Comparative genetics of Montana and arctic grayling, Thymallus arcticus
by Jeremiah Cornelius Lynch

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Zoology
Montana State University

Abstract:
An investigation was made of the biochemical genetic variation within and among four populations of the arctic grayling, Thymallus articus. Two populations surveyed were representative of the form found in the main range of the species, northern Canada and Alaska, and two populations were representative of the disjunct Montana form of Thymallus articus. Estimates of these parameters were obtained from a starch gel electrophoretic survey of thirty-five enzyme loci and protein loci. The percent polymorphic loci (12.5 percent) and average heterozygosity (2.7-3.I percent) are intermediate in the range estimated for salmonid species and may reflect the limited habitat diversity of grayling compared with other salmonid species.

No relationship between genetic variability and enzyme function was identified for this species. Both a rapidly evolving set and a slowly evolving set of proteins appeared to be present.

Comparisons among the four populations were based on allelic protein variation at eight loci. Results of genetic similarity and genetic distance calculations indicate that genetic divergence has taken place between the arctic form and Montana form of T. arcticus, which may warrent subspecific status for the two forms.

STATEMENT OF PERMISSION TO COPY

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at Montana State University, I agree that the Library shall make it freely available for inspection. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by my major professor, or, in his absence, by the Director of Libraries. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature Jeremiah C. Lynch

Date 9/12/77

COMPARATIVE GENETICS OF MONTANA AND ARCTIC GRAYLING, *THYMALLUS ARCTICUS*

by

Jeremiah C. Lynch

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Zoology

Approved:

E R Hyse
Chairperson, Graduate Committee

James M. Pickett
Head, Major Department

Henry L Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

September, 1977

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my major professor, Dr. Ernest R. Vyse, for his guidance, assistance and continued support throughout this study.

Special thanks are extended to John D. Varley (Yellowstone Park Fisheries Management) for his interest in the study and assistance in obtaining samples; Dr. David G. Cameron for his assistance throughout this study and constructive review of this manuscript; Dr. Fred W. Allendorf (University of Montana) for the use of his computer program for analysis of the data; and Dr. Calvin Kaya for his review of this manuscript.

Finally, I would like to thank my wife, Teri, for the special support she has given me throughout this study.

TABLE OF CONTENTS

TABLE OF CONTENTS
(Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES
(Continued)

ABSTRACT

An investigation was made of the biochemical genetic variation within and among four populations of the arctic grayling, *Thymallus arcticus*. Two populations surveyed were representative of the form found in the main range of the species, northern Canada and Alaska, and two populations were representative of the disjunct Montana form of *Thymallus arcticus*. Estimates of these parameters were obtained from a starch gel electrophoretic survey of thirty-five enzyme loci and protein loci. The percent polymorphic loci (12.5 percent) and average heterozygosity (2.7-3.1 percent) are intermediate in the range estimated for salmonid species and may reflect the limited habitat diversity of grayling compared with other salmonid species.

No relationship between genetic variability and enzyme function was identified for this species. Both a rapidly evolving set and a slowly evolving set of proteins appeared to be present.

Comparisons among the four populations were based on allelic protein variation at eight loci. Results of genetic similarity and genetic distance calculations indicate that genetic divergence has taken place between the arctic form and Montana form of *T. arcticus*, which may warrent subspecific status for the two forms.

INTRODUCTION

Distribution

Thymallus arcticus, the arctic grayling, is a freshwater fish which inhabits cold or arctic regions. The native range of the species is holarctic, occurring in northern drainages of North America and Eurasia. In Eurasia, it is found from the Kara and Ob Rivers, in the western U.S.S.R. to the eastern Siberian Coast (including all streams draining into the Bering Sea, and the Penzhina River draining into the sea of Okhotosk), south to northern Mongolia and the Yalu River (Walters 1955, Scott and Crossman 1973).

In Canada and Alaska, *T. arcticus* occurs from Vansittart Island off the Melville Peninsula; south along the west coast of Hudson Bay to the Owl River, Manitoba; west throughout the Northwest and Yukon Territories to the Bering Sea drainages in Alaska; south in Saskatchewan to Reindeer Lake but absent in most of the Churchill River; south to Central Alberta; in northern British Columbia from the Pease and Stikine River north (Walters 1955, Slastenenko 1950, Scott and Crossman 1973). Figure 1 shows the distribution of *Thymallus arcticus* in Canada and Alaska.

In the contiguous United States *Thymallus arcticus* was indigenous in Michigan and Montana. Isolated populations were present in Michigan in the upper part of the Lower Peninsula, and in the Otter

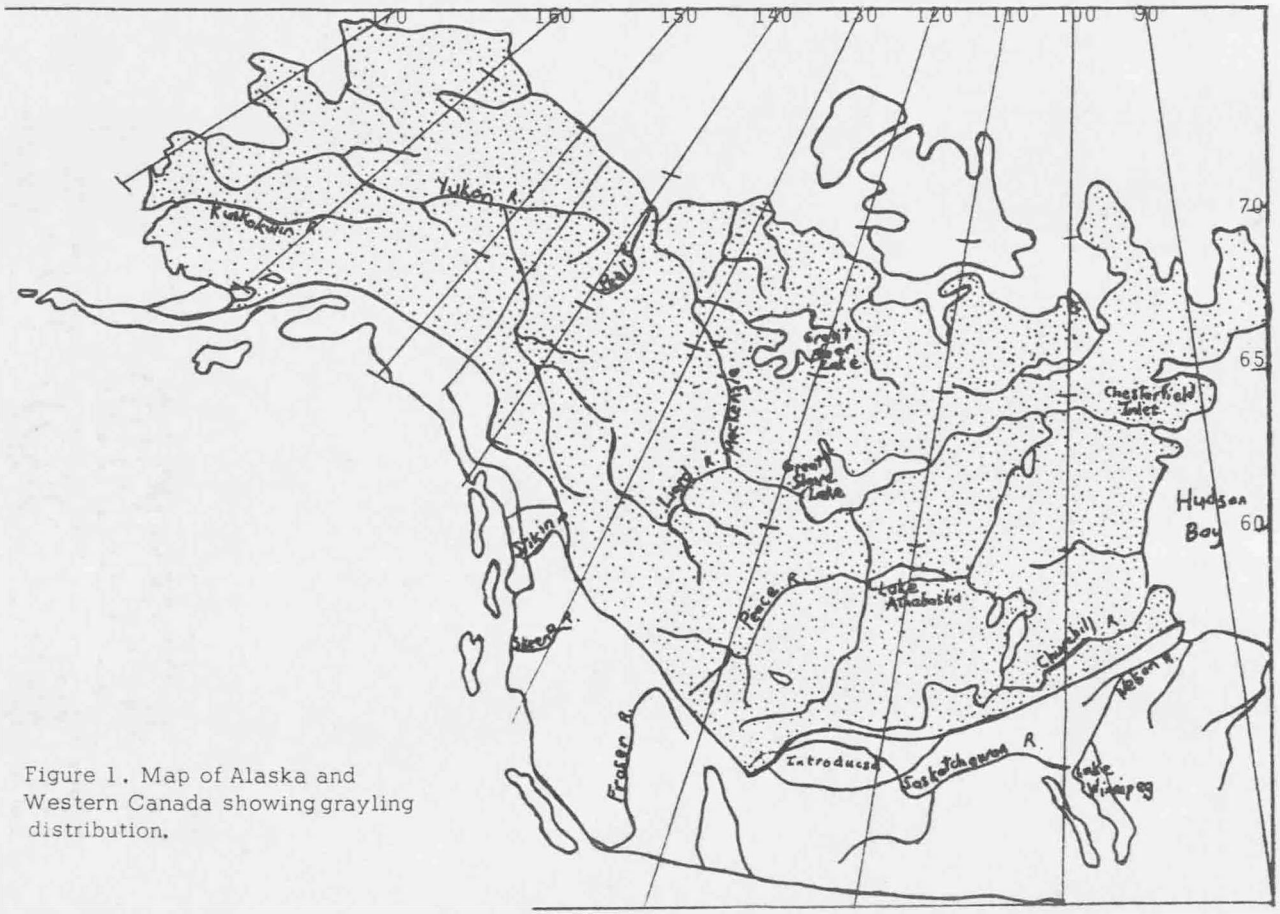


Figure 1. Map of Alaska and Western Canada showing grayling distribution.

River of the Upper Peninsula (Hubbs and Lagler 1949). The Michigan form, however, has been extinct since 1936 (Scott and Crossman 1973, U.S. Dept. of Interior 1966). Another population was found in Montana in the headwaters of the Missouri River above the Great Falls (Henshall 1906). This southward extension and the subsequent establishment of these two populations was evidently the result of glacial action.

In Montana, the original range as described by Henshall (1906) has been greatly reduced. The decline of the species has been reported and reaffirmed by various investigators (Kelly 1931, Brown 1943, Nelson 1954, 1956). The present distribution in native rivers and streams is described by Brown (1971) with the statement that "a few are found in the Sun, Big Hole, Red Rock, and Madison Rivers." Grayling are entirely absent from the Missouri River, the Gallatin River and the main stem of the Jefferson River. Two small remnant populations remain in the Jefferson tributaries: one in the Big Hole River and the other in the Red Rock Lakes area (Nelson 1954). The range of indigenous populations of *Thymallus arcticus* in Montana is shown in Figure 2.

There has been widespread transplanting of Canadian stocks into Montana populations (McPhail and Lindsey 1971) and hatchery planting from a single source (the Red Rock Lakes) into all but one of the

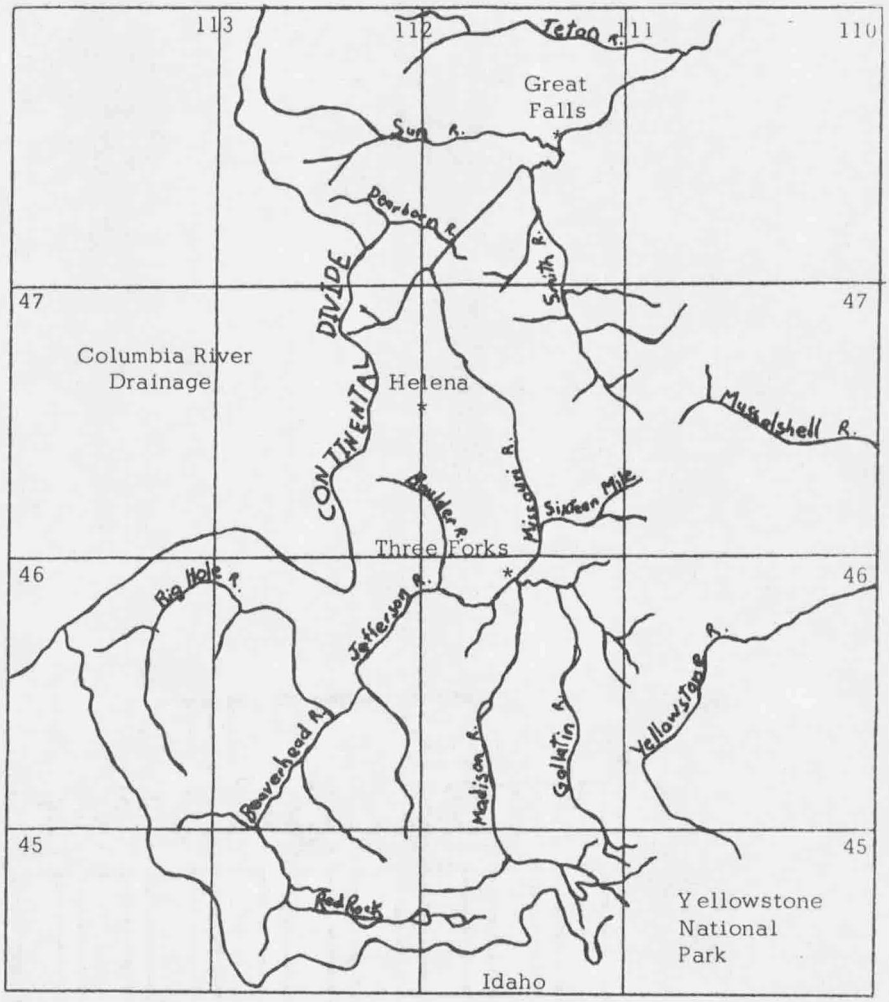


Figure 2. Distribution of indigenous grayling in Montana.

natural populations (Kelly 1931). At this time reproducing grayling populations are known to exist in 39 lakes and 14 streams in western Montana on both sides of the Continental Divide (Holten 1971). The only indigenous Montana population known not to be contaminated by plantings is the Red Rock Lakes population (Nelson 1954).

In Yellowstone National Park the grayling occurred naturally in the Madison River system and the Gallatin River. It is no longer present in the Gallatin River and is rare in the Madison River (Dean and Varley 1974). Transplanting of grayling to lakes in Yellowstone National Park has been described by Kruse (1959). Successful self-propagating populations were established in Grebe Lake, Wolf Lake, and Ice Lake, above the Virginia Cascades on the Gibbon River, and Cascade Lake in the Yellowstone drainage.

Taxonomy

The graylings are soft rayed teleost fish belonging to the order Isospondyli, suborder Salmonoidei. Their further taxonomic classification has been one fraught with confusion. The genus *Thymallus* was separated from the genus *Salmo* (Curvier 1829), but their family classification was debated for some time by taxonomists and remains unresolved today (Scott and Crossman 1973). Some authors have assigned all three geographic groups (Arctic, Asian, and Montana-Michigan) to the Salmonidae (Boulenger 1895, Regean 1914) while others (Jordan and

Everman 1896, Berg 1940, 1955) have placed the graylings in a separate family, the Thymmallidae. The most recent classification, based on osteological characteristics (Norden 1961), places the grayling in the sub-family Thymallinae, of the family Salmonidae. Four species are recognized: *T. brevirostris* (Mongolia), *T. thymallus* (Europe), *T. nigrescens* (Lake Kosogol, Mongolia) and *T. arcticus* (eastern Siberia and North America).

The status of the various forms of *T. arcticus* has been debated for some time. For decades it was considered that the North American graylings consisted of three species: *T. signifer* (Richardson 1823) found in northern Canada and Alaska, *T. tricolor* (Cope 1865) found in Michigan, and *T. montanus* (Milner 1873) found in Montana. This classification was principally based on geographic isolation, and several morphological characteristics (size and shape of dorsal fin, maxillary length, and color variation). More recently, *T. signifer* has been considered conspecific with *T. arcticus* (Pallas), and the other American forms relegated subspecific status (Walters 1955). Walters showed that the Canadian and Alaskan form was identical with two Asiatic forms (*T. a. pallasi* and *T. a. gruberi natio mertensi*) and suggested that they be designated as *Thymallus arcticus signifer* (Richardson 1823). Walters (1955) further recognized the Montana-Michigan form as another subspecies *tricolor*. The validity of the

North American subspecies has not been adequately demonstrated (Scott and Crossman 1973, Norden 1961). At the present time no subspecies should be recognized within *T. arcticus* until further evidence warrants such distinction (McPhail and Lindsey 1971).

Electrophoresis

Proteins are ampholytes and, therefore, may carry a net negative or positive charge. The net charge depends on the ionization of 1) free carboxyl groups ($COOH^-$) of glutamic acid and aspartic acid residues and 2) free amino groups (NH_3^+) of lysine and arginine (and to a lesser extent histidine). The net charge of the protein depends on which group predominates. The degree of ionization depends on the pH of the protein solution. In a buffer of high pH the acidic groups are progressively neutralized by the alkali component of the buffer, thus allowing the basic groups to predominate. This results in the protein molecule having a net negative charge. At a low pH the reverse occurs, and the protein will have a net positive charge. At a certain pH, the isoelectric point, the positive and negative charges are balanced and there is no net charge.

Electrophoresis manipulates the ampholytic behavior of proteins by applying an electrical field to a solution of proteins, separating them on the basis of their net charge. If the pH is less than the isoelectric point of the protein, it will migrate toward the cathode

and if the pH is greater than the isoelectric point of the protein, it will migrate toward the anode. The rate of migration depends on the number of charges, the molecular size and on the voltage applied. Therefore, by the selection of the appropriate buffer and electrical current, protein differences can be determined.

Electrophoretic techniques vary as to the supporting media used. Starch gel was employed in the present study. Isozyme separation using starch gel depends not only on net ionic charge, but to a lesser extent on differences of molecular size. Starch gel acts as a molecular sieve, mechanically separating molecules of different sizes by affecting their rate of migration (Smithies 1955).

Variation in Natural Populations

The development of starch gel electrophoresis (Smithies 1955), and the introduction of simple staining techniques for the detection of specific enzyme activity (Hunter and Markert 1957), has allowed the visualization of individual proteins, hence, single gene products. The combination of these techniques provided a means by which heterogeneity of proteins and enzymes could easily be detected. This allowed the characterization at the molecular level of the amount of genetic variability in populations and an estimate of the extent of genetic divergence among closely related species (Gottlieb 1971).

Kimura and Crow (1964) hypothesized that the number of alleles that can be maintained at a single locus in a finite population is large. Shaw (1965) pointed out that isozymes which vary within populations is the rule rather than the exception. Approximately 100 loci are known to have electrophoretic variants in populations of many organisms, including man, *Drosophila*, ants, fish, mice, frogs and many plant species (Gottlieb 1971). The amount of such genic variation, measured by the proportion of polymorphic loci (common allele frequency less than or equal to 0.99) can be determined directly from electrophoretic analysis.

In theory, a large number of structurally different alleles may be generated by independent mutations within the confines of a single gene (Harris 1976). A certain proportion of these mutations can be expected to result in a substitution affecting the net charge of the protein. Such a mutation would be reflected in the mobility of the protein. The probability that such a mutation will occur is calculated to be 25-30% (Shaw 1965, Nei 1975). This means that a large number of amino acid substitutions go undetected since they do not result in a charge change. The estimates of genetic variability based on electrophoretically detectable differences may, therefore, be conservative (Harris and Hopkinson 1976, Nei 1975).

Data from a random sample of loci coding for proteins can be extrapolated to estimate the amount of genetic variability in the entire genome (Lewontin and Hubby 1966). The estimates of the amount of polymorphism in the sample can be used to calculate the proportion of polymorphic loci and individual heterozygosity in a species. These estimates can provide a basis for comparison between species (Utter *et al.* 1973) and may also be used to compare populations within species.

The level of genetic polymorphism has been estimated for a variety of species: man - 25 percent of 12 loci were polymorphic (Harris 1966) and more recently man - 31 percent of 71 loci (Harris and Hopkinson 1972); *Mus musculus* - 30 percent polymorphic (Selander *et al.* 1969) and 40 percent polymorphic (Selander and Yang 1969); *Peromyscus polionotus* - 23 percent polymorphic (Selander *et al.* 1971); quail, *Coturnix coturnix* - 54 to 58 percent polymorphic (Baker and Manwell 1967); pheasant, *Phasianus colchicus* - 43 percent polymorphic (Baker *et al.* 1966); *Drosophila* (variety of species) - 30 to 67 percent polymorphic (Lewontin and Hubby 1966, Prakash *et al.* 1969, Ayala *et al.* 1970, Berger 1970, O'Brien and MacIntyre 1969). In fish specifically, estimates are: *Astynax* - 29 to 41 percent for inland populations and 0 to 20 percent for cave dwellers (Avise and Selander 1972), herring - 45 percent polymorphic (Altukhov *et al.* 1972), brook

trout, *Salvelinus fontinalis* - 38 percent polymorphic (Wright and Atherton 1970); chum salmon - 11 to 18 percent polymorphic (Altukhov *et al.* 1972); rockfish, *Sebastes* (variety of species) - 4 to 8 percent polymorphic (Johnson *et al.* 1973), Pacific salmon - 8 to 13 percent (Utter *et al.* 1973); rainbow trout - 26 percent (Utter *et al.* 1973). In surveying the literature as a whole, approximately 30 percent of the structural gene loci are polymorphic (Gottlieb 1971; Nei 1975). Heterozygosity varies considerably with locus (King and Wilson 1975, Nei and Roychoudhury 1974, Selander and Johnson 1973, Utter *et al.* 1973). The existence of locus dependent rates of change is well illustrated by amino acid sequencing data in diverse organisms (Dickerson 1972), which indicates that proteins evolve at different rates.

Gene loci are polymorphic when allele substitution is in transition, when balancing selection stabilizes frequencies, or when a mutant allele becomes frequent by chance. Since each locus may undergo allele substitution independently, a high degree of interlocus variation in heterozygosity may result. Interlocus variation may also be produced if the mutation rate or the type and intensity of natural selection varies among loci (Nei 1975). That loci variation and, hence, protein heterogeneity is present in a wide variety of vertebrate species was shown by Selander and Johnson (1973).

Interlocus variation has also been found in many species of fish (Utter *et al.* 1973).

This interlocus variation can be separated into two groups, a rapidly evolving set and a slowly evolving set. The rapidly evolving set, including plasma proteins and esterases, accumulates electrophoretically detectible substitutions at a rate tenfold greater than the slower set, which includes enzymes involved in metabolic pathways (Sarich 1977). Correlations have been proposed between enzyme function and heterozygosity (Gillispie and Kojima 1968, Kojima *et al.* 1970, Johnson 1971, 1974, Powell 1975), and more recently a relationship between heterozygosity and quaternary structure has been proposed (Ward 1977). The basis for the difference in heterozygosity between loci is not adequately resolved, but estimates of average heterozygosity or genetic distance would be overestimated or underestimated if the proteins chosen did not include an adequate mixture of both sets. In the present study a large number of loci have been examined with no preference given to either the rapidly evolving or the slower evolving set of proteins.

Nei and Roychoudhury (1974) have suggested that to estimate the average heterozygosity per locus, a large number of loci rather than a large number of individuals should be used. Avise and Ayala (1975) state that with respect to genetic similarity, the variance about

individuals is small relative to the variance about loci; therefore, the precision of these estimates is much more dependent on the number of loci than on the numbers of individuals sampled. If the intent is to predict Hardy-Weinberg equilibrium as well as estimate average heterozygosity, a relatively large number of individuals should be examined for each polymorphic locus (Nei 1975). If both of these estimated are within the scope of a study, a large number of individuals should be screened at a large number of loci with the hope of minimizing any bias.

A great deal of data has been collected as to the genetic variability of fish proteins (deLigny 1969, Kirpichnikov 1973). Protein heterogeneity appears to be present in nearly every species studied.

A reliable set of proteins which have been studied in other fish species, and reported in the literature, were chosen for analysis so that reliable estimates of average heterozygosity and Hardy-Weinberg frequencies could be calculated. In addition, comparisons can be made to the published results for other species. The availability of substrate for staining and the clarity of resolution were the final factors in determining what proteins were included.

