, it is necessary to know both the amount and pattern of genetic variation. Studies using biochemical genetics methods have become increasingly important in fisheries management in the last two decades. Biologists have long recognized that salmonids form phenotypically (physically) recognizable subdivided populations. Homing behavior allows them to mix in feeding and rearing areas, while remaining reproductively isolated (Ricker 1972; Smith 1985).

Since the effect of environmental conditions is such a large

component of the observed variation among stocks, it was very difficult before protein analysis to determine what proportion of the differences is genetic and thus to identify discrete stocks. With this knowledge it is possible to understand the natural system, make recommendations for ongoing management, and determine the direction for future study.

Amount of variation

North Slope Arctic char have more genetic variation than might be expected given the relatively narrow range of northern waters they inhabit and the harsh environmental conditions encountered. With a percent average heterozygosity per locus of 5.1%, they are typical of fish species in general (H = 5.1%: Nevo 197'8); are at the upper end of the range observed in other salmonid fishes; and are higher than most other Arctic char populations that have been studied (Table 7).

Generally, Arctic char populations that have been studied are non-migratory, and have been profoundly influenced by repeated glaciation (Behnke 1972; Saunders and McKenzie 1971). Glacial action can cause loss of whole populations; result in small, isolated populations with low effective population size and the potential for inbreeding problems; and limit the number of fish founding new populations. All of these conditions lead to loss of variation and loss of variants. Northern Alaska Table

Table 7. Average heterozygosity (% H) in ten groups of **salmonid** fishes.

Species	Number of Populations	% H	Source of data
Brook char	8	8.1	Stoneking et al. 1981
Rainbow trout	55	6.0	Leary and Allendorf 1982
Arctic char (N. Slope)	15	5.1	This study
Arctic char (N. America)	5	2.9	Kornfield et al. 1981
Pacific salmon (5 species)	41	2.8	Allendorf and Utter 1979
Brown trout (Sweden)	38	2.5	Ryman 1983
Atlantic salmon (Sweden)	6	2.3	Stahl 1981
Arctic char (Norway)	15	1.7	Hindar et al. 1986
Arctic char (Sweden)	10	1.1	Andersson et al. 1983
Arctic char (Ireland)	9	1.1	Ferguson et al. 1981

Arctic char have probably benefitted both from the ice-free refuge during the last glaciation (McPhail and Lindsey 1970) and their anadromous life history strategy which permits migration among drainages. Both factors have likely contributed to maintenance of relatively high population sizes (preventing loss of variation) and the ability to successfully colonize this area.

Genetic similarity among populations

The genetic identity (Nei 1972; 1978) values among North Slope Arctic char populations are high, indicating fairly recent common ancestry. North Slope Arctic char populations exhibit a level of divergence typical of locally adapted populations as opposed to different species or even subspecies. Preliminary studies with the Arctic char-Dolly Varden char complex indicate that despite the number of different "morphotypes" and life history strategies, the overall genetic similarities are typically above 0.950, even between species (Robb Leary, University of Montana, personal communication).

High similarity values, though, do not imply lack of significant differences between Arctic char populations. The relationships between populations apparent from the clustering and multidimensional scaling figures indicate the distinctness of the populations and the complexity of the relationships between them. Comparison of these figures to the map of sample sites

indicates that there is no simple correlation between genetic relationships and geographical proximity.

It is not possible to determine the underlying cause of the observed relationships among North Slope Arctic char populations from protein studies. Selection, migration, mutation, behavioral isolation, founder effects, random genetic drift (chance changes due to small populations size) and combinations of these and other forces may all contribute.

We cannot, for instance, determine at this time whether or not non-migratory (resident) populations of Arctic char were included among our samples. While we have no evidence of mixed populations within samples, we cannot tell if, for example, the population sampled from the Canning Marsh Fork or Ribdon River are actually non-anadromous.

Sampling for this project was designed to collect anadromous char from their spawning streams to determine whether or not populations from different drainages are genetically distinct. If they are reproductively isolated, we should be able to demonstrate significant genetic differences among populations from different drainages. Since adult Arctic char are known to "visit" or overwinter in non-natal drainages, juveniles were collected. Juveniles of resident and **anadromous** forms are essentially indistinguishable morphologically.

