The population genetics of Arctic grayling (*Thymallus arcticus*) of Montana

by

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B.S., University of California, Davis 1976

Presented in partial fulfillment of the requirements

for the degree of

Master of Arts

University of Montana, Missoula

1986

Approved by:

Chairman, Board of Examiners

Dean, Graduate School

Date

Everett, Rebecca Jane, M.A., December 1986 Zoology

Population Genetics of Arctic Grayling
(Thymallus arcticus) of Montana (82 pp.)

Director: Dr. Fred W. Allendorf FWA

Electrophoretic and morphological methods were used to describe the amount and pattern of genetic variation in populations of Arctic grayling from Montana. Samples representing Alaska and Canada populations were used for comparison.

The amount of genetic variation, measured as average heterozygosity, was 3.28% in Montana populations, 2.11% in Alaska fish, and 5.96% in the Canada-derived stock. Of 34 loci studied, 7.8% were polymorphic in Montana populations versus 11.8% in the Canada sample.

Montana grayling were historically a river-dwelling species. Current stocks are mainly in lakes, and are of hatchery origin or have been supplemented by stocking. It was expected that all populations would be very similar genetically due to the shared common origin or that differences among populations would reflect adaptations to different environmental conditions.

The genetic identities among all populations studied are high. Nevertheless, there are significant differences in allele frequencies among populations. Regardless of habitat type, the hatchery populations are most closely related to one another whereas the Big Hole River Drainage populations, with native fish, are distinct.

There is no evidence of introgression with transplanted Arctic stocks. High frequencey variants observed in the non-native populations would make mixed stocks detectable.

The amount of variation in seven morphological traits, measured as a coefficient of variation, was uncorrelated with average heterozygosity. The relationships of populations indicated by morphological data showed little or no correspondence with stocking records or isozyme data.

Management strategies for conservation of this threatened species should reflect the presence of genetically unique stocks that should be preserved.

ACKNOWLEDGMENTS

Training a graduate student in population genetics takes a lot of time and effort; I would like to thank my major professor, Fred Allendorf, and Kathy Knudsen, Robb Leary, and Steve Phelps of the Genetics Lab for teaching me. Thanks also to my committee, Drs. Andy Sheldon, Kathy Peterson, and Craig Spencer.

Kathy Knudsen, Tom Deveny, Debbie Burgoon, and Melinda Everett helped me collect samples. Other samples were provided by Rocky Holmes of the Alaska Department of Fish and Game; Bill Hill and Dick Oswald of Montana Department of Fish, Wildlife and Parks; and the Harold Wetzsteon family of Wisdom, Montana.

Financial support was arranged by Fred Allendorf, and came from the Bechtel Trout Fund; the Graduate School and the Zoology Department of University of Montana; the U.S. Fish and Wildlife Service and Yellowstone National Park; and the Montana Department of Fish, Wildlife and Parks. George Holton of MDFWP did a lot to make the study successful.

Thanks to fellow graduate students, special friends, and the faculty and staff of the Zoology and Wildlife Departments for making graduate school such a pleasant experience.

Most important are the thanks I owe my parents, Richard and Margaret Everett, who have been very patient and supportive through my long college career.

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INTRODUCTION

The evolutionary potential of a species determines its capacity to adapt when challenged by change in the environment. The ability of a species to evolve is limited by the amount of genetic variation it has (Frankel and Soule' 1981). Since man is accelerating the rate of environmental change, it has become increasingly important to conserve biological diversity. Conservation of organisms requires quantification of genetic variation and an understanding of how it is distributed within and among populations (Allendorf and Utter 1979; Ryman 1983). This type of analysis can identify reproductively or ecologically specialized populations, or suggest whether all or only some existing populations include most of the diversity typical of the species. Many unique gene pools have been lost in western North America salmonids because of inadequate understanding of population structure (Behnke and Zarn 1975).

Arctic grayling (Thymallus arcticus) are members of the family Salmonidae and live in freshwater drainages in northern Asia and North America. A disjunct population exists in Montana, and another survived in Michigan until it was extirpated in 1936. These two southern populations are thought to have been separated from northern populations since the Wisconsin glaciations. It has been postulated that the encroachment of man with the accompanying habitat alteration, pollution, introduction of exotic fishes, exploitation of fish stocks, and an

overall lack of genetic diversity caused the extinction of the Michigan grayling and the decline of the Montana grayling (Vincent 1962). Though they may be well-suited to the environment where they evolved, grayling may not have enough genetic variability to persist where rapid environmental changes occur.

Arctic grayling are considered a "species of special interest or concern" in Montana by the U.S. Fish and Wildlife Service (USFWS) and by the Montana Department of Fish, Wildlife, and Parks (MDFWP) (Holton 1980). The Federal Register (1985) assigns them to Category 2, which proposes that it may be appropriate to list them as an Endangered Species pending further biological research and field study. The original range of Arctic grayling in Montana and Wyoming was the tributaries of the Missouri river upstream from Great Falls (Henshall 1906) (Figure 1). With the influx of white man and the subsequent environmental changes, populations of grayling declined, and efforts to conserve them began. They were raised in hatcheries, transplanted into isolated barren lakes, and grayling were introduced from the Arctic into Montana waters at least once. These early conservation efforts resulted in many grayling stocks of mixed origin in Montana and Wyoming. Varley (in Peterson 1981) estimated that grayling populations in Montana, as of 1975, have been reduced to four percent of their original river range in spite of all the programs to preserve them.

The American Fisheries Society classified stream-dwelling grayling as a separate taxa and listed them as a "species of special concern", different from those populations that had been established in lakes (Deacon et al. 1979). While there are still grayling in some rivers

and streams and in several lakes in Montana and Wyoming, it was not known if the remaining populations in streams or the stream-derived stocks planted in lakes were unchanged genetically or how they are currently related to each other. Transplanting bottlenecks, adaptation to lake habitats, or introgression of Arctic fish, as well as mixing of subpopulations from different areas, may have altered the gene pools. It is also not known if the sources of the lake populations (primarily Madison River tributaries) were historically different genetically from the other river populations or if current differences between lake and stream populations have resulted from divergence subsequent to their isolation from each other.

Many biologists believe that the southern fluvial grayling are genetically distinct from the Arctic populations. Differences in length of life, age at maturity, and growth rate have been documented (Tryon 1947). Electrophoretic differences have also been reported (Lynch and Vyse 1979). McPhail and Lindsey (1970) mention introductions of grayling from the Arctic to Montana. Though planting records show one introduction of grayling into certain lakes in Montana in 1952, it has been unclear whether or not these Arctic fish were mixed with Montana populations. It was also not known whether or not such introductions would have altered the genetic character of the southern stock of Montana and Wyoming.

Recently several stocks were used to try to restore grayling into Canyon Creek, a rehabilitated stream (one with the non-native species of fish poisoned out), in Yellowstone National Park (USFWS Annual Project Technical Reports, YNP, 1976 - 1980). Because these efforts were

repeatedly unsuccessful, it has been suggested that adaptation to a lacustrine environment has resulted in loss of the tendency to hold stream position, indicating divergence between the lake and original stream "ecotypes" (Jones et al. 1977). Questions have been raised regarding the most useful source of grayling for successful transplantation into streams in the native range of the species.

The current population genetic structure of grayling in Montana and Wyoming cannot be expected to represent historical conditions because of the extensive transplantation of fish within the states, and the expected divergence of stocks in isolated refuges. However, because current populations were drawn mainly from stocks that are now extinct, the ones remaining provide the only possible information on their progenitors. Differences between populations could provide data to suggest which populations might be more successfully used in restoration programs. If stream-dwelling grayling are indeed different from the present "lake" stocks, it could be that they would be better suited in some way to re-populate a stream. If Arctic fish have been mixed with southern populations the resulting stocks would not be appropriate for a native fish restoration program.

Many populations of salmonids are distinct in morphological and life history traits over relatively small geographic areas (Ryman 1983, 1981; Behnke 1972). Early taxonomists and biologists relied on morphometric measurements and meristic counts to make inferences about systematic and historical relationships between populations. Several studies have shown that much morphological variation is causally related to chemical and physical factors in the environment, e.g. salinity or

temperature (Hubbs 1922; Taning 1950; Barlow 1961; Fowler 1970). More recent work includes ecological and behavioral traits to describe variation in conspecific populations (Behnke 1972). These characteristics also are influenced to an unknown degree by environmental parameters.

Biochemical genetic methods allow collection of data that expresses direct relationships among populations at the level of the gene. It is possible to quantify the amount of genetic variation in populations, how they compare to other populations, and how they compare to other taxa. It is also possible to quantify the way in which variation is partitioned within and among populations of a species (Nei 1973) and to compare the population structure among species (Allendorf et al. 1979; Ryman 1983; Gyllensten 1985).

There is general concordance between electrophoretic classification of species and classifications by more traditional methodologies, including cytotaxonomic and karyotypic studies (Avise 1974). Few studies, however, address the consistency between biochemical and morphological analysis of the relationships of conspecific populations or consider the information these analyses provide on the amount of variability in natural populations.

Objectives

I initiated a study of the population genetics of Arctic grayling in Montana because efforts to re-introduce them into their native stream habitat were generally unsuccessful. It was suggested that the transplant stocks had become adapted to lake conditions, and could not survive in rivers and streams. Genetic differences between lake and stream "ecotypes" were suspected, as well as an overall lack of genetic diversity in this glacial relict species. Also, questions have arisen regarding past introductions of Arctic stock into Montana waters, which were possibly mixed in with native stocks.

The objectives of this study were to use electrophoretic and morphological methods to (1) characterize the population genetic structure of Arctic grayling, that is, to determine how much genetic variation there is and how it is distributed within and between populations; (2) determine if riverine grayling are genetically distinct from lacustrine populations; and (3) find out if grayling brought down from the Arctic are detectably different from southern stocks and if these stocks have been mixed.

METHODS AND MATERIALS

Samples and their stocking history

I studied enzyme and morphological variation in fourteen populations of Arctic grayling. Figure 1 shows sampling locations, and Table 1 lists names, locations, sample sizes, and year of capture for each population. Montana and Wyoming samples were obtained from twelve populations; nine are from lakes, and the other three are from a creek, a river, and a canal. The grayling in Fuse Lake, Montana were used to represent the grayling of Canada, and a sample was obtained from Alaska.

Grayling are native in two of the lakes sampled, Red Rocks Lake and Elk Lake, in the Red Rocks River Drainage. They also are native in the Big Hole River, its tributaries, and probably the lakes on the tributaries (including Miner, Bobcat, and Mussigbrod). It is not known if the Steel Creek fish sampled were residents or had migrated up from the Big Hole River less than 0.5 km away. The other lake and canal populations were established, and in many cases maintained, by stock transplanted from "nursery lakes". These lakes were originally stocked mainly from Madison River tributaries, but also from the Red Rocks Lake population (MDFWP records; Randall 1978).

Fuse Lake, Montana has a population of grayling that was established in 1952 from a Canada source. This stock originated in the Northwest Territories in the Fond du Lac region of the Athabasca Drainage, but was actually brought here from a secondary population in

Saskatchewan. The sample from Alaska was sent by biologists from the Alaska Department of Fish and Game; it is from a natural population in the Chena River and Badger Slough from the interior of the state, near Fairbanks.

Sampling

Samples were collected using gill nets, electroshocking equipment, hook-and-line, and other methods. Whole fish were brought on ice to the lab where they were treated in three ways: 1) sampled, frozen at -80 degrees C, thawed, and run on gels, 2) frozen at -40 degrees C, sampled, and run, or 3) frozen at -40 degrees C, sampled, frozen at -80 degrees C, thawed and run. Differences in enzyme activity between samples seemed to correspond more to length of time in storage and number of times they were re-used than to the method of preparation. Whole fish were stored frozen for eventual dissection for meristic counts.

Electrophoresis

I used horizontal starch-gel electrophoresis to identify protein products of gene loci. The methods used are those of Utter et al. (1974). Buffers and staining procedures are after Allendorf et al. (1977). Isozyme nomenclature is that of Allendorf et al. (1983). Gel buffers included: AC (Clayton and Tretiak 1972) pH 6.1-6.6; AC+ (same as AC, plus 30 mg nicotinamide adenine dinucleotide (NAD) and one drop of 2-mercaptoethanol); RW (Ridgway et al 1970) pH 8.5; MF (Markert and Faulhaber 1965) pH 8.7). I tested over 25 enzymes for activity and resolution with various buffers, and chose 34 loci coding for seventeen enzymes to use in statistical analyses (Tables 2 and 3). The ones

chosen are the ones for which I have complete data sets and consistent results, including good resolution and a repeatable pattern of expression.

Two populations samples, Fuse Lake and Red Rocks Lake, were used to determine the expression of enzyme loci in several tissues. Muscle, liver, and eye tissue were used for analyses in all populations.

Inferences were made regarding enzyme expression based on 1) assumptions of parallel expression with that of other salmonids with experimentally determined patterns of inheritance, 2) comparisons based on expression in different tissues, and 3) on the known molecular subunit structure of the enzymes, e.g. dimeric or tetrameric. I measured the mobilities of enzymes relative to the common homologous (orthologous) loci in rainbow trout.

Meristic counts

The term meristic is used in the broad sense to denote countable vs. measurable morphological traits. I counted meristic characters for ten populations of Arctic grayling: nine samples were from Montana and Wyoming, including the Fuse Lake sample of Canadian origin, and the other was from Interior Alaska. The characters studied included anal, dorsal, pectoral,and pelvic rays; gillrakers; vertebrae; and pored scales in the lateral line. I dissected the specimens to make the counts, and used a dissecting microscope for magnification. I recorded bilateral characters separately as left and right counts, and also distinguished counts of upper and lower gillrakers. In most analyses, however, right and left counts, and upper and lower counts were

combined, for a total of seven characters.