Objectives

The North American forms of *Thymallus arcticus* have been isolated since before the last Wisconsin glaciation (Vincent 1962). Isolated

populations were established in favorable habitats south of the main range of *Thymallus arcticus*. For populations to become genetically differentiated, evolutionists believe they must be completely isolated from one another. This isolation may occur geographically or reproductively (Dobzhansky 1951, 1970). Complete isolation has occurred in the case of *Thymallus arcticus*, between the allopatric populations, which at one time shared the same gene pool, but have since become isolated from one another. Therefore, an opportunity for evolutionary divergence of the various stocks has been present. An estimation of the amount of divergence which has taken place could be obtained by a survey of electrophoretic differences (discussed previously). However, the comparison of the three american forms of *T. arcticus* meets with some difficulty. The Michigan form has been extinct since 1936 (Scott and Crossman 1973, U.S. Dept. of the Interior 1966) and thus its divergence from the other two cannot be determined. The Montana form as described by Henshall (1906) dealt almost exclusively in rivers and streams, hence it was an adfluvial form (stream dwelling-stream spawning) and only secondarily a lacustrine form (lake dwelling-stream spawning). From a genetic and taxonomic point of view, the study of the adfluvial form may be impossible due to the reduced numbers (Brown 1971), the contamination of independent populations by the introduction of Canadian stocks

(McPhail and Lindsey 1971), and the universal planting of lacustrine forms from a single donor (Red Rock Lakes) into discrete adfluvial populations. The Red Rock Lakes population is a pure deme of the Montana form of *Thymallus arcticus*, with no transplants of any other stocks having taken place (Nelson 1954). The Red Rock Lakes population, although known to be native, is a restricted headwaters population and may have been genetically distinct in its own right. The use of it as a donor stock in widespread planting may have diluted the enzootic populations in rivers and streams throughout the native range. The validity of a genetic basis for the adfluvial and lacustrine behavioral differences has not been studied, however, the innate genetic control of migration in other salmonid species has been suggested (Raleigh 1967, Brannon 1967, Northcote 1969, Raleigh and Chapman 1971). If there are distinct genetically controlled behavioral differences between the two forms, it may be suggested that the lacustrine form, historically rare in the Montana range of the species, is now widespread while the historically common adfluvial type is threatened with extinction.

In the present study, the Grebe and Wolf Lakes populations, which were established by transplanting from a stock derived from the Madison River system (Kelly 1931, Kruse 1959), were used to represent the Montana form. Grayling were not native to either of these lakes,

but both were stocked with grayling derived only from the Madison River system. Eggs taken from grayling native to Meadow Creek in the Madison River drainage were reared in the State Fish and Game's Anaconda hatchery and the progeny were planted in Georgetown Lake (Kelly 1931). Subsequent to this planting, eggs taken from these Georgetown grayling were transplanted to these lakes in Yellowstone Park (Kruse 1958).

The data obtained from these demes is, therefore, presumed to be of an adfluvial form which have since become adapted to a lacustrine existence. The data obtained can be used to estimate the amount of divergence which has taken place between the Arctic form and the Montana forms of *Thymallus arcticus*. This estimate of the amount of genetic divergence can be based on the genetic characters of the populations obtained through electrophoretic data. The use of the Madison River population itself is a practical impossibility due to the diminished numbers of individuals in this declining population (Dean and Varley 1974).

In addition to the comparison of the Arctic form and the Montana form, the study can also determine if genetic differentiation has occurred within two Montana populations, the Grebe Lake and Wolf Lake populations. Salmonids have a tendency to evolve genetically discrete, ecologically specialized populations with differentiation based on life history characters such as time and place of spawning

(Behnke 1972). As previously mentioned, innate genetic control of migration habits in salmonids has been suggested. The strong homing behavior of most salmonids is an important factor in the generation and maintenance of this genetic diversity (Allendorf *et al.* 1971). Such appears to be the case with these populations, the Wolf Lake population sampled was an outlet spawning population while the Grebe Lake population sampled was an inlet spawning population. Thus, the Grebe and Wolf Lakes populations have partially established ethological reproductive isolation, which may be the first step in genetic isolation. The amount of genetic divergence between these two populations thus gives an estimate of the amount of genetic change, at the structural gene level, that has accompanied this event. If the magnitude of the divergence between these populations and the Canadian populations is large, the evolutionary position of these forms could be clarified.

The phenotypic frequencies revealed by patterns of proteins on electrophoretic gels, can be interpreted in terms of genotypic frequencies and the population allelic frequencies, which are the parameters of evolutionary genetics. With these two parameters of the demes known, the inlet and outlet spawning populations can be compared with one another and with the Arctic form with regard to the unique proportion of the genome that distinguishes these

populations, and the level of heterozygosity under the different environments. Both of these are important properties of diverging genetic systems.

Electrophoretic techniques provide considerable information to help elucidate evolutionary relationships among closely related species (Avise 1974). Biochemical genetic variation among closely related populations can be used to examine systematic relationships (Nei 1975, Sarich 1977). The use of such data to determine taxonomic status has been successfully applied to salmonid species (Payne *et al.* 1971, Nyman 1972, Utter *et al.* 1973, Reinitz 1974). The data obtained in the present study will contribute to the data needed to help clarify the confused taxonomic position that presently exists through the subspecies classification of *Thymallus arcticus*. The results will hopefully provide some evidence as to whether subspeciation has occurred between the Arctic and Montana forms of *Thymallus arcticus*.

MATERIALS AND METHODS

Sampling of Populations

Discrete populations of either the Canadian form or the Montana form of *Thymallus arcticus* were chosen for analysis. Populations, which according to Montana Fish and Game stocking records, were known not to be a mixture of both forms were sampled as representative of the Montana form. The Canadian arctic population was assumed to be native, but designation of any population as a Montana form had to be confirmed from transplant records.

The populations used as representatives of the Canadian form were taken from the Donnelly River, N.W.T. and Fuse Lake, Montana. The Donnelly River lies in the MacKenzie River drainage and grayling are native to its waters (McPhail and Lindsey 1970). Fuse Lake in Granite County, Montana, is an alpine lake which had no native fish fauna. Fuse Lake was stocked in 1930 with grayling from the Saskatchewan River drainage (State Fish and Game Records) with no subsequent plantings, thus it is a pure population of the Canadian form. The Saskatchewan River population in turn was derived from a Canadian Arctic grayling population (Lindsey 1956).

The Montana form was sampled from Grebe and Wolf Lakes populations in Yellowstone National Park. Grebe and Wolf Lakes are connected by five hundred meters of the Gibbon River which flows from Grebe Lake

into Wolf Lake. Both lakes have inlet and outlet spawning adapted populations of grayling. In sampling these populations, only the outlet spawning Wolf Lake population and the inlet spawning Grebe Lake population were sampled in order that any genetic differences associated with this spawning behavior could be estimated in the electrophoretic survey.

The grayling were collected either by angling or by use of an electric backpack shocker. The number of individuals collected, the date, the method, and the location site are listed in Table 1.

Upon capture the weight and length of the fish were recorded. Blood samples were taken by making a longitudinal incision from the isthmus to the abdominal region of the fish, opening the pericardial sac with an incision and removing approximately 1 ml of blood. The blood was placed in a plastic tube and either centrifuged at 5000 g for approximately 3 minutes when done in the field, or the tube of blood was placed on ice and transported to the laboratory where centrifuging was done. After centrifugation the serum was separated from the blood cells, stored in a microfuge tube and both were immediately frozen.

Tissue samples of the liver, muscle, heart and eye were taken and placed immediately on dry ice and were transferred to a freezer maintained at -50°C upon return to the laboratory. After removal

Table 1. Collection data for the four populations of *Thymallus arcticus*.

Population	Date	Number of Individuals	Ancestral Stock
Grebe Lake[a] Y.N.P.	6/25/75	30	Madison River System
	6/11/76	30	
	6/17/76	18	
	6/7/77	22	
Wolf Lake[b] Y.N.P.	6/25/75	8	Madison River System
	7/22/75	12	
	6/10/76	10	
	5/24/77	22	
	5/31/77	8	
Donnelly River N.W.T.	9/1/76	44	Native
Fuse Lake Mont.	8/15/75	19	MacKenzie River

[a]Inlet Creek
[b]Outlet Creek

of tissue samples the sex of the individual was noted when it could be determined, otherwise it was classified as immature.

Sample Preparation

Tissue extracts were prepared by grinding the tissue in an equivalent volume of buffer (.01 M Tris; .001 M EDTA; 5×10^{-5} M NADP; pH adjusted to 6.8), in a glass homogenizer. The homogenate was then centrifuged at 15,000 g for 20 minutes in a refrigerated centrifuge maintained at -10°C. The supernatant was then removed for electrophoresis or stored at -50°C for later use.

Electrophoresis

Horizontal starch gel electrophoresis was used in the electrophoretic analysis of proteins. The starch gels were 11.2% hydrolyzed starch (Electrostarch C. Madison, Wisconsin). The starch was mixed thoroughly with the appropriate amount of buffer in a side arm flask and placed on a hot plate with a magnetic stirrer, with additional hand shaking, until the solution boiled. The rate of stirring was increased as the viscosity of the fluid increased to keep the fluid homogeneous. A vacuum was then applied to the flask for approximately 60 seconds to remove air bubbles. The hot starch solution was poured on glass plates 25 cm by 18 cm. Plexiglass strips 1.5 cm wide and 1 cm thick, held in place by large paper clamps, were used to form

the edges of the gel. After an initial cooling (1 to 2 hours at room temperature) the gels were covered with plastic wrap without trapping any bubbles and stored in a refrigerator. Gels were used for electrophoresis any time up to 24 hours later.

Prior to application of samples to the gel, 26 sample slots .5 x 1 cm were made across the gel by a slot former. This allowed each sample slot to be completely separated by .5 cm of gel. A filter paper wick (approximately 1 cm x .5 cm) was soaked in a sample supernatant, blotted on filter paper to remove excess, and placed in the sample slot by the use of small forceps.

Plastic buffer trays (15 cm x 5 cm x 3 cm) were filled with 250 ml buffer. Disposable cloths (Handy Wipes) 25 cm wide, were used as the electrode sponges. The sponges were placed 3 cm from the ends of the gel, and the entire top of the apparatus was covered with plastic wrap. A direct electrical current, the voltage of which varied as to buffer system used was applied to the gel. The gel was cooled by a pan of ice suspended 2 mm over the entire gel surface by plexiglass strips.

Electrophoresis was continued until a marker (Bromophenol Blue) reached the anodal sponge. The buffer systems employed, voltage used, and length of run are listed in the Appendix.

After electrophoresis was completed, the gels were sliced into .20-.25 cm thick slices by a stainless steel blade. The top slice was always discarded due to distortion and the bottom 3 slices were stained by the appropriate procedure for visualization of the proteins desired. The staining procedures for different protein systems are listed in the Appendix. After the appropriate time for adequate development of the zymogram, the gels were washed three times in distilled water and fixed in a methanol/water/acetic acid (5/5/1) solution.

Permanent records of the zymograms were made by keeping a written record of the results, and photographing each gel on 35 mm Kodak High Contrast Copy or Panotomic X film. The latter type appeared to produce better results. The fixed gels were covered with Saran Wrap and refrigerated for later comparison with the photographs.

Qualitative Analysis

Isozymes are defined as multiple molecular forms of an enzyme occurring in a single individual or in different members of the same species (Markert 1968). Such isozymes may occur together in the same cell, but there may also be marked differences in isozyme patterns between tissues (Harris 1975). Several authors (Manwell and Baker 1970, Harris 1976) have discussed the fact that isozyme systems may be generated in a variety of ways. The various causes of isozymes may

be classified into 3 main categories (Harris 1969, 1975; Hopkinson and Harris 1971): 1) occurrence of multiple gene loci coding for structurally distinct polypeptide chains of the enzyme; 2) occurrence of multiple allelism at a single locus determining structurally distinct versions of a particular polypeptide chain; 3) occurrence of so-called "secondary isozyme formation due to past translational modifications of the enzyme structure. Furthermore, apparent variation observed on zymograms of starch gel electrophoresis may be artifacts due to storage and preservation of material (Eppenberger *et al.* 1971), or it may be the result of differential activation of genes due to environmental conditions (Hochachka and Somero 1971).

Stringent criteria must be used in analyzing biochemical variation of zymograms before one can assume that there is a genetic basis for the variation. The strongest data for determining if the variation has a genetic basis comes from breeding experiments and the subsequent analysis of progeny from parents having known biochemical differences. In the absence of such data, as is the case in the present study, other criteria must be imposed as suggested by various authors (Utter *et al.* 1973, Avise and Smith 1974, Gall *et al.* 1976). The criteria used in the present study to verify the genetic basis of observed variation were:

1) The banding pattern observed was typical for the presumed structure of the enzyme found in closely related species.

2) The pattern was interpretable on the basis of a simple genetic hypothesis; with typical homozygotes and heterozygotes being expressed.

3) The observed genotypic class proportions were in close agreement with those expected on the basis of Hardy-Weinberg equilibrium; any significant deviations from the predicted values must be explained.

4) Patterns must be repeatable upon subsequent sampling of the same individual, with parallel expression in other tissues to confirm any polymorphism.

Variation which did not conform to all of these criteria could not be conclusively verified to be genetic. However, if the evidence presented was strongly suggestive of a genetic basis, it was included in the analysis.

Since isozymes may have differential tissue expression, several different tissues were analyzed to determine the pattern of such expression. The tissues examined for a given enzyme system are summarized in Table 2. This allowed the determination of an added number of loci whose protein may be expressed in one tissue and not another (eg. LDH-5 locus). A variety of buffer systems were employed in early screening runs since variation may be expressed in one buffer

Table 2. Proteins surveyed, tissues examined, and buffer systems employed in electrophoretic analysis of *Thymallus arcticus*.

Protein	E.C. No.	Abbreviation	Tissue					Buffer System
			Liver	Muscle	Heart	Eye	Serum	
Alcohol Dehydrogenase	(1.1.1.1)	(ADH)	+					C
Alpha-glycerophosphate Dehydrogenase	(1.1.1.8)	(AGPD)						C,D,F
Esterase	(3.1.1.1)	(EST)					+	I
Glucose-6-Phosphate Dehydrogenase	(1.1.1.49)	(G-6-PD)	+	+	+	+		A,F
Glutamate Oxaloacetate Transaminase	(2.6.1.1)	(GOT)	+	+	+	+		C,H
Hexose-6-Phosphate Dehydrogenase	(1.1.1.47)	(H-6-PD)	+	+	+	+		A,F
Hexokinase	(2.7.1.1)	(HK)	+					G
Isocitrate Dehydrogenase	(1.1.1.42)	(IDH)	+	+				B,D
Lactate Dehydrogenase	(1.1.1.27)	(LDH)	+	+	+	+	+	F,G,H
Malate Dehydrogenase	(1.1.1.37)	(MDH)	+	+	+	+	+	A,B,D
Malic Enzyme	(1.1.1.40)	(ME)	+	+				A,B

Table 2. (Continued)

Protein	E.C. No.	Abbre-viation	Tissue					Buffer System
			Liver	Muscle	Heart	Eye	Serum	
Phosphoglucomutase	(2.7.5.1)	(PGM)	+	+				A,E
Transferrin		(TFN)					+	I
Tetrazolium Oxidase		(TO)	+	+				A,F
Serum Proteins		(SP)					+	I
Sorbitol Dehydrogenase	(1.1.1.14)	(SDH)	+					H
Xanthine Dehydrogenase	(1.2.3.2)	(XDH)	+	+				A,F

but not another (Utter *et al.* 1973). The different buffer systems employed for resolution of a given protein are listed in Table 2.

Salmonid fish are believed to have undergone extensive gene duplication and, therefore, may be tetraploid organisms (Ohno 1969). Grayling are salmonids, hence in the genetic interpretation of electrophoretic patterns, it is important to realize that there may be two or more gene loci which determine the primary structure of proteins. The presence of such duplicated loci has been found to exist in many salmonids (Allendorf *et al.* 1975, Bailey *et al.* 1970, Morrison and Wright 1966, Wolf *et al.* 1970). Therefore, grayling would be assumed to have similarly duplicated loci, complicating the analysis of zymogram patterns. Genetic interpretation was further classified by determining if the loci were duplicated which explained observed branding patterns of these loci. This allowed variation to be attributed to either multiple loci or to multiple alleles at a single locus.

All of the above possibilities were considered and the criteria imposed before the genetic basis of variability was assumed.

Nomenclature

The system of nomenclature follows that of Richmond (1972) and Prakash, Lewontin, and Hubby (1969). Each locus was named using an abbreviation of the protein name. When proteins of two or more loci

with identical substrate specificities were observed, the loci were numbered according to the migration rate of the protein products from the origin, i.e., the loci whose protein had the slowest migration was assigned numeral one, the next fastest the numeral two, and so on. When allelic variation was found at a locus, the most common allele was assigned a number 1.00 and all other alleles were assigned numbers that represented their proteins migration distance relative to the most common allele's protein, i.e., if a protein migrated a distance half as far, its allele would be labeled 0.50 or if one migrated a distance one and a half times as far, its allele would be designated 1.50.

RESULTS

Electrophoretic Phenotypes of Monomorphic Proteins

Lactate dehydrogenase - (LDH) is a tetramer composed of subunits of equal size (Appella and Markert 1961). LDH isozymes have been resolved electrophoretically into two main types designated A and B (Markert 1962). In mammals thus far studied, five principal isozymes are usually found which result from the random combination of these subunits A and B, into all possible tetrameric structures. Shaw and Barto (1963) provided genetic confirmation of the hypothesis that the subunits were under distinct genetic control by showing that each was controlled by separate gene loci.

An additional isozyme, designated C, has been observed in mature testis extracts of mammals and birds (Blanco and Zinkham 1963, Goldberg 1963). It has subsequently been shown to be coded by a separate locus (Blanco *et al.* 1964). Isozyme patterns of teleost fish provide evidence of an additional LDH locus, formerly designated E, in these animals (Markert and Faulhaber 1965, Whitt 1969, 1970, Markert and Holmes 1969, Shaklee *et al.* 1973). This locus is now considered to be homologous to the C locus of birds and mammals, with tissue expression varying with the fish studied (Markert *et al.* 1975). Genetic studies of electrophoretic variants have established the existence of this locus (Whitt *et al.* 1971).

The isozyme is expressed predominantly in nervous tissue, especially in the retina of the eye (Goldberg 1966, Whitt 1969, 1970, Whitt and Horowitz 1970, 1972).

In the case of salmonid fish, especially the trout, a complex pattern of more than fifteen isozymes is observable, with genetic studies showing that trout LDH is determined by at least five distinct loci (Morrison and Wright 1966, Morrison 1970, Massaro and Markert 1968, Utter *et al.* 1973). Relative to this observation is the hypothesis of Ohno *et al.* (1968), based on cytological evidence, that salmonids are tetraploids. This indicates that there has been duplication and subsequent divergence of A and B loci. The validity of the existence of duplicated loci is supported by the molecular hybridization and immunochemical studies of Massaro and Markert (1968). Therefore, the loci in salmonids are comprised of the duplicated A (A and A') and B (B and B') loci and an additional C locus. The complex zymogram patterns of LDH isozymes are explained by: five isozymes containing A and A' subunits, five isozymes containing B and B' subunits and hybrid isozymes containing B, B' and C subunits.

The various loci have differential tissue expression. The B and B' are expressed in most tissues (see Massaro and Markert 1968), except the liver where the B' predominates (Utter and Hodgins 1972).

The A and A' loci are expressed primarily in the skeletal muscle (Massaro and Markert 1968, Utter *et al.* 1973). The C locus is expressed in the eye and other neural tissue (Horowitz and Whitt 1972).

The isozyme patterns found in *T. arcticus* were typical of that found in other salmonids (Fig. 3). Five loci designated LDH-1, LDH-2, LDH-3, LDH-4, and LDH-5 (equivalent to A, A', B, B' and C, respectively) in order of increasing anodal migration, were expressed. In muscle tissue, 5 isozymes resulting from the combination of LDH-1 and LDH-2 subunits and 5 isozymes resulting from the combination of LDH-3 and LDH-4 subunits were expressed on LDH zymograms. The A group predominated as expected for salmonids. In heart, serum and eye, five isozymes resulting from the combination of LDH-3 and LDH-4 subunits (B group) were expressed. An additional locus LDH-5 was expressed in eye tissue which is the C locus common to salmonids (Horowitz and Whitt 1972). In liver, the LDH-3 locus predominated, exhibiting a single band in most buffer systems. However, in buffer system A five isozymes could be resolved, thus both the LDH-3 and LDH-4 loci are expressed in the liver. The predominance of the LDH-3 locus (B) is in contrast to the predominance of the LDH-4 (B') locus in the liver of other salmonids (Utter and Hodgins 1972, Utter *et al.* 1973).

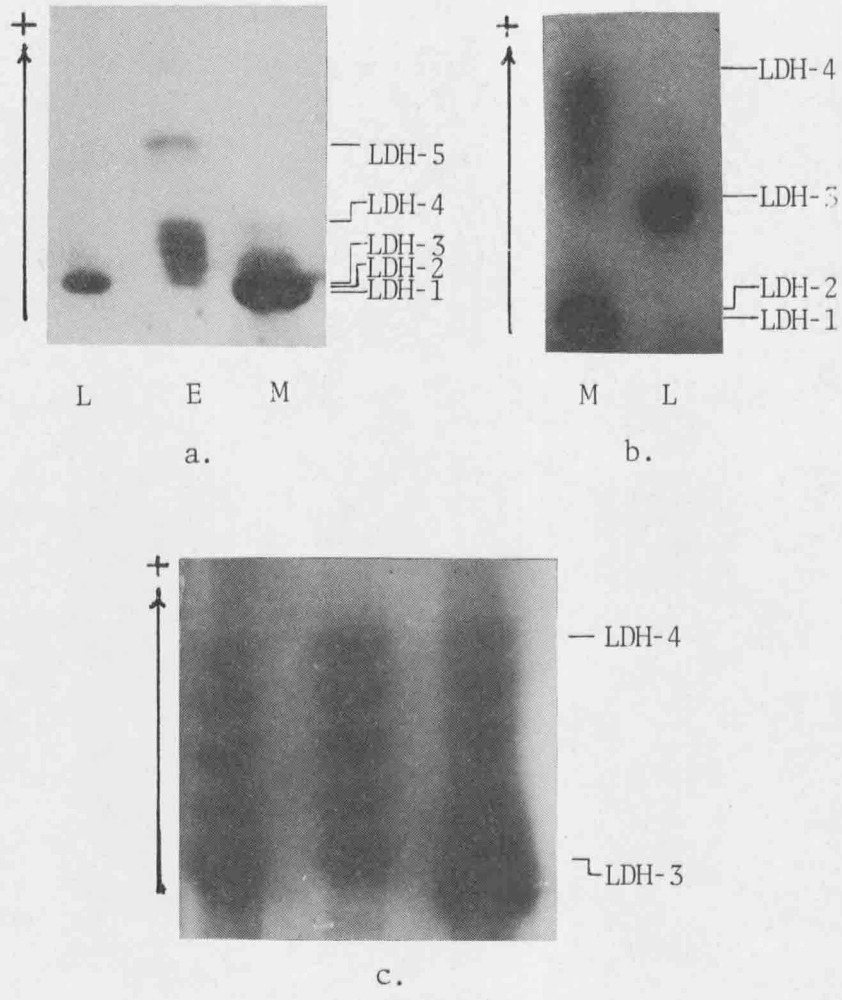


Figure 3. Lactate Dehydrogenase (LDH). Tissue distribution and the effects of different buffers on resolution of the LDH isozymes.

a. Liver, eye and muscle tissue of the same fish. Buffer system H.
b. Muscle and liver tissue from the same fish. Buffer system G.
c. Liver tissue from three individuals. Buffer system A.

No allelic variation was found at any of the loci in any of the populations examined. Individuals were homozygous at all loci, therefore, eleven isozyme types were expressed. The fewer number of isozymes resulted from the apparent lack of heterotetramers being formed between the LDH-5 and LDH-3 or LDH-4 loci. The number of LDH isozymes in *T. arcticus* is evidence that gene duplication has taken place at the A and B loci of LDH.

