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Introgressive hybridization and variation in Salmo clarki and S. gairdneri in Montana

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INTROGRESSIVE HYBRIDIZATION AND VARIATION IN *Salmo clarki* AND *S. gairdneri* IN MONTANA

By

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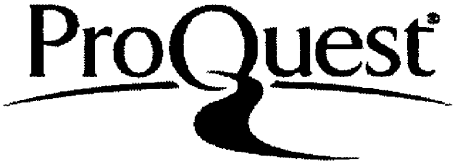

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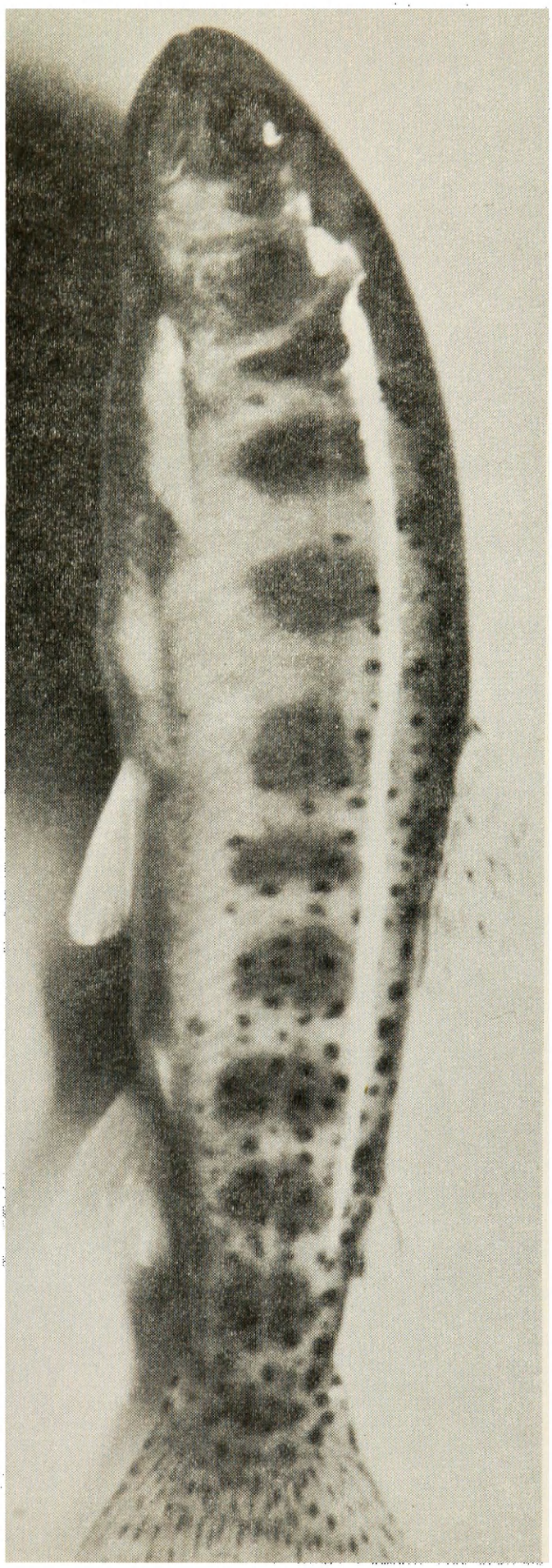

Photo showing a westslope cutthroat trout taken from Lolo Creek.

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Chapter I

INTRODUCTION

Distribution of *Salmo clarki* Richardson

The cutthroat trout, *Salmo clarki*, is a polytypic species which, historically, had a very wide distribution. Behnke (personal communication) states, "The original distribution of cutthroat trout occurred in coastal streams from Prince William Sound, Alaska, to the Eel River in northern California. In the interior regions, the range included the South Saskatchewan drainage, the upper Columbia and upper Missouri basins, the Snake River segment of the Columbia drainage, above and below Shoshone Falls, the upper Colorado and Rio Grande systems, the South Platte and Arkansas drainages in Colorado, and the Great Basin (Bonneville, Lahontan, and Alvord basins)." The first reports of the presence of cutthroat trout in Montana came from the Pacific Railroad surveys of 1853 to 1855, at which time the species inhabited all waters in the mountainous regions of western Montana.

Presently the distribution of "pure" native cutthroat trout in Montana is restricted to small relict populations in the extreme headwaters of mountain streams. This drastic decline in the range of the cutthroat trout can be attributed to the introduction of several exotic species of trout in streams throughout the state (Hanzel 1960).

According to Hanzel, rainbow trout, *Salmo gairdneri*, and brown trout, *Salmo trutta*, were both introduced into Montana in 1891 and

brook trout, Salvelinus fontinalis, were introduced in 1894. Brown trout now predominate in larger streams, and brook trout occupy the smaller streams at lower elevations, as well as creeks and lakes at higher elevations. Both occupy waters formerly used by the cutthroat trout. However, it is the rainbow trout that presents the most serious threat to the continued survival of the native cutthroat trout. The widespread and massive stocking of rainbow trout, the similarity of the feeding habits of the rainbow trout and the cutthroat trout (Brown 1971) and the production of viable hybrids between the two species explains why the introduction of the rainbow trout has been more important than any other single factor in causing the widespread disappearance of pure populations of native cutthroat trout in Montana.

Hybridization Between S. clarki and S. gairdneri

In some coastal drainages, from northern California to southern Alaska, rainbow trout and cutthroat trout are sympatric and yet exhibit almost complete reproductive isolation (Behnke personal communication). However, where rainbow trout have been introduced into interior drainages in which subspecies of S. clarki are indigenous, hybrids are readily produced between the two species. According to Hubbs (1955), it is not unusual for the introduction of a new species to an area to be associated with the production of hybrid individuals.

The tendency for the production of hybrids between cutthroat and rainbow trout is enhanced by the very similar spawning patterns

of the two species. Both spawn in the spring of the year and prefer localities characterized by relatively swift current, loose gravel about ½ inch in diameter, and water with a dissolved oxygen content of at least 7 p.p.m. (Brown 1971, Dietz 1971).

The contamination of the gene pools of cutthroat trout is an obvious consequence of hybridization with the rainbow trout. Because the preservation of native species is generally considered desirable, this creates a problem for fisheries management.

Management of *S. clarki* in Montana

In the past, when a species or subspecies of fish has become endangered, state or federal agencies have attempted to develop management programs intended to increase the range of the endangered species. Programs involving the creation of new habitat, the elimination of introduced species, the construction of barriers to upstream migration, and the introduction of the species or subspecies concerned into previously barren waters have been used to modestly expand the range of the greenback cutthroat trout, the Gila trout, and the Apache trout (Behnke, personal communication). However, programs of this type for the conservation of the cutthroat trout in Montana cannot be implemented until a procedure is established by which pure populations can be distinguished from those containing genes introduced through hybridization with other species of trout.

Taxonomy of *S. clarki* in Montana

Miller (1950) presents a list of subspecies for *S. clarki* in

which the Yellowstone cutthroat trout is classified as S. clarki lewisi. Behnke (personal communication) states, "According to the rules of nomenclature, the name lewisi is assigned to a cutthroat trout taken at Great Falls, Montana, in the Missouri River ... Thus the name applies to all native cutthroat trout of the upper Missouri River basin (above Yellowstone drainage) and any other geographical groups of cutthroat trout which are taxonomically similar to a degree that no consistent difference can be demonstrated in any characters ..." Zimmerman (1965) stated that cutthroat trout from western Montana could not be differentiated from S. clarki lewisi from east of the Continental Divide except possibly for minor differences. He concluded that cutthroat trout from western Montana, as well as those from eastern Montana should be considered S. clarki lewisi. Both Hanzel (1960) and Brown (1971) have avoided the problem of the subspecific classification of S. clarki in Montana; they do not use subspecific names.

The difficulties surrounding the subspecific classification of S. clarki, as well as many other species, results from the continuity of the evolutionary process. Mayr (1971) states, "All findings agree that in every actively evolving genus there are populations that are hardly different from each other, others that are as different as subspecies, others that have almost reached species level, and finally still others that are full species." For two populations that are geographical isolates Mayr states that mutation, recombination and selection will be different and independent in the two areas and that an increasing genetic divergence between the two populations is

inevitable.

To what degree such a genetic divergence occurs depends upon several factors, one of which is the amount of time the populations involved were geographically isolated. Svardson (1961) found that during various Pleistocene glaciations the production of sibling species of sculpin (Myoxocephalus) and smelt (Osmerus) occurred in Eurasia in as short a time as 60,000 years due to geographic isolation. The cutthroat trout populations of the headwaters of various major drainages in Montana may have been genetically isolated from each other for several thousands of years and the Yellowstone cutthroat has likely been geographically isolated from other populations of cutthroat trout in Montana since before the last glaciation, and perhaps as long as 25,000 to 50,000 years (Behnke, personal communication). Because different populations of cutthroat have been isolated for different lengths of time, it should be possible to find populations of native cutthroat that hardly differ from each other, others that are as different as subspecies and others which have almost reached the species level. This complicates the task of categorizing populations of cutthroat trout in Montana.

Difficulties in the Use of Classical Methods of Taxonomy for Fish

In the past, taxonomists have utilized meristic characters, spotting pattern and coloration for the categorization of fish populations. Lists of characters typical of cutthroat and rainbow trout, such as those in Table 1 (from Schreck & Behnke 1971), are used to make such determinations. As can be seen from the table,

there is an overlap between S. clarki and S. gairdneri for the ranges of each of the meristic characters listed. Thus it would be impossible to classify those fish that were intermediate with respect to many of these meristic characters. Several studies have established that meristic characters exhibit phenotypic variations in fish due to environmental changes during early developmental stages (Hubbs 1922, Taning 1952, McHugh 1954, Lindsey 1958, Seymour 1959, Barlow 1961, Garside 1966). No doubt these environmentally induced variations account in part for the problems involved in using meristic characters to distinguish the two species of trout. Jordan and Evermann (1937) pointed out that coloration in Salmoninae is subject to great variation and that consequently this character rarely assists in distinguishing between the species. When dealing with subspecies of cutthroat trout, Zimmerman (1965) found coloration to be so variable that it was useless as a primary means of taxonomic separation.

Meristic characters, coloration and spotting patterns may be successfully used by experienced personnel to distinguish species of Salmo, if the populations sampled are "pure". However, this is seldom the case in Montana, since hybridization between S. clarki and S. gaidneri is so widespread (Hanzel 1960, Brown 1971).

The problem then becomes whether or not meristic characters, spotting patterns and coloration can be used to consistently detect hybrids in a given population of trout. After studying hundreds of specimens of natural fish hybrids representing dozens of interspecific and often intergeneric combinations in several families, Hubbs (1940) found that as a general rule the systematic characters

of fishes show blending inheritance, the phenotypes of hybrids appearing intermediate to those of the parents. He believed this type of inheritance also operated when subspecies and races of fishes were crossed and when backcrosses were made. This would indeed be expected for polygenic characters. Anderson (1953) found the effects of hybridization under natural conditions to be difficult to detect. He believed that backcrosses tend to resemble the recurrent parent so strongly as to pass unnoticed by naturalists and monographers.

Several factors would be involved in the dispersion and frequency of genes that have entered a population as a result of introgression, that is, as a result of genes of a different species entering a gene pool by the backcrossing of F_1 hybrids. These factors would thereby regulate the ease with which hybridization could be detected. For adjacent populations of rainbow and cutthroat trout with a zone of overlap, for example, the magnitude of introgressive hybridization would depend upon the fitness of the hybrids in the parental environments, as well as the length of time that the two populations had been in contact. In the case of hybridization following the introduction of one species within the range of distribution of another, the number of individuals introduced becomes an important additional factor in determining the dispersion and frequencies in the population of the native species of genes introduced by introgression.
In light of the variation in the amount of introgressive hybridization which may occur in populations of trout, the inherent variability of meristic characters, spotting patterns and coloration in trout, and the apparent mode of blending inheritance of these

characteristics, it would appear impossible for even the expert fish taxonomist to detect introgressive hybridization in trout populations by the use of classical methods of taxonomy.

Electrophoretic Detection of Intraspecific and Interspecific Variation in Fish Populations

Northcote et. al. (1970) used starch gel electrophoresis to investigate phenotypic variation in lactate dehydrogenase present in the liver of rainbow trout taken from above and below a waterfall on Kokanee Creek in British Columbia. One form was predominant in the population of trout from above the falls and another was predominant in fish below the falls. Meristic characters also showed significant differences between trout from above and below the falls. Several other studies have successfully used electrophoresis to investigate intraspecific variation in fish populations (Ridgeway et. al. 1970, Wright et. al. 1970, Eckroat 1971, Payne et. al. 1971, Nyman and Pippy 1972, Stegeman and Goldberg 1972, Morgan and Koo 1973).

Nyman (1970) crossed Atlantic salmon (S. salar) and brown trout (S. trutta) and examined the resulting hybrids using both biochemical and morphological methods. The analysis of protein variation by means of starch gel electrophoresis revealed "more or less complete summations" of the parental patterns in the F_1 hybrid in the case of 17 of the systems examined. Nyman points out that most protein systems were more reliable than morphological characters in identifying F_1 hybrids, but that their efficiency was equally low for the detection of F_2 hybrids. Other studies have also used electrophoresis

for the examination of phenotypes resulting from the hybridization between species of fish (Hitzeroth et. al. 1968, Chen and Tsuyuki 1971, Morrison 1970, Wheat et. al. 1971, Whitt et. al. 1971, Metcalf et. al. 1972, Whitt et. al. 1973). A more extensive review of serological and biochemical studies on fish populations is given by de Ligny (1969).

The present study used electrophoretic techniques to determine the amount of intraspecific variation within and differentiation between selected populations of cutthroat trout in Montana, and to find biochemical interspecific differences between rainbow and cutthroat trout. Starch gel electrophoresis was chosen for this study because of the success with which this technique has been used in the past. It is hoped that this study will help clarify the taxonomic status of the cutthroat trout in Montana, and prove useful in implementing programs for the management of native cutthroat trout.

Chapter II

MATERIALS AND METHODS

Sampling of Trout Populations

In choosing the sites from which to take samples of trout, a number of factors were considered. Several of the major subdrainages of the Clark Fork of the Columbia River were chosen for sampling in order to determine the amount of genetic variation between populations from different geographical areas. For the same reason, samples were also taken from the headwaters of the Missouri River and from the headwaters of the Yellowstone River. In all, populations of three types of trout, that are visually distinguishable, were sampled--westslope cutthroat, Yellowstone cutthroat and rainbow trout.

An attempt was also made to locate streams with a high probability of containing "pure" populations of native cutthroat trout. National Forest Maps of the U. S. Department of Agriculture, Forest Service, were consulted in order to determine sites suitable for sampling. By their use, barriers to the movement of trout within streams were located. Beaver dams, steep gradients (500 to 1500 ft/mi) and waterfalls (of 4 ft or greater) constitute such barriers (Hanzel 1960). Stocking records were obtained from the Montana State Department of Fish and Game to confirm suspected introductions of exotic species into certain streams and lakes. Samples were then taken upstream from barriers in streams which had no records of being stocked with rainbow trout. This sampling procedure was followed in order to minimize the possibility that introgression had occurred

between populations of the native cutthroat trout located upstream to barriers and rainbow trout which may have moved into waters below such barriers. In addition, a few sites, such as those on Rock Creek and Odell Creek, were chosen because of the high probability that they contained rainbow and cutthroat hybrids. The sites sampled in this study are described in Table 2 and shown in Fig. 1. Trout were also collected from two state hatcheries in Montana. Two stocks were sampled at the hatchery at Big Timber: cutthroat trout from McBride Lake (24) and Yellowstone cuttthroat trout from Yellowstone Lake (25). At the hatchery at Arlee, stocks of rainbow trout (26) and westslope cutthroat trout (27) were sampled. The brood stock of westslope cutthroat trout at Arlee was taken from Hungry Horse Creek and another nearby stream in the Flathead drainage.