For dorsal and anal fins I made counts of all detectable rays. The posterior ray, which is usually two branches with a common base, was counted as one ray as in Hubbs and Lagler (1970). For pectoral and pelvic fins, I counted the large leading ray as one, disregarding any ray anterior to it. For gillrakers on the first branchial arch, I counted upper and lower rakers separately; any ray in the angle of the first arch was arbitrarily assigned to the count of the lower raker. All rudiments were included. I counted forked rakers as two if they forked at the base, and as one if they branched distal the base. I counted the pored scales in the lateral line anterior to the hypural plate, as described in Hubbs and Lagler (1970). For vertebrae counts, I excluded those in the urostyle.

RESULTS: POPULATION GENETICS

The amount of genetic variation in populations can be quantified and comparisons among populations can be made. Study of variation within populations can indicate whether separate samples from a population are homogeneous; whether the genotypes are in random mating proportions; and can be used as a reference for comparison to other populations. Analyses of the variation among populations can determine whether they are different statistically; provide an index of how similar they are on a pairwise basis; and provide information on the genetic relationships among populations. The overall structuring of the populations can then be determined regarding the amount of the observed variation that is due to differences among individuals within populations as opposed to the amount of the total variation that is due to differences among populations or other subdivisions of the species. This information can then be used for comparison with other taxa.

Genotypic proportions

Some populations were sampled twice, so they were tested for differences of allele frequencies between years; there are no significant differences between these samples when chi-square values and degrees of freedom for each locus are summed and tested for each population. The data were consequently pooled for all analyses. The Alaska sample was composed of fish from both the Chena River (N = 28)

and Badger Slough (N = 10), which are connected bodies of water. There were no significant differences in allele frequencies between these two samples so they were combined.

Genotypic distributions at individual variable loci were tested for conformity to random mating (Hardy-Weinberg) proportions using multiple simultaneous chi-square tests for goodness of fit (Tables 4, 5, 6). The genotypes of all loci studied were in random mating proportions when chi-square values and degrees of freedom were combined over non-duplicated, variable loci in each population.

Because some duplicated loci have not diverged (isoloci) and consequently have alleles in common, it is not possible to determine whether one or both of the pair are varying. Thus, allele frequencies for isoloci are usually calculated as if they are single, tetrasomic loci. I tested duplicated loci for conformance to expected tetrasomic frequencies using the chi-square statistic. The degrees of freedom were established for each locus tested by requiring a minimum expected value of 1.0 for each cell (Lewontin and Felsenstein 1965); cells were combined to meet this minimum.

At Gap3,4, the observed genotypes of only four of the twelve populations with variation fit the expected values for tetrasomic inheritance (Table 7). In the native Montana/Wyoming populations the pattern at Gap3,4 could be ascribed to variation at only one locus; nine out of the eleven samples with variation fit a disomic model (Table 8) for Gap3. In the Fuse Lake (Canada) population, however, the observed pattern of variation would not fit a model suggesting that only one of the pair were variable, but it also did not fit the model of

tetrasomic inheritance. The other loci studied in the Fuse Lake population were in random mating proportions so it is unlikely that more than one population was included in the sample.

For the other duplicated loci (Mdh1,2; Mdh3,4; Aat3,4; and Idh3,4), observed frequencies were not significantly different than expected values for a tetrasomic model excepting Mdh3,4 in the Chena River sample ($P < .05$) (Table 9). This locus was not easily scored because resolution was poor and banding intensities are important in a duplicated system; this could be the source of the deviation from expected values. It could also have been due to sampling error, with only a few families represented.

Amount of genetic variation

The amount of genetic variation was estimated by determining the percent of loci that are polymorphic (P), and the mean percent of heterozygous loci per individual (H) (Table 10). P is the percent of loci in which the frequency of the common allele is less than or equal to 0.99. Average heterozygosity was estimated using the observed allele frequencies and expected Hardy-Weinberg proportions averaged over all loci except for those that are duplicated. For isoloci I used observed heterozygosity, defined as the proportion of individuals in the sample having copies of more than one allele; variation at these loci could not definitely be attributed to one or the other of the duplicated pair.

The percent of loci that were polymorphic ranged from 3.0 to 11.8. Most populations have variation at Sod1 and Gap3,4. Fuse Lake, the Canada-derived population, has the greatest number of variable loci. Miners Lake has a high frequency of a Pgm1 variant, and the Big Hole River has a unique muscle Ck1 variant at low frequencies. The mobilities of the variants for Idh3,4 in the Rogers Lake and Fuse Lake samples are similar, but not necessarily the same; the frequency of the variant was low in Rogers Lake, and good comparisons could not be made.

The average heterozygosity per individual varied from 0.62 to 5.96%. The lake populations established from the Madison River and Red Rocks Lake stock have an average heterozygosity of 4.0%. The Sunnyslope Canal population, also derived from Madison stock, has only 3.1% average heterozygosity. The Big Hole River populations (Bobcat, Steel, Mussigbrod, Miner Lakes; Big Hole River) have been repeatedly planted with Madison/Red Rocks stock, but are lower in variability (2.0%).

With an average heterozygosity per individual of 6.0%, the Fuse Lake population has the highest amount of variation measured. The Alaska population, Chena River, has a calculated heterozygosity of 2.1. This is an underestimate of the variability of this population as it was not possible to score Pgm-3,4, which was highly variable. This locus appears to be duplicated (as evidenced by the banding intensities) as is Pgm3,4 in rainbow trout (Allendorf et al. 1983). In both species there are at least three alleles, resolution is poor, and banding intensities are hard to interpret reliably in a duplicated locus producing a monomeric enzyme.

Allele frequencies

Allele frequency data for gene loci were compared to test for genetic differences between the fourteen populations sampled. I used multiple simultaneous chi-square tests for goodness of fit to make pairwise comparisons of polymorphic loci. The chi-square values and the degrees of freedom for all variable loci in each pair of populations were summed. The significance level was modified according to Cooper (1968) because the tests are not independent. The probability value, .05, was divided by 91, the number of pairwise comparisons made, requiring a calculated probability of .001 for significance (Table 11).

Most of the differences between Montana populations are the result of differences in allele frequencies at one or two variable loci. The Fuse Lake population, with the Canada-derived stock, had more variable loci and significant differences at three to five loci when compared to other populations. The Alaska sample was significantly different at two to four loci, and at the unscored Pgm-3,4 locus which was variable only in that population.

The Chena River, Fuse Lake, and Sunnyslope Canal populations are significantly different in allele frequencies from all other populations. The Big Hole River population is significantly different from all populations outside that drainage, and is different from the Miner Lake population within the drainage because of the high frequency of a Pgm1 variant in Miner Lake grayling. Pgm1 data are not available for the Mussigbrod Lake population (also from the Big Hole River Drainage); the variant at this locus is not detectable on AC buffer gels, and tissue samples for this population were discarded before I

determined that there is a variant detectable only on high pH gels.

Though all native Montana/Wyoming populations studied have been planted at least once with large numbers of fish originating from Grebe Lake stock (MDFWP stocking records), the Grebe Lake population is still significantly different from the Big Hole River and the Miner Lake populations from the Big Hole Drainage, and from the Sunnyslope Canal population.

Genetic identity

I used the method of Nei (1972) to measure the genetic identity between populations. 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. This similarity value is scaled from 0.0 to 1.0; 0.0 corresponds to complete allelic substitution at all loci, and 1.0 to pairs of populations that are electrophoretically identical. I observed no complete allele substitutions among populations. The identities among Arctic grayling are very high, all at least 0.980. Genetic distance is calculated as the negative natural log of the genetic identity and is reported with standard errors (Table 12).

Cluster analysis

I used a cluster analysis of both Nei's (1972) genetic identity and Rogers' (1972) genetic similarity to make dendrograms (Figures 2 and 3) to show relationships between populations. The average linkage method of cluster analysis was used on weighted averages of the similarity values because the sample sizes were unequal. Rogers' similarities are calculated using the geometric distances between the allele frequency vectors of all loci of the populations being compared. They give the same general relationships between populations as Nei's index, but exaggerate the differences between very similar populations.

The values used reflect analysis of 34 loci in all populations excepting Mussigbrod Lake, for which 33 loci were examined. Native Montana/Wyoming populations in lakes (hatchery transplants) cluster first, followed by Big Hole River Drainage populations, then Miner Lake (BHR), Chena River (Alaska), and Fuse Lake (Canada) populations. The divergent Sunnyslope Canal population clusters with Alaska.

Gene diversity analysis

Partitioning genetic variation makes it possible to determine whether the total genetic variation (H_T) in the populations studied is a result of differences between individuals within subpopulations (H_S) or differences among subpopulations (D_{ST}). The relative amount of divergence (G_{ST}) among stocks compared to the total amount of genetic variation is then D_{ST} / H_T (Nei 1973). For this analysis, the variation at Gap3 and Gap4 was treated as if all the variation was at Gap3. In the Fuse Lake sample, where the observed variation could not be fitted

to one locus, the real values were estimated by including the relatively rare, anomalous genotype with the genotype most similar to it. Since this method of lumping the genotypes together changes the allele frequencies very slightly, and it is allele frequencies that are used to calculate expected heterozygosities for this analysis, little error was introduced by using this approximation.

Calculations were made to compare the amount of differentiation between populations to the total variation among all populations studied. With the Canada-derived and Alaska populations, about 31 percent of the total variation in the Arctic grayling sampled is due to differences among populations as opposed to differences between individuals within populations. When only the native Montana/Wyoming populations were included in the analysis, the amount of the total variation attributable to variation between populations is 25%. If only native Montana/Wyoming lake populations of hatchery origin are assessed, only four percent of the total variation is due to differences among populations, as substantiated by their close clustering on dendrograms of genetic relationships.

RESULTS: ISOZYMES

This section is a summary of my results with enzyme electrophoresis of thirty-four loci coding for seventeen enzymes in Arctic grayling. No breeding experiments have been done with genetic markers in Arctic grayling; I used the literature available on other salmonids as a reference with which to compare the tissue expression, evidence for duplication, and variablity of Arctic grayling enzymes.

The names of enzymes studied, their abbreviations, subunit structure, and enzyme commission (E.C.) numbers are listed in Table 2. The loci examined in all populations, mobilities measured relative to the homologous loci in rainbow trout, tissues for which isozyme mobilities were measured, and buffers used are presented in Table 3. Tissue expression (muscle, liver, eye, heart, brain, kidney, stomach, spleen) of enzymes were studied using Fuse Lake (Canada-derived) and Red Rocks Lake samples of Arctic grayling (Table 13); muscle, liver, and eye tissue were used in all 14 populations.

I did not get good activity and repeatable electrophoretic results when staining for the following enzymes: esterase, hexose-6-phosphate, mannose phosphate isomerase, sorbitol dehydrogenase, and glucose-6-phosphate dehydrogenase; they are excluded from the analysis due to poor resolution (e.g. faint activity or smearing of bands) or lack of a repeatable pattern of expression. Some enzymes are unstable in storage, and repeated use of tissue samples demonstrates changes that

occur during chemical breakdown that do not have a genetic basis.

The enzymes that are monomorphic at all loci in all populations of grayling studied are discussed first, and those enzymes with at least one locus polymorphic follow.

Monomorphic enzymes

Adenylate kinase

Formation of two bands on gels in all tissues in all individuals, suggests a minimum of two loci coding for adenylate kinase in grayling. This pattern is consistent with that of rainbow trout. No variation was observed and no additional conclusions can be drawn.

Alcohol dehydrogenase

A single, liver-specific locus for alcohol dehydrogenase has been demonstrated in rainbow trout (Allendorf et al. 1975). Alcohol dehydrogenase causes a single, cathodally migrating band when liver tissue of Arctic grayling is run on electrophoretic gels.

Xanthine dehydrogenase and 6-Phosphogluconate dehydrogenase

XDH and 6PG are expressed as single, monomorphic anodally migrating bands in Arctic grayling, as well as in rainbow trout. Since there was no variation, no conclusions can be drawn about the number of loci coding for these enzymes.

Glycerol-3-phosphate dehydrogenase

Engel et al. (1971) reported that there are three G3P loci in rainbow trout (and other species of fish). Later authors determined that, with appropriate buffers, two loci are typical (Allendorf et al. 1975) and that additional bands observed were more than likely artifactual (May et al. 1979). A single, cathodal band band was seen in all grayling, intermediate in mobility to the two bands seen in rainbow trout. Another possible zone of activity, more anodal, was noted but was obscured by negatively staining SOD activity. No variation was seen, so no further conclusions can be drawn about this enzyme.

Glucose-6-phosphate isomerase

Avise and Kitto (1973) found three loci for GPI in rainbow trout, with two major zones of activity and different tissue expression. They concluded that at least one of the two ancestral loci (typical of other teleosts) had re-duplicated and diverged. They did not have inheritance data to determine whether the most anodal zone of activity was composed of co-migrating products of two loci, or if it was just one locus, with the other member of the presumed pair silenced. In all Arctic grayling I have studied, four GPI loci are apparent; all loci and their heterodimers are expressed in muscle and faintly in heart tissue, as in rainbow trout, and the expression of the two most anodal loci in Arctic grayling correspond to that of Gpi3 in all other tissues of rainbow trout. The fourth locus was not detected by Lynch and Vyse (1979) in their work with Arctic grayling, though they sampled two of the same

populations described here. This locus is only slightly anodal to Gpi3 and without good resolution it could go undetected.

Peptidases

Very little comprehensive genetic work has been done with fish peptidases. Understanding of these enzymes is complicated by the lack of total substrate specificity. Wyban's work (1982) with Japanese medaka, Orizias latipes, shows a pattern of expression for certain peptidases similar to the one I found in Arctic grayling.

Wyban (1982) assigned names to peptidases based on their apparent orthologies with human peptidases, based on substrate specificity and biochemical similarities. He showed that the electrophoretic phenotype, using glycyl-leucine as a substrate, corresponds to the expression of Peptidase A and Peptidase S in all tissues, and the expression of Peptidase C in eye and brain tissue. Using leucyl-glycyl-glycine as the substrate, he demonstrated that there were two zones of activity in all tissues, corresponding to Peptidase B, and again, Peptidase S.