In other salmonid populations that have been studied, e.g., rainbow trout (Allendorf and Utter 1979) and brown trout (Ryman and Stahl 1981), only a small percentage of the divergence among populations is due to the ecological distinction between resident and migratory forms. Resident populations of North Slope Arctic char could either be composed of a separately evolved group with physiological or behavioral isolating mechanisms from migratory groups, or they could have arisen independently in various drainages where condition made it unfavorable or impossible to migrate. Thus, resident groups could either resemble each other across the North Slope, or could most closely resemble the migratory groups in their drainage, with local divergence due to selection or genetic drift (random changes) in presumably small populations.

The taxonomy of Arctic char has still not been fully resolved. Based on counts of gill rakers and pyloric caeca, McPhail (1961) identified three forms, two of them from the North American Arctic region. His Eastern form is lacustrine and the Western form is generally anadromous. McCart and Craig (1971), using the same morphological features, identified both forms in the Sagavanirktok River Drainage. Bain (1974) determined that resident and non-resident forms in the Babbage River Drainage are both derived from the Western form of Arctic char.

Apparently, genetic differences detected between resident and migratory forms can be due either to separate evolutionary lines or recent reproductive isolation. Recent divergence may be due to behavioral or physical isolation, which allows genetic differences to accumulate.

Though we are reasonably sure that we have sampled Arctic char from their natal streams, we cannot be sure, then, that we have studied only **anadromous** stocks. This might explain the unexpected relationships among samples within drainages as illustrated by the dendrogram (Figure 3). Though some clusters of populations on the dendrogram may be explained by this possibility, it is certainly possible that similarities among geographically isolated groups may be due to selection, founding events, or by chance convergence of electrophoretic phenotypes at structural loci.

For example, Anaktuvuk and Firth River Arctic char, which show up as a cluster on the dendrogram of genetic similarities, may or may not be closely related **phylogenetically**. They may not be genetically similar over the major portion of the genome. The enzyme loci we study may be selectively neutral, and similarities among populations may be due to random forces leading to their inclusion in the population. Major differences may exist at loci controlling characters subjected to dissimilar selective forces imposed by different environmental conditions. What the

dendrogram does present is the divergence pattern observed for selectively neutral or nearly neutral loci among populations characterized by a very restricted amount of gene flow.

Also, because the computer program uses averages between populations to link them to each other, the dendrogram is not a direct reflection of pairwise tests of heterogeneity between population samples. It is not necessary to conclude that relationships among populations that cluster together on the dendrogram have biological meaning. Perhaps the multidimensional scaling plot is a better illustration of the true relationships among the populations studied; the populations actually are quite distinct from each other, as evidenced by the fact that they do not cluster tightly together.

Heterogeneity

Tests of genetic similarity between populations use all gene loci studied. Because over half the genes tested are **electrophoretically** indistinguishable in all the char populations, similarity calculations show the high degree of relatedness we have discussed. Heterogeneity tests, however, use only the gene loci that are variable to test for differences between populations, and consequently magnify the differences between them. Almost all North Slope Arctic char populations are significantly genetically distinct from each other. This

information indicates that fish from different drainages are not freely interbreeding, and are most likely true to their spawning streams.

Genetic diversity

Knowing that Arctic char populations are genetically heterogeneous is not helpful unless we know at what level of the population structure they vary. Our results show that most of the diversity in North Slope Arctic char is between individuals within subpopulations, and that a seemingly small percent is due to differences between subpopulations. However, our data are more informative when related to the structures of other Arctic char populations and other **salmonids**.

Gene diversity analysis (Nei 1973) uses **electrophoretic** data to determine how much variation there is within each population of a species studied versus how much difference there is among populations or groups of populations. **Salmonid** populations are typically subdivided genetically (Allendorf and Utter 1979; Behnke 1972; Ryman and Stahl 1981) but there is considerable difference in how divergent subpopulations are from each other (Ryman 1983). Gyllensten (1986) has found correspondence between life history strategies in fish species, e.g., whether they are marine, **anadromous**, or freshwater forms, and the pattern of genetic diversity.

Much of the observed pattern among different ecological forms probably reflects population size and lack of barriers to migration. Freshwater fish obviously live in **relatively small** populations, and the combination of isolated bodies of water and tendency of salmonids to home results in reproductive isolation. While anadromous **salmonids** also exhibit strong homing behavior, they have more opportunities (less barriers) to stray. Further, migratory species may have been able to avoid some of the effects of repeated glaciation. The North Slope of Alaska was ice-free during the last Wisconsin glaciation (McPhail and Lindsey 1970). Hence, North Slope populations may have been able to maintain population size and consequently, genetic variation.