Trout were collected by several methods. Those collected at hatcheries were taken by a dip net. Those collected in Yellowstone Lake were taken with a gill net. In streams, trout were collected either by angling or the use of an electric backpack shocker (whenever the site to be sampled was not more than five miles from the end of a forest road). In this manner a total of 547 trout were taken from 25 different locations during 1972 and 1973.

Treatment of Captured Trout

The trout were transported alive in a bucket of stream water to the place where they were to be processed. This was usually in an open area near the stream, where the necessary equipment could be more easily used. The fish were then anesthetized in a solution of

tricaine methane sulfonate (M.S.-222) and their total length was recorded. In order to record characteristic spotting and color patterns for later examination, color slides of all fish were taken while they were still alive using 126 Ektachrome film.

Blood samples were taken from captured trout by making a longitudinal incision from the isthmus to a line connecting the most posterior points of the pectoral fins and inserting a capillary pipette directly into the pericardial cavity for the collection of approximately 1 ml of blood. The blood was placed in 1 ml plastic tubes and centrifuged in a Fisher centrifuge at 5000 g for 2 minutes when the procedure was preformed in the laboratory. In the field, a D. C. centrifuge powered by a 12-volt airplane battery was used. After centrifugation the serum was separated from the blood cells and stored in 0.5 ml Beckman microfuge tubes (Beckman, Pequannock, N. J.). The serum was immediately frozen by placing it in a vacuum flask containing dry ice and was later transferred to a freezer which maintained a temperature of -40^{0}C. The cells were washed and centrifuged with Bacto hemogluttination buffer (Difco Laboratories, Detroit, MI.) twice. A few drops of deionized water was then added, and the cells were transferred to a capped microfuge tube and frozen. Note was made of the sex of the fish after it had been bled. Those fish that could not be sexed without the use of a microscope were classified as "immature".

Electrophoretic Techniques

In an electric field, proteins migrate at a rate dependent upon

their charge-to-mass ratio. By using a starch gel as a supporting medium, proteins may also be separated if they vary in molecular size. This is due to the porous structure of the gel which acts as a molecular sieve (Smith 1968).

Vertical starch gel electrophoresis (Smithies 1959) was used to examine the proteins in the serum of trout. Approximately 44 g of hydrolyzed starch (Electrostarch Co., Madison, WI.) and 400 ml of an appropriate buffer were mixed in a 2 litre conical flask. The starch gel was made in the manner described by Smithies (1959) and then poured into Hiller starch gel trays (O. Hiller, Madison, WI.). Two trays used were as described by Smithies (1959) with the addition of a cooling chamber on one surface of each tray. After pouring the gel into these trays, plastic covers with two rows of slot formers were applied to the gel surface. The third tray, with cooling chambers on each side of the gel, was as described by Azen and Smithies (1969). A plastic and glass cover was applied to the third apparatus after pouring the gel, and only one row of slots could be formed. The gels were allowed to cool for 1 h before removing the gel covers. Each slot former made 16 slots, 4 mm wide x 1 mm across x 3 mm deep.

Serum was loaded into the slots in each gel with a 1 ml tuberculin syringe and a 25-gauge needle. Molten petroleum jelly was used to cover the slots and plastic wrap was used to cover the exposed portion of each gel to prevent dehydration. The gel tray was then set vertically in the lower electrode chamber. Filter paper was used to form a bridge between the two compartments of each

electrode chamber, and also used to connect the upper electrode chambers to the exposed upper end of each gel. 500 volts of direct current was then passed through the gels for 4 h. The amperage at the beginning of electrophoresis ranged from 30 to 50 ma, depending upon the buffer used. Coolant at 4^0C was passed through the cooling chambers during the operation of the apparatus. The two end slots of each row were not used. Using the three sets of starch gel apparatus, 70 serum samples could be processed at one time.

At the end of the 4 h period of electrophoresis, gels were carefully removed from the trays with a spatula and placed in a container to be stained.

Serum Proteins Chosen for Study

Serum proteins, esterases, hemoglobins and lactate dehydrogenases (LDH) stained clearly and were examined in this study. In Table 3 are shown the buffers used to separate these proteins and in Table 4 are shown the stains used to identify them.

The serum protein transferrin was identified by the iron stain developed by Ornstein (no date) shown in Table 4. The gels remained in this stain for 18 h, after which distinct white bands were visible. The gels were then stained in amido black as described in Table 4. Only the white bands already present stained clearly, the remaining proteins having diffused in the gel while in the iron stain. It seems likely that the white bands represented the original position of the transferrins, which had formed a complex in the iron stain too large to diffuse in the same way as the other proteins.

The banding pattern of each gel was recorded by photographing with 35 mm Kodak High Contrast Copy film. The gels were placed in plastic wrap to prevent dehydration and were stored in a refrigerator for later examination.

Numerical Taxonomy

The phenetic relationships between populations (that is, their similarities based on a set of phenotypic characteristics) were examined by means of squared Euclidean distance, a measure of difference described by Sneath and Sokal (1973). The formula for the calculation of squared Euclidean distance (Δ^2) between the two populations (j and k) is

$$\Delta^2_{jk}=\sum^{n}(X_{ij}-X_{ik})^2$$

where X is the frequency of occurrence of the ith character and n is the number of characters used in the comparison. By this method, pairs of populations are compared with respect to their known characteristics, so that they can be ordered in a hierarchy on the basis of the degree of dissimilarity between them.

A computer program was written to calculate squared Euclidean distance (Δ^2). The distances were used to construct a t x t matrix, where t is the number of operational taxonomic units, or OTU's, involved in the study. An OTU is the lowest ranking taxon employed--in this case a population. Only one diagonal half of such a matrix is used since the two halves contain identical information.

From the various hierarchical grouping strategies given by

Sneath and Sokal (1973), the UPGMA (unweighted pair-group method using arithmetic averages) was chosen because of the advantages it possesses.

The first step in UPGMA is to locate from the matrix, populations which are reciprocally least dissimilar (i.e. produce the smallest Δ^2), thereby forming the first groups of populations in the hierarchy. When a group is formed it is added to the matrix, and the average distance of this group to all remaining populations is calculated. Further fusions between populations and groups of populations that are reciprocally least dissimilar are subsquently then made at successively greater levels of dissimilarity, until the hierarchy is completed.

The formula

$$\Delta^2_{JK}=\left(\frac{1}{t_J\ t_K}\right)\sum^{n}\Delta^2_{jk}$$

is used to compute the average squared Euclidean distance, Δ^2_{JK}, between any two groups of populations, J and K, where t_J is the number of populations in group J, and t_K the number of populations in group K. This formula must be used when no previous comparison between the populations of the two groups has been made. However, if the values of Δ^2 between groups J and L and between groups J and M have been calculated, and if groups L and M fuse to form a new group, K, the formula

$$\Delta^2_{JK}=\frac{t_L(\Delta^2_{JL})+t_M(\Delta^2_{JM})}{t_L+t_M}$$

may be used to calculate the squared Euclidean distance between groups

J and K. This avoids the need to calculate the values of Δ^2 between all pairs of populations in groups J and K. This formula can also be used when J is a single population rather than a group of populations.

A hierarchy produced in the manner described above can be represented diagrammatically in a phenogram (a dendrogram indicating phenetic relationships), so that the results can be easily examined.

For the purpose of grouping phenotypes, information analysis as described by Sneath and Sokal (1973) was used. However, instead of using an agglomerative sorting strategy, in which t separate entities are grouped into successively fewer sets until a single set containing all t entities is formed, a divisive sorting strategy was employed, as suggested by Lambert and Williams (1966). In a divisive sorting strategy, t entities in a set are subdivided into subsets, which in turn are subdivided to some preset level of information or until there are t separate entities.

The first step in the analysis is the construction of an n x t matrix, where n is the number of characters observed in the total number of phenotypes, t. Each entry in the matrix, X_{ij}, therefore indicates if the ith character (in this case a protein), is present or absent in a given phenotype.

Next using the general formula

$$I_H = nt_H \ln t_H - \sum^{n} [a_{iH} \ln a_{iH} + (t_H - a_{iH}) \ln (t_H - a_{iH})]$$

where t_H is the number of phenotypes in taxon H (initially the entire matrix) and a_{iH} is the number of phenotypes in taxon H possessing the ith character, a value for the total amount of information in the

matrix is calculated.

For each character under consideration, the matrix is then divided into two taxa--one, J, possessing the positive state and the other, K, the negative state for the ith character, (i.e. one matrix in which the character is present and the other in which it is absent). Using the above formula, the total amount of information in the taxon possessing the positive state for the ith character, I_J, and the total amount of information in the taxon possessing the negative state for the ith character, I_K, are calculated for each of the characters incorporated in the matrix.

By using the formula

$$\Delta I_i = I_H - (I_J + I_K)$$

where ΔI_i equals the increase in information created by joining taxa J and K for a given character and I_H is the total information contained in the matrix created by joining taxa J and K, the character for which ΔI is maximal can be found. On the basis of the presence or absence of this character in the phenotype, the first division in the hierarchy is made at level of information I_H: one of the branches gives rise to all phenotypes in which it is absent. The two taxa formed in the manner described are each more homogenous (and therefore possess less information) than any other two taxa that could be created by a single division of the matrix. Both contain phenotypes resembling one another more than any other possible grouping of phenotypes based on the presence or absence of one character.

The formation of the hierarchy continues by treating the

matrices resulting from the first division in the same manner as was the original matrix. That is, each of these new matrices is divided into two parts on the basis of the presence or absence of one of the remaining characters: that for which ΔI_i is greatest. The characters used to make the second division in one of the matrices may differ from that used in the other. This process is repeated until all characters have been incorporated into a particular sequence of the hierarchy or until a calculated value of I_H drops below a predetermined level of significance. This can be determined from a table of chi-square values, because 2I is approximately distributed as chi-square with n degrees of freedom, where n equals the number of characters used.

This process results in the formation of a monothetic sequential key which allows phenotypes to be grouped at appropiate levels of information, I_H, on the basis of the degrees of relatedness between them.

Chapter III

RESULTS

Appearance of Sampled Trout

From an examination of field notes and color slides of the trout sampled, it appeared that populations 1-16, 18-20, and 22 (Fig. 1 and Table 2), as well as the stock of westslope cutthroat trout at the Montana State Fish Hatchery at Arlee (27), contained mainly trout with spotting patterns, coloration and shapes similar to those depicted by Brown (1971) as the westslope cutthroat trout. However, these characters were highly variable in most of the populations sampled.

Trout from Lower Elliot Lake (16) had spots confined exclusively to posterior of the dorsal fin, while those from Straight Creek (6) were heavily spotted over the entire body with the exception of the belly area. Fish in the remaining populations listed were a mixture of trout with either of these two extremes of spotting pattern and trout with intermediate spotting patterns. The size of spots found in these populations varied considerabl . For example, trout from Deer Creek (4) that had a body length of approximately 17 cm had spots from less than 1 mm to 3 mm in diameter.

The coloration of the trout from the listed populations generally appeared to vary in accordance with the density of the canopy of the stream from which they were taken. For example, Bear Trap Creek (12) had an open canopy and the fish taken from it were of a silvery color, while Little Stony Creek had a tight canopy and trout there were

considerably darker in color. Within-stream variation in color was also apparent. Trout visually classified as rainbow trout from Rock Creek (17) and those from the hatchery at Arlee (26) did not differ in spotting pattern, coloration or spot size to any considerable extent.

Trout taken from Yellowstone Lake (23) and the stock from Yellowstone Lake at the Montana State Fish Hatchery at Big Timber (25) were typical of the trout depicted as Yellowstone cutthroat trout by Brown (1971). The majority of trout from Overwhich Creek (21) also matched the description of Yellowstone cutthroat trout.

The stock of trout from McBride Lake at the hatchery at Big Timber (24) did not uniformly resemble westslope cutthroat trout or Yellowstone cutthroat trout, but appeared to be a mixture of these forms and their intermediates.

The Serum Proteins-Qualitative Analysis

Patterns of hemoglobins obtained from the washed cells of rainbow trout taken from the hatchery at Arlee differed from those of cutthroat trout from the same source. Proteins migrating anodally appeared to be identical in the two species while those migrating cathodally showed extensive differences. As indicated by Fig. 2 and 3, the rainbow trout possesses four proteins (L,N,S and U) not present in the cutthroat trout, and the cutthroat trout possesses five proteins (M,O,R,T and V) not present in the rainbow trout. Two proteins (P and Q) migrating cathodally were common to both. Very similar differences between the patterns of migration of hemoglobins in rainbow and cutthroat trout were demonstrated by Tsuyuki *et. al.* (1965). Unfor-

tunately, consistently good separations of the bands of hemoglobin proved impossible in the case of most of the populations sampled, and therefore further analysis of this protein was not attempted.

Nine distinct forms of lactate dehydrogenase (LDH) were observed in the serum of the trout sampled following electrophoresis and appropiate staining. Some stained weakly, and all nine were not invariably detectable. Massaro and Markert (1968) surveyed the LDH isozymes of certain salmonids, and found that the blood of rainbow trout usually contained five forms of LDH which they called group b. However, other groups of five LDH isozymes were sometimes present in the blood, perhaps as a result of leakage of the highly soluble enzyme from other tissues. The presence of up to nine forms of LDH in the serum of the trout sampled in this study might be explained on the basis of two groups of five isozymes having one form of the enzyme in common.

Because of the variability in the staining intensity of the isozymes of LDH, and the consequent problems encountered in their detection, variation in this enzyme was not considered in subsequent analysis.

Esterases were polymorphic in some populations sampled, but monomorphic in most. This result differs from that of Nyman (1971), who concluded that esterases were monomorphic in rainbow trout. In all, six esterases were distinguished by means of electrophoresis of serum and appropriate staining in this study (Fig. 3). These were numbered 4-9 in order of decreasing rate of anodal migration. Four different banding patterns for esterases were observed--4,5; 4,5,7,9;

4,5,6,8 and 6,8 (Fig. 4). In Table 5 are shown the frequencies of these phenotypes in the various populations sampled.

Esterase phenotype 4,5 had a high frequency of occurrence in both westslope cutthroat and rainbow trout. It occurred with a frequency of 1.00 in all but three of the populations visually classified as westslope cutthroat trout, and occurred wtih frequencies of 1.00 and 0.68 in the two populations visually classified as rainbow trout. Phenotype 4,5,7,9 was unique to trout visually classified as rainbow trout, and phenotype 6,8 was unique to those populations visually classified as Yellowstone cutthroat trout.

The hypothesis was tested that the esterases observed in this study were controlled by three alleles at a single locus, with each allele responsible for a pair of bands seen on the starch gel. The observed frequencies of esterase bands observed in the serum of trout from Overwhich Creek matched exactly the frequencies expected according to the Hardy-Weinberg Law, and a chi-square test revealed that in Congdon Creek, frequencies did not differ significantly from those expected according to the Hardy-Weinberg Law ($p > 0.3$). Observed frequencies of esterases in rainbow trout from the hatchery at Arlee, also match frequencies expected according to the Hardy-Weinberg Law ($p > 0.1$). These findings are consistent with the proposed hypothesis. Esterases 4 and 5 appear to be the products of one allele, with one of them probably a breakdown product of the other. Esterases 6 and 8 appear to be the products of a second allele, and 7 and 9 the products of a third.

Since esterases 4 and 5 were observed with 7 and 9 in population

26 and with 6 and 8 in populations 19 and 21, it appears that the alleles controlling the esterases were codominant. Nyman (1971) crossed chum with sockeye salmon and concluded that their esterases were also inherited in a codominant fashion.