In Arctic grayling, with glycyl-leucine as the substrate, there are three zones of activity and expression in the same tissues as Japanese medaka. The most cathodal region is less active and does not resolve well. The same zone stains using leucyl-glycyl-glycine (LGG) as substrate, suggesting that this could be Peptidase S. Two bands are the common phenotype with LGG as substrate in Arctic graying. In both species, there is a very anodal zone with faint activity that Wyban equates to the locus coding for leucyl amino peptidase.

Because there is no conclusive evidence that these are the peptidases of medaka, the loci examined using glycyl-leucine as substrate have been called Gl1 and Gl2. The two actively staining bands on gels with leucyl-glycyl-glycine added as substrate are referred to as Lgg1 and Lgg2. Johnson (1984) has determined that the phenotype expressed for GL1 has a genetic basis in trout. Robb Leary (personal communication) has seen variation in salmonids indicating a dimeric subunit structure for these peptidases.

Polymorphic enzymes

Aspartate aminotransferase

The pattern of variation of aspartate aminotransferase in Arctic grayling is very similar to that of rainbow trout. There are three zones of activity; two zones migrate anodally, and another migrates cathodally. It was presumed that the component that migrated cathodally was mitochondrial because its activity was markedly influenced by freeze-thawing. Tissue expression of this dimeric enzyme indicated at least two mitochondrial loci in grayling: one predominant in muscle, eye, and brain and the other in liver. Heart tissue expressed both loci equally. These loci were not routinely examined due to the difference in expression with different storage regimes.

Of the zones that migrate anodally, one is predominant in muscle tissue (Aat3,4) and the other zone is composed of two loci, one predominant in liver (Aat1) and the other predominant in eye tissue (Aat2). The muscle-predominant locus is a duplicated dimer according to inheritance studies with cutthroat trout (Allendorf and Utter 1976);

this is corroborated by observations with rainbow trout (Allendorf 1975) and Arctic grayling (this study), as evidenced by the banding patterns in populations having variation.

Breeding studies with chum salmon (Oncorhynchus keta) (Allendorf et al. 1975) have confirmed that the other cytosolic loci, with the eye- and liver-predominant expression, have diverged. In both rainbow trout and Arctic grayling the pattern of mobilities and the tissue dominance of Aat1 and Aat2 are also conserved. For Arctic grayling, these loci have been named to correspond to the nomenclature used in rainbow trout.

Creatine kinase

The banding patterns corresponding to CK activity do not conform to the usual pattern for a dimeric molecule. Ferris and Whitt (1978) determined that CK heterodimers are not manufactured in vivo. Utter et al. (1979) proposed that the pattern generally observed in trout could be explained by post-translational modifications of the enzyme. There are no heterodimers formed between alleles or between loci. Therefore, the three-banded pattern common in rainbow trout muscle is the result of the overlap in mobility of Ck1 and Ck2, each with two bands. Heterozygotes for rainbow trout Ck1 have five bands.

In Arctic grayling, there is generally a single, relatively cathodal band in all tissues which was designated Ck1. The Big Hole River population has a variant phenotype (Figure 4), such that there were two, equally active bands seen for some individuals. In eye and brain tissue, a single band was seen in all individuals of all populations, and was designated Ck3 to conform with the terminology used

in rainbow trout.

Glyceraldehyde-3-phosphate dehydrogenase

GAP produces two major zones of activity on electrophoretic gels of rainbow trout and Arctic grayling. The more cathodal zone of activity is apparently composed of two divergent loci with different tissue expression; these loci do not resolve well using our methods. The second, anodal zone resolves well, and is apparently composed of two diverged loci, referred to as Gap3,4. Most Arctic grayling populations studied are highly variable for these loci.

The phenotype generally seen for Gap3 and Gap4 in rainbow trout is a five-banded, fixed heterozygote pattern. If you assume that this is the ancestral pattern for salmonids, it would be reasonable to conclude that Arctic graying are losing the expression of Gap3 via expression of a null allele; this is the common phenotype in some populations, including the Alaska sample. The other possible explanation of the observed phenotypes is that variants at this locus co-migrate, possibly due to a single charge difference between the two molecules.

In native Montana/Wyoming graying the banding pattern observed for Gap3,4 could be explained by variation of a single locus of the pair. However, the pattern observed in the Fuse Lake population (Figure 5) would not fit this model; both loci are apparently variable.

Isocitrate dehydrogenase

IDH in rainbow trout and Arctic graying can be generalized to two zones of activity. Inheritance studies with rainbow trout have shown that the cathodal zone, which is predominant in skeletal muscle is probably mitochondrial, is produded by two disomic loci which have diverged to cause a fixed heterozygote effect in these tissues (Reinitz 1977). Other breeding studies (Allendorf and Utter 1973; Reinitz 1977) have shown that the anodal zone of acivity is coded for by two disomic loci that have not diverged.

Arctic grayling appear to have the same pattern of IDH expression as rainbow trout; two diverged loci, Idh1 and Idh2, are predominant in muscle tissue. Idh3,4, isoloci, are strongly expressed in liver tissue. In native Montana/Wyoming grayling and Alaska grayling studied, no variation has been observed at Idh1 or Idh2. The Fuse Lake (Canada) population is highly variable at the most cathodal locus, designated Idh1 (Figure 6). This variant was not observed by Lynch and Vyse (1979) when they studied this population. Some Idh3,4 variation has been observed, making it possible to deduce from the banding pattern and staining intensity that it is also a duplicated locus in Arctic grayling.

No fast-migrating IDH variants such as those seen by Lynch and Vyse (1979) in their Grebe Lake samples were seen in my sample from that lake. Fast IDH "variants" for Idh3,4 (liver) were seen in the first sample taken from the Big Hole River, but subsequent investigation by re-running samples showed that these bands were formed by breakdown in storage. No such "variants" were seen in the subsequent sample of the

same population. This type of change has been documented before when tissue, especialy liver, is stored; anodally migrating components from the soluble form are observed (Harris and Hopkinson 1976).

Lactic dehydrogenase

This enzyme has been described extensively for rainbow trout (Wright et al. 1975; Allendorf 1975; Bailey, Tsuyuki, and Wilson 1976). Five loci in three zones of activity are typical. The most cathodal zone is coded for by two diverged loci (Ldh1 and Ldh2) which are predominant in muscle tissue. The second zone of activity is seen in most tissues, and has diverged (Ldh3 and Ldh4). The locus that predominates in the liver of salmonids has been named Ldh4. Ldh5 is a single locus which is seen in eye tissue (Morrison and Wright 1966).

Wright et al. (1975) found that in Arctic grayling the tissue expression for muscle and liver loci, as regards their relative mobility, were reversed. This was also observed for Salvelinus. These authors also noted that only one muscle band was expressed in vivo, which agrees with my results. I found variation for LDH in muscle tissue in four of the populations I studied, and the intensity of the bands from the common to the variant were symmetrical, indicating that only one locus is producing the active product. In brown trout, Ldh1 segregates for a null allele (Allendorf et al. 1977). In Arctic grayling, this locus could be homozygous for a null allele. Arbitrarily, the "silent" locus has been designated as Ldh1. The locus active in the muscle has been designated as Ldh2, and was measured relative to the Ldh2 locus in rainbow trout.

Malate dehydrogenase

The phenotypic expression of MDH indicates that is composed of two duplicated loci, predominant in different tissues, in the soluable fraction of cells (Bailey et al. 1970) plus other loci in the mitochondrial fraction (Clayton et al. 1975). This is the same pattern of expression observed by Massaro (1973) for Arctic grayling. Allendorf et al. (1975) report that the anodal locus in rainbow trout may not duplicated based on observed banding intensities for individuals with variation. However, in Arctic grayling (this study) where variation has been observed for both cytosolic loci, both appear to be duplicated, again, as evidenced by intensities of banding patterns (Figure 7).

Malate dehydrogenase (NADP-dependent)

NADP-dependent malate dehydrogenase (also referred to as malic enzyme, or ME) has two zones of activity. Cross et al. (1979) demonstrated that in brook trout (Salvelinus fontinalis) and Atlantic salmon (Salmo salar), the cathodal locus for ME is coded in the mitochondrial fraction of the cells. In Arctic grayling, this finding is not contradicted in that there are no heterodimers between the cathodal and anodal loci. Genetic variation for the mitochondrial locus has been described for rainbow trout (May et al. 1982); it is apparently a duplicated locus in this species. In Arctic grayling, I observed no variation at this locus, designated Mel,2, so it was counted as one monomorphic locus.

In sea-run cutthroat trout (Campton 1981), the anodal pair of loci has apparently diverged; variation has been observed at one locus of the duplicated pair, Me3. It is a single locus that forms heterotetramers with Me4. These loci are not identically expressed in liver and muscle tissue. The "liver" band changes corresponding to presense of the variant to cause a complicated banding pattern.

Due to lack of resolution, it is not possible to reliably score Me3,4 in Arctic grayling. Most individuals showed a five-banded phenotype, but the bands did not have a consistent pattern of intensity. Re-running samples, running second samples from the same individuals, and changing the pH of the buffers did not resolve the problem. Single bands in a few individuals in one population (Rogers Lake) further complicate interpretation of the observed pattern. Lynch and Vyse (1979) note a rare variant for this locus in their sample from Grebe Lake. Since the Rogers Lake population was begun from Grebe Lake stock, it is possible that this variant is real. However, in neither study could the problem be resolved. For this reason, Me3,4 was excluded from the quantitative analyses.

Phosphoglucomutase

Three zones of activity for this enzyme are found in rainbow trout (Roberts et al. 1969). With inheritance studies (Allendorf et al. 1982) it was found that the two zones of activity that migrate cathodally on low pH gels are produced by two single loci, Pgm1 and Pgm2. The anodal zone is produced by isoloci, Pgm3,4, which are difficult to score; there are more than one allele, dosages for a

duplicated locus are hard to score for a monomeric isozyme, and the products of these loci are unstable in frozen storage. In most of the Arctic grayling samples studied, Pgm3,4 was expressed as a single, poorly resolving band. Arctic grayling from Alaska, however, were highly variable at this locus, and also had more than two alleles. Consequently it was not scored for the Chena River population.

The pattern observed in Arctic graying for Pgm1 and Pgm2 fits the model of inheritance for rainbow trout at these loci, though the tissue distribution is somewhat different between species. In general, the cathodal zone in grayling has a two-banded pattern in all populations on low pH gels. There are also two bands apparent when muscle tissue was run on gels made with an alkaline buffer, and a variant allele at Pgm1 was observed in relatively high frequency in one population, Miners Lake. When liver tissue was run on alkaline gels and stained for PGM activity, three bands formed; it is assumed that the third band is an artifact of breakdown because of the buffer-specificity of the bands combined with the information from other salmonids, which have two loci.

Lynch and Vyse (1979) reported two different low frequency variants for Pgm1 in two different populations of Arctic grayling. In both populations with variation, alternate homozygotes were reported. It seems highly unlikely that in samples where the frequency of the variants was less than 3% that alternate homozygote phenotypes would be observed.

If the pH of the gel stained for PGM activity is about 6.7, the bands corresponding to the activity of Pgm1 and Pgm2 bands are at the origin. Some individuals from Red Rocks Lake and Rogers Lake populations have an additional cathodal band not seen in other samples. Without breeding data from families with the variant, it would be difficult to determine what the underlying basis of the observed phenotype might be.

Superoxide dismutase

Using breeding experiments with rainbow trout, it has been determined that the SOD locus generally observed in liver tissue (Sod1) is a single locus (Utter, Mighell, and Hodgins 1971). Sod1 is highly variable in most Arctic grayling populations I studied, and resembles the pattern observed in rainbow trout. It was not variable in the Canada-derived population I studied, but variation has been seen in another Canadian population by Lynch and Vyse (1979), who suggested that lack of variation at this locus in Fuse Lake grayling may be due to a founder effect. The mobilities of the variants I observed in the Alaska population were indistinguishable from those of Montana and Wyoming samples.

RESULTS: MERISTIC COUNTS

The number of meristic characters I used for analysis was seven; the counts for bilateral characters were combined, as were the upper and lower gillraker counts, because they are highly correlated. Among the seven combined traits used in the analyses, only 2 of 21 possible pairs were significantly correlated when corrections were made for the number of tests (Cooper 1968): anal ray counts were correlated with dorsal ray counts, and dorsal ray counts were correlated with pelvic ray counts (Pearson's correlation: $P < .001$ and $P < .002$; SPSS Inc. 1983). Gillraker numbers were correlated with anal ray counts if no correction was made for the number of tests. These traits have been determined to have relatively high heritabilities in rainbow trout (Leary 1985), and should be useful to discriminate among stocks.

Means and standard deviations were determined for each trait in each population, and a grand average for each trait was calculated (Table 14). Comparisons of the distributions of counts among populations were made for each character. To make multivariate comparisons between populations using meristic traits, discriminant function analysis was used for classification and to determine the relationships between populations. Finally, to estimate the amount of variation in populations, using meristic characters, coefficients of variation were calculated.

Distribution of characters

The distributions of traits were not normal, as evidenced by visual inspection of frequency histograms for these discontinuous measurements. Also, the variances of the characters that I measured were not homogeneous among populations (Box's M: $P < .05$; SPSS Inc. 1983) despite efforts to normalize the data using transformations, so non-parametric methods were used for comparisons.

There are significant differences in distribution for each trait when sampled over all populations (Kruskal-Wallis: $P < .01$; SPSS Inc. 1983). When the distribution of meristic traits is tested pairwise between groups, there are significant differences (Mann-Whitney U test: $P < .05$; SPSS Inc. 1983) for at least one trait in all pairs. Separate probabilities for each trait for each pairwise comparison were combined ($P = -2 \Sigma \ln P$ tested against a chi-square distribution: Soka and Rohlff 1981) and forty-two of the forty-five population pairs showed a significant difference in distribution of the meristic characters studied (Table 15). Results for each trait tested pairwise over ten populations are reported (Tables 16, 17, 18, 19). Pectoral rays, dorsal rays, and anal rays are most frequently different when counts are compared between populations.