Malate dehydrogenase (MDH) - Two major classes of malate dehydrogenase isozymes are found to exist in vertebrates, the mitochondrial form and the supernatant (or cytoplasmic) form (Kitto and Kaplan 1966, Siegal and England 1961). Both have a dimeric structure and are coded by distinct nuclear genes (Shows *et al.* 1970, Wheat *et al.* 1971).

Three types of supernatant MDH isozymes AA, AB, BB, coded by 2 gene loci A and B, are found in many species of fish (Wheat and Whitt 1971, Clayton *et al.* 1971). The investigations of Bailey *et al.* (1970) indicated that this was the case in salmonids. In the salmonids, not only is there evidence of genetic variation of the B form of supernatant MDH (Syln'ko 1972, Utter *et al.* 1973, Clayton *et al.* 1975, Bailey *et al.* 1970, Aspinwall 1974), but there is also evidence of duplication of the B locus (Bailey *et al.* 1970, Utter and Hodgins 1972, Allendorf 1973). Bailey *et al.* (1970) provided evidence from

isozyme dosage studies which suggested that the A locus is also duplicated in the brown trout (*Salmo trutta*), while Aspinwall (1974) suggested that this was also true for pink salmon (*Oncorhynchus gorbuscha*). However, this has not been found in other salmonid species (Syln'ko 1976, Clayton *et al.* 1975, Allendorf 1973).

There is tissue specific expression of the A and B loci with the B form predominating in skeletal muscle and the A form predominating in the liver (Clayton *et al.* 1975, Allendorf *et al.* 1973, Allendorf 1973, Utter and Hodgins 1972, Bailey *et al.* 1970).

Massaro (1973) studied the MDH isozymes of grayling and reported that they possess three major isozymes of supernatant MDH in skeletal muscle and eye tissue, which may correspond to the AA, AB, and BB isozymes present in other salmonids as reported by Bailey *et al.* (1970). In the present study, tissue samples of liver, skeletal muscle, heart muscle, eye and serum were surveyed for supernatant MDH activity. The results are shown in Figure 4. A single band of strong intensity was found in liver samples, whereas 3 bands were expressed in all other tissues surveyed. It is postulated that there are two loci MDH_s-1 and MDH_s-2, corresponding to the A and B loci, respectively, of other salmonids, coding for supernatant MDH isozymes in *T. arcticus*. The A form, encoded by the MDH_s-1 locus, is predominant in the liver, while both forms A and B, (B encoded by the MDH_s-2

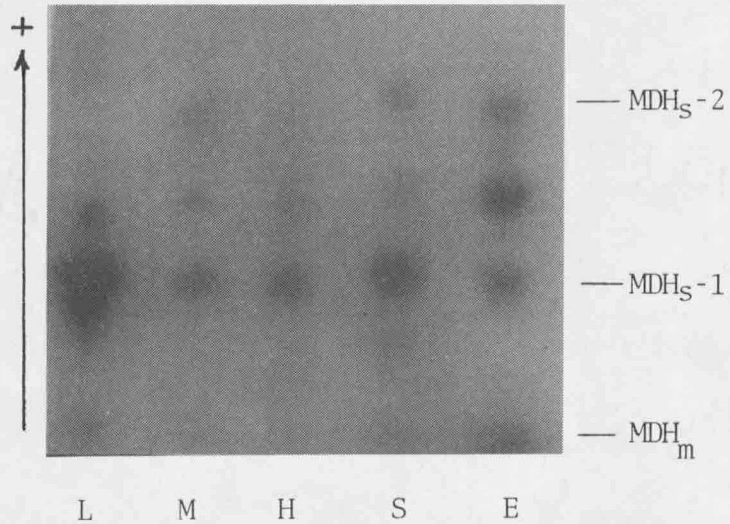


Figure 4. Tissue distribution of Malate Dehydrogenase (MDH) from the same fish. Samples are of liver, muscle, heart, serum and eye tissue. The MDH_m isozyme near the origin is common to all tissues but stains weakly in muscle, heart and serum samples. MDH_S-1 predominates in liver tissue. Both MDH_S-1 and MDH_S-2 are more equally expressed in muscle, heart and serum tissue.

locus) are expressed in the other tissues surveyed. The three banded phenotype seen for this dimeric enzyme is best explained by the random combination of subunit products from two loci with different alleles. This results in a fixed heterozygote effect with every individual exhibiting the three banded phenotype.

No variation at either locus was found in any of the populations studied. Since grayling possess no polymorphism at the loci coding for both the A type and B type subunits, it was not possible to determine if duplication has taken place at these two loci.

A fourth band of activity was also found on zymograms stained for MDH activity. This isozyme had a shorter migration distance and weaker staining intensity than the other forms. This isozyme is believed to be the mitochondrial form of MDH (designated MDH_m) based on correlation with zymograms of other salmonids (Syln'ko 1976, Aspinwall 1973, Bailey *et al.* 1970, Bailey *et al.* 1969), and is controlled by a separate gene locus (discussed previously). No variation was observed for this isozyme in any of the populations studied.

Glutamate-oxaloacetate transaminase (GOT) - There are two distinct forms of glutamate-oxaloacetate transaminase in vertebrate cells (Moore and Lee 1960), both of which have a dimeric structure (DeLorenzo and Ruddle 1970). One of the forms of GOT is found in the mitochondrial fraction while the other one occurs in the supernatant (or

cytoplasmic) fraction of the cell. The supernatant form migrates anodally at a neutral pH, while the mitochondrial form migrates cathodally (Schmidtke and Engel 1972). The supernatant form of GOT has been shown to be coded for by two loci in several fish species (Schmidtke and Engel 1972). In salmonids, GOT has been reported to be coded by two disomic loci in brown trout (Schmidtke and Engel 1972), chum salmon (Allendorf *et al.* 1975, May *et al.* 1975) and cutthroat trout (Allendorf and Utter 1976).

In the present study, grayling were surveyed for GOT activity in liver, skeletal muscle, heart muscle, and eye tissue. The results are shown in Figure 5. The buffer system employed in the separation of GOT isozymes had a high pH (8.6), which results in the anodal migration of both forms (mitochondrial and supernatant).

The results indicate that there are at least two loci (GOT_s-1 and GOT_s-2) coding for the supernatant form of GOT and two loci (GOT_m-1 and GOT_m-2) coding for the mitochondrial form.

There are two bands of activity for the mitochondrial form with no heterodimer formed between the two. This result is best explained by the presence of 2 loci (GOT_m-1 and GOT_m-2) coding for distinct polypeptide subunits which do not interact with one another to form a heterodimer consisting of a subunit from each locus. No variation was observed at either locus in any of the populations surveyed.

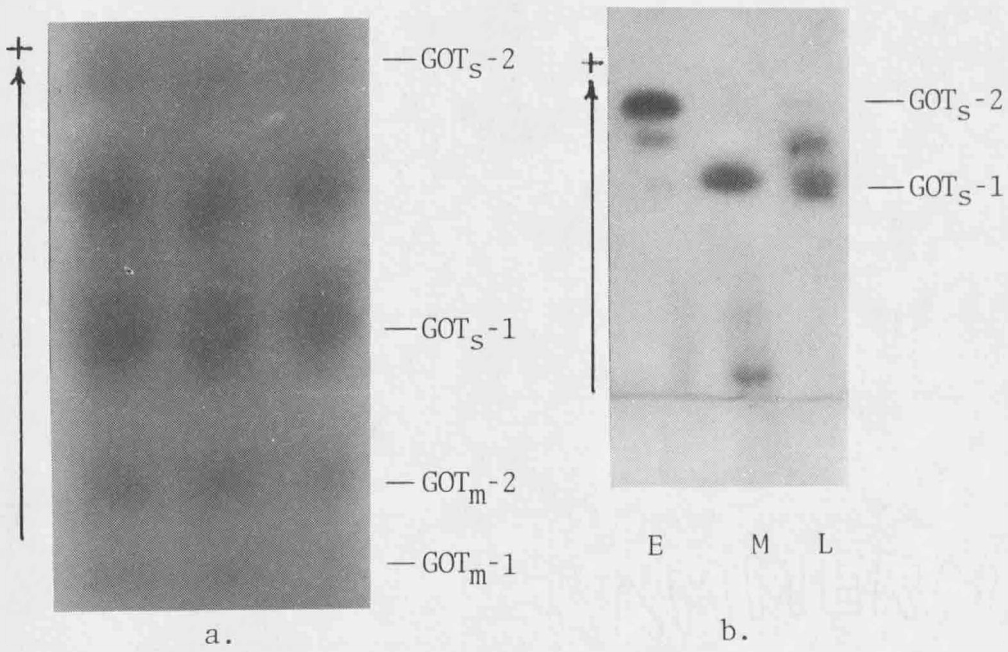


Figure 5. Glutamate oxaloacetate transaminase (GOT) tissue distribution.
a. Liver samples from three fish with expression of two GOT_S and two GOT_m loci.
b. Tissue distribution of GOT isozymes from one fish: eye, muscle, liver. GOT_m isozymes did not stain on this gel.

The supernatant GOT exhibited different patterns in the various tissues examined. In the liver a three banded phenotype with asymmetrical staining intensity was found in all individuals. This pattern may be explained by the presence of two loci with different alleles coding for subunits of different electrophoretic mobility. This fixed heterozygote effect is evidence for the presence of a duplicated locus (Allendorf *et al.* 1975). In eye tissue a similar three banded pattern was the phenotype of all individuals, however, the asymmetry of staining intensity was in the opposite direction (Figure 5b). This pattern again suggests a duplicated locus. The asymmetry of intensity cannot be explained by the postulate that one of the loci is monomorphic for the common allele while the other is polymorphic since: 1) all individuals express the same pattern which would not be expected if the locus was polymorphic (some would be symmetrical), and 2) there is opposite asymmetry in a different tissue of the same individual. The lack of parallel expression between eye and liver suggests that the pattern is the result of differential activation of the two loci in these different tissues.

In muscle, only one band of supernatant GOT activity was expressed. The band had the same electrophoretic mobility as the GOT_S-1 homozygote in liver. There is evidence in some salmonids that this band may represent a distinct locus (Allendorf personal

comm., Allendorf and Utter 1976). However, the lack of variation at this locus does not allow the confirmation of this postulate in grayling. Therefore, the conservative estimate of two supernatant GOT loci and two mitochondrial GOT loci was used in the present study.

Alcohol dehydrogenase - (ADH) has been shown to have a dimeric structure in vertebrates (Butler *et al.* 1969). It is believed to be under the control of a single gene locus in salmonids (Allendorf *et al.* 1975). The enzyme in trout appears to be negatively charged, migrating to the cathode. In most salmonids studied, ADH appears as a single band with little or no variation within the species (Kristiansson and McIntyre 1965, Gall *et al.* 1977, Utter *et al.* 1973, Allendorf *et al.* 1975).

In *T. arcticus* liver samples were examined for ADH activity. In all individuals ADH was expressed as an identical single band of activity, migrating cathodally. On the basis of these results it was postulated that ADH is encoded by a single locus in grayling.

The ADH locus was monomorphic and identical in all populations surveyed with no variation between populations.

Xanthine dehydrogenase - (XDH) is represented in salmonids by a single band of activity which migrates anodally (Kristiansson *et al.* 1976, Allendorf *et al.* 1975, Allendorf 1973). Since no variation

exists at this locus in the species studied, no conclusions can be made as to whether XDH is encoded by a single locus or duplicated locus (Allendorf *et al.* 1975).

In *T. arcticus* a single band of XDH activity was found which migrated anodally. All populations were monomorphic with no variation detected between populations. It is assumed that the enzyme is coded for by a single locus. However, the possibility of two loci with identical electrophoretic gene products cannot be excluded.

Sorbitol dehydrogenase - (SDH) is presumed to have a tetrameric structure in vertebrates (Opt'Hof 1969, Opt'Hof *et al.* 1969, Engel *et al.* 1970). Engel *et al.* (1970) proposed that this is also the structure of SDH found in rainbow trout, and has reported the presence of a polymorphism. Invariant multi-banded phenotypes have been reported in various other salmonid species (May *et al.* 1975, Khanna *et al.* 1975, Allendorf *et al.* 1975, Utter *et al.* 1973). The patterns in these species is indicative of two disomic loci fixed for alleles coding for subunits of different electrophoretic mobility (Allendorf *et al.* 1975, Utter *et al.* 1973). This postulate is in contrast to that of Engel *et al.* (1970) which proposed a tetrasomic mode of inheritance for a single locus based on the phenotypic distribution of isozyme patterns with no breeding experiments performed. Allendorf *et al.* (1975) on the basis of preliminary inheritance

data of a variant in cutthroat trout, provide further evidence in support of the former postulate.

In the present study, grayling exhibited a distinct single band phenotype of SDH activity, which migrated anodally. No intropopulational or interpopulational variation was observed. This result allows no conclusions to be drawn as to the presence of a duplicated locus in *T. arcticus* as proposed for rainbow trout (Allendorf *et al.* 1975). For the purposes of this study the conservative estimate of one locus coding for SDH was used.

Isocitrate dehydrogenase (mitochondrial form) - (IDH) exists in distinct mitochondrial (here designated IDH_m) and supernatant (IDH_s) forms which are determined by distinct gene loci in vertebrates (Henderson 1968). Wolf *et al.* (1970) showed that IDH_m manifests a dimeric structure in electrophoretic studies of rainbow trout (*Salmo gairdneri*). Engel *et al.* (1971) showed that in some diploid groups of fish, a single gene locus presumably coded for IDH_m, whereas in some tetraploid groups two different gene loci were responsible for the fixed heterozygote pattern observed on zymograms. Allendorf *et al.* (1975) reported that in rainbow trout (*Salmo gairdneri*) IDH_m is also represented by three nonvariant bands indicating the presence of two monomorphic disomic loci with common alleles coding for subunits of different electrophoretic mobilities. IDH_m activity is best visualized on zymograms of heart muscle extracts.

In grayling, heart muscle extracts were surveyed for IDH_m activity. IDH_m phenotypes were represented by three invariant bands which indicate the presence of two monomorphic disomic loci, IDH_m-1 and IDH_m-2, with common alleles coding for subunits of different electrophoretic mobilities. The pattern observed is evidence that the IDH_m locus in *T. arcticus* is duplicated as presumed to be the case in rainbow trout (Allendorf *et al.* 1975). All individuals expressed the same phenotype in all populations studied.

Alpha-glycerophosphate dehydrogenase - (AGPDH) is a dimeric molecule for which genetic variation has been described in various species of fish (Aspinwall 1972, Utter and Hodgins 1972, Allendorf *et al.* 1975, Engel *et al.* 1971, Johnson *et al.* 1970, McCabe *et al.* 1970). Engel *et al.* (1971) proposed the existence of three different gene loci (A, B, and C) coding for AGPDH in the brown trout (*Salmo trutta*) and the rainbow trout (*Salmo gairdneri*) with the random combination of subunits from alleles at the various loci accounting for the complex zymogram patterns. Other authors (Aspinwall 1972, Utter and Hodgins 1972) have proposed the existence of a single locus, two codominant allele system in other salmonids on the basis of electrophoretic results. Aspinwall (1972) suggested the presence of a single locus in salmonids may be due to a "silencing" of duplicated loci in salmonids. However, Allendorf *et al.* (1975)

provided evidence that the buffer system employed in the electrophoresis of AGPDH determines the number of isozymes and hence, the number of loci which are detected. A low pH phosphate buffer allows the detection of an added number of loci which are not detected with the use of a high pH tris-borate system.

In the present study, three loci (AGPDH-1, AGPDH-2 and AGPDH-3) with alleles coding for proteins of different electrophoretic mobilities are postulated to exist in *T. arcticus*. The zymograms obtained reflect the presence of these three loci with active dimers formed from the subunits of the different loci, resulting in six isozymes being formed. Liver and muscle tissue extracts exhibited parallel expression of the identical number of isozymes. Examination of this isozyme system with the high pH buffer system resulted in the detection of only the AGPDH-1 locus. Further studies using the low pH buffer system resulted in the detection of the two additional loci. These findings are similar to those of Engel *et al.* (1971) and reflect the differences which are observed depending on the buffer system employed as suggested by Allendorf *et al.* (1975).

No allelic variation at any of the AGPDH loci was detected in any of the populations surveyed.

Esterase - The banding patterns observed in liver samples of *Thymallus arcticus* were inconsistent in number and intensity. Due

to the inconsistency, the liver esterases were not dealt with in any detail.

The esterase of the serum was represented by a single invariant band in all populations. It was assumed the band was representative of a single gene locus.

Hexokinase - (HK) has been reported to be represented by a single band in rainbow trout (Allendorf 1973). Liver samples of *T. arcticus* surveyed for hexokinase actively exhibited an identical single band in all populations examined. It is assumed the band is representative of a single gene locus.

Electrophoretic Phenotypes of Polymorphic Proteins

Tetrazolium oxidase (Indophenol oxidase) - TO is the designation for an enzyme first described by Brewer (1967) which oxidized reduced tetrazolium dyes resulting in an achromatic region against the colored background of reduced dye on electrophoretic zymograms. Intraspecific variants of TO were found to exist in fifteen species of Pacific rockfish (*Sebastodes*) (Johnson *et al.* 1970b). Interspecies variation of TO has been reported to exist in the salmonids (Utter 1971, Utter *et al.* 1973, May *et al.* 1975). The existence of intraspecies TO polymorphisms have also been found in the rainbow trout (Utter 1971, Utter *et al.* 1973, Cederbaum and Yoshida 1972, Utter and Hodgins 1972, Allendorf 1973) and Chinook salmon (Utter

1971, Kristiansson and McIntrye 1976). The patterns observed appear to reflect one locus with two alleles encoding a dimeric protein (Utter 1971, Cederbaum and Yoshida 1972).

In *T. arcticus* individuals exhibited either one zone or three zones of activity (Figure 6) similar to the patterns found in other salmonids (discussed previously). Parallel tissue expression was found in liver and muscle extracts of the same individual. No interpopulational variation was found.

The common allele ($TO^{1.00}$), and a variant allele ($TO^{.50}$), were found in the Donnelly, Wolf and Grebe populations. Homozygotes (1.00/1.00) for the common allele expressed a single band of activity which migrated the farthest anodally (Fig. 6), heterozygotes (1.00/.50) exhibited a 3 banded pattern with a heterodimeric zone of greater intensity, and homozygotes (.50/.50) for the variant allele exhibited a single band with the slowest migration. It is assumed that the patterns reflect a single locus, two codominant allele system similar to that suggested for rainbow trout (Utter 1971, Cederbaum and Yoshida 1972).

No variation was found in the Fuse Lake population with all individuals being homozygous for the common allele. The lack of variation in this population is presumably attributable to a founder effect.

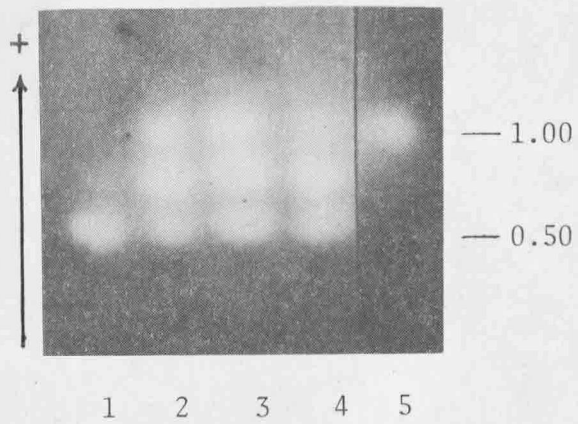


Figure 6. Tetrazolium Oxidase (TO) polymorphism. Column 1 (0.50/0.50) homozygote. Column 2, 3, 4, (1.00/0.50) heterozygotes. Column 5 (1.00/1.00) homozygote.

Phosphoglucomutase - (PGM) isozyme variation was first studied in human populations by Spencer *et al.* (1964). Further genetic studies in humans showed the PGM activity could be divided into three zones, with each zone apparently controlled by an independent locus (Parrington *et al.* 1968). Roberts *et al.* (1969) reported finding three distinct zones of activity on PGM zymograms of *Salmo gairdneri*, and postulated the existence of three loci, PGM-1, PGM-2 and PGM-3 (in order of increasing anodal migration of the coded protein). Polymorphism of PGM have been found in rainbow trout (Roberts *et al.* 1969, Utter *et al.* 1972, 1973) and in various species of salmon (Kristiansson and McIntyre 1976, May *et al.* 1975, Utter *et al.* 1973, Utter and Hodgins 1970).

In the present study, liver and muscle tissue extracts were surveyed for PGM activity. Three zones of activity were detected on PGM zymograms of liver samples, all of which migrate anodally (Figure 7). Three loci (PGM-1, PGM-2 and PGM-3) were postulated to be encoding PGM subunits in *T. arcticus*. Only PGM-1 and PGM-2 were expressed in muscle tissue. The faint staining of the PGM-3 in liver tissue and its absence in muscle tissue is consistent with the findings of other authors (Roberts *et al.* 1969, Hopkinson and Harris 1968).

PGM-1 and PGM-3 were monomorphic in all populations studied, represented by single invariant bands. PGM-2 was polymorphic in the

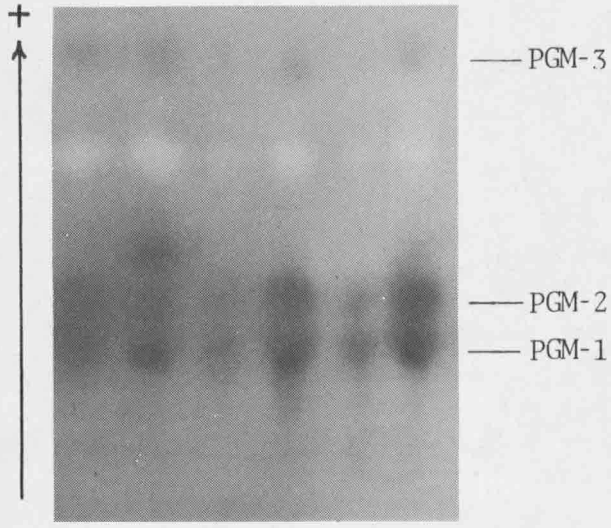


Figure 7. Phosphoglucomutase (PGM). The products of three PGM loci appear to be present in liver samples. PGM-1, PGM-2 and PGM-3. PGM_2 is polymorphic, PGM-1 and PGM_3 are monomorphic.

Donnelly River population, but was monomorphic in the other populations surveyed. Two alleles, PGM-$2^{1.00}$ and PGM-$2^{1.25}$, were present in the Donnelly population. Homozygous individuals for either allele were represented by a single band while heterozygous individuals exhibited two bands of activity (Figure 8) which is typical of a monomeric protein. The patterns found are similar to those reported in other salmonids (noted above). The existence of the polymorphism at the PGM-2 locus clarifies the interpretation of zymogram patterns and strengthens the validity of the three loci postulate.