Several serum proteins were observed when the starch gel was stained with amido black (Fig. 5), but only seven of these could be detected with any consistency (see Fig. 7). These were named C,D,E,F, G, H and Y in order of decreasing rate of migration toward the anode. The frequency of occurrence of these proteins is given in Table 5, and Table 6 lists the 36 phenotypes of serum proteins that were observed.

From Table 5 it appears that protein C only occurred with a frequency greater than 0.05 (that is, in more than 5% of the fish sampled) in those populations visually classified as Yellowstone cutthroat trout. Protein D occurred, with varying frequencies, in all but one population. Protein E was present in six of the 21 populations visually classified as westslope cutthroat trout and it occurred with a frequency greater than 0.73 in all populations visually classified as rainbow or Yellowstone cutthroat trout. Protein F occurred with a frequency greater than 0.37 in all population visually classified as westslope trout and with a frequency less than 0.18 in all populations visually classified as rainbow or Yellowstone cutthroat trout. Protein G occurred in high frequencies in all populations classified as rainbow or westslope cutthroat trout, but was totally absent in two of the three populations visually classified as Yellowstone cutthroat trout. Protein H was absent in 17 of the 21

populations classified as westslope cutthroat trout and was absent in all populations visually classified as Yellowstone cutthroat trout. It was present with a frequency greater than 0.86 in both populations classified as rainbow trout. Protein Y occurred with a frequency of 1.00 in 19 of the 27 populations sampled, and with a frequency greater than 0.4 in five of the remainder.

The Serum Proteins-Quantitive Analysis

On the basis of a chi-square contingency test, no signigicant differences were found between the sexes with respect to the banding frequencies of serum proteins in the 52 rainbow trout taken from the hatchery at Arlee ($p > 0.8$). This was the only population tested in this way. All other samples consisted mainly of immature fish whose sex could not be accurately determined in the field.

Relationships between the populations sampled, based on the frequencies of occurrence of individual serum proteins and esterases, are shown by the phenogram in Fig. 8. In Fig. 9 is shown a phenogram based on the frequencies in the samples of the phenotypes created by a consideration of all of the serum proteins examined in this study. The frequencies of phenotypes resulting from a consideration of the esterases as well as the serum proteins (Table 7) were not used in the construction of this phenogram, because variation in the esterases added too many new phenotypes to the total already recognized. Preliminary analysis indicated that the inclusion of the esterase variation resulted in each population becoming so unique as to cause a breakdown in the grouping strategy so that unrelated groups of

populations were formed. Squared Euclidean distance, Δ^2, was the measure of difference and UPGMA the grouping strategy employed in both phenograms. In order to attach some level of statistical significance to the relationships drawn in Figs. 8 and 9, chi-square contingency tests were used to compute the probability (p) of the chance occurrence of the observed amount of variation between populations or groups of populations. Those probabilities below the 0.05 level are indicated on the phenograms. The tests compared numbers of individuals in two populations or groups or populations possessing the various proteins or phenotypes considered in the construction of the phenogram. Therefore, the validity of the phenogram itself was not tested, since it was constructed on the basis of the frequencies of proteins or phenotypes, without regard for sample size.

Both of the phenograms generally support the groupings of populations made on the basis of a visual examination of the sampled trout. The three types of trout recognized by visual inspection occur in separate groups in the phenograms. The phenogram in Fig. 8, which is based on banding frequencies, joins the group of all populations containing trout visually classified as westslope cutthroat trout (1-16, 18-20, 22 and 27) with the two populations containing trout visually classified as rainbow trout (17 and 26) at $\Delta^2=2.43$. This group of populations in turn joins with all populations containing trout visually classified as Yellowstone cutthroat trout or their hybrids (21 and 23-25) at $\Delta^2=4.53$.

The phenogram in Fig. 9, which is based on the phenotypic frequencies of the serum proteins, joins all but two of the populations

containing trout visually classified as westslope cutthroat trout with the two populations of trout visually classified as rainbow trout at Δ^2= 0.76. This group of populations in turn joins with two of the three populations containing trout visually classified as Yellowstone cutthroat trout at Δ^2=1.01.

The relationship shown by both phenograms of the three types of trout recognized on the basis of a visual inspection is surprising in that the logical conclusion to be drawn is that rainbow trout are biochemically more closely related to westslope cutthroat trout than are Yellowstone cutthroat trout. Yet Yellowstone cutthroat and westslope cutthroat trout are both classified as S. clarki, while rainbow trout are given the separate specific designation of S. gairdneri.

Further examination of Fig. 8, the phenogram based on protein frequencies, reveals that of the group of populations 5, 9-15, 18 and 27 (the sample of westslope cutthroat trout obtained from the hatchery at Arlee) formed at Δ^2=0.06, only populations 9 and 10 contained trout with proteins found in high frequencies in rainbow or Yellowstone cutthroat trout. Only three trout possessed such proteins in these two populations. There was a geographical relationship between a majority of the populations in this group. Populations 9-14 all occurred in streams which flow into the Blackfoot River above its junction with the Clearwater River (Fig. 1). In Fig. 8, populations 1-4 are grouped at Δ^2=0.04. All of these samples were taken in the vicinity of Thompson Falls, Montana.

From Fig. 8, which is based on protein frequencies, it appears that trout from Odell Creek (22), which is located at the headwaters

of the Missouri River east of the Continental Divide, are more similar to the group of populations of westslope cutthroat from west of the Continental Divide than they are to cutthroat trout from Yellowstone Lake, in the Missouri drainage.

Further examination of Fig. 9, the phenogram based on phenotypic frequencies, reveals many of the same relationships shown by Fig. 8, which is based on protein frequencies. In Fig. 9 populations 5, 11-14, 18 and 27 again group at a low value of Δ^2, but populations 9, 10 and 15 are excluded. As in Fig. 8, populations 1-4 group at a low value of Δ^2, population 22 taken from east of the Continental Divide groups at a low value of Δ^2 with populations of westslope cutthroat trout taken from west of the Continental Divide, and populations visually classified as rainbow trout are more closely related to the populations of westslope cutthroat trout than are populations of Yellowstone cutthroat trout.

The phenogram in Fig. 9, which is based on phenotypic frequencies, in differs from that in Fig. 8, based on protein frequencies, in that populations populations 7 and 20, containing trout visually classified as westslope cutthroat trout, join all other groups of populations at a high value of Δ^2. Furthermore, in Fig. 9 population 21 containing trout visually classified as Yellowstone cutthroat trout, joins directly with populations 17 and 26, containing trout visually classified as rainbow trout, whereas in Fig. 8 population 21 grouped first with populations 23, 24 and 25, which also contained trout visually classified as Yellowstone cutthroat trout or their hybrids.

It should be noted that Fig. 9, unlike Fig. 8, was constructed from data that distinguish between the situations in which rare genes

are dispersed among many trout or concentrated in a few, possibly of a different species.

Phenotypic Analysis by Means of Information Theory

In Fig. 10 a monothetic sequential key is presented for the various phenotypes of trout observed in this study. In this key, the ultimate position of phenotypes is determined by the presence or absence of particular serum proteins and esterases. The result is the placing of phenotypes in groups which contain a lower total level of information (I) than any other groups which could possibly be formed from the same phenotypes.

Since 2I is approximately distributed as chi-square with n degrees of freedom, where n equals the number of characters used, if two groups join at a given level of information, it is possible to attach a level of significance to the relationship given. Since ten characters were used in the construction of the key, groups which join at $I > 9.15$ differ at or below the 0.05 level of significance. Only groupings that occur at $I > 9.15$ are shown.

Table 7 gives the number of individuals in each sample of trout possessing a particular phenotype. From this table, it can be seen that the phenotypes of the first group on the left of the key in Fig. 10 were found only in trout from Congdon Creek (19), which were visually classified as westslope cutthroat trout. The phenotypes of the second group were found only in fish visually classified as Yellowstone cutthroat trout. The same is true for the third, fourth and fifth groups of phenotypes, with the exception of phenotype 47 in

the fourth group, which was found in a single trout from Congdon Creek (19). The phenotypes of all trout from populations 23-25 were found in the second, third and fourth groups. Therefore, all phenotypes common to Yellowstone cutthroat trout were found in groups 2-5 of Fig. 10, indicating that esterases 6 and 8 were present in all Yellowstone cutthroat trout sampled in this study. The only fish visually classified as other than Yellowstone cutthroat trout to possess esterases 6 and 8 were seven individuals from Congdon Creek (19) and those at the hatchery at Big Timber (24).

All phenotypes in the sixth and seventh group in the key were found only in the rainbow trout from the hatchery at Arlee. Phenotypes in the eighth group were found in populations 8, 17 and 26. Populations 17 and 26 consist of trout visually classified as rainbow trout. Trout from Greenough Creek (8) contained only one of the phenotypes in the eighth group, and that was present in only a single individual.

With the exception of the single trout from Greenough Creek, phenotypes of the sixth, seventh and eighth groups, were found only in those trout visually classified as rainbow trout. Thus phenotypes lacking esterases 6 and 8 and possessing serum proteins E and H appear very common to fish visually classified as rainbow trout. Furthermore, all sampled fish lacking esterases 6 and 8 and possessing esterases 7 and 9 and serum proteins E, G and H were visually classified as rainbow trout. Of the 71 trout of populations 17 and 26, which were visually classified as rainbow trout, 57 had phenotypes found in the sixth and eighth groups. Seven of the remaining trout

from these populations had phenotypes found in the tenth group, which contained only these phenotypes.

Phenotypes of the ninth group were found only in populations visually classified as westslope cutthroat trout--namely 2-4, 19 and 22. However, these phenotypes occurred only rarely in the populations containing them.

Phenotypes in the eleventh group were found only in populations 2 and 4, which were visually classified as westslope cutthroat trout. However, only one trout from each population possessed either of the phenotypes in this group.

Phenotypes in the twelfth, thirteenth and fourteenth groups represented 361 of the 386 fish from populations visually classified as westslope cutthroat trout. Only three trout from a population not so classified were included in these groups, and all three were from Rock Creek (17). Field notes taken at their capture revealed that two of these three fish appeared as rainbow trout but had a bright orange cutthroat slash mark, indicating that they were probably hybrids between rainbow and westslope cutthroat trout. The third fish appeared to be a westslope cutthroat trout--the only one in the 15 fish sampled to appear so. Thus, all the sampled fish with phenotypes in the twelfth, thirteenth and fourteenth groups were visually classified as westslope cutthroat trout or as hybrids between westslope cutthroat trout and rainbow trout.

A total of eight fish possessed the phenotypes found in the fifteenth group. Seven were visually classified as rainbow trout, and one was classified as westslope cutthroat trout.

In summary, the key in Fig. 10 separates all fish visually classified as Yellowstone cutthroat trout from all fish classified as rainbow trout and from most fish classified as westslope cutthroat trout at a high level of information (I=326). Furthermore, the majority of fish classified as rainbow trout are also separated from those visually classified as westslope cutthroat trout at a high level of information (I=174). All other divisions simply separate groups containing rare phenotypes from those containing common phenotypes.

Chapter IV

DISCUSSION

Sibley (1962) made the following observation: "Since protein molecules are the principle morphological units of the animal body at the molecular level of organization, it follows that their form and structure are as relevant as sources of genetic and phylogenetic information as are the muscles, bones, organs, skin, hair, feather, and other structures which themselves are composed largely or entirely of protein molecules." If the relationships established by classical procedures are valid, it should be possible to confirm them by biochemical analysis. In this study, the three types of trout recognized by means of classical procedures are indeed separable on the basis of their biochemical differences--a high correlation exists between the groupings of populations made from a consideration of the frequencies of individual proteins or overall protein phenotypes and those made on the basis of a visual examination of the trout.

The phenograms in Figs. 8 and 9 quantify the relationships between the populations of trout sampled in this study. Although each incorporates certain information omitted by the other, both are based on the same data (the proteins present in the individual trout) and it is therefore not surprising that they indicate similar relationships. The least anticipated conclusion drawn from an examination of the phenograms is that those populations visually classified as rainbow trout are biochemically more closely related to those visually classified as westslope cutthroat trout than are those visually classified as Yellowstone cutthroat trout.

The key in Fig. 10, separated those phenotypes characteristic

of Yellowstone cutthroat trout from those characteristic of rainbow and westslope cutthroat trout at almost twice the level of information at which phenotypes characteristic of rainbow trout are separated from those characteristic of westslope cutthroat trout. This result places in doubt the validity of the currently accepted classification based on classical procedures, which indicates that westslope cutthroat and Yellowstone cutthroat trout are members of the species Salmo clarki, while rainbow trout are member of the species S. gairdneri. More extensive biochemical analysis might verify the current classification, but until such a time the question of its validity remains. A similar situation is already known to exist in the Salmonidae, in that the masu salmon (Oncorhynchus masou) is biochemically more closely related to the rainbow trout than to other species of the genus Oncorhynchus (Utter et. al. 1973, Tsuyuki and Roberts 1966).

A number of explanations are possible for the observed relationships between the rainbow, Yellowstone cutthroat and westslope cutthroat trout. One possibility is that barriers to gene flow between populations of rainbow trout in the lower Columbia River and those of westslope cutthroat trout in the upper Columbia River have been less effective than barriers to gene flow between the former populations and those of the upper Snake River from which the Yellowstone cutthroat trout was apparently derived. If this were the case, and if different environmental conditions in the upper Snake and upper Columbia Rivers favored different genotypes, then the observed relationship might be predicted.

Another possibility is that the populations of cutthroat trout

with large spots now found in the upper Snake River and in Yellowstone Lake actually invaded the Snake River from the headwaters of a river system other than the Columbia River, thus accounting for the radical biochemical differences between the Yellowstone cutthroat and the westslope cutthroat trout.

A third explaination for the observed relationships between the three types of trout is that the populations of Yellowstone and westslope cutthroat trout sampled merely represent two extremes of a continuum that exists within S. clarki. If this were the case, the sampling of a wider range of populations of S. clarki, followed by the type of grouping strategy that has been performed in this study, would be expected to show that populations of Yellowstone cutthroat trout and westslope cutthroat trout group before either join with populations of rainbow trout.

Many other plausible explanations for these results could no doubt be given. However, in the absence of further research it would not be possible to determine which, if any, was correct.

Several other relationships between populations are apparent from the phenograms in Fig. 8 and 9, and are worthy of note. As previously mentioned, populations 1-4 exist within a circle 25 mi in diameter in the area of Thompson Falls. In both phenograms these populations group at a low squared Euclidean distance (Δ^2) and chi-square tests indicate no significant differences between them with respect to the frequencies of proteins or phenotypes. In Fig. 8 populations 9-14 group at a low value of Δ^2 and chi-square tests show that they do not differ significantly. This group of populations

occurred in streams which were tributaries to the upper Blackfoot River. In Fig. 9 a similar relationship between these populations can be seen, although populations 9 and 10 are omitted from the group. Thus the method of biochemical analysis used in this study appears to group populations according to their locations to some extent--certainly to a greater extent than expected by the use of meristic characters, spotting patterns and coloration of sampled trout. However, factors unique to a given stream could easily confuse the geographical relationships between populations. This could explain the omission of population 5 from the group containing populations 1-4, and explain why populations 6 and 7, or 18 and 19, did not group at a lower value of Δ^2 in the phenograms.