Multivariate comparison

To compare populations using meristic variables I used discriminant function analysis. Discriminant function analysis depends on the assumptions that the group dispersion (covariance) matrices between groups are equal and that the distributions of traits are normal. These

conditions were not met, but discriminant function analysis is sufficiently robust that the results are still meaningful, though probability values generated are not appropriate.

The functions created are linear combinations of the original variables, evaluated at the centroid of the multivariate distribution of characters for each population. Using these functions, which maximize the between-population variability, it is possible to calculate similarity or distance values between populations.

Discriminant function analysis of seven characters in ten populations makes correct classification of individuals to known groups approximately sixty percent of the time. Transformations of data did not appreciably improve the percent of correct classification. Mahalanobis' distance is the method of discriminant analysis used, and the first six functions it produced explained ninety-eight percent of the variation in the data. These functions were used as variables to calculate Euclidean distances, expressed as similarity values between populations, pairwise.

The Euclidean distances were then used for cluster analysis with the average linkage method, unweighted (Cluster: SPSS Inc. 1983), and a dendrogram was produced (Figure 8). It represents the relationships of ten populations based on similarities of seven meristic traits. Several native Montana/Wyoming populations cluster first, as pairs, then as a group with the two Big Hole River populations. The Alaska and Canada-derived populations cluster next, though the distances between them are nearly as great as those between their cluster and that of the Montana/Wyoming populations. Finally, two Montana/Wyoming populations

that are closely related to the first four "hatchery" populations cluster together.

Averages of meristic character counts and averages of the square roots of the counts were used for the same type of analyses; Euclidean distances between populations are calculated and then used in a cluster analysis. The dendrograms produced bore little resemblance to the relationships between populations as determined by electrophoresis, stocking records, and by using discriminant scores.

Amount of variation

To evaluate the amount of variation in populations using meristic characters, a multivariate coefficient of variation (CVp) was calculated for each group. The formula is that of Van Valen (1978) as used by Winans (1985):

$$CVp = \text{sqrt} \left(\Sigma s^2 / \Sigma x^2 \right) X\ 100$$

where Σs^2 equals the sum over all traits of the squared standar deviations and Σx^2 equals the sum over all traits of the squared mean (Table 14).

A comparison was then made of the amount of variability in populations; CVp was used as a measure of morphological variability, and average heterozygosity per locus was used as a measure of isozyme variation. There is no correlation of the amount of meristic and enzyme variation (Spearman's rank test: $P > .64$; SPSS Inc. 1983) measured by this method.

DISCUSSION

Montana's Arctic grayling are below average in the amount of genetic variation that they have compared to other fish species. Genetic variation in populations is important because environmental alteration is inevitable and populations need to be responsive to change. With less genetic variation there is a reduced potential to adapt to changing environments. While the immediate consequences of low genetic variation are not known for Arctic grayling, in other species genetic variation is related with growth rate, developmental stability, survivorship, and the ability to compete (Frankel and Soule' 1981; Mitton and Grant 1984).

Even with the low levels of genetic diversity in grayling, the observed variability does provide important information about the relationships between populations. Though the genetic similarities measured are relatively high, there are significant genetic differences between populations. A priori knowledge of historical distribution and stocking records is not sufficient to explain the observed amount and pattern of genetic variation. Since mixing of populations via hatchery propagation was widespread it was expected either that Montana populations would be homogeneous, or that selection in differing habitats would cause a certain pattern of divergence relating to ecotype. It was further suspected that grayling from Canada had been mixed with native Montana/Wyoming grayling.

Amount of genetic variation

Vincent (1962) suggested that southern Arctic grayling were probably low in genetic variation as they did not readily adapt to changes in the environment. It was assumed that this was related to the fact that the southern populations (of Michigan and Montana/Wyoming) are likely glacial relicts. Small populations could have been isolated in headwater drainages. Low initial numbers would mean that, by chance, only a little of the variation typical of the species might have been included in the founding stocks. Genetic bottlenecks, caused by population crashes, have the same effect, i.e. loss of genetic variation, if the small effective population size is chronic.

Grayling in Montana have less genetic variation than grayling representing Canada (this study; Lynch and Vyse 1979). The amount of variation in Montana grayling is not unusual relative to Alaska populations (this study; Hop 1985) or that of Lake Baikal (Kartavtsev and Mamontav 1983), nor are they exceptionally low in variation compared to many other salmonid species (Allendorf and Utter 1979).

Montana/Wyoming populations

According to Montana Department of Fish, Wildlife, and Parks records, the Grebe Lake stock was established from two Madison River tributary populations. Other populations I studied are more likely a mixture of stocks, either because grayling were native (Red Rocks and Elk Lake) or because Grebe as well as Red Rocks fish were used for propagation. The Grebe Lake population currently has less genetic variation than several other Madison/Red Rocks lake populations, and

also has less variant alleles than some other populations.

Arctic grayling were introduced in the Sunnyslope Canal from native Montana/Wyoming stocks. The Canal is seasonally dewatered, and major fluctuations in the grayling population size are not unlikely. Genetic drift is an important factor in small populations, and bottlenecks, especially if chronic, cause loss of alleles and loss of heterozygosity. The Canal population is low in genetic variation and has only two variable loci. The frequency of the allele that is common for one of these loci in most Montana/Wyoming population is rare in the Canal sample. The result is that this population has significantly different allele frequencies from all other populations. Though it clusters with the Alaska sample on the dendrogram of genetic similarities, it is unlikely that this similarity is biologically meaningful. If the Pgm3,4 locus could be scored in the Alaska sample, it is unlikely that the Canal and River populations would cluster together.

Generally, Madison/Red Rocks populations have higher average heterozygosities than do those of the Big Hole River Drainage. There is no way of knowing whether there has been a difference in the amount of variation present in these populations for a long time, or if this represents more recent divergence among stocks. That would suggest that either the lake populations have "gained" variation through the mixing of stocks, or the Big Hole River populations are losing variation throughout the drainage.

Relative to other salmonids

It is possible that the low levels of genetic variability observed in Montana/Wyoming populations are typical of the species in general. The average amount of variation in native Montana/Wyoming grayling is low compared to published data for fish in general, including marine and freshwater species; it is comparable to that of other grayling populations (excepting the Fuse Lake population) and other salmonid species. Since the data from Fuse Lake grayling provide only a point estimate of the variation in Canada grayling, it could be atypical of populations across the major range of the species. The values of heterozygosity determined for grayling from Montana, Alaska, and Lake Baikal (Table 20) are not unusual compared to those of Pacific salmon (Oncorhynchus spp.), Atlantic salmon (Salmo salar), and Arctic char (Salvelinus alpinus).

Gyllensten (1985) has shown that marine species usually have higher average heterozygosity than do anadromous or freshwater species. Generally, marine species are composed of large panmictic populations with extensive gene flow. Many salmonids have highly structured populations usually consisting of isolated populations with low effective population sizes (Utter et al. 1978; Allendorf et al. 1976; Ryman 1983) and little gene flow due to homing behavior (Ricker 1972; Smith 1985).

Genetic drift is a strong force in small populations; heterozygosity is lost in populations where low numbers are chronic, and loss of alleles is likely with a genetic bottleneck. Arctic grayling populations, especially Montana and Alaska populations, have a low

proportion of polymorphic loci. These populations have half the amount of polymorphism typical of fish (Nevo 1978), and have considerably less than rainbow trout, which have about one third of their loci polymorphic (Allendorf and Phelps 1980). Because most Montana/Wyoming grayling populations have half the genetic variation and half the percent loci polymorphic that the Canada sample (Fuse Lake) has, it is possible that more genetic variation is typical; that would suggest the populations that have been studied in Montana, Alaska, and Lake Baikal have lost variation due to small population sizes of the founding populations, probably related to repeated glaciations over their range.

Genetic divergence among native populations

Discussion of divergence among Arctic grayling populations is complicated by the extensive hatchery propagation and transplantation of stocks from location to location. Sufficient variation persists among groups to make it possible to characterize them using differences in allele frequencies. Added information is available from rare alleles peculiar to certain populations.

Though the populations I studied did appear to be mating at random, there are examples in the literature of reproductively isolated sympatric populations in several salmonid species (Allendorf et al. 1976; Behnke 1972) and specifically in grayling in Alaska (Hop 1985). This was not the case with any population I examined, although a few individual loci (e.g. Mdh3,4 in Chena River sample) were not in random mating proportions. Despite mixing of native Montana/Wyoming stocks,

there is no indication of reproductive or behavioral separation among them. Also, there is no suggestion that transplanted northern fish were co-existing with native fish.

Montana/Wyoming populations

Differences between the Madison/Red Rocks-derived lake populations as a group and the Canal and Big Hole River Drainage populations are evident when a dendrogram of genetic similarities is examined. Also, the Big Hole River population has a creatine kinase variant seen in no other populations, and lactate dehydrogenase variation, present in four other Montana/Wyoming populations, is not seen in the River population.

When the amount of variation is partitioned among native populations, it can be seen that the lake populations in Montana and Wyoming, which were begun or mixed repeatedly with progeny from the Madison River and Red Rocks Drainage populations, are not greatly diverged from each other. They are considerably different from both the Sunnyslope Canal and the Big Hole River populations. However, there is no indication that the Canal population and the River population are especially similar to each other.

Grayling are native in the Red Rocks River Drainage, but Red Rocks Lake and its tributaries have been planted repeatedly with grayling, including Grebe Lake grayling (listed in Randall 1978). The Red Rocks population is not significantly different in allele frequencies at variable loci from the other "founding" population, Grebe Lake, when the significance level is modified to reflect the number of pairwise tests (91) performed. If only this pair is considered, to represent the two

drainages of origin, they are significantly different. This result could reflect historical differences in Madison vs. Red Rocks Drainage populations.

The Big Hole River population is responsible for a large amount of the measurable differentiation among the Montana/Wyoming grayling populations. My sample came from the upstream reach of the Big Hole River. Although this section of the river and the tributary populations have been planted with Madison River-derived hatchery grayling at least once, the plants either were not successful, or did not contribute overwhelmingly to the spawning population.

This possibility is not without support from other studies on the effects of stocking. Wishard et al. (1984) report that none of their analyses indicate that the relationship among populations of "redband" rainbow trout in Idaho are influenced by hatchery planting. Rather, the genetic relationships of populations clearly follow geographic patterns. They concluded that this reflected the natural history of these populations.

In the case of Arctic grayling, there is no way of knowing if (1) Madison River and Big Hole River stocks have been divergent for a long time, (2) lake populations established by propagation do not actually represent the Madison River stocks from which they were originally drawn, (3) the surviving Big Hole population has changed significantly from its original genetic composition, or 4) the Big Hole River populations now represent a mixture like neither the historical populations nor the hatchery stock.

Without concurring data from other populations in the drainage it would be tempting to postulate that this river population was divergent because of selection in a stream habitat. With the similarities between populations within the drainage it is more parsimonious to presume that the differences correspond to historical relationships among populations, rather than rapid selection in populations related by hatchery stocking. Further, it seems unlikely that selection was occurring drainage-wide in the Big Hole region since we are comparing fish from river, stream, pond, and lake outlet habitats.

However, there is no way, using electrophoretic methods, to prove or disprove whether or not the Big Hole River population is ecologically specialized for rivers. While electrophoretic analysis is capable of testing whether or not two groups of fish were likely to have been drawn from the same sampling distribution, it detects less than a third of protein variation, and is only sampling a portion of the genome. Since "ecological specialization" involves complex combinations of polygenic characteristics, it is unlikely that this analysis would detect that type of variation.

Canada and Alaska populations

Variation in the Canada-derived population and the Alaska population contribute to a large degree to the total variation and to the variation among populations studied. Fuse Lake's Canada-derived population is significantly different in allele frequencies at variable loci from all other populations. The fact that they are different agrees with the work of Lynch and Vyse (1979), who suggested that

grayling from the Arctic are probably a different subspecies.

However, the levels of divergence observed in Arctic grayling better approximate those found in fish populations from different drainages rather than different subspecies (cf. Turner 1983). Though this is not reasonable in light of the distribution of grayling in North America, it is clear that, to the limits of this analysis, these groups have a relatively low level of divergence compared to other fish.

The only documented plants of grayling from the Arctic to Montana were to two isolated mountain lakes, Fuse Lake and one adjoining it (MDFWP records). There are no records of transplantations from Arctic stock into other waters. Unlike Lynch and Vyse (1979), I found that Fuse Lake fish are highly variable compared to other grayling, having over twice the level of variability as, for instance, the Big Hole River population. These Canada-derived grayling are also readily identifiable because isocitrate dehydrogenase and malate dehydrogenase variants, present in high frequency, are unique to this population. The absence of these alleles in all other grayling samples from Montana and Wyoming indicates that these populations do not contain genetic material from grayling derived from Fuse Lake or probably from the Canadian Arctic.

The sample of grayling from Alaska has a relatively low level of genetic variation and is significantly different in allele frequencies from southern grayling populations and from the Canada-derived population. It has two variants in high frequency not present in the Montana/Wyoming samples; Mdh3,4 and the unscorable Pgm3,4. Variation observed in the Chena River/Badger Slough sample closely resembles that seen by Hop (1985) in several other Interior Alaska samples.

Interestingly, Alaska grayling are no more similar, as measured by Nei's index of genetic identity, to the Canada-derived populations than they are to the Montana/Wyoming fish.

Geological history

It is possible that after the glaciations the waters of interior Alaska were recolonized by southern grayling populations or by headwater transfer via the Peel River, leading to early divergence. The McKenzie River Drainage populations in Canada, from which the Fuse Lake population was derived, could have been repopulated by grayling from the northern (Bering) glacial refuge (McPhail and Lindsey 1970). This idea is supported by electrophoretic data from work with lake whitefish populations (Franzin and Clayton 1977). It is also supported by studies of grayling lateral line counts (McCart and Pepper 1979), though I have found that lateral line counts are not reliable in predicting relationships of populations (see Table 19).

