

THE GENETICS OF NATIVE AND STOCKED BROOK TROUT POPULATIONS
IN EAST TENNESSEE

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DEDICATION

This thesis is dedicated to my parents, Frank and Nancy Kriegler, who have always encouraged me and who understand the importance of education. Also to Tania, who loves me and who will continue to supported me as I progress in my career.

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ABSTRACT

Previously native southern Appalachian brook trout were shown to be genetically distinct from northern derived hatchery populations. In this study diagnostic allozyme loci and mitochondrial DNA restriction sites were used to determine the genetic origin of thirty-nine Tennessee brook trout populations outside of the Great Smoky Mountain National Park. Allozyme data revealed that 22 populations are putative native, eight are hatchery derived, and nine are hybrids. Genetic origins generally agreed with stocking histories. The genetic diversity among all populations was high. Hatchery derived, hybrid, and putative native populations are all polytypic. These findings have direct implications for the future management of native brook trout in Tennessee.

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Chapter 1

INTRODUCTION

Background

Brook trout (*Salvelinus fontinalis*) are the only stream trout native to the Appalachian mountains. The historic range of brook trout extends across eastern Canada from Manitoba to the Atlantic Ocean, through New England, and south into the southern Appalachian mountains. Southern Appalachian brook trout are at the extreme portion of their native range (MacCrimmon and Campbell 1969).

The 200 million year old southern Appalachian mountains have a unique geological history. The high peaks divided by low river valleys subject many plant and animal species to the forces of geographic isolation and the interruption of gene flow (Brooks 1971). Thus, through mutation, genetic drift, and natural selection many species have become endemic to the southern Appalachians. The fish fauna in this region is very specious mostly due to the diverse geological features of the Appalachian mountains. (Jenkins et al. 1972, Starns and Etnier 1985).

Glaciation during the Pleistocene played an important role in the evolution and distribution of many species. Many plants and animals responded to changing environments as the ice moved south. Cooling during glacial advances allowed eastern coldwater fish to move south. Large rivers that served as routes for dispersal warmed and became

uninhabitable for cold water fish as the glaciers retreated. Many coldwater fish species such as brook trout were confined to relict populations. Brook trout south of the Tennessee and Roanoke River drainages were isolated in mountainous populations (Jenkins et al. 1972, Starns and Etnier 1985). Similar events also occurred in western North America, affecting western trout species (Behnke 1992).

Today brook trout in Tennessee are confined to high elevation headwater streams typically above 800 meters (Bivens et al. 1985). They are geographically isolated from other populations in the region as well as from northern populations. Geographic isolation has potentially caused significant divergence between brook trout populations. Although many fish cut off by glacial retreat have not evolved significantly (Jenkins et al. 1972), genetic studies (Stoneking et al. 1981, McCracken et al. 1993) and limited morphological data (King 1937, Lennon 1967) indicate that brook trout of the southern Appalachians have differentiated significantly from northern brook trout.

Southern Appalachians brook trout experienced a severe decline in distribution during the early part of this century due to the effects of logging, fires, railroad construction and the introduction of exotic rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) (King, 1937, Bivens 1984, Larson and Moore 1985, Fausch 1988, DeWald and Wilzbach 1992). Currently, there are about 90

brook trout populations in Tennessee (Bivens 1984) which appear to be relatively stable (Strange and Habera 1992). Stocking programs were initiated early in this century to combat the range decline as well as to supplement the sport fishery. Several hundred thousand brook trout were stocked in east Tennessee from three state hatcheries within the past 40 years (Tennessee Wildlife Resource Agency stocking records). Brook trout stocking was halted in Tennessee in the mid 1980's although stocking still occurs in neighboring states (North Carolina, South Carolina, and Georgia).

Hatchery Introductions

Most brook trout stocking in Tennessee and many other places in North America involved hatchery brook trout derived from northeastern U.S. populations. Hatchery strains are used for stocking because they have been selected for increased growth and size as well as behaviors preferred by anglers. (Flick and Webster 1962, 1964). In addition, wild brook trout raised in hatcheries have greater susceptibility to disease and lower catchability than hatchery strains. For these reasons, well established strains of hatchery fish were primarily used for stocking in Tennessee. Some brook trout populations also were reclaimed with wild fish transferred from other populations (Bivens 1984).

There has been substantial concern that the stocking of non-native trout may have had detrimental genetic and

ecological effects on native salmonid species (Butler 1975, Krueger and Menzel 1979, Hulett and Leider 1984, Petrosky and Bjornn 1984, Vincent 1984, Fausch 1988, Martin et al. 1992). Introduction of hatchery-produced salmonids into wild populations has largely been halted, although, this practice continues and is the source of much debate (Martin et al. 1992, Daley 1993).

The potential genetic consequences of stocking non-native strains include the introduction of non-native alleles and the breaking up of native co-adapted gene complexes (Ferguson 1990). Potentially harmful genetic effects of hatchery introductions on native salmonids have been documented for several species (Krueger et al 1989, Evans and Willox 1991, Forbes and Allendorf 1991, Waples 1991).

Ecological interactions between native and hatchery brook trout could also have affected the genetic structure of native populations (Krueger and Menzel 1979). Hatchery introductions may reduce effective population sizes of salmonid populations (Hulett and Leider, 1989, Waples 1991). Random effects such as genetic drift become more important in smaller populations (Utter and May 1976). In addition hatchery introductions may alter mating strategies and selection regimes of wild populations (Waples 1991).

Previous Genetic Studies

Several studies have attempted to determine how unique southern Appalachian brook trout are compared to northern brook trout. Morphological studies (King 1937, Lennon 1967, Harris et al. 1978) have not been conclusive; however, King (1937) and Lennon (1967) noted that the "speckled trout" of the southern Appalachians generally have more numerous and smaller red spots on the side, smaller body sizes and longer fins in general compared to their northern counterparts. In addition there may be other behavioral and ecological differences between northern and southern Appalachian brook trout (Harris et al. 1978, Lennon 1967).

Brook trout genetic systems have been studied extensively with allozyme electrophoresis and more recently with mitochondrial DNA (Quattro and Morgan 1990). Many earlier allozyme studies focused on the pattern of inheritance of enzymatic loci (Morrison 1970, Wright 1970, Wright et al. 1975, May et al. 1979, Stoneking et al. 1979, Wright et al. 1980). Brussard and Nielsen (unpublished) were the first to address the question of whether northern and southern brook trout differ taxonomically. They examined 5 northern populations and a population from Great Smoky Mountains National Park (GRSM) and concluded that the population from GRSM was genetically distinct perhaps warranting taxonomic distinction. Subsequently, a more extensive study by Harris et al. (1978) examined 34

populations from Utah, Wisconsin, Pennsylvania, West Virginia, Virginia, North Carolina, South Carolina, and Tennessee. They concluded that the southern Appalachian brook trout populations were not distinct from northern populations. The polyploid nature of salmonids and the fact that many loci are duplicated (Ohno 1970, Allendorf and Thorgaard 1984) complicates salmonid isozyme banding patterns (Waples and Abersold 1990) and this confounded the studies of Harris et al. (1978) and Brussard and Nielsen (unpublished) (see discussion in Stoneking et al. 1981).

In Stoneking et al. (1981) inheritance data was used to correctly interpret the duplicated loci banding patterns of many enzymes. In this study five northern populations and three southern populations were examined at 39 loci. Stoneking et al. (1981) concluded that genetic evidence does suggest that southern populations of brook trout are distinct from their northern counterparts.

Stocking histories were not examined by Stoneking et al. (1981), Harris et al. (1978), or Brussard and Nielsen (unpublished). However, several southern Appalachian streams included in Stoneking et al. (1981) and Harris et al. (1978) had been stocked. Neglecting stocking histories almost certainly confounded the study by Harris et al. (1978). The Tennessee streams examined by Stoneking et al. (1981), Blockstand Creek and Rocky Fork, have been stocked and this knowledge would have strengthened their results.

Stocking records were considered in a recent study by McCracken et al. (1993) of brook trout in GRSM. In this study 34 allozyme loci were examined in five putatively unstocked populations, three hatchery or hatchery derived populations, and three stocked and presumably hybrid populations. Average genetic identities between the putative native populations and the hatchery populations ($\bar{I}=0.906 \pm 0.024$) are significantly lower than between putative native populations ($\bar{I}=0.985 \pm 0.017$) and between hatchery populations ($\bar{I}=0.986 \pm 0.003$). These genetic identities suggest levels of differentiation between hatchery and native populations that are typical of subspecies in other fish taxa (McCracken et al. 1993).

In addition, several allozyme loci contained alleles in hatchery and stocked GRSM populations not present in putative native (unstocked) populations. Unique hatchery alleles were found for enzymatic loci of: creatine kinase (CK-A2*78), l-lactate dehydrogenase (LDH-B1*67), malate dehydrogenase, (SMDH-B1,2*145), malic enzyme (mMEP-1,2*88), and fumarate hydratase (FH-1,2*148). All hatchery populations examined by McCracken et al. (1993) were fixed for CK-A2*78 and all unstocked populations examined were fixed for CK-A2*100.

