AN ELECTROPHORETIC STUDY OF BROOK TROUT (*Salvelinus fontinalis*) FROM HEADWATER STREAMS OF THE BLUE RIDGE PARKWAY

A Thesis

by

LONNIE NEWELL SHULL III

Submitted to the Graduate School

Appalachian State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 1995

Major Department: Biology

AN ELECTROPHORETIC STUDY OF BROOK TROUT (*Salvelinus fontinalis*) FROM HEADWATER STREAMS OF THE BLUE RIDGE PARKWAY

A Thesis

by

LONNIE NEWELL SHULL III

August 1995

APPROVED BY:

Gary L. Walker
Chairperson Thesis Committee

William E. Dobson
Member Thesis Committee

J. Kenneth Shull
Member Thesis Committee

Richard N. Henson
Chairperson Biology Department

Joyce V. Lawerence
Dean of Graduate Studies and Research

ABSTRACT

AN ELECTROPHORETIC STUDY OF BROOK TROUT (*Salvelinus fontinalis*)

FROM HEADWATER STREAMS OF THE

BLUE RIDGE PARKWAY (July 1995)

Lonnie N. Shull III, B. S., The Citadel

M.S., Appalachian State University

Thesis Chairperson: Gary L. Walker

The brook trout (*Salvelinus fontinalis* Mitchell) is the only salmonid native to the southeastern United States. Human activities, such as logging, construction, fires, over-fishing and the introduction of non-native rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), have led to a restriction in the range of *Salvelinus fontinalis* in the southern Appalachian Mountains. Southern brook trout populations stocked with brook trout of northeastern origin exposed the southern populations to non-native genotypes. Previous studies have identified a marker for southern Appalachian brook trout at the CK-A2* locus. This study attempted to identify the genetic differences within and among populations of brook trout, and to identify populations of southern Appalachian brook trout based on CK-A2*, from streams located within holdings of the Blue Ridge Parkway.

Brook trout were collected from ten streams, representing four major river systems. Two of these, the New River and Watauga River, are located west of the Eastern Continental divide while the Roanoke River and Yadkin River flow east to the Atlantic Ocean. Standard horizontal starch gel electrophoresis techniques were used in this study, with fifteen enzyme systems surveyed and 29 loci scored. It was found that six of the ten streams surveyed contained the CK-A2*100 allele indicating the presence of southern Appalachian brook trout. Two streams from the Watauga River were fixed for CK-A2*100 suggesting these streams likely contain native populations of southern Appalachian brook trout. Three streams from the New River had frequencies of CK-A2*100 that ranged from 0.786 to 0.250. The only population located east of the continental divide to possess the CK-A2*100 allele was Garden Creek (CK-A2*100 = 0.250) in the Yadkin River drainage. All other eastern draining streams were fixed for CK-A2*78. The results of this study suggest that the natural range of southern Appalachian brook trout in western North Carolina and southwestern Virginia may not extend east of the Eastern Continental divide.

ACKNOWLEDGMENTS

I would like to thank the following persons for their particular input on this project. First and foremost I would like to thank Dr. Gary L. Walker, who secured the grant for this study and asked me if I would be interested in attempting it. His time, patience, knowledge, and guidance kept me in graduate school at my darkest moments. I would not be here if It were not for Gary.

I would also like to thank my parents, for their support (emotional, financial and other-wise) during my academic career.

A special thanks goes to Stan Guffey for help with lab techniques, scoring, data interpretation and general insight towards this project.

I would like to thank Dr. Bill Dobson, Dr. Kenneth Shull, and Dr. Wayne VanDevender for their help in the preparation of this thesis. Their input, and knowledge gave me new insights and directions that I would not have explored on my own. Ms. Betsy Harris deserves a word of thanks because she always knew where I could find that one special item that I needed "5 minutes ago". I also appreciate the help of the entire faculty of the Appalachian State University Biology Department, who either gave insight and (or) who put up with my antics while I was here. I would like to thank the National Park Service, the Blue Ridge Parkway, and

all of the associated personnel who helped me complete this project, but especially Ms. Bambi Teague, for her patience on the completion of this report.

A thank you is also extended to everyone who helped in the field and lab especially Ed Lickey and Kerry Heafner, who made the rigors of lab fun and exciting. Others who helped in field or lab include A. Atkins, Dana Tamashiro, Gibbs Smith, John Welch, Tim Smith and David Yale. A special thanks also goes to John Young who helped motivate me during my time in graduate school.

DEDICATION

I would like to dedicate this paper to my Parents. Thank you for being there when I needed you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The brook trout (*Salvelinus fontinalis* Mitchell) is the only salmonid native to the southeastern United States. The natural range of this species extends from northeastern Canada south to the Appalachian mountains of North Carolina, Tennessee and Georgia. Brook trout require clean, cold water to survive and reproduce, and at the southern limit of their natural range they are restricted to cold, headwater streams above 900 meters in elevation (Jones 1975; McCracken *et al.* 1993). These fish were originally found at elevations as low as 490 meters. However, logging, construction, fires, over-fishing and the introduction of non-native rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) have restricted the range of *Salvelinus fontinalis* in the southern Appalachian Mountains (King 1937; Lennon 1967; Jones 1975; Kelly *et al.* 1980).

Brook trout belong to the family Salmonidae which includes salmon, trout, char, whitefish and grayling. The family Salmonidae is among the most primitive of the fishes with bony skeletons and the lineage Salmoniformes can be traced back 100 million years (Willers 1991). The family Salmonidae is confined to the holarctic realm, the northern latitudes of North America and Eurasia. Fishes in this family can live in fresh water, or are anadromous (Behnke, from Stolz and Schnell 1991). Salmonids are identified by an elongate body, an adipose fin, a single dorsal fin, paired fins placed low on the body, and a lack of spines. Salmonids also have small

cycloid scales loosely embedded in the skin. These fish not only have teeth on the jaw, but also have patches of teeth on the vomer (Rohde et al. 1994).

The brook trout are in the genus *Salvelinus* and thus are not true trout, but are actually char. Fish in this genus are characterized by a boat-shaped vomer, and light spots on a dark-colored body. True trout and salmon have dark spots on a light-colored body. In addition, brook trout have a white margin on all but the dorsal fin, and heavy vermituclation.

Brook trout are a generalized species, and can live in a variety of natural habitats, from lakes to rivers and small streams. Some brook trout populations are anadramous and travel to salt water for part of their life cycle. The preferred water temperatures for brook trout is between 5° C and 19° C. However, brook trout can survive in water with temperatures between near 0° C and 25° C (Jenkins and Burkhead 1994). Brook trout in the mid-Atlantic region spawn from late October through November (Rohde *et al.* 1991). These reproductive activities are triggered by a reduction in day length and stream temperatures (Jenkins and Burkhead 1994).

In the southeastern United States brook trout are found in the Appalachian Mountains of North Carolina, Tennessee and Virginia. Several populations have been reported in South Carolina and Georgia, however it is uncertain if brook trout are native to watersheds east of the continental divide south of the James River in Virginia. These populations are regarded as probable introductions in the Savannah, Broad, Catawba and Pee-Dee (Yadkin) Rivers (Smith, 1907; Menhinick 1991).

The geological history of North America and the southern Appalachian Mountains has undoubtedly affected the range and distribution of brook trout. Most waters inhabited by salmonids have contained these species for less than 40,000 years (Willers 1991). Before the Pleistocene epoch, brook trout (or their ancestors) were probably restricted to the cold waters of northern latitudes. During this epoch, four major glacial advances and retreats changed the landscape of North America. As glaciers advanced, northern latitudes were covered with glacial ice and large river systems cooled, facilitating the southward migration of many cold water species. With glacial retreat, the rivers which served as migration corridors warmed and brook trout in the southern limits of the species range were isolated in cold, headwater steams of the southern Appalachian Mountains. Thus many cold water species common in more northern latitudes are also found in the southern Appalachian Mountains of North Carolina and Virginia.

Pleistocene glaciation events altered the drainage patterns of many rivers in the central and southern Appalachian Mountains. For example, the New River system which originates in Northwestern North Carolina and southwestern Virginia, flows north into the Ohio River complex which in turn flows west into the Mississippi River. The Allegheny River which flows southwest from southern Pennsylvania and New York State also empties into the Ohio River system. However, before the Wisconsin glaciation, the New River is thought to have been part of a larger river system, the Teays River, which probably joined the Mississippi in southern Illinois. The Allegheny River is also believed to have flowed north to the

Pittsburgh River approximately where Lake Erie is today. The Pittsburgh River is thought to have emptied into the Gulf of St. Lawrence. During the Wisconsin glaciation the path of the Allegheny River was blocked by glacial ice and the course of this river is thought to have reversed. The Teays River was thought to have been impounded by glacial ice to form Teays Lake. When the glacial ice melted, the Teays and Allegheny Rivers formed the lower Ohio River system. Countless other smaller changes in drainage patterns of eastern North America's rivers probably occurred during this time (Hocutt *et al.* 1978).

All of these geologically induced changes in the range and distribution of brook trout likely influenced the genetic structure within and among populations of brook trout. Southern populations of brook trout became isolated from the main species range. This isolation could have affected the genetic structure of brook trout populations in several ways. Southern populations would have likely been influenced by founder effects in small, colonizing populations. Genetic drift (due to restricted gene flow), likely has also occurred in small, relict populations of brook trout in the southern Appalachian Mountains isolated from the main species range. Another factor that could alter the genetic population structure of southern Appalachian brook trout populations is selection for specific traits that would be beneficial for survival in small, headwater streams.

Bailey and Smith (1981) theorized that two brook trout refugia may have existed during the Wisconsin glaciation based on the distribution patterns of brook trout in North America. One of these refugia is proposed to have existed east of the

Appalachian Mountains (Atlantic refugium) while the other likely existed west of the Appalachian Mountains as the Mississippi refugium. Today in North Carolina and Virginia, brook trout are known to occur in watersheds located east and west of the continental divide. Historical records indicate that brook trout are probably native to the Tennessee River system in North Carolina, and to the New River located in both North Carolina and Virginia.

Around the turn of the century, fisheries managers began to notice a marked decline in brook trout population sizes in the southern Appalachian Mountains. These reductions were mainly due to destructive logging practices, the construction of railroads, fires, and over-fishing (King 1937; Lennon 1967; Jones 1975; Kelly *et al.* 1980). To supplement these declines, managers began aggressive stocking programs which introduced hatchery brook trout from populations native to the northeastern United States and the rainbow trout (*Oncorhynchus mykiss*) which is native to the western United States. Both of these actions had detrimental effects on southern Appalachian brook trout populations. Unknown to fisheries managers of the time, stocking with brook trout of northeastern origin exposed the southern populations to non-native genotypes (McCracken *et al.* 1993). Brook trout are unable to compete for resources with rainbow trout except at the upper-most headwaters of streams (Nagel 1991). The wild populations used as the original stock of many early hatchery strains is undocumented. The early stocking histories of many streams in the southern Appalachian Mountains of North Carolina and Virginia is likewise poorly documented (McCracken *et al.* 1993).

Previous Genetic Studies

A large body of data exists concerning Salmonid genetics. Indeed, only *Drosophila* and *Homo sapiens* have been the subject of more studies regarding population genetics. Because of this plethora of information, only studies directly pertinent to this investigation have been included. The family Salmonidae is of a tetraploid origin. This tetraploid condition causes many loci to exhibit duplication and overlapping of allozyme banding patterns within and among genera and species. Stoneking *et al.* (1981a) used loss of expression in overlapping aspartate aminotransferase (AAT) loci for brook trout muscle tissue to hypothesize that salmonids are of an autotetraploid origin, and are losing expression (by formation of null alleles) of many duplicated genes.

In many cases, it has been demonstrated that the addition of hatchery fish into a wild population severely affects the genetic structure of that population. Vincent (1960) studied growth and behavior of hatchery brook trout compared to wild brook trout and noted that wild brook trout were more wary, had greater stamina, and could tolerate higher water temperatures than domesticated brook trout. Hatchery brook trout grew faster under hatchery conditions, but this effect was minimized under simulated stream conditions. Krueger and Menzel (1979) noted differences between wild and domesticated brook trout at two of three biochemical loci examined.

Lennon (1967) suggested that brook trout from populations located along the southern margin of the species range possibly constitute a separate subspecies or even a separate species. These inferences were based on morphometric data, but

little genetic information was offered to support this hypothesis. Two early genetic studies resulted in separate conclusions regarding the genetic relatedness of southern Appalachian brook trout populations. Brussard and Nielsen (1976) surveyed six populations of wild brook trout, including one population from the Great Smoky Mountains National Park (GSMNP). Using starch gel electrophoresis (18 allozyme loci) they determined that southern brook trout populations were genetically distinct from northern populations. Harris *et al.* (1978) surveyed 35 population (29 allozyme loci) from Georgia to New York and populations from Wisconsin and Utah, and determined that there were no significant genetic differences between northern and southern brook trout populations. Since brook trout are a tetraploid derived species, and many loci show duplication, overlapping and complexing between enzyme products. The studies by Brussard and Nielsen (1976) and Harris *et al* (1978) had difficulty in interpreting the isozyme banding patterns, and interpreted banding patterns based on Hardy-Weinberg expectations (May *et al.* 1979).

May *et al.* (1979) used inheritance data, based on the genotypic composition of offspring compared to their parents, to help clarify isozyme banding patterns in brook trout. With this information, Stoneking *et al.* (1981b) used starch gel electrophoresis (39 loci) to survey eight populations of "wild" brook trout across a wide geographic area including three populations from the Great Smoky Mountains National Park. This study found a mean genetic similarity of 0.890 between northern and southern brook trout populations. Most of the differences found by Stoneking *et al.* (1981b) were at the creatine kinase - A2 and glucose-6-phosphate isomerase -2

loci, in which southern populations have alleles not present in any northern population.

Stoneking *et al.* (1981b) did not account for stocking histories of the streams surveyed from the Great Smoky Mountains National Park, which would have helped explain the presence of northern alleles in southern populations. McCracken *et al.* (1993) used starch gel electrophoresis (34 loci) to survey streams within the GSMNP and demonstrated that the genetic differences between streams is related to their stocking histories. This study also described the second creatine kinase loci in brook trout muscle tissue (CK-A2*) locus as diagnostic for southern Appalachian brook trout. Native southern populations are fixed for CK-A2*100, northern (hatchery) populations are fixed at CK-A2*78 and hybrid populations possess both alleles and are designated CK-A2*100/78. Nei's index of genetic similarity for native southern Appalachian brook trout populations compared to hatchery derived populations was 0.906.

Perkins *et al.* (1993) examined brook trout from 24 wild populations in New York State using starch gel electrophoresis (68 loci) and found Nei's genetic distance estimates ranged from 0.001 to 0.094. These populations were from both sides of the eastern Continental divide. No northern populations of brook trout possessed the CK-A2*100 allele that McCracken described as the marker for southern Appalachian brook trout. Perkins *et al.* (1993) also suggested that southern brook trout were more similar to brook trout from west of the continental divide in New York State than brook trout from east of the continental divide in New York State.

Purpose of This Study

The goal of this study was to identify streams located within holdings of the Blue Ridge Parkway that contained brook trout and to describe the genetic differences within and among these populations. Furthermore, this study was conducted to establish which of these streams contained native southern Appalachian brook trout as designated by the CK-A2*100 allele as designated by McCracken *et al.* (1993). Finally this study was conducted in hopes of providing some insight towards the original natural range of brook trout within the central and southern Appalachian Mountains of North Carolina and Virginia, based on the genetic differences within and among populations of brook trout from the Blue Ridge Parkway drainages.

MATERIALS AND METHODS

Collection of Samples

Although the Blue Ridge Parkway traverses an extensive geographic area, the number of streams that are suitable for trout are relatively few, mainly due to the Parkway's narrow boundaries along ridge tops. Many streams along the Blue Ridge Parkway are important to conservation concerns because they represent the uppermost headwaters of drainages. Twenty streams which were thought to have high potential as brook trout habitat, based on the following criteria, were sampled in this study. First streams of large volume, compared to other streams within the same watershed, were prioritized. Second, a significant length of the stream was to be located on property administered by the Blue Ridge Parkway. Third, a stream needed a relatively large population of brook trout, with a high percentage of brook trout greater than one year old (age class 1+). These criteria were selected in order to minimize biological impact to these streams' trout populations and so management questions posed by this study could be addressed. These 20 streams represent four major river drainages along the Blue Ridge Parkway. Two of these rivers, the Roanoke and the Yadkin, originate east of the continental divide and flow to the Atlantic Ocean. The New River and the Watauga River flow west to the Mississippi River and eventually into the Gulf of Mexico.

Most collections were made by electrofishing, using a custom-made electroshocker loaned from Western Carolina University, (powered by a 120-volt

Honda generator). Voltages of between 500 and 700 volts AC according to the specific conductivity of a stream, were used at most sties. Moody's Mill Creek alone was sampled by angling. An attempt was made to collect ten fish from each population, however this sample size was not always realized. Often sample sizes were constrained by low overall population densities of brook trout, which was likely related to the small flow volume of most headwater streams. All individuals sampled were greater than 110 mm in length to assure all fish were from age classes of one year or greater. Finglerling brook trout or brook trout that are less than one year in age (age class <1) were not used due to the difficulty of dissecting tissue from these individuals. Forty brook trout were obtained from Cedar Springs Trout Farm in Rural Retreat, Virginia as stock standards.

All collected fish were sacrificed for muscle, heart and liver tissue. These tissues were placed in 1.8 ml cryovials and stored on ice during *in situ* processing of tissue. The tissue samples were then quick frozen in liquid nitrogen for transport to Appalachian State University (A.S.U.), or placed directly in a -80° C ultra-cold freezer. Two samples provided by a local landowner from Moody's Mill Creek were stored in a household freezer for approximately four months until they could be processed. This storage had no apparent effect on enzyme banding pattern resolution. Eye and muscle tissue were used for electrophoretic analysis at A.S.U., while heart and liver samples were saved for mtDNA analysis at the University of Tennessee at Knoxville.

Starch Gel Electrophoresis

Standard horizontal starch gel electrophoresis techniques were used in this study (Hillis and Moritz, 1989, Werth, 1985). Fifteen enzyme systems were surveyed and 29 loci were scored. A list of enzyme systems surveyed in this study and the tissue sources of each is shown in Table 1. Two gel and electrode buffer systems were used, amine-citrate (morpholine citrate), pH 6.1 (Clayton and Tretiak 1972) and lithium hydroxide (LiOH), pH 8.3 (Selander *et al.* 1971). Malate dehydrogenase, malate dehydrogenase ($NADP^+$) and peptidase were resolved on morpholine citrate pH 6.1. All other systems were run on LiOH pH 8.3.

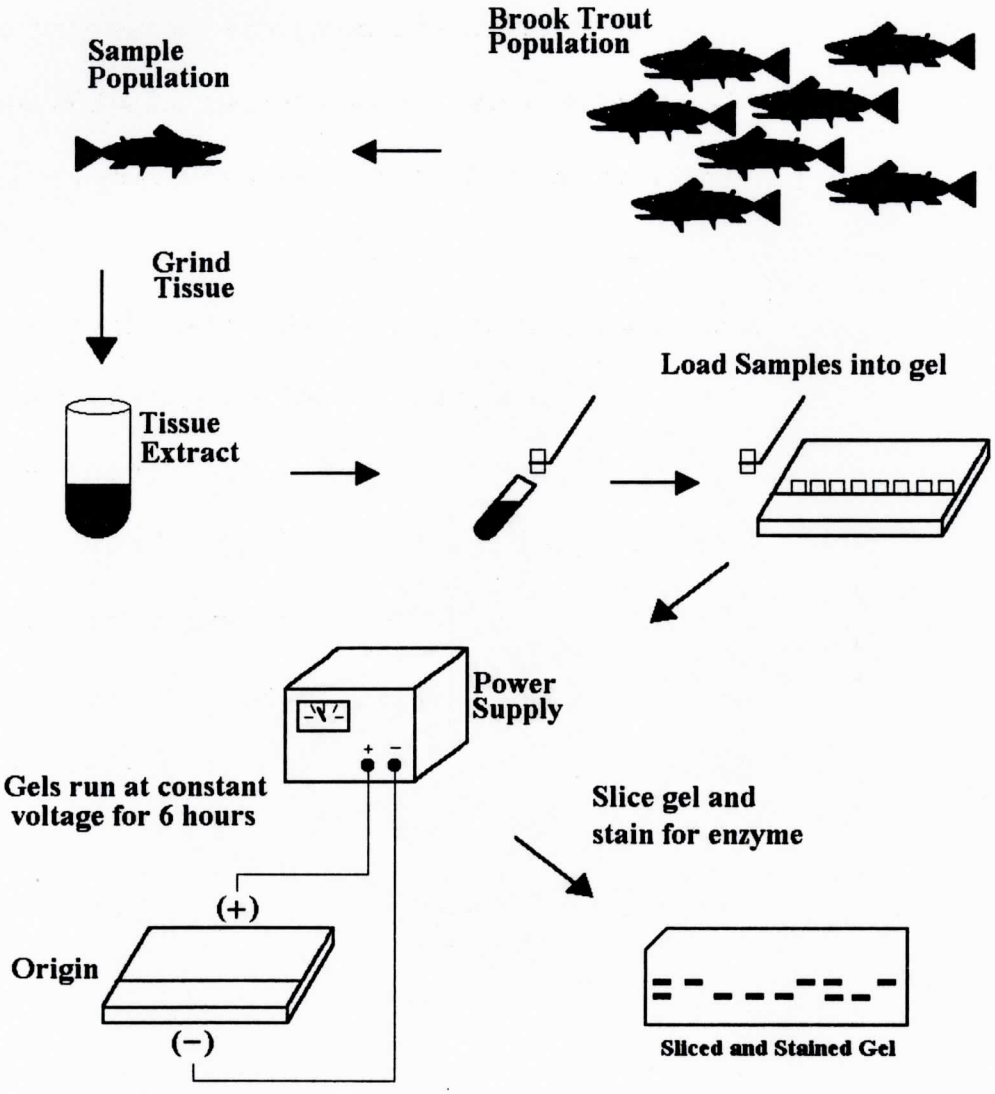
Gels were prepared one day prior to use. A schematic diagram of the starch gel electrophoresis process in shown in Figure 2. Buffers were prepared according to protocol listed in Appendix A. In a 1000 ml sidearm Erlenmeyer flask, 400 ml of gel buffer were added to 17 grams Sigma starch and 30 grams of Electrostarch. This was swirled until suspended. The starch and buffer solution was vigorously swirled over a 5000 BTU Bunsen burner until the onset of polymerization. Heating was continued for 35-40 seconds after the solution started to boil. Next, air bubbles were removed from the polymerized gel solution by degassing for 50 seconds using a vacuum aspirator. The solution was then slowly poured into a 400 ml gel mold. Dirt particles and air bubbles were removed using a pasteur pipet. Gels were allowed to cool for approximately one hour and then wrapped in plastic wrap and were stored at room temperature overnight prior to use. Gels were then placed in a refrigerator for 1 hour to cool them to operating temperature. During this time

Table 1. List of enzyme systems surveyed, number of loci, locus designation, and tissue sources for brook trout electrophoresis.

Enzyme or Other Protein	Enzyme Number	Number Of Loci	Locus	Tissue[a]
Aspartate aminotransferase	2.6.1.1	2	sAAT-3*;AAT-4*	E
		2	sAAT-1,2*	M
Creatine kinase	2.7.3.2	1	CK-B*	E
		2	CK-A1*;CK-A2*	M
Esterase	3.1.1.-	1	EST-1*	E
Fumarate hydratase	4.2.1.2	2	FH-1,2*	M
Glucose-6-Phosphate isomerase	5.3.1.9	3	GPI-1*;GPI-2*; GPI-3*	M
Glycerol-3-phoshpate Dehydrogenase	1.1.1.8	1	G3PDH-1*	M
L-Lactate Dehydrogenase	1.1.1.27	2	LDH-A1*;LDH-A2*	M
		2	LDH-B*;LHD-C*	E
Malate dehydrogenase	1.1.1.37	2	MDH-B1,2*; MDH-B3,4*	M
Malic enzyme (NADP+)	1.1.1.40	3	mMEP-1*;mMEP-2*	M
			sMEP-1*	M
Mannose-6-phosphate isomerase	5.3.1.8	1	MPI-1*	E
Dipeptidase	3.4.-.-	1	PEPA-1*	M
General Protein	No Number	2	PROT-1*;PROT-2*	M

[a]E= Eye tissue; M=muscle tissue

Figure 1. Schematic diagram of the major steps involved in starch gel electrophoresis.


Brook Trout
Population
Sample
Population
Grind
Tissue
Tissue
Extract
Load Samples into gel
Power
Supply
Gels run at constant
voltage for 6 hours
Slice gel and
stain for enzyme
(+)
Origin
(−)
Sliced and Stained Gel

tissue samples were removed from storage at -80° C and allowed to thaw at room temperature. Between 16 and 18 individuals and two standards were prepared for each gel. Eye and muscle tissue samples were prepared by adding an approximately equal volume of distilled water to tissue samples in cryovials, and were then homogenized using a glass stirring rod. Homogenized samples were stored on ice until all samples were prepared. At this time, 0.2 ml of ground sample tissue was placed into prechilled spot plates in preparation for loading into gels. The remaining homogenized tissue samples were returned to storage at -80° C in case additional analysis were necessary.

Current was applied to the gels for six hours at 50 mA, as this amperage was determined in preliminary runs to provided the best banding resolution. After six hours, gels were removed from the current and all excess gel was trimmed away using an exacto-knife or micro-spatula. Gels were then sliced into one millimeter thick sections using a gel-slicer fitted with a G-string banjo wire. Top slices were saved and used for dipeptidase (morpholine-citrate) or fluorescent esterase (LiOH), as the two systems showed superior resolution on this section of the gel. Slices were placed into appropriately labeled 15 cm x 20 cm plastic gel boxes and placed into a refrigerator until the application of stains. All stains followed recipes and protocols found in Hillis and Moritz (1990), except that the reducible dye MTT was substituted for NBT. Fluorescent esterase followed the recipe listed in Wendel and Weeden (1989). Creatine kinase was prepared and applied as an agar overlay. All stain recipes are listed in Appendix A.

After staining, gels were placed into an incubator preheated to 37° C. All gels were photographed and scored immediately after development. Scoring was consistent with McCracken *et al.* (1993), with the most common allele in the southern Appalachian populations designated *100. Alternative alleles were designated based on their electrophoretic mobility relative to the *100 allele, with faster alleles receiving a number greater than *100 and slower alleles receiving a number less that *100. After development, all stain solutions were discarded in a hazardous waster container. Gels were then rinsed with cold water and fixed overnight by immersion in 25-50 ml of a fixative solution comprised of a 5:5:1 mixture of methanol, water and glacial acetic acid. Fixed gels were wrapped in plastic wrap, labelled, and placed inside a sealed plastic container in a refrigerator for extended storage.

Description of Statistics

Allele Frequencies

Average allele frequencies are one method used to quantify the amount of genetic variation at a particular locus, within and among populations. For any population, the frequency of an allele is equal to the percentage of that allele at that locus.

Hardy-Weinberg Equilibrium

If the allele frequencies of a specific locus within a population can be determined, the Hardy-Weinberg equilibrium can be used to predict the expected occurrence of genotypic combinations within that population. The principle of a Hardy-Weinberg equilibrium generally states that allele frequencies will remain in a stable equilibrium with the genotypic frequencies of $AA=p^2$, $Aa=2pq$ and $aa=q^2$, where p is the frequency of allele A and q is the frequency of allele a. There are several assumptions that must be met before in order to maintain this equilibrium. These assumptions are:

1) organism must reproduce sexually

2) organism must be diploid

3) generations cannot overlap

4) all pairings for mating must be random

5) population size must be very large

6) no differential migration

7) no differential natural selection

8) mutation rate must be small enough to ignore

It is then possible using the Chi-square goodness-of-fit test, to see if the observed frequency of genotypic combinations deviate from expected genotypic frequencies. If populations are not in equilibrium, one can assume that one or more of the assumptions has been violated.

F-statistics

F-statistics also know as fixation indices, (Wright 1965) are a widely used description of the degree of genetic differentiation within and among subpopulations, when compared to the total sample population. F-statistics are based on observed allele frequency deviations from expected Hardy-Weinberg genotypic frequencies within and among subpopulations, as compared to the total sample population. By comparing these features, inferences as to the genetic architecture of subpopulations and the total sample population can be made. F-statistics are calculated based on the following levels of heterozygosity:

Ho = The observed heterozygosity of an individual within a subpopulation.

Hs = The expected heterozygosity of an individual within a subpopulation based on a Hardy-Weinberg equilibrium.

Ht = The expected heterozygosity of an individual in the total population if unstructured, calculated from the allele frequencies averaged across subpopulations.

In their simplest form, fixation indices can be calculated from the following equation.

$$F = \frac{(Hs - Ho)}{Hs}$$

F = 0 indicates no deviation from expected,

F = 1 indicated total deviation from expected

F-statistics are also used to describe the different levels of genetic variation within and among subpopulations. If the total sample population is subdivided, then an excess or deficiency of heterozygotes may result within subpopulations, when compared to what would be expected for a panmitic, total sample population. A subdivided population can be divided into three levels of complexity; individual organisms (i), subpopulations within the total population (s), and the total sample population (t). Three fixation indices are used to characterize the amount of genetic variation within these subdivisions.

F_{is} measures the levels of heterozygosity due to differentiation of individuals within subpopulations and is calculated using the formula:

$$F_{is} = \frac{(Hs - Hi)}{Hs}$$

where Hi is the observed heterozygosity of an individual in the subpopulation.

F_{st} measures the deviation from Hardy-Weinberg expectations based on levels of heterozygosity as a result of genetic differentiation among subpopulatons and is calculated from the following formula,

$$F_{st} = \frac{(Ht - Hs)}{Ht}$$

F_{it} measures the deviation from Hardy-Weinberg expectations based on levels of heterozygosity for an individual relative to the total sample population. Fit is described by the equation written below:

$$F_{it} = \frac{(Ht - Ho)}{Ht}$$

Wright's three F-statistics are related by the following equation:

$$(1-F_{it}) = (1-F_{is})\ (1-F_{st})$$

where the genetic variation within the total population (F_{it}) is equal to the genetic variation within subpopulations (F_{is}) times the genetic variation among the subpopulations (F_{st}), (Wright 1965).

G - Statistics

G - Statistics (Sokal and Rohlf 1981) are used as a goodness-of-fit test of allele frequency homogeneity when compared with Hardy-Weinberg expectations. With a *G* value = 0, the observed allele frequencies are exactly equal to Hardy-Weinberg expectations. The greater the value of *G* the greater the deviation from a Hardy-Weinberg equilibrium. The value of *G* can be used as a test for homogeneity by testing *G* for significance using the Chi square goodness-of-fit or the log likelihood test (Sokal and Rohlf 1981) with the null hypothesis that $G = 0$. If a significant *G* value is found, this represents a heterogenous (non-randomly mating) total sample population.

Nei's Genetic Distances

Genetic distance (D) is the degree of genomic difference between populations as measured by allele frequency differences (Nei, 1972). Estimates of genetic distance represent the number of gene or codon substitutions per locus that have occurred since the populations under consideration have diverged. With protein electrophoresis not all codon differences present in a population are detectable, so only differences detectable by the technique are used. As a result, measures of genetic distance are usually an underestimate of the true genetic divergence between populations. When calculated, a genetic distance of 0.00 indicates that two populations are identical in terms of allele frequencies, while a value of 1.00 indicates total divergence. Genetic distances can then be used to construct a dendogram or a phylogenetic tree showing genetic relatedness, using the unweighted pair-group method cluster analysis (UPGMA).

All statistical analyses were calculated using the "Gene's in Populations" computer program (May *et al.* 1992).

RESULTS

Description of Sample Sites

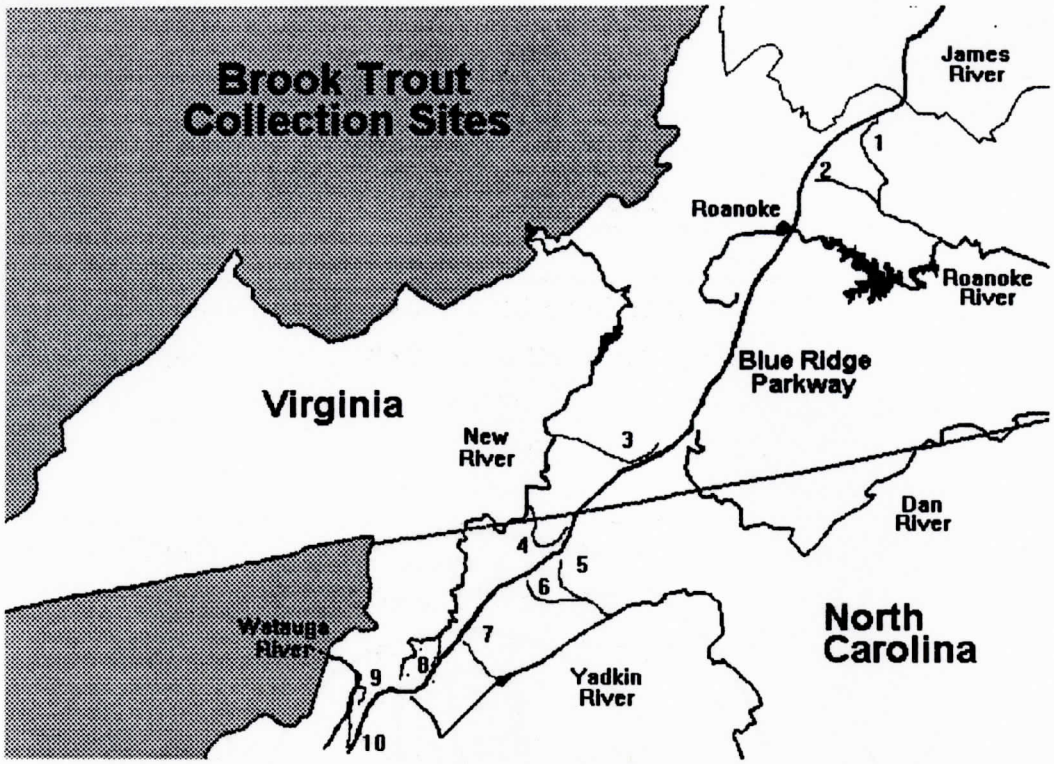
Brook trout were collected from 10 of 20 streams that met the selected criteria based on minimum biological impact to these streams and how best to answer management question posed by this study. The location of these creeks relative to the continental divide and watershed is shown in Figure 2. Four other streams had populations of brook trout, which were not collected due to low population densities, or had no fish in the +1 years age class. Six streams contained only brown trout or rainbow trout or both. One stream contained no trout at all in the area sampled. A summary of sample stream status relative to trout presence or absence is listed in Table 2.

Roanoke River Drainage

Big Stony Creek, Va.

Big Stony Creek was the northern-most sample site of this study. It borders Blue Ridge Parkway holdings and U.S. Forest Service land. This stream was sampled at 37° 28′ 30″ north latitude and 79° 33′ 30″ west longitude. This small creek averages approximately 3 meters in width and 40 cm in depth at the sample site. Big Stony Creek is managed as a wild trout stream by Virginia Department of Fish and Game. It received one reported stocking of brook trout (40 to 80 fish), 10 to 15 years ago (Blue Ridge Parkway Personnel, personal communication). Fingerling brook trout were observed indicating natural reproduction is taking place

Figure 2. Distribution of brook trout collection sites from the Blue Ridge Parkway in North Carolina and Virginia.



Population number = Population name

1 = Big Stony Creek	2 = Little Stony Creek
3 = Bull Head Creek	4 = Garden Creek
5 = Falls Creek	6 = Laurel Fork Va.
7 = Big Pine Creek Tributary	8 = Goshen Creek
9 = Cannon Branch	10 = Moody's Mill Creek

Table 2. Results of electrofishing from Blue Ridge Parkway streams.

Stream	State	B.R.P. Milepost	Observation
Roanoke River Drainage			
Big Stony Creek	Virginia	86.0	10 Brook trout
Little Stony Creek	Virginia	86.9	6 Brook trout
Lower Rock Castle Creek	Virginia	167.0	Brown & Rainbow trout
New River Drainage			
Laurel Fork	Virginia	174.0	7 Brook trout
Round Meadow Creek	Virginia	179.0	<1 yr Brooks
Mayberry Creek	Virginia	180.0	<1 yr Brooks
Chestnut Creek	North Carolina	216.0	<1 yr Brooks
Big Pine Creek (Trib.)	North Carolina	226.0	6 Brook trout
Laurel Creek	North Carolina	250.0	Brown trout
Meadow Fork	North Carolina	248.0	No trout
Goshen Creek	North Carolina	286.0	11 Brook trout
Yadkin River Drainage			
Bullhead Creek	North Carolina	232.0	7 Brook trout
Garden Creek	North Carolina	235.0	10 Brook trout
Basin Creek	North Carolina	241.0	Rainbow trout
Cove Creek	North Carolina	244.0	Rainbow trout
Falls Creek	North Carolina	268.0	7 Brook trout
Watauga River Drainage			
Sims Branch	North Carolina	295.0	Brown trout
Cannon Branch	North Carolina	296.0	10 Brook Trout
Bee Tree Creek	North Carolina	297.0	Brown Trout (1 Brook trout)
Moody's Mill Creek	North Carolina	297.0	6 Brook Trout

in Big Stony Creek. Big Stony Creek had an elevation of approximately 750 meters. Ten brook trout were collected from Big Stony Creek.

Little Stony Creek, Va.

Little Stony Creek was sampled at 37° 25′ 30″ north latitude and 79° 35′ 30″ west longitude. This was a small stream averaging 3 meters wide and 50 cm deep. Little Stony Creek is managed as a wild trout stream by the Virginia Department of Fish and Game. At one time this stream contained no trout and was subsequently stocked to create a trout fishery. Because of these factors population densities were quite low and only six brook trout were collected from Little Stony Creek. Few brook trout in age class <1 were found indicating little natural reproduction. At the sample site this creek had an elevation of 780 meters and carried a high sediment load. Little Stony Creek and Big Stony Creek merge at and elevation of 330 meters 4.5 km downstream from our sample site off Virginia Highway 43.

New River

Laurel Fork, VA.

Laurel Fork Creek flows parallel to the Parkway for 3 km. This study sampled Laurel Fork at 80° 24′ 0″ north latitude and 36° 46′ 30″ west longitude. This stream averaged approximately 4 meters wide and 20 cm deep. Seven brook trout were collected from this site. The elevation at this site was calculated from

topographical maps to be approximately 980 meters. This stream is stocked by the Virginia Department of Fish and Game and fingerling brook trout were present.

Big Pine Creek Tributary, NC.

A small, north-flowing unnamed tributary of Big Pine Creek was sampled after no 1+ year age class brook trout were found in Big Pine Creek itself. It is likely that no brook trout were found in Big Pine Creek due to heavy fishing pressure. The sample site was located at 36° 28′ 30″ north latitude and 81° 59′ 0″ west latitude. Big Pine Creek tributary was 1.5 meters wide and 30 cm deep at the sample site. Many fingerling brook trout were found in this stream. Only six brook trout were collected from Big Pine Creek Tributary since few age class 1+ were found. This possibly due to the small size of this stream. Big Pine Creek is stocked by the North Carolina Wildlife Resources Commission.

Goshen Creek, NC.

Goshen Creek was sampled at 38° 30′ 0″ North latitude and 81° 10″ 0″ west longitude. Goshen Creek was the largest creek sampled in this study, averaging 5 meters wide and 50 cm deep. Eleven brook trout were collected from Goshen Creek and fingerling brook trout were also present. Goshen Creek is stocked by the North Carolina Wildlife Resources Commission and brook trout were living sympatricly with rainbow trout in this stream. The elevation at the sample site was 1100 meters.

Yadkin River

Bullhead Creek, NC.

Bullhead Creek was sampled above Stone Mountain State Park at 81° 05′ 30″ north latitude and 36° 25′ 30″ west longitude. This stream had the largest brook trout (271 mm) collected in this study. Individuals of the brook trout population in this stream also had the largest average length of any stream in this investigation. Population densities were relatively low in Bullhead Creek and only six brook trout were collected after shocking approximately 600 meters of the stream. Elevation at the sample site was 860 meters. Bullhead Creek has been stocked by the North Carolina Wildlife Resources Commission.

Garden Creek, NC.

Garden Creek was sampled below Devil's Garden overlook above Stone Mountain State Park. The sample site was located at 36° 25′ 30″ north latitude and 81° 6′ 30″ west longitude. Sampling took place at the extreme headwaters where Garden Creek averaged only 2 meters wide and 20 cm deep. Designated as wild trout water by the North Carolina Wildlife resources Commission, Garden Creek contained a healthy population of brook trout and ten fish were collected. Many fingerling brook trout were found indicating natural reproduction is taking place at this site. Some stocking has taken place on Garden Creek (Joe Mickey, NCWRC, personal communication). Elevation at the sample site was estimated at 860 meters.

Garden Creek is located 3 km from Bullhead Creek and these two streams join at an elevation of 430 meters.

Falls Creek, NC.

Falls Creek was sampled at the Cascades overlook located in E.B. Jeffress Park at 36° 14′ 45″ north latitude and 81° 27′ 30 west longitude. Falls Creek was very small, with a depth of 20 cm and 50 cm wide. Due its small size, only seven brook trout were collected from Falls Creek. Falls Creek was sampled above a 25 m waterfall that is presumably a barrier from down stream migration. Elevation at the sample site was 1050 meters. Falls Creek was reported to have been stocked by North Carolina Wildlife Resources Commission but it is unclear if this stocking event took place above the cascades.

Watauga River

Cannon Branch, NC.

Cannon Branch is a tributary of Boone Fork Creek and was sampled off Old Shull's Mill road in Julian Price Park. This sample site was located at 36° 9′ 30 north latitude and 81° 43′ 30″ west longitude. At the sample site Cannon Branch was not a continuously flowing creek, but a collection of small pools one meter wide and 5 to 10 meters long. It did, however, contain a healthy population of brook trout and 10 brook trout were collected. The elevation at the sample site was 950 meters.

Moody's Mill Creek, NC.

Moody's Mill Creek drains the northern face of Grandfather Mountain. Moody's Mill Creek forms the south-western border of Julian Price Park and is adjacent to a private land holding. This creek was sampled after the main collection dates when the landowners found out about this project through an article published in the Mountain Times and contacted Appalachian State University. The sample site was located at 81° 46′ 0″ north latitude and 36° 08′ 0″ west longitude. Moody's Mill Creek is 1.5 meters wide and 20 cm deep. Six fish were collected by angling from Moody's Mill Creek between January and April of 1994. The Moody's Mill Creek site is located at an elevation of 1150 meters.

Results of Allozyme Analysis

A total of 21 loci were found to be consistently scorable and were used for allozyme analysis. Of these, nine loci were found to be polymorphic and twelve were monomorphic. sAAT-1,2* were over-lapping polymorphic loci that were consistently scorable but were excluded from our analysis, because it was impossible to assign the variation observed in sAAT-1,2* to either of the loci, and the computer program "Gene's in populations" (May *et al.* 1992) is not capable of analyzing over-lapping loci.

The CK-A2*100 allele which is diagnostic of the native southern Appalachian brook trout as described by McCracken *et al.* (1993) was found in six of the ten streams surveyed. CK-A2*100 was found in all western draining streams

and in one eastern draining stream, Garden Creek, at a frequency of 0.25. Of the western draining streams, only Cannon Branch and Moody's Mill Creek were fixed for CK-A2*100, representing pure populations of native southern Appalachian brook trout. Both of these streams are in the Watauga River drainage. All three of the remaining western draining streams, located within the New River drainage, were found to contain hybrid populations of brook trout with varying frequencies of CK-A2*100 ranging from 0.786 to 0.250. A summary of the allele frequencies found for CK-A2*100/78 is shown in Figure 3.

This study is the first to describe the locus AAT-4* for eye tissue as semi-diagnostic for southern Appalachian brook trout. This locus was fixed for AAT-4*100 in all populations west of the continental divide except for Big Pine Creek Tributary. All populations east of the continental divide were fixed for AAT-4*88 with the exception of Garden Creek which was fixed for AAT-4*100. The frequency of AAT-4* alleles for all populations is shown in Figure 4. A pattern similar to that of CK-A2*100 can be seen, with the 100 variant of the allele being held only in populations west of the Eastern continental divide with the exception of Garden Creek. It is also important to note that no heterozygotes were found for AAT-4*. This locus may represent the expression of a mitochondrial AAT locus (Stanley Z. Guffey, personal communication). The lack of heterozygotes explains the deviation from Hardy-Weinberg equilibrium at this locus in the Big Pine Creek population.

Figure 3. Pie diagrams of allele frequencies at CK-A2* in brook trout populations from the Blue Ridge Parkway.

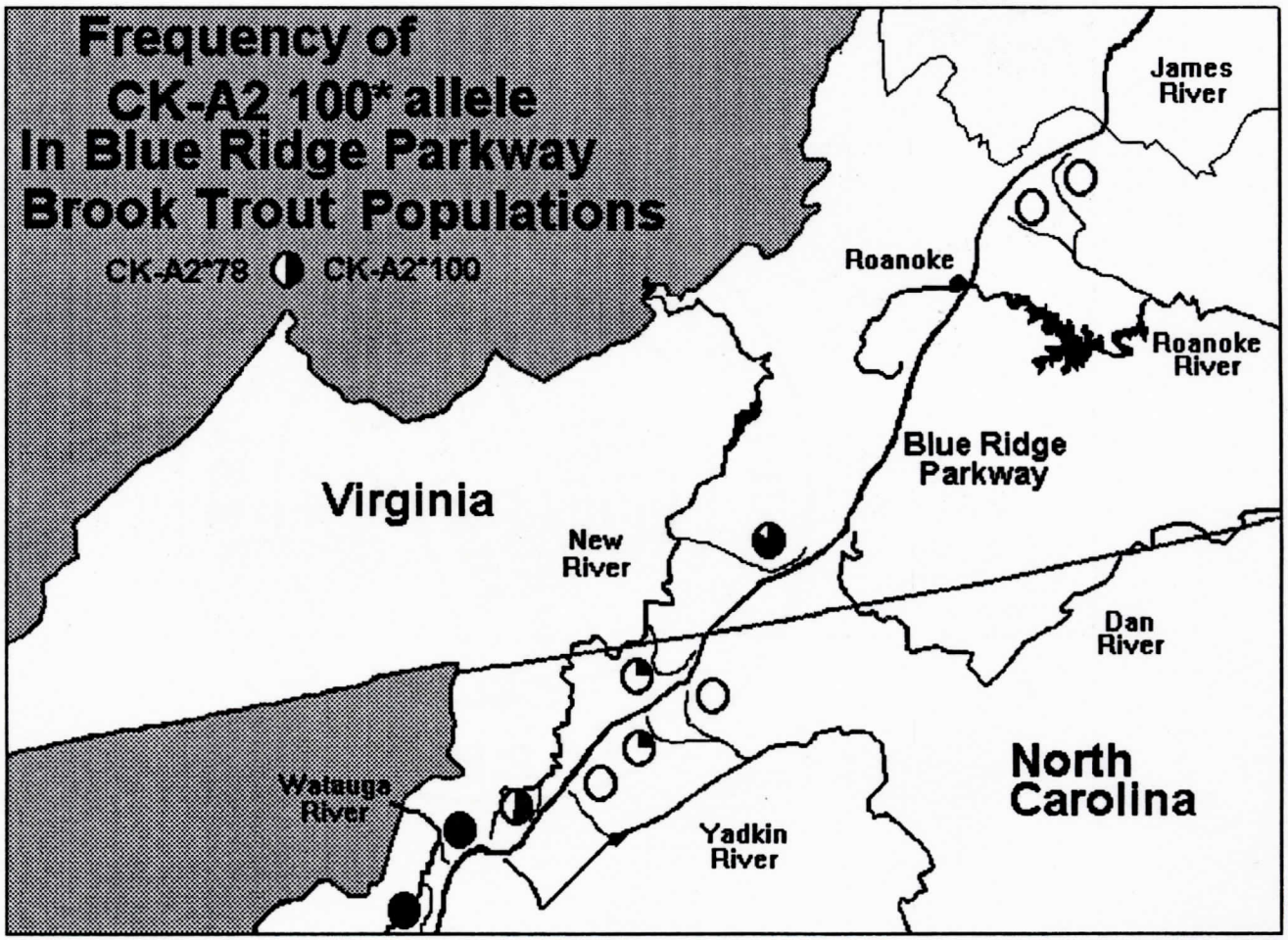
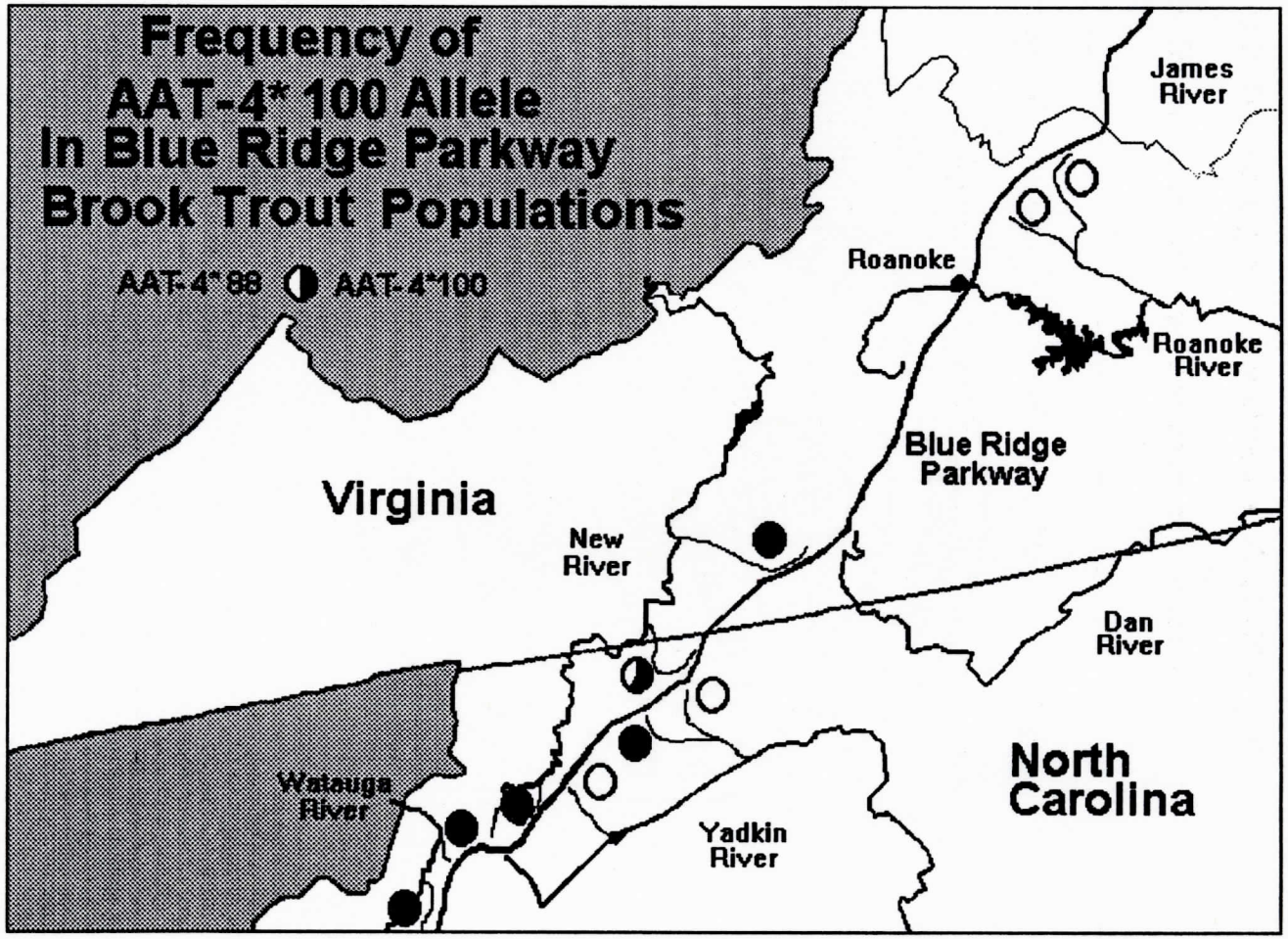

Frequency of
CK-A2 100* allele
In Blue Ridge Parkway
Brook Trout Populations
CK-A2*78
CK-A2*100
James River
Roanoke
Roanoke River
Blue Ridge Parkway
Virginia
New River
Dan River
North Carolina
Watauga River
Yadkin River

Figure 4. Pie diagrams of allele frequencies at AAT-4* in brook trout populations from the Blue Ridge Parkway.


Frequency of
AAT-4* 100 Allele
In Blue Ridge Parkway
Brook Trout Populations
AAT-4*88
AAT-4*100
James River
Roanoke
Roanoke River
Blue Ridge Parkway
Virginia
New River
Dan River
North Carolina
Watauga River
Yadkin River

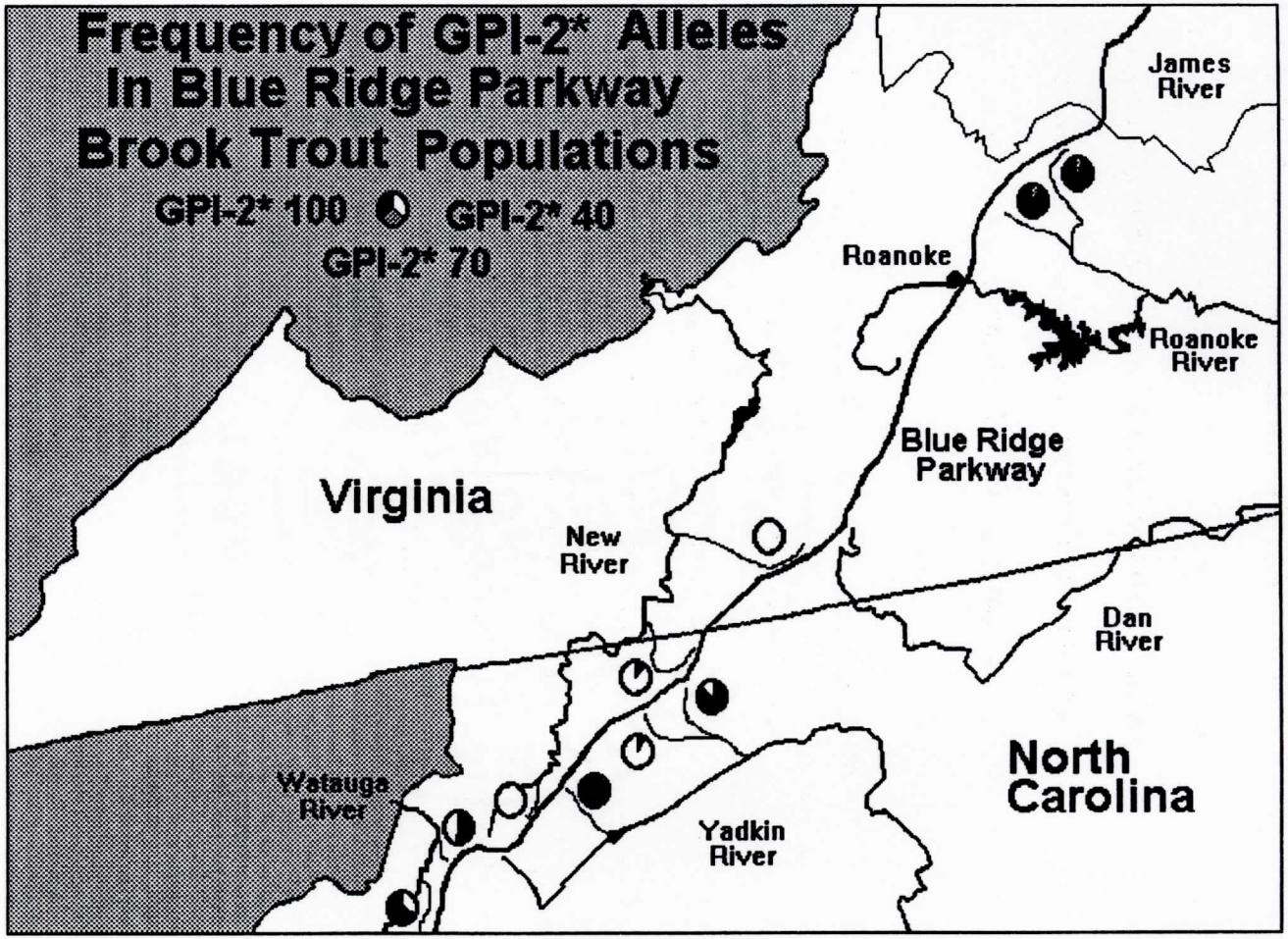
It was shown that the GPI-2* locus may be semi-diagnostic for southern Appalachian brook trout, with the 40 allele representing southern Appalachian brook trout and the 100 allele representing the eastern draining or stocked populations. A map of GPI-2* frequencies is shown in Figure 5. The highest levels of GPI-2*100 west of the continental divide were found in the putative native population of Cannon Branch and Moody's Mill Creek. This locus does not appear diagnostic in studies done by the University of Tennessee at Knoxville where frequencies of GPI-2*100 are higher in native southern Appalachian brook trout populations (McCracken *et al.* 1993, Kreigler 1993).

No other loci exhibited patterns with regard to allele frequency and stream position relative to the continental divide. All allele frequency data is shown in Appendix B.

Fixation Indexes(F_{is})

F-statistics were calculated for all natural populations (i.e., brook trout standards were excluded because they do not represent a natural population undergoing random mating) at all polymorphic loci with the exception of the overlapping loci AAT-1,2*. F_{it}, F_{is} and F_{st} were calculated for each polymorphic locus across all populations. Negative F_{is} values, indicating an excess of heterozygotes, were found for the following loci, CK-A2*, GPI-2* and GPI-3*. Positive Fis values, indicating a deficiency of heterozygotes, were found for LDH-A2*, sMDH-3,4*, mMEP-1*, AAT-4* and FH-1,2*. For all loci, F_{st} values are

Figure 5. Pie diagrams of allele frequencies at GPI-2* in brook trout populations from the Blue Ridge Parkway


Frequency of GPI-2* Alleles
In Blue Ridge Parkway
Brook Trout Populations
GPI-2* 100
GPI-2* 40
GPI-2* 70
James River
Roanoke
Roanoke River
Blue Ridge Parkway
Virginia
New River
Dan River
North Carolina
Watauga River
Yadkin River

larger than F_{is} values indicating that a majority of the genetic differentiation is among brook trout populations rather than within populations. CK-A2* and GPI-2* have the greatest difference between F_{st} and F_{is} values. Results from these tests are listed in Table 3. F-statistics were also calculated with populations pooled by watershed and by location relative to the continental divide. All F_{is} values were positive indicating some degree of reduction in total expected heterozygosities. Total F_{is} values are shown in Table 4. Average F_{it} was 0.598 at the individual population level. Average F_{is} was 0.140 and an F_{st} value of 0.533. When stream populations were pooled at the watershed level, F_{it} values remained similar to individual populations at 0.588, but the values for F_{is} increased to 0.378 and that of F_{st} decreased to 0.338. Finally, when all populations were pooled into two groups relative to position east or west of the eastern continental divide, the value of F_{it} equaled 0.595, while the value of F_{is} increased to 0.461 and the value of F_{st} further decreased to 0.249. When the brook trout standards are included, F_{it} becomes 0.607 with F_{is} equaling 0.181 and F_{st} becomes 0.520.

Average heterozygosities across all loci were calculated for natural populations. Observed average heterozygosity (H_o) was estimated to be 0.061. This was less than the average expected heterozygosity (H_s) of 0.071. Three loci had higher levels of H_o than expected, CK-A2*, GPI-2* and GPI-3*. However, only one of these, GPI-2*, varied significantly from expected with a H_o of 0.249 and a Hs of 0.213, and this difference might be explained due to the relatively small population sizes of this study. All other loci showed a deficiency of heterozygotes

Table 4. Hierarchial average F_{is} values for populations, watersheds and east and west of the Continental divide for brook trout populations from the Blue Ridge Parkway.

	F_{is}	F_{it}	F_{st}
Populations	0.140	0.598	0.533
Watersheds	0.378	0.588	0.338
East vs. West	0.461	0.595	0.249
With Standards	0.181	0.607	0.520

Table 3. Average F_{is}, F_{it} and F_{st} values for all polymorphic loci in brook trout population from the Blue Ridge Parkway.

Locus	F_{is}	F_{it}	F_{st}
CK-A2*	-0.006	0.663	0.665
LDH-A2*	0.330	0.759	0.589
sMDH-3,4*	0.317	0.529	0.311
mMEP-1*	0.198	0.604	0.507
GPI-2*	-0.166	0.523	0.591
GPI-3*	-0.014	0.110	0.122
sAAT-4e*	1.000	1.000	0.903
FH-1,2*	0.077	0.295	0.237
Average	0.140	0.598	0.533

Table 5. Ht, Hs and Ho values for all polymorphic loci in brook trout populations from the Blue Ridge Parkway

Locus	Ht	Hs	Ho
CK-A2*	0.473	0.158	0.159
LDH-A2*	0.330	0.136	0.080
sMDH-3,4*	0.354	0.244	0.166
mMEP-1*	0.423	0.209	0.168
GPI-2*	0.521	0.213	0.249
GPI-3*	0.180	0.158	0.160
sAAT-4e*	0.493	0.048	0.000
FH-1,2*	0.439	0.335	0.310
Average[a]	0.153	0.071	0.061
Variance	0.002	0.001	0.000
Std. Err	0.046	0.024	0.022

[a]averaged across all 21 loci.

relative to expected Hardy-Weinberg values with a range of -0.002 to -0.078. These values are shown in Table 5.

Nei's genetic identities were calculated for all natural populations and are shown in Table 6. Similarities between all populations ranged from 0.988 to 0.785 with all eastern draining populations being more similar than all western draining populations with the exception of Garden Creek. Genetic similarities between eastern draining populations ranged from 0.988 to 0.861. The eastern draining Garden Creek was more similar to western draining populations. Values for genetic similarities of western draining populations and Garden Creek ranged from 0.912 to 0.967. This was due to the presence of CK-A2*100 and AAT-4*100 in Garden Creek and the absence of these alleles in all other eastern populations. For populations located west of the continental divide, similarities ranged between 0.896 and 0.993. The largest difference was between Big Pine Creek tributary and Moody's Mill Creek while the greatest similarity was between Laurel Fork and Goshen Creek. Dendrograms based on Nei's genetic distance values using Unweighted Pair Group Method Analysis were calculated for all natural populations alone (Figure 6) and with all natural populations and the brook trout standards (Figure 7). The natural populations separated into two major groups, at a distance of 0.155. These two divisions represent populations east and west of the continental divide, with the exception of Garden Creek, an eastern draining stream, which grouped with the western draining watersheds. All genetic distances from Figure 6 and Figure 7 are shown in Table 7 and Table 8.

Table 6. Nei's genetic similarities between streams calculated from allele frequency differences.

Pop.[a]	1	2	3	4	5	6	7	8	9
2	**0.988**	*	*	*	*	*	*	*	*
3	0.981	0.957	*	*	*	*	*	*	*
4	0.893	0.877	**0.861**	*	*	*	*	*	*
5	0.985	0.980	**0.970**	**0.862**	*	*	*	*	*
6	0.828	0.831	0.787	0.967	0.824	*	*	*	*
7	0.869	0.886	0.828	0.912	0.900	**0.921**	*	*	*
8	0.846	0.857	0.804	0.974	0.836	**0.993**	**0.920**	*	*
9	0.820	0.824	0.785	0.919	0.842	0.974	0.921	0.960	*
10	0.864	0.869	0.812	0.950	0.842	0.964	0.896	0.960	**0.964**

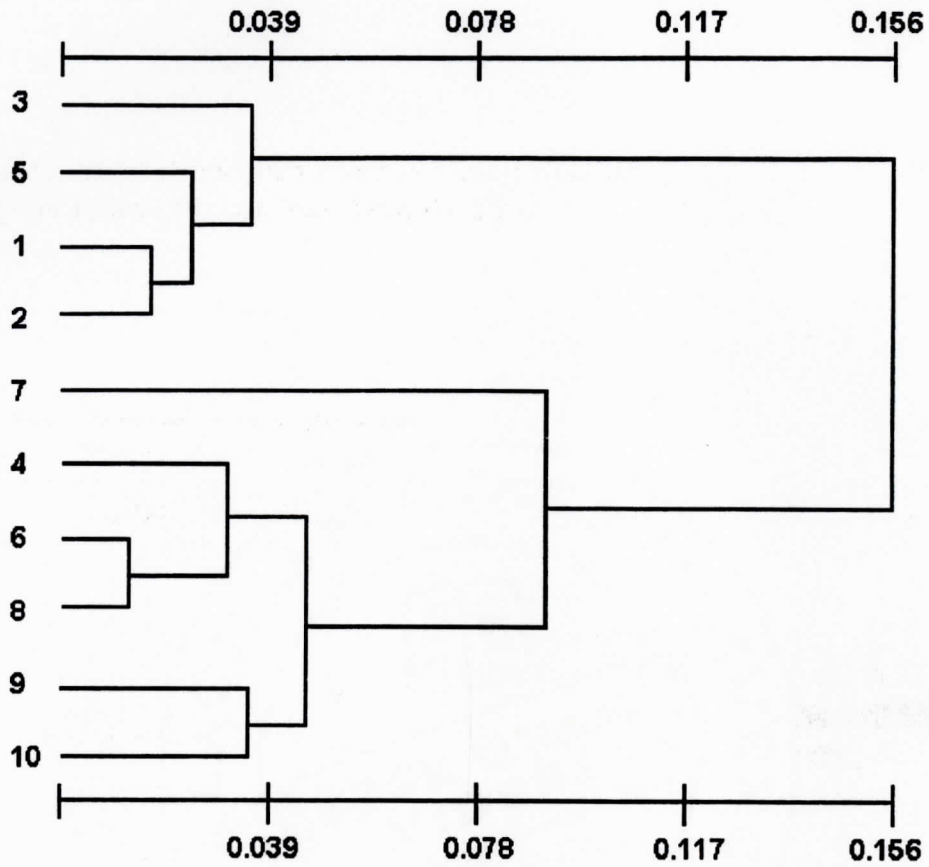
[a]Pop = Population Number

Population number = Population name

1= Big Stony Creek
2 = Little Stony Creek
3 = Bull Head Creek
4 = Garden Creek
5 = Falls Creek
6 = Laurel Fork Va.
7 = Big Pine Creek Tributary
8 = Goshen Creek
9 = Cannon Branch
10 = Moody's Mill Creek

Bold Numbers represent streams in the same watershed

Figure 6. UPGMA generated dendogram for all natural populations of brook trout from the Blue Ridge Parkway.



Population number = Population name

1 = Big Stony Creek	2 = Little Stony Creek
3 = Bull Head Creek	4 = Garden Creek
5 = Falls Creek	6 = Laurel Fork Va.
7 = Big Pine Creek Tributary	8 = Goshen Creek
9 = Cannon Branch	10 = Moody's Mill Creek

Table 7. Nei's genetic distances for dendogram generated using UPGMA for all natural populations of brook trout from the Blue Ridge Parkway.

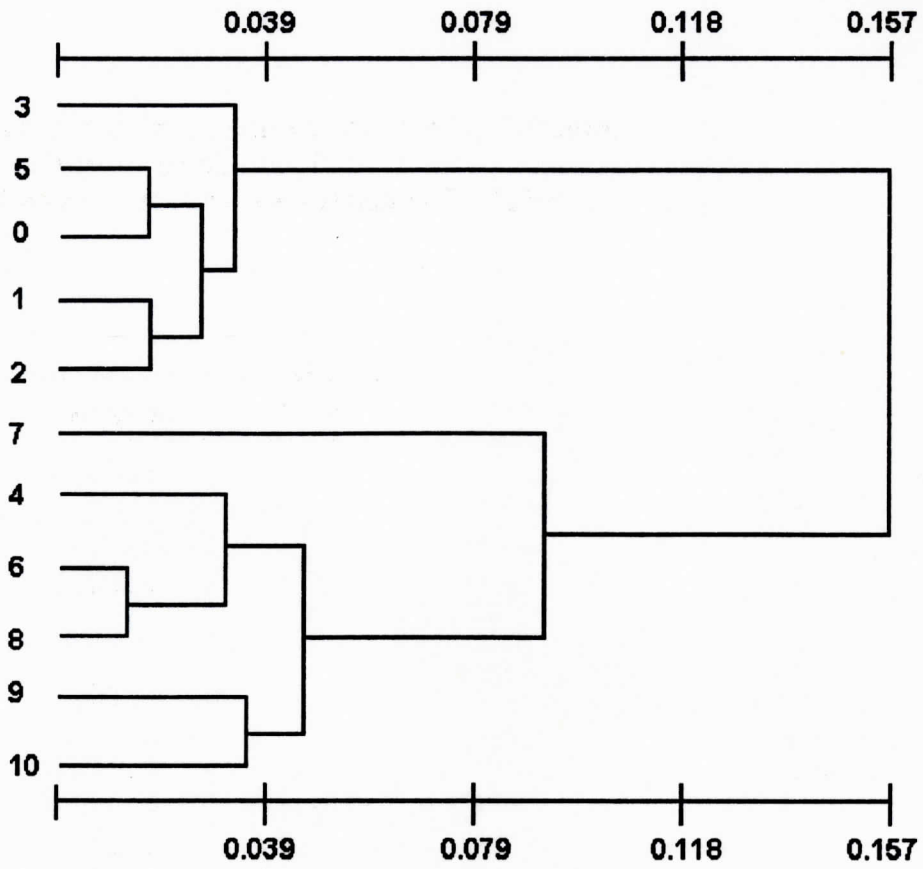
Pop[a] 1	Pop[a] 2	Merge at	Node
6	8	0.007	A
1	2	0.012	B
5	B	0.017	C
4	A	0.030	D
3	C	0.031	E
9	10	0.036	F
D	F	0.046	G
7	G	0.086	H
E	H	0.156	I

[a]Pop = Population Number

Population number = Population name

1= Big Stony Creek	2 = Little Stony Creek
3 = Bull Head Creek	4 = Garden Creek
5 = Falls Creek	6 = Laurel Fork Va.
7 = Big Pine Creek Tributary	8 = Goshen Creek
9 = Cannon Branch	10 = Moody's Mill Creek

Figure 7. UPGMA generated dendogram for all natural populations of brook trout from the Blue Ridge Parkway with brook trout hatchery standards included.



Population number = Population name

1 = Big Stony Creek	2 = Little Stony Creek
3 = Bull Head Creek	4 = Garden Creek
5 = Falls Creek	6 = Laurel Fork Va.
7 = Big Pine Creek Tributary	8 = Goshen Creek
9 = Cannon Branch	10 = Moody's Mill Creek

Table 8. Nei's genetic distances for dendogram generated using UPGMA for all natural populations of brook trout from the Blue Ridge Parkway with brook trout hatchery standards included.

Pop 1	Pop 2	Merge at	Node
6	8	0.007	A
0	5	0.011	B
1	2	0.012	C
B	C	0.028	D
4	A	0.030	E
3	D	0.033	F
9	10	0.036	G
E	G	0.046	H
7	H	0.086	I
H	I	0.157	J

[a]Pop = Population Number

Population number = Population name

0 = Brook Trout Standards
1 = Big Stony Creek
2 = Little Stony Creek
3 = Bull Head Creek
4 = Garden Creek
5 = Falls Creek
6 = Laurel Fork Va.
7 = Big Pine Creek Tributary
8 = Goshen Creek
9 = Cannon Branch
10 = Moody's Mill Creek

When brook trout hatchery standards are included in the calculations the genetic distance between the eastern and western population groups increases slightly to 0.157. No changes occurred in the branching patterns of the dendrogram for populations west of the continental divide, however populations east of the continental divide were shown to group around the brook trout standards. In neither dendrogram did eastern populations group according to watershed or preliminary stocking information. The failure to assort by watershed or stocking history and along with grouping around the brook trout standards may be due to small sample sizes for many of the eastern draining populations and the relative large sample size of Brook trout standards when compared to the small sample sizes for the natural populations.

Allele frequency heterogeneity (*G*) was calculated using the computer program "Gene's in Populations" May *et al.* (1992) for the null hypothesis that Fst = 0 indicating no deviation from Hardy-Weinberg expected values. Comparisons were calculated for the total population, and the subsets of putative native, putative hybrid and putative hatchery categories, based on allele frequency data of the CK-A2 locus. Populations fixed for the CK-A2*100 allele were grouped as putative native and populations fixed for the CK-A2*78 allele were grouped as putative hatchery. All other populations were grouped as putative hybrids. All pair-wise combinations of these subsets were tested for allele frequency heterogeneity.

It was found that *G* values indicating significant allele frequency heterogeneity were found for the total population, for all subsets and for all pair-wise comparisons of these subsets. The highest average Fst for all polymorphic loci was for all

populations with an average Fst = 0.520. Fst values from these comparisons, total *G* values and number of loci showing significant heterogeneity are listed in Table 9.

Table 9. Average F_{st} and *G*-tests for interpopulation heterogeneity for all polymorphic brook trout loci examined. *G*-test for the null hypothesis that $F_{st} = 0$.

Populations Compared (Sample size)	Average F_{st}	Total *G*	Total df	Number of loci showing significant heterogeneity p<0.05	p<0.01
Putative Native (*N = 2*)	0.154	24.499	9	2	1
Putative Hybrid (*N = 4*)	0.316	152.196	30	7	5
Putative Hatchery (*N = 5*)	0.217	167.882	48	6	3
Native + Hybrid (*N = 6*)	0.332	255.211	50	8	5
Native + Hatchery (*N = 7*)	0.478	587.281	84	7	7
Hybrid + Hatchery (*N = 8*)	0.514	825.685	120	6	5
West of Continental Divide (*N = 5*)	0.303	193.860	40	8	7
East of Continental Divide (*N = 5*)	0.448	277.970	36	7	7
All Populations (*N = 11*)	0.520	1034.515	150	8	7

DISCUSSION

The goal of this study, as mandated by the Natural Resources Division of the Blue Ridge Parkway, was to characterize the genetic variation within and among populations of brook trout from streams with headwaters on the Blue Ridge Parkway for management purposes. Furthermore, this study attempted to identify populations of southern Appalachian brook trout as designated by the CK-A2*100 allele described by McCracken *et al.* (1993). It was found that six of the ten streams surveyed contained the CK-A2*100 allele indicating the presence of southern Appalachian brook trout or hybrids derived from them. Cannon Branch and Moody's Mill Creek were fixed for CK-A2*100 suggesting these streams likely contain native populations of southern Appalachian brook trout. The only population located east of the continental divide to possess the CK-A2*100 allele was Garden Creek (CK-A2*100 = 0.250). All other eastern draining streams were fixed for CK-A2*78. The frequency of CK-A2*100 within putative hybrid populations of brook trout ranged from 0.250 to 0.786. This data indicates that the eastern continental divide may be a natural barrier to brook trout dispersal.

At least two hypotheses exist as to the original status of native southern Appalachian brook trout within the New River drainage (Stan Guffey, 1994 personal communication). One hypothesis suggests that all populations within the New River were originally native southern Appalachian brook trout and were fixed for CK-A2*100. The alternative is that the New River represents a hybrid zone and that

brook trout populations within the New River would have had varying frequencies of CK-A2*100 and CK-A2*78. Based on our preliminary data, support could be given to the hypothesis that the New River represents a hybrid zone between the two conditions. However, all of our sample sites within the New River had a history of stocking, and unpublished data from Stan Guffey at the University of Tennessee at Knoxville indicate that other streams from the New River are indeed fixed for CK-A2*100 representing native southern Appalachian brook trout.

Two other loci AAT-4 and GPI-2 were shown to have allele frequency differences relative to population position east or west of the eastern continental divide. However, these loci can only be considered as semi- diagnostic for southern Appalachian brook trout. More extensive examination of AAT-4 should be undertaken to discern whether the pattern observed in this study persists throughout the southern extent of the brook trouts' range. In GPI-2 populations east and west of the continental divide were not fixed for a single allele, but alternative alleles were held in higher frequencies on opposite sides of the continental divide. Kreigler (1993) hypothesized that native brook trout populations from south of the French Broad River in Tennessee, a western draining watershed, are fixed for GPI-2*70, while populations north of this watershed carry other alleles at varying frequencies with the GPI-2*100 allele being most common. This study found that the most common allele east of the continental divide was GPI-2*100, while a third allele GPI-2*40, was most common in populations west of the continental divide. It is also interesting to note that the only watershed in which the current study found GPI-2*70 was in

Big Stony Creek and Little Stony Creek, both of which are tributaries to the Roanoke River, an eastern draining watershed several hundred Km north of the French-Broad River.

AAT-4* described by this study as a semi-diagnostic locus between native southern Appalachian brook trout, resolved to a faint but scorable second locus in brook trout eye tissue. This locus was fixed in all populations except Big Pine Creek tributary. No heterozygotes were found at this locus and AAT-4* as described by this study may represent a mitochondrial expression of AAT. Mitochondrial forms of AAT are known (Hillis and Moritz 1990) and mitochondrial DNA does not undergo recombination (Brown 1983, from Ryman and Utter 1987). This may explain the deviation from a Hardy-Weinberg equilibrium for the AAT-4* locus in the Big Pine Creek Tributary population, which is known to be heavily stocked. Since mitochondrial DNA is maternally inherited, a mitochondrial AAT-4* locus might also explain the differences between the relatively low frequency of CK-A2*100 (the marker locus for southern Appalachian brook trout) and AAT-4* which was fixed for the 100 allele in Garden Creek. If a few large, gravid females from a brook trout population west of the continental divide were transferred by humans into Garden Creek, and mated with eastern males, then the result could be a maternal founder effect with regard to mitochondrial DNA (and AAT-4*). Disparity between mtDNA haplotype and allele frequencies of nuclear genes is not uncommon (Harrison 1989). Further investigation of this locus and mtDNA haplotyping for Garden Creek should be undertaken.

Deviations from Hardy-Weinberg genotypic expectations other than those caused by linkage are normally interpreted as a violation of one or more of the assumptions such as random mating, no natural selection, and/or large population size. Deviations from expected Hardy-Weinberg equilibria were found in six cases from five populations at four loci. Brook trout standards were found to deviate from Hardy-Weinberg at two loci, LDH-A2* and MDH-3,4*. It seems likely that, due to aquaculture practices, that this "population" is not undergoing random mating. All natural populations that significantly deviated from Hardy-Weinberg expectations were located west of the continental divide. As mentioned earlier, the deviation for Big Pine Creek tributary at AAT-4* might be due to a mitochondrial expression of AAT at this locus. Deviations in other western populations may be due to small population sizes due to range restriction by habitat destruction or encroachment of the exotic species, rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). Deviations from expected Hardy-Weinberg values in putative hybrid streams, Laurel Fork and Big Pine Creek Tributary, could possibly be due to habitat destruction or non-random mating after stocking events. Both putative native populations deviated from Hardy-Weinberg expectations at only one locus in each population.

Average F_{is} and F_{st} values calculated from allele frequency data indicate that substantial genetic differentiation has occurred within and among populations of brook trout from streams with headwaters on the Blue Ridge Parkway. Average F_{is} (the measure of genetic variation within populations) was found to be 0.140 for all

natural populations. This value indicates an overall deficiency of heterozygotes within populations. These values are substantially lower than the observed value for average F_{st} of 0.533 (the measure of genetic differentiation between populations). McCracken *et al.* (1993), found F_{st} values among all populations of brook trout from the GSMNP to be 0.320 (N=11, range 0.041-0.862). Our values were higher than these reported averages, but less than F_{st} = 0.617 (7 loci) that Kreigler (1993) found in a study of brook trout populations in Tennessee outside the GSMNP. McCracken *et al.* (1993), used 16 polymorphic loci as compared to the 9 used in this study and McCracken et al. did not sample streams across as wide a geographic area as the current study. High F_{st} values of this study could be explained by a principle known as the Wahlund effect (Wahlund, 1928). If the total sample population is not a continuous, randomly breeding population, but instead is subdivided into smaller units for breeding purposes, then the level of observed homozygosity in the total population will be greater than that predicted by a Hardy-Weinberg equilibrium. It is logical to assume that populations of brook trout separated by watersheds and by a larger barrier, the continental divide, represent isolated breeding populations. Further, most streams were sampled at their extreme headwaters and gene flow among populations at these sites is unlikely. This can be inferred from relatively large genetic differentiation across very short geographical distances. The largest genetic distance, based on Nei's genetic similarity, for streams on the same side of the continental divide was between Garden Creek and Bullhead Creek with a similarity of 0.861. Geographically, these two streams were very close to each other

at a distance of only 1.6 km. It is likely that a combination of all of these factors account for the large F_{st} values observed in this study.

When populations are pooled to the level of watershed, F_{is} becomes 0.378 and F_{st} becomes 0.328 (Table 4). This suggests that there is more genetic differentiation between streams within the same watershed than between the watersheds themselves. However, this information is best considered relative to the observation that all the streams sampled within the New River watershed are putative hybrid populations which would tend to increase F_{IS} values. This is due to the introduction of additional alleles into these populations which might tend to increase heterozygosity and F_{IS}.

G values indicating significant allele frequency heterogeneity were found within the total population and for all subsets of populations. The lowest F_{st} value (F_{st} = 0.154) for these pair-wise comparisons was for putative native populations of southern Appalachian brook trout. The highest F_{st} value (Fst=0.514) was for the subsets of hybrid + hatchery populations. This may be attributed to the assumption that the subsets of hybrid + hatchery populations contain individuals from both sides of the continental divide. This is consistent with the observations of McCracken *et al.* (1993), except that the F_{st} values for all subsets were much larger. In contrast, Kreigler (1993) found the largest F_{st} value (Fst=0.623) for putative native populations. This could be explained by the observation that many of the native populations in that study were from different watersheds across a wide geographical area. The small sample populations and relatively few study streams from each

watershed, resulting from the mandate of the present study, could contribute to smaller observed F_{st} values.

Based on Nei's index of genetic similarity, comparisons among all streams were found to be closet between to populations on the same side of the continental divide, with the exception of Garden Creek, which was more similar to populations located west of the continental divide (Table 5). Among populations other than Garden Creek, genetic similarities east of the continental divide relative to each other ranged from 0.957 to 0.988. Genetic similarities for populations located west of the continental divide ranged from 0.896 to 0.993. Among eastern and western populations values ranged from 0.785 to 0.974. Stoneking *et al.* (1981b) found genetic similarities for brook trout population sampled across a wide geographic area ranged from 0.993 to 0.852. Perkins *et al.* (1993) reported genetic similarities for populations of brook trout east of the continental divide in New York State and Pennsylvania range from 0.999 to 0.935. When populations from the Allegheny River which is west of the continental divide are also compared, then the similarity for these streams range from 0.99 to 0.904. McCracken *et al.* (1993) reported genetic similarity between native populations of brook trout west of the continental divide to range between 1.000 and 0.962. When putative hybrid populations of brook trout were also included the similarity ranged between 1.000 and 0.959. When hatchery fish are added to the analysis, then similarities range from 0.987 to 0.855. These values are similar in all cases to genetic similarities found by this study.

A dendrogram for all natural populations using UPGMA based on Nei's genetic similarities was constructed (Figure 6). A tree was generated with two main branches, which joined at a distance of 0.156. Each branch represents populations on the same side of the continental divide, with the exception of Garden Creek which was found to group within the western branch. All streams in the eastern branch joined at a distance no further than 0.031. Within the western cluster Garden Creek was found to associate with two streams from the New River (Goshen and Laurel Fork Creek) at a distance of 0.030. Big Pine Creek joined the cluster for the western populations and Garden Creek at a distance of 0.086. These results are consistent to those found by Perkins *et al.* (1993), which found that brook trout populations located on the same side of the continental divide form closely associated branches when dendograms are created. The branches of dendograms created by this study separate further than ones found by Perkins *et al.* (1993) due to the presence of alleles from southern brook trout populations that are not found in populations further north.

The study of fish dispersal in the central Appalachians is a perplexing problem for biogeographers who must often rely on geological literature to determine past drainage relationships as routes of dispersal (Hocutt *et al.* 1978). The Eastern Continental divide may represent a barrier to brook trout migration and dispersion and is thought to have been stable south of Roanoke, Virginia in the Blue Ridge Mountains throughout the Pleistocene. North of Roanoke, the divide between the Roanoke River and New River may have fluctuated between 64 and 120 km since

the Tertiary period (Hocutt 1978). Further the dispersal patterns of brook trout in the Eastern United States may have been influenced by glacial advance and retreat. Bailey and Smith (1981) theorized that two brook trout refugia may have existed during the Wisconsin glaciation based on brook trout's distribution patterns in North America. One of these refugia probably existed east of the Appalachian Mountains (Atlantic refugium) while the other might have existed west of the Appalachian Mountains as the Mississippi refugium. Perkins *et al.* (1993) used allozyme data and found that brook trout populations in New York state fit this proposed model of refugia dispersal. Genetic distances of 0.940 were observed between brook trout populations from the eastern drainages and the west draining Allegheny River. These values indicate populations that are more genetically similar than populations located east and west of the continental divide, from south of the New River in Virginia. Also CK-A2*100 was not found in any brook trout populations examined by Perkins *et al* (1993).

Today the western draining Allegheny River flows south from south-western New York state and Pennsylvania and eventually empties into the Ohio River. The New River that originates in Northwestern North Carolina and southwestern Virginia flows north and also joins the Ohio River. However, the drainage patterns of the rivers from the central Appalachian mountains has dramatically changed since the Pliocene. Hocutt *et al* (1978) has proposed the following scenario for the creation of present drainage patterns for central Appalachian rivers. During the Pliocene, what is now the New River system was thought to be part of a larger series of drainages

known as the Teays River system. The Teays River flowed west through central Ohio and Indiana where it may have joined the Mississippi River in southern Illinois. The current Allegheny River probably flowed north into what used to be the Pittsburgh River, approximately where the Lake Erie is today. The Pittsburgh River is thought to have emptied into the Gulf of St. Lawrence. During the Pleistocene, the main channel of the Teays River was impounded by glacial ice forming Teays Lake which probably also blocked the northern and eastern tributaries of the Teays River. These tributaries would have backed up and found new outlets to the southwest and eventually formed the lower Ohio River. This most likely occurred during the Kansasan glaciation. Also at this, time the flow of the Pittsburgh River was most likely was diverted, along with the diverted Allegheny River complex and these two rivers are thought to have reversed their flow south to the Monongahela River. After the final retreat of the ice from this area, the Allegheny-Upper Ohio River came into existence and joined with the New River system to form the lower Ohio River (Hocutt *et al.* 1978).

The genotypic makeup of brook trout populations from the New River and Allegheny River (Perkins *et al.* 1993) are quite dissimilar based on allele frequency data from CK-A2* and other loci. Based on the genotypic differences and geological information regarding the former flow patterns of the major river systems in relation to glacial advance and retreat, it seems likely that a third brook trout refugium existed during the Wisconsin glaciation in addition to the two described by Bailey and Smith (1981). This southern refugium included all western draining streams

south of and including the New River system. The existence of an isolated southern refugium for brook trout would explain genotypic differences between brook trout populations from the New River and Allegheny Rivers. Initial differences between these refugia could be increased or maintained by genetic drift or natural selection.

Management Implications

Proper management of a fishery requires an understanding of the biological principles that effect the resource. For almost a century, fisheries managers have managed trout populations based on maintaining large populations of catchable fish (Allendorf *et al.* 1987). Brook trout in hatcheries were bred for traits that would help them survive under hatchery conditions, without regards for the natural history, ecology, or the genetic make up of this species (Vincent 1960). As a result many streams were stocked without knowledge of how the genetic make up of these streams was being effected. With the advent of modern biochemical techniques, it is now possible to ascertain how past stocking events have effected the genetic make up of many streams. In the past 15 years, a large body of information regarding the genetic makeup of this species has been established. Fisheries managers should use this information, as well as the information included here, when formulating management practices. These management practices should emphasize the preservation of the genetic makeup of this species based on it's natural history in conjunction with maintaining healthy populations sizes. This should be done to preserve that natural variation that is present in our streams.

The results of this study suggest that the natural range of southern Appalachian brook trout in western North Carolina and southwestern Virginia may not extend east of the Eastern Continental divide. It is unclear if brook trout are native to the Yadkin River drainage in western North Carolina. Brook trout have been eliminated from suitable habitat in many locations west of the continental divide, or have had the integrity of native gene pools violated by the introduction of hatchery brook trout. Based on these results, and the mission of the National Park Service, Blue Ridge Parkway, to protect native species, management strategies in regard to native brook trout are potentially complex.

Proposed Management Strategies

1). Streams identified as putative native should be given special consideration and protection. For example, regulations regarding these streams to prohibit fishing or catch and release regulations may be appropriate. No brook trout from populations originating east of the continental divide should be stocked into these streams, and if possible, barriers to prevent the invasion of rainbow trout, or non-native brook trout should be constructed on these streams.

2). Streams identified with putative hybrid populations could be managed in several ways. In establishing native southern Appalachian brook trout in streams west of the continental divide, two approaches could be taken. Streams could be left alone (not stocked) to try to allow native alleles to re-establish themselves in the populations. This would be cost effective, but may not achieve the desired result in a timely fashion. Once alleles are established in a population, it becomes difficult

for natural processes (genetic drift, selection) to remove them even if they have selective disadvantages.

Alternatively, streams could be stocked with native southern Appalachian brook trout to try to re-establish native populations. If this is done however, several guidelines must be followed to assure that the established gene pools resemble the original gene pools as closely as possible. Stocked brook trout should be from a stream fixed for CK-A2*100 within the same watershed. A third option open to managers could be to continue to manage hybrid streams located west of the continental divide as "put and take" fisheries and to continue to stock these streams with hatchery brook trout containing the CK-A2*78 allele. However, this option is undesirable if one wishes to re-establish native gene pools.

3). Streams fixed for CK-A2*78 can continue to be managed in their present conditions, since few estimated genotypic differences exist between wild brook trout populations in these streams and genotypic frequencies of hatchery populations. However, this study only represents a small proportion of the actual genetic composition of this species. For example, other genetic differences among populations of brook trout may exist but were undetected by this project. A conservative approach would be to treat all streams within a watershed as discrete units. Introduction of hatchery brook trout (or wild brook trout from other watersheds), should be kept to a minimum if at all possible. This would reduce the probability of introduction of non-native genes into these populations.

Literature Cited

Allendorf, F.W., N. Ryman, and F.M. Utter. 1987. Genetics and fishery management; past present and future. pp. 1-19 *in* N. Ryman and F.W. Utter editors. Population genetics and fishery management. University of Washington Press Seattle, Washington.

Bailey, R.M. and G.R. Smith. 1981. Origin and geography of the fish fauna of the Laurenttian Great Lakes basin. Canadian Journal of Fisheries and Aquatic Sciences. 38:1539-1561.

Behnke, R.J. 1991. The ancestry of trout pages 2-9 *in* J. Stolz, and J. Schnell, editors. Trout. Stackpole Books, Harrisburg, Pennsylvania.

Brown W.M. 1983. Evolution of Animal mitrochondiral DNA. *in* N. Ryman and F. Utter editors. 1987. Population genetics and fishery management. University of Washington Press. Seattle, Washington.

Brussard, P.F. and D.F. Nielsen. 1976. Allozyme variation in the brook trout *Salvelinus fontinalis*: a preliminary report. Unpublished report to Great Smoky Mountains National Park. 11 pp.

Clayton, J.W. and D.N. Tretiak. 1972. Amine-citrate buffers and pH control in starch gel electrophoresis. Journal of the Fisheries Research Board of Canada 29:1169-1172.

Hillis, D.M., and C. Moritz. 1990. Molecular systematics. Sinauer Associates Inc., Sunderland, Mass.

Harris, J.R., D. Estes, W.F. Brandes, E. Shipp, and D.Y. White. 1978. Systematics of the Brook Trout *Salvelinus fontinalis*, in the Great Smoky Mountains National Park. Part I Electrophoresis: Part II Meristic; Final Report to the National Park Service. Tennessee Tech Cooperative Fisheries Unit. Cookville, Tennessee.

Harrison, R.F. 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. Trends in ecology and evolution 4:6-11.

Hocutt, C.H., R.F. Denoncourt, and J.R. Stauffer Jr. 1978. Fishes of the Greenbrier River West Virginia, with drainage history of the central Appalachians. Journal of Biogeography 5:59-80.

Jenkins, R.E. and N.M. Burkhead. 1994. Freshwater fishes of Virginia. Americans Fisheries Society, Bethasda Maryland.

Jones, R.D. 1975. Regional distribution trends of the trout resource. Pages 1-10 *in* Southeastern trout resource: ecology and management symposium proceedings. Southeastern Forest Experimental Station, Asheville, North Carolina.

Kelly, G.A., J.S. Griffith, and R.D. Jones. 1980. Changes in Distribution of trout in Great Smoky Mountains National Park, 1900-1977. U.S. Fish and Wildlife Service Technical Paper 102.

King, W. 1937. Notes on native speckled and rainbow trout in the streams at Great Smoky Mountains National Park. Journal of the

Tennessee Academy of Science 12:351-361.

Kreigler, F.W. 1993. Diferentation between stocked and native brook trout populations in east Tennessee. M.S. thesis University of Tennessee Knoxville. Knoxville, Tennessee.

Krueger C.C. and B.W. Menzel. 1979. Effects of stocking on genetics of wild brook trout populations. Transactions of American Fisheries Society 108:277-287.

Lennon, R.E. 1967. Brook trout of Great Smoky Mountains National Park. U.S. Fish and Wildlife Service Technical Paper 15.

May, B., J.E. Wright, and M. Stoneking. 1979. Joint segregation of biochemical loci in Salmonidae: Results from experiments with Salvelinus and review of the literature on other Species. Journal of the Fisheries Research Board of Canada 36:1114-1128.

May, B., C.C. Krueger, and W. Eng. 1992. Genes in populations: A computer program for analysis of genetic data. Cornell University, unpublished program.

McCracken, G.F., C.R. Parker, and S.Z. Guffey. 1993. Genetic differentiation and hybridization between stocked hatchery and native brook trout in Great Smokey Mountains National Park. Transactions of the American Fisheries Society 122:533-542.

Menhinick, E.F. 1991. The Freshwater fishes of North Carolina. North Carolina Wildlife Resources Commission, Raleigh, North Carolina.

Nei, M. 1972 Genetic distance between populations. Amercian Naturalist 106:283-292.

Nagel, J.W. 1991. Is the decline of brook trout in the southern Applalachians resulting from competitive exclusion and/or extinction due to habitat fragmentation ? Journal of Tennessee Academy of Science 66:141-143.

Perkins, D.L., C.C. Krueger and B. May. Heritage brook trout in Northeastern USA: Genetic variability within and among populations. Transactions of the American Fisheries Society 122:515-532.

Rhode, F.C., R.G. Arndt, D.G. Lindquist, and J.F. Parnell. 1994. Freshwater Fishes of the Carolinas, Virginia, Maryland and Delaware. The University of North Carolina Press, Chapel Hill, North Carolina.

Ryman, N. and F.W. Utter editors. 1987. Population genetics and fishery management. University of Washington Press Seattle, Washington.

Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson, and J.B. Gentry. 1971. Biochemical polymorphism and Systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). *in* Studies in Genetics VI. University of Texas Publishers.

Smith, H.M. 1907. The Fishes of North Carolina. N.C. Geological and Economic Survey, Vol. 2. 453 p.

Soltis, D.E. and P.S. Soltis editors. 1989. Isozymes in plant biology. Discorides Press. Portland, Oregon.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry, 2nd edition. Freeman San Francisco, CA.

J. Stolz, and J. Schnell, editors. Trout. Stackpole Books, Harrisburg, Pennsylvania.

Stoneking, M.D., B. May, and J.E. Wright. 1981a. Loss of duplicate gene expression in salmonids: Evidence for a null allele polymorphism at the duplicate Aspartate Aminotransferase loci in brook trout (*Salvelinus fontinalis*). Biochemical Genetics 19:1063-1077.

Stoneking, M.D., D.J. Wagner, and A.C. Hildebrand. 1981b. Genetic evidence suggesting subspecific differences between northern and southern populations of brook trout (*Salvelinus fontinalis*). Copeia 1981:810-819.

Vincent, R.E. 1960. Some influences of Domestication upon three stocks of brook trout (*Salvelinus fontinalis* Mitchell). Transactions of American Fisheries Society 89:35-52.

Willers, W.B. 1991. Trout biology. Lyons and Burford Publishers, New York.

Wahlund, S. 1928. The combination of populations and the appearence of correlation examined from the standpoint of the study of heredity. Hereditas 11:65-106 (in German).

Wendel, J.F. and N.F. Weeden. 1989. Visualization and interpretation of plant isozymes *in* Soltis, D.E. and P.S. Soltis editors. 1989. Isozymes in plant biology. Discorides Press. Portland, Oregon.

Werth, C.R. 1985. Implementing an isozyme laboratory at a field station. Virginia Journal of Science 36:53-76.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395-420.

Appendix A

Enzyme Stain Recipes, Gel and Electrode Buffers:

Appendix A: Enzyme Stain Recipes

Modified from Hillis and Moritz (1990), and Wendel and Weeden (1989).

1. Aspartate Aminotransferase, AAT (E.C. 2.6.1.1)

Buffer	0.2 *M* Tris-HCl, pH 8.0	25 ml
	L-aspartic acid	0.12 g
	α-Ketoglutaric acid	0.05 g

Readjust pH to 8.0 with 4.0 *N* NaOH. Then add just before staining:

	pyridoxal 5-phosphate	0.005 g
	fast blue BB salt	0.05 g

2. Creatine Kinase, CK (E.C. 2.7.3.2)

To Flask A add the following

Buffer	0.2 *M* Tris-HCl, pH 7.0	15 ml
	0.1 M $MgCl_2 6\ H_20$	1.0 ml
	adenosine 5'-diphosphate	0.03 g
	D(+)-glucose	0.05 g
	hexokinase	40 NAD units
	phosphocreatine	0.05 g
	G6PDH	40 NAD units
When ready to stain add:	1% NAD	1 ml
	1% NADP	1 ml
	1% MTT	1 ml
	1% PMS	1 ml
	To flask B add the following:	
Buffer	0.2 *M* Tris-HCl, pH 7.0	35 ml
	agar	0.4 g

When ready to stain, heat solution in flask b until boiling and allow to cool in ice water to 50° C. Add solution in flask A to flask B and slowly pour over gel as to avoid air bubble formation.

3. Esterase, Est (E.C. 3.1.1.-) Florescent

To flask A add the following:

Buffer	0.05 Na-acetate pH 5.0	15 ml
	Agar	0.2 g

To flask B add the following:

Buffer	0.05 M Na-acetate pH 5.0	15 ml
	4-Methylumbelliferyl acetate dissolved in 3 ml of acetone	0.01 g

When ready to stain, heat solution in flask b until boiling and cool in ice water to 50° C. Quickly add solution in flask A to flask B and slowly pour over gel as to avoid air bubble formation.

4. Fumarate Hydratase, FH (E.C. 4.2.1.2)

Buffer	0.2 M Tris-HCl pH 8.0	25 ml
	fumaric acid	0.025 g
	malic dehydrogenase	75 units
When ready to stain add:	1% NAD	0.5 ml
	1% MTT	0.5 ml
	1% PMS	0.5 ml

5. General Proteins, GP (Nonspecific)

Stock solution:

	naphthol blue black (amido black)	1.0 g
	Stain fixing solution	500 ml

Pour 50 ml of solution over gel and let stain for 20 minutes at room temperature. Pour off staining solution and rinse in fixative until gel background is pale. The stain may be reused.

7. Glucose-6-phosphate Isomerase, GPI (E.C. 5.3.1.9)

Buffer	0.2 *M* Tris-HCl, pH 7.0	25 ml
	0.1 *M* $MgCl_2 \cdot 6\ H_2O$	2.5 ml
	$_D$-fructose-6-phosphate	0.02 g
	G6PDH	20 NAD units
When ready to stain add:	1% NAD	1 ml
	1% NADP	1 ml
	1% MTT	0.5 ml
	1% PMS	0.5 ml

8. Glycerol-3-phosphate Dehydrogenase, G3PDH (E.C. 1.1.1.8)

Buffer	0.2 *M* Tris-HCl, pH 8.0	25 ml
	DL-α glycerophosphate, pH 8.0	0.5 g
	0.1 *M* $MgCl_2 \cdot 6\ H_2O$	0.5 ml
When ready to stain add:	1% NAD	0.5 ml
	1% MTT	0.5 ml
	1% PMS	0.5 ml

9. $_L$-Lactate Dehydrogenase, LDH (E.C. 1.1.1.27)

Buffer	0.2 *M* Tris-HCl, pH 8.0	25 ml
	1.0 *M* lithium lactate, pH 8.0	4 ml
When ready to stain add:	1% NAD	0.5 ml
	1% MTT	0.5 ml
	1% PMS	0.5 ml

10. Malate Dehydrogenase, MDH (E.C. 1.1.1.37)

Buffer	0.2 *M* Tris-HCl, pH 8.0	25 ml
	2.0 *M* $_{DL}$-malic acid	2.5 ml
When ready to stain add:	1% NAD	0.5 ml
	1% MTT	0.5 ml
	1% PMS	0.5 ml

11. Malate Dehydrogenase ($NADP^+$), ME (E.C. 1w.1.40)

Buffer	0.2 *M* Tris-HCl, pH 8.0	25 ml
	0.1 *M* $MgCl_2 \cdot 6\ H_2O$	0.5 ml
	2.0 M $_{DL}$-malic acid pH 8.0	2.5 ml
When ready to stain add:	1% NADP	0.5 ml
	1% MTT	0.5 ml
	1% PMS	0.5 ml

12. Mannose-6-phosphate Isomerase, MPI (E.C. 5.3.1.8)

Buffer	0.2 *M* Tris-HCl, pH 8.0	25 ml
	0.1 *M* $MgCl_2 \cdot 6\ H_2O$	0.5 ml
	$_D$-mannose-6-phosphate	
	Glucose-6-phosphate isomerase	25 units
	G6PDH	20 units
When ready to stain add:	1% NAD	1.0 ml
	1% NADP	0.5 ml
	1% MTT	0.5 ml
	1% PMS	0.5 ml

13. Peptidase, Pep (E.C. 3.4.-.-)

Buffer	0.1 *M* KH_2PO_4 pH 7.0	25 ml
	0.1 *M* $MgCl_2 \cdot 6\ H_2O$	0.5 ml
	10mg/ml L-leucine-	
	β-naphthylamide HCl	0.05 ml

Pour solution over gel and incubate at 37° C for 30-60 minutes then add the following: Black K salt 0.15 g
Continue incubation.

Gel and Electrode Buffers:

Lithium Hydroxide

Solution A (electrode)	Molarity	Amount Per Liter
Lithium hydroxide	0.03	1.20 g
Boric acid	0.19	11.89 g
pH 8.1		

Solution B		
Tris	0.05	6.2 g
Citric acid (monohydrate)	0.008	1.6 g
pH 8.4		

Gel is 1 part solution A and 9 parts
solution b with finally of pH 8.3.

Morpholine Citrate

Stock Solution:	Molarity	Amount Per Liter
citric acid (monohydrate)	0.04	8.4 g

Adjust to pH 6.1 using ≈10-15 ml/l of
N-(3-aminopropyl)-morphline

Electrode: Undiluted stock solution
Gel: 1:19 dilution of stock solution
Gels are hazardous and should be handled with protective gloves.

Appendex B

Allele Frequency Data

Appendix B. Allele Frequencies for all loci.

	Pop 0	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	Pop 10
CK-A1*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
CK-A2* 78	1.00	1.00	1.00	1.00	0.75	1.00	0.21	0.75	0.46	0.00	0.00
100	0.00	0.00	0.00	0.00	0.25	0.00	0.79	0.25	0.55	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.30	0.00	0.43	0.50	0.36	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.38	0.00	0.34	0.38	0.50	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.20	0.00	-0.27	-0.33	0.27	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
LDH-B1*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
LDH-A2* 78	0.68	0.00	0.00	0.00	0.00	0.36	0.14	1.00	0.00	0.50	0.08
100	0.32	1.00	1.00	1.00	1.00	0.64	0.86	0.00	1.00	0.50	0.92
Ho	0.11	0.00	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.20	0.17
Hs	0.43	0.00	0.00	0.00	0.00	0.46	0.25	0.00	0.00	0.50	0.15
Fis	0.76	0.00	0.00	0.00	0.00	0.07	1.00	0.00	0.00	0.60	-0.09
N	19	10	6	7	10	7	7	6	11	10	6
sMDH-1,2*-100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
sMDH-3,4*100	0.71	1.00	0.92	1.00	1.00	0.64	0.50	0.75	0.55	0.35	1.00
145	0.18	0.00	0.08	0.00	0.00	0.36	0.50	0.25	0.46	0.65	0.00
0	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho	0.16	0.00	0.17	0.00	0.00	0.14	0.14	0.17	0.55	0.50	0.00
Hs	0.45	0.00	0.15	0.00	0.00	0.46	0.50	0.38	0.50	0.46	0.00
Fis	0.65	0.00	-0.09	0.00	0.00	0.69	0.71	0.56	-0.10	-0.10	0.00
N	19	10	6	7	10	7	7	6	11	10	6
mMEP-1* 88	0.61	0.50	0.50	1.00	0.00	0.64	0.00	0.25	0.00	0.15	0.00
100	0.24	0.50	0.50	0.00	1.00	0.36	1.00	0.75	1.00	0.85	1.00
76	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho	0.58	0.40	0.33	0.00	0.00	0.14	0.00	0.50	0.00	0.30	0.00
Hs	0.55	0.50	0.50	0.00	0.00	0.46	0.00	0.38	0.00	0.26	0.00
Fis	-0.05	0.20	0.33	0.00	0.00	0.69	0.00	-0.33	0.00	-0.18	0.00
N	19	10	6	6	10	7	7	6	11	10	6

	Pop 0	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	Pop 10
mMEP-2*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
mMEP-3*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
PEP*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
GPI-1*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
GPI-2* 40	0.07	0.00	0.00	0.14	0.85	0.00	1.00	0.83	1.00	0.55	0.42
70	0.00	0.05	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100	0.87	0.95	0.83	0.86	0.15	1.00	0.00	0.17	0.00	0.45	0.58
115	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho	0.13	0.10	0.33	0.29	0.30	0.00	0.00	0.33	0.00	0.30	0.83
Hs	0.24	0.10	0.28	0.25	0.26	0.00	0.00	0.28	0.00	0.50	0.49
Fis	0.44	-0.05	-0.20	-0.17	-0.18	0.00	0.00	-0.20	0.00	0.39	-0.71
N	15	10	6	7	10	7	7	6	11	10	6
GPI-3* 95	0.00	0.00	0.08	0.14	0.00	0.00	0.00	0.00	0.23	0.30	0.17
100	1.00	1.00	0.92	0.86	1.00	1.00	0.93	1.00	0.77	0.70	0.83
105	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00
Ho	0.00	0.00	0.17	0.29	0.00	0.00	0.14	0.00	0.27	0.40	0.33
Hs	0.00	0.00	0.15	0.25	0.00	0.00	0.13	0.00	0.35	0.42	0.28
Fis	0.00	0.00	-0.09	-0.17	0.00	0.00	-0.08	0.00	0.22	0.05	-0.20
N	15	10	6	7	6	7	7	6	11	10	6
G3PD-1*100	0.87	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
105	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6

	Pop 0	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	Pop 10
EST-1*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
CK-B*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
LDH-C*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
MPI-1*100	0.91	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
105	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	17	10	6	7	10	7	7	6	11	10	6
sAAT-3e*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	19	10	6	7	10	7	7	6	11	10	6
sAAT-4e* 88	1.00	1.00	1.00	1.00	0.00	1.00	0.00	0.40	0.00	0.00	0.00
100	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.60	1.00	1.00	1.00
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.00	0.00	0.00
Fis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
N	17	10	6	7	10	7	7	5	11	10	6
FH-1,2*100	0.50	0.55	1.00	0.25	0.40	0.64	0.64	1.00	0.77	0.65	0.83
148	0.50	0.45	0.00	0.75	0.60	0.36	0.36	0.00	0.23	0.35	0.17
Ho	0.29	0.50	0.00	0.50	0.80	0.43	0.14	0.00	0.09	0.30	0.33
Hs	0.50	0.50	0.00	0.38	0.48	0.46	0.46	0.00	0.35	0.46	0.28
Fis	0.43	-0.01	0.00	-0.33	-0.67	0.07	0.69	0.00	0.74	0.34	-0.20
N	14	10	6	6	5	7	7	6	11	10	6
Avg Hs	0.12	0.05	0.05	0.04	0.05	0.09	0.08	0.09	0.08	0.12	0.06
std err	0.04	0.03	0.03	0.02	0.03	0.04	0.04	0.04	0.04	0.05	0.03
Avg Ho	0.07	0.05	0.05	0.05	0.07	0.05	0.04	0.07	0.06	0.10	0.08
std err	0.03	0.03	0.02	0.03	0.04	0.03	0.02	0.04	0.03	0.04	0.04

Vita

Lonnie N. Shull III was born on 15 April 1969 in Charleston, South Carolina. His parents are Dr. L. N. Shull Jr. and Mrs. Mary Ann Shull. He has two siblings Beth and Edwin. He has lived in Lenoir, North Carolina since 1974. He attended Hibriten High School in Lenoir from 1982 until 1987. He then entered The Citadel in Charleston, SC in the fall of 1987. While at The Citadel, Lonnie attained the rank of sergeant, was a member of the Bond Volunteers, and Beta-Beta-Beta. Lonnie graduated from The Citadel in May of 1991. Upon graduation from The Citadel, Lonnie enrolled in the masters program of the Appalachian State University Biology department where he graduated from in 1995. At Appalachian Lonnie was awarded several teaching assistantships and a research assistantship. His research was funded by a $2000.00 grant from the National Park Service. Lonnie presented his research at the 1994 Association of Southeastern Biologist meeting in Orlando Florida, and at the 1994 Society of Ecology and Evolution meeting in Athens, Georgia.