MORPHOLOGY

Amount of variation within populations

Morphological methods provide no discernible pattern in the amount of genetic variation in Arctic grayling stocks compared to electrophoretic analysis. When meristic counts are summarized over populations as a multivariate coefficient of variation and compared to average heterozygosity, there is no statistical relationship in the amount of variation. Further, I can see no pattern in the amount of variation among the populations, relative to each other, in light of

what I know about their relationships based on stocking records and historical distribution.

Variation among populations

There are significant differences between populations as measured by meristic counts. The populations that are not significantly different for meristic counts are populations that have high genetic similarities using electrophoretic data, but not all populations with high genetic similarities cluster together. When a dendrogram calculated from meristic characters is studied (Figure 8), the pattern has similarities to electrophoretic results, as well as contradictions. The Grebe Lake population was a major source of hatchery stocks, and is genetically similar to those populations with a hatchery origin. The expectation is that these populations would be most similar morphologically. Instead, Grebe Lake grayling are the most divergent from that hatchery group. Since the first four populations that cluster are in a variety of lake habitats, it is not obvious what is causing the observed pattern of relationships based on morphological data.

SUMMARY AND IMPLICATIONS FOR MANAGEMENT

Arctic grayling are a species of special concern in Montana. The populations are much reduced from their original range in the headwater tributaries of the Missouri River. This study was undertaken to examine morphological and protein variation in grayling in order to better understand the amount and pattern of variation in remaining stocks. Samples from an Alaska population and one originally from Canada were used for comparison with the Montana populations.

Genetic variation of proteins was studied using electrophoretic methods. The amount of genetic variation observed in Arctic grayling from Montana and Wyoming is less than that of most fish species and is at the low end of the normal range for salmonids. There is considerably less genetic variability in native Montana grayling than in the population from Fuse Lake, Montana that was introduced there from Canada. The amount of genetic variation detected in native Montana/Wyoming graying is relatively low; there are large differences in allele frequencies among the populations studied.

The genetic differences among native Montana/Wyoming populations are high relative to many other species. The lake populations that are entirely of hatchery origin or were repeatedly mixed with hatchery stocks are most closely related to each other. The Sunnyslope Canal population was started from the same stock, but has diverged. A major difference among native Montana/Wyoming grayling shows up in the

differences between the hatchery stocks and the Big Hole River Drainage populations, where stocking was supplementary to existing populations.

The Alaska and Fuse Lake populations are significantly different in allele frequencies of variable loci from all other populations studied. Nevertheless, the overall genetic similarity of the northern and southern populations is relatively high.

The amount of variation in grayling populations determined using meristic counts of seven traits does not correspond with the results of isozyme analysis. There are significant differences in meristic counts between populations, but the relationships among populations with this method is not concordant with what is known from protein variation and stocking records.

Management Implications

It is imperative that efforts be made to minimize further losses in current stocks because Arctic grayling already have a relatively low level of genetic variation. Any program attempting to propagate these fish for restoration or supplementation should use proper practices to avoid directional selection, loss of alleles, and loss of heterozygosity.

It is important that as many of the remaining stocks as possible be preserved since much of the variation in Arctic grayling populations is distributed among populations. These individual populations should be treated as gene banks that can be used to create hatchery stocks or to provide different varieties of fish to be used under various environmental conditions.

The Big Hole River grayling population is significantly different genetically from stocks of hatchery origin. It is not possible, however, to attribute this difference specifically to adaptations to river versus lake habitats. The Big Hole River population is genetically similar to other populations within the drainage, some of which are also lake inhabitants. The pattern of variation may well be geographical. Hatchery grayling planted in the Big Hole River Drainage have apparently not replaced the native stocks.

The Canada-derived population in Fuse Lake, Montana has alleles at high frequency that are not observed in native populations. This would make introgression between northern and southern stocks readily detectable. There is no indication that any native stocks I studied were mixed with Canada fish. A native fish restoration effort could make use of any of the populations studied excepting that of Fuse Lake.

Any further loss of genetic variability in Arctic grayling is undesirable. Each stock is particularly valuable because any given population is not representative of the evolutionary potential of the entire species. The remaining stocks must be preserved to guarantee the future of Arctic grayling in Montana.

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TABLE 1

Population, location, number of grayling sampled, and year

Populations	Location	Number sampled	Year of sample
Big Hole River	Beaverhead County, Montana	45	1983,1984
Bobcat Lake	Beaverhead County, Montana	5	1983
Chena River	North Star Borrough, Alaska	38	1984
Elizabeth Lake	Glacier County, Montana	13	1983,1984
Elk Lake	Beaverhead County, Montana	50	1983
Fuse Lake	Granite County, Montana (transplanted from Canada)	92	1980,1983
Grebe Lake	Yellowstone National Park	41	1983
Lake Agnes	Beaverhead County, Montana	36	1984
Miner Lake	Beaverhead County, Montana	15	1984
Mussigbrod Lake	Beaverhead County, Montana	14	1983
Red Rocks Lake	Beaverhead County, Montana	29	1983,1984
Rogers Lake	Flathead County, Montana	43	1982,1984
Steel Creek	Beaverhead County, Montana	4	1983
Sunnyslope Canal	Teton County, Montana	41	1983
Total		466	

TABLE 2

Enzymes, abbreviations, subunit structure (Harris and Hopkinson 1976, 1977), and enzyme commission numbers (Recommendations of the Nomenclature Committee of the International Union of Biochemistry. Enzyme Nomenclature. 1984. Academic Press, N.Y.).

Enzyme	Symbol	Subunit	E.C. number
Adenylate kinase	AK	Monomer	2.7.4.3
Alcohol dehydrogenase	ADH	Dimer	1.1.1.1
Aspartate aminotransferase	AAT	Dimer	2.6.1.1
Creatine kinase	CK	Dimer	2.7.3.2
Glucose-6-phosphate isomerase	GPI	Dimer	5.3.1.9
Glyceraldehyde-3-phosphate dehydrogenase	GAP	Tetramer	1.2.1.12
Glycerol-3-phosphate dehydrogenase	G3P	Dimer	1.1.1.8
Glycyl-leucine peptidase	GL	Dimer*	3.4.11
Isocitrate dehydrogenase	IDH	Dimer	1.1.1.42
Lactate dehydrogenase	LDH	Tetramer	1.1.1.27
Leucyl-glycyl-glycine peptidase	LGG	Dimer*	3.4.13
Malate dehydrogenase	MDH	Dimer	1.1.1.37
Malate dehydrogenas (NADP)	ME	Tetramer	1.1.1.40
Phophoglucomutase	PGM	Monomer	5.4.2.2
6-Phosphogluconate dehydrogenase	6PG	Dimer	1.1.1.44
Superoxide dismutase	SOD	Dimer	1.15.1.1
Xanthine dehydrogenase	XDH		1.1.1.204

* Robb Leary (personal communication)

TABLE 3

Loci and mobilities measured relative to common homologous loci in rainbow trout using these tissues and buffers (see Methods).

Loci	Relative Mobility	Tissues	Buffers
Ak1,Ak2	100,100	Muscle	AC
Adh1	-189	Liver	RW
Aat1,Aat2	211,136	Liver,Eye	AC
Aat3,4	85	Muscle	AC
Ck1	116(103)	Muscle	RW
Ck3	100	Eye	RW
Gpi1,Gpi2	100,145	Muscle	RW
Gpi3,Gpi4	100,108	Muscle, Eye	RW
Gap3,4	null(88),89(null)	Eye	AC+
G3p1	60	Liver	AC
Gl1,Gl2	108,108	Eye,Liver	MF
Idh1,Idh2	450(225),212	Muscle	AC+
Idh3,4	100(83)	Liver	AC
Ldh2	85(131)	Muscle	RW
Ldh3,Ldh4,Ldh5	132,76,94	Eye	RW
Lgg1,Lgg2	150,178	Muscle	MF
Mdh1,2	140(214)	Liver	AC
Mdh3,4	98(119)	Muscle	RW
Me1,2	120	Muscle	AC
Pgm1,Pgm2,Pgm3,4	-44,0,100	Liver	AC
Pgm1,Pgm2	85(49),103	Muscle	RW
6Pg1	92	Muscle	AC+
Sod1	145(97)	Liver	AC
Xdh1	98	Liver	RW

Gpi4 measured relative to Gpi3; Pgm1 relative to Pgm2 of rainbow trout

TABLE 4

Observed genotypes for Sod1 (with expected values); chi-square (X^2) with one degree of freedom; observed proportion heterozygous (h).

Population	number of fish	common allele frequency	Genotypes AA	AA'	A'A'	X^2	h
Big Hole R	45	.922	38 (38.2)	7 (6.5)	0 (0.0)	.3	.156
Bobcat L.	5	.900	4 (4.1)	1 (0.9)	0 (0.0)	.0	.200
Chena R.	38	.211	1 (1.7)	14 (12.6)	23 (23.7)	.5	.368
Elizabeth	18	.528	5 (5.0)	9 (9.0)	4 (4.0)	.0	.500
Elk Lake	50	.650	23 (21.1)	19 (22.8)	8 (6.1)	1.4	.380
Fuse Lake	92	1.000	92 (92.0)	0 -	0 -	-	.000
Grebe L	41	.768	23 (24.2)	17 (14.6)	1 (2.2)	1.1	.415
L.Agnes	36	.653	16 (15.4)	15 (16.3)	5 (4.3)	.2	.417
Miner Lake	15	.633	5 (6.0)	9 (7.0)	1 (2.0)	1.2	.600
Mussigbrod	14	.885	11 (11.0)	3 (2.8)	0 (0.2)	.2	.214
Red Rocks	29	.552	9 (8.8)	14 (14.3)	6 (5.9)	.0	.483
Rogers Lake	43	.581	14 (14.5)	22 (20.9)	7 (0.0)	.1	.512
Steel Creek	4	.875	3 (3.1)	1 (0.9)	0 (0.1)	.1	.250
Sunnyslope C.	41	.122	1 (0.6)	8 (8.8)	32 (31.6)	.3	.195

TABLE 5

Observed genotypes of Ldh2 with expected values, chi-square values (X^2) with one degree of freedom, and observed proportion heterozygous (h).

Population	number of fish	common allele frequency	Genotypes AA	Genotypes AA'	Genotypes A'A'	X^2	h
Elk Lake	50	.990	49 (49.0)	1 (1.0)	0 (0.0)	0.0	.020
L. Agnes	36	.986	35 (35.0)	1 (1.0)	0 (0.0)	0.0	.028
Red Rocks	29	.982	28 (28.0)	1 (1.0)	0 (0.0)	0.0	.034
Rogers	43	.953	39 (39.1)	4 (3.8)	0 (0.1)	0.1	.093

TABLE 6

Observed genotypes of variable loci (expected values), chi-square values (X^2) with 1 degree of freedom, and observed proportion heterozygous (h).

Locus	Population	N	common allele frequency	Genotypes AA	Genotypes AA'	Genotypes A'A'	X^2	h
Ck1	Big Hole R.	45	.967	42 (42.0)	3 (2.9)	0 (0.1)	.1	.067
Idh1	Fuse Lake	92	.647	43 (38.5)	33 (42.0)	16 (11.5)	4.2*	.359
Pgm1	Miner Lake	15	.700	6 (7.4)	9 (6.3)	0 (1.3)	2.8	.600

* significant at $P < .05$

TABLE 7

Observed genotypes for Gap3,4 with values expected using a tetrasomic model of inheritance and Hardy-Weinburg proportions, chi-square values (x^2), and observed proportion heterozygous (h).

Population	number of fish	allele frequency	Genotypes					degrees of freedom	x^2	h
			AAAA	AAAA'	AAA'A'	AA'A'A'	A'A'A'A'			
Big Hole River	45	.878	27 (26.7)	14 (14.8)	4 (3.1)	0 (0.3)	0 (0.0)	2	.59	.400
Bobcat Lake	5	.800	1 (2.0)	4 (2.1)	0 (0.8)	0 (0.1)	0 (0.0)	2	3.29	.800
Chena River	38	1.000	38 (38.0)	0 -	0 -	0 -	0 -	-	0	.000
Elizabeth L.	18	.583	2 (2.1)	2 (6.0)	14 (6.4)	0 (3.0)	0 (0.5)	3	12.58**	.889
Elk Lake	49	.643	6 (8.4)	16 (18.6)	27 (15.5)	0 (5.7)	0 (0.8)	3	16.13**	.878
Fuse Lake	89	.556	1 (8.5)	22 (27.2)	62 (32.5)	4 (17.3)	0 (3.4)	4	47.98***	.989
Grebe Lake	41	.683	4 (8.9)	22 (16.6)	15 (11.5)	0 (3.6)	0 (0.4)	3	9.51*	.902
L. Agnes	36	.722	7 (9.8)	18 (15.1)	11 (8.7)	0 (2.2)	0 (0.2)	3	4.41	.806
Miner Lake	15	.950	13 (12.2)	1 (2.6)	1 (0.2)	0 (0.0)	0 (0.0)	1	4.15	.133
Mussigbrod L.	14	1.000	14 (14.0)	0 -	0 -	0 -	0 -	-	.00	.000
Red Rocks L.	28	.571	1 (3.0)	6 (9.0)	21 (10.1)	0 (5.0)	0 (0.9)	4	20.12***	.964
Rogers Lake	43	.692	5 (9.9)	23 (17.5)	15 (11.7)	0 (3.5)	0 (0.4)	3	8.87*	.884
Steel Creek	4	.875	2 (2.4)	2 (1.3)	0 (0.3)	0 (0.0)	0 (0.0)	1	.70	.500
Sunnyslope C.	41	.683	6 (8.9)	18 (16.6)	17 (11.5)	0 (3.6)	0 (0.4)	3	7.69	.854

* $P < .050$ ** $P < .010$ *** $P < .001$

TABLE 8

Observed genotypes for Gap3 (with expected values) under the assumption that only this locus is variable in native Montana/ Wyoming populations. Chi-square values (X^2), probability with 1 degree of freedom, and proportion expected to be heterozygous (h).