The total gene diversity (as average heterozygosity per locus) in several stocks of Arctic char and other salmonid fishes are summarized in Table 8. The table also shows the amount of the total diversity due to differences among individuals, and the percent of the variation due to differences among populations. Our data for North Slope char have been included for comparison. As evident from the table, Arctic char populations from Europe and eastern North America that have been studied have not only less variation, but also the pattern of variation derives from large differences between groups.

Table 8. Distributions of **electrophoretically** detectable gene diversity (Nei 1973) among Arctic char and other **salmonid** species. Total diversity is divided into that due to variation within each population versus that due to differences between populations (expressed as a percent).

Species	Data ^a source	No. popns	No, loci	Total diversity	Diversity within	% between
Arctic char (Sweden)	1	10	37	.011	.008	27.3
Arctic char (N. Am.)	2	5	26	.061	.029	52.4
Arctic char (Ireland)	3	9	27	.018	.011	38.9
Arctic char (N. Alaska)	4	15	41	.060	.055	8.3
Chinook salmon	5	80	17	.099^b	.081	18.2
Chum salmon	6	13	12	.213 ^b	.208	2.3
Sockeye salmon	7	13	26	.047	.043	8.5
At. salmon (Sweden)	8	6	45	.025	.023	8.0

^a **1** - Andersson et al. 1983. **5** - Wilmot unpublished summary.
2 - Kornfield et al. 1981. **6** - Wishard 1981.
3 - Ferguson et al. 1981. **7** - Grant et al. 1980.
4 - This study. **8** - Stahl 1981.

^b Amount of variation is overestimated when only variable loci are included in the analyses.

North Slope Arctic char do not have the magnitude of difference between groups exhibited by non-migratory char, but do compare with the population structure of **anadromous** pacific salmon. This fact is particularly relevant because genetic stock identification methods have been successfully applied to these **salmonids**, and can give us an indication of whether this method can be applied to mixed stocks of North Slope Arctic char.

Genetic stock identification

The basis of genetic stock identification is **electrophoretically** detectable differences in genotype frequencies between stocks. To do genetic stock identification (**GSI**) there must be sufficient detectable genetic variation in the stocks to be studied. Variation between groups of populations, e.g., between those of major drainages, should be relatively high combined with a low within-group variability. **Also**, the baseline should represent the major populations contributing to the mixed stock to be analyzed.

Genotype frequency estimates are made for major stocks expected to contribute to the mixed stock (baseline data) and for samples taken from the mixed stocks. Maximum likelihood estimates of proportional contribution of different populations to the mixed stock are then made (**Milner** et al. 1981), based on the patterns observed in baseline samples.

Genetic stock identification is a useful tool in the study of fish populations. The GSI method has shown very good agreement with other methods of determining stock composition such as scale pattern analysis and coded wire tagging. It is currently used by the Washington Department of Fisheries, Olympia; Canadian Department of Fisheries and Oceans, British Columbia; and the National Marine Fisheries Service, Seattle.

This method may be useful for discrimination of populations of Arctic char in mixed populations using the offshore waters of the Beaufort Sea. Our data indicate that North Slope char have a relatively large amount of genetic variation; that there are significant differences among populations; and that the observed variation is partitioned such that there is as much difference between char from different drainages as there is among populations of sockeye and chum salmon where genetic stock identification has been used successfully. As such, we can anticipate successful application of this technique to the identification of char at specific offshore sites.

Recommendations

We have determined that North Slope char have a relatively large amount of genetic variation, and that populations we sampled are genetically distinct from each other. From this we know that different stocks are currently reproductively isolated from each other. Since they mix to some unknown degree in feeding areas, the differences that have been established between stocks are maintained by homing behavior. Populations of each drainage are probably discrete, locally adapted units. It is not clear at this time how non-migratory forms are related to anadromous stocks.

It is unlikely that loss of any one stock would be mitigated by substitution of another. While the actual loci we have studied may be selectively neutral, underlying variation that is marked by these loci may be highly selected for in different environments, corresponding generally to different drainages. As such, Arctic char stocks of the North Slope should be managed as individual, unique gene pools. Further study will make the relationships of anadromous and resident forms of this species more clear. With additional effort, using GSI methods, it would also be possible to understand more about the Arctic char in its migratory phase in the offshore areas.

Strategy

To expand our understanding of the genetic diversity and population structure of North Slope Arctic char, we should continue to use the techniques of biochemical genetics. Further work is needed to understand the relationships among populations, and to improve the data base for genetic stock identifications of migratory offshore stocks.