Isocitrate dehydrogenase (supernatant form) - The supernatant form of isocitrate dehydrogenase (IDH_2) behaves electrophoretically as a dimeric molecule (Henderson 1968, Darnall and Klotz 1972). Allelic variation of IDH_2 isozymes has been described in a variety of salmonid species (Allendorf *et al.* 1975, May *et al.* 1975, Allendorf 1973, Ropers *et al.* 1973, Engel *et al.* 1975, Wolf *et al.* 1970) which confirm the dimeric structure in salmonid fish. The mode of inheritance of IDH_s was initially proposed to be tetrasomic in rainbow trout (Wolf *et al.* 1970) on the basis of phenotypic expression alone. Allendorf (1973) and Allendorf and Utter (1973) described a system of IDH_s isozymes identical to that found by Wolf *et al.* (1970). Based on the number of bands and relative dosage, a two locus, four

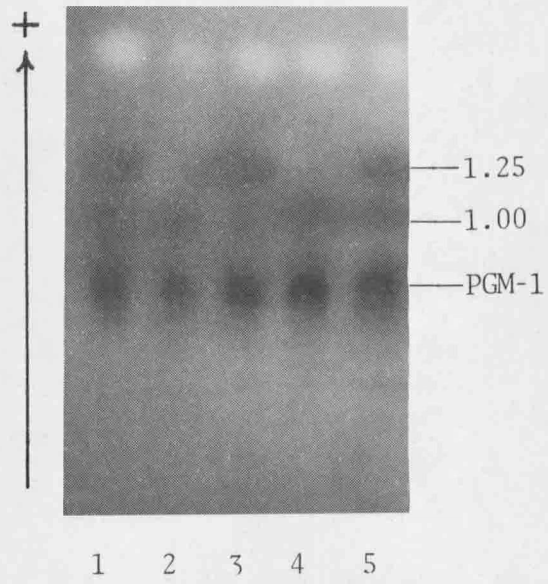


Figure 8. Phosphoglucomutase (PGM). PGM-1, cathodal, is monomorphic. PGM_2 is polymorphic. Column 2 and 3 (1.00/1.00) homozygotes. Column 1 and 4 (1.00/1.25) heterozygotes. Column 3 (1.25/1.25) homozygote.

allele system was proposed to be encoding IDH_s in these individuals. Breeding experiments verified that in the stock of rainbow trout examined by these authors, IDH_s followed a disomic mode of inheritance with the locus having been duplicated. Subsequent to these findings, breeding experiments were performed with the stock examined by Wolf *et al.* (1970) which conclusively verified that, indeed, IDH_s followed a disomic mode of inheritance (Engel *et al.* 1975).

Liver tissue extracts of *T. arcticus* were examined for IDH_s activity and revealed the system depicted in Figure 9. The IDH_s patterns were polymorphic in three of the populations surveyed. The expression of only three phenotypes in any one population is indicative of a single disomic locus with a common and a variant allele. This hypothesis of a single disomic locus for *T. arcticus* is in contrast to the two disomic loci postulated for rainbow trout (Allendorf and Utter 1973, Engel *et al.* 1975). The IDH_s locus does not appear to have undergone gene duplication as in rainbow trout (Allendorf *et al.* 1975). A single disomic locus encoding IDH_s has been reported in chum salmon (*Oncorhynchus keta*) and confirmed through breeding experiments (May *et al.* 1975).

Allelic variation was observed at the IDH_s locus in the Donnelly River, Wolf Lake, and Grebe Lake populations. The common allele designated $IDH_s^{1.00}$ encoded subunits which were electrophoretically

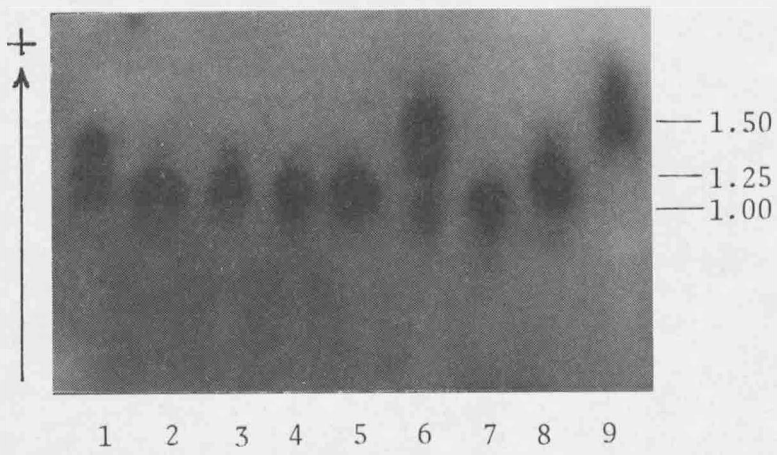


Figure 9. Isocitrate Dehydrogenase (IDH) is polymorphic. Three populations have an IDH variant and one allele common to all populations. Column 1 (1.25/1.00) heterozygote. Column 2 3, 4, 5, 7 (1.00/1.00) homozygotes. Column 6 (1.50/1.00) heterozygote. Column 8 (1.25/1.25) homozygote. Column 9 (1.50/1.50) homozygote. Samples 1, 2, 3, 8 are from the Donnelly river population. Samples 4, 5, 6, 7, 9 from the Grebe lake population.

identical in all populations. A variant allele, designated $IDH_s^{1.25}$, was unique to the Donnelly River population. A homozygous individual for the variant allele (1.25/1.25) expressed a single band of activity with a greater migration distance than a homozygous individual for the common allele (1.00/1.00). A heterozygous individual (1.00/1.25) exhibited a three banded pattern with symmetrical staining intensity, characteristic of a dimeric molecule.

Another variant allele, $IDH_s^{1.50}$, was unique to the two Yellowstone Park populations. The isozyme of an individual homozygous for this allele had a characteristic migration distance greater than either the 1.00/1.00 individuals or the 1.25/1.25 individuals. A heterozygous individual for this variant (1.00/1.50) again expressed a three banded phenotype. As noted in Figure 9 a 1.00/1.50 individual has an asymmetrical staining intensity which may reflect a differential activation of the two alleles or differential activity of these allele products.

Only 1.00/1.00 individuals were found in the Fuse Lake population which may again reflect a founder effect.

Transferrin - (Tfn) is a monomeric B-globulin which exhibits a high degree of polymorphism in most vertebrate species (Manwell and Baker 1970). The existence of an extensive amount of genetic variation has been reported in teleosts, with more than thirty species

being polymorphic (reviewed by Kirpichnikov 1973, DeLigny 1969). The majority of these polymorphisms reflect codominant inheritance of alleles associated with a single locus (Kirpichnikov 1973). Inheritance studies verified the interpretation of one polymorphic disomic locus (Valenta *et al.* 1976, Utter *et al.* 1973). The large amount of variability of transferrin is well documented in salmonid species (Hershberger 1970, Wright and Atherton 1970, Moller 1970, Utter *et al.* 1970, Utter and Hodgins 1972, Eckroat 1973, Allendorf 1973, Utter *et al.* 1973). In all cases there is a simple additive pattern in heterozygotes for a transferrin polymorphism. The presence of three or more alleles has been observed in rainbow trout and various salmon species (Utter *et al.* 1970, 1973, Utter and Hodgins 1972, Reichenbach-Klinke 1973) and also in brook trout (Eckroat 1973).

Serum samples of individual grayling were analyzed for transferrin phenotypes. The transferrin bands (Figure 10) were identified as the bands having an electrophoretic mobility similar in position to those of rainbow trout and salmon (Utter *et al.* 1970, 1973, Reichenbach-Klinke 1973). It is assumed that these bands in *T. arcticus* are also transferrins because of this electrophoretic identity and the taxonomic relationship of these species.

Transferrin was found to be polymorphic in the Donnelly River, Wolf Lake, and Grebe Lake populations. On the basis of observed

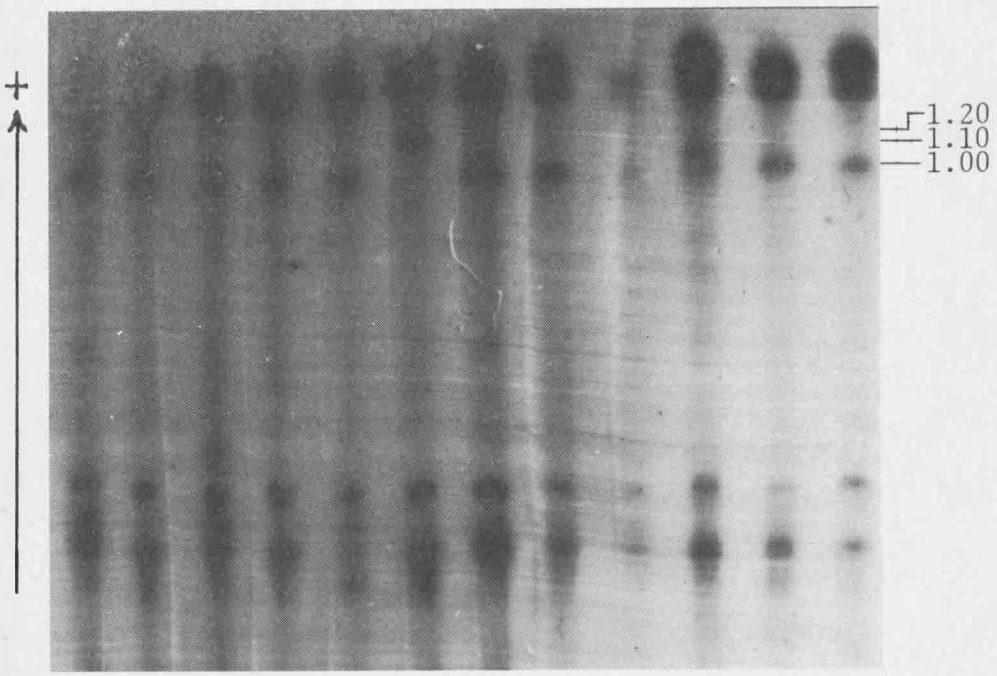


Figure 10. Transferrin polymorphism (Tfn). Two alleles were found in the Donnelly river population and three in the Yellowstone Park populations. Columns 1, 2, 3, 4, 5 7, 8, 9, 11, 12 (1.00/1.00) homozygotes. Column 6 (1.20/1.20) homozygote. Column 10 (1.10/1.00) heterozygote. Column 1, 3, 7 Donnelly river. All others from Grebe lake.

phenotypes and the monomeric structure of Tfn, three codominant alleles ($Tfn^{1.00}$, $Tfn^{1.10}$, and $Tfn^{1.20}$ in order of increasing anodal migration) at a single disomic locus were postulated to encode Tfn in the two Yellowstone Park populations. Six phenotypes of either one or two of three different bands are expected to result from the codominant expression of three alleles. The six phenotypes accordingly are designated 1.00/1.00, 1.00/1.10, 1.00/1.20, 1.10/1.10, 1.10/1.20 and 1.20/1.20. Four of these phenotypes are shown in Figure 10. The only phenotype not found in the individuals sampled was 1.10/1.20. The lack of observation of this phenotype is most likely the result of the small sample size resulting in no detection of 1.10/1.20 whose maximum expected value is .013.

Electrophoretically identical alleles to $Tfn^{1.00}$ and $Tfn^{1.10}$ were expressed in the Donnelly River population, but the $Tfn^{1.20}$ allele was absent. The three expected phenotypes (1.00/1.00, 1.00/1.10 and 1.10/1.10) reflecting two codominant alleles were observed.

The common allele $Tfn^{1.00}$ was the only one expressed in the Fuse Lake population, thus only the common phenotype (1.00/1.00) was observed. The lack of variation at the Tfn locus in this population is presumably due to a founder effect.

Glucose and hexose 6-phosphate dehydrogenase - Two major electrophoretically distinct components of glucose 6-phosphate dehydrogenase

activity are found in vertebrate organisms. One is highly specific for the utilization of glucose 6-phosphate with NADP as substrate and coenzyme, respectively (Noltman and Kuby 1963). The second form is distinguished by its ability to catalyze the oxidation of glucose 6-phosphate, galactose 6-phosphate, 2-deoxyglucose 6-phosphate and glucose as substrates, with either NAD or NADP functioning as the coenzyme (Shaw 1966, Ohno *et al.* 1966, Beutler and Morrison 1967, Shaw and Koen 1968). The isozyme with glucose 6-phosphate specific dehydrogenase activity is designated glucose 6-phosphate dehydrogenase (G6PD), while the enzyme with the broader range of substrate specificity is designated hexose 6-phosphate dehydrogenase (H6PD) for convenience of distinction (Shaw 1966, Ohno *et al.* 1966, Shaw and Koen 1968).

Vertebrate G6PD and H6PD isozymes have been shown to be encoded by distinct gene loci. G6PD is sex linked in mammals (Kirkman and Hendrickson 1963, Richardson *et al.* 1971) but is under autosomal gene control in birds and fish (Manwell and Baker 1969, Yamauchi and Goldberg 1973). G6PD is localized in the nuclear and cytoplasmic fractions of the cell (Beutler and Morrison 1967). In contrast, H6PD is autosomally inherited in mammals (Shaw 1966, Ohno *et al.* 1966, Shaw and Koen 1968) and is localized in the microsomal fraction of the cell (Beutler and Morrison 1967, Metzger *et al.* 1965).

G6PD exists as two catalytically active forms, dimer and tetramer (Bonsignore *et al.* 1970), being mostly tetrameric in some organisms (Yamauchi and Goldberg 1973) but dimeric in others (Shaw and Koen 1968). In contrast H6PD exhibits a dimeric structure as evidenced in zymograms of allelic variants (Stegeman and Goldberg 1971).

The tissue distribution of G6PD appears ubiquitous in vertebrate cells (Beutler and Morrison 1967). H6PD is most active in the liver and kidney (Beutler and Morrison 1967) with activity in other tissues only five to ten percent of that in liver extracts (Stegeman and Goldberg 1971).

The occurrence of both G6PD and H6PD in the livers of salmonids has been clearly demonstrated by various authors (Stegeman and Goldberg 1971, Shatton *et al.* 1971, Ohno *et al.* 1966). The exact number of isozymes in the multi-banded zymograms of rainbow trout is, however, not known. Stegeman and Goldberg (1971, 1972) demonstrated that in the genus *Salvelinus* G6PD appears to be tetrameric (exhibiting five bands) with H6PD existing as a dimeric molecule. The existence of two codominant gene loci with different alleles has been postulated to be determining the G6PD bands in trout (Ohno 1966, Yamauchi and Goldberg 1973, Stegeman and Goldberg 1971). However, Cederbaum and Yoshida (1975) recently proposed that G6PD in rainbow trout liver may be encoded by a single gene locus, two allele system,

with posttranslational modification of the enzyme accounting for the complex banding patterns. On the basis of allelic variation studies by Stegeman and Goldberg (1971, 1972), H6PD is believed to be the product of a single autosomal gene locus.

Electrophoretic analysis of G6PD activity in liver tissue extracts of *T. arcticus* revealed that this tissue contained four distinct zones of activity when glucose 6-phosphate was used as substrate in staining (Figures 11 and 13). The band appearing second from the cathodal end (liver samples) was identified as H6PD, exhibiting broad substrate specificity indicated by staining with galactose 6-phosphate and glucose with NAD or NADP as the coenzyme (Figure 11). Therefore, both forms of G6PD are present in *T. arcticus* as in other vertebrates.

Three zones of G6PD activity, each represented by a single band, is the common phenotype. The middle zone of G6PD activity is greater in staining intensity than either of the other two zones (Figure 11, erythrocytes, and Figure 13). This phenotypic pattern is suggestive of two loci with alleles encoding subunits of different electrophoretic mobility for a dimeric molecule. The two loci are designated G6PD-2 and G6PD-3 in order of increasing anodal migration. A verification of this model of inheritance was obtained by finding individuals in the Grebe and Wolf Lake populations possessing electrophoretic patterns as shown in lane two and four of Figure 12. These

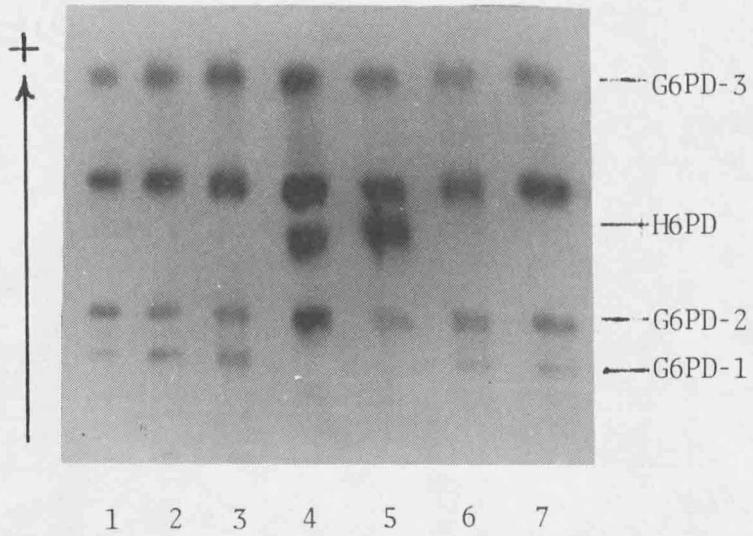


Figure 11. Glucose-6-Phosphate Dehydrogenase (G6PD) and Hexose-6-Phosphate Dehydrogenase (H6PD) expression in erythrocytes and eye tissue. Liver tissue samples have the same banding pattern as erythrocytes. Column 1, 2, 3, 6 and 7 are eye samples from individual fish. Column 4 and 5 erythrocytes from individual fish.

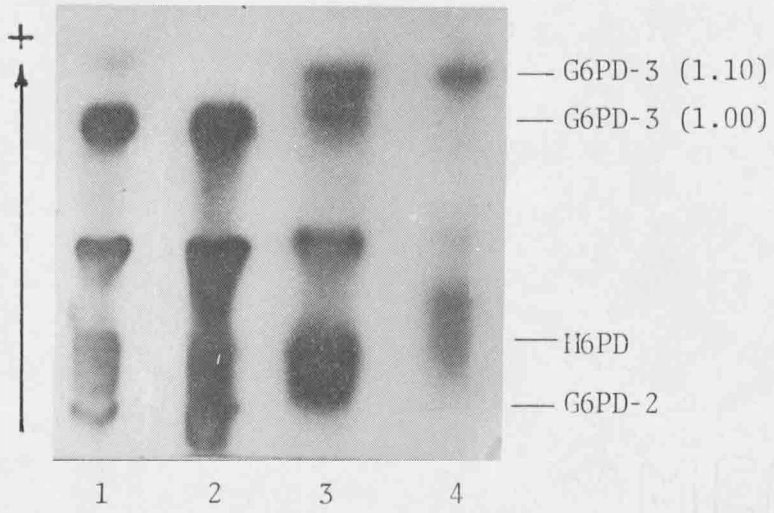


Figure 12. Glucose-6-Phosphate Dehydrogenase-3 (G6PD-3) is polymorphic in the Yellowstone Park populations. Column 1 mixture of homogenates applied to column 2 and 4. Column 2 G6PD-3 (1.00/1.00) homozygote. Column 3 G6PD-3 (1.00/1.10) heterozygote. Column 4 G6PD-3 (1.10/1.10) homozygote. Liver samples.

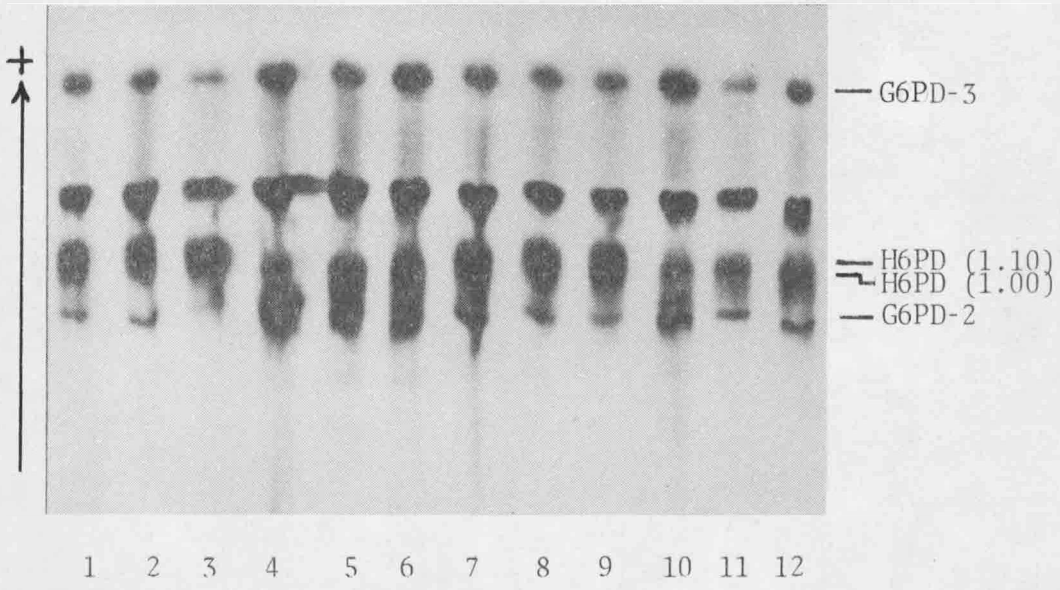


Figure 13. Hexose-6-Phosphate Dehydrogenase (H6PD) polymorphism. Donnelly River samples have a slightly faster anodal migrating form than the Grebe Lake (YNP) samples. Columns 1, 2, 3, 7, 8 and 9 (1.10/1.10) homozygotes from Donnelly River. Columns 4, 5, 6, 10, 11 and 12 (1.00/1.00) homozygotes from Grebe Lake. Liver homogenates.

patterns indicate the presence of a variant allele at the G6PD-3 locus. A homozygous individual for the variant allele (designated G6PD-$3^{1.10}$) is represented by a single band with a greater electrophoretic mobility than an individual homozygous for the common allele (G6PD-$3^{1.00}$). Heterozygotes (1.00/1.10) exhibit a three banded phenotype characteristic of the dimeric structure with a band intermediate between the two homozygote bands. The various isozyme patterns are depicted in Figure 12.

The presence of this polymorphism provides additional evidence to support the two locus postulate. Since a heterodimer is formed of subunits from the two loci, a band of greater electrophoretic mobility in the heterodimeric region would be expected in a homozygous individual for the variant allele at the G6PD-3 locus. Furthermore, an individual heterozygous for the variant G6PD-3 allele would have two heterodimeric bands, formed between subunits of the G6PD-2 and the two G6PD-3 alleles. Figure 12 clearly shows this to be the case. These results are found whether NADP is used in the grinding solution or not, although the presence of NADP does enhance resolution. The data is in agreement with the two codominant loci postulated by Ohno *et al.* (1966) for rainbow trout and Stegeman and Goldberg (1971) for brook trout. The resolution obtained in these zymograms of *T. arcticus* is much more convincing than those

used by Cederbaum and Yoshida (1975) in postulating the presence of a single locus.

Analysis of eye tissue and red blood cells resulted in the parallel expression of the two G6PD, loci with the heterodimeric region again evident (Figure 11). The lack of H6PD activity in the eye tissue clarifies interpretation of liver zymograms since one can assume that none of the activity in this region is due to G6PD.

An additional band close to the origin was expressed in eye tissue and red blood cells, zymograms (Figure 11). No distinct heterodimeric region was formed between this band and the subunits of G6PD-2 or G6PD-3. The band may represent a dissociation product of the G6PD-2 but its consistent presence in all samples in equal intensity and absence in liver suggests an additional locus, designated G6PD-1, which is active in these tissues but not in the liver.