Population	number of fish	common allele frequency	Genotypes AA	AA'	A'A'	X^2	h
Big Hole River	45	.756	27 (25.7)	14 (16.6)	4 (2.7)	1.12	.369
Bobcat Lake	5	.600	1 (1.8)	4 (2.4)	0 (0.8)	2.22	.480
Chena River	38	1.000	38 (38.0)	0 -	0 -	-	.000
Elizabeth L.	18	.167	2 (0.5)	2 (5.0)	14 (12.5)	6.48*	.278
Elk Lake	49	.286	6 (4.0)	16 (20.0)	27 (25.0)	1.96	.408
Grebe Lake	41	.366	4 (5.5)	22 (19.0)	15 (16.5)	1.00	.464
L. Agnes	36	.444	7 (7.1)	18 (17.8)	11 (11.1)	.01	.494
Miner Lake	15	.900	13 (12.2)	1 (2.7)	1 (0.1)	5.95*	.180
Mussigbrod L.	14	1.000	14 (14.o)	0 -	0 -	-	.000
Red Rocks L.	28	.143	1 (0.6)	6 (6.8)	21 (20.6)	.44	.245
Rogers Lake	43	.384	5 (6.3)	23 (20.4)	15 (16.3)	.74	.473
Steel Creek	4	.750	2 (2.2)	2 (1.5)	0 (0.3)	.44	.375
Sunnyslope C.	41	.366	6 (5.5)	18 (19.0)	17 (16.5)	.12	.464

* $P < .050$

TABLE 9

Observed genotypes for duplicated loci with values expected using a tetrasomic model of inheritance and Hardy-Weinburg proportions; degrees of freedom; chi-square values (x^2); and the observed proportion heterozygous (h).

Locus	Population	N	frequency	Genotypes					df	x^2	h
				AAAA	AAAA'	AAA'A'	AA'A'A'	A'A'A'A'			
Aat3,4	Chena River	38	.993	37 (37.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1	.00	.026
Idh3,4	Fuse Lake	91	.986	86 (86.1)	5 (4.8)	0 (0.1)	0 (0.0)	0 (0.0)	1	.11	.055
Idh3,4	Rogers Lake	43	.994	42 (42.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1	.00	.023
Mdh1,2	Fuse Lake	91	.821	43 (41.4)	33 (36.0)	13 (11.7)	2 (1.7)	0 (0.1)	3	.59	.527
Mdh3,4	Chena River	38	.849	24 (19.7)	7 (14.1)	5 (3.8)	2 (0.4)	0 (0.0)	2	10.29*	.368

* $P < .010$

TABLE 10

Percent of loci polymorphic (P) and percent average heterozygosity per individual (H) in 14 populations of at 34 loci.

Population	P	H
Big Hole River	8.8	1.79
Bobcat Lake	5.9	2.88
Chena River (Alaska)	5.9	2.11
Elizabeth Lake	5.9	4.08
Elk Lake	8.8	3.98
Fuse Lake (Canada)	11.8	5.96
Grebe Lake	5.9	3.70
Lake Agnes	8.8	3.78
Miner Lake	8.8	2.99
Mussigbrod Lake	3.0	.62
Red Rocks Lake	8.8	4.40
Rogers Lake	8.8	4.37
Steel Creek	5.9	2.12
Sunnyslope Canal	5.9	3.14
Mean	7.4	3.28

TABLE 11

Chi-square values with significance level of loci pairwise between fourteen populations of Arctic grayling above the diagonal and degrees of freedom / number of loci contributing to the difference below the diagonal.

Populations	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
(1) Big Hole R.	-----	0.9 NS	115.8 *	40.5 *	38.7 *	108.3 *	20.4 *	28.3 *	45.7 *	5.1 NS	51.3 *	45.9 *	0.5 NS	123.0 *
(2) Bobcat Lake	3/0	-----	38.4 *	5.9 NS	3.7 NS	28.2 *	1.5 NS	2.9 NS	8.5 NS	5.9 NS	6.7 NS	2.9 NS	0.2 NS	33.5 *
(3) Chena River	5/3	4/2	-----	55.8 *	86.5 *	321.4 *	93.1 *	68.5 *	52.3 *	44.9 *	67.3 *	69.6 *	27.8 *	45.2 *
(4) Elizabeth L.	3/2	2/1	4/3	-----	2.2 NS	118.1 *	7.4 NS	4.0 NS	21.6 *	24.1 *	0.6 NS	3.4 NS	5.5 NS	24.2 *
(5) Elk Lake	4/2	3/0	5/3	3/0	-----	144.2 *	4.2 NS	1.3 NS	43.2 *	9.9 NS	3.0 NS	4.7 NS	3.5 NS	53.0 *
(6) Fuse Lake	6/5	5/2	7/5	5/3	6/3	-----	105.2 *	127.8 *	166.9 *	61.4 *	140.4 *	162.0 *	32.0 *	280.8 *
(7) Grebe Lake	3/2	2/0	4/3	2/1	3/0	5/3	-----	3.9 NS	37.2 *	13.4 NS	11.6 NS	11.8 NS	1.8 NS	69.3 *
(8) Lake Agnes	4/2	3/0	5/3	3/0	3/0	6/4	3/0	-----	30.8 *	15.5 NS	5.1 NS	3.1 NS	2.6 NS	47.7 *
(9) Miner Lake	4/2	3/1	5/4	3/2	4/2	6/5	3/2	4/2	-----	6.4 NS	34.1 *	38.1 *	5.4 NS	65.1 *
10) Mussigbrod Lk.	3/0	2/1	3/2	2/2	3/2	5/4	2/1	3/2	2/1	-----	27.5 *	22.0 *	3.6 NS	67.8 *
11) Red Rocks Lake	4/2	3/1	5/3	3/0	3/0	6/3	3/1	3/0	4/2	3/2	-----	3.6 NS	6.2 NS	31.1 *
12) Rogers Lake	5/3	4/0	6/3	4/0	6/4	4/2	4/0	5/2	5/2	4/2	4/0	-----	4.5 NS	41.3 *
13) Steel Creek	3/0	2/0	4/2	2/0	3/0	5/2	2/0	3/0	3/0	2/0	3/0	4/0	-----	28.2 *
14) Sunnyslope C.	3/2	2/1	4/2	2/1	3/1	5/3	2/1	3/1	3/3	2/2	3/1	4/2	2/1	-----

NS not significantly different * P <.001 (significance level adjusted (Cooper 1968) for 91 pairwise comparisons)

TABLE 12

Genetic identities (Nei 1972) of 14 pairs of Arctic grayling populations (above the diagonal), using 34 enzyme loci. Genetic distances with standard deviation (below the diagonal).

Populations	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
(1) Big Hole R.	------	1.000	.992	.997	.998	.996	.999	.999	.998	1.000	.997	.998	1.000	.990
(2) Bobcat Lake	.000 (.010)	------	.992	.998	.999	.997	1.000	.999	.997	1.000	.997	.998	1.000	.991
(3) Chena River	.008 (.013)	.008 (.015)	------	.996	.995	.985	.994	.996	.996	.986	.995	.996	.993	.998
(4) Elizabeth L.	.003 (.012)	.002 (.015)	.004 (.027)	------	1.000	.995	.999	1.000	.997	.991	1.000	1.000	.997	.997
(5) Elk Lake	.002 (.011)	.001 (.014)	.005 (.027)	.000 (.024)	------	.996	1.000	1.000	.997	.995	1.000	1.000	.999	.996
(6) Fuse Lake	.004 (.022)	.003 (.013)	.015 (.023)	.005 (.024)	.004 (.024)	------	.997	.996	.992	.989	.995	.995	.996	.986
(7) Grebe Lake	.001 (.010)	.000 (.013)	.006 (.027)	.001 (.021)	.000 (.022)	.003 (.023)	------	1.000	.997	.997	.999	.999	.999	.994
(8) Lake Agnes	.001 (.018)	.001 (.013)	.004 (.023)	.001 (.023)	.000 (.022)	.004 (.023)	.000 (.020)	------	.998	.996	.999	1.000	.999	.996
(9) Miner Lake	.002 (.018)	.003 (.011)	.004 (.024)	.003 (.022)	.003 (.021)	.008 (.018)	.003 (.020)	.002 (.020)	------	.998	.996	.998	.998	.994
10) Mussigbrod	.001 (.005)	.001 (.009)	.015 (.018)	.009 (.011)	.005 (.009)	.011 (.008)	.003 (.007)	.004 (.008)	.002 (.008)	------	.991	.994	1.000	.980
11) Red Rocks Lk.	.003 (.012)	.003 (.015)	.005 (.027)	.000 (.028)	.000 (.024)	.005 (.024)	.001 (.021)	.001 (.023)	.004 (.022)	.009 (.011)	------	1.000	.997	.997
12) Rogers Lake	.002 (.020)	.002 (.014)	.004 (.025)	.000 (.025)	.000 (.024)	.005 (.022)	.001 (.023)	.000 (.024)	.002 (.021)	.006 (.009)	.000 (.025)	------	.998	.997
13) Steel Creek	.000 (.009)	.000 (.011)	.007 (.015)	.003 (.014)	.001 (.013)	.004 (.011)	.001 (.012)	.001 (.012)	.002 (.010)	.001 (.008)	.003 (.014)	.002 (.013)	------	.991
14) Sunnyslope C.	.010 (.031)	.009 (.018)	.002 (.030)	.003 (.032)	.006 (.032)	.014 (.026)	.006 (.032)	.004 (.031)	.006 (.025)	.020 (.021)	.003 (.032)	.003 (.027)	.008 (.018)	-----

Table 13

Tissue expression of gene loci of Arctic grayling from Montana and Canada. Activity: + = active, ++ = more active, - = no activity, and nd = no data.

Loci	Muscle	Liver	Eye	Heart	Brain	Kidney	Stomach	Spleen
Adk1	++	+	+	+	+	+	+	+
Adk2	++	+	+	+	+	+	+	+
Adh1	-	+	-	-	-	-	-	-
Aat1	-	++	-	-	-	+	-	nd
Aat2	-	-	++	-	++	-	-	nd
Aat3,4	++	-	+	++	+	+	+	nd
Ck1	++	+	+	+	+	nd	+	nd
Ck3	-	-	++	-	+	+	-	-
Gpi1+2	++	-	-	+	-	-	-	-
Gpi3+4	+	+	++	+	+	+	+	+
G3p1	+	+	nd	nd	nd	nd	nd	nd
Gap1	++	+	-	-	-	-	-	-
Gap2	-	+	-	++	-	-	-	-
Gap3,4	-	+	++	-	++	+	+	+
Gl1	-	-	++	-	++	-	-	-
Gl2	++	++	++	++	++	++	++	++
Idh1+2	++	-	-	++	-	+	+	-
Idh3,4	-	++	+	-	+	+	+	+
Ldh2	++	-	-	-	-	-	-	-
Ldh3	+	+	++	++	+	+	++	+
Ldh4	+	++	+	+	+	++	+	+
Ldh5	-	-	++	-	-	-	-	-
Lgg1+2	++	++	++	++	++	++	++	++
Mdh1,2	+	++	+	++	+	+	+	+
Mdh3,4	++	+	+	++	+	+	+	+
Me1,2	++	-	+	++	+	+	+	+
Me3,4	+	++	-	+	-	-	-	+
Pgm1+2	++	++	-	+	++	+	+	+
Pgm3,4	+	+	+	+	+	+	+	+
6Pg1	+	++	+	+	+	+	+	+
Sod1	+	+	+	+	+	+	+	+
Xdh1	-	+	-	-	-	-	-	-

TABLE 14

Mean, standard deviation, and number sampled for each character over all populations studied. Paired fin rays, gillrakers, and pored scales in the lateral line were summed (right + left counts). A multivariate coefficient of variation (CVp) was calculated over all characters for each population.

Population	Anal rays	Dorsal rays	Pectoral rays	Pelvic Rays	Gillrakers	Vertebrae	Pored scales	CVp
Agnes Lake								4.49
Mean	15.06	23.31	33.06	20.69	37.59	59.37	176.06	
St Dev	.80	1.18	1.21	.80	2.27	1.24	7.40	
Number	35	35	35	35	34	35	35	
Big Hole River								3.11
Mean	14.48	22.42	31.03	20.39	39.78	58.85	175.97	
St Dev	.67	.90	1.02	.70	2.18	.80	6.08	
Number	33	33	33	33	32	33	32	
Chena River								3.44
Mean	14.42	24.32	30.62	21.05	36.83	59.46	179.95	
St Dev	.65	1.03	1.23	.97	2.35	.77	6.96	
Number	36	37	37	37	36	37	37	
Elk Lake								4.21
Mean	14.73	22.98	32.39	20.35	37.02	58.67	175.45	
St Dev	.60	.38	1.06	.63	1.80	1.23	7.07	
Number	49	49	49	49	48	49	49	
Fuse Lake								3.53
Mean	15.50	24.68	32.13	20.65	38.32	58.65	175.28	
St Dev	.63	.79	1.38	.84	2.28	.84	6.78	
Number	30	31	30	31	28	31	25	

TABLE 14 (continued)

Population	Anal rays	Dorsal rays	Pectoral rays	Pelvic Rays	Gillrakers	Vertebrae	Pored scales	CVp
Grebe Lake								
Mean	14.80	23.43	35.12	20.49	38.42	59.17	173.59	3.47
St Dev	.56	.78	1.55	.75	1.97	.97	6.20	
Number	41	40	41	41	38	41	39	
Elizabeth Lake								
Mean	14.56	23.28	32.50	20.50	38.47	59.61	175.50	2.85
St Dev	.70	.57	1.04	.71	1.77	.61	6.45	
Number	18	18	18	18	17	18	18	
Miners Lake								
Mean	14.93	22.53	30.80	20.27	40.57	59.00	174.60	4.71
St Dev	.46	.52	1.26	.70	1.45	.85	9.66	
Number	15	15	15	15	14	15	15	
Red Rocks Lake								
Mean	14.69	22.83	31.83	20.48	37.90	59.28	176.52	3.53
St Dev	.66	.80	1.26	.69	1.57	1.10	6.19	
Number	29	29	29	29	29	29	29	
Rogers Lake								
Mean	13.74	22.33	34.07	20.77	37.79	58.63	179.54	4.30
St Dev	.76	1.10	2.24	.95	2.28	1.36	6.64	
Number	43	42	43	43	42	43	41	
Total								
Mean	14.65	23.22	32.55	20.58	38.03	59.02	176.43	3.76
St Dev	.80	1.13	1.99	.81	2.26	1.09	7.06	.62
Number	329	329	330	331	318	331	320	10

TABLE 15

Mann-Whitney U tests on meristic count data for seven traits between 10 populations. Probability values for the seven tests were combined and tested for significance against a chi-square distribution (Sokal and Rohlff 1981) (above the diagonal). The number of characters that are significantly different pairwise (P < .05) are below the diagonal.