Populations 5, 11-14, 18 and 27 join at a low value of Δ^2 in both phenograms. Each individual in the samples taken from these seven populations was visually classified as a westslope cutthroat trout, and none expressed biochemical phenotypes characteristic of trout visually classified as rainbow or Yellowstone cutthroat trout. Stocking records of the Montana Department of Fish and Game give no indication that rainbow or Yellowstone cutthroat trout were stocked in any of the streams from which these samples were taken. State records also indicate that the brood stock of westslope cutthroat trout at the hatchery at Arlee (27) is the only one considered "pure" in the state hatchery system. All phenotypes that occurred in populations 5, 11-14, 18 and 27 were found in the twelfth, thirteenth and fourteenth groups of the key in Fig. 10. In view of these facts it seems reasonable to refer to these seven populations as consisting

of "pure" westslope cutthroat trout; that is, westslope cutthroat trout not possessing in high frequencies genes normally found in such frequencies only in rainbow or Yellowstone cutthroat trout. In short, these populations of westslope cutthroat trout show no evidence of introgressive hybridization with trout of other types.

It is of some interest that several of these populations of "pure" westslope cutthroat trout, notably 11 and 12, contained fish with a silvery coloration and small spots dispersed over most of the body. These traits are characteristic of the rainbow trout. Therefore, on the basis of color and spotting pattern, it might be concluded that these fish were hybrids, since each of them also possessed the bright slash mark characteristic of the cutthroat trout. However, the biochemical analysis of the trout from these populations revealed no indication of the presence of hybrids. Martin and Richmond (1973) collected a sample of two species of darter (Percidae), 11.6% of which appeared on the basis of morphological characteristics to be hybrids. However, a biochemical analysis of the sample revealed that less than 3% were hybrids. This would appear to indicate that "pure-bred" fish may often be mistaken for hybrids on the basis of morphological criteria. The alternative explanation that could be drawn from the results--that hybrids are more likely to be detected by an examination of gross phenotype than by the biochemical analysis of direct gene products--seems less likely.

On the supposition that populations 5, 11-14, 18 and 27 in fact contain "pure" westslope cutthroat trout, attention should now be given to the remaining 14 populations visually classified as

westslope cutthroat trout. In Fig. 8, populations 9, 10 and 15 join at a low value of Δ^2 with the group of seven populations considered to be "pure". This implies that little or no introgression with other types of trout has occurred in these three populations. All of the phenotypes that were found in these populations occur in the twelfth, thirteenth and fourteenth groups in the key in Fig. 10 as did those of the supposedly "pure" populations of westslope cutthroat trout. Stocking records indicate that both Chamberlin Creek (9) and Arrastra Creek (10) have had rainbow trout introduced into their lower reaches. The Little Blackfoot River (15) has never been stocked with rainbow or Yellowstone cutthroat trout.

There remain 11 populations of trout visually classified as westslope cutthroat trout. Of these, six (2-4, 8, 19 and 22) yielded trout which possessed proteins present in high frequencies only in rainbow or Yellowstone cutthroat trout (see Fig. 6) and which were completely absent in the seven populations considered to be "pure" westslope cutthroat trout. A plausible explanation for this result is that hybridization between westslope cutthroat trout and Yellowstone cutthroat or rainbow trout occurrred in these populations at some time in the past, and that the foreign genes and proteins have been retained. A similar conclusion was drawn by Payne *et. al.* (1972), following the finding that several specimens of the brown trout (*S. trutta*) taken in their native habitat possessed proteins found in high frequencies only in the Atlantic salmon (*S. salar*). Crosses between the two species confirmed that proteins characteristic of both parental types were present in the serum of the F_1 hybrids, and the probability

therefore appeared high that brown trout found to possess proteins normally occurring in high frequencies only in the Atlantic salmon were hybrids.

An alternative explaination to introgressive hybridization is that proteins normally present only in rainbow or Yellowstone cutthroat trout are typical of populations of westslope cutthroat trout, but in frequencies too low to be detected in relatively small samples. High frequencies of such proteins might be explained on the basis of the operation of natural selection in populations subject to atypical environmental conditions, or on the basis of genetic drift in populations isolated from others by a barrier to gene flow or founded by a small number of migrant individuals. However, evidence in favor of these hypotheses is lacking.

On the other hand, there is some reason to believe that the proteins normally found only in the other types of trout occurred in populations visually classified as westslope cutthroat trout as a result of hybridization. The stocking records of the Montana Department of Fish and Game indicate that Crystal Lake, which empties into Deer Creek (4), was stocked wtih rainbow trout from 1949 to 1953. Deer Creek was also scheduled to be heavily stocked with cutthroat trout several years ago, although there is no record that the stocking actually took place. Three of the 25 trout taken from Deer Creek had proteins not commonly found in fish visually classified as westslope cutthroat trout. In 1948, Cherry Creek (3) was also stocked with rainbow trout, and one of the 14 fish in the sample from Cherry Creek possessed a protein not normally present in westslope

cutthroat trout. Chippy Creek (2) was stocked in 1950 with cutthroat trout from the hatchery at Arlee. Prior to 1968, such trout were derived from a brood stock obtained from a number of sources including Georgetown Lake which had itself been stocked with Yellowstone cutthroat trout. Three of the 20 fish taken from Chippy Creek had proteins not common in westslope cutthroat trout. Medicine Lake, which empties into Congdon Creek (19), was stocked between 1948 and 1957 with cutthroat trout from the Montana State Fish Hatchery at Anaconda. These trout were known to be Yellowstone cutthroat trout. Ten of the 20 trout taken from Congdon Creek had proteins not characteristic of westslope cutthroat trout. No stocking records were found for Odell Creek (22) or Greenough Creek (8) each of which had trout with proteins not characteristic of westslope cutthroat trout. However, both creeks empty into rivers or lakes that contain rainbow trout and neither has any apparent barriers to the upstream migration of these trout.

There is no record of stocking for the remaining five populations of trout visually classified as westslope cutthroat trout. However, one of these occurred in Big Rock Creek (1), and grouped with other populations (2-4) in nearby creeks which had been stocked. Of the rest, three (6, 7 and 20) had unusually low frequencies of protein Y. The samples from Lolo Creek (7) and Straight Creek (6) were taken above waterfalls and were, therefore, from isolated populations. Thus the unusually low frequency of the protein Y in these populations could be explained on the basis of genetic drift. However, no such physical barrier was found on Moose Creek (2), although one could have been present that was not detected. Of course, natural selection

operating against protein Y in the populations of trout in these three streams is an alternate explaination for its low frequency.

Genetic drift or natural selection might also explain the unusually high frequency of two phenotypes (45DGY and 45DY) in the remaining population visually classified as westslope cutthroat trout--that from Elliot Lake (16), which is isolated from populations of trout in the stream draining the lake.

It was previously noted that in the phenogram based upon the frequencies of overall protein phenotypes (Fig. 9), the population in Overwhich Creek (21) grouped with those classified as rainbow trout (17 and 26) even though its trout were visually classified as Yellowstone cutthroat trout. However, a chi-square test did indicate that the phenotypic frequencies in the population from Overwhich Creek differed significantly ($p < 0.001$) from those of rainbow trout. The reason for this apparently anomalous situation appears to be that the population in Overwhich Creek has resulted from hybridization between introduced Yellowstone cutthroat trout and native westslope cutthroat trout. Records of the Montana Department of Fish and Game verify that Yellowstone cutthroat trout from the Montana State Fish Hatchery at Hamilton were stocked in Overwhich Creek. Since rainbow trout appear to group at a position intermediate to westslope and Yellowstone cutthroat trout on the basis of the phenotypic frequencies, it is not surprising that hybrids between Yellowstone and westslope cutthroat trout would also be located at an intermediate position in the phenogram.

It has also been mentioned that cutthroat trout from the

hatchery at Big Timber (24), which are derived from a brood stock taken from McBride Lake, appear from visual inspection to possess spotting characteristics of both Yellowstone and westslope cutthroat trout--the size of spots varied from large to small. Yet these trout group closely with those populations considered to be Yellowstone cutthroat trout in the phenograms in Figs. 8 and 9. Behnke (personal communication) has suggested that McBride Lake may have had an indigenous population of cutthroat trout before Yellowstone cutthroat trout were introduced. This would be a feasible explaination for the appearance of the trout presently found in McBride Lake, if the indigenous trout population of this lake consisted of a form of cutthroat trout with small spots, such as the westslope cutthroat trout. However, the esterase phenotype 4,5 was absent from the sample of trout from the hatchery at Big Timber, and it therefore seems unlikely that the indigenous fish of McBride Lake were westslope cutthroat trout. Possibly the indigenous trout were derived from the Snake River, in which exist populations of cutthroat trout with small spots. If biochemical analysis of the trout from the Snake River bore out this hypothesis, it would reinforce the value of biochemical analysis in providing insight into taxonomic relationships. Unfortunately, populations of cutthroat trout in the Snake River were not sampled for this study.

Behnke (personal communication) has stated that, on the basis of an analysis of meristic characters, the westslope cutthroat trout and trout from Silver Creek in the upper Missouri drainage are more closely related to each other than are either to Yellowstone cutthroat trout.

This relationship is also indicated in the phenograms in Figs. 8 and 9 where the population in Odell Creek (22) in the upper Missouri drainage groups with westslope cutthroat trout rather than with the Yellowstone cutthroat trout. Zimmerman (1965) could find no significant meristic differences between cutthroat trout from the upper Missouri River and those from western Montana. It would therefore seem appropriate to include westslope cutthroat trout with cutthroat trout from the headwaters of the Missouri River under the name Salmo clarki lewisi. The implication of these findings is that cutthroat trout originating in the upper Columbia drainage were forced from glacial lakes into the upper Missouri drainage. This suggestion was originally made by Behnke (personal communication). It had previously been believed that cutthroat trout found in the upper Missouri drainage entered the drainage via Yellowstone Lake (Jordan and Evermann 1937).

At this point, it is appropriate to discuss the value of the key of phenotypes encountered in trout sampled for this study (Fig. 10). The key gives an indication of the relative importance of the various proteins described in this study for the purpose of identifying trout as Yellowstone cutthroat, westslope cutthroat or rainbow trout, or hybrids thereof.

Esterases 6 and 8 are of the greatest importance in distinguishing Yellowstone cutthroat trout from westslope cutthroat and rainbow trout. No fish visually classified as a Yellowstone cutthroat trout lacked these esterases. Furthermore, no trout visually classified as westslope cutthroat or rainbow trout lacked esterases 4 and 5. Only in trout taken from streams in which Yellowstone cutthroat trout had

been planted although westslope cutthroat trout were already present--namely Overwhich Creek (21) and Congdon Creek (19)--did esterases 4, 5, 6 and 8 occur together. Esterases 7 and 9 occurred only in trout visually classified as rainbow trout with the exception of one individual visually classified as a westslope cutthroat trout. Thus, it would seem that esterases 4 and 5 are characteristic of westslope cutthroat and rainbow trout, esterases 7 and 9 are characteristic of rainbow trout, and esterases 6 and 8 are characteristic of Yellowstone cutthroat trout. Since the esterases appear to be controlled by three codominant alleles at a single locus they are very useful for detecting hybridization between the various types of trout. For example, the population in Overwhich Creek (21) would appear to have resulted from hybridization between Yellowstone and westslope cutthroat trout, because esterases 4, 5, 6 and 8 are all present.

Protein F was found only in those trout visually classified as cutthroat trout, or in rainbow trout possessing the bright cutthroat slash mark and therefore clearly hybrids. Protein H was not found in any trout visually classified as Yellowstone cutthroat trout, and the same was true of protein G except that it was present in trout from Overwhich Creek, presumably as a result of hybridization. Protein C never occurred in trout visually classified as rainbow trout, and protein E occurred only rarely in trout visually classified as westslope cutthroat trout. Only proteins D and Y appeared to be frequent in all three types of trout.

It would not always be possible to classify a fish as a westslope cutthroat, Yellowstone cutthroat or rainbow trout, or a hybrid, only on the basis of the proteins found in its serum. However, when the

serum proteins and enzymes are considered in addition to general morphology, it is likely that a better understanding of the genetic make-up of a population and of its taxonomic relationships with other populations would be obtained than if a judgement was based on either type of evidence alone.

As a final topic for discussion, it might be worthwhile to speculate upon the chances for survival of the westslope cutthroat trout. Behnke (1973) has stated that at least 99% of the native populations of S. clarki in the interior regions of the U.S.A. have been lost in the last 100 years, and Brown (1971) also concluded that a major reduction in the number of native cutthroat trout in Montana had occurred in that period.

Miller (1957) found that the home range for cutthroat trout in a small stream in Alberta was about 9-18 m in length. All stages of the life cycle occurred in the home range. Even when displaced by high waters, trout returned to their home range whenever possible. When a half-mile section of stream was poisoned below an area populated with cutthroat trout for several years in succession, only about 12 to 50 trout drifted down from upstream into this area in any given year. Such an area would normally have a population of about 500 cutthroat trout. Upstream migration of cutthroat trout also appeared to be very limited. Assuming that similar circumstances are found in the small high mountain streams of Montana, one would rarely expect to find movement of great distances by westslope cutthroat trout under normal conditions in these streams. Therefore if hybridization with rainbow trout, which would presumably migrate upstream only far enough

to find suitable breeding grounds, occurred in the lower sections of various streams, one would expect the upstream flow of genes from rainbow trout to be a very slow process. However, the foreign genes might eventually reach the headwaters of the streams in which hybridization occurred, thereby contaminating the populations of westslope cutthroat trout in these streams. Selection against the foreign genes might occur in the upper reaches of a stream where conditions could be very different from those in the normal habitat of the rainbow trout.

A similar situation was investigated by Hagen (1967), who found that hybridization between the marine threespine stickback (Gasterosteus trachurus) and the freshwater threespine stickleback (G. leiurus) readily occurred in an environment intermediate to the one preferred by each species and hybrids did not appear to be selected against in this area. However, outside the narrow zone of hybridization, hybrid fish appeared to be selected against strongly.

If, in fact, hybrids between rainbow and westslope cutthroat trout were selected against in the upper reaches of small mountain streams, the presently surviving populations of westslope cutthroat trout would appear to be in less danger than one might expect.

Whether or not the streams and lakes of Montana should be managed to protect native species of fish is a matter of personal judgement. If the goal of management is to encourage the proliferation of trout with maximal growth rates and catchability, perhaps the planting of rainbow trout and other exotic species in state waters should be continued. If, however, the goal is to maintain the genetic

diversity already present in populations of trout in Montana, then more extensive programs of management should be instituted to protect the remaining species of native trout. Such programs might involve the planting of native westslope cutthroat trout in barren waters, and the reclaimation of streams from which the westslope cutthroat trout has been displaced by exotic species.

The need to maintain the genetic diversity of native species should be apparent to all who possess a basic understanding of fisheries management. It is to be hoped that the state and federal agencies which control the waters of Montana will endeavor to preserve and expand the range of distribution of the most important game fish native to Montana--the westslope cutthroat trout.

Chapter V

SUMMARY

Intraspecific and interspecific variation was investigated in the westslope cutthroat and Yellowstone cutthroat trout, S. clarki, and the rainbow trout, S. gairdneri. The extent of introgressive hybridization between these species was also examined. In all, 547 trout were taken from populations at 25 different locations in Montana and Yellowstone National Park. Information obtained in this study revealed the following:

1. The spotting pattern, coloration and spot size of westslope cutthroat trout varied greatly both within and between populations. There was no evidence that this variation was the result of hybridization with trout of another type. Coloration of the trout appeared to be correlated with the density of the canopy over the stream from which they were taken.

2. Starch gel electrophoresis revealed that serum proteins, esterases, lactate dehydrogenases and hemoglobins were polymorphic in several of the populations sampled. However, only the serum proteins and esterases were used in a subsequent analysis of variation within and between populations. Six esterases, apparently controlled by three codominant alleles at a single locus, and seven serum proteins were distinguished by means of electrophoresis.

3. No significant differences were found between the sexes with respect to the frequencies of the serum proteins in rainbow trout taken from the Montana State Fish Hatchery at Arlee.

4. The three types of trout recognized by visual inspection--

westslope cutthroat, Yellowstone cutthroat and rainbow trout--were distinguished on the basis of the frequencies of individual serum proteins and their overall phenotypes. Relationships between populations of these three types of trout, measured in terms of squared Euclidean distance and shown in phenograms, revealed that populations visually classified as westslope cutthroat trout were biochemically more closely related to those visually classified as rainbow trout than to those visually classified as Yellowstone cutthroat trout, even though both types of cutthroat trout are presently classified as Salmo clarki while rainbow trout are classified as S. gairdneri.

5. Cutthroat trout from the headwaters of the Missouri River were found to be genetically more similar to westslope cutthroat trout taken from west of the Continental Divide in Montana than to Yellowstone cutthroat trout from Yellowstone Lake, in the Missouri drainage. This suggests that populations of cutthroat trout in the headwaters of the Missouri River were derived from populations of westslope cutthroat trout in western Montana rather than from cutthroat trout that passed through Yellowstone Lake.

6. In general, populations of westslope cutthroat trout in close proximity showed a considerable degree of genetic similarity.

7. In a monothetic sequential key of the phenotypes of sampled trout constructed on the basis of information theory, phenotypes characteristic of Yellowstone cutthroat trout were separated from those characteristic of westslope cutthroat and rainbow trout at almost twice the level of information that separated phenotypes characteristic of rainbow trout from those characteristic of westslope cutthroat

trout.

8. The presence in populations of trout visually classified as westslope cutthroat trout of proteins usually found in high frequencies only in Yellowstone cutthroat or rainbow trout was believed to be the result of introgressive hybridization following the stocking of one of these exotic species.

9. Of the 21 populations sampled which were visually classified as westslope cutthroat trout, eight were suspected on the basis of biochemical evidence, of containing hybrid trout. Of the eight streams involved, six had records of being stocked in the past with either rainbow or Yellowstone cutthroat trout, and it is highly likely that unrecorded introductions of exotic species of trout were made in the remaining two streams. On the basis of the results obtained in this study, the stocking of exotic species of trout is almost certainly the greatest immediate threat to native populations of westslope cutthroat trout.

10. When serum proteins and enzymes are considered in addition to general morphology, a better understanding of the genetic make-up of populations and their taxonomic relationships is likely to be obtained than if either type of evidence is considered alone.

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Table 1. Characters typical of cutthroat and rainbow trout.

	S. clarki, cutthroat trout		*S. gairdneri*, rainbow trout	
Character	Mean	Range	Mean	Range
Vertebrae	62	60-64	63.5	61-65
Scales in lateral line	170	140-200	133	110-150
Scales above lateral line	40	32-48	28.5	25-32
Gill rakers	19	15-23	19	16-22
Pyloric caeca	40	30-50	50	35-70
Pelvic rays	9	8-10	10	9-11

Table 2. Locations and physical characteristics of sites sampled.

Map Ref.	Location	Canopy	Stream bottom *	Map coordinates
1	Big Rock Creek	Moderate coniferous and deciduous	Rubble	T25N, R26W, sec. 34
2	Chippy Creek	Moderate coniferous and deciduous	Rubble	T24N, R26W, sec. 32 & 33
3	Cherry Creek	Tight coniferous	Rubble and sand	T20N, R29W, sec. 14
4	Deer Creek	Moderate coniferous	Rubble	T46N, R30W, sec. 22 & 15
5	Ward Creek	Tight coniferous	Rubble	T46N, R29W, sec. 31
6	Straight Creek	Moderate coniferous and deciduous	Rubble	T13N, R26W, sec. 10
7	Lolo Creek	Moderate coniferous	Rubble	T10N, R24W, sec. 2
8	Greenough Creek	Tight deciduous	Rubble and gravel	T12N, R17W, sec. 21
9	Chamberlin Creek	Open	Rubble and gravel	T14N, R13W, sec. 9
10	Arrasta Creek	Moderate coniferous	Rubble	T15N, R10W, sec. 24
11	Poorman Creek	Moderate coniferous and deciduous	Gravel, sand and clay	T14N, R9W, sec. 36
12	Bear Trap Creek	Open	Clay	T15N, R6W, sec. 27
13	Shave Gulch Creek	Tight deciduous	Gravel	T15N, R6W, sec. 21
14	Alice Creek	Open	Rubble	T16N, R7W, sec. 14
15	Little Blackfoot River	Open to moderate deciduous	Rubble and sand	T7N, R7W, sec. 2 & 3
16	Lower Elliot Lake	Open	Bedrock	T7N, R11W, sec. 29
17	Rock Creek	Open	Boulders and rubble	T7N, R16W, sec. 7
18	Little Stony Creek	Tight deciduous and coniferous	Rubble and gravel	T6N, R17W, sec. 3 & 4
19	Congdon Creek	Tight coniferous	Rubble	T4N, R17W, sec. 2 & 3
20	Moose Creek	Moderate coniferous	Boulders, rubble and gravel	T2N, R17W, sec. 4
21	Overwhich Creek	Open	Rubble	T1S, R20W, sec. 28, 33, & 34
22	Odell Creek	Moderate coniferous	Rubble and gravel	T14S, R1W, sec. 31
23	Yellowstone Lake	Open	Sand	

* Classification system used by Welch (1952).

Table 3. (A) Electrophoretic conditions for the proteins examined. (B) Constituents of buffers.

(A)

Protein	Gel Buffer	Electrode Buffer	pH	Voltage	Time
Serum proteins	tris-borate-EDTA	tris-borate-EDTA	7.0	500	4 h
Esterase	tris-borate-EDTA	tris-borate-EDTA	7.0	500	4 h
Lactate dehydrogenase	tris-citrate	tris-borate	8.6	500	4 h
Hemoglobin	tris-borate-EDTA	tris-borate-EDTA	8.6	500	4 h
Transferrin	tris-borate-EDTA	tris-borate-EDTA	7.0	500	4 h

(B)

Tris-borate-EDTA, pH 7.0	tris (hydroxymethyl) aminomethane	24.24 g
	ethylenediamine tetraacetic acid	2.40 g
	boric acid	95.50 g
	distilled water to	4.00 l
Tris-citrate, pH 8.6	tris (hydroxymethyl) aminomethane	38.40 g
	citric acid, monohydrate	3.30 g
	distilled water to	3.60 l
	borate-lithium hydroxide, pH 8.6 to	4.00 l
Borate-lithium hydroxide, pH 8.6	boric acid	47.20 g
	lithium hydroxide	9.60 g
	distilled water to	4.00 l
Tris-borate-EDTA*, pH 8.6	tris (hydroxymethyl) aminomethane	109.00 g
	boric acid	30.90 g
	ethylenediamine tetraacetic acid	5.84 g
	distilled water to	4.00 l

*For electrode chamber, diluted 1:7; for gel, diluted 1:20.

Table 4. Staining procedures for proteins.

Proteins	Stains	
Serum proteins	Saturated solution of napthol blue-black (Sigma Chemical Co., St. Louis, Mo.) in methanol, water, and acetic acid (50:50: 1 by volume).	200.0 ml
	Stained for 4 minutes.	
	Destained in solvent.	
Esterase	fast blue RR salt	200.0 mg
	1% alpha-napthyl acetate (in acetone and water, 1:1 by volume)	6.4 ml
	0.1 M tris-HCl buffer, pH 7.0	24.0 ml
	distilled water to	200.0 ml
	Stained for 30 m and fixed in methanol, water, and acetic acid (50:50:1 by volume).	
Lactate dehydrogenase	0.1 M tris-HCl buffer, pH 8.0	40.0 ml
	lithium lactate	1.9 g
	beta-diphosphopyridine nucleotide	60.0 mg
	phenazine methosulfate	15.0 mg
	nitro blue tetrazolium	40.0 mg
	distilled water to	200.0 ml
	Stained for 30 m and fixed in methanol, water, and acetic acid (50:50:1 by volume).	
Hemoglobin	o-dianisidine	0.2 g
	conc. HCl	10.0 ml
	0.1 M sodium acetate-HCl buffer, pH 5.7	20.0 ml
	95% ethanol	60.0 ml
	distilled water to	200.0 ml
Transferrin	The following solutions mixed in the proportions 1:1:20.	
	(a) 2,4-dinitroso-1,3-napthalenediol (Eastman # 9503)	25.0 mg
	absolute ethanol to	10.0 ml
	(b) hydrquinone	1.0 g
	absolute ethanol to	10.0 ml
	(c) sodium acetate trihydrate	32.0 g
	glacial acetic acid	14.0 ml
	distilled water to	200.0 ml
	Stained overnight.	

Table 5. Banding frequencies of serum proteins and esterases.

POPULATION	BANDING FREQUENCIES OF PROTEINS										n
	C	D	E	F	G	H	Y	45	68	79	
1	-	0.75	-	0.89	1.00	-	1.00	1.00	-	-	28
2	0.05	0.80	0.05	0.85	0.95	0.05	1.00	1.00	-	-	20
3	-	0.86	0.07	0.79	0.93	-	1.00	1.00	-	-	14
4	0.04	0.68	0.04	0.96	1.00	0.04	1.00	1.00	-	-	25
5	-	0.27	-	1.00	1.00	-	1.00	1.00	-	-	15
6	-	0.91	-	0.82	0.91	-	0.41	1.00	-	-	22
7	-	-	-	1.00	1.00	-	0.09	1.00	-	-	11
8	-	0.78	0.11	0.89	1.00	0.11	1.00	1.00	-	-	9
9	-	0.43	-	0.96	1.00	0.07	1.00	1.00	-	-	28
10	-	0.47	-	0.93	1.00	-	0.87	1.00	-	0.05	15
11	-	0.29	-	1.00	1.00	-	1.00	1.00	-	-	21
12	-	0.25	-	1.00	1.00	-	1.00	1.00	-	-	16
13	-	0.31	-	0.94	1.00	-	1.00	1.00	-	-	16
14	-	0.30	-	1.00	1.00	-	1.00	1.00	-	-	20
15	-	0.54	-	0.92	1.00	-	1.00	1.00	-	-	13
16	-	1.00	-	0.38	0.62	-	1.00	1.00	-	-	24
17	-	0.60	0.73	0.13	0.80	0.87	0.93	1.00	-	-	15
18	-	0.21	-	1.00	1.00	-	1.00	1.00	-	-	14
19	-	0.70	0.30	0.95	1.00	0.10	0.70	1.00	0.35	-	20
20	-	0.36	-	1.00	1.00	-	0.05	1.00	-	-	22
21	0.12	1.00	0.96	0.04	0.72	-	0.16	0.16	1.00	-	25
22	-	0.48	0.39	0.96	1.00	-	1.00	1.00	-	0.09	23
23	0.24	1.00	0.82	0.18	-	-	1.00	-	1.00	-	17
24	0.04	0.96	1.00	0.04	-	-	1.00	-	1.00	-	24
25	0.08	1.00	0.96	0.17	-	-	1.00	-	1.00	-	24
26	-	0.73	0.89	-	0.64	0.88	0.96	0.75	-	0.32	56
27	-	0.20	-	0.90	1.00	-	1.00	1.00	-	-	10

Table 6. Phenotypic frequencies of serum proteins.

Phenotype	Population													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
GHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DEGHY	-	-	-	-	-	-	-	.11	-	-	-	-	-	-
DEY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DEHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EGHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FGY	.21	.15	.14	.28	.73	-	.09	.22	.50	.40	.71	.75	.69	.70
DFGY	.68	.60	.57	.56	.27	.27	-	.67	.39	.40	.29	.25	.25	.30
DGY	.07	.10	.21	.04	-	.05	-	-	.04	.07	-	-	.06	-
DY	-	-	-	-	-	.09	-	-	-	-	-	-	-	-
DEGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DEFGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CDGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CDEY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CDEGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CDFGY	-	-	-	.04	-	-	-	-	-	-	-	-	-	-
DFGHY	-	-	-	.04	-	-	-	-	-	-	-	-	-	-
EFGY	-	.05	-	.04	-	-	-	-	-	-	-	-	-	-
CDFY	-	.05	-	-	-	-	-	-	-	-	-	-	-	-
DGHY	-	.05	-	-	-	-	-	-	-	-	-	-	-	-
GY	.04	-	-	-	-	-	-	-	-	-	-	-	-	-
EY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DEFY	-	-	.07	-	-	-	-	-	-	-	-	-	-	-
FGHY	-	-	-	-	-	-	-	-	.07	-	-	-	-	-
CDY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DFY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DE	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FG	-	-	-	-	-	.09	.91	-	-	.13	-	-	-	-
DFG	-	-	-	-	-	.41	-	-	-	-	-	-	-	-
DG	-	-	-	-	-	.09	-	-	-	-	-	-	-	-
DEG	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DEFG	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CDE	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DFGH	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FGH	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Phenotype	Population												
	15	16	17	18	19	20	21	22	23	24	25	26	27
GHY	-	-	.07	-	-	-	-	-	-	-	-	.11	-
EHY	-	-	.13	-	-	-	-	-	-	-	-	.16	-
DEGHY	-	-	.47	-	-	-	-	-	-	-	-	.52	-
DEY	-	-	.07	-	-	-	.04	-	.59	.88	.75	.09	-
DEHY	-	-	-	-	-	-	-	-	-	-	-	.09	-
EGHY	-	-	.07	-	-	-	-	-	-	-	-	.02	-
FGY	.46	-	.07	.79	.20	-	-	.48	-	-	-	-	.80
DFGY	.46	.38	-	.21	.20	.05	-	.09	-	-	-	-	.10
DGY	.08	.25	-	-	-	-	-	.04	-	-	-	-	.10
DY	-	.38	-	-	-	-	-	-	-	-	-	-	-
DEGY	-	-	-	-	.05	-	-	.04	-	-	-	-	-
DEFGY	-	-	-	-	.15	-	-	.30	-	-	-	-	-
CDGY	-	-	-	-	-	-	.04	-	-	-	-	-	-
CDEY	-	-	-	-	-	-	-	-	.12	.04	.04	-	-
CDEGY	-	-	-	-	-	-	.04	-	-	-	-	-	-
CDFGY	-	-	-	-	-	-	-	-	-	-	-	-	-
DFGHY	-	-	-	-	.05	-	-	-	-	-	-	-	-
EFGY	-	-	-	-	.05	-	-	.04	-	-	-	-	-
CDFY	-	-	-	-	-	-	-	-	-	-	-	-	-
DGHY	-	-	-	-	-	-	-	-	-	-	-	-	-
GY	-	-	-	-	-	-	-	-	-	-	-	-	-
EY	-	-	-	-	-	-	-	-	-	.04	-	-	-
DEFY	-	-	-	-	-	-	-	-	.12	.04	.17	-	-
FGHY	-	-	-	-	-	-	-	-	-	-	-	-	-
CDY	-	-	-	-	-	-	-	-	.18	-	.04	-	-
DFY	-	-	-	-	-	-	-	-	.06	-	-	-	-
DE	-	-	-	-	-	-	.60	-	-	-	-	-	-
FG	-	-	-	-	.05	.64	-	-	-	-	-	-	-
DFG	-	-	-	-	.15	.32	-	-	-	-	-	-	-
DG	-	-	-	-	-	-	-	-	-	-	-	-	-
DEG	-	-	-	-	-	-	.20	-	-	-	-	-	-
DEFG	-	-	-	-	.05	-	.04	-	-	-	-	-	-
CDE	-	-	-	-	-	-	.04	-	-	-	-	-	-
DFGH	-	-	-	-	.05	-	-	-	-	-	-	-	-
FGH	-	-	.07	-	-	-	-	-	-	-	-	-	-
DHY	-	-	.07	-	-	-	-	-	-	-	-	-	-

Table 7. Frequencies of serum protein and esterase phenotypes.

No.	Phenotype	Population													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	45GHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	45EHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	45DEGHY	-	-	-	-	-	-	-	1	-	-	-	-	-	-
4	45DEY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	45DEHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	45EGHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	45FGY	6	3	2	7	11	-	1	2	14	5	15	12	11	14
8	45DFGY	19	12	8	14	4	6	-	6	11	6	6	4	4	6
9	45DGY	2	2	3	1	-	1	-	-	1	1	-	-	1	-
10	45DY	-	-	-	-	-	2	-	-	-	-	-	-	-	-
11	45DEGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	45DEFGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	45CDFGY	-	-	-	1	-	-	-	-	-	-	-	-	-	-
14	45DFGHY	-	-	-	1	-	-	-	-	-	-	-	-	-	-
15	45EFGY	-	1	-	1	-	-	-	-	-	-	-	-	-	-
16	45CDFY	-	1	-	-	-	-	-	-	-	-	-	-	-	-
17	45DGHY	-	1	-	-	-	-	-	-	-	-	-	-	-	-
18	45GY	1	-	-	-	-	-	-	-	-	-	-	-	-	-
19	45DEFY	-	-	1	-	-	-	-	-	-	-	-	-	-	-
20	45FGHY	-	-	-	-	-	-	-	-	2	-	-	-	-	-
21	45DFG	-	-	-	-	-	9	-	-	-	-	-	-	-	-
22	45FG	-	-	-	-	-	2	10	-	-	2	-	-	-	-
23	45DFGH	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	45DHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	45DG	-	-	-	-	-	2	-	-	-	-	-	-	-	-
26	45FGH	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	4579GHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	4579EHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	4579DEGHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	4579DEY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	4579EGHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	4579FGY	-	-	-	-	-	-	-	-	-	1	-	-	-	-
33	4579DEFGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	68DE	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	68DEG	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	68DEFG	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	68CDGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	68CDE	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	68DEY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	68CDEGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	68CDY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	68CDEY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	68DEFY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	68DFY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	68EY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	4568DE	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	4568DEGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	4568DEFGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	4568FGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	4568DFGHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	4568DEFG	-	-	-	-	-	-	-	-	-	-	-	-	-	-

No.	Phenotype	Population												
		15	16	17	18	19	20	21	22	23	24	25	26	27
1	45GHY	-	-	1	-	-	-	-	-	-	-	-	3	-
2	45EHY	-	-	2	-	-	-	-	-	-	-	-	5	-
3	45DEGHY	-	-	7	-	-	-	-	-	-	-	-	19	-
4	45DEY	-	-	1	-	-	-	-	-	-	-	-	4	-
5	45DEHY	-	-	-	-	-	-	-	-	-	-	-	5	-
6	45EGHY	-	-	1	-	-	-	-	-	-	-	-	-	-
7	45FGY	6	-	1	11	2	-	-	11	-	-	-	-	8
8	45DFGY	6	9	-	3	4	1	-	2	-	-	-	-	1
9	45DGY	1	6	-	-	-	-	-	1	-	-	-	-	1
10	45DY	-	9	-	-	-	-	-	-	-	-	-	-	-
11	45DEGY	-	-	-	-	-	-	-	1	-	-	-	-	-
12	45DEFGY	-	-	-	-	1	-	-	5	-	-	-	-	-
13	45CDFGY	-	-	-	-	-	-	-	-	-	-	-	-	-
14	45DFGHY	-	-	-	-	-	-	-	-	-	-	-	-	-
15	45EFGY	-	-	-	-	1	-	-	1	-	-	-	-	-
16	45CDFY	-	-	-	-	-	-	-	-	-	-	-	-	-
17	45DGHY	-	-	-	-	-	-	-	-	-	-	-	-	-
18	45GY	-	-	-	-	-	-	-	-	-	-	-	-	-
19	45DEFY	-	-	-	-	-	-	-	-	-	-	-	-	-
20	45FGHY	-	-	-	-	-	-	-	-	-	-	-	-	-
21	45DFG	-	-	-	-	3	7	-	-	-	-	-	-	-
22	45FG	-	-	-	-	1	14	-	-	-	-	-	-	-
23	45DFGH	-	-	-	-	1	-	-	-	-	-	-	-	-
24	45DHY	-	-	1	-	-	-	-	-	-	-	-	-	-
25	45DG	-	-	-	-	-	-	-	-	-	-	-	-	-
26	45FGH	-	-	1	-	-	-	-	-	-	-	-	-	-
27	4579GHY	-	-	-	-	-	-	-	-	-	-	-	3	-
28	4579EHY	-	-	-	-	-	-	-	-	-	-	-	4	-
29	4579DEGHY	-	-	-	-	-	-	-	-	-	-	-	10	-
30	4579DEY	-	-	-	-	-	-	-	-	-	-	-	2	-
31	4579EGHY	-	-	-	-	-	-	-	-	-	-	-	1	-
32	4579FGY	-	-	-	-	-	-	-	-	-	-	-	-	-
33	4579DEFGY	-	-	-	-	-	-	-	2	-	-	-	-	-
34	68DE	-	-	-	-	-	-	11	-	-	-	-	-	-
35	68DEG	-	-	-	-	-	-	5	-	-	-	-	-	-
36	68DEFG	-	-	-	-	-	-	1	-	-	-	-	-	-
37	68CDGY	-	-	-	-	-	-	1	-	-	-	-	-	-
38	68CDE	-	-	-	-	-	-	1	-	-	-	-	-	-
39	68DEY	-	-	-	-	-	-	1	-	10	21	18	-	-
40	68CDEGY	-	-	-	-	-	-	1	-	-	-	-	-	-
41	68CDY	-	-	-	-	-	-	-	-	2	-	1	-	-
42	68CDEY	-	-	-	-	-	-	-	-	2	1	1	-	-
43	68DEFY	-	-	-	-	-	-	-	-	2	1	4	-	-
44	68DFY	-	-	-	-	-	-	-	-	1	-	-	-	-
45	68EY	-	-	-	-	-	-	-	-	-	1	-	-	-
46	4568DE	-	-	-	-	-	-	4	-	-	-	-	-	-
47	4568DEGY	-	-	-	-	1	-	-	-	-	-	-	-	-
48	4568DEFGY	-	-	-	-	2	-	-	-	-	-	-	-	-
49	4568FGY	-	-	-	-	2	-	-	-	-	-	-	-	-
50	4568DFGHY	-	-	-	-	1	-	-	-	-	-	-	-	-
51	4568DEFG	-	-	-	-	1	-	-	-	-	-	-	-	-

Figure 1. Map of western Montana showing sampling locations. Dotted line represents Continental Divide. C--Clark Fork River, M--Missouri River, Y--Yellowstone River. Sampling locations are listed in Table 2.

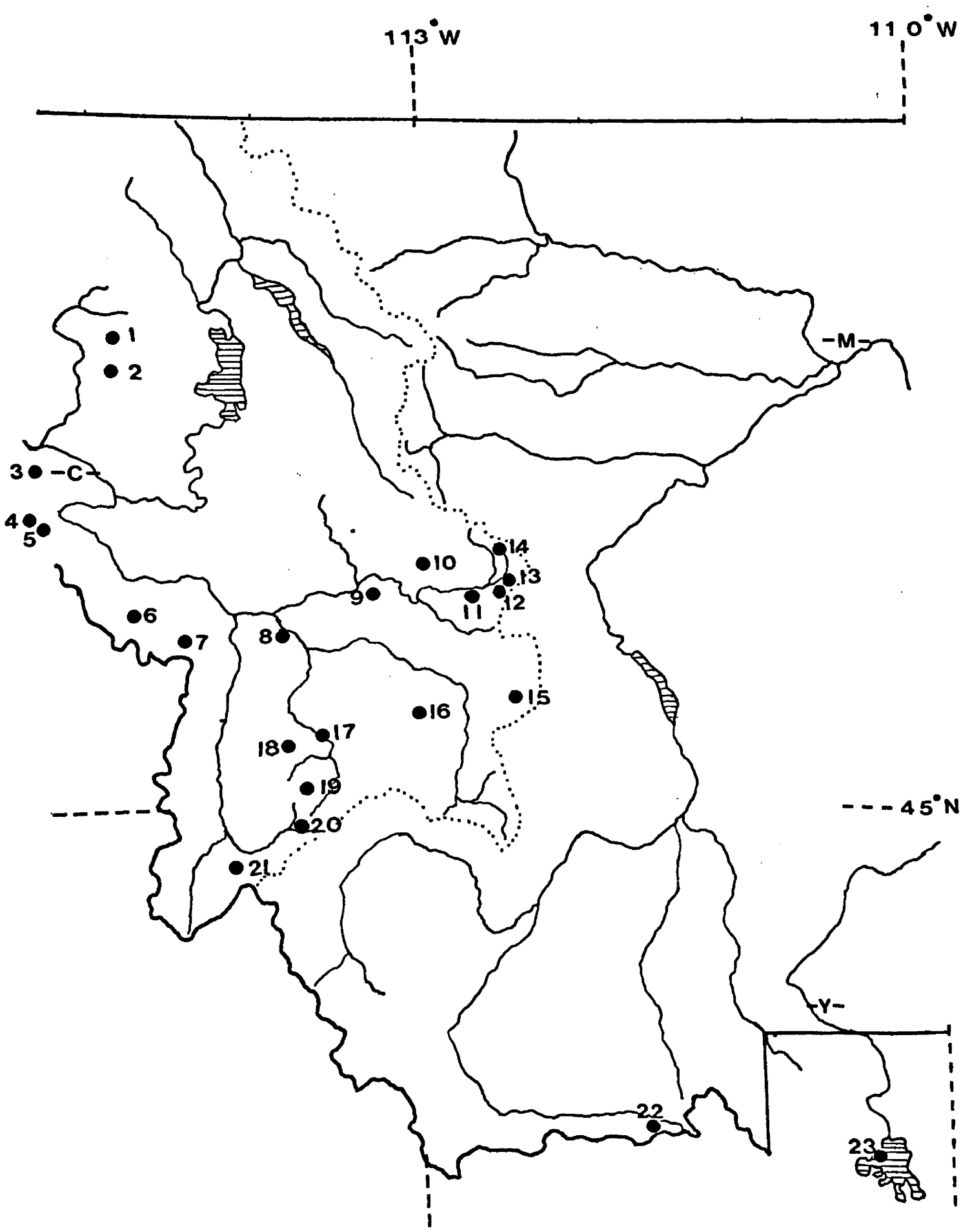
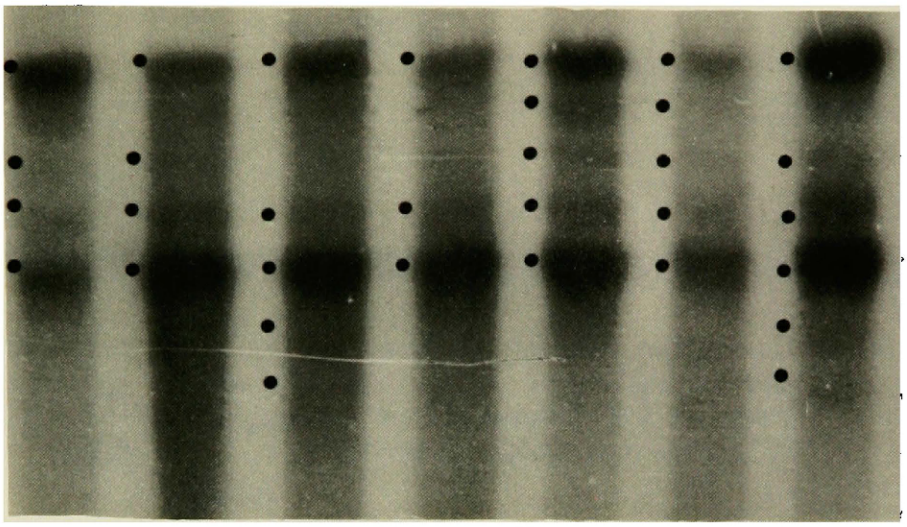
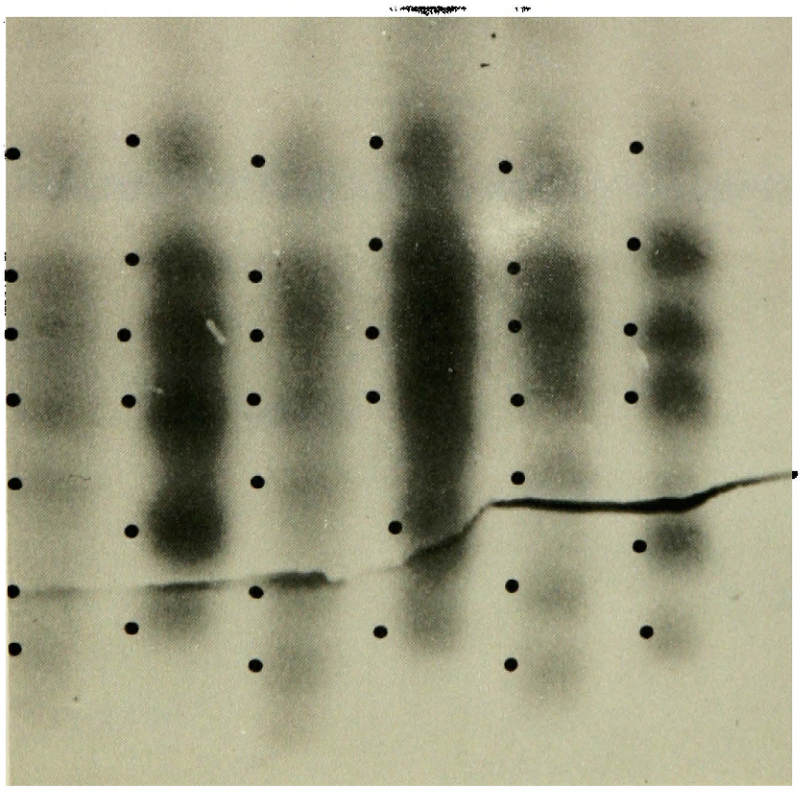

113°W
110°W
45°N
-M-
-C-
-Y-
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23

Figure 2. Top: starch gel showing lactate dehydrogenases found in S. clarki and S. gairdneri. Phenotype of column 1: 1,3,4,5. 2: 1,3,4,5. 3: 1,4,5,6,7. 4: 1,4,5. 5: 1, 2,3,4,5. 6: 1,2,3,4,5. 7: 1,3,4,5,6,7.

Bottom: starch gel showing hemoglobin phenotypes. Serum samples 1,3,5: S. clarki, samples 2,4,6: S. gairdneri. Phenotype of columns 1,3 and 5: M,O,P,Q, R,T,V. Phenotype of columns 2,4 and 6: L,N,P,Q,S,U.

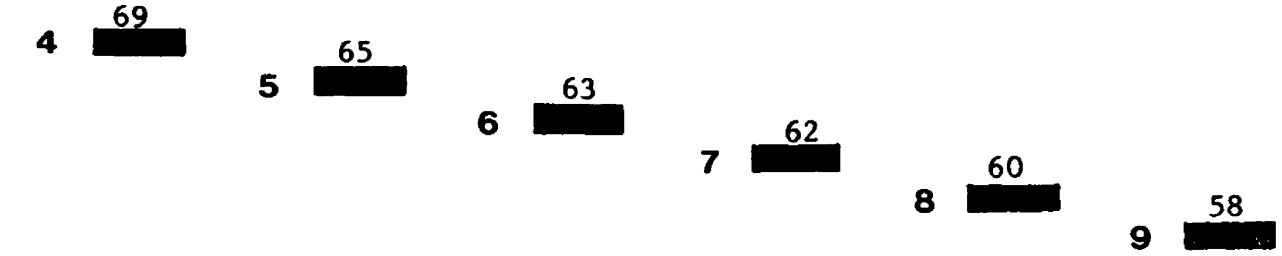


1 2 3 4 5 6 7

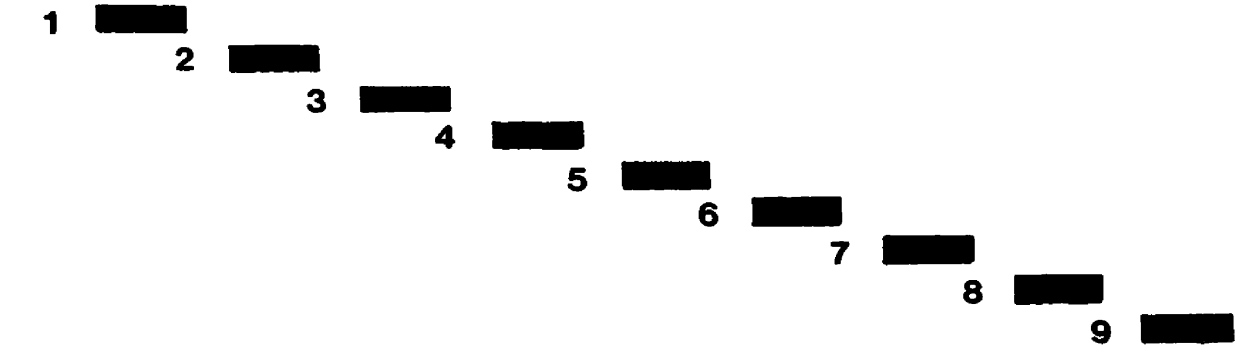


1 2 3 4 5 6

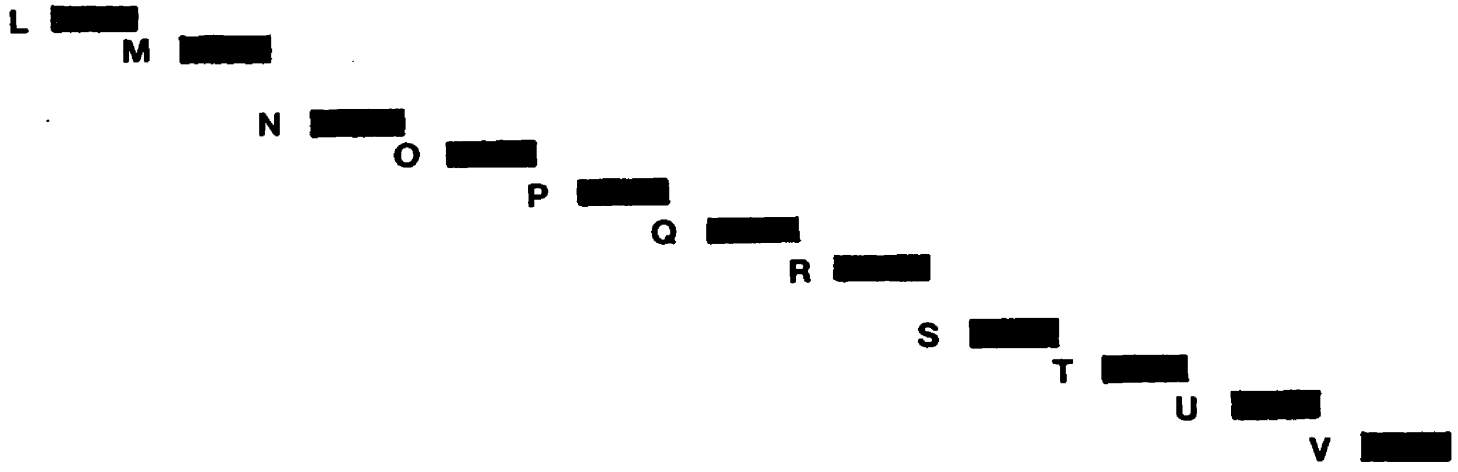
Figure 3. Variation in the esterases, lactate dehydrogenases and hemoglobins in S. clarki and S. gairdneri. Numbers represent rates of migration of the esterases relative to serum protein C.



ESTERASE



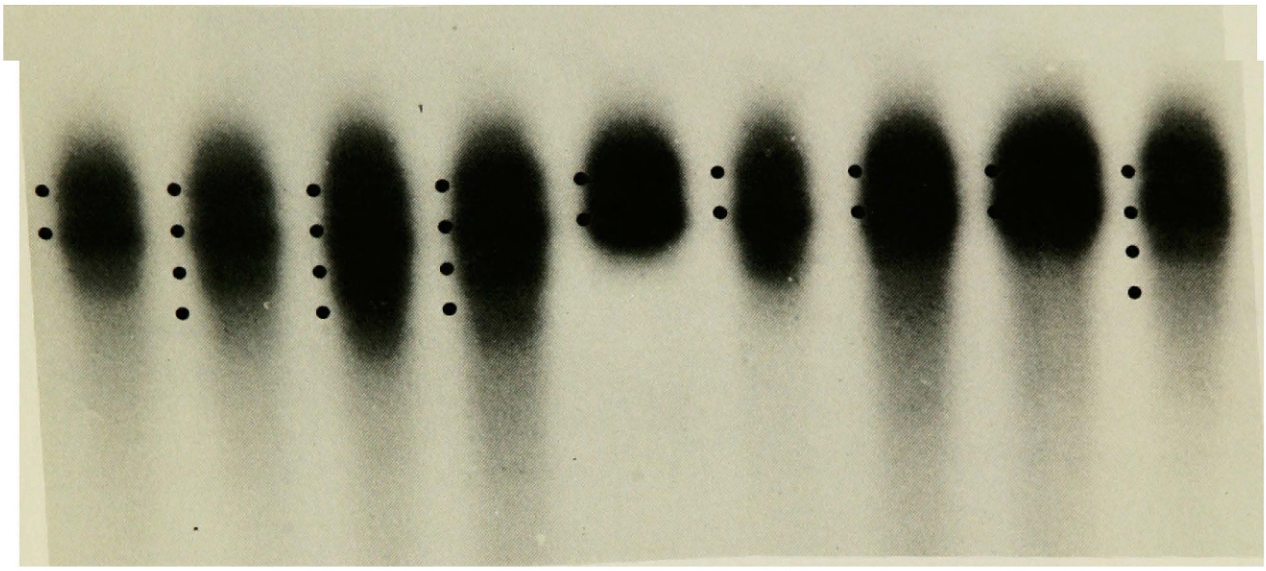
L D H



HEMOGLOBIN

Figure 4. Top: starch gel showing esterase phenotypes found in S. gairdneri. Phenotype of columns 1,5,6,7 and 8: 4,5. Phenotype of columns 2,3,4 and 9: 4,5,7,9.

Bottom: starch gel showing esterase phenotypes found in S. clarki. Phenotype of column 1: 4,5,6,8. 2: 6,8. 3: 4,5. 4: 4,5. 5: 4,5,6,8. 6: 4,5. 7: 4,5,6,8.


1
2
3
4
5
6
7
8
9

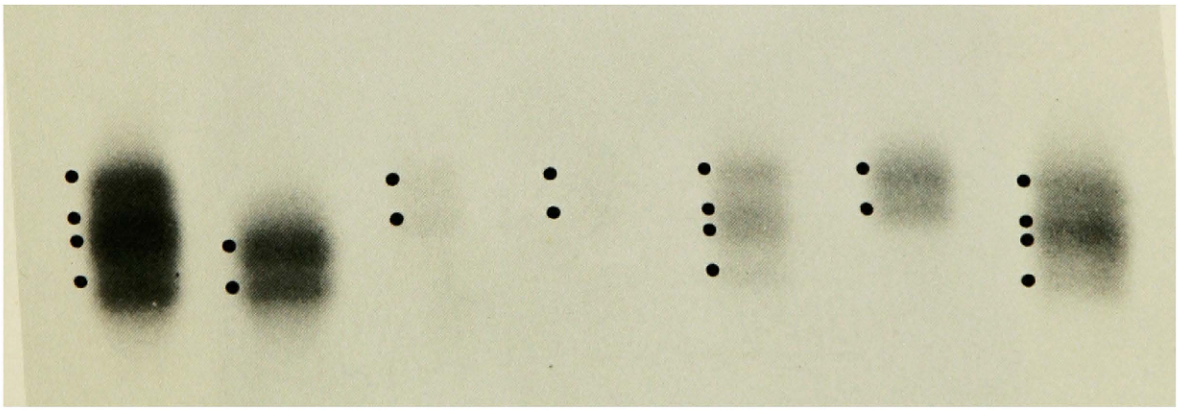

1
2
3
4
5
6
7

Figure 5. Starch gel showing serum protein phenotypes. Serum samples 1-4: *S. clarki*, samples 5 and 6: *S. gairdneri*. Phenotype of column 1: F,G,Y. 2: D,F,G,Y. 3: F,G,Y. 4: F,G,Y. 5: D,E,Y. 6: D,E,G,H,Y.

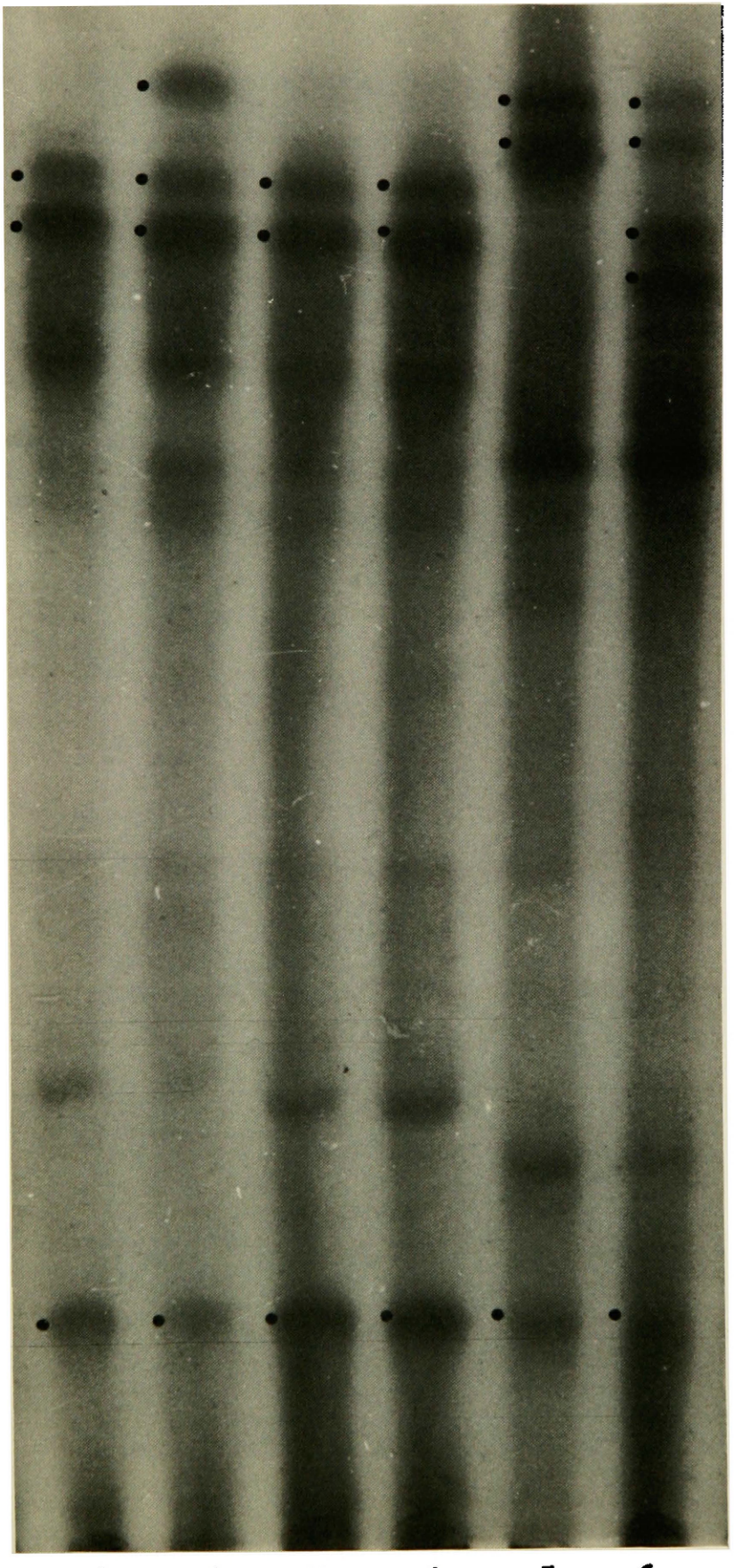

1
2
3
4
5
6

Figure 6. Starch gel showing serum protein phenotypes. Serum samples 1, 3-5: S. clarki, sample 2: S. gairdneri. Phenotype of column 1: D,E,F,G,Y. 2: D,E,G,H,Y. 3: F,G,Y. 4: F,G. 5: D,F,G,H,Y.

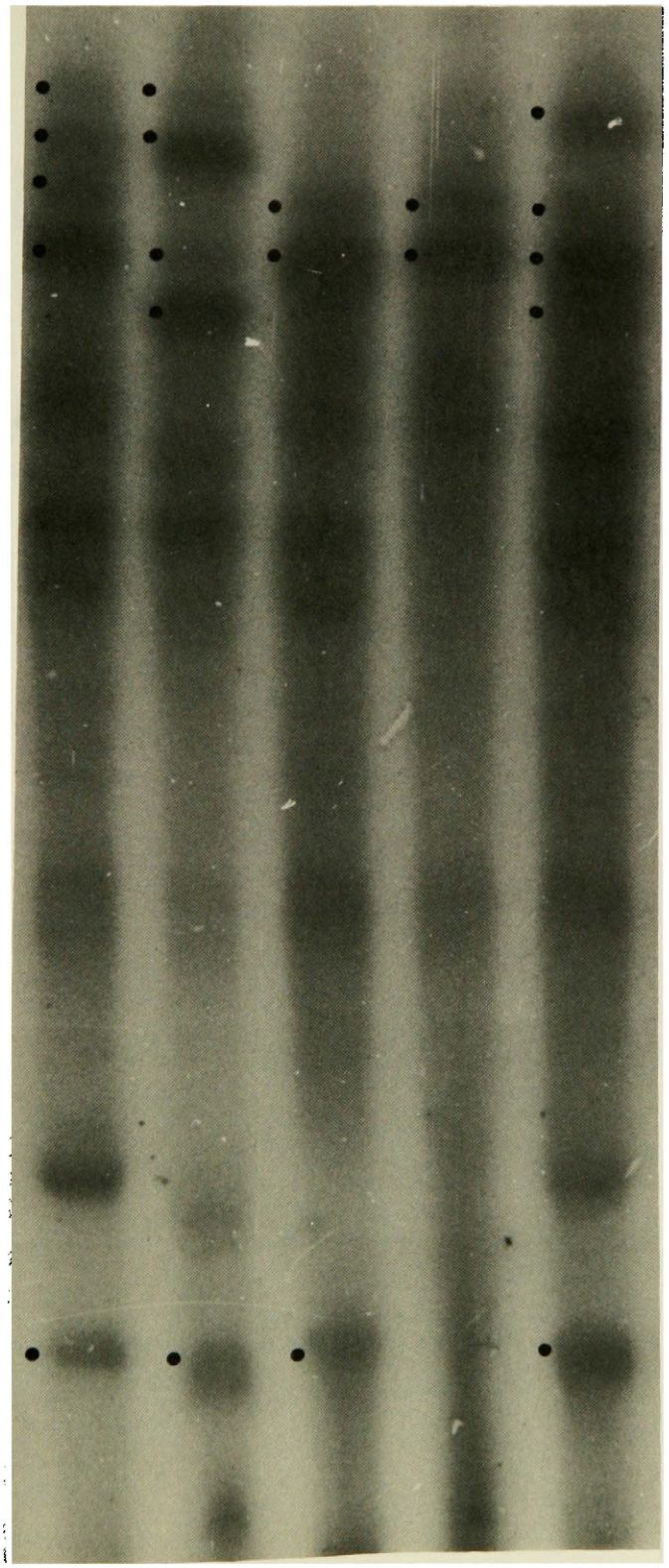
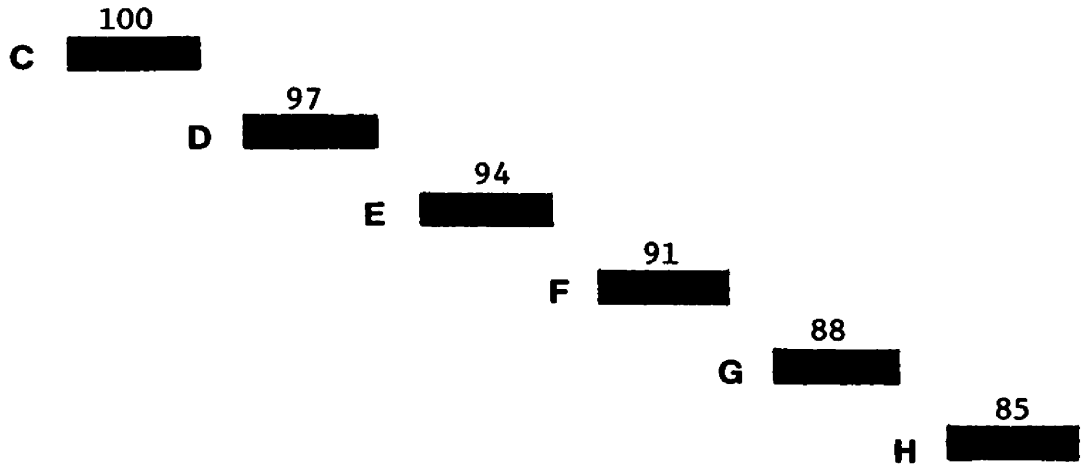

1
2
3
4
5

Figure 7. Variation in the serum proteins in S. clarki and S. gairdneri. Numbers represent rates of migration of the serum proteins relative to that of protein C.