Many stocks of Arctic char living on the North Slope are not **anadromous**. Since we have no method of discriminating among resident and **anadromous** juveniles, we have assumed that those in the rivers are anadromous. To get a complete picture of the resource, we should consider deliberately sampling resident populations, e.g. , those fish associated with springs or lakes.

It is important that we identify and sample additional populations making major contributions to the Beaufort Sea admixture, as it is an important assumption of the **GSI** model that all major contributors to a mixed stock be represented in the baseline. The baseline should particularly be expanded to include more samples from subpopulations from other tributaries in the drainages we have already begun studying.

An important consideration in doing genetic stock identification is that each analysis of a sample from the

offshore stocks estimates, with predetermined expectations of precision and accuracy, the percent composition at only one point in space and time. Distribution of offshore stocks of fish is related to environmental conditions which are highly variable from year to year (Dick Marshall, USFWS, Anchorage, Alaska). Also, Arctic char are highly mobile in offshore areas, so estimates should be made of stock composition at several times during the short summer feeding season.

While we will surely know more about the use of offshore areas by anadromous char than we did before, it must be realized that there will be considerable variation, regardless of study method used, between data from different years and different areas and at different times during the season. This means that stock identification must be done on a site-specific basis, with repeated sampling during the summer, and that data from more than one year will be required to establish the pattern of use by the fish.

Even though the composition of stocks using the offshore area at any given area may change, baseline data can be used in more than one year. By doing offshore sampling and identifying the origin of the populations that are represented, we may find specifically which stocks are at risk at specific sites at several different times of the season.

Additional study would provide the opportunity to gain a more thorough understanding of the population structure of Arctic char in an area which, at this time, is relatively untouched by development . With an appropriate sampling strategy over space and time, genetic stock identification could yield data appropriate for site-specific approach to determining the use and timing of individual stocks in offshore waters. Knowledge of the natural system will afford us the information needed to address present concerns , and the basis for future conservation and management .

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Appendix 1. Gene frequencies of variable 10cI in 15 populations of Arctic char from the North Slope of Alaska and Canada. Variants of duplicated 10cI were arbitrarily assigned to one locus of the duplicate pair. Names of enzyme loci (abbreviated here) are in Table 2. ND: no data.

LOCI		Populations														
		<u>AIC</u>	<u>ANA</u>	<u>BAB</u>	<u>CAN</u>	<u>ECH</u>	<u>EGA</u>	<u>FIX</u>	<u>HUI</u>	<u>HUZ</u>	<u>HUJ</u>	<u>IVI</u>	<u>KAV</u>	<u>KON</u>	<u>MAF</u>	<u>KIB</u>
HEX1	100	.525	.859	.530	1.000	1.000	.806	1.000	1.000	.973	1.000	1.000	1.000	1.000	.638	1.000
	67	.475	.141	.470	--	--	.194	--	--	.027	--	--	--	--	.362	--
	N	4(I)	39	33	20	23	31	40	13	37	59	49	40	40	29	40
AC03	100	.550	.403	.700	.476	.478	.557	.392	.545	.435	.491	.561	.463	.550	.552	.587
	115	.175	.292	.028	.262	.109	.243	.243	.182	.242	.280	.204	.137	.163	.190	.113
	130	.278	.305	.272	.262	.413	.200	.365	.273	.323	.229	.235	.400	.287	.258	.300
	N	40	36	35	21	23	35	37	11	31	59	49	40	40	29	40
AAT1	100	1.000	.975	1.000	1.000	1.000	.986	.897	ND	ND	ND	ND	1.000	1.000	.983	ND
	33	--	.025	--	--	--	.014	.103	--	--	--	--	--	--	.017	--
	N	40	40	33	20	22	35	39	--	--	--	--	37	40	29	--
AAT3	100	.937	.925	1.000	.796	.935	.939	.950	ND	ND	ND	.980	1.000	.887	.931	.950
	75	.063	.075	--	.204	.065	.061	.037	--	--	--	.020	--	.113	.069	.050
	129	--	--	--	--	--	--	.013	--	--	--	--	--	--	--	--
	N	40	40	35	27	23	41	40	--	--	--	50	40	40	29	40
GP11	100	1.000	.950	1.000	1.000	1.000	.950	1.000	1.000	1.000	1.000	.990	1.000	1.000	.983	.925
	55	--	.050	--	--	--	.050	--	--	--	--	.010	--	--	.017	.075
	N	40	40	35	27	24	41	40	15	36	59	50	40	40	29	40
GP13	100	.775	.900	.628	.815	.896	.829	.763	.967	.843	.847	.920	.987	.700	.931	.667
	96	.225	.100	.372	.185	.104	.171	.237	.033	.157	.153	.080	.013	.300	.069	.333
	N	40	40	35	27	24	41	40	15	35	59	50	40	40	29	39
GAP3	100	.566	.934	ND	.780	.792	.500	.967	.455	.365	.283	.766	.750	.730	.250	.730
	null	.434	.066	--	.220	.208	.500	.033	.545	.635	.717	.234	.250	.270	.250	.270
	N	38	38	--	25	24	32	15	11	26	53	47	32	37	28	37
IDH2	100	1.000	1.000	1.000	.978	1.000	1.000	.963	1.000	.986	1.000	.980	.975	1.000	1.000	.950
	220	--	--	--	.022	--	--	.037	--	.014	--	.020	.025	--	--	.050
	N	40	37	35	23	24	35	40	15	36	59	50	40	40	29	40
IDH3	100	.888	1.000	1.000	.950	.978	.986	.949	1.000	.894	.983	1.000	.913	.937	.966	1.000
	80	.112	--	--	.050	.022	.014	.051	--	.106	.017	--	.087	.063	.034	--
	N	4(I)	39	35	20	23	35	39	11	33	59	49	40	40	29	40

Appendix 1. Cent I nued.

LOCI	Population															
	AIC	ANA	BAB	CAN	ECH	EGA	FIR	HU1	HU2	HU3	IVI	KAV	KON	MAF	RIB	
LDN5	100	.947	1.000	.986	.979	.978	.929	.963	1.000	.936	.966	.970	.987	.937	.983	.925
	97	.053	--	.014	.021	.022	.071	.037	--	.064	.034	.030	.013	.063	.017	.075
	N	38	35	35	24	23	35	40	15	39	59	50	40	40	29	40
MDH1	100	1.000	.956	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
	128	--	.044	--	--	--	--	--	--	--	--	--	--	--	--	--
	N	37	34	35	20	23	35	40	13	37	59	49	40	40	29	40
MEE3	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.972	.975	1.000	1.000	1.000	1.000	1.000
	69	--	--	--	--	--	--	--	--	.028	.025	--	--	--	--	--
	N	40	40	35	23	24	35	40	15	36	59	50	40	40	29	40
PGM2	100	1.000	1.000	1.000	ND	ND	1.000	.987	1.000	.986	.983	1.000	1.000	1.000	1.000	1.000
	88	--	--	--	--	--	--	.013	--	.014	.017	--	--	--	--	--
	N	40	35	28	--	--	39	40	15	36	59	50	40	40	26	40
6Pg1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.990	1.000	1.000	1.000	.987
	95	--	--	--	--	--	--	--	--	--	--	.010	--	--	--	.013
	N	40	40	35	23	24	35	40	15	36	59	50	40	40	29	40
SDH1	100	.987	.886	1.000	.880	.913	.975	1.000	.929	1.000	.972	ND	1.000	.963	.897	1.000
	43	.013	.114	--	.120	.087	.025	--	.071	--	.028	--	--	.037	.103	--
	N	40	35	34	25	23	20	39	7	17	36	--	39	40	29	40
SOD1	100	.950	1.000	.956	.976	.978	.943	.987	.885	.903	.915	.969	1.000	.975	.983	.887
	115	.050	--	.015	.024	.022	.057	.013	.115	.097	.085	.031	--	.025	.017	.113
	87	--	--	.029	--	--	--	--	--	--	--	--	--	--	--	--
	N	40	35	34	21	23	35	40	13	36	59	49	40	40	29	40
Xxol	100	.750	.727	.780	.737	.932	.629	.778	ND	ND	ND	.804	ND	.676	.714	.809
	86	.250	.273	.220	.263	.068	.371	.222	--	--	--	.196	--	.324	.286	.191
	N	40	33	25	19	22	35	36	--	--	--	46	--	37	28	34

AIC = Aichilik; ANA = Anaktuvuk; BAB = Babbage; CAN = Canning; ECH = Echooka; ECA = Egakarak; FIR = Firth;
 HU1 = Hula Hula Site 1; HU2 = Nula Hula Site 2; HU3 = Hula Hula Site 3; IVI = Ivishak; KAV = Kavik; KON =
 Kongacut; MAF = Canning Marsh Fork; RIB = Ribdon