Populations	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1) Big Hole R.	------	***	***	***	***	***	***	NS	***	***
(2) Chena River	5	------	***	***	***	***	***	***	***	***
(3) Elizabeth L.	4	5	------	***	***	***	NS	***	NS	***
(4) Elk Lake	4	6	3	------	***	***	***	***	**	***
(5) Fuse Lake	3	5	3	3	------	***	***	***	***	***
(6) Grebe Lake	4	6	1	4	4	------	***	***	***	***
(7) Lake Agnes	5	4	1	3	4	1	------	***	**	***
(8) Miner Lake	1	4	4	3	3	3	3	------	***	***
(9) Red Rocks L.	3	5	1	3	3	3	1	2	------	***
10) Rogers Lake	5	4	4	6	3	5	5	4	3	------

NS no significant difference * (P < .050) ** (P < .010) *** (p < .001)

TABLE 16

Mann-Whitney U tests on meristic count data between 10 populations. Significant difference for anal rays (above the diagonal) and for dorsal rays (below the diagonal).

Populations	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1) Big Hole R.	------	***	NS	*	***	*	**	*	NS	***
(2) Chena River	NS	------	NS	*	***	**	***	**	NS	***
(3) Elizabeth L.	***	***	------	NS	***	NS	*	NS	NS	***
(4) Elk Lake	***	***	**	------	***	NS	NS	NS	NS	***
(5) Fuse Lake	***	NS	***	***	------	***	*	**	***	***
(6) Grebe Lake	***	***	NS	***	***	------	NS	NS	NS	***
(7) Lake Agnes	**	***	NS	NS	***	NS	------	NS	NS	***
(8) Miner Lake	NS	***	***	***	NS	***	**	------	NS	***
(9) Red Rocks L.	NS	***	*	NS	***	**	NS	NS	------	***
10) Rogers Lake	NS	***	***	***	***	***	***	NS	NS	------

NS not significant * P < .050 ** P < .010 *** P < .001

TABLE 17

Mann-Whitney U tests on meristic count data between 10 populations. Significant difference for pectoral rays (above the diagonal) and for pelvic rays (below the diagonal).

Populations	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1) Big Hole R.	------	NS	***	***	***	***	***	NS	**	***
(2) Chena River	**	------	***	***	***	***	***	NS	***	***
(3) Elizabeth L.	NS	*	------	NS	NS	***	NS	***	NS	***
(4) Elk Lake	NS	***	NS	------	NS	***	**	**	*	***
(5) Fuse Lake	NS	NS	NS	NS	------	***	**	**	NS	***
(6) Grebe Lake	NS	**	NS	NS	NS	------	***	***	***	**
(7) Lake Agnes	NS	NS	NS	*	NS	NS	------	***	***	***
(8) Miner Lake	NS	**	NS	NS	NS	NS	NS	------	*	***
(9) Red Rocks L.	NS	*	NS	NS	NS	NS	NS	NS	------	***
10) Rogers Lake	*	NS	NS	**	NS	NS	NS	*	NS	------

NS not significant * P < .050 ** P < .010 *** P < .001

TABLE 18

Mann-Whitney U tests on meristic count data between 10 populations. Significant differences for vertebrae (above the diagonal) and pored scales in the lateral line (below the diagonal).

Populations	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1) Big Hole R.	------	**	***	NS	NS	NS	*	NS	*	NS
(2) Chena River	*	------	NS	***	***	NS	NS	NS	NS	**
(3) Elizabeth L.	NS	*	------	***	***	NS	NS	*	NS	*
(4) Elk Lake	NS	**	NS	------	NS	*	**	NS	**	NS
(5) Fuse Lake	NS	*	NS	NS	------	**	**	NS	**	NS
(6) Grebe Lake	NS	***	NS	NS	NS	------	NS	NS	NS	*
(7) Lake Agnes	NS	*	NS	NS	NS	NS	------	NS	NS	**
(8) Miner Lake	NS	NS	NS	NS	NS	NS	NS	------	NS	NS
(9) Red Rocks L.	NS	*	NS	NS	NS	*	NS	NS	------	*
10) Rogers Lake	*	NS	NS	**	NS	***	*	NS	NS	------

NS not significant * P < .050 ** P < .010 *** P < .001

TABLE 19

Mann-Whitney U tests on meristic count data between 10 populations. Significant difference for gillrakers (below the diagonal).

Populations	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1) Big Hole R.	------									
(2) Chena River	***	------								
(3) Elizabeth L.	*	**	------							
(4) Elk Lake	***	NS	**	------						
(5) Fuse Lake	NS	**	NS	**	------					
(6) Grebe Lake	*	**	NS	**	NS	------				
(7) Lake Agnes	***	NS	NS	NS	NS	NS	------			
(8) Miner Lake	NS	***	**	***	**	***	***	------		
(9) Red Rocks L.	**	*	NS	*	NS	NS	NS	***	------	
10) Rogers Lake	***	NS	NS	*	NS	NS	NS	***	NS	------

NS not significant * P < .050 ** P < .010 *** P < .001

Table 20

Estimates for the amount of genetic variation, measured as average heterozygostiy per individual (H) for various taxa.

Taxa	number of populations	minimum loci	H	source of data
Fish	51 species	varies	.051	Nevo (1978)
Salmonids				
Pacific salmon (5 sp.)	41	30	.028	Allendorf, Utter 1979
Brook char	8	39	.081	Stoneking et al.1981
Arctic char (Sweden)	15	42	.017	Hindar et al. 1986
Arctic char (Norway)	10	37	.008	Andersson et al.1983
Brown trout (Sweden)	38	35	.025	Ryman 1983
Atlantic salmon	2	30	.024	Allendorf and Utter 1979
Cutthroat trout				
Interior	19	30	.010	Leary and Allendorf 1982
Coastal	6	30	.063	Allendorf and Utter 1979
Rainbow trout	55	30	.060	Leary and Allendorf 1982
Grayling				
Montana grayling	2	33	.028	Lynch and Vyse 1979
Canada grayling	1	33	.032	"
Fuse Lake grayling*	1	33	.000	"
Baikal grayling	1	22	.025	Kartavtsev, Mamontov 1983
Alaska grayling	4	30	.021	Hop 1985
Montana grayling	12	34	.032	this study
Alaska grayling	1	34	.021	"
Fuse Lake grayling*	1	34	.060	"

* Canada stock transplanted to Montana

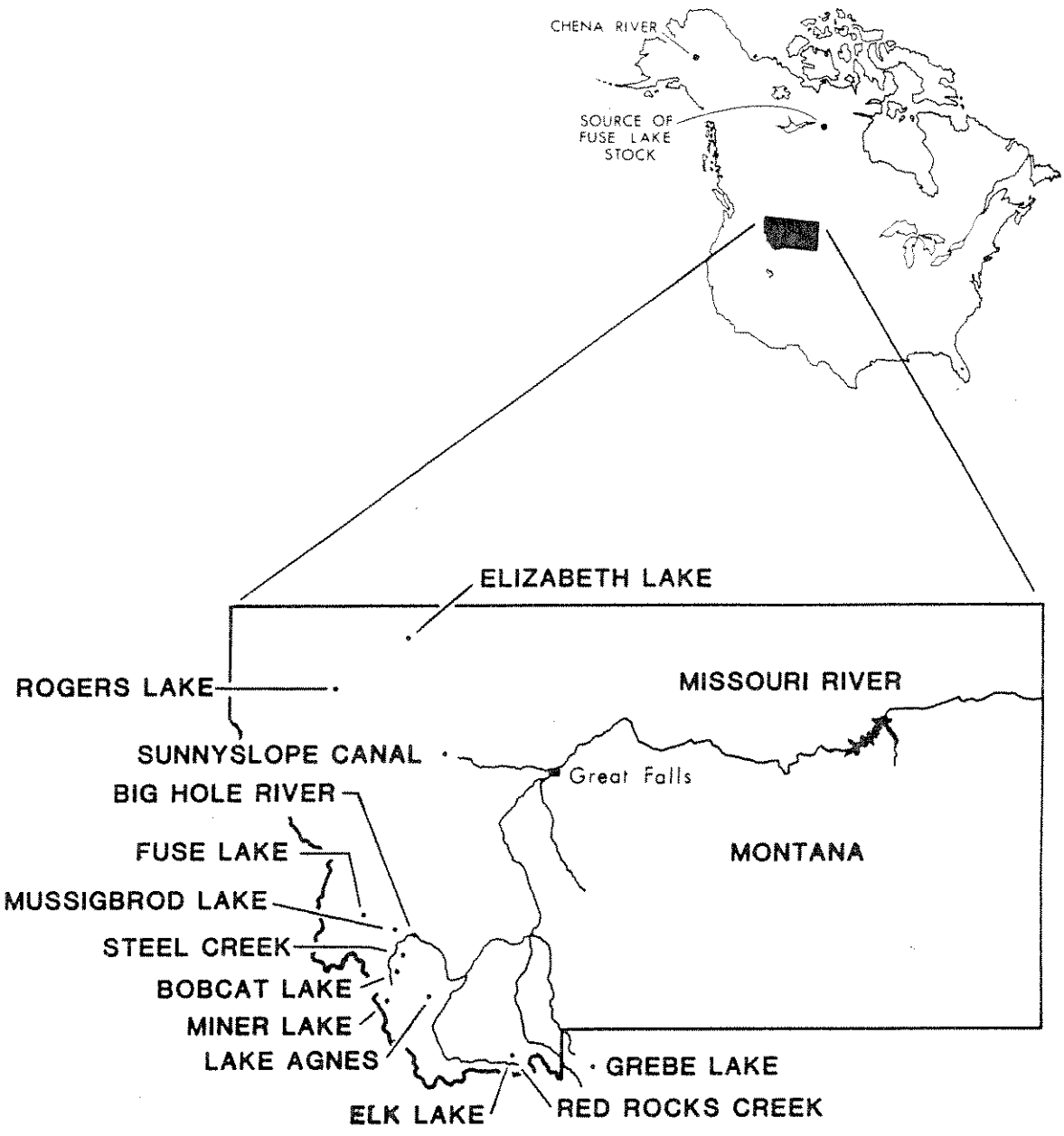

Figure 1. Arctic grayling were native to the tributary rivers of the Missouri River upstream from Great Falls, Montana. Samples of grayling for this study came from the locations labelled on the map.

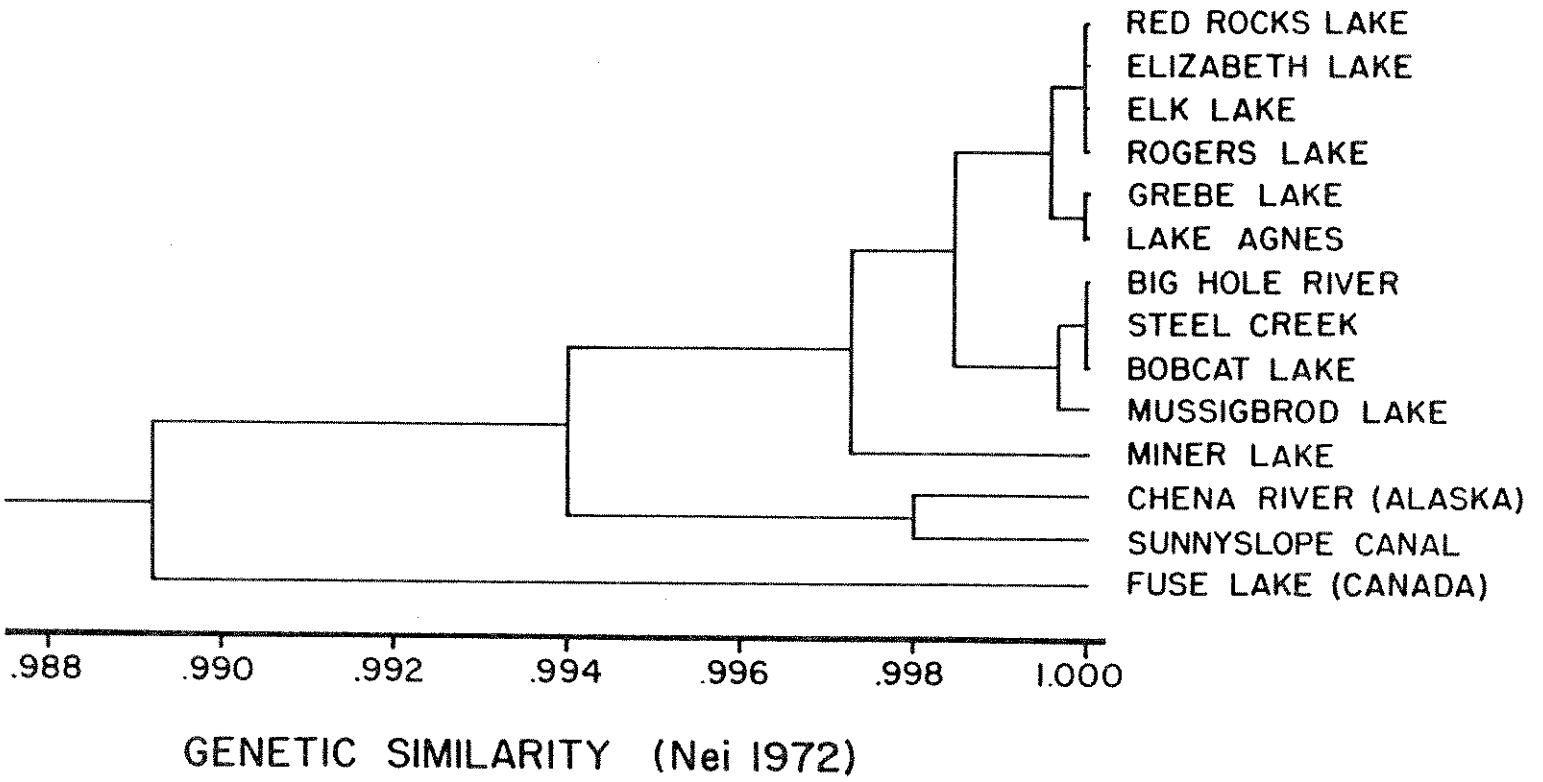

Figure 2. Genetic identities among Arctic grayling populations were calculated using the method of Nei (1972).

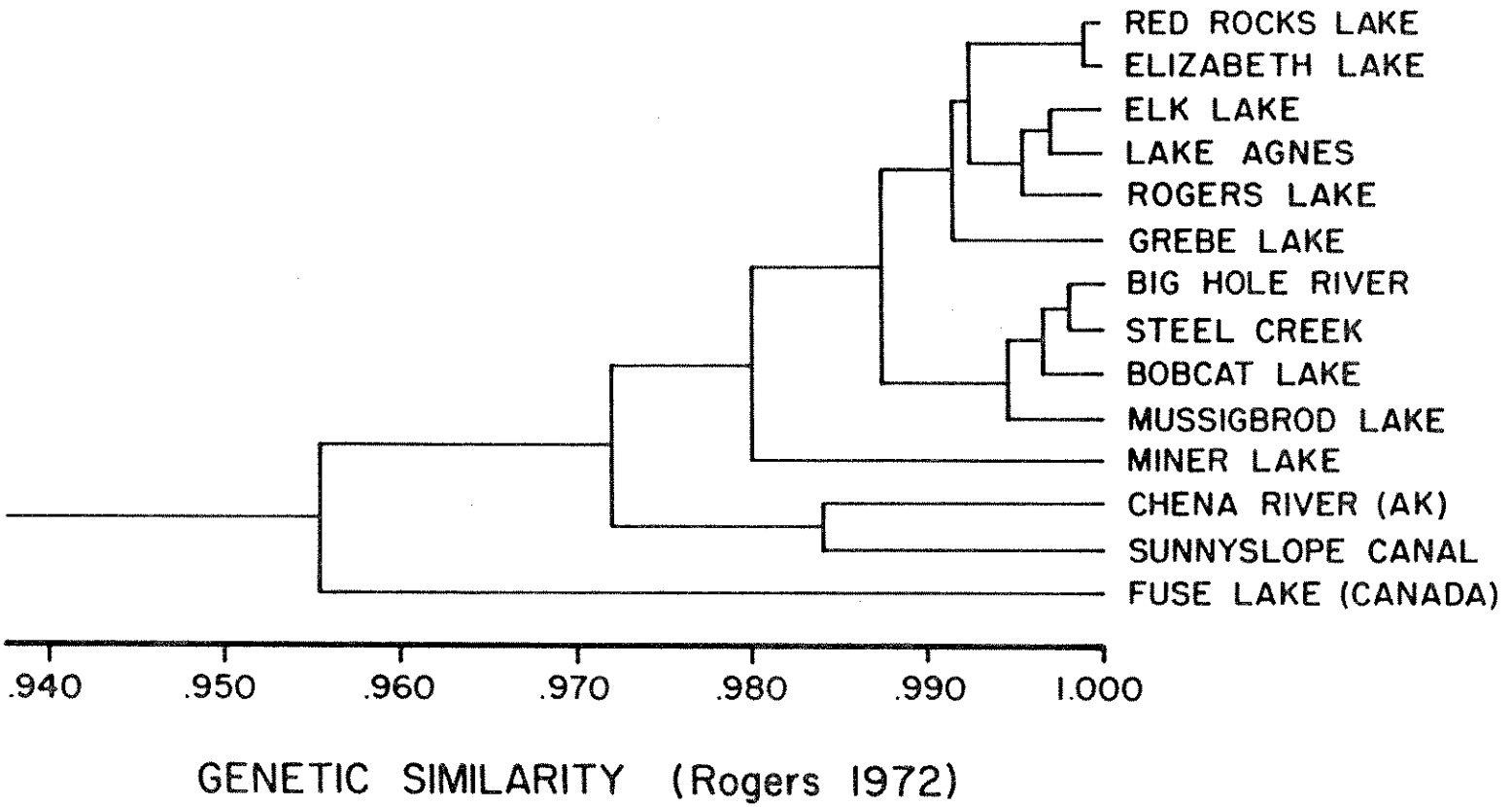

Figure 3. Genetic similarities among Arctic grayling populations were calculated using the method of Rogers (1972).

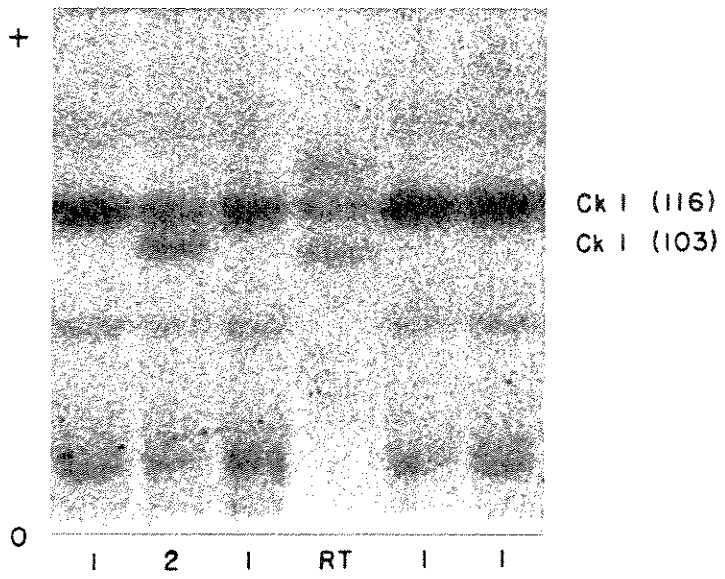

Figure 4. Muscle CK variation in Arctic grayling from the Big Hole River, Montana. (1 = common homozygote; 2 = heterozygote) Mobilities of enzymes are measured relative to the Ck1 locus of rainbow trout (RT).

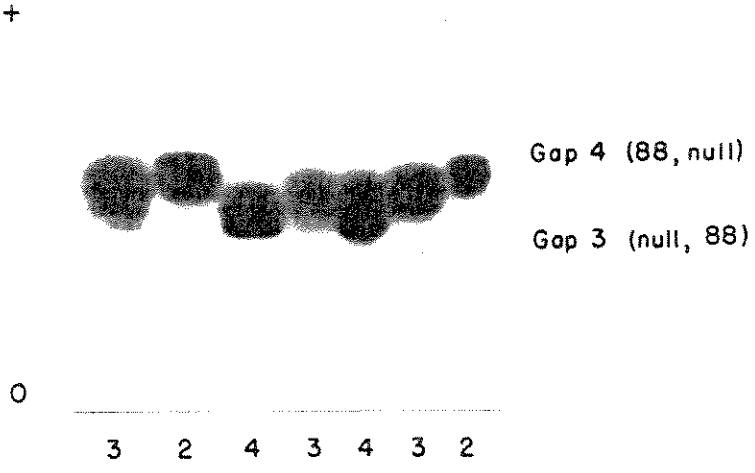

Figure 5. Eye GAP variation in Fuse Lake, Montana grayling. (3 = common phenotype, homozygous for the common allele; 2 = heterozygous null at Gap3; 4 = heterozygous null at Gap4) A homozygous null phenotype for Gap3 (not shown) is seen in native Montana populations.

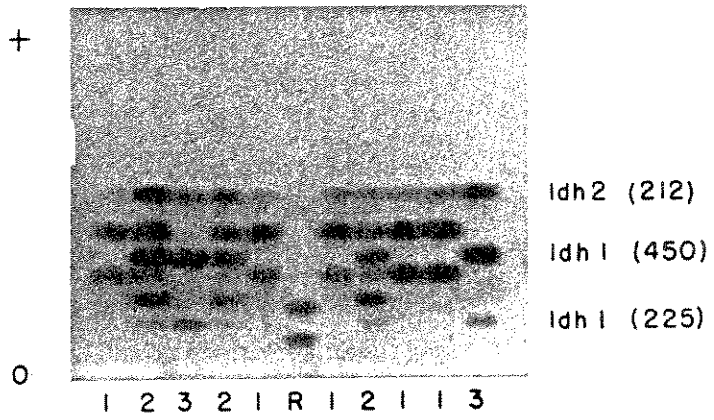

Figure 6. Muscle IDH phenotypes in Arctic grayling from Fuse Lake, Montana. (1 = common homozygote for Idh1 (450/450); 2 = heterozygote for Idh1 (450/225); 3 = alternate homozygote for Idh1 (225/225).} Mobilities of enzymes are measured relative to rainbow trout (R).

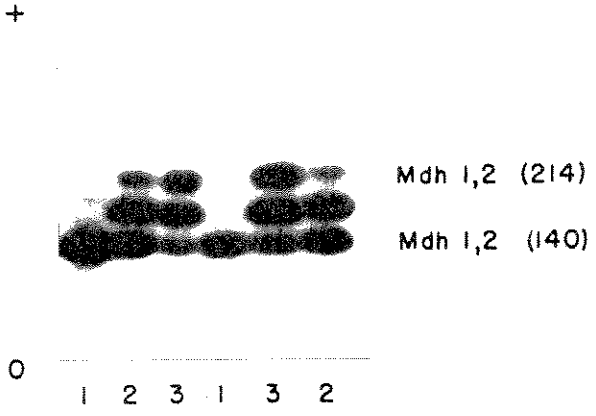

Figure 7. Liver MDH phenotypes in Arctic grayling from Fuse Lake, Montana. Band intensities show that this is a duplicated locus in grayling. (1 = common homozygote; 2 = one copy of the alternate allele (214); 3 = two copies of the alternate allele)

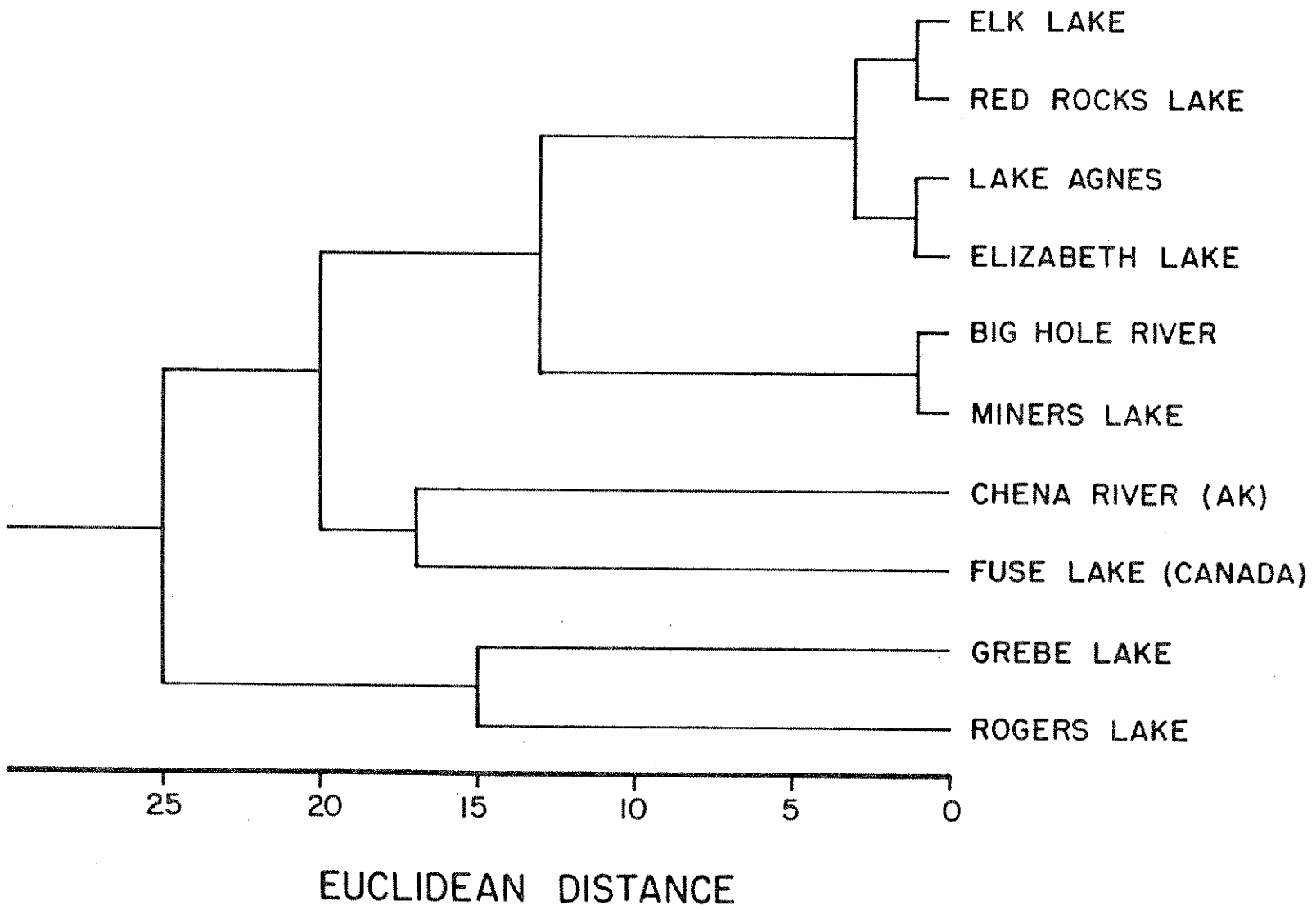

Figure 8. Euclidean distances among Arctic grayling populations were calculated by using a clustering program on morphological count data.