The allozyme data of McCracken et al. (1993) has been corroborated with unpublished mitochondrial DNA (mtDNA) data of Dr. John Hayes (former postdoctoral researcher in Dr.

Gary McCracken's laboratory) that also indicate that native southern brook trout are distinct at levels typically found between subspecies (Hayes, personnel communication). Of 18 restriction enzymes examined, mitochondrial DNA haplotypes of the native southern populations showed fixed differences from hatchery populations at three sites.

The work of McCracken et al. (1993) and Hayes has identified genetic systems that are apparently diagnostic for native southern Appalachian brook trout and northern derived hatchery strain brook trout. Use of these diagnostic markers for management purposes is essential. Stocking records may not be reliable because unrecorded brook trout stockings have likely occurred (Bivens pers. comm.) and stockings may not result in reproduction of catchable fish.

Purpose of Study

The purpose of this study was to use the genetic systems described to determine the origin of 39 brook trout populations in East Tennessee outside of GRSM. Because native brook trout management has been emphasized by fisheries managers, information about the genetic origins of brook trout populations is necessary for intelligent management of this resource. In addition, several biological questions related to stocked and hybrid populations must be addressed in implementing an informed brook trout management plan.

Chapter 2

METHODS

Brook Trout Tissue Collections

Brook trout were collected during the summer and fall of 1991 and 1992. Collections were primarily made by Jim Habera as part of the University of Tennessee Department of Forestry, Wildlife and Fisheries, and the Tennessee Wildlife Resource Agency (TWRA) cooperative Wild Trout Project (Strange and Habera 1992, Strange and Habera 1993).

Most brook trout were obtained from populations sampled quantitatively (Strange and Habera 1992). Several streams were sampled only for tissue (Table 2.1). A sample size of between 15 and 20 fish was targeted for each population. Only one fish was encountered in a half mile section of Rough Ridge Creek in which rotenone was applied to remove rainbow trout. Collections of 25 or more fish were made from Rocky Fork, Blockstand Creek, and Brookshire Creek because they were targeted for mtDNA analysis.

A total of 39 brook trout populations was sampled during this study. These included many in which active management has taken place or is scheduled. The US Forest Service and TWRA have cooperated for several years to restore the native range of brook trout in the Cherokee National Forest. This is typically accomplished by removing encroaching rainbow trout populations upstream of barriers to prevent re-invasion. An effort was also made to obtain

Table 2.1. Thirty-nine brook trout populations in East Tennessee sampled for genetic analysis

Stream	County	Sample Size	Date Sampled	Quan. Data
<u>TELLICO RIVER</u>				
Bald River headwaters (BLD)	Monroe	17	8/15/92	yes
Brookshire Creek (BRO)	Monroe	25	4/14/92	no
Henderson Branch (HEN)	Monroe	17	8/23/91	no
Meadow Branch (MBR)	Monroe	18	8/16/91	yes
Roaring Branch (ROR)	Monroe	9	6/5/92	no
Rough Ridge Creek (RRC)	Monroe	1	5/26/92	no
Sugar Cove Creek (SCO)	Monroe	4	5/26/92	no
<u>FRENCH BROAD RIVER</u>				
Brown Gap Creek (BGC)	Cocke	16	7/3/91	yes
Middle Prong of Gulf Creek (MPG)	Cocke	18	9/13/91	yes
Norton Creek (NOR)	Sevier	12	7/16/92	no
<u>NOLICHUCKY RIVER</u>				
Blockstand Creek (BLK)	Greene	30	2/14/92	no
Davis Creek (DAV)	Greene	12	7/8/92	yes
Higgins Creek (HIG)	Unicoi	10	9/9/92	yes
Jones Branch (JBR)	Unicoi	21	6/28/91	yes
Rock Creek (RCK)	Unicoi	16	6/21/91	yes
Rocky Fork (RFK)	Unicoi	31	10/10/91 & 2/14/92	yes
Round Knob Branch (RKB)	Greene	12	7/9/92	no
West Fork of Dry Creek (DRY)	Greene	6	8/7/92	yes

Table 2.1 Continued.

Stream	County	Sample Size	Date Sampled	Quan. Data
<u>SOUTH FORK OF THE HOLSTON RIVER</u>				
Birch Branch (BIR)	Johnson	18	8/21/91	yes
East Fork of Beaverdam (EFB)	Johnson	10	8/19/92	yes
Gentry Creek (GEN)	Johnson	20	6/17/92	yes
Hoot Owl Hollow (HOH)	Johnson	10	8/17/92	yes
Jim Wright Branch (JWB)	Johnson	17	8/28/91	yes
Johnson Blevins Branch (JBB)	Johnson	12	8/27/91	yes
<u>WATAUGA RIVER</u>				
Bill Creek (BIL)	Carter	16	7/31/91	yes
Clarke Creek (CLK)	Carter	15	7/20/92	yes
Cress Branch (COR)	Johnson	20	5/28/92	no
Cook Branch (CKB)	Carter	20	7/27/92	no
Cove Creek (COV)	Carter	18	7/17/91	yes
Doe River headwaters (DOE)	Carter	20	7/16/91	no
Furnace Creek (FUR)	Johnson	10	8/18/92	yes
George Creek (GEO)	Carter	18	9/10/91	yes
Leonard Branch (LEO)	Carter	15	7/28/92	yes
Little Laural Branch (LLB)	Carter	15	8/18/92	yes
Little Stony Creek (LST)	Carter	15	7/21/92	yes
Middle Branch (MID)	Carter	17	7/26/91	yes
North Fork of Stony Creek (NFS)	Carter	18	8/13/91	yes
Tiger Creek (TIG)	Carter	17	8/30/91	yes
Toms Branch (TOM)	Carter	21	8/25/91	yes

samples from within all of the major Tennessee watersheds known to have brook trout.

Brook trout were collected with standard backpack electrofishing gear. Fish were overdosed with tricaine methanesulfonate (MS-222) and sacrificed for tissue. Most fish were greater than 120 mm because of the difficulty of dissecting liver tissue from smaller fish. Liver, muscle, and eye tissues were held on wet ice and frozen in liquid nitrogen within a few hours of dissection. Samples were transferred to a freezer and stored at -80°C until they could be prepared.

Allozyme Analysis

Muscle tissue was prepared for allozyme analysis by homogenizing 0.1 to 1.0 gram of muscle tissue with an equal volume of an allozyme grinding buffer (10 mMolar tris: 1 mMolar EDTA, pH 6.8). The homogenate was placed on ice for at least 15 minutes and then spun in a cold centrifuge for approximately 20 minutes at 14,000 RPM. The supernatant was then transferred to a clean cryotube and frozen until used.

Horizontal starch gel electrophoresis techniques, enzyme stains, and gel buffer recipes followed standard procedures used in Dr. Gary McCracken's laboratory (Appendix A). Equipment and apparatus specifications can be found in McCracken and Wilkinson (1988). Figure 2.1 is a schematic representation of the major steps involved in starch gel electrophoresis.

Starch Gel Electrophoresis

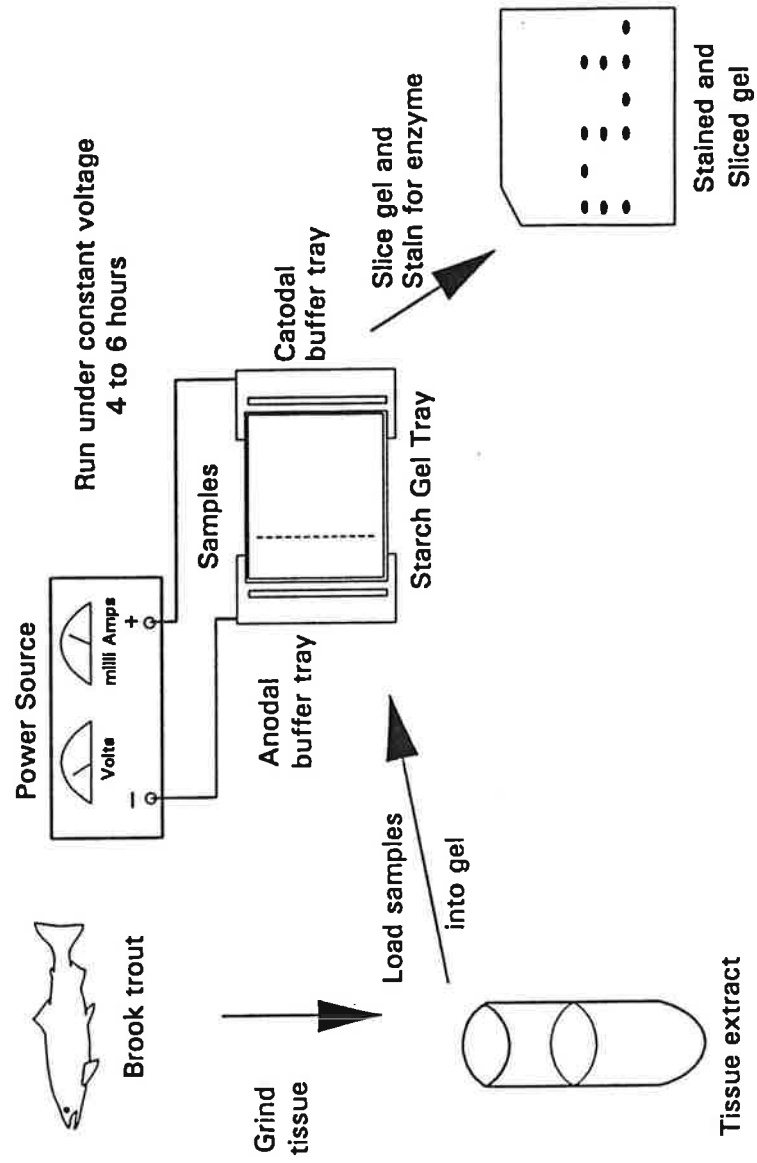


Figure 2.1. Schematic diagram of the major steps involved in starch gel electrophoresis.

All populations were surveyed for nine enzymes which are encoded by 18 presumptive loci (Table 2.2). Most alleles identified corresponded to electrophoretic alleles identified by McCracken et al. (1993) for GRSM brook trout. Glucose-6-phosphate isomerase (GPI*) alleles corresponded to alleles described by Stoneking et al. (1981) and Morgan and Baker (1991). Supernatant aspartate aminotransferase (sAAT-1,2*) alleles corresponded to those described by Wright et al. (1980) and variation at this isolocus system was treated as in Krueger et al. (1989). Diagrams of phenotypes corresponding to allelic products for the enzyme systems investigated appear in Appendix B.

Most gels were photographed after scoring. Alleles were designated on data sheets with letters and were converted into numeric allelic designations for purposes of analysis (Appendix C). Data were entered into a lotus spreadsheet and analysis was performed with the microcomputer program "Genes in Populations" by B. May, C.C Krueger, and W. Eng of Cornell University.

Mitochondrial DNA Analysis

An ongoing mtDNA study of brook trout hybridization was extended to include several populations from this study. DNA extractions and mtDNA analysis generally followed standard protocols (Lansman et al. 1981) as compiled by Dr. John Hayes (Appendix D). Figure 2.2 is a schematic diagram of the major steps involved in mtDNA analysis.

Table 2.2. Enzymes, locus designations and electrophoresis buffers. Enzyme numbers follow the recommendations of IUBNC (1984). All enzymes investigated were in muscle tissue. Buffer systems used were CT (Clayton and Tritiak 1972 as modified by May et al. 1979) and RSL (Ridgeway et al. 1970).

Enzyme	Enzyme number	Number of loci	Locus	Buffer System
supernatant aspartate aminotransferase	2.6.1.1	2	<u>SAAT-1,2*</u>	RSL
Creatine kinase	2.7.3.2	2	<u>CK-A1*</u> , <u>CK-A2*</u>	RSL
Esterase	3.1.1.-	2	<u>EST-1*</u> , <u>EST-2*</u>	RSL
Fumerate hydratase	4.2.1.2	2	<u>FH-1,2*</u>	RSL
L-lactate dehydrogenase	1.1.1.27	2	<u>LDH-A1*</u> , <u>LDH-A2*</u> , <u>LDH-B1*</u>	RSL
Malate dehydrogenase	1.1.1.37	2	<u>MDH-B1,2*</u>	CT
Malic enzyme	1.1.1.40	3	<u>mMEP-1*</u> , <u>mMEP-2*</u> , <u>mMEP-3*</u>	CT
Glucose-6-phosphate isomerase	5.3.1.9	3	<u>GPI-1*</u> , <u>GPI-2*</u> , <u>GPI-3*</u>	RSL
Dihydrolipoamide dehydrogenase	1.8.1.4	1	<u>DDH*</u>	CT

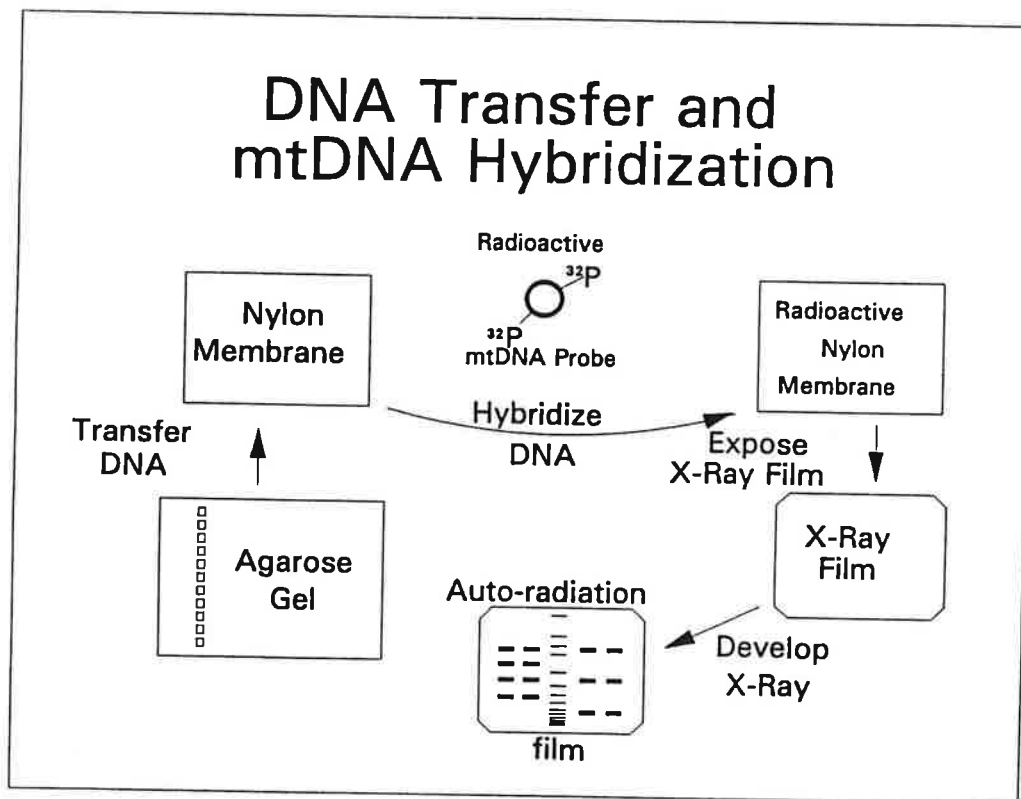
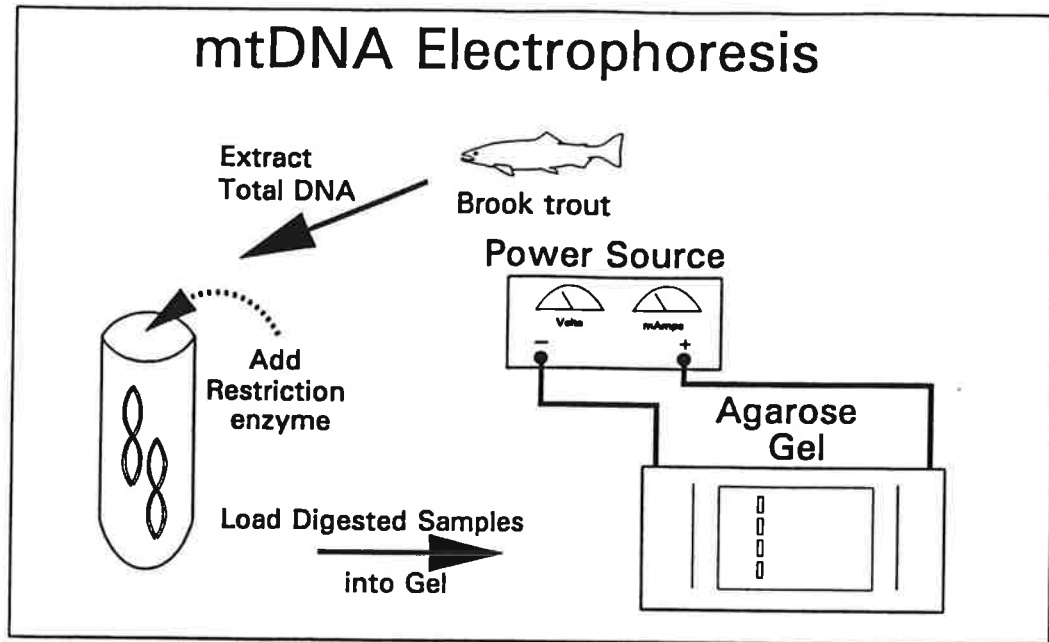


Figure 2.2. Schematic diagram of the major steps involved in mitochondrial DNA analysis.

Stocking Records

Stocking records were examined from three state fish hatcheries (Appendix E). Weekly fish output forms from the Erwin, Tellico, and Buffalo Springs hatcheries are on file at the Tennessee Wildlife Resource Agency Region IV office in Morristown, Tennessee. These files include the years 1952 to present for the Tellico hatchery, 1950 to present for the Erwin hatchery, and 1972 to present for the Buffalo Springs hatchery. Brook trout stockings were generally of catchable fish. Fingerlings and eyed eggs were also stocked and these are noted. Large fingerlings, however, were not distinguished from catchable fish for the purpose of this thesis. Additional information about brook trout stocking and transplantation efforts came from Bivens (1984).

Chapter 3

RESULTS

Allozyme Data

Allele frequencies, average overall individual heterozygosities (H_o), average fixation indexes (F_{is}), and the number of polymorphic loci for each population are reported by major watershed (Tables 3.1-3.5 and Figures 3.1-3.5). Deviations from Hardy-Weinberg expected genotype distributions were found in single populations at FH-1,2* in Rocky Fork and at LDH-B1* in Rocky Fork and Blockstand Creek. Deviation at LDH-B1* occurred because LDH-B1*100/117 heterozygotes were not detected. Average F_{is} values and H_o estimates for Rocky Fork and Blockstand were calculated without LDH-B1*. With LDH-B1*, average F_{is} and H_o estimates were +0.322 and 0.159 ± 0.005 for Blockstand Creek and +0.406 and 0.179 ± 0.003 for Rocky Fork respectively.

Only two populations contained loci polymorphic for more than two alleles which required pooling of alleles for purposes of analysis. The two rare alternate alleles at GPI-3* in the Jones Branch population and the two alternate alleles at GPI-2* in the Rocky Fork population were appropriately pooled.

Genetic Origins of Populations

The 39 populations were grouped into three putative categories (Table 3.6) based on the information in McCracken et al. (1993) on genetic origins of native, hatchery and

Table 3.1. Allele frequencies at polymorphic loci from seven brook trout populations in the Tellico River watershed.

Locus	Allele	Populations						
		BLD	BRO	HEN	MBR	ROR	RRC	SCO
<u>SAAT-1,2*</u>		(17)	(24)	(16)	(16)	(9)	(1)	(4)
	<u>*118</u>	0.828	0.657	0.979	0.469	0.639	0.5	0.188
	<u>*100</u>	0.132	0.343	0.021	0.531	0.361	0.5	0.822
<u>CK-A2*</u>		(17)	(25)	(17)	(17)	(9)	(1)	(4)
	<u>*100</u>	1.00	0.940	0.588	--	--	0.5	--
	<u>*78</u>	--	0.060	0.412	1.00	1.00	0.5	1.00
<u>FH-1,2*</u>		(17)	(25)	(17)	(16)	(9)	(1)	(4)
	<u>*148</u>	--	--	0.188	0.382	--	--	--
	<u>*100</u>	1.00	1.00	0.822	0.618	1.00	1.00	1.00
<u>LDH-B1*</u>		(17)	(25)	(17)	(16)	(9)	(1)	(4)
	<u>*117</u>	--	--	--	--	--	--	--
	<u>*100</u>	1.00	0.98	0.706	0.706	0.222	1.00	0.125
<u>SMDH-B1,2*</u>	<u>*67</u>	--	0.02	0.294	0.294	0.778	--	0.875
		(17)	(25)	(17)	(16)	(9)	(1)	(4)
	<u>*145</u>	--	--	0.147	0.471	--	--	--
<u>*100</u>		1.00	1.00	0.853	0.529	1.00	1.00	1.00

Table 3.1. Continued.

Locus	Allele	Population							
		BLD	BRO	HEN	MBR	ROR	RRC	SCO	
<u>mmEP-1*</u>		(17)	(25)	(17)	(16)	(9)	(1)	(4)	
	<u>*100</u>	1.00	0.92	0.765	0.382	1.00	1.00	1.00	
	<u>*88</u>	--	0.08	0.235	0.618	--	--	--	
<u>GPI-1*</u>		(17)	(25)	(17)	(17)	(9)	(1)	(4)	
	<u>*150</u>	--	--	--	0.324	--	--	--	
	<u>*125</u>	--	--	--	--	0.722	--	--	
	<u>*100</u>	1.00	1.00	1.00	0.676	0.278	1.00	1.00	
<u>GPI-2*</u>		(17)	(25)	(17)	(17)	(9)	(1)	(4)	
	<u>*121</u>	--	--	--	--	--	--	--	
	<u>*100</u>	--	0.280	1.00	0.794	1.00	0.5	0.750	
	<u>*70</u>	1.00	0.720	--	--	--	--	--	
	<u>*39</u>	--	--	--	0.206	--	0.5	0.250	
<u>GPI-3*</u>		(17)	(25)	(17)	(17)	(9)	(1)	(4)	
	<u>*105</u>	--	--	--	--	--	--	--	
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	<u>*95</u>	--	--	--	--	--	--	--	
<u>**Avg F_i</u> <u>**H_o</u> <u>SE</u> <u># loci polymorphic</u> <u>**Calculated without SAAT-1,2*</u>		--	+ .123	+ .281	+ .193	+ .256	--	--	
		--	0.075	0.154	0.265	0.069	--	--	
		--	0.037	0.056	0.060	0.047	--	--	
		0	5	6	7	2	--	3	

Table 3.2. Allele frequencies at polymorphic loci from three East Tennessee brook trout populations in the French Broad watershed and Little Muncy Creek in PA.

Locus	Allele	French Broad Populations			Little Muncy Creek	
		BGC	MPG	NOR	MUN	
<u>SAAT-1,2*</u>		(16)	(18)	(12)	(0)	
	<u>*118</u>	0.833	0.635	--	--	
	<u>*100</u>	0.167	0.365	1.00	--	
<u>CK-A2*</u>		(16)	(18)	(12)	(5)	
	<u>*100</u>	1.00	1.00	--	--	
	<u>*78</u>	--	--	1.00	1.00	
<u>FH-1,2*</u>		(16)	(18)	(12)	(5)	
	<u>*148</u>	--	--	0.125	--	
	<u>*100</u>	1.00	1.00	0.825	1.00	
<u>LDH-B1*</u>		(16)	(18)	(12)	(5)	
	<u>*117</u>	--	--	--	--	
	<u>*100</u>	1.00	1.00	0.675	1.00	
	<u>*67</u>	--	--	0.325	--	
<u>SMDH-B1,2*</u>		(16)	(18)	(12)	(5)	
	<u>*145</u>	--	--	--	--	
	<u>*100</u>	1.00	1.00	1.00	1.00	

Table 3.2. Continued.

Locus	Allele	French Broad Populations				Little Muncy Creek	
		BGC	MPG	NOR		MUN	
<u>mMEP-1*</u>	<u>*100</u>	(16)	(18)	(12)		(5)	
	<u>*88</u>	1.00	1.00	0.083		1.00	
		--	--	0.917		--	
<u>GPI-1*</u>	<u>*150</u>	(16)	(18)	(12)		(5)	
	<u>*125</u>	--	--	--		--	
	<u>*100</u>	--	--	--		--	
		1.00	1.00	1.00		1.00	
<u>GPI-2*</u>	<u>*121</u>	(16)	(18)	(12)		(5)	
	<u>*100</u>	--	--	--		--	
	<u>*70</u>	--	--	0.925		1.00	
	<u>*39</u>	1.00	1.00	--		--	
		--	--	0.075		--	
<u>GPI-3*</u>	<u>*105</u>	(16)	(18)	(12)		(5)	
	<u>*100</u>	--	--	--		--	
	<u>*95</u>	1.00	1.00	1.00		1.00	
		--	--	--		--	
Avg F_b		--	--	--		--	
H_o		--	--	0.083		--	
SE		--	--	0.002		--	
# loci polymorphic		1	1	4		0	
** Calculated without <u>sAAT-1,2*</u>							

Table 3.3. Allele frequencies at polymorphic loci from populations from the Nolichucky River watershed.

Locus	Allele	Populations							
		BLK	DAV	DRY	HIG	JBR	RCK	RFX	RKB
<u>SAAT-1,2*</u>		(30)	(15)	(6)	(10)	(21)	(16)	(29)	(12)
	<u>*118</u>	0.644	0.967	0.458	0.30	0.429	0.140	0.552	1.00
	<u>*100</u>	0.366	0.033	0.542	0.70	0.571	0.860	0.448	--
<u>CK-A2*</u>		(29)	(15)	(6)	(10)	(21)	(16)	(31)	(12)
	<u>*100</u>	0.534	1.00	--	--	--	--	0.435	0.792
	<u>*78</u>	0.466	--	1.00	1.00	1.00	1.00	0.565	0.208
<u>FH-1,2*</u>		(30)	(15)	(6)	(10)	(21)	(16)	(31)	(12)
	<u>*148</u>	0.017	--	--	--	0.286	0.656	0.194	--
	<u>*100</u>	0.983	1.00	1.00	1.00	0.714	0.344	0.806	1.00
<u>LDH-B1*</u>		(27)	(15)	(6)	(10)	(21)	(16)	(28)	(12)
	<u>*117</u>	0.556	--	--	--	--	--	0.304	--
	<u>*100</u>	0.222	1.00	0.750	0.75	0.738	0.469	0.571	0.958
	<u>*67</u>	0.222	--	0.250	0.25	0.262	0.531	0.125	0.042
<u>SMDH-B1,2*</u>		(30)	(15)	(6)	(10)	(21)	(16)	(31)	(12)
	<u>*145</u>	0.067	--	--	--	0.333	0.344	0.177	--
	<u>*100</u>	0.933	1.00	1.00	1.00	0.667	0.656	0.823	1.00

Table 3.3. Continued.

Locus	Allele	Populations									
		BLK	DAV	DRY	HIG	JBR	RCK	RFK	RKB		
<u>mMEP-1*</u>		(30)	(15)	(6)	(10)	(21)	(16)	(31)	(12)		
	<u>*100</u>	0.933	1.00	0.250	0.50	0.50	--	0.839	1.00		
	<u>*88</u>	0.067	--	0.750	0.50	0.50	1.00	0.131	--		
<u>GPI-1*</u>		(30)	(15)	(6)	(10)	(19)	(16)	(31)	(12)		
	<u>*150</u>	--	--	--	--	--	--	--	--		
	<u>*125</u>	--	--	--	--	--	0.219	--	--		
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	0.718	1.00	1.00		
<u>GPI-2*</u>		(30)	(15)	(6)	(10)	(19)	(16)	(31)	(12)		
	<u>*121</u>	--	--	--	--	--	--	0.048	--		
	<u>*100</u>	0.483	1.00	1.00	1.00	0.421	1.00	0.661	1.00		
	<u>*70</u>	0.517	--	--	--	0.579	--	0.290	--		
	<u>*39</u>	--	--	--	--	--	--	--	--		
<u>GPI-3*</u>		(30)	(15)	(6)	(10)	(19)	(16)	(31)	(12)		
	<u>*105</u>	--	--	--	--	0.132	--	--	--		
	<u>*100</u>	1.00	1.00	1.00	1.00	0.605	1.00	1.00	1.00		
	<u>*95</u>	--	--	--	--	0.263	--	--	--		
	**Avg F_L	+0.287	--	+0.111	-0.257	+0.235	+0.180	+0.282	-0.220		
**H _o		0.167	--	0.083	0.138	0.265	0.213	0.189	0.063		
SE		0.066	--	0.063	0.091	0.060	0.070	0.057	0.052		
# loci polymorphic		7	0	3	3	7	5	7	2		

** Calculated without SAAT-1,2*

Table 3.4. Allele frequencies at polymorphic loci from six brook trout populations in the South Fork of the Holston River watershed.

Locus	Allele	Populations					
		BIR	EFB	GEN	HOH	JBB	JWB
<u>SAT-1,2*</u>		(18)	(10)	(20)	(10)	(12)	(17)
	<u>*118</u>	1.00	1.00	1.00	1.00	0.979	1.00
	<u>*100</u>	--	--	--	--	0.021	--
<u>CK-A2*</u>		(18)	(10)	(20)	(10)	(12)	(17)
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00
	<u>*78</u>	--	--	--	--	--	--
<u>FH-1,2*</u>		(18)	(10)	(20)	(10)	(12)	(17)
	<u>*148</u>	--	--	--	--	--	--
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00
<u>LDH-B1*</u>		(18)	(10)	(20)	(10)	(12)	(17)
	<u>*117</u>	--	--	--	--	--	--
	<u>*100</u>	1.00	1.00	1.00	0.950	1.00	1.00
<u>SMDH-B1,2*</u>	<u>*67</u>	--	--	--	0.050	--	--
		(18)	(10)	(20)	(10)	(11)	(17)
	<u>*145</u>	--	--	--	--	--	--
<u>*100</u>		1.00	1.00	1.00	1.00	1.00	1.00

Table 3.4. Continued.

Locus	Allele	Populations					
		BIR	EFB	GEN	HOH	JBB	JWB
<u>mMEP-1*</u>		(18)	(10)	(20)	(10)	(11)	(17)
	<u>*100</u>	1.00	1.00	1.00	0.850	1.00	1.00
	<u>*88</u>	--	--	--	0.150	--	--
<u>GPI-1*</u>		(18)	(10)	(20)	(10)	(12)	(17)
	<u>*150</u>	--	--	--	--	--	0.059
	<u>*125</u>	--	--	--	--	--	--
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	0.941
<u>GPI-2*</u>		(18)	(10)	(20)	(10)	(10)	(17)
	<u>*121</u>	--	--	--	--	--	--
	<u>*100</u>	0.972	1.00	0.450	0.700	1.00	1.00
	<u>*70</u>	--	--	--	--	--	--
	<u>*39</u>	0.028	--	0.550	0.300	--	--
<u>GPI-3*</u>		(18)	(10)	(20)	(10)	(12)	(17)
	<u>*105</u>	--	--	--	--	--	--
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00
	<u>*95</u>	--	--	--	--	--	--
**Avg F_L		-0.029	--	-0.212	+0.221	--	--
** H_o		0.007	--	--	0.075	--	0.007
SE		0.007	--	--	0.041	--	0.007
# loci polymorphic		1	0	1	2	1	1
**Calculated without <u>SAAT-1,2*</u>							

Table 3.5. Allele frequencies at polymorphic loci for 15 brook trout populations in the Watauga River watershed.

Locus	Allele	Populations									
		BIL	CKB	CLK	COR	COV	DOE	FUR	GEO		
<u>SAAT-1,2*</u>	<u>*118</u>	(16) 1.00	(20) 0.713	(15) 1.00	(21) 1.00	(18) 1.00	(20) 1.00	(10) 1.00	(18) 1.00		
	<u>*100</u>	--	0.288	--	--	--	--	--	--		
<u>CK-A2*</u>		(16)	(20)	(15)	(21)	(18)	(20)	(10)	(18)		
	<u>*100</u>	1.00	0.80	1.00	1.00	1.00	1.00	1.00	1.00		
	<u>*78</u>	--	0.20	--	--	--	--	--	--		
<u>FH-1,2*</u>	<u>*148</u>	(16) --	(20) 0.175	(15) --	(21) --	(18) --	(20) --	(10) --	(18) --		
	<u>*100</u>	1.00	0.825	1.00	1.00	1.00	1.00	1.00	1.00		
<u>LDH-B1*</u>		(16)	(20)	(15)	(21)	(18)	(20)	(10)	(18)		
	<u>*117</u>	--	--	--	--	--	--	--	--		
	<u>*100</u>	1.00	0.675	1.00	1.00	1.00	1.00	1.00	1.00		
	<u>*67</u>	--	0.325	--	--	--	--	--	--		
<u>SMDH-B1,2*</u>		(16)	(20)	(15)	(21)	(18)	(20)	(10)	(18)		
	<u>*145</u>	--	0.125	--	--	--	--	--	--		
	<u>*100</u>	1.00	0.875	1.00	1.00	1.00	1.00	1.00	1.00		

Table 3.5. Continued.

Locus	Allele	Populations									
		BIL	CKB	CLX	COR	COV	DOE	FUR	GEO		
<u>mMEP-1*</u>	(16)	(16)	(20)	(15)	(21)	(18)	(20)	(10)	(18)		
	<u>*100</u>	1.00	0.375	1.00	1.00	1.00	1.00	1.00	1.00		
	<u>*88</u>	--	0.625	--	--	--	--	--	--		
<u>GPI-1*</u>	(16)	(16)	(20)	(15)	(21)	(18)	(20)	(10)	(18)		
	<u>*150</u>	--	--	--	--	--	--	--	--		
	<u>*125</u>	--	--	--	--	--	--	--	--		
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
<u>GPI-2*</u>	(16)	(16)	(20)	(15)	(21)	(18)	(20)	(10)	(18)		
	<u>*121</u>	--	--	--	--	--	--	--	--		
	<u>*100</u>	1.00	0.925	1.00	1.00	1.00	1.00	1.00	1.00		
	<u>*70</u>	--	--	--	--	--	--	--	--		
	<u>*39</u>	--	0.075	--	--	--	--	--	--		
<u>GPI-3*</u>	(16)	(16)	(20)	(15)	(21)	(18)	(20)	(10)	(18)		
	<u>*105</u>	--	--	--	--	--	--	--	--		
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
	<u>*95</u>	--	--	--	--	--	--	--	--		
**Avg F_L		--	+0.119	--	--	--	--	--	--		
** H_o		--	0.206	--	--	--	--	--	--		
SE		--	0.063	--	--	--	--	--	--		
# loci polymorphic		0	7	0	0	0	0	0	0		
**Calculated without <u>sAAT-1,2*</u>											

Table 3.5. Continued.

Locus	Allele	Populations						
		LEO	LLB	LST	MID	NFS	TGR	TOM
<u>SRAT-1,2*</u>		(15)	(15)	(15)	(17)	(18)	(17)	(21)
	<u>*118</u>	0.967	1.00	1.00	1.00	1.00	1.00	1.00
	<u>*100</u>	0.033	--	--	--	--	--	--
<u>CK-A2*</u>		(15)	(15)	(15)	(17)	(18)	(17)	(21)
	<u>*100</u>	1.00	1.00	0.867	1.00	1.00	1.00	1.00
	<u>*78</u>	--	--	0.133	--	--	--	--
<u>FH-1,2*</u>		(12)	(15)	(15)	(17)	(18)	(17)	(21)
	<u>*148</u>	--	--	--	--	--	--	--
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<u>LDH-B1*</u>		(15)	(15)	(15)	(17)	(18)	(17)	(21)
	<u>*117</u>	--	--	--	--	--	--	--
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	<u>*67</u>	--	--	--	--	--	--	--
<u>SMDH-B1,2*</u>		(15)	(15)	(15)	(17)	(18)	(17)	(21)
	<u>*145</u>	--	--	--	--	--	--	--
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 3.5. Continued.

Locus	Allele	Populations							
		LEO	LLB	LST	MID	NFS	TGR	TOM	
<u>mMEP-1*</u>	<u>*100</u>	(15) 1.00	(15) 1.00	(15) 1.00	(17) 1.00	(18) 1.00	(17) 1.00	(21) 1.00	
	<u>*88</u>	--	--	--	--	--	--	--	
<u>GPI-1*</u>	(15)	(15)	(15)	(15)	(17)	(18)	(17)	(21)	
	<u>*150</u>	0.467	--	--	--	--	--	--	
	<u>*125</u>	--	--	--	--	--	--	--	
<u>GPI-2*</u>	<u>*100</u>	0.533	1.00	1.00	1.00	1.00	1.00	1.00	
	(15)	(15)	(15)	(15)	(17)	(18)	(17)	(21)	
	<u>*121</u>	--	--	--	--	--	--	--	
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
<u>GPI-3*</u>	<u>*70</u>	--	--	--	--	--	--	--	
	<u>*39</u>	--	--	--	--	--	--	--	
	(15)	(15)	(15)	(15)	(17)	(18)	(17)	(21)	
	<u>*105</u>	--	--	--	--	--	--	--	
	<u>*100</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	<u>*95</u>	--	--	--	--	--	--	--	
	(15)	(15)	(15)	(15)	(17)	(18)	(17)	(21)	
	<u>*105</u>	--	--	--	--	--	--	--	
**Avg F _t		+0.143	--	-0.154	--	--	--	--	
**H _o		0.067	--	0.033	--	--	--	--	
SE		0.050	--	0.033	--	--	--	--	
# loci polymorphic		2	0	1	0	0	0	0	
**ditto									

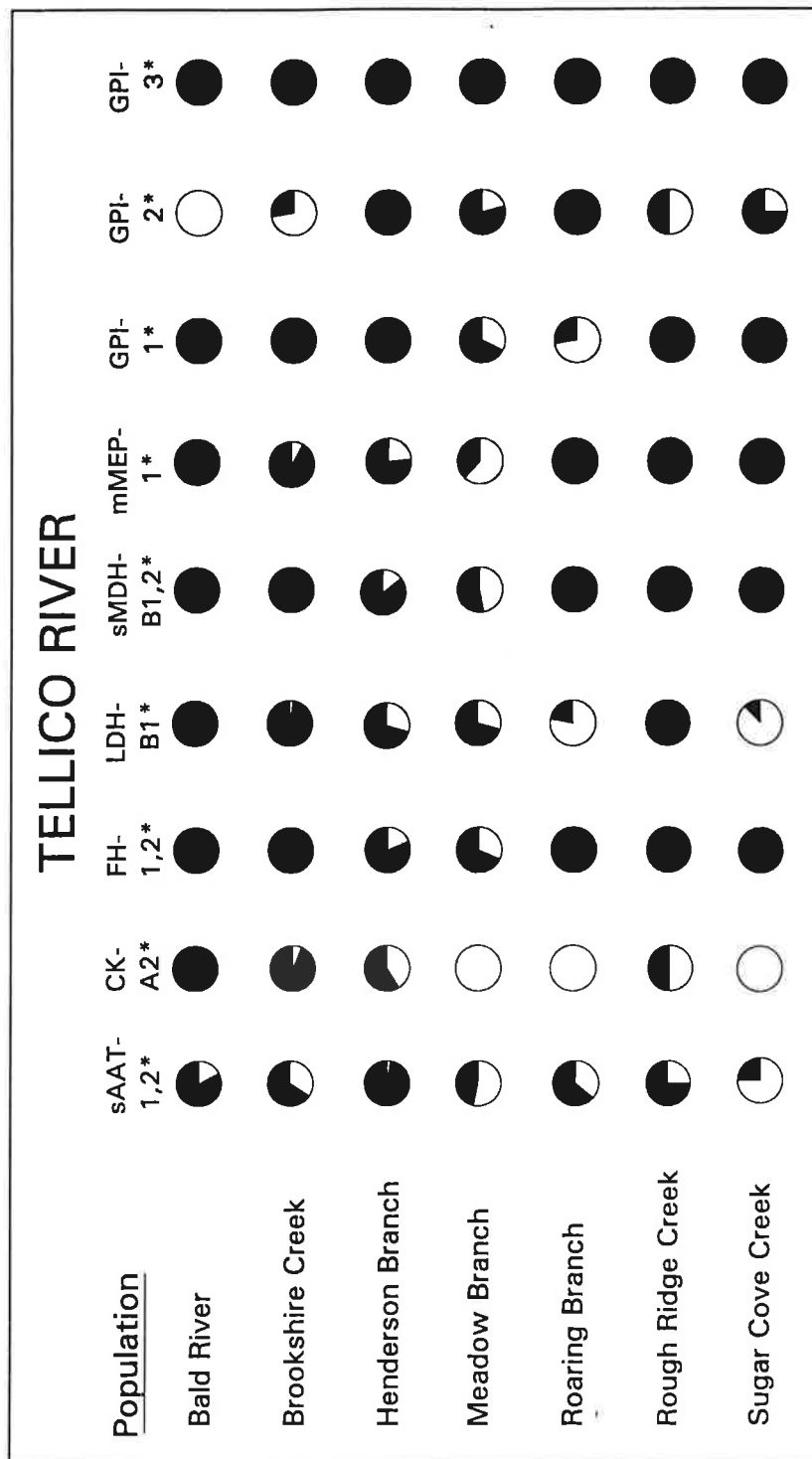


Figure 3.1. Pie diagrams of allele frequencies at polymorphic loci in six brook trout populations from the Tellico River watershed. Dark shaded areas represent the frequency of the common allele in native southern Appalachian brook trout.

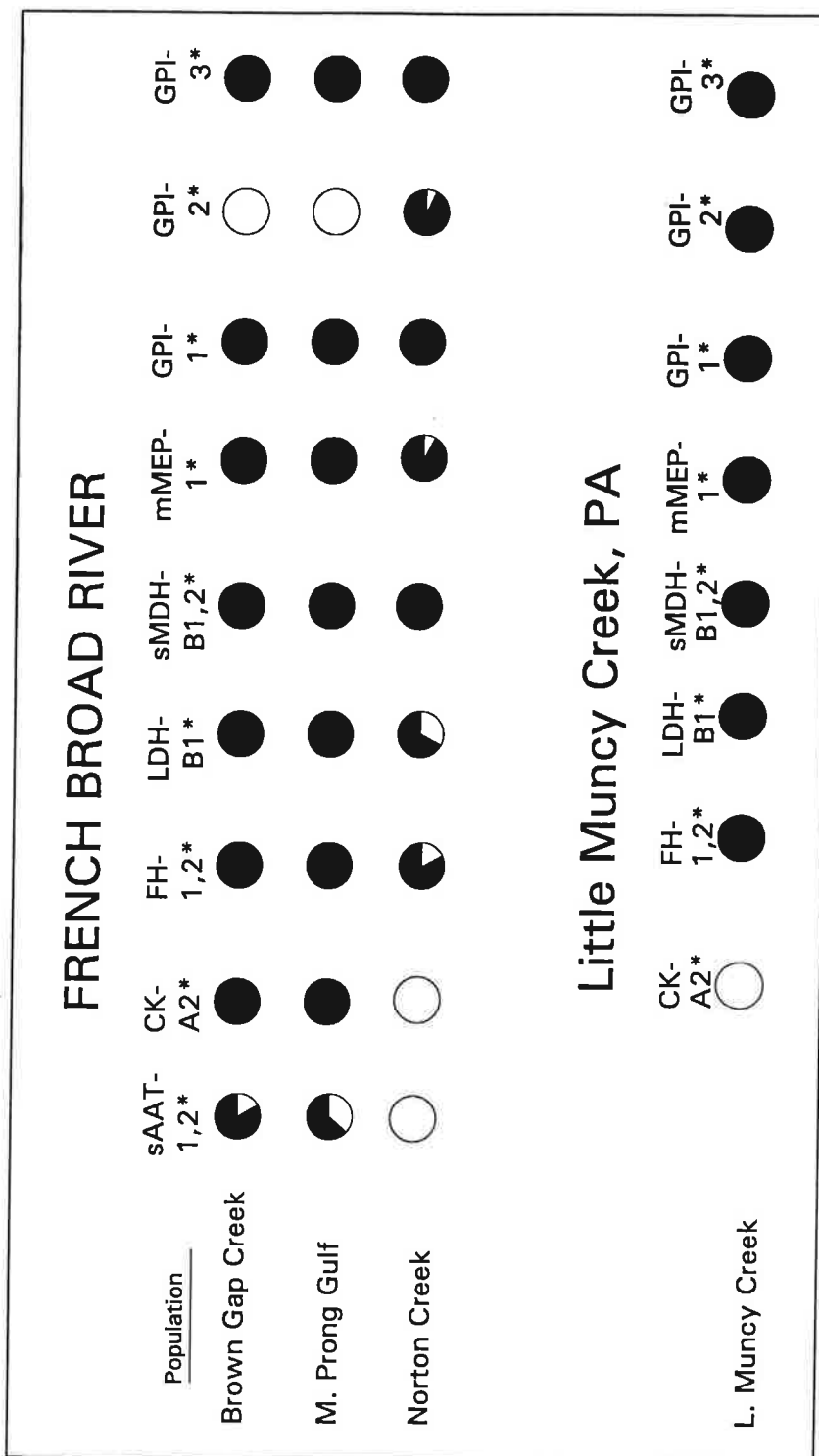


Figure 3.2. Pie diagrams of allele frequencies at polymorphic loci in three brook trout populations from the French Broad River watershed and Little Muncy Creek in Pennsylvania. Dark shaded areas represent the frequency of the common allele in native southern Appalachian brook trout.

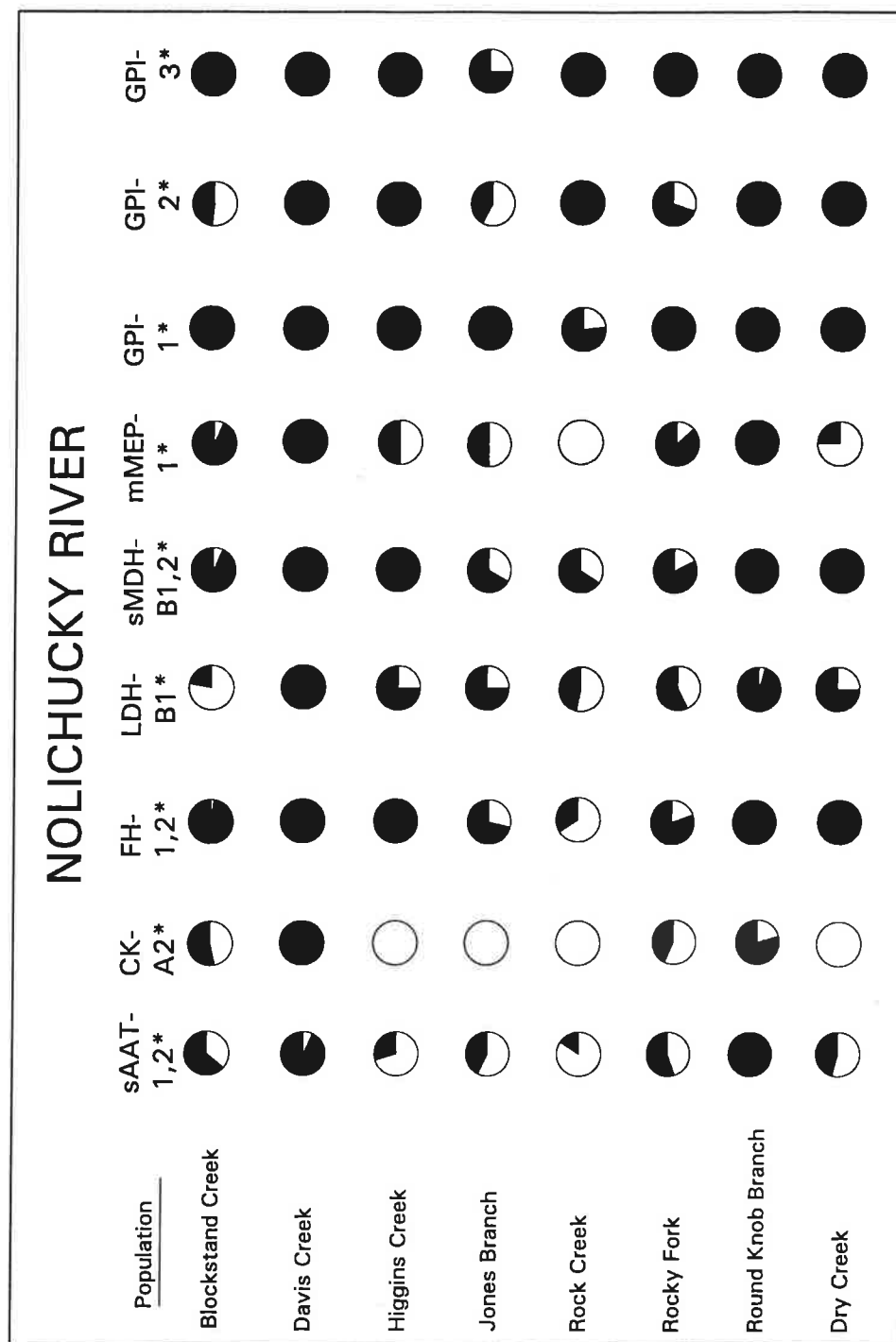


Figure 3.3. Pie diagrams of allele frequencies at polymorphic loci in eight brook trout populations from the Nolichucky River watershed. Dark shaded areas represent the frequency of the common allele in native southern Appalachian brook trout.

SOUTH FORK OF THE HOLSTON RIVER

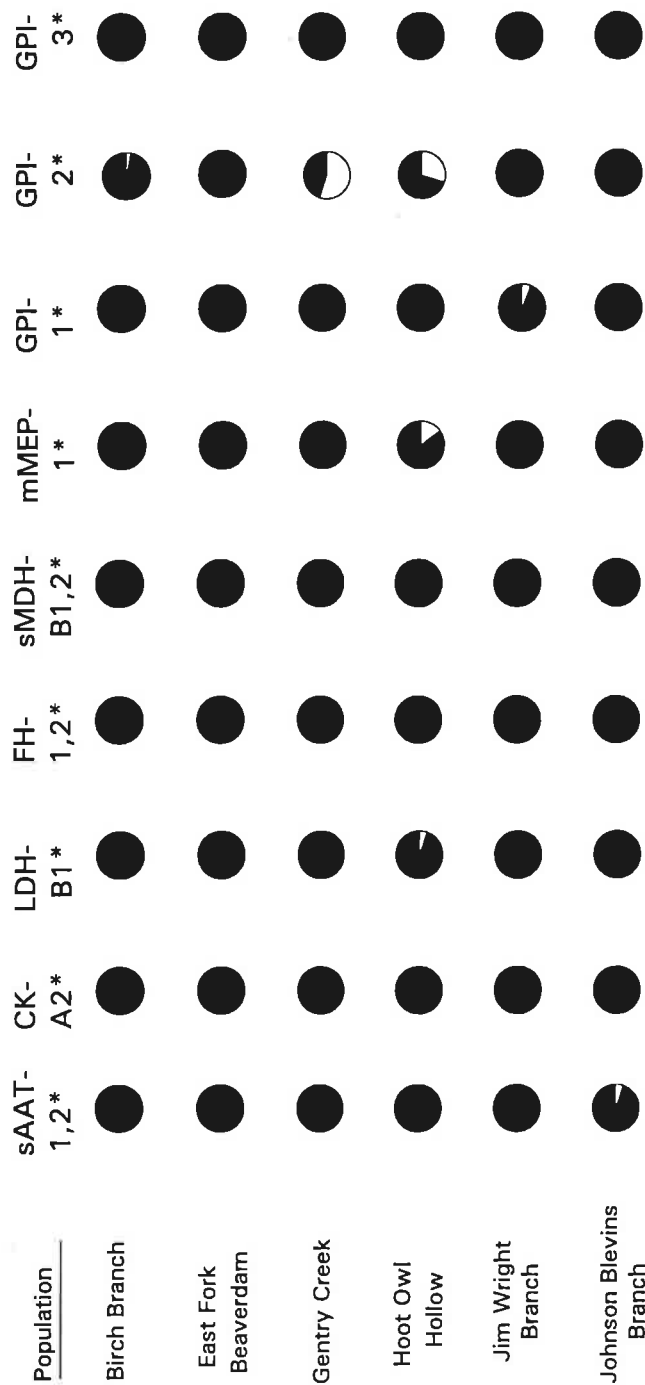


Figure 3.4. Pie diagrams of allele frequencies at polymorphic loci in six brook trout populations from the South Fork of the Holston River watershed. Dark shaded areas represent the frequency of the common allele in native southern Appalachian brook trout.

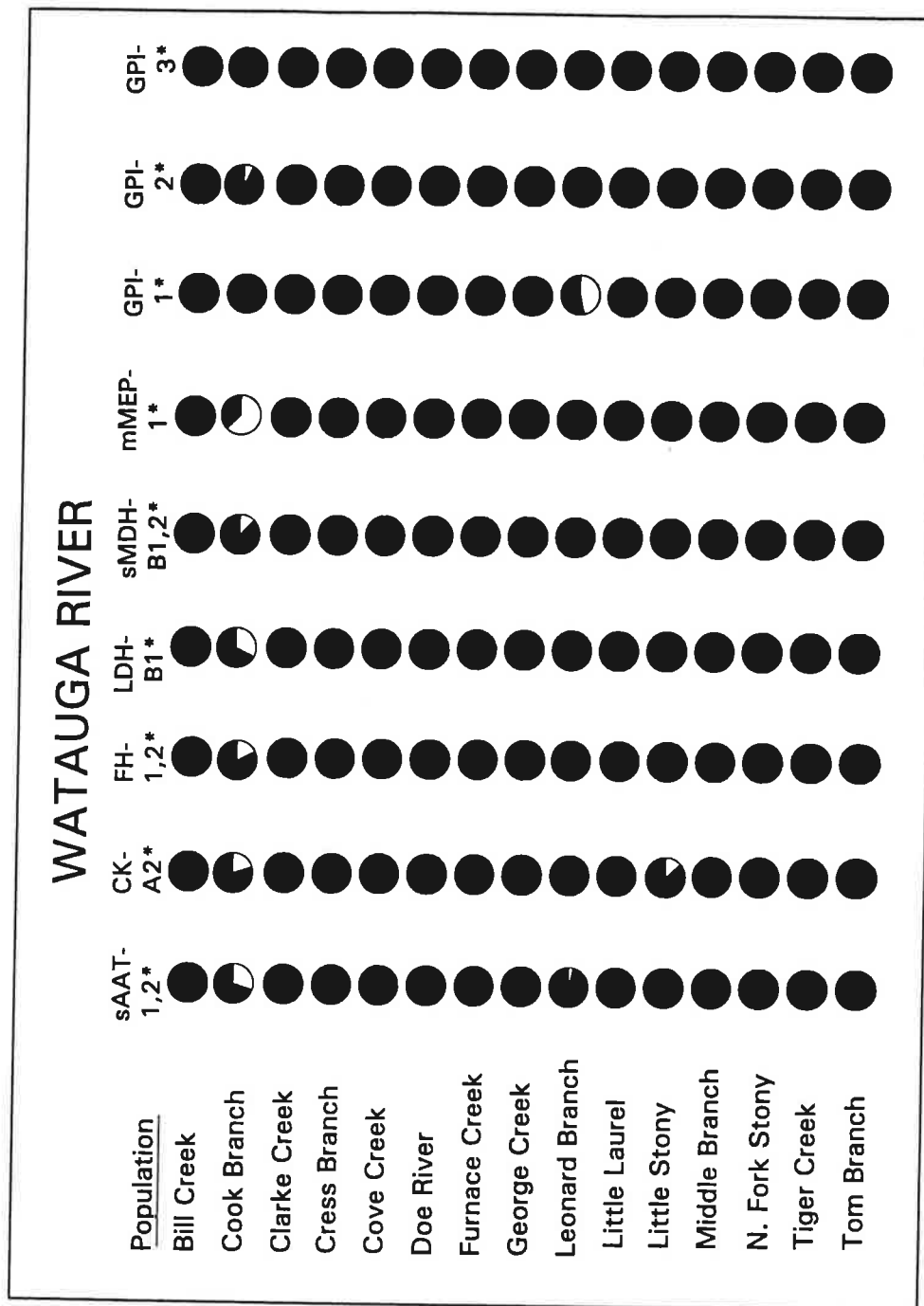


Figure 3.5. Pie diagrams of allele frequencies at polymorphic loci in 15 brook trout populations from the Watauga River watershed. Dark shaded areas represent the frequency of the common allele in native southern Appalachian brook trout.

Table 3.6. Genetic Origins of 39 Brook Trout Populations in Tennessee.

Watershed	Stream	(N)	Origin
TELLICO RIVER	Bald river headwaters (DOE)	17	Native
	Brookshire Creek (BRO)	25	Hybrid
	Henderson Branch (HEN)	17	Hybrid
	Meadow Branch (MBR)	18	Hatchery
	Roaring Branch (ROR)	9	Hatchery
	Rough Ridge Creek (RRC)	1	Hybrid
	Sugar Cove Creek (SCO)	4	Hatchery
FRENCH BROAD RIVER	Brown Gap Creek (BGC)	16	Native
	M. Prong of Gulf Creek (MPG)	18	Native
	Norton Creek (NOR)	12	Hatchery
NOLICHUCKY RIVER	Blockstand Creek (BLK)	30	Hybrid
	Davis Creek Creek (DAV)	12	Native
	Higgins Creek (HIG)	10	Hatchery
	Jones Branch (JBR)	21	Hatchery
	Rock Creek (RCK)	16	Hatchery
	Rocky Fork Creek (RFK)	31	Hybrid
	Round Knob Branch (RKB)	12	Hybrid
	West Fork of Dry Creek (DRY)	6	Hatchery
SOUTH FORK OF THE HOLSTON RIVER	Birch Branch (BIR)	18	Native
	East Fork of Beaverdam (EFB)	10	Native
	Gentry Creek (GEN)	20	Native
	Hoot Owl Hollow (HOH)	10	Hybrid
	Johnson Blevins Branch (JBB)	12	Native
	Jim Wright Branch (JWB)	17	Native

Table 3.6. Continued.

Watershed	Stream	(N)	Origin
WATAUGA RIVER	Bill Creek (BIL)	16	Native
	Clarke Creek (CLK)	15	Native
	Cress Branch (COR)	20	Native
	Cook Branch (CKB)	20	Hybrid
	Cove Creek (COV)	18	Native
	Doe River headwaters (DOE)	20	Native
	Furnace Creek (FUR)	10	Native
	George Creek (GEO)	18	Native
	Leonard Branch (LEO)	15	Native
	Little Laurel Branch (LLB)	15	Native
	Little Stony Creek (LST)	15	Hybrid
	Middle Branch (MID)	17	Native
	North Fork of Stony Creek (NFS)	18	Native
	Tiger Creek (TGR)	17	Native
	Toms Branch (TOM)	21	Native

hybrid GRSM brook trout. Populations fixed for native alleles over all loci were presumed native. Populations fixed for the CK-A2* hatchery allele were presumed to be hatchery-derived. Populations with individuals heterozygous for CK-A2* were designated as hybrids. One population, Hoot Owl Hollow, was determined to be hybrid based on presumptive hatchery alleles present at mMEP-1* and LDH-B1*. The individual from Rough Ridge Creek was excluded from analysis, however, it was heterozygous at CK-A2* and therefore came from a hybrid population. Putative hatchery-derived and hybrid populations were often polymorphic at several diagnostic loci, whereas most putative native populations were monomorphic for all the loci. Twenty-two populations are putatively native, nine are hybrid, and eight are putative hatchery derived.

Genetic Diversity

Average standard genetic variance (F_{st}) and G-statistic analysis of genetic differentiation (χ^2 tables in Sokal and Rohlf 1981) among populations were examined among all 38 populations and among the subsets of hatchery, putative native, and hybrid populations (Table 3.7)

Table 3.7 Standardized genetic variation for East Tennessee brook trout populations by genetic origin. G-test for the null hypothesis that $F_{st}=0$.

Comparison	Number of loci	Average F_{st}	Total G	Total df
All 38 Populations	10	0.617	5531*	703
Hatchery Derived (8 populations)	10	0.2324	598*	133
Putative Native (22 populations)	6	0.623	1025*	336
Hybrid (8 populations)	8	0.241	701*	126

* $P<0.001$

Hatchery, hybrid and native populations

Significant genetic differentiation occurred at all nine of the polymorphic loci ($P<0.001$) among all hatchery derived populations with sample sizes of ten or more. G-statistics were significant ($P<0.001$) in all pair-wise comparisons of hatