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ELECTROPHORETIC ANALYSIS AND POPULATION GENETICS OF SELECTED
ENZYMES OF THE BROOK TROUT (SALVELINUS FONTINALIS) IN THE
GREAT SMOKY MOUNTAINS NATIONAL PARK

A Thesis
Presented to
the Faculty of the Graduate School
Tennessee Technological University
by
Elizabeth Shipp

In Partial Fulfillment
of the Requirements for the Degree
MASTER OF SCIENCE
Biology

August 1979

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ELECTROPHORETIC ANALYSIS AND POPULATION GENETICS OF SELECTED
ENZYMES OF THE BROOK TROUT (SALVELINUS FONTINALIS) IN THE
GREAT SMOKY MOUNTAINS NATIONAL PARK

Elizabeth Shipp

Master of Science in Biology

Because of the severe decline in both the number and the range of the brook trout, Salvelinus fontinalis (Mitchill), in the Great Smoky Mountains National Park, this study was proposed to obtain information about their taxonomic status. Samples from thirty two populations of brook trout from nine states and the Park were collected. These were subjected to starch gel electrophoresis in an effort to assay their genetic makeup. Eight enzymes from this study and six from a parallel study were examined and found to code for an estimated 29 genetic loci. Nei's coefficient, a statistical test which compares each population to every other one for all loci examined, indicated no specific or sub-specific differentiation. Reduced genetic variation was observed in brook trout collected from the Park. It is concluded that no significant genetic differentiation exists between brook trout from the Great Smoky Mountains National Park and other brook trout populations.

CERTIFICATE OF APPROVAL OF THESIS

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ACKNOWLEDGMENTS

The author wishes to express her sincerest appreciation to her major professor, Dr. John W. Harris, for his guidance and support. Also, a special note of thanks is expressed to Dr. R. Don Estes for his aid in this project. The continuing encouragement of Dr. Gordon E. Hunter has been invaluable throughout the author's graduate work.

This project was funded by the USDI National Park Service through a contract with the Tennessee Cooperative Fishery Research Unit, Tennessee Technological University. Unit cooperators are the Tennessee Wildlife Resources Agency, U.S. Fish and Wildlife Service, and Tennessee Technological University.

Thanks are extended to Alan Kelly of the U.S. Fish and Wildlife Service and to all biologists who helped in the collection of brook trout.

Sincere appreciation is expressed to William Brandes for his partnership and pioneer work in this project.

Finally, the author would like to express deepest gratitude to her parents who have offered lifelong encouragement and love.

TABLE OF CONTENTS

	Page
LIST OF TABLES	v
LIST OF FIGURES	vi
Chapter	
1. INTRODUCTION	1
2. MATERIALS AND METHODS	11
Sample Collection	11
Sample Preparation	11
Gel Preparation	14
Electrophoresis	15
Staining Procedures	15
Nomenclature	16
3. RESULTS	20
Variant Enzymes	20
Nonvariant Enzymes	27
Additional Enzymes Investigated	28
Genetic Similarity	28
4. DISCUSSION	32
LITERATURE CITED	37

LIST OF TABLES

Table	Page
1. Illustration of the Application of Chi-Square	7
2. Brook Trout Collected	12
3. Tissues and Staining Solutions Used for Electrophoresis	17
4. Summary of Electrophoretic Data on Brook Trout Obtained from this Study	24
5. Gene Frequencies at the H6PD-1 Locus	25
6. Summary of Electrophoretic Data on Brook Trout Obtained by Brandes (1978)	29
7. Genetic Similarity Matrix based on Gene Frequencies Using Nei's Coefficient of Genetic Similarity	30

LIST OF FIGURES

Figure		Page
1.	Expected Banding Patterns for a Dimeric Enzyme that is Specified by a Gene with Two Alleles, A and B	5
2.	H6PD Phenotypes in Brook Trout Liver Samples	21
3.	Nonvariant Enzymes Investigated: GDH, TO, SDH	22
4.	Nonvariant Enzymes Investigated: G6PD, EST, DIA, XDH	23
5.	Frequency Distribution of Coefficients of Genetic Similarity between Populations of Sunfish	33

Chapter 1

INTRODUCTION

The brook trout (Salvelinus fontinalis) is the only salmonid fish native to the southern Appalachian region. Once abundant in the streams of the Great Smoky Mountains National Park, brook trout are found now mostly in the headwaters. Among the causes for their decline, King (1937) listed widespread logging, with its attendant clearing; railroad building; frequent fires; harmful fishing practices, including use of nets and dynamite; and the introduction of rainbow trout (Salmo gairdneri). The stocking of rainbow trout began soon after 1900 (Lennon 1967). This species thrived and gradually invaded and occupied brook trout habitat in the headwaters. By as early as 1942, rainbow trout dominated the Park streams (Jones, unpublished). Lennon observed that remnant populations of brook trout are restricted to less than favorable habitat and have failed to extend their range downstream. They are not abundant when found alone, and their numbers are even smaller when found with rainbow trout.

According to Lennon (1967), brook trout of the Park are typically small and short-lived. Male brook trout outnumber females two to one. The fecundity of these females is lower than that of brook trout from Wisconsin and

Canadian waters. Red spots on the sides of the Park brook trout are smaller and more numerous than those on northern fish. A comparison of relative sizes of certain body parts of brook trout from the Park and from a Canadian population indicates that there may be some significant differences between them. Southern brook trout are highly susceptible to furunculosis and ulcer diseases in contrast to brook trout from Pennsylvania. Hatchery-reared, native brook trout are able to survive and reproduce in Park streams, whereas attempts to stock New England brook trout in the Park repeatedly failed. Conversely, the native Park fish are unable to survive when stocked in streams of the Shenandoah National Park under favorable conditions. The foregoing observations led Lennon to conclude that the Park fish are a southern Appalachian strain of brook trout. Further consideration led to the hypothesis that the Park trout are a distinct subspecies or possibly even a distinct species.

The purpose of this study was to use the process of electrophoresis to obtain information about the taxonomic status of the brook trout in the Great Smoky Mountains National Park. Electrophoresis provides an effective tool for delineating the genetic diversity present between populations of living organisms. Among fish studies published, there appears to be an increasing number which use electrophoretic data to define, or to support definition of, new species or new subspecies (Payne et al. 1971).

Electrophoresis is the separation of proteins by an electric field in some medium, such as a gel of hydrolyzed potato starch. Particular proteins (usually enzymes) can be visualized as bands on the gel with the application of histochemical staining methods. A given protein molecule has a net charge that is dependent upon the arrangement of the amino acids in its three-dimensional structure. This charge causes it to migrate in an electric field toward the pole that is oppositely charged. The greater the charge on the molecule, the faster its rate of migration.

The rate of migration of a given protein molecule in a given medium depends not only upon its net charge, but also upon the size and shape of the molecule. Starch gel has a sieving effect because the pore size of the gel approaches that of proteins. This matrix nature of the medium maintains the position of the protein molecule after electrophoresis is terminated (May 1975).

The banding patterns that result from staining for specific proteins are under genetic control. A gene is a stretch of DNA that specifies a particular polypeptide. The location on the chromosome of that particular segment of DNA in relation to the total DNA is called the locus for that gene. Different forms of a gene that occur at the same locus are called alleles. Alleles differ from one another by one or more nucleotides, causing the protein products of the two alleles to differ. This difference may or may not

cause a difference in the migration rates, depending on the charges of the affected amino acids.

If an individual has two copies of the same allele of a given gene, it is said to be homozygous for that gene. If it has one copy of one allele and one copy of another allele of the gene, it is heterozygous. Several different alleles of the same gene may be present in a population. For example, if there are two alleles of a given gene, one may specify a fast migrating polypeptide and the other a slow. A gene that has only one allele is termed monomorphic, and the polypeptide which it specifies is visualized as a single band on the gel. If a gene has more than one allele, it is referred to as polymorphic. Multimolecular forms of an enzyme are called isozymes. These different molecular forms which may be specified by different alleles of a polymorphic gene are visualized in electrophoresis as different bands.

The majority of enzymes that have been used for electrophoresis catalyze specific biochemical reactions. Some of these are made up of more than one copy of a given polypeptide chain with the number of subunits usually being constant for a specific enzyme. If the protein consists of a single subunit, it is called a monomer; a dimer consists of two subunits; a tetramer has four subunits. Different alleles of a gene may specify different forms of the subunit. There is usually random combination of these subunits to produce the enzyme. To illustrate this, suppose

an individual is heterozygous for a gene which specifies a dimeric enzyme and that the gene has two alleles, A and B, which specify subunits A and B. The forms of the enzyme present would be represented by bands AA, AB, and BB in a 1:2:1 ratio. The banding patterns expected for the different genotypes are illustrated as follows: (Figure 1):

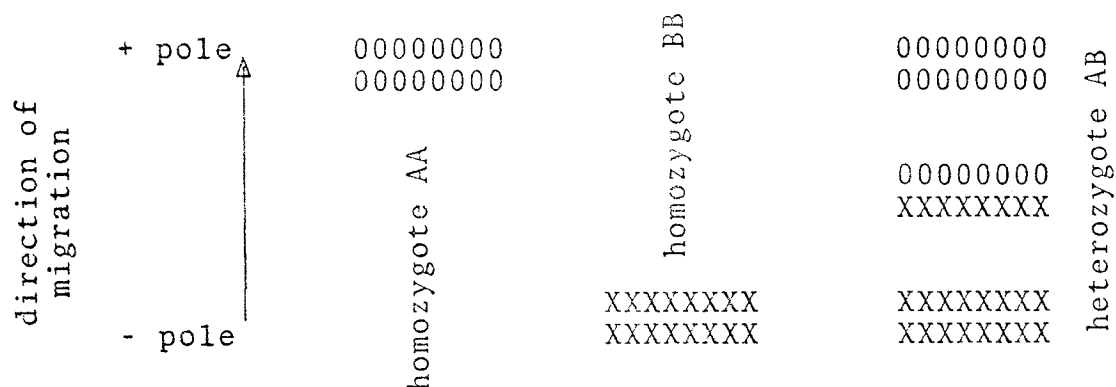


Figure 1. Expected Banding Patterns for a Dimeric Enzyme that is Specified by a Gene with Two Alleles, A and B

For a given population, it is possible to convert the electrophoretic results into allele frequency data. Assume that a population is represented by a sample of 24 individuals (N) and that there are equal numbers of the three phenotypes observed. The frequency (p) of allele A would be determined by:

$$p_A = \frac{2AA + AB}{2N} = \frac{2(8) + 8}{2(24)} = 0.50, \quad (1)$$

and the frequency (q) of allele B would be:

$$q_B = \frac{2BB + AB}{2N} = \frac{2(8) + 8}{2(24)} = 0.50 = 1 - p. \quad (2)$$

When a genetic system involves only two alleles at a given locus, that system may reach a Hardy-Weinburg Equilibrium and remain as such until one or more of the following factors acts to disturb the allele frequency:

natural selection

artificial selection

mutation

exchange of breeding adults between populations

random genetic drift.

A population is said to be in Hardy-Weinburg Equilibrium when the proportions of observed genotypes do not differ significantly from those expected based upon the calculated allele frequencies. The genotypic proportions that would be expected are calculated by inserting allele frequencies into a binomial expansion. The binomial expansion for two alleles would be $(p + q)^2$ or $p^2 + 2pq + q^2$ (Burns 1976). For the example cited above, the expected proportions and number of individuals of each genotype are as follows:

$$p^2 = (.50)(.50) = .25 = .25(24) = 6, \quad (3)$$

$$2pq = 2(.50)(.50) = .50 = .50(24) = 12, \quad (4)$$

$$q^2 = 2(.50)(.50) = .25 = .25(24) = 6. \quad (5)$$

In order to determine whether the deviations in observed numbers from those expected occurred merely by chance, the chi-square test is applied. The formula for chi-square is:

$$\chi^2 = \sum \left[\frac{(o - c)^2}{c} \right] \quad (6)$$

where o = observed numbers, c = calculated (expected) numbers, and Σ indicates that the bracketed quantity is to be summed. The following table illustrates the application of chi-square to the sample being used (Table 1):

Table 1. Illustration of the Application of Chi-Square

Class	Observed o	Calculated c	Deviation (o - c)	Squared Deviation (o - c) ²	$\frac{(o - c)^2}{c}$
AA	8	6	2	4	.666
AB	8	12	-4	16	1.333
BB	8	6	2	4	.666
					2.665

A chi-square table (Burns, 1976) is consulted and it is found that, with two degrees of freedom, the probability that the discrepancy between the observed and calculated number of individuals in each genotypic class occurred only by chance is approximately 0.30. Because statisticians usually choose the 0.05 value as the level of significance, the 0.30 probability indicates that there is a good fit

between the observed and calculated results for our sample (Burns 1976).

For a detailed genetic analysis of population structure, a large number of loci must be examined (Nei 1973; Lewontin 1974). Eight different enzymes which were found to be specified by fifteen different loci were examined in this investigation: hexose-6-phosphate dehydrogenase (H6PD), glucose-6-phosphate dehydrogenase (G6PD), sorbitol dehydrogenase (SDH), xanthine dehydrogenase (XDH), glutamate dehydrogenase (GDH), esterase (EST), tetrazolium oxidase (TO), and diaphorase (DIA). Six additional enzymes, representing 14 loci, were examined in a parallel study by another investigator (Brandes 1978; Harris et al. 1978): isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucose isomerase (PGI), and phosphoglucomutase (PGM).

Electrophoretic study of a variety of enzymes present in tissues obtained from individuals of selected populations provides information about genotype and gene frequencies in those populations. Many methods have been developed to quantify and express in a single parameter the amount of genetic differentiation among populations (Ayala 1975). The terms most commonly used in electrophoretic studies are genetic identity, I , and genetic distance, D , proposed by Nei (1972). Let X and Y be two different populations, and K a given locus. The normalized

probability that two alleles, one from each population, are identical is

$$I_k = \frac{\sum x_i y_i}{(\sum x_i^2 \sum y_i^2)^{1/2}} \quad (7)$$

where x_i and y_i are the frequencies of the i^{th} allele in populations X and Y. The mean genetic identity over all loci studied is given by

$$I = \frac{I_{xy}}{(I_x \cdot I_y)^{1/2}} \quad (8)$$

where I_{xy} , I_x , and I_y are the arithmetic means, over all loci, of $\sum x_i y_i$, $\sum x_i^2$, and $\sum y_i^2$, respectively. The average genetic differentiation, or genetic distance, between the two populations is estimated by

$$D = -\log_e I \quad (9)$$

where genetic distance values range from 0 to 1, with 1 indicating genetic identity.

As a result of extensive use of Nei's coefficient and other similarity coefficients in the last few years, certain criteria have been established which enable delineation of genera, species, subspecies, and local populations. Conspecific populations of a wide variety of organisms generally show similarity coefficient values in the 0.90's (Selander and Johnson 1973). Avise and Smith

(1974) concluded that the coefficient of genetic similarity which defines subspecies level occurs at 0.85 for the various species of the genus Lepomis.

Allendorf (1975) compared 38 different populations of anadromous and resident rainbow trout in Washington state and calculated genetic similarities of 0.90 or higher between all populations sampled. He concluded that the range of S values in the study populations was well within that reported for other conspecific populations of vertebrates.

Of the eight enzymes studied, only one, H6PD, was found to be polymorphic. In their study of brook trout, Stegeman and Goldberg (1971) found H6PD to be a dimer coded for by a single locus. H6PD is present in both liver and kidney of several vertebrates (Ohno et al. 1966; Shaw and Koen 1968). Stegeman and Goldberg (1972) reported the occurrence of seven known alleles for the H6PD gene in brook trout from Canadian waters.

Chapter 2

MATERIALS AND METHODS

Sample Collection

The brook trout used in this study were collected by electrofishing, utilizing lightweight, backpack A.C. electrofishing units. The fish were returned live to the vehicle and stored on ice until they reached the laboratory, at which time they were stored at -20°C . In some cases, the fish were stored in dry ice before arrival at the laboratory.

Table 2 contains a list of locations from which the brook trout were taken. Twenty-five fish from each location were chosen as a representative population.

Sample Preparation

The frozen fish were allowed to partially thaw and tissue extracts of liver were prepared by combining equal amounts of tissue and grinding solution (0.01 M tris - 0.0001 M EDTA, pH 6.8). Each extract was prepared in a 12 X 75 mm glass tube using a glass rod. The grinding and squashing process breaks the cell membranes, releasing the soluble enzymes into the grinding solution. The extracts were then centrifuged for 15 minutes at 9750 g to separate

Table 2. Brook Trout Collected

Location	No. of Samples	Date
Utah		
Blacksmith Fork River	30	July '77
Wisconsin		
Lawrence Creek	50	15 July '77
New York		
Cat Pond	25	1 February '78
Pennsylvania		
Bellefonte Hatchery	20	9 August '77
Brenner Creek	35	7 February '78
John Summerson Creek	35	7 February '78
West Virginia		
White's Run Creek	30	10 August '77
Senaca Creek	20	10 August '77
Horsecamp Creek	34	10 August '77
Virginia		
Chestnut Creek	45	13 June '77
Low Place Run	31	31 July '77
Helton Creek	45	13 June '77
Stewart Creek	52	14 June '77
North Carolina		
Pisgah Hatchery	50	17 June '77
Rough Butt Creek	32	24 August '77

Table 2. (continued)

Location	No. of Samples	Date
Tennessee		
Fagall Branch	13	21 June '77
Chestnut Branch	10	21 June '77
Birch Branch	24	22 June '77
Rough Ridge Creek	61	6 July '77
	25	13 July '77
Rocky Fork Creek	57	7 February '78
	25	7 February '78
Great Smoky Mountains National Park		
Dunn Creek	26	August '77
Enlow Creek	15	August '77
Dule Creek	52	August '77
Goshen Creek	24	August '77
Bunches Creek	24	August '77
Forney Creek	28	August '77
Flat Creek	24	August '77
Indian Camp Creek	24	August '77
Eagle's Rock Prong	26	August '77
Georgia		
Dover Creek	50	11 July '77
Board Camp Creek	51	11 July '77
Popcorn Creek	50	12 July

the broken tissues from the soluble proteins. The supernatant was poured off into small vials and closed with rubber stoppers appropriately marked as to sample location, sample number, and tissue. These samples were then frozen at -40°C for a period varying from a few hours to several months before electrophoresis was performed on them.

Gel Preparation

The techniques described in this and the following two sections are modified from procedures observed at the Savannah River Ecology Laboratory which are, in turn, based upon those described by Selander et al. (1971).

Each gel was prepared by combining 42 grams of hydrolyzed potato starch (Electrostarch Co., Madison, Wisconsin, Lot #307) with 400 ml of the gel buffer in a 2000 ml Pyrex boiling flask. The gel buffer used (0.03 M - 0.005 M citric acid, pH 8.5) was described by Ridgeway et al. (1970). This mixture was heated to boiling while it was swirled continuously. It was allowed to boil for a few moments after which it was degassed under the vacuum of a water aspirator for approximately one minute to remove any air bubbles. In the case of NADP-dependent enzyme systems, NADP was added after degassing. The starch preparation was poured into a rectangular plexiglass gel mold (18 X 21 X 1 cm). The gel was allowed to cool and solidify and was either used that day or kept overnight after being wrapped in plastic wrap to prevent drying out.

Electrophoresis

The gel was cut approximately 5 cm from the cathodal end with a thin spatula. Filter paper wicks (Whatman #3) which were cut into 4 X 9 mm pieces were dipped into partially thawed extracts and placed vertically in the gel cut. Each gel can hold 24 samples. The gel mold was placed on top of two plexiglass buffer trays in which the positive and negative electrodes are contained. Thin sponge cloths were utilized to conduct the electric current from the electrode buffer to the appropriate ends of the gel. The electrode buffer (0.06 M lithium hydroxide - 0.3 M boric acid, pH 8.1) was described by Ridgeway et al. (1970). A current was then applied across the gel by means of a constant voltage power unit. The wicks were removed after 10 to 15 minutes of electrophoresis and the two sections of the gel placed firmly together. A plastic ice tray was placed on top of the gel and electrophoresis was carried out inside a refrigerator maintained at 4°C. Both of these procedures prevent overheating. Electrophoresis required 3.5 hours at 250 volts.

Staining Procedures

After electrophoresis is completed, approximately 3 cm of the gel is cut off from each end. The remaining cathodal and anodal pieces were placed on a plexiglass cutting board which had raised edges along its two side

margins that were approximately 1/16 in high. The gel was sliced horizontally by drawing a wire through it. Usually four slices could be obtained from each gel. The slices were placed into individual staining trays (Ulchek Plastic Co. No. A401) with the side of the slice nearest the center of the gel facing upward. The appropriate stain (Table 3) was poured onto the gel and the tray was put in an incubator maintained at 37°C. After sufficient time had elapsed for a good banding pattern to become visible, the stain was poured off and the gel was fixed with approximately 30 ml of a 5:5:1 methanol, water, glacial acetic acid mixture. The fix halts the staining process and toughens the gel so that it can be handled.

Nomenclature

The nomenclature used in this study is that proposed by Allendorf and Utter (1977). An abbreviation is chosen to designate each protein. In the case of multiple forms of the same enzyme, a hyphenated numeral is included; the form with the least anodal migration is designated one, the next, two, and so on. Allelic variants are designated according to their relative electrophoretic mobility. One allele (generally the most common one) is arbitrarily designated 100. This unit distance represents the migration distance of the isozyme coded for by this allele. Other alleles are then assigned a numerical value representing their mobility relative to this unit distance. For example, a single gene

Table 5. Tissues and Staining Solutions Used for Electrophoresis (NAD = nicotinamide adenine dinucleotide, NADP = Nicotinamide adenine dinucleotide phosphate, NBT = Nitro blue tetrazolium, PMS = Phenazine methosulfate, NADH = Nicotinamide adenine dinucleotide reduced)

Enzyme	Staining Solution	
Diaphorase (DIA)	1	mg 2, 6-dichlorophenolindolphenol
	2.5	mg NADH
	10	mg MTT tetrazolium
	50	ml gel buffer
Esterase (EST)	5	ml 1% α -naphthyl-acetate in 1:1 acetone and water
	150	mg Fast Blue BB salt
	50	ml gel buffer
Glutamate dehydrogenase (GDH)	2	g L-glutamic acid
	10	mg NBT
	5	mg PMS
	5	mg NAD
	31	ml Phosphate B
	18	ml distilled water
Glucose-6-phosphate dehydrogenase (G6PD)	200	mg Glucose 6-phosphate
	10	mg NBT
	5	mg PMS
	5	mg NADP
	4	ml 0.5 M Tris HCl (pH 7.1)
	30	ml distilled water

Table 3. (continued)

Enzyme	Staining Solution	
Sorbitol dehydrogenase (SDH)	100	mg D-sorbitol
	2.5	mg MTT
	10	mg NBT
	5	mg PMS
	5	mg NAD
	50	ml 0.2 M Tris HCl (pH 8.0)
Xanthine dehydrogenase (XDH)	10	mg Hypoxanthine
	10	mg NBT
	5	mg PMS
	5	mg NAD
	50	ml 0.2 M tris HCl (pH 8.0)
Hexose-6 phosphate dehydrogenase (H6PD)	15	mg D-Galactose (6-phosphate)
	22.5	mg NBT
	2.5	mg PMS
	27	mg NADP
	38.3	ml 0.1 M tris 8.0
	5	ml 0.1 M MgCl ₂
Tetrazolium oxidase (TO)	Shows as a light band on the GDH slice	

codes for the enzyme hexose-6-phosphate dehydrogenase. The allele which specifies an enzyme migrating one-half as fast as the most common allele product would be designated H6PD-1(50).

Chapter 3

RESULTS

The results of the electrophoretic investigation of the 800 brook trout examined in this study are shown in Figures 2, 3, and 4 and Tables 4 and 5. The figures show a schematic representation of the banding patterns observed. Table 4 is a summary of the electrophoretic data collected.

Variant Enzymes

Interpretation of hexose-6-phosphate dehydrogenase (H6PD) was a relatively simple procedure due to the fact that all banding patterns were either a single band, representing a homozygote, or three bands, representing a heterozygote (Figure 2). In addition, H6PD appears to be a stable enzyme and there were few problems with resolution of samples. A total of four alleles were found in the populations surveyed (Table 5), with the most common allele, H6PD-1(100), predominating the Great Smoky Mountains National Park fish. Five of the eight Park populations were fixed for this allele and those populations exhibiting polymorphism had high frequencies for this allele: the Goshen Creek population had a frequency of 0.98 ($P > 0.99$), Flat Creek 0.98 ($P > 0.99$), and Indian Camp Creek 0.80 ($P > 0.50$) for the H6PD-1(100) allele.

H6PD-1(200)

H6PD-1(150)

H6PD-1(100)

H6PD-1(50)

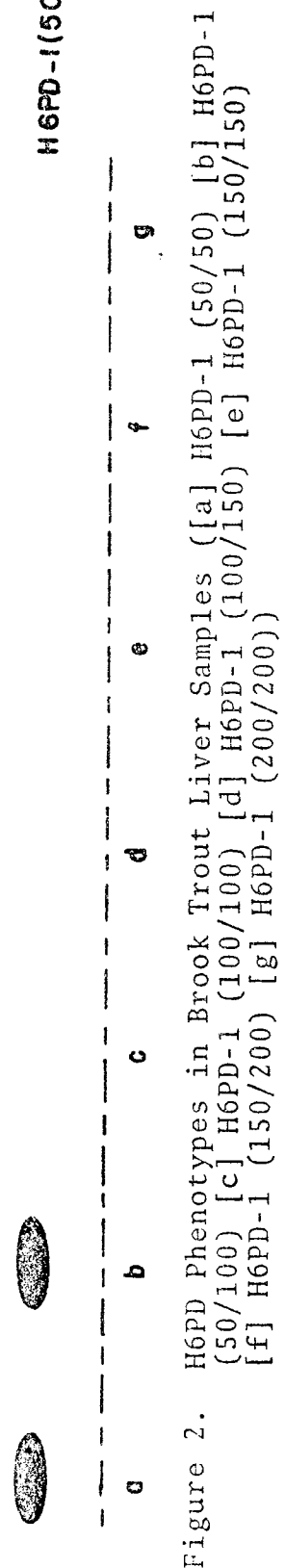


Figure 2. H6PD Phenotypes in Brook Trout Liver Samples ([a] H6PD-1 (50/50) [b] H6PD-1 (50/100) [c] H6PD-1 (100/100) [d] H6PD-1 (100/150) [e] H6PD-1 (150/150) [f] H6PD-1 (150/200) [g] H6PD-1 (200/200))

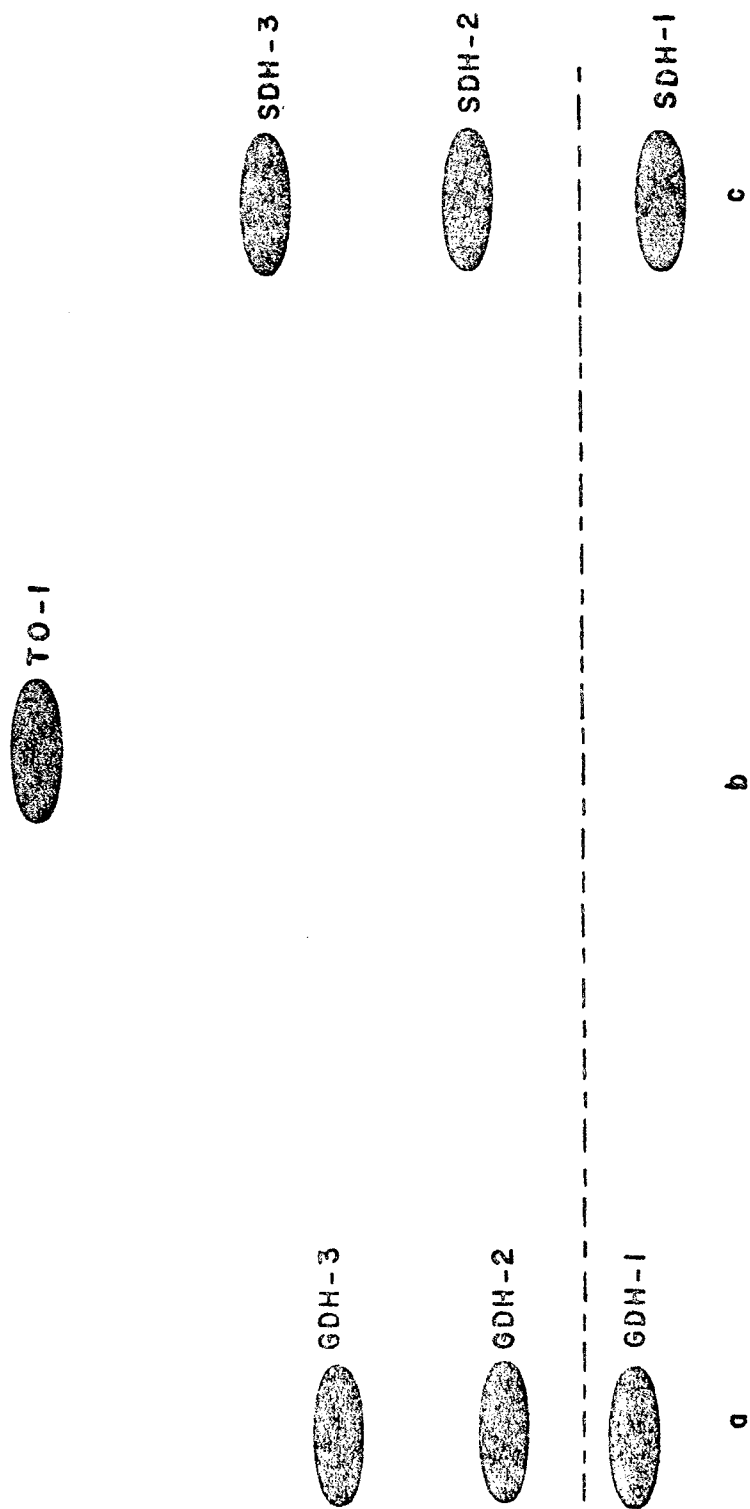


Figure 3. Nonvariant Enzymes Investigated: (a) GDH, (b) TO, (c) SDH

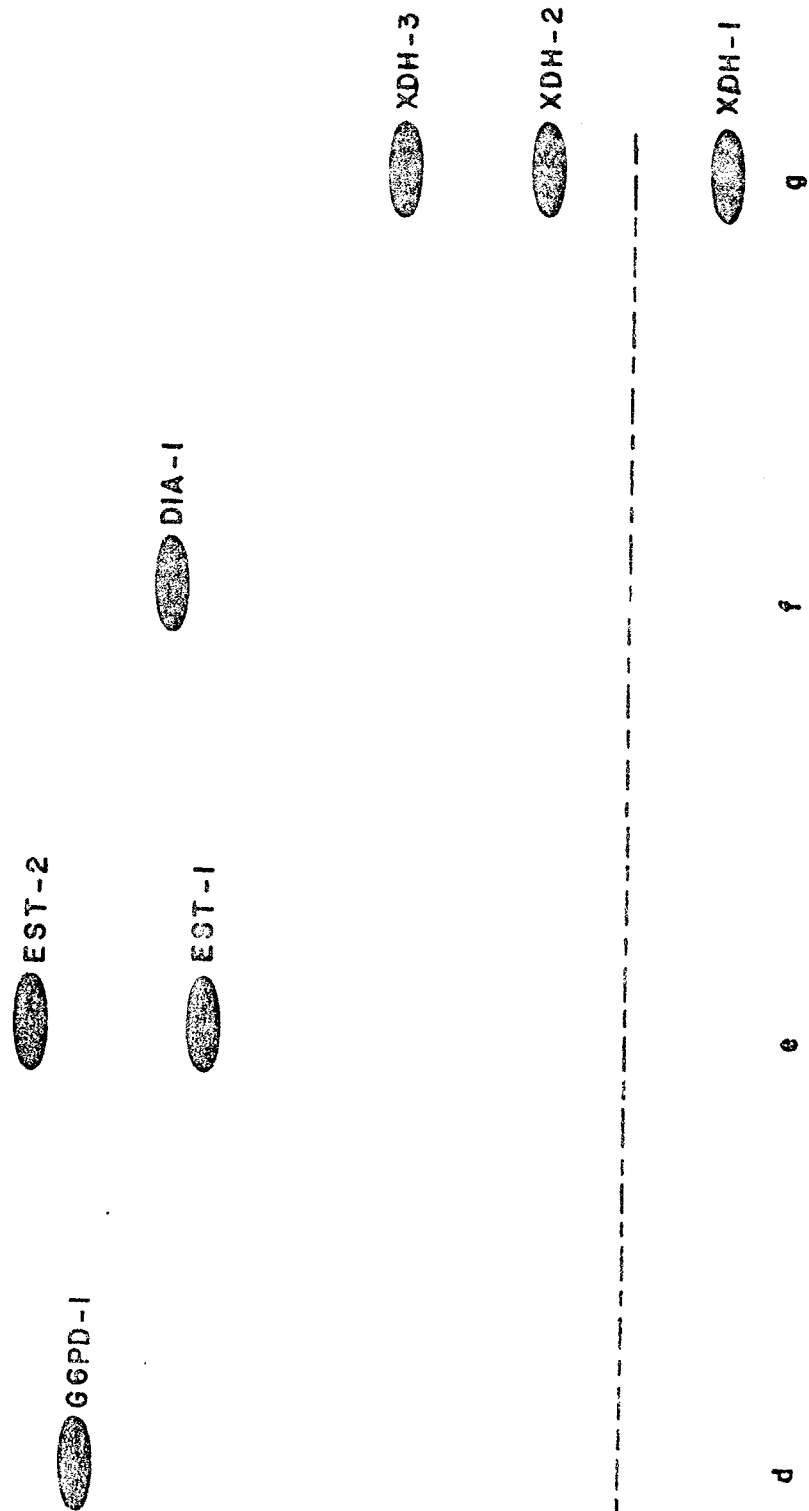


Figure 4. Nonvariant Enzymes Investigated: (d) G6PD, (e) EST, (f) DIA, (g) XDH

Table 4. Summary of Electrophoretic Data on Brook Trout Obtained from this Study (N_t = no. alleles, h = avg. proportion heterozygous individuals at each locus)

Locus	Populations Sampled	Individuals Sampled	Proportion of Populations Polymorphic*		N_t	h
			Criterion 1	Criterion 2		
H6PD-1	32	800	0.360	0.450	4	0.085
DIA-1	32	800	0.	0.	1	0.
G6PD-1	32	800	0.	0.	1	0.
EST-1	32	800	0.	0.	1	0.
EST-2	32	800	0.	0.	1	0.
TO-1	32	800	0.	0.	1	0.
XDH-1	32	800	0.	0.	1	0.
XDH-2	32	800	0.	0.	1	0.
XDH-3	32	800	0.	0.	1	0.
GDH-1	32	800	0.	0.	1	0.
GDH-2	32	800	0.	0.	1	0.
GDH-3	32	800	0.	0.	1	0.
SDH-1	32	800	0.	0.	1	0.
SDH-2	32	800	0.	0.	1	0.
SDH-3	32	800	0.	0.	1	0.

*Criterion 1: the frequency of the most common allele < 0.95 ;
 Criterion 2: the frequency of the most common allele < 0.99 .

Table 5. Gene Frequencies at the H6PD-1 Locus

Population	H6PD-1(200)	H6PD-1(150)	H6PD-1(100)	H6PD-1(50)	P of a larger χ^2
Utah					
Blacksmith Fork River			0.10	0.90	0.99
Wisconsin					
Lawrence Creek			0.23	0.77	0.70
New York					
Cat Pond			1.00		
Pennsylvania					
Bellefonte Hatchery					
Brenner Creek	0.36		0.61	0.03	0.80
John Summerson Creek			0.11	0.89	0.50
				1.00	
West Virginia					
Senaca Creek					
Horsecamp Creek			0.08	0.92	0.99
White's Run Creek			0.01	0.99	0.99
				1.00	
Virginia					
Low Place Run					
Chestnut Creek			0.03	0.97	0.99
Helton Creek			1.00		
Stewart Creek			1.00		
North Carolina					
Pisgah Hatchery					
Rough Butt Creek			1.00	1.00	

Table 5. (continued)

Population	H6PD-1(200)	H6PD-1(150)	H6PD-1(100)	H6PD-1(50)	P of a larger χ^2
Tennessee					
Birch Branch			1.00		
Fagall Branch			1.00		
Chestnut Branch			1.00		
Rocky Fork Creek	0.14	0.66	0.20		0.05
Rough Ridge Creek		0.15	0.85		0.05
Great Smoky Mountains National Park					
Dunn Creek			1.00		
Enloe Creek			1.00		
Dude Creek			1.00		
Goshen Creek		0.02	0.98		0.99
Bunches Creek			1.00		
Forney Creek			1.00		
Flat Creek		0.02	0.98		0.99
Indian Camp Creek			0.80	0.20	0.50
Eagle's Rock Prong			1.00		
Georgia					
Dover Creek			0.38	0.62	0.70
Board Camp Creek			0.54	0.46	0.95
Popcorn Creek			1.00		

All Tennessee populations except Rocky Fork Creek had high frequencies for this allele also. The Rocky Fork Creek population revealed the presence of an allele that was found in no other population studied. This allele specified the fastest migrating isozyme, and was given the designation H6PD-1(200). Rocky Fork was also one of the two populations that contained three alleles. (The other population was Bellefonte Hatchery in Pennsylvania). All other populations contained only one or two alleles.

The other populations that had high frequencies for the H6PD-1(100) allele were Cat Pond in New York, Rough Butt Creek in North Carolina, Bellefonte Hatchery in Pennsylvania, Chestnut Creek, Helton Creek, and Stewart Creek in Virginia, and Popcorn Creek in Georgia.

Nonvariant Enzymes

Glucose-6-phosphate dehydrogenase was represented by a single invariant band (Figure 4). This enzyme is considered by Stegeman and Goldberg (1971) to be a primary isozyme of H6PD and can usually be visualized on the same gel with H6PD. While G6PD can catalyze the oxidation of galactose 6-phosphate, as does H6PD, better resolution of G6PD banding patterns can be obtained using glucose 6-phosphate as the substrate.

Glutamate dehydrogenase, sorbitol dehydrogenase and xanthine dehydrogenase were all represented by three invariant bands (Figures 3 and 4). Each band was assumed to

be coded for by a different gene. In a similar study with brown trout, GDH and XDH were reported to be represented by a single band that was coded for by one gene (Allendorf et al. 1977). Allendorf reports the presence of two fixed loci for SDH in a study using rainbow trout (Allendorf 1973). Tetrazolium oxidase occurred as a single invariant band, assumed to represent one locus.

Esterase was present in the form of two invariant bands, representing two loci (Figure 4), although Brussard and Neilsen (unpublished) reported the existence of three loci in brook trout. Two loci appear to be operative for diaphorase. However, only one gave a consistently readable banding pattern which appeared as a single invariant band, as was reported for brown trout by Allendorf et al. (1977).

Additional Enzymes Investigated

A summary of the data collected from a parallel study by another investigator, Brandes (1978), is shown in Table 6. Of six enzymes studied, three were polymorphic, IDH, PGI, and LDH.

Genetic Similarity

Estimates of genetic similarity using the method described by Nei (1972) were computed with the gene frequencies for 29 loci (Table 7). Fifteen loci were from this study and fourteen were from the parallel study by

Table 6. Summary of Electrophoretic Data on Brook Trout Obtained by Brandes (1978) (N_t = no. alleles, h = avg. proportion heterozygous individuals at each locus)

Locus	Populations Sampled	Individuals Sampled	Proportions of Populations Polymorphic*		N_t	h
			Criterion 1	Criterion 2		
IDH-1	32	800	0.	0.	1	0.
IDH-2	32	800	.371	.457	4	.148
LDH-3	32	800	.058	.088	3	.031
LDH-4	32	800	.029	.029	2	.066
MDH-1	32	800	0.	0.	1	0.
MDH-2	32	800	0.	0.	1	0.
PGM-1	32	800	0.	0.	1	0.
PGM-2	32	80	0.	0.	1	0.
PGM-3	32	800	0.	0.	1	0.
PGI-1	28	675	.357	.428	4	.093
PGI-2	28	675	.071	.071	2	.019
PGI-3	28	675	.035	.045	3	.012
6PGD-1	32	800	0.	0.	1	0.
6PGD-2	32	800	0.	0.	1	0.

*Criterion 1: the frequency of the most common allele <0.95 ;
 Criterion 2: the frequency of the most common allele <0.99 .

Table 7. Genetic Similarity Matrix Based on Gene Frequencies Using Nei's Coefficient of Genetic Similarity

Lawrence Cr. VT	99
Cat Pond NY	97 97
Brenner Cr. PA	98 99 95
John Summerson Cr. PA	96 97 99 97
Bellefonte Hatchery PA	98 98 99 97 99
Seneca Cr. WV	98 99 95 00 97 97
White's Run WV	98 99 95 00 96 96 00
Horseshoe Cr. WV	97 97 94 97 94 95 97 97
Low Place Run VA	98 99 95 00 96 96 00 00
Chestnut Cr. VA	96 97 99 97 00 99 97 97 94 97
Helton Cr. VA	95 97 98 97 00 98 97 96 93 97 00
Stewart Cr. VA	96 97 99 97 00 99 97 95 94 97 00
Pisgah Hatchery NC	00 99 96 98 94 97 98 98 97 98 94
Rough Butt Cr. NC	91 92 92 92 92 92 92 92 92 92 92
Rocky Fork Cr. TN	91 95 95 94 96 96 94 94 90 94 96 95 96 92 90
Rough Ridge Cr. TN	91 95 95 94 96 96 94 94 90 94 96 95 96 92 90
Birch Branch TN	95 97 98 97 00 98 97 00 98 97 97 97 97 97 97
Chestnut Branch TN	92 94 95 94 96 96 94 93 90 93 97 97 96 91 93 89 93 97
Fagall Branch TN	95 97 98 97 00 98 97 00 98 97 96 94 96 00 00 00 94 97 92 97 97 98
Eagles Rock Prong CSNCP	93 94 95 94 96 96 94 93 90 94 97 96 96 92 00 94 97 92 97 97 97
Forney Cr. CSNCP	93 95 95 94 97 96 94 93 90 94 97 97 97 97 92 00 89 92 92 92 92 92
Flat Cr. CSNCP	93 95 95 94 97 96 94 94 90 94 97 97 97 97 92 00 89 92 92 92 92 92
Bunches Cr. CSNCP	93 94 95 94 96 96 94 93 90 94 97 96 96 92 00 85 99 99 96 93 97
Coshen Cr. CSNCP	93 97 98 97 99 99 99 97 97 97 95 96 92 00 89 92 92 92 92 92 92
Dude Cr. CSNCP	94 95 95 95 95 96 94 94 90 94 97 97 97 97 97 95 96 93 93 93 93
Indian Camp Cr. CSNCP	94 95 95 95 95 96 95 94 91 91 95 96 96 96 96 96 96 96 96 96 96
Enloe Cr. CSNCP	93 94 95 94 96 96 94 94 90 94 97 97 97 97 97 97 97 97 97 97 97
Dunn Cr. CSNCP	95 97 98 97 99 98 97 97 93 93 90 94 97 97 97 97 97 97 97 97 97
Popcorn Cr. GA	92 94 95 94 96 96 95 94 93 90 93 93 97 97 97 97 97 97 97 97 97
Dover Cr. GA	97 96 95 95 95 95 95 95 95 95 95 95 95 95 95 95 95 95 95 95 95
Board Camp Cr. GA	96 99 99 99 99 99 99 99 99 99 99 99 99 99 99 99 99 99 99 99 99
Blacksmith Fork	
Lawrence Cr.	
Cat Pond	
Brenner Cr.	
John Summerson Cr.	
Bellefonte Hatchery	
Seneca Cr.	
White's Run	
Horseshoe Cr.	
Low Place Run	
Chestnut Cr.	
Helton Cr.	
Stewart Cr.	
Pisgah Hatchery	
Rough Butt Cr.	
Rocky Fork Cr.	
Rough Ridge Cr.	
Birch Branch Cr.	
Chestnut Branch Cr.	
Fagall Branch TN	
Eagles Rock Prong	
Forney Cr.	
Flat Cr.	
Bunches Cr.	
Coshen Cr.	
Dude Cr.	
Indian Camp Cr.	
Enloe Cr.	
Dunn Cr.	
Popcorn Cr.	
Dover Cr.	

*All values were between 0.89 and 1.00. In this table a value of 00 represents 1.00, total genetic identity. All other values are preceded by 0.; therefore the value 99 is read 0.99.

Brandes (1978). Estimates of genetic similarity ranged from 0.89 to 1.00. Rocky Fork Creek, Tennessee, was the only population whose similarity value fell below the 0.90 level when compared with the other populations examined. It showed a value of 0.89 when compared with three populations from the Great Smoky Mountains National Park. The nine brook trout populations from the Park displayed similarity values of 0.96 or higher when compared with each other. In comparing Park populations to all other populations (except Rocky Fork Creek, Tennessee), the coefficient values were 0.90 or above.

Chapter 4

DISCUSSION

The values generated by use of Nei's coefficient indicate that there are no significant genetic differences between the populations examined, with the possible exception of fish from Rocky Fork Creek, Tennessee. As described in the Introduction, it has generally been observed that continuous populations of vertebrates have coefficients of genetic similarity in the range of 0.90 and above. Avise (1974) has graphically represented the coefficient values obtained for various species of the genus Lepomis. As illustrated in Figure 5, values of genetic similarity in the 0.90's indicate that the populations sampled belong to the same subspecies.

In the present study, no unique alleles were observed in the brook trout populations from the Park. This fact, along with values calculated by use of Nei's coefficient, leads to the conclusion that there are no significant taxonomic differences between the Park fish and the other brook trout populations examined. It is unlikely that the brook trout from the Great Smoky Mountains National Park belong to a different subspecies, based on the data from the 32 populations studied.

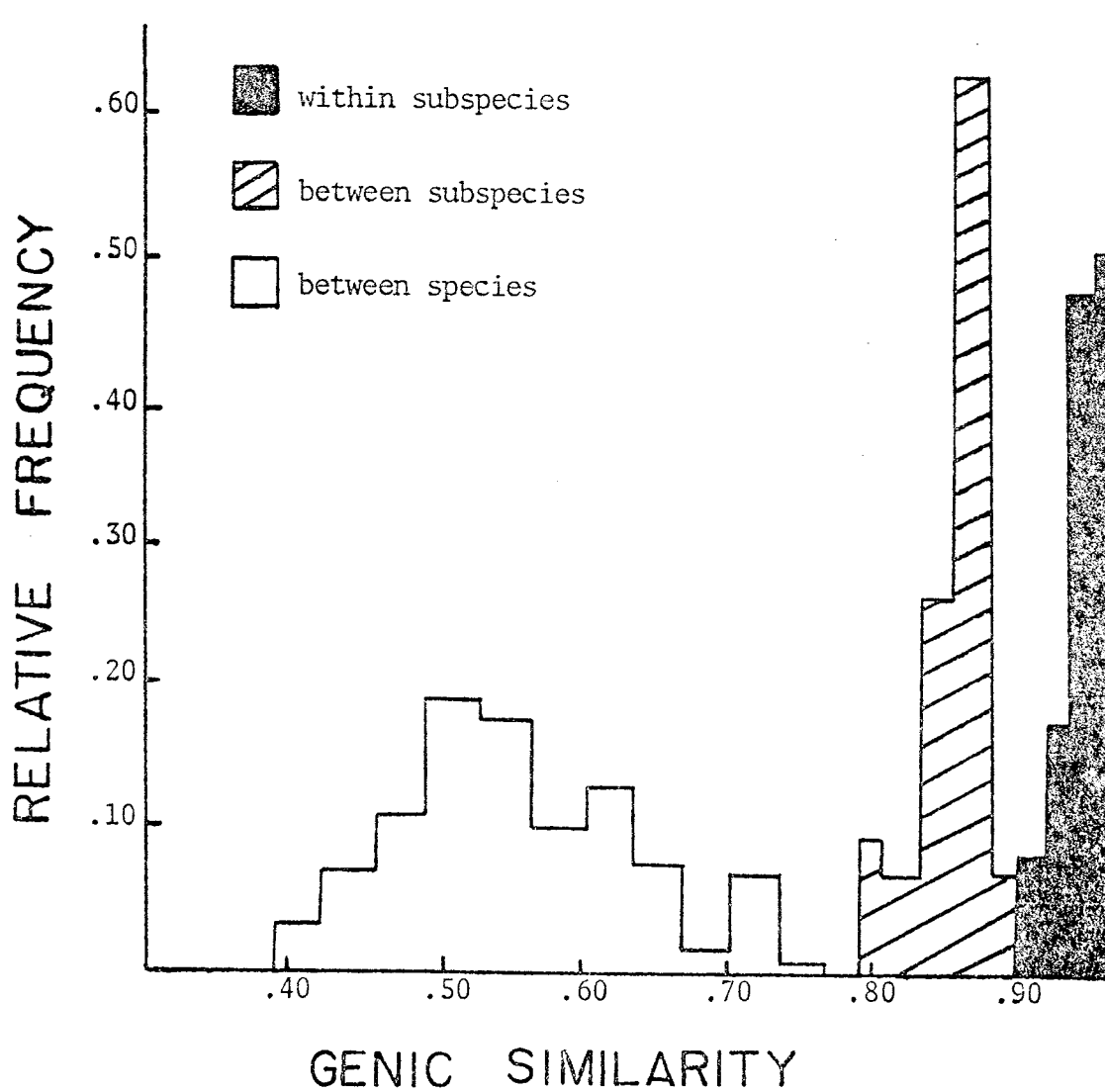


Figure 5. Frequency Distribution (expressed as percentages) of Coefficients of Genetic Similarity between Populations of Sunfish (from Avise 1974)

Several interesting observations were made about Rocky Fork Creek, Tennessee. A unique allele for H6PD was found in this population. Brandes (1978) reported unusual IDH phenotypes in Rocky Fork Creek individuals. Furthermore, coefficient of similarity values as low as 0.89 were observed when comparing Rocky Fork to the various populations in West Virginia, Tennessee, and the Park. These observations suggest that the genetic constitution of the Rocky Fork fish may differ from that of the other populations studied. This possibility should be examined further.

The Park populations examined in this study exhibited only a small amount of genetic variation for the 29 loci under consideration. All of them were fixed at all loci examined except for three populations which showed small degrees of polymorphism at the H6PD-1 locus. Genetic variation among organisms sharing a common gene pool is necessary before evolutionary change can occur. If all of the loci relevant to a particular trait lack genetic variation, then no scheme of selection can be successful (Allendorf and Utter 1977). Studies with a variety of organisms have shown that reduction in genetic variability often results in reduced viability of the population (Hershberger et al. 1976). The rainbow trout has historically been the most successful salmonid species in adapting to new environmental conditions. This greater adaptability is very likely due to the greater genetic variation throughout the genome (Allendorf and Utter 1977).

Loss in genetic variation is largely attributable to a limited population size. The magnitude of this loss in a single generation is inversely proportional to the size of that population as expressed by the following equation:

$$\Delta F = \frac{1}{2N_e} \quad (10)$$

where F is the inbreeding coefficient, which represents the proportional loss in genetic variation, and N_e is the effective size of the population (Crow and Kimura 1970). A major factor which influences N_e is the relative number of males and females in a population. The effective population size of a population with different numbers of males and females is expressed by the relationship:

$$\frac{1}{N_e} = \frac{1}{4N_f} + \frac{1}{4N_m} \quad (11)$$

where N_f and N_m are numbers of females and males in the population, respectively (Crow and Kimura 1970). As stated in the Introduction, Lennon (1967) reported that male brook trout in the Park outnumber females two to one. Hence, the effective size of the population is reduced.

Eaton (1970) discusses the probability that a small isolated population that happens to be cut off by barriers or distance from other populations of the same species may develop homozygosity for a number of genes by means of genetic drift. Assuming equal frequency to begin with, if

the population remains very small, the probability that, in a few generations, the two alleles will still be present and equally frequent approaches zero because of the strong effect of random chance.

The low level of genetic variability of the Park brook trout is probably a significant factor in their decline in number in Park streams. Without genetic variation, the Park populations cannot adapt to new environmental conditions. This has led to their gradual replacement by more genetically diverse, and hence more successful, rainbow trout.

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