In summary, the G6PD-1 and G6PD-2 loci were monomorphic in all populations. The G6PD-3 locus was monomorphic in the Donnelly and Fuse populations, but was polymorphic in the Grebe and Wolf Lake populations.

A region of H6PD activity was expressed in all populations surveyed. As noted in Figure 13, H6PD activity is represented by an area of diffuse staining. It is postulated that a single locus is represented by this H6PD activity, although it appears two bands

are present at times. As shown in Figure 13, the H6PD band in Donnelly River and Fuse Lake individuals has a characteristic migration distance greater than the individuals from the Yellowstone populations. A mixture of an individual sample from each population results in an area staining which is clearly additive of the areas stained in individual samples. The results provide evidence that the H6PD locus is fixed for alleles which code for subunits of different electrophoretic mobility. The allele in the Grebe and Wolf Lake populations is designated $H6PD^{1.00}$ and that in the Donnelly River and Fuse Lake populations is $H6PD^{1.10}$. No intrapopulational variation was found to exist.

Malic enzyme (NADP - MDH) - Malic enzyme otherwise known as NADP dependent malate dehydrogenase exists in mammalian tissue in two forms, supernatant and mitochondrial (Henderson 1966, Shows *et al.* 1970). Evidence from electrophoretic studies of the mouse suggest a tetrameric structure for both the supernatant (ME_s) and mitochondrial (ME_m) form of this enzyme (Shows and Ruddle 1968, Baker and Mintz 1969, Povey *et al.* 1975). The mitochondrial form migrates less anodally than the soluble form in some species (Cohen and Omen 1972) but may move a greater distance in others (Henderson 1966).

Two autosomal gene loci (ME_s and ME_m) determine the soluble and mitochondrial forms, respectively (Cohen and Omen 1972, Povey *et al.* 1975).

The ME systems have not been as well studied in teleosts. The lack of variation in species studied has not allowed determination of the molecular structure in teleost fish (Utter *et al.* 1973, Allendorf 1973, Kristiansson and McIntyre 1973). Frydenberg and Simonsen (1973), on the basis of electrophoretic patterns, suggested that a simpler molecule existed in the teleost, *Zoarces*, than the tetramer found in the house mouse (Shows and Ruddle 1968, Povey *et al.* 1975). They considered the enzyme in *Zoarces* to be controlled by a single locus. Allendorf *et al.* (1975) have reported a different band of activity present in muscle than in liver in rainbow trout, but the exact number of loci was not determined.

Liver and muscle tissue extracts were surveyed for ME activity in *T. arcticus*. An identical phenotypic pattern (Figure 14) was expressed in both tissues. A group of five bands which migrates further than any MDH isozymes was identified as the supernatant form of ME (ME_s). A slower diffuse staining band with a migration distance intermediate between MDH_s and MDH_m isozymes was identified as the mitochondrial form of ME (ME_m).

The presence of five bands in every individual indicates the presence of two loci with alleles encoding subunits of different electrophoretic mobility for a tetrameric molecule. The two loci were designated ME_s-1 and ME_s-2. The intensity of the individual

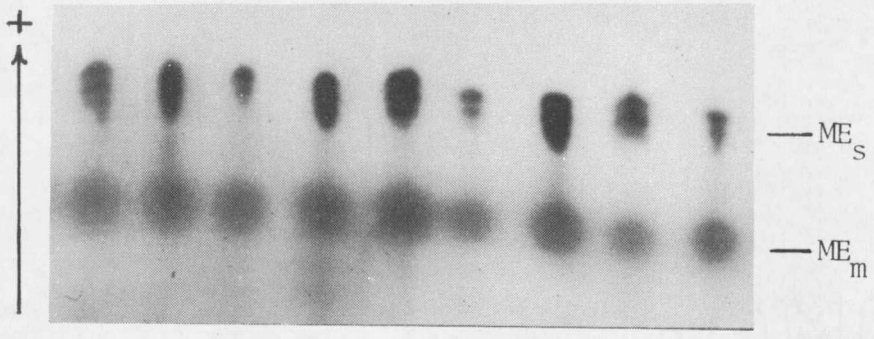


Figure 14. Malic enzyme (M.E.). The supernatant form of M.E. appears to have a multimeric structure perhaps produced by loci which are differentially active. Liver homogenates. The mitochordrial form appears to be monomorphic.

bands varied with the individual sample suggesting that one of the loci may be polymorphic while the other is monomorphic. A rare variant was also identified in one individual in the Grebe Lake population. Since variation is suspected but the number of alleles is not known, these loci were excluded from the quantitative calculations. However, the presence of two loci coding for a tetrameric molecule is suggested. The structure is similar to that reported in other vertebrates (discussed previously). The ME_s locus appears to be duplicated in *T. arcticus*.

The ME_m band was electrophoretically identical in all populations. Since no variation is present, nothing can be deduced about the structure of this molecule. One structural gene probably codes for this enzyme.

Serum proteins - Teleost species have been shown to possess intraspecies specificity of plasma protein patterns (Woods and Engle 1957, Tsuyki and Roberts 1966). Although fish serum proteins are as yet imperfectly studied from a genetic point of view (Kirpichnikov 1973), they have been used successfully in work on intraspecific systematics (Lukjanenko and Popov 1969, Wright and Hasler 1967, Reinitz 1973, Booke 1964, DeLigny 1969). In analyzing serum proteins for genetic variation, it must be noted that serum protein patterns resolved electrophoretically vary with physiological and environmental

conditions (Booke 1964, Thurston 1967). A principal variation noted is related to sex and maturity in females (reviewed by DeLigny 1969, Feeney and Brown 1974). The non-genetic variation of serum proteins appears to involve quantitative changes rather than the presence or absence of bands (Booke 1964, Thurston 1967, Feeney and Brown 1974, DeLigny 1969). As DeLigny (1969) suggests, in any study of variation in non-identified components of serum proteins, sufficient population data and analysis of the composition of the samples with regard to sex, maturity, and development stage appears an absolute requirement. Furthermore, a good resolution of the pattern which allows a sharp distinction between individual fractions is needed in order to avoid wrong interpretation of the observed variation.

The plasma proteins of fish as yet do not appear to have accurate classification. No conclusions as to similarity to human plasma fractions can be made (Feeney and Brown 1974). Recent attempts at classification of the various fractions of plasma proteins of various salmonid species have been done (Perrier *et al.* 1973, Reichenbach-Klinke 1973). However, it was beyond the scope of the present study to attempt to identify and biochemically characterize the various fractions of serum proteins observed on starch gels of *Thymallus arcticus*.

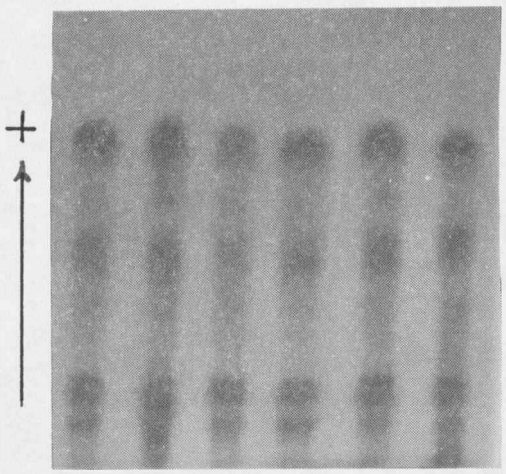
Serum samples of the four populations of *Thymallus arcticus* under investigation were subjected to electrophoresis and stained with a general protein stain. Electropherograms of the serum proteins are shown in Figures 14 and 15. Samples were normally frozen before electrophoresis but subsequent studies revealed that freezing and thawing had no effect on the electrophoretic patterns of the serum proteins. Electropherograms of serum samples revealed 6 zones of staining as shown diagrammatically in Figure 16. Zone 2 was identified as transferrin which has been dealt with previously. The only other zone adequately resolved was Zone 5. The resolution of this zone was excellent and the number of bands could be determined precisely. Individuals exhibited from three to five bands in this region. A total of twelve different electrophoretic phenotypes were observed as depicted in Figure 17. Further analysis revealed that the zone could be further divided into two groups designated A (most anodal), B (least anodal). Group A was found to be represented by two intense staining bands in all individuals in both the Grebe Lake and Wolf Lake populations whose expression appeared independent of the B group (Figure 17). It was postulated that these bands represented two gene loci, SP-2 and SP-3, in order of increasing anodal migration, encoding proteins of different electrophoretic mobility.

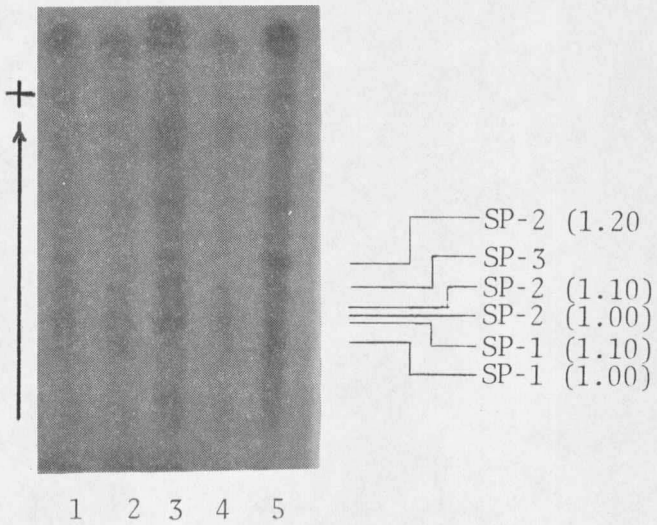
Individuals from the Donnelly River population, however, exhibited either two or three bands in this region. One band of

Figure 15. Electropherograms of serum protens.

a. Phenotypes of the Yellowstone Park populations. All individuals are homozygous at the SP-2 (1.00/1.00) locus and the SP-3 (1.00/1.00) locus. The individuals are polymorphic at the SP-1 locus: Column 1 - homozygote (1.10/1.10); column 2 and 4 - homozygote (1.00/1.00); column 3, 5 and 6 - heterozygotes (1.00/1.10).

b. Phenotypes of Donnelly River individuals. All individuals are homozygous (1.00/1.00) at the SP-3 locus. Individuals are polymorphic at the SP-1 and SP-2 loci: Columns 1 and 3 - SP-1 (1.10/1.10), SP-2 (1.10/1.20); column 2 - SP-1 (1.10/1.00), SP-2 (1.10/1.10); column 5 - SP-1 (1.10/1.00), SP-2 (1.10/1.20); column 4 - Yellowstone Park individual - SP-1 (1.00/1.00), SP-2 (1.00/1.00), SP-3 (1.00/1.00).


+
SP-3
SP-2
SP-1 (1.10)
SP-1 (1.00)
1 2 3 4 5 6


+
SP-2 (1.20
SP-3
SP-2 (1.10)
SP-2 (1.00)
SP-1 (1.10)
SP-1 (1.00)
1 2 3 4 5

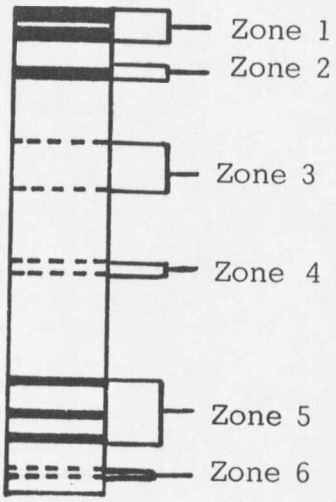


Figure 16. Diagrammatic representation of electrophoretic pattern of serum proteins of *Thymallus arcticus*. Six zones, numbered in order of decreasing anodal migration, are normally observed. Relative intensities are depicted in the diagram.

Figure 17. The twelve observed phenotypic patterns of electropherograms of grayling serum proteins in Zone 5. Columns 1-9, Donnelly River; Columns10-12, Yellowstone Park populations. The genotypes of the individuals are: 1- SP-1(1.00/1.00),SP-2 (1.10/1.10) ; 2- SP-1(1.00/1.10),SP-2(1.10/1.10) ; 3-SP-1 (1.10/1.10), SP-2(1.10/1.10) ; 4- SP-1(1.00/1.00), SP-2 (1.20/1.20) ; 5- SP-1(1.00/1.10), SP-2 (1.20/1.20) ; 6-SP-1 (1.10/1.10), SP-2(1.20/1.20) ; 7- SP-1(1.00/1.00), SP-2 (1.10/1.20) ; 8- SP-1(1.00/1.10), SP-2(1.10/1.20) ; 9- SP-1 (1.10/1.10), SP-2(1.10/1.20) ; 10- SP-1(1.00/1.00), SP-2 (1.00/1.00) ; 11- SP-1(1.00/1.10), SP-2(1.00/1.00) ; 12- SP-1(1.10/1.10), SP-2(1.00/1.00). All individuals are homozygous for the common allele (1.00/1.00) at the SP-3 locus.

strong intensity was consistent in all samples, and was electrophoretically identical to the SP-3 band of the Yellowstone populations (Figure 17), and was designated as such. Two other bands were also observed in this region, one which had a slightly faster migration distance than the SP-3 band and one which had a slightly slower migration distance, intermediate between the SP-3 and the SP-2 band of the Yellowstone individuals. These bands are considered to be the equivalent of the bands produced by the SP-2 locus in the Yellowstone populations, since no band equivalent in migration distance to the SP-2 band of the Yellowstone populations is found in the Donnelly population. Individuals exhibited either an intense staining fast band, an intense staining slow band, or had both bands present in weaker intensity. These results lead to the interpretation of a one locus, two allele system encoding a monomeric protein, with heterozygous individuals expressing two weaker staining bands, the mobilities of which are identical to the bands expressed in both types of homozygous individuals. The allele common to the Yellowstone populations was designated $SP\text{-}2^{1.00}$, those in the Donnelly River population $SP\text{-}2^{1.10}$ and $SP\text{-}2^{1.20}$ in order of increasing anodal migration. On the basis of this hypothesis, allele frequencies were determined for the two alleles in the Donnelly River population.

In the Group B region, individuals in all populations, except the Fuse Lake population, exhibited either one or two bands of

activity (Figure 17). A single locus, two allele system encoding a monomeric protein was postulated to be responsible for this pattern of variation, with homozygous individuals exhibiting a single band of strong intensity and heterozygous individuals having two bands of weaker intensity. This locus was designated SP-1 with the two alleles termed SP-$1^{1.00}$ and SP-$1^{1.10}$ in order of increasing anodal migration.

Evidence in support of this genetic interpretation lies in the expression of the twelve expected phenotypes. Nine phenotypes would be expected to result in the Donnelly River population, and three in the Wolf and Grebe Lake populations, as is indeed the case.

Fuse Lake individuals expressed an identical SP-3 band as that found in both the Yellowstone Park and Donnelly River populations. The Fuse Lake population was not polymorphic at the SP-2 locus, but appeared fixed for the SP-$2^{1.10}$ allele which was present in the Donnelly populations, exhibiting a band electrophoretically identical to that observed in the Donnely population. At the SP-1 locus the Fuse Lake population was fixed for the 1.10 allele with no apparent variation.

As previously noted, any study of variation of serum protein patterns must have sufficient population data to determine if the variation has a basis other than genetic. In the present case,

records were kept as to the sex, age and reproductive condition of the individuals sampled. No correlation existed between these factors and the pattern exhibited by the individual.

Since the variation observed for these serum proteins has not been adequately studied in related species, the quantitative analysis of these systems will be dealt with separately.

Quantitative Analysis of Genetic Variability

Allele frequencies of thirty-five enzyme and protein loci are presented in Table 3, for the four populations of *Thymallus arcticus* surveyed. The table shows the number of alleles detected by electrophoresis at each locus. The heterozygosity at each locus is also calculated. Homozygosity at a locus (j) is defined as E x^2 where x is the frequency of the i-th allele. Heterozygosity for the locus (h) is defined as 1-E x^2 (Nei 1975). The allele frequencies used are those obtained directly from the observed data.

The enzymes and proteins surveyed in this study were divided into three groups. Group I includes those enzymes involved in glucose metabolism. Group II includes non-glucose metabolizing enzymes and Group III includes non-enzymatic proteins.

The genetic basis of isozyme polymorphisms scored on the gels were not verified directly by progeny studies. The genetic interpretation of the observed variation in simple Medelian terms is

Table 3. Allele frequencies and degree of heterozygosity in 35 loci examined in four populations of *Thymallus arcticus*. (h = heterozygosity)

Locus	Alleles	Grebe	Wolf	Donnelly	Fuse
	Group I. Glucose Metabolizing Enzymes				
AGPD-1	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
AGPD-2	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
AGPD-3	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
Hexokinase-1	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
H6PD-1	1.00	1.00	1.00	----	----
	1.25	----	----	1.00	1.00
(h)		0.0	0.0	0.0	0.0
IDH_s-1	1.00	0.98	0.98	0.97	1.00
	1.10	----	----	0.03	----
	1.20	0.02	0.02	----	----
(h)		0.04	0.04	0.06	0.0
IDH_m-1	1.00	1.00	1.00	1.00	1.00
$(h)^m$		0.0	0.0	0.0	0.0
IDH_m-2	1.00	1.00	1.00	1.00	1.00
$(h)^m$		0.0	0.0	0.0	0.0
G6PD-1	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
G6PD-2	1.00	0.92	0.98	1.00	1.00
	1.10	0.08	0.02	----	----
(h)		0.14	0.04	0.0	0.0

Table 3. (Continued)

Locus	Alleles	Grebe	Wolf	Donnelly	Fuse
LDH-1	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
LDH-2	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
LDH-3	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
LDH-4	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
LDH-5	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
MDH_s-1	1.00	1.00	1.00	1.00	1.00
$(h)^s$		0.0	0.0	0.0	0.0
MDH_s-2	1.00	1.00	1.00	1.00	1.00
$(h)^s$		0.0	0.0	0.0	0.0
MDH_m-1	1.00	1.00	1.00	1.00	1.00
$(h)^m$		0.0	0.0	0.0	0.0
ME_m-1	1.00	1.00	1.00	1.00	1.00
$(h)^m$		0.0	0.0	0.0	0.0
PGM-1	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
PGM-2	1.00	1.00	1.00	0.89	1.00
	1.10	----	----	1.10	----
(h)		0.0	0.0	0.20	0.0
PGM-3	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0

Table 3. (Continued)

Locus	Alleles	Grebe	Wolf	Donnelly	Fuse
	Group II. Non-glucose Metabolizing Enzymes				
ADH-1	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
XDH-1	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
SDH-1	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
GOT_s-1	1.00	1.00	1.00	1.00	1.00
$(h)^s$		0.0	0.0	0.0	0.0
GOT_s-2	1.00	1.00	1.00	1.00	1.00
$(h)^s$		0.0	0.0	0.0	0.0
GOT_m-1	1.00	1.00	1.00	1.00	1.00
$(h)^m$		0.0	0.0	0.0	0.0
GOT_m-2	1.00	1.00	1.00	1.00	1.00
$(h)^m$		0.0	0.0	0.0	0.0
EST-1	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0
TO-1	1.00	0.65	0.56	0.65	1.00
	0.50	0.35	0.44	0.35	----
(h)		0.46	0.49	0.46	0.0

Table 3. (Continued)

Locus	Alleles	Grebe	Wolf	Donnelly	Fuse
	Group III. Nonenzymatic Proteins				
TFN-1	1.00	0.87	0.75	0.81	1.00
	1.10	0.11	0.17	0.19	----
	1.20	0.02	0.08	----	----
(h)		0.22	0.41	0.30	0.0
SP-1	1.00	0.60	0.51	0.65	----
	1.10	0.40	0.49	0.35	1.00
(h)		0.48	0.50	0.45	0.0
SP-2	1.00	1.00	1.00	----	----
	1.10	----	----	0.30	1.00
	1.20	----	----	0.70	----
(h)		0.0	0.0	0.42	0.0
SP-3	1.00	1.00	1.00	1.00	1.00
(h)		0.0	0.0	0.0	0.0

supported by agreement between the observed and expected proportions of genotypes in the populations, assuming Hardy-Weinberg equilibrium. Table 4 shows the correspondence of observed genotype frequencies to those expected on the basis of Hardy-Weinberg equilibrium. The class sizes in several systems were small and required grouping for adequate analysis (Sokal and Rohlf 1969). The differences observed from Hardy-Weinberg equilibrium are not significant. The tetrazolium oxidase polymorphism in the Donnelly River population exhibits a slight but not statistically significant deficiency of heterozygotes. These results and moreover the similarity of the protein patterns with those exhibited in other fish species whose genetics have been thoroughly studied, appear to make the proposed genetic interpretations sound.

The serum proteins of Zone 5 on zymograms of *T. arcticus* are an exception to the above criteria as previously noted. The serum proteins of fish are as yet imperfectly studied from a genetic standpoint (Kirpichnikov 1975). However, in the present study the resolution of a number of these proteins exhibited such clarity that postulates as to their genetic basis are reasonable. Since the electrophoretic patterns allowed such interpretations, they were included in a second set of quantitative data. The allele frequencies at the proposed loci are, therefore, included in Table 3.

Table 4. Correspondence of observed genotype frequencies to those expected on the basis of Hardy-Weinberg equilibrium for the polymorphic loci of *Thymallus arcticus*.[a]

Locus	Population	Chi-Square[b]	(DF)	Probability of Greater Chi-Square
TFN-1	Grebe	.0013	1	P = .97
	Wolf[c]	----	-	----
	Donnelly	.2496	1	P = .62
TO-1	Grebe	.1124	2	P = .95
	Wolf	.1115	2	P = .95
	Donnelly	3.4550	2	P = .18
SP-1	Grebe	.1817	1	P = .67
	Wolf	.0568	2	P = .97
	Donnelly	.5958	2	P = .75
SP-2	Donnelly	.0854	1	P = .77
PGM-2	Donnelly	.0030	1	P = .96
G-6-PD-2	Grebe	.0003	1	P = .99
	Wolf[c]	----	-	----

[a] IDS-1 not included since class sizes were too small for adequate analysis.

[b] Continuity correction factor used since number of classes less than 3.

[c] Locus not included for this population since class sizes too small for adequate analysis.

The genetic variation of a population is usually measured by the proportion of polymorphic loci (P) and the average heterozygosity per locus (H). These two parameters were used to measure the intrapopulational genetic variation in the present study. A locus was here defined as polymorphic in a population if the frequency of the commonest allele is equal to or less than 0.99. Average heterozygosity is the mean of the heterozygosity over all loci examined. Average heterozygosity (H) was estimated using the method of Nei (1975):

$$H = \sum_{L=1}^{r} h_L / r$$

where gene frequencies for r loci are studied, and subscript L refers to the Lth locus. Sampling variance of H was also calculated for each population. Several other measures of genetic variation are less satisfactory for most purposes (Nei 1975). These parameters for the four populations studied are summarized in Table 5. Percent polymorphic loci and average heterozygosities were calculated separately for Group I and Group II enzymes. In addition these parameters were calculated for Group II and Group III enzymes and proteins treated as an entire set. Finally, these parameters were calculated for the total number of loci surveyed in the study, one including the general serum proteins and one excluding them.

Table 5. Estimates of genetic variability in *Thymallus arcticus*.

Population	Number of Individuals	Number of Loci	% Polymorphic Loci	Average Heterozygosity
Grebe	102			
Group I		22	9.1	.0081 (±.0065)
Group II		9	11.1	.0507 (±.0507)
Group II & III[a]		13	23.1	.0893 (±.0998)
Total[b]		32	12.5	.0268 (±.0161)
Total[a]		35	14.3	.0382 (±.0197)
Wolf	58			
Group I		22	9.1	.0039 (±.0024)
Group II		9	11.1	.0547 (±.0547)
Group II & III[a]		13	23.1	.1069 (±.0566)
Total[b]		32	12.5	.0303 (±.0195)
Total[a]		35	14.3	.0418 (±.0223)
Donnelly	63			
Group I		22	9.1	.0118 (±.0094)
Group II		9	11.1	.0508 (±.0508)
Group II & III[a]		13	30.8	.1259 (±.0555)
Total[b]		32	12.5	.0318 (±.0177)
Total[a]		35	17.1	.0542 (±.0230)
Fuse	20			
Group I		22	0.0	.0000
Group II[a]		13	0.0	.0000
Total[b]		32	0.0	.0000
Total[a]		35	0.0	.0000

[a]Including SP-1, SP-2, SP-3.

[b]Excluding SP-1, SP-2, SP-3.

DISCUSSION

Genetic Variability of *Thymallus arcticus*

Estimates of genetic variation in populations are based on the amount of heterogeneity detected in structural gene products, mainly proteins and enzymes. Genetic variation has been studied in many organisms although the number of loci is not always large. In the present study, thirty-five enzyme and protein loci in *Thymallus arcticus* were surveyed electrophoretically. The number of loci is considerably large in comparison with the number of loci surveyed in comparable studies (reviewed by Powell 1975). This heterozygosity estimate is extrapolated to the entire genome of *T. arcticus*. One must keep in mind the limitations of electrophoretic surveys in studying molecular variation in populations. These limitations are repeatedly discussed in current literature on the subject and need not be discussed here.

The percent polymorphic loci (common allele 0.99 or less) and average heterozygosity for outbreeding organisms appears, usually, to be 25-50 percent and 5-15 percent, respectively (Selander 1976, Nei 1975). These estimates vary considerably with the organism studied. In general, vertebrates appear less variable than invertebrates, which may reflect the smaller population sizes of vertebrates (Nei 1975). In vertebrate species the percent polymorphic loci

ranges from 20-35 percent, and average heterozygosity 3-8 percent. These estimates are taken from the averages of these parameters for various vertebrate groups (Selander 1976, Nei 1975).

Estimates for fish are highly heterogeneous. In Table 6 the variability estimates for *T. arcticus* are compared with those of other fish species. The values obtained for grayling are within the range of those found in other fish species (with or without SP-1, SP-2, and SP-3), but at the lower end of this range. The standard errors for the average heterozygosity estimates are large, which appear to be common in electrophoretic surveys (Nei 1975).

The comparisons of variability between *T. arcticus* and other salmonid species become more reliable when the general serum proteins are excluded since the loci sampled are more homologous. The importance of comparing similar loci is evident in Table 5, since the heterogeneity of loci surveyed can greatly bias the estimates of variability. Therefore, in any interspecies comparisons, the groups of loci studied should be similar to eliminate potential bias. The estimates of proportion of polymorphic loci (12.5 percent) and proportion of the genome heterozygous (2.7-3.1 percent) for *Thymallus arcticus* are in the mid-range of estimates for all salmonid species (Table 6). The variability estimate is higher than that found in salmon species (Utter *et al.* 1973, Altukhov *et al.* 1972), but lower than that found in rainbow trout (Utter *et al.* 1973).

Table 6. Amount of polymorphism and the degree of heterozygosity in some fish species.

Species	Number of Loci	% Polymorphic Loci	Average Heterozygosity	Reference
Thymallus arcticus	32	12.5	2.9	
Zoarces viviparus	32	28-31	8.9	Frydenberg and Simonsen 1973
Astyanax mexicanus				
surface dwelling	17	29-41	11.2	Avise and Selander
cave dwelling	17	0-29	3.6	1972
Sebastes alutus	25	8	3.8	Johnson *et al.* 1973
Sebastes caurinus	25	4	1.8	"
Sebastes elongatus	24	8	3.2	"
Pacific salmon (variety of species)	19-23	8.7-13	0.6-1.8	Utter *et al.* 1973
Salmo gairdneri	19-23	26	3.7	Utter *et al.* 1973

Within the salmonidae, the higher genetic variability found in rainbow trout may be a reflection of the habitat diversity of this species when contrasted with the more specialized habitats of Pacific salmon (Utter *et al.* 1973). In light of this possibility, the lower variability estimates for grayling may reflect the less diverse habitat of this species, as compared to rainbow trout. This hypothesis is supported by the restriction of grayling to northern latitudes, their failure to survive both in modified habitats and in transplants into new waters (Vincent 1962). This hypothesis is suggestive of a selective advantage for the protein polymorphisms observed. However, the results in no way rule out the possibility of the protein polymorphisms being selectively neutral. Other possible reasons for the low amount of genetic variability in *T. arcticus* may be similar to those suggested by Johnson *et al.* (1973) for *Oncorhynchus*, that either the species has evolved in an environment where survival depends on a unique genotype, or that the genome became fixed for an optimal genotype and very little change is needed for survival in a slowly changing environment.

The lack of variability observed in the Fuse Lake population is apparently due to a founder effect, which reduces variability through sampling error (Nei 1975). The Grebe and Wolf Lake populations, with a similar transplant origin, may have experienced a similar

"bottleneck" in their establishment. The variability found in these populations is not significantly different from the variability found in the Donnelly population in the main range of the species, suggesting that this is not the case. These populations have maintained a level of variability common to the species.

In surveys estimating average heterozygosity, it becomes apparent that heterozygosity varies considerably among loci, with enzymes having different levels of genetic variation (Selander 1975, Powell 1975, Selander and Johnson 1973, Ayala 1974). This high degree of interlocus variation is theoretically expected if each locus undergoes gene substitution independently at a low rate (Nei 1975). The relative degrees of variability of any particular enzyme tends to cut across taxonomic lines, some proteins are almost universally variable while others are rarely polymorphic. Gillispie and Kojima (1968) attempted to account for this degree of variation by speculating that the degree of variation in enzymes varies according to function. These authors divided enzymes into two groups, those involved in glucose metabolism (Group I) and those enzymes not involved in glucose metabolism (Group II). A great deal of data from diverse organisms appears to support this idea as reviewed by Powell (1975). Kojima *et al.* (1970) subsequent to the above hypothesis, suggested that the greater variability of Group II may

be due to the fact that these enzymes act on a variety of substrates of varying concentrations, many of which originate external to the organism. The Group I enzymes, however, act on a single substrate whose concentration is relatively constant. The conclusion being that enzymes in Group II should be more genetically variable. Johnson (1973) proposed a similar hypothesis to account for variation in *Drosophila*. The hypothesis of Gillispie and Kojima (1968) is supported by data on gene diversity in some species (Kojima *et al.* 1970, Ayala and Powell 1972, Cohen *et al.* 1973), but not in others (Nair *et al.* 1971, Frydenberg and Simonsen 1973).

In addition, primary structure (Zouros 1975) and the quaternary structure (Ward 1977) of the protein have been proposed as important factors determining the extent of polymorphism. Selander (1976) provides an excellent review concerning these proposed hypotheses and the degree of polymorphism.

The most pragmatic way to examine this problem, as Nei (1975) suggests, is to use a wide variety of organisms. Since the present study presents data from *Thymallus arcticus*, which may serve to help elucidate the question by adding data to that already collected for other organisms, the enzymes studied were divided into two groups as suggested by Kojima *et al.* (1970). The enzymes surveyed were classified as Group I (glucose metatrolizing enzyme) and

Group II (non glucose metabolizing) as shown in Table 3. Non-enzymatic proteins of serum were reported separately and classified as Group III.

The percent polymorphic loci and average heterozygosities for the Group I and Group II enzymes of *T. arcticus* are summarized in Table 5. The percent polymorphic loci for the Group I (9.1) and Group II (11.1) do not appear to be significantly different. The average heterozygosities for the Group I and Group II enzymes appear different, but, the large standard errors suggest that this difference is not significant. On the basis of the data obtained, it is concluded that there is no significant difference in heterozygosity between the Group I and Group II enzymes in *T. arcticus*. The data do not support the suggestion that levels of variability between Group I and Group II are different.

In a few cases the enzyme classification in the present study may be challenged: XDH is generally classified in Group II since it has variable substrates (Glassman 1965), but was classified in Group I in *Drosophila* (Gillispie and Langley 1974); TO is classified as Group II although it has an unknown function. When acceptable reclassifications are made, the conclusion remains the same. There is no evidence that the glucose metabolizing enzymes are less variable than other enzymes in *Thymallus arcticus*.

An important consideration in any electrophoretic survey is the set of loci used in estimating average heterozygosity of a population. Since there is considerable interlocus variation, the set of proteins examined may bias the results. Sarich (1977) has shown that there appears to exist a rapidly evolving set of proteins (eg. esterases, transferrin, plasma proteins and many of the class II and II enzymes) (Gillispie and Kojima 1968, Kojima *et al.* 1970, Johnson 1974), and a set of slowly evolving loci (enzymes of complex metabolic pathways). The data obtained in the present study allow a comparison of such sets to be made. The enzymes and proteins of Group II and Group III were considered as one set while the Group I enzymes were considered as another (those involved in complex metabolic pathways). The results are shown in Table 5 for the populations of *T. arcticus*. The percent polymorphic loci and average heterozygosity for the former set, 23-31 percent and 9-12 percent, respectively, are significantly higher than for the Group I proteins, 9 percent and 0.4-1 percent, respectively. The existence of a rapidly evolving set of proteins and a slowly evolving set is supported by the results in *T. arcticus*. These results emphasize the importance of surveying a wide variety of loci in any electrophoretic survey. If one set or the other was consistently used in estimating heterozygosity, the results would be biased.

Genetic Divergence Between Populations of *Thymallus arcticus*

Protein electrophoresis allows quantification of the amount of genetic differences between populations based on a sample of the genome. An inherent bias of electrophoresis, however, is that only structural genes which code for soluble proteins are sampled. Thus regulatory genes and other structural genes are not sampled, which means that only a minimal estimate of genetic differentiation between different taxa can be made.

Electrophoretic data consists of allele and genotype frequencies determined from a sample of a population. A common index which summarized this information into a common meter of genetic divergence between population is genetic distance. Genetic distance is the genetic difference between populations as expressed by a function of gene frequencies. The measure of genetic distance used in the present study was that proposed by Nei (1971, 1972, 1973), by which the average number of codon differences per locus can be estimated from the gene frequency data. The method can be applied to any pair of taxa whether they are local populations, species, or genera (Nei 1975). The standard genetic distance as proposed by Nei (1972) was the measure used in the present study, where the normalized genetic identity of genes between two populations at the j locus is defined as:

$$I = \frac{\Sigma xy}{\sqrt{(\Sigma x^2 y^2)}}$$

where $\underline{x}_i$ and $\underline{y}_i$ represent the frequencies of the ith allele in populations x and y. For all loci in a sample the genetic identity is defined as:

$$I = \frac{Jxy}{\sqrt{(JxJy)}}$$

where Jx, Jy, and Jxy are the arithmetic means over all loci of x^2, y^2, and x, y, respectively. The genetic distance is defined as:

$$D = \log_e I$$

which estimates the accumulated number of codon differences per locus since the time of divergence of two populations.

The genetic distances among the various populations of *Thymallus arcticus* are summarized in Table 7.

Another index commonly employed is Rogers similarily coefficient (S) (Rogers 1972). Estimates of I and S are calculated from the same data and are fairly similar although S gives lower numerical values than I. The values of S among the populations of *Thymallus arcticus* are also summarized in Table 7. The Rogers similarity coefficients were also calculated, so that comparisons with values obtained in other species by authors employing this index, could be made.

In analyzing the significance of the amount of genetic divergence between populations, the question of how much genetic differentiation occurs during speciation, which is the most important question in

Table 7. Indices of similarity[a] (below diagonal) and genetic distance[b] (above diagonal) for four populations of *Thymallus arcticus*.

	Total (without serum)			
	Grebe	Wolf	Donnelly	Fuse
Grebe	.0000	.0007	.0335	.0368
Wolf	.9922 (.9993)	.0000	.0337	.0401
Donn.	.9597 (.9670)	.9587 (.9668)	.0000	.0054
Fuse	.9511 (.9638)	.9470 (.9606)	.9786 (.9946)	.0000

	Total (with serum)			
	Grebe	Wolf	Donnelly	Fuse
Grebe	.0000	.0007	.0359	.0406
Wolf	.9917 (.9993)	.0000	.0359	.0405
Donn.	.9363 (.9647)	.9343 (.9647)	.0000	.0183
Fuse	.9097 (.9602)	.9071 (.9603)	.9420 (.9818)	.0000

[a]Roger's Coefficient of Genetic Similarity (Nei's Coefficient of Similarity)

[b]Nei's Measure of Standard Genetic Distance

evolutionary genetics, must be addressed. The most common mode of speciation is geographic isolation, which is the usual prerequisite to genetic divergence and hence, speciation. Two stages may be recognized in the process of geographic speciation (Ayala *et al.* 1974): 1) populations become isolated by geographic barriers and accumulate genetic differences, 2) reproductive isolating mechanisms are developed. The second stage begins when genetically differentiated populations regain geographic contact. A strategy employed to attempt to determine the proportion of gene loci altered during the speciation process involves assaying populations which appear to be in various stages of the speciation process (Avise 1976).

Ayala *et al.* (1974) have done the most extensive study of genetic differentiation during geographic speciation using populations of *Drosophila willistoni*. Estimates were made of levels of genetic differentiation between populations at five levels of evolutionary divergence: 1) geographic populations within a taxon ($I = 0.970 \pm .006$), 2) subspecies, in the first stage of geographic speciation ($I = 0.795 \pm 0.013$), 3) semispecies, in the second stage of speciation ($I = 0.798 \pm 0.026$), 4) sibling species ($I = 0.517 \pm 0.024$), 5) non-sibling species ($I = 0.352$).

Many studies exist on genetic differentiation between vertebrate populations in early stages of evolutionary divergence (reviewed by

Ayala 1975). The results of such studies in several species of fish are summarized in Table 8. The degree of genetic differentiation between subspecies in fish species is comparable to that in *Drosophila willistoni*, representing an eightfold increase over the average distance between local populations. However, general conclusions about genetic differentiation during speciation are not warranted due to the tremendous heterogeneity of the biological world (Avise 1976). Mayr (1963) stressed the point that species are not characterized by a given number of counted gene differences. It is highly unlikely that all speciation events will involve the same amount of genetic change. Even when new species arise according to the general model of geographic speciation, the amount of genetic change involved may vary from one case to another. A case in point are the estimates of genetic divergence in nine genera of California minnows (Avise and Ayala 1976, Avise *et al.* 1975). Little genetic differentiation existed between local populations (I = 0.99). The average genetic identity between nine species is I = 0.592 ± 0.023. However, the most genetically similar species are *Hesperoleucus symmetricus* and *Lavina exilicavda* with I = 0.946 and D = 0.055. The two species have virtually identical genetic constitutions at 23 out of 24 loci, but are highly differentiated at a single locus which is indeed species diagnostic for most populations (Ayala 1975). Utter *et al.*

Table 8. Genetic similarities between populations at different stages of evolutionary divergence in several groups of fishes.

Group	Local Populations	Subspecies	Species	Genera	Source
Lepomis	0.97[a]	0.85[a]	0.54 ± .016	----	Avise and Smith 1974
Cyprinodon	----	----	0.89 ± .016[a]	----	Turner 1974
Sciaenidae	----	----	----	0.17 ± .027[a]	Shaw 1970
California minnows	0.99	----	0.59 ± .029	----	Avise and Ayala 1975
Salmonidae	----	----	0.46 ± .032[a]	----	Utter *et al.* 1973

[a]Similarities calculated using methods other than Nei's (1972).

(1973) have studied allozyme differentiation in two species of trout (*Salmo*) and six species of salmon (*Oncorhynchus*). The average similarity (index similar to methods of Nei and Rogers) between the six salmon species is 0.422 ± 0.04; that between the two trout species is 0.90. The average genetic similarity between all eight species is 0.456 ± 0.032.

In general, little genetic differentiation exists between local populations within a species. Subspecies, representing populations in the first stage of speciation, show moderate but substantial degrees of genetic differentiation. These observations are pertinent to the present study since local populations and geographically isolated populations of *T. arcticus* were surveyed for the amount of genetic differentiation. It is assumed that since the Canadian populations and Montana populations have been isolated for more than 7,000 years (Vincent 1962), that they represent populations in the first stage of speciation. To determine what level of evolutionary divergence these two forms actually represent, the best indication of divergence would be the magnitude of the differentiation between local populations and the geographically isolated populations. Two factors which support this line of reasoning are 1) the apparent heterogeneity of the degree of genetic differentiation involved in the speciation process in diverse organisms (previously discussed)

and 2) no comparable estimates of the amount of genetic differentiation between local populations or subspecies in salmonid species are presently available.

The Grebe and Wolf Lakes populations represent local populations of *T. arcticus*. The results (Table 7) indicate that little genetic divergence has occurred between these populations (I = .99 and D = .0007). The populations are, however, reproductively isolated by means of an ethological barrier. The populations studied at Grebe Lake is inlet spawning while the Wolf Lake population studied is outlet spawning adapted. A proposed genetic basis for such behavioral characteristics has been discussed previously. Apparently, reproductive isolation through ethological mechanisms does not require changes in a substantial proportion of the genome, as suggested in the case of *Drosophila paulistorium* (Ayala 1975). An alternative explanation as offered by Ayala (1975) may be that the completion of such reproductive isolation may require genetic changes, but not in the class of genes studied by electrophoretic techniques. The changes required may involve other types of structural or regulatory genes.

Wilson *et al.* (1974 a,b) have suggested that there may be two types of molecular evolution, one involving structural genes, which goes on at a more or less constant rate, and a second for regulatory

genes, which are primarily responsible for reproductive incompatabilities and morphological evolution. They further point out that evolutionary divergence as measured by protein differentiation on one side and by morphological divergence and reproductive incompatibility on the other do not always go hand in hand. A great deal of morphological divergence and reproductive incompatibility with only moderate protein differentiation is observed among mammals, while the reverse is observed in some amphibians and birds.

The suggestion of changes in regulatory genes controlling the reproductive behavior of *T. arcticus* is a plausible explanation. This may help to explain why there is little genetic differentiation at the structural gene level despite the probable ethological isolating mechanism present in the Grebe and Wolf Lakes populations.

Although at present there are no good estimates of the proportion of the genome encoding soluble gene products detected by electrophoresis, protein differences within particular animal groups correspond closely to levels of morphological and other divergence described by classical systematists (Avise 1974). To this extent structural genes provide information which is of evolutionary significance.

The Fuse Lake and Donnelly River populations were also considered local populations since their origins were both in the same geographical range. The genetic divergence (0.032) between these populations

is considerably larger than between the local Montana populations (.0007). It is believed that this estimate of the divergence is not entirely representative of the distance between local populations of the Canadian form due to the "bottleneck" effect on the Fuse Lake population. The possible loss of alleles at some loci ($PGM\text{-}2^{1.10}$, $IDH_s^{1.10}$, $SP\text{-}2^{1.20}$) in the Fuse Lake population, which are present at relatively high frequencies in the Donnelly River population, results in the Fuse Lake population being fixed for the alleles common to both forms. This results in a greater genetic distance between the Canadian populations than might normally be found. This idea is supported by the observation that the Fuse Lake population contains alleles common to the Donnelly population at the H6PD and SP-2 loci. While there is complete separation of the Montana and Arctic populations at these loci.

The genetic distance between the geographically isolated populations of the Montana and Canadian forms (Table 7) is substantial in comparison to the divergence between local populations. The genetic distance between the geographically isolated populations (.056-.077) and the local populations (.001) represents a magnitude greater than that between local populations and subspecies in other organisms (discussed previously). A substantial degree of genetic differentiation at the structural gene level has accumulated between the Montana

and Arctic forms of *T. arcticus* since their separation from a common ancestor. The two forms appear to be well advanced into the first stage of geographic speciation, representative of the subspecies level. The genetic similarity between the two forms (0.91-0.94) is not much greater than the similarity (0.90) between two salmonids at the species level, *Salmo gairdneri* and *Salmo clarkii* (Utter *et al.* 1973).

Taxonomic Considerations

Phylogenetic relationships can be inferred to some extent by studying morphological affinity, however, the morphological affinity of taxa does not necessarily represent the real phylogeny (Nei 1975). Sokal and Sneath (1963) stressed the separation of the phenetic (similarity) and phyletic (phylogeny) relationships. Numerical taxonomy applied to morphological characters gives only the phenetic relation of taxa (Nei 1975). Genetic distance may be used to study the phylogeny of a group of taxa producing a more reliable and quantitative phylogenetic tree. This method allows differentiation to be studied at the codon level. The probability of back mutations or parallel mutations at a codon is negligibly small unless evolutionary time is very large (Nei 1975). This method, therefore, has a great advantage over the method of comparative morphology, in which

divergence and convergence in morphological changes may make the result uncertain (Sokal and Sneath 1963).

A dendrogram of the four populations of *Thymallus arcticus* surveyed in the present study is presented in Figure 18. The tree is constructed from the genetic distances estimated in the present study. The populations were grouped using the unweighted pair-group method of clustering of Sokal and Sneath (1963).

The genetic distances estimated in the present study allows the phylogenetic position of the two forms of *Thymallus arcticus* to be estimated. At the present time no subspecies are recognized within *T. arcticus* (McPhail and Lindsey 1970). The genetic distance found in the present study suggests that such a distinction may be warranted. The Montana form of *T. arcticus* appears genetically distinct from the Arctic form deserving subspecific status. These findings appear congruent with those previously proposed by various authors on the basis of morphological characteristics (see introduction). It is proposed that the Montana form of *T. arcticus* be recognized as the subspecies *montanus* (nomenclature of Milner 1874). Further, the Arctic form should be designated as the subspecies *signifer* (nomenclature of Richardson 1823).