100
C
97
D
94
E
91
F
88
G
85
H

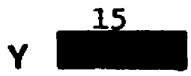

15
Y

Figure 8. Phenogram showing relationship between populations based upon the frequency of occurrence of the serum proteins. The numbers to the left of a bar represents the probability that the two populations or groups of populations joined by the bar were identical with respect to the frequencies of serum proteins and esterases. Δ^2 represents squared Euclidean distance.

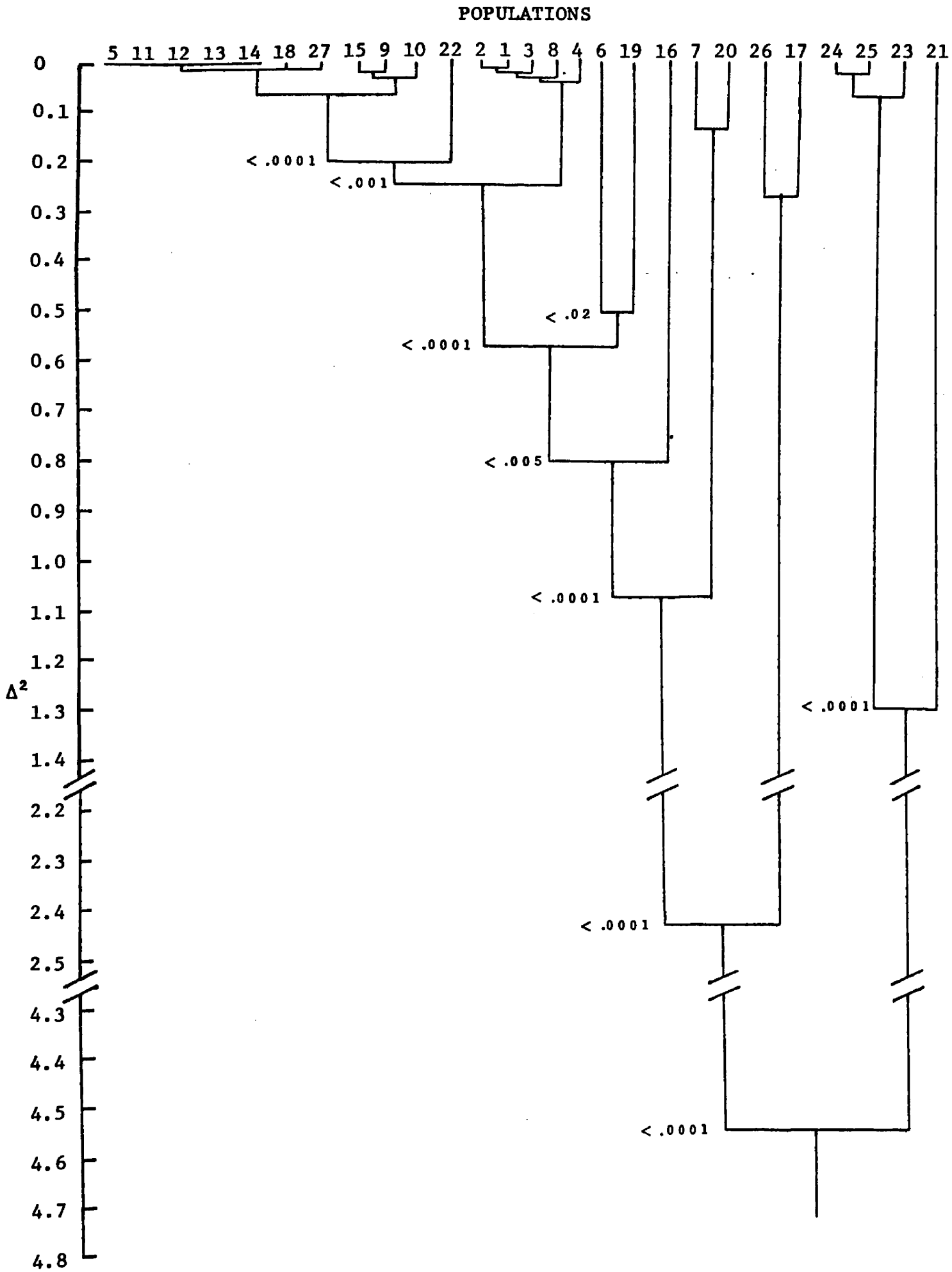

POPULATIONS
5 11 12 13 14 18 27 15 9 10 22 2 1 3 8 4 6 19 16 7 20 26 17 24 25 23 21
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
Δ²
1.3
1.4
2.2
2.3
2.4
2.5
4.3
4.4
4.5
4.6
4.7
4.8
< .0001
< .001
< .02
< .0001
< .005
< .0001
< .0001
< .0001
< .0001

Figure 9. Phenogram showing relationships between populations based upon the frequencies of serum protein phenotypes. The numbers to the left of a bar represents the probability that the two populations or groups of populations joined by the bar were identical with respect to the frequencies of protein phenotypes. Δ^2represents squared Euclidean distance.

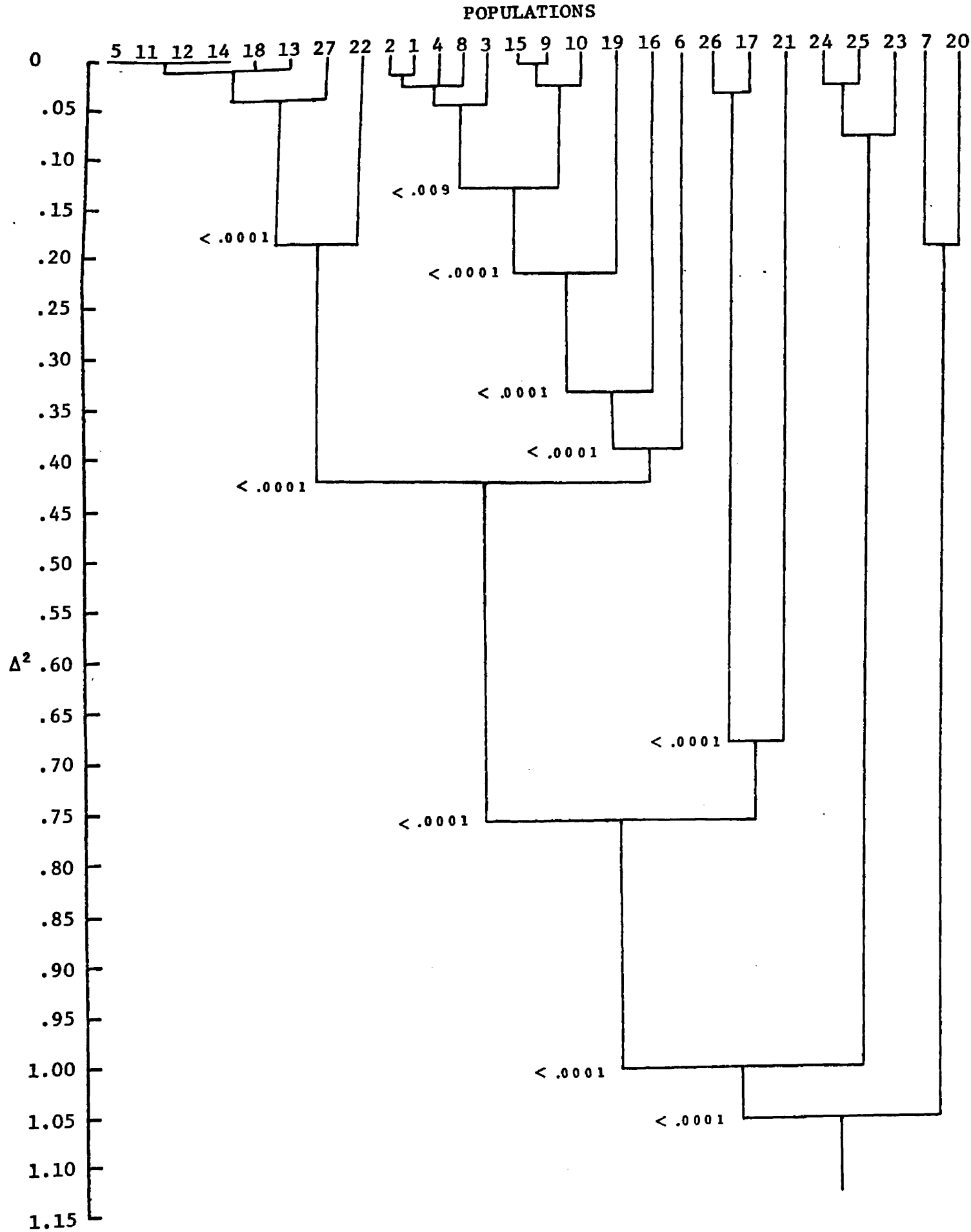
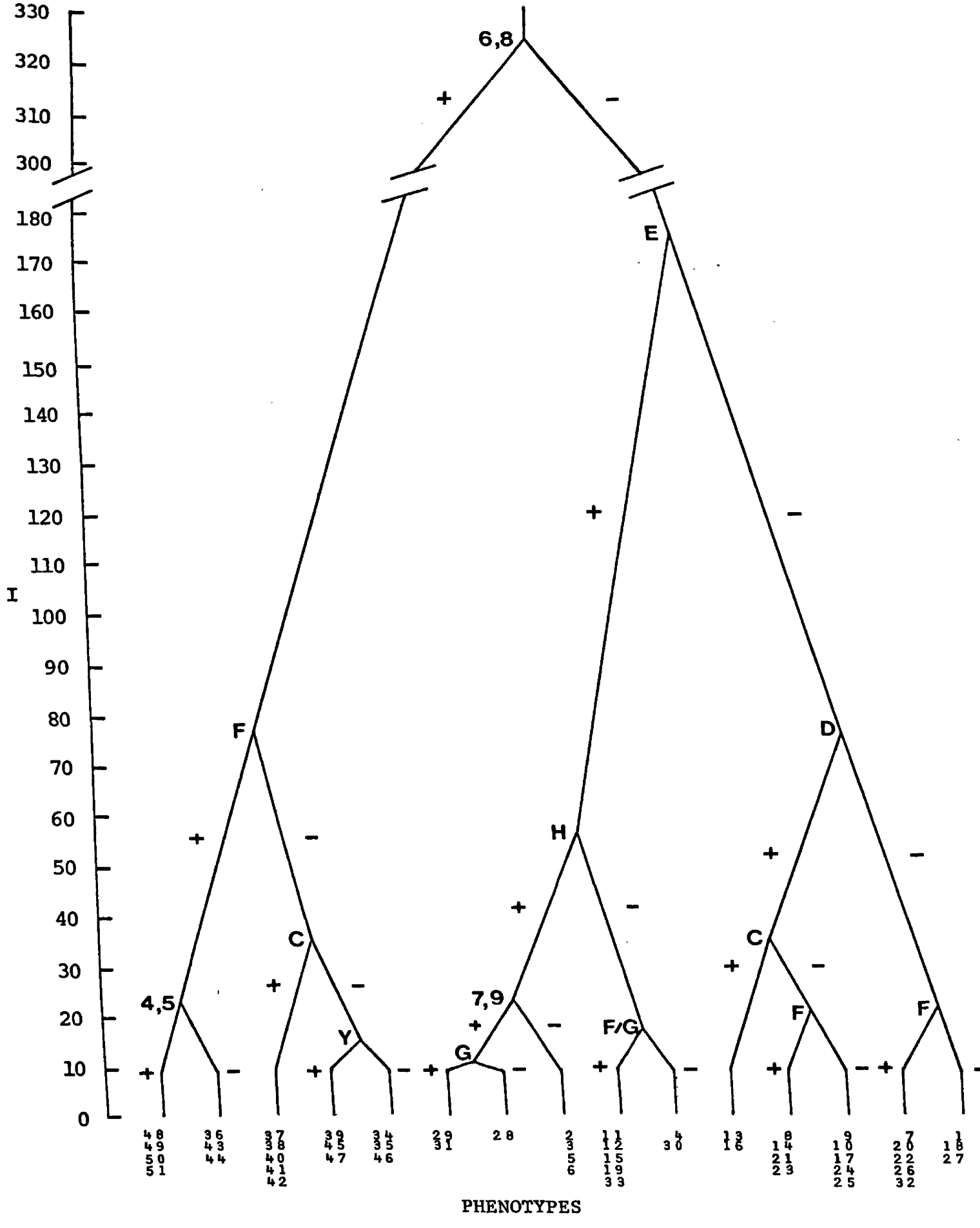

POPULATIONS
5 11 12 14 18 13 27 22 2 1 4 8 3 15 9 10 19 16 6 26 17 21 24 25 23 7 20
0
.05
.10
.15
.20
.25
.30
.35
.40
.45
.50
.55
Δ² .60
.65
.70
.75
.80
.85
.90
.95
1.00
1.05
1.10
1.15
< .009
< .0001
< .0001
< .0001
< .0001
< .0001
< .0001
< .0001
< .0001

Figure 10. Monothetic sequential key of the phenotypes of sampled trout. I represents the total information content of the groups of phenotypes. The numbers at the bottom of the key represent the phenotypes listed in Table 7. The numbers and letters at divisions in the key represent serum proteins or esterases observed in this study.


330
320
310
300
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
I
6,8
E
F
D
H
C
4,5
7,9
Y
G
F/G
C
F
F
48 49 50 51
36 43 44
37 38 40 41 42
39 45 47
34 35 46
29 31
28
2 3 5 6
11 12 15 19 33
4 30
13 16
8 14 21 23
9 10 17 24 25
7 20 22 26 32
1 18 27
PHENOTYPES