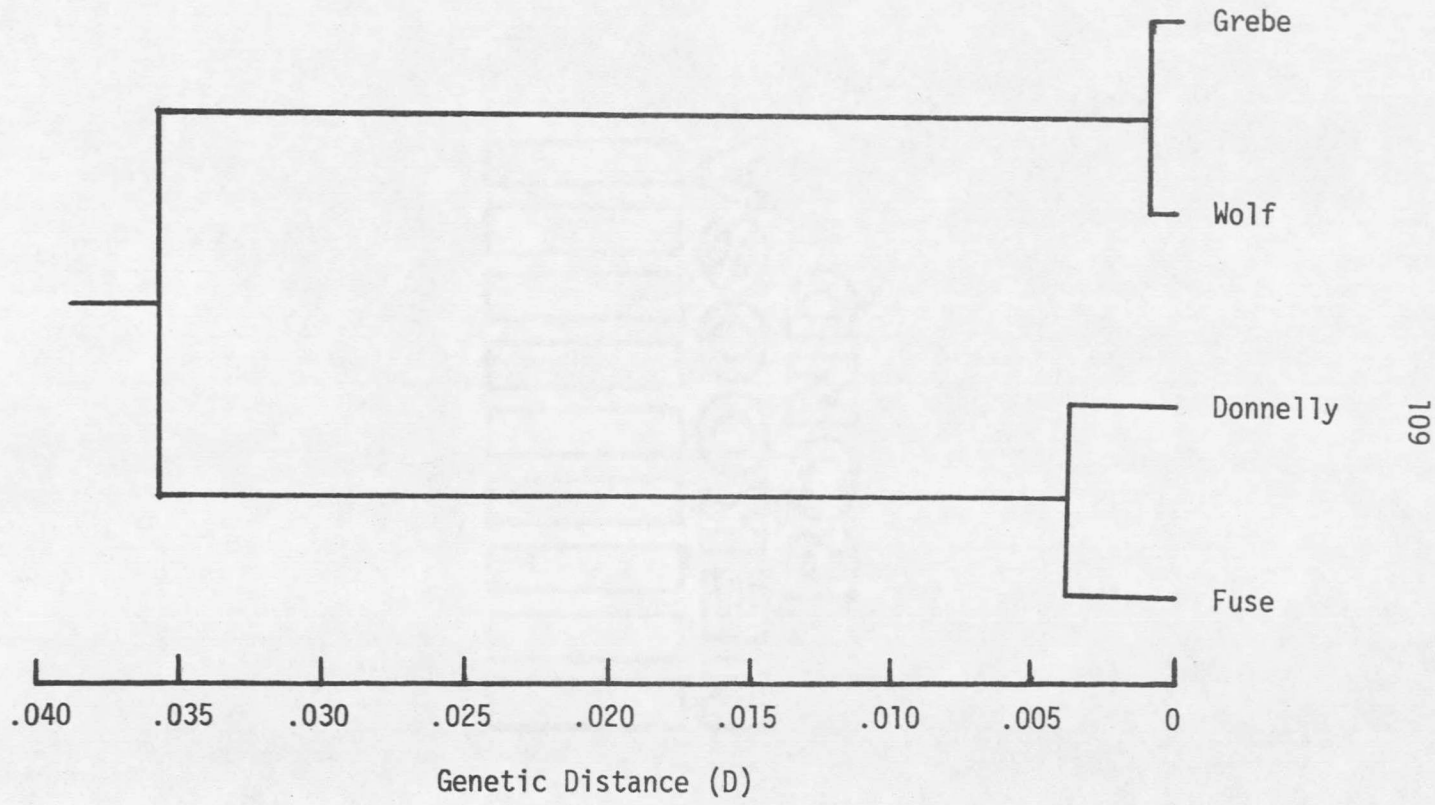


Figure 18. Dendrogram for four populations of *T. arcticus*. Genetic distance is represented on the horizontal axis.

APPENDIX

Buffer Systems

Buffer System		pH	Power (V)	Time (hr)	Reference
A. Poulik					
Electrode:	0.3 M Borate	8.2			
Gel:	0.076 M Tris 0.005 M Citrate	8.7	250	3	Selander *et al.* 1971
B. Continuous Tris Citrate I					
Electrode:	0.223 M Tris 0.086 M Citrate	6.3			
Gel:	0.008 M Tris 0.003 M Citrate	6.7	170	3	Selander *et al.* 1971
C. Continuous Tris Citrate II					
Electrode:	0.687 M Tris 0.157 M Citrate	8.0			
Gel:	22.890 M Tris 5.220 M Citrate	8.0	100	4	Selander *et al.* 1971
D. Sodium Phosphate					
Electrode:	0.04 M NaH_2PO_4 0.06 M NaH_2PO_4	8.3			
Gel:	Dilute Elec 10:1	8.3	150	5	May *et al.* 1975
E. Potassium Phosphate					
Electrode:	0.138 M KH_2PO_4 0.062 M NaOH	6.7			
Gel:	Dilute Elec 19:1	6.7	120	5	Selander *et al.* 1971

Buffer System		pH	Power (V)	Time (hr)	Reference
F. Tris-Borate EDTA					
Electrode:	0.5 M Tris 0.65 M Borate 0.02 M EDTA	8.0			
Gel:	Dilute Elec 9:1	8.0	200	4	Selander *et al.* 1971
G. Tris-Borate EDTA					
Stock:	0.9 M Tris 0.5 M Borate 0.02 M EDTA	8.6			
Gel:	Dilute 20:1		250	3	Markert and Faulhaber 1965
Electrode:	Dilute 4:1				
H. Ridgeway Buffer					
Electrode:	.06 M LiOH 0.3 M Borate	8.3			
Gel:	.03 M Tris .005 M Citrate	8.0	250	3	Ridgeway *et al.* 1970
I. Lithium Hydroxide					
Stock Sol. A:	.03 M LiOH .19 M Borate	8.1			
Stock Sol. B:	.05 M Tris .008 M Citrate	8.4			
Electrode:	Stock Solution A				
Gel:	1:9 Mixture Stock Solution A and B		350	3	Selander *et al.* 1971

Staining Procedures

(Selander *et al.* 1971, Shaw and Prasad 1965)

IDH

0.2 M tris-HCl (pH 8.0)	50 ml
0.25 M manganese chloride	0.2 ml
0.10 M tri-sodium DL-isocitric acid	3 ml
NADP	10 mg
NBT	5 mg
PMS	7 mg

Incubate 30-60 mintues (dark)

MDH

0.2 M tris-HCl (pH 8.0)	30 ml
2.0 M malate	5 ml
H_2O	20 ml
NAD	10 mg
NBT	20 mg
PMS	5 mg

Incubate 1-2 hours (dark)

GOT

0.2 M tris-HCl (pH 8.0)	50 ml
Pyridoxial-5'-phosphate	0.5 mg
-aspartic acid	200 mg
Fast blue BB	150 mg
-ketoglutarate	100 mg

Incubate 10-15 minutes

α-GPD

0.2 M tris-HCl (pH 8.0)	50 ml
0.1 M $MgCl_2$	1 ml
Disodium DL-glycerophosphate	50 mg
NAD	20 mg
NBT	13 mg
PMS	4 mg

Incubate 1-2 hours (dark)

PGM

0.2 M tris-HCl (pH 8.0)	5 ml
H_2O	25 ml
0.05 M disodium-D-glucose-1-phosphate	5 ml
0.0005 M dipotassium-D-glucose-1,6-diphosphate	5 ml
0.1 M $MgCl_2$	5 ml
10 units/ml H_2O glucose-6-phosphate dehydrogenase	4 ml
NADP	5 mg
MTT	5 mg
PMS	2 mg

Incubate 1 hour (dark)

LDH

0.2 M tris-HCl (pH 8.0)	20 ml
H_2O	30 ml
1.0 M lactate	4.5 ml
NAD	20 mg
NBT	4 mg
PMS	8 mg

Incubate 1-2 hours (dark)

ME

0.2 M tris-HCl (pH 8.0)	30 ml
2.0 M malate	5 ml
H_2O	20 ml
NADP	10 mg
NBT	20 mg
PMS	5 mg

Incubate 5-6 hours (dark)

G6PD

0.2 M tris-HCl (pH 8.0)	45 ml
0.25 M disodium glucose-6-phosphate	5 ml
NADP	15 mg
NBT	10 mg
PMS	2 mg

Incubate 6 hours (dark)

H6PD

Same as G6PD but substitute disodium galactose-6-phosphate for glucose-6-phosphate

TO

0.2 M tris-HCl (pH 8.0)	50 ml
NBT (MTT)	15 mg
PMS	10 mg

Incubate 1-2 hours (light)

Esterase

0.1 M sodium phosphate	4 ml
H_2O	45 ml
Napthyl proprionate or B Napthyl proprionate (1 gm/100 ml acetone)	1 ml
Fast blue RR	25 mg

XDH

0.2 tris-HCl (pH 8.0)	50 ml
Hypoxanthine	25 mg
NAD	10 mg
NBT	10 mg
MTT	10 mg
PMS	5 mg

Incubate 3 hours (dark)

Serum Proteins

2% Buffalo Black NBT in fixing solution

Stain 20 minutes at 20°C. Wash gel several times in fixing solution.

ADH

.5 M KPO_4 (pH 7.0)	5 ml
H_2O	40 ml
95% ethanol	5 ml
NAD	30 mg
NBT	20 mg
PMS	5 mg

Incubate 3-5 hours (dark) (migrates cathodally)

SDH

0.2 M tris-HCl (pH 8.0)	50 ml
Sorbitol	10 mg
NAD	10 mg
MTT	5 mg
PMS	2 mg

Incubate 3 hours (dark)

Hexokinase

0.2 M tris-HCl (pH 8.0)	10 ml
H_2O	40 ml
Glucose	45 mg
.1 M $MgCl_2$	10 ml
G6PD	40 units
NADP	15 mg
ATP	15 mg
NBT	10 mg
PMS	2 mg

Incubate 3 hours (dark)

LITERATURE CITED

Allendorf, F. W. (1973) Genetic variation, inheritance, and preliminary population distribution of some proteins of *Salmo gairdneri*. M.S. Thesis, Univ. of Washington.

Allendorf, F. W. and F. M. Utter. (1973) Gene duplication within the family Salmonidae: Disomic inheritance of two loci reported to be tetrasomic in rainbow trout. Genetics 74: 647-654.

Allendorf, F. W. and F. M. Utter. (1976) Gene duplication in the family Salmonidae. III. Linkage between two duplicated loci coding for aspartate aminotransferase in the cutthroat trout (*Salmo clarki*). Hereditas 82: 19-24.

Allendorf, F. W., F. M. Utter and B. P. May. (1975) Gene duplication within the family Salmonidae: II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations. *In* Markert, C. L. (ed), Isozymes IV. Genetics and Evolution. Acad. Press, New York. pp. 415-432.

Allendorf, F. W., N. Ryman, A. Stennek and G. Stahl. (1976) Genetic variation in Scandanavian brown trout (*Salmo trutta* L.): Evidence of distinct sympatric populations. Hereditas 83:1; 73-82.

Altukhov, J. P., E. A. Salmenkova, V. T. Omelchenko, T. D. Sachko, V. I. Slynko. (1972) Number of mono- and polymorphic loci in the population of tetraploid salmon species *Oncorhynchus keta*. Genetica 8(2): 67-75.

Appella, E. and C. L. Markert. (1961) Dissociation of lactate dehydrogenase into subunits with guanidine hydrochloride. Biochem. Biophys. Res. Commun. 6: 171-176.

Aspinwall, N. (1973) Inheritance of alpha-glycerophosphate dehydrogenase in the pink salmon, *Oncorhynchus gorbuscha* (Walbaum). Genetics 73: 639-643.

Aspinwall, N. (1974) Genetic analysis of duplicate malate dehydrogenase loci in the pink salmon, *Oncorhynchus gorbuscha*. Genetics 76: 65-72.

Avise, J. C. (1974) Systematic value of electrophoretic data. System. Zool. 23: 465-481.

Avise, J. C. (1976) Genetic differentiation during speciation. *In* Ayala, F. J. (ed), Molecular Evolution. Sinauer Assoc., Sunderland, Mass.

Avise, J. C. and F. J. Ayala. (1976) Genetic differentiation in speciose versus depauperate phylads: Evidence from the California minnows. Evolution 30: 46-58.

Avise, J. C. and R. K. Selander. (1972) Evolutionary genetics of cave-dwelling fishes of the genus *Astyanax*. Evolution 26: 1-19.

Avise, J. C. and M. H. Smith. (1974) Biochemical genetics of sunfish. I. Geographic variation and subspecific intergradation in the bluegill, *Lepomis macrochirus*. Evolution 28: 42-56.

Avise, J. C., J. J. Smith, and F. J. Ayala. (1975) Adaptive differentiation with little genic change between two native California minnows. Evolution 29: 3; 411-426.

Ayala, F. J. (1975) Genetic differentiation during speciation. *In* Dobzhansky, T., M. K. Hecht and W. C. Steere (eds), Evolutionary Biology, Plenum Press, New York.

Ayala, F. J. and J. R. Powell. (1972) Enzyme variability in the *Drosophila willistoni* group. VI. Levels of polymorphism and the physiological function of enzymes. Biochem. Genet. 7: 334-345.

Ayala, F. J., M. L. Tracey, D. Hedgecock, and R. C. Richmond. (1974) Genetic differentiation during the speciation process in *Drosophila*. Evolution 28(4): 576-592.

Bailey, G. S., G. T. Cocks, and A. C. Wilson. (1969) Gene duplication in fishes: Malate dehydrogenase of salmon and trout. Biochem. Biophys. Res. Comm. 34: 605-612.

Bailey, G. S., A. C. Wilson, J. E. Halver, and C. L. Johnson. (1970) Multiple forms of supernatant malate dehydrogenase in salmonid fishes. J. Biol. Chem. 245(22): 5927-5940.

Baker, C. M. A. and C. Manwell. (1967) Molecular genetics of avian proteins. VIII. Egg white proteins of the migratory quail *Coturnix coturnix* - new concepts of "hybrid vigour". Comp. Biochem. Physiol. 23: 21-42.

Baker, C. M. A., C. Manwell, R. F. Labisky, and J. A. Harper. (1966) Molecular genetics of avian proteins. V. Egg, blood and tissue proteins of the ring-necked pheasant, *Phasianus colchicus* L. Comp. Biochem. Physiol. 17: 467-499.

Baker, W. W. and B. Mintz. (1969) Subunit structure and gene control of mouse NADP malate dehydrogenase. Biochem. Genet. 2: 351.

Behnke, R. J. (1972) The systematics of salmonid fishes of recently glaciated lakes. J. Fish. Res. Bd. of Canada 29: 639-671.

Berg, L. (1940) Classification of fishes, both recent and fossil. Dok. Zool. Inst. Akad. Nauk. SSSR. 5(2): 85-517.

Berg, L. (1955) Classification of fishes, both recent and fossil. Ed. 2. Dok. Zool. Inst. Akad. Nauk SSSR. 20: 1-286.

Berger, E. (1970) A comparison of gene-enzyme variation between *Drosophila melanogaster* and *D. simulans*. Genetics 66: 667-683.

Beutler, E. and M. Morrison. (1967) Localization and characteristics of hexose-6-phosphate dehydrogenase (glucose dehydrogenase). J. Biol. Chem. 242: 5298.

Blanco, A. and W. H. Zinkham. (1963) Lactate dehydrogenases in human testes. Science 139: 601-602.

Blanco, A., W. H. Zinkham, and L. Kupchyk. (1964). Genetic control and ontogeny of lactate dehydrogenase in pigeon testes. J. Exp. Zool. 156: 137-152.

Booke, H. E. (1964) A review of variations found in fish serum proteins. N.Y. Fish and Game Jour. 11: 47-57.

Boningsore, A., R. Cancedda, A. Nicolini, G. Damiani and A. DeFlora. (1971) Metabolism of human erythrocyte glucose-6-phosphate dehydrogenase. VI. Interconversion of multiple molecular forms. Arch. Biochem. and Biophys. 147: 493-501.

Boulenger, G. A. (1895) Remarks on some cranial characters of the salmonids. Proc. Zool. Soc. London for 1895. pp. 299-302.

Brannon, E. L. (1967) Genetic control of migrating behavior of newly emerged sockeye salmon fry. Int. Pacific Salmon Fish. Comm. Progr. Rept. 16: 31 pp.

Brewer, G. J. (1967) Achromatic regions of tetrazolium stained starch gels: inherited electrophoretic variation. Amer. J. Hum. Genet. 19: 674-680.

Brown, C. J. D. (1943) Age and growth of Montana grayling. J. Wildl. Mgmt. 7(4): 353-364.

Brown, C. J. D. (1971) Fishes of Montana. Big Sky Books, Montana State University, Bozeman. 207 pp.

Butler, P. J. G., H. Jornvall and J. I. Harris. (1969) FEBS Lett. 2: 239.

Cederbaum, S. E. and A. Yoshida. (1972) Tetrazolium oxidase polymorphism in rainbow trout. Genetics 72: 363-367.

Cederbaum, S. E. and A. Yoshida. (1976) Glucose 6-phosphate dehydrogenase in rainbow trout. Biochem. Genet. 14: 245.

Clayton, J. W., D. N. Tretiak and A. H. Kooyman. (1971) Genetics of multiple malate dehydrogenase isozymes in skeletal muscle of walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Bd. Can. 28: 1005-1008.

Clayton, J. W., D. N. Tretiak, B. N. Billeck and P. Ihssen. (1975) Genetics of multiple supernatant and mitochondrial malate dehydrogenase isozymes in rainbow trout (*Salmo gairdneri*). *In* Isozymes IV: Genetics and Evolution, (ed. C. L. Markert). Acad. Press, New York. pp. 415-432.

Cohen, P. T. W. and G. S. Omenn. (1972) Genetic variation of the cytoplasmic and mitochondrial malic enzymes in the monkey *Macaca nemestrina*. Biochem. Genet. 7: 289-301.

Cohen, P. T. W., G. S. Omenn, A. G. Motulsky, S. Chen, and E. R. Giblett. (1973) Restricted variation in glycolytic enzymes of human brain and erythrocytes. Nat. New. Biol. 241: 228-233.

Cope, E. D. (1865) Partial catalogue of the cold blooded vertebrata of Michigan, Part II. Proc. Acad. Natur. Sci. Philadelphia 17: 78-88.

Curvier, G. (1829) Le regne animal distribue d'apres son organization. Paris 2: 1-306.

Darnall, D. W. and I. M. Klotz. (1972) Protein subunits: A table (revised edition). Arch. Biochem. Biophys. 149: 1-14.

Dean, J. D. and J. D. Varley. (1974) Annual Project Report - 1973 - Fishery Management Program - Yellowstone National Park. U.S. Fish and Wildlife Service Processed Rpt. 170 pp.

DeLigny, W. (1969) Serological and biochemical studies on fish populations. Oceanographic Mar. Biol. Ann. Rev. 7: 411-513.

DeLorenzo, R. J. and F. H. Ruddle. (1970) Glutamic-oxaloacetic transaminase (GOT) genetics in *Mus musculus*: Linkage, polymorphism and phenotypes of the GOT-2 and GOT-1 loci. Biochem. Genet. 4: 259-273.

Dickerson, R. E. (1972) The structure and history of an ancient protein. Sci. Amer. 226(4): 58-72.

Dobzhansky, T. (1951) Genetics and the Origin of the Species. 3rd ed. Columbia Univ. Press, New York.

Dobzhansky, T. (1970) Genetics of the Evolutionary Process. Columbia Univ. Press, New York.

Eckroat, L. R. (1973) Allele frequency analysis of five soluble protein loci in brook trout, *Salvelinus fontinalis* (Mitchill). Tran. Amer. Fish. Soc. 102: 335-340.

Engel, W., J. Faust and U. Wolf. (1971) Isoenzyme polymorphism of the sorbitol and the NADP-dependent isocitrate dehydrogenases in the fish family Cyprinidae. Anim. Blood Grps. Biochem. Genet. 2: 127-133.

Engel, W. J., J. Opt'Hof and U. Wolf. (1970) Genduplikation durch polyploide evolution: die isoenzyme der sorbit dehydrogenase bei herrings und lachsartigen fischen (*Isospondyli*). Humangenetik 9: 157-163.

Engel, W., J. Schmidtke and U. Wolf. (1971) Genetic variation of α-glycerophosphate dehydrogenase isoenzymes in clupeoid and salmonid fish. Experentia 27(12): 1489-1491.

Engel, W., J. Schmidtke and U. Wolf. (1975) Diploid-tetraploid relationships in teleostean fishes. *In* Markert, C. L. (ed), Isozymes IV: Genetics and Evolution. Acad. Press, New York, pp. 449-462.

Eppenberger, H. M., A. Scholl and H. Ursprung. (1971) FEBS Letters 14: 317-319.

Feeney, R. E. and W. D. Brown. (1974) Plasma proteins in fish. *In* M. Florkin and B. T. Scheer (eds), Chemical Zoology. VIII. Acad. Press, New York.

Frydenberg, O. and V. Simonsen. (1973) Genetics of *Zoarces* populations. V. Amount of protein polymorphism and degree of genic heterozygosity. Hereditas 75: 221-232.

Gall, G. A. E., C. A. Busack, R. C. Smith, J. R. Gold, and B. J. Kornblatt. (1976) Biochemical genetic variation in populations of golden trout, *Salmo aquabonita*. J. of Hered. 67: 330-335.

Gillispie, J. H. and K. Kojima. (1968) The degree of polymorphisms in enzymes involved in energy compared to that in non-specific enzymes in two *Drosophila ananassae* populations. Proc. Natl. Acad. Sci. US. 61: 582-585.

Gillispie, J. H. and C. H. Langley. (1974) A general model to account for enzyme variation in natural populations. Genetics 76: 837-848.

Glassman, E. (1965) Genetic regulation of xanthine dehydrogenase in *Drosophila melanogaster*. Fed. Proc. 24: 1243-1251.

Goldberg, E. (1963) Lactic and malic dehydrogenases in human spermatozoa. Science 139: 602-603.

Goldberg, E. (1966) Lactate dehydrogenase of trout: hybridization *in vivo* and *in vitro*. Science 151: 1091-1093.

Gottlieb, L. D. (1971) Gel electrophoresis: New approach to the study of evolution. Bioscience 21: 939-944.

Harris, H. (1966) Enzyme polymorphisms in man. Proc. Roy. Soc. Ser. B. 164: 298-310.

Harris, H. (1969) Genes and isozymes. Proc. Roy. Soc. Lond. B. 174: 1.

Harris, H. (1975) The Principles of Human Biochemical Genetics. 2nd ed. North Holland, Amsterdam.

Harris, H. and D. A. Hopkinson. (1972) Average heterozygosity per locus in man: an estimate based on incidence of enzyme polymorphism. Ann. Hum. Genet. 36: 9-20.

Harris, H. and D. A. Hopkinson. (1976) Handbook of Enzyme Electrophoresis in Human Genetics. North Holland, Amsterdam.

Henderson, N. S. (1965) Isozymes of isocitrate dehydrogenase: subunit structure and intracellular location. J. Exp. Zool. 158: 263-274.

Henderson, N. S. (1966) Isozymes and genetic control of NADP-malate dehydrogenase in mice. Arch. Biochem. Biophys. 117: 28.

Henderson, N. S. (1968) Intracellular location and genetic control of isozymes of NADP-dependent isocitrate dehydrogenase and malate dehydrogenase. Ann. N.Y. Acad. Sci. 151: 429-440.

Henshall, J. A. (1906) A list of the fishes of Montana. Bull. Univ. of Montana, No. 34(11): 12 pp.

Hersberger, W. K. (1970) Some physiochemical properties of transferrins in brook trout. Trans. Am. Fish. Soc. 99: 207.

Hitzeroth, H., J. Klose, S. Ohno, and U. Wolf. (1968). Asynchronous activation of parental alleles at the tissue specific gene loci observed on hybrid trout during early development. Biochem. Genet. 1: 287-300.

Hochachka, P. W. and G. A. Somero. (1971) Biochemical adaptation to the environment. *In* Hoar, W. S. and D. Randall (eds), Fish Physiology, Vol. 6, Acad. Press, New York. pp. 99-156.

Holton, G. D. (1971) Montana grayling: the lady of the streams. Montana Outdoors Sept/Oct: 18-23.

Hopkinson, D. A. and H. Harris. (1968) A third phosphoglucomutase locus in man. Ann. Hum. Genet. Lond. 30: 167.

Hopkinson, D. A. and H. Harris. (1971) Recent work on isozymes in man. Ann. Rev. Genet. 5: 5.

Horowitz, J. J. and G. S. Whitt. (1972) Evolution of a nervous system specific lactate dehydrogenase isozyme in fish. J. Exp. Zool. 180: 13-32.

Hubbs, C. L. and K. F. Lagler. (1949) Fishes of the Great Lakes region. Cranbook Inst. Sci. Bull. No. 26. 186 pp.

Hunter, R. L. and C. L. Markert. (1957) Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science 125: 1294-1295.

Johnson, A. G., F. M. Utter and H. O. Hodgins. (1970a) Electrophoretic variants of L-alpha-glycerophosphate dehydrogenase in Pacific ocean perch (*Sebastodes glutus*). J. Fish. Res. Bd. Can. 27: 943-945.

Johnson, A. G., F. M. Utter and H. O. Hodgins. (1970b) Interspecific variation of tetrazolium oxidase in *Sebastodes* (rockfish). Comp. Biochem. Physiol. 37: 281-286.

Johnson, A. G., F. M. Utter and H. O. Hodgins. (1973) Estimates of genetic polymorphism and heterozygosity in three species of rockfish (genus *Sebastes*). Comp. Biochem. Physiol. 44B: 397-406.

Johnson, G. B. (1971) Metabolic implications of polymorphism as an adaptive strategy. Nature 232: 347-349.

Johnson, G. B. (1974) Enzyme polymorphism and metabolism. Science 184: 28-37.

Jordan, D. S. and B. W. Evermann. (1923) American Food and Game Fishes. Doubleday, Page Co., New York. 574 pp.

Kelly, J. L. (1931) Nation watches Montana grayling. Mt. Wildl. 3(8): 9-11.

Khanna, N. D., R. K. Juneja, B. Larsson, and B. Gahne. (1975) Electrophoretic studies on proteins and enzymes in the Atlantic salmon, *Salmo salar* L. Swed. J. Ag. Res. 5: 185-192.

Kimura, M. and J. F. Crow. (1964) The number of alleles that can be maintained in a finite population. Genetics 49: 725-738.

King, M. and A. C. Wilson. (1975) Evolution at two levels: molecular similarities and biological differences between humans and chimpanzees. Science

Kirkman, H. and K. M. Hendrickson. (1963) Sex linked electrophoretic difference in glucose-6-phosphate dehydrogenase. Am. J. Hum. Genet. 15: 241.

Kirpichnikov, V. S. (1973) Biochemical polymorphism and microevolution processes in fish. *In* Schroder, J. H. (ed), Genetics and Mutagenesis of Fish, Springer-Verlag, New York. pp. 223-241.

Kitto, G. B. and N. O. Kaplan. (1966) Purification and properties of chicken heart mitochondrial and supernatant malic dehydrogenases. Biochemistry 5: 3966-3980.

Kojima, K., J. Gillispie, and Y. N. Tobari. (1970) A profile of *Drosophila* species' enzymes assayed by electrophoresis. I. Number of alleles, heterozygosities, and linkage disequilibrium in glucose-metabolizing systems and some other enzymes. Biochem. Genet. 4: 627-637.

Kristiansson, A. C. and J. D. McIntyre. (1976) Genetic variation in chinook salmon (*Oncorhynchus tshawytscha*) from the Columbia River and three Oregon coastal rivers. Trans. Am. Fish. Soc. 105(6): 620-623.

Kruse, T. E. (1959) The grayling of Grebe Lake, Yellowstone National Park, Wyoming. U.S. Fish and Wildlife Service, Fish Bull. 149, vol. 59: 307-351.

Lewontin, R. C. and J. L. Hubby. (1966) A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics 54: 595-609.

Lindsey, C. C. (1956) Distribution and taxonomy of fishes in the MacKenzie drainage of British Columbia. J. Fish. Res. Bd. Can. 13: 759-798.

Lukjanenko, V. T. and A. V. Popov. (1969) Proteins of the serum in two allopatric populations of the Siberian sturgeon *Acipenser baeri* Br. Dokl. Acad. Nauk. 186(1): 233-235.

Manwell, C. and C. M. A. Baker. (1969) Hybrid proteins, heterosis, and the origin of species. I. Unusual variation of polychaete Hyalinoecia "nothing dehydrogenases" and of quail *Coturnix* erythrocyte enzymes. Comp. Biochem. Physiol. 28: 1007.

Manwell, C. and C. M. A. Baker. (1970) Molecular Biology and Origin of Species: Heterosis, Protein Polymorphism, and Animal Breeding. Sidgwick and Jackson, London.

Markert, C. L. (1962) *In* Metcoff, J. (ed), Hereditary, Developmental and Immunological Aspects of Kidney Disease. Northwestern Univ. Press, Illinois. pg. 54.

Markert, C. L. (1968) Molecular basis for isozymes. Ann. N.Y. Acad. Sci. 151: 14-40.

Markert, C. L. and I. Faulhaber. (1965) Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool. 159: 319-332.

Markert, C. L. and R. S. Holmes. (1969) Lactate dehydrogenase isozymes of the flatfish, Pleuronectiformes: kinetic, molecular, and immunochemical analysis. J. Exp. Zool. 171: 85-104.

Massaro, E. J. (1973) Tissue distribution and properties of the lactate and malate dehydrogenase isozymes of the grayling *Thymallus arcticus* (Pallas). J. Exp. Zool. 186: 151-158.

Massaro, E. J. and C. L. Markert. (1968) Isozyme patterns of salmonid fishes: evidence for multiple cistrons for lactate dehydrogenase polypeptides. J. Exp. Zool. 168: 223-228.

May, B. M., F. M. Utter and F. W. Allendorf. (1975) Biochemical genetic variation in pink and chum salmon: Inheritance of intraspecies variation and apparent abscence of interspecies introgression following massive hybridization of hatchery stocks. J. Hered. 66: 227-232.

Mayr, E. (1963) Animal Species and Evolution. Harvard Univ. Press, Cambridge, Mass.

McCabe, M. M., D. M. Dean and C. S. Olson. (1970) Multiple forms of 6-phosphogluconate dehydrogenase and alpha glycerophosphate dehydrogenase in the shipjack tuna, *Katsuwonus pelamis*. Comp. Biochem. Physiol. 34: 755-757.

McPhail, J. D. and C. C. Lindsey. (1970) Freshwater fishes of northwest Canada and Alaska. Fish. Res. Bd. of Canada Bull No. 173.

Metzger, R. P., S. Wilcox and A. Wick. (1965) Subcellular distribution and properties of hepatic glucose dehydrogenases of selected vertebrates. J. Biol. Chem. 240(7): 2767.

Milner, J. W. (1873) Notes on the graylings of North America. *In* Freshwater Fisheries of the United States. U.S. Govt. Printing Office, Washington, D.C. pp. 730-742.

Moller, D. (1970) Transferrin polymorphism in Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Can. 27: 1617-1625.

Moore, B. W. and R. H. Lee. (1960) Chromatography of rat liver soluble proteins and localization of enzyme activity. J. Biol. Chem. 234: 1359.

Morrison, W. J. (1970) Nonrandom segregation on two lactate dehydrogenase subunit loci in trout. Trans. Amer. Fish. Soc. 99: 193-206.

Morrison, W. J. and J. E. Wright. (1966) Genetic analysis of three lactate dehydrogenase systems in trout: Evidence for linkage of genes coding subunits A and B. J. Exp. Zool. 163: 259-270.

Nair, P. S., D. Brncic and K. Kojima. (1971) II. Isozyme variations and evolutionary relationships in the *mesophragmatica* species group of *Drosophila*. Studies in Genetics VI. (Univ. Texas Publ. No. 7103) pp. 17-28.

Nei, M. (1971) Interspecific gene differences and evolutionary time estimated from electrophoretic data on protein identity. Amer. Nat. 105: 385-398.

Nei, M. (1972) Genetic distance between populations. Amer. Nat. 106: 283-292.

Nei, M. (1975) Molecular Evolution and Population Genetics. North Holland, Amsterdam.

Nei, M. and R. Chakraborty. (1973) Genetic distance and electrophoretic identity of proteins between taxa. J. Molec. Evol. 2: 323-328.

Nei, M. and A. K. Roychoudhury. (1974) Genic variation within and between the three major races of man, Caucasoids, Negroids, and Mongoloids. Amer. J. Hum. Genet. 26: 421-443.

Nei, M. and A. K. Roychoudhury. (1974) Sampling variances of heterozygosity and genetic distance. Genetics 76: 379-390.

Nei, M., T. Maruyama and R. Chakraborty. (1975) The bottleneck effect and genetic variability in populations. Evolution 29: 1-10.

Nelson, P. H. (1954) Life history and management of the American grayling (*Thymallus signifer tricolor*) in Montana. J. Wildl. Mgmt. 18(3): 324-342.

Nelson, P. H. (1956) The grayling - an endangered species. Mt. Wildl. 6(1): 20-21.

Noltman, E. A. and S. A. Kuby. (1963) D-glucose-6-phosphate and 6-phosphogluconate dehydrogenases. *In* Boyer, P., H. Lardy, and R. Myrback (eds), The Enzymes, Vol. 7. Acad. Press, New York.

Norden, C. R. (1961) Comparative osteology of representative salmonid fishes with particular reference to the grayling (*Thymallus arcticus*) and its phylogeny. J. Fish. Res. Bd. Can. 18: 679-791.

Northcote, T. G. (1969). Patterns and mechanisms in the lakeward migratory behavior of juvenile trout. *In* Symposium on Salmon and Trout in Streams. Northcote, T. G. (ed), Univ. Brit. Col., Vancouver. pp. 183-204.

Nyman, L. (1972) A new approach to the taxonomy of the *Salvelinus alpinus* species complex. Rep. Invt. Freshw. Res. Drottingholm 52: 103-131.

O'Brien, S. J. and R. J. MacIntyre. (1969) An analysis of gene-enzyme variability in natural populations of *Drosophila melanogaster* and *Drosophila simulans*. American Naturalist 103: 97-113.

Ohno, S., U. Wolf, and N. B. Atkin. (1968) Evolution from fish to mammals by gene duplication. Hereditas 59: 169-187.

Ohno, S., H. W. Payne, M. Morrison and E. Beutler. (1966) Hexose-6-phosphate dehydrogenase found in human liver. Science 153: 1015.

Ohno, S., J. Muramato, J. Klein and N. B. Atkin. (1969) Diploid-tetraploid relationship in Clupeoid and Salmonid fish. Chromosomes Today 2: 139-147.

Opt'Hof, J. (1969) Isoenzymes and population genetics of sorbit dehydrogenase (E.C.:1.1.1.14) in swine (*Sus scrofa*). Humangenetik 7: 258-259.

Opt'Hof, J., U. Wolf and W. Krone. (1969) Studies on isozymes of sorbitol dehydrogenase in some vertebrate species. Humangenetik 7: 178-182.

Parrington, J. M., G. Cruickshank, D. A. Hopkinson, E. B. Robson, and H. Harris. (1968) Linkage relationships between the three phosphoglucomutase loci PGM, PGM_2 and PGM_3. Ann. Hum. Genet. 32: 37.

Payne, R. H., A. R. Child, A. Forrest. (1971) Geographical variation in the Atlantic salmon. Nature 231: 250-252.

Perrier, H., J. P. Delcroix, C. Perrier, and J. Gros. (1973) An attempt to classify the plasma proteins of the rainbow trout (*Salmo gairdneri* Richardson) using disc electrophoresis, gel filtration and salt solubility fractionation. Comp. Biochem. Physiol. 46(b): 475-482.

Povey, S., D. E. Wilson and H. Harris. (1975) Subunit structure of soluble and mitochondrial malic enzyme: demonstration of human mitochondrial enzyme in human-mouse hybrids. Ann. Hum. Genet. Lond. 39: 203.

Powell, J. (1975) Protein variation in natural population of animals. Evolutionary Biology, Vol. 8. Plenum Press, New York. pp. 79-119.

Prakash, S., R. C. Lewontin and J. L. Hubby. (1969) A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. Genetics 61: 841-858.

Raleigh, R. F. (1967) Genetic control in the lakeward migrations of sockeye salmon, *Oncorhynchus nerka*, fry. J. Fish. Res. Bd. Can. 24: 2613-2622.

Raleigh, R. F. and D. W. Chapman. (1971) Genetic control in lakeward migrations of cutthroat trout fry. Trans. Amer. Fish. Soc. 100(1): 33-40.

Reagan, C. T. (1914) The systematic arrangement of the fishes of the family Salmonidae. Ann. Mag. Nat. Hist. 13(8): 405-408.

Reinitz, G. L. (1974) Introgressive hybridization and variation in *Salmo clarkii* and *S. gairdneri* in Montana. M.S. Thesis, Univ. of Montana, Missoula.

Richardson, J. R. (1823) Notice of the fishes. *In* Franklin, J. (ed), Narrative of a Journey to the Shores of the Polar Sea in the years 1819, 1820, 1821, 1822. John Murray, London. pp. 705-728.

Richmond, R. C. (1972) Enzyme variability in the *Drosophila willistoni* group. III. Amounts of variability in the superspecies *D. paulistorum*. Genetics 70: 87-112.

Ridgeway, G. J., S. W. Sherburne and R. D. Lewis. (1970) Polymorphism in the esterases of Atlantic herring. Trans. Amer. Fish. Soc. 99: 147-151.

Riechenbach-Klinke, H. H. (1973) Investigations on the serum polymorphism of trout and carp. *In* Schroder, J. H. (ed), Genetics and Mutagenesis of Fish, Springer-Verlag, Berlin. pp. 315-318.

Roberts, F. L., J. F. Wohnus and S. Ohno. (1969) Phosphoglucomutase polymorphism in the rainbow trout, *Salmo gairdneri*. Experentia 25(10): 1109-1110.

Rogers, J. S. (1972) Measures of genetic similarity and genetic distance. Studies in Genetics VII (Univ. Texas Publ. No. 7213). pp. 145-153.

Ropers, H. H., W. Engel, and U. Wolf. (1973). Inheritance of the s-form of NADP-dependent isocitrate dehydrogenase polymorphism in rainbow trout. *In* Schroder, J. H. (ed), Genetics and Mutagenesis of Fish, Springer-Verlag, New York. pp. 319-327.

Sarich, V. M. (1977) Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. Nature 265: 24-28.

Schmidtke, J. and W. Engel. (1972) Duplication of the gene loci coding for the supernatant aspartate aminotransferase by polyploidization in the fish family Cyprinidae. Experientia 28: 976-978.

Scott, W. B. and E. J. Crossman. (1973) Freshwater fishes of Canada. Fish. Res. Bd. Can. Bull. 184: 300-305.

Selander, R. K. (1976) Genic variation in natural populations. *In* Ayala, F. J. (ed), Molecular Evolution. Sinaeur Assoc., Sunderland, Mass.

Selander, R. K. and W. E. Johnson. (1973) Genetic variation among vertebrate species. Ann. Rev. Ecol. Syst. 4: 75-91.

Selander, R. K. and S. Yang. (1969) Protein polymorphism and genic heterozygosity in a wild population of the house mouse (*Mus musculus*). Genetics 63: 653-667.

Selander, R. K., W. G. Hunt and S. Y. Yang. (1969) Protein polymorphism and genic heterozygosity in two European subspecies of the house mouse. Evolution 23: 379-390.

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson and J. B. Gentry. (1971) Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old field mouse (*Peromyscus polionotus*). Studies of Genetics VI. (Univ. of Texas Publ. No. 7103) pp. 49-90.

Shaklee, J. B., K. L. Kepes, and G. S. Whitt. (1973) Specialized lactate dehydrogenase isozymes: the molecular and genetic basis for the unique eye and liver LDHs of Teleost fishes. J. Exp. Zool. 185: 217-240.

Shatton, J. B., J. E. Halver and S. Weinhouse. (1971). Glucose (hexose-6-phosphate) dehydrogenase in liver of rainbow trout. J. Biol. Chem. 246: 4878.

Shaw, C. R. (1965) Electrophoretic variation in enzymes. Science 149: 936-943.

Shaw, C. R. (1966) Glucose-6-phosphate dehydrogenase homologous molecules in deer mouse and man. Science 153: 1013.

Shaw, C. R. (1970) How many genes evolve? Biochem. Genet. 4: 275-283.

Shaw, C. R. and E. Barto. (1963) Genetic evidence for the subunit structure of lactate dehydrogenase. Proc. Natl. Acad. Sci. U.S. 50: 211-214.

Shaw, C. R. and L. Koen. (1968) Glucose-6-phosphate dehydrogenase and hexose-6-phosphate dehydrogenase of mammalian tissues. Ann. N.Y. Acad. Sci. 151: 149.

Shaw, C. R. and R. Prasad. (1970) Starch gel electrophoresis of enzymes - a compilation of recipes. Biochem. Genet. 4: 297-320.

Shows, T. B. and F. H. Ruddle. (1968) Malate dehydrogenase. Evidence for tetrameric structure in *Mus musculus*. Science, N.Y. 160: 1356.

Shows, T. B., V. M. Chapman, and F. H. Ruddle. (1970) Mitochondrial malate dehydrogenase and malic enzyme: Mendelian inherited electrophoretic variants in the mouse. Biochem. Genet. 4: 707-718.

Siegel, L. and S. Englard. (1961) Beef heart malate dehydrogenase: properties of the enzyme purified from extracts of acetone dried powder. Biochim. Biophys. Acto 54: 67-76.

Slastenenko, E. P. (1958). The Freshwater Fishes of Canada. Kiev Printers, Toronto.

Smithies, O. (1955). Zone electrophoresis in starch gels: group variations in the serum proteins of normal adults. Biochem. J. 61: 629-641.

Sokal, R. and F. J. Rohlf. (1969) Biometry. The Principles and Practice of Statistics in Biological Research. W.H. Freeman, San Francisco.

Sokal, R. R. and P. H. A. Sneath. (1963). Principles of Numerical Taxonomy. W.H. Freeman, San Francisco.

Spencer, N., D. A. Hopkinson and H. Harris. (1964) Phosphoglucomutase polymorphism in man. Nature 204: 742-745.

Stegeman, J. J. and E. Goldberg. (1971) Distribution and characterization of hexose-6-phosphate dehydrogenase in trout. Biochem. Genet. 5: 579.

Stegeman, J. J. and E. Goldberg. (1972) Inheritance of hexose-6-phosphate dehydrogenase polymorphism in brook trout. Biochem. Genet. 7: 279-288.

Syln'ko, V. I. (1976) Electrophoretic analysis of isoenzymes of malate dehydrogenase from fish of the Salmonidae family. Doklady Akad. Nauk SSSR, 226(2): 448-451.

Thurston, R. V. (1967) Electrophoretic patterns of blood serum proteins from rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Can. 24: 2169-2188.

Tsuyuki, H. and E. Roberts. (1966) Interspecies relationships within the genus *Oncorhynchus* based on biochemical systematics. J. Fish. Res. Bd. Can. 23: 101-107.

Turner, B. J. (1974) Genetic divergence of Death Valley pupfish species: Biochemical vs. morphological evidence. Evolution 28: 281-294.

U.S. Department of Interior. (1966) Rare and endangered fish and wildlife of the United States. Bur. Spt. Fish. Wildl. Res. Pub. 24 Sheet F-7.

Utter, F. M. (1971) Tetrazolium oxidase phenotypes of rainbow trout (*Salmo gairdneri*) and Pacific salmon (*Oncorhynchus* spp.). Comp. Biochem. Physiol. 39B: 891-895.

Utter, F. M. and H. O. Hodgins. (1970) Phosphoglucomutase polymorphism in sockeye salmon. Comp. Biochem. Physiol. 86(1): 195-200.

Utter, F. M. and H. O. Hodgins. (1972) Biochemical genetic variation at six loci in four stocks of rainbow trout. Tran. Amer. Fish. Soc. 101: 494-502.

Utter, F. M., W. E. Ames, and H. O. Hodgins. (1970) Transferrin polymorphism in coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Bd. Can. 27: 2371-2373.

Utter, F. M., F. W. Allendorf, and H. O. Hodgins. (1973) Genetic variability and relationships in Pacific salmon and related trout based on protein variations. Syst-Zool. 22: 257-270.

Vincent, R. E. (1962) Biogeographical and ecological factors contributing to the decline of arctic grayling, *Thymallus arcticus* Pallas, in Michigan and Montana. Ph.D. Thesis, Univ. of Mighigan.

Walters, V. (1955) Fishes of western Arctic America and eastern Arctic Siberia: Taxonomy and zoogeography. Bull. Amer. Mus. Nat. Hist. 106(5): 255-368.

Ward, R. D. (1977) Relationship between enzyme heterozygosity and quaternary structure. Biochem. Genet. 15: 123-135.

Wheat, T. E. and G. S. Whitt. (1971) *In vivo* and *in vitro* molecular hybridization of malate dehydrogenase isozymes. Experentia 27: 647-648.

Wheat, T. E., W. F. Childers, E. T. Miller and G. S. Whitt. (1971) Genetic and *in vitro* molecular hybridization of malate dehydrogenase isozymes in interspecies bass (*Micropterus*) hybrids. Anim. Blood Groups Biochem. Gen. 2: 3-14.

Whitt, G. S. (1969) Homology of lactate dehydrogenase genes: E gene function in the nervous system. Science 166: 1156-1158.

Whitt, G. S. (1970) Developmental genetics of the lactate dehydrogenase isozymes of fish. J. Exp. Zool. 175: 1-36.

Whitt, G. S. and G. M. Booth. (1970) Localization of lactate dehydrogenase activity in the cells of the fish (*Xiphophorus helleri*) eye. J. Exp. Zool. 174: 215-224.

Whitt, G. S. and J. J. Horowitz. (1970) Evolution of retinal specific lactate dehydrogenase isozyme in teleosts. Experentia 26: 1302-1304.

Wilson, A. C., L. R. Maxson, and V. M. Sarich. (1974a) Two types of molecular evolution: Evidence from studies of interspecific hybridization. Proc. Natl. Acad. Sci. 71: 2843-2847.

Wilson, A. C., V. M. Sarich and L. R. Maxson. (1974b) The importance of gene rearrangement in evolution: Evidence from studies on rates of chromosomal, protein and anatomical evolution. Proc. Natl. Acad. Sci. 71: 3028-3030.

Wolf, U., W. Engel, J. Faust. (1970) Zum mechanisms der diploidisierung in der wirbeltierevolution: Koexistenz von tetrasomen und disomen genloci der isocitratdehydrogenase bei der regenhogenforelle, *Salmo irrideus*. Humangenetik 9: 150-156.

Woods, K. and R. Engle. (1957) Phylogenesis of plasma proteins and plasma cells. I. Starch gel zone electrophoresis of sera from marine invertebrates and fishes. Biol. Bull. 113(2): 362-363.

Wright, J. E. and L. M. Atherton. (1970) Polymorphism for LDH and transferrin loci in brook trout populations. Trans. Amer. Fish. Soc. 99(1): 179-192.

Wright, T. D. and A. D. Hasler. (1967) An electrophoretic analysis of the effects of isolation and homing behavior of the serum proteins of the white bass (*Roccus chrysops*) in Wisconsin. Amer. Natur. 101(921): 401-413.

Yamuchi, T. and E. Goldberg. (1973) Glucose-6-phosphate dehydrogenase from brook, lake and splake trout: an isozymic and immunological study. Biochem. Genet. 10: 121.

Zouros, E. (1975). Electrophoretic variation in allozymes related to function or structure? Nature 254: 446-448.

Lynch, Jeremiah C
Comparative genetics of Montana and arctic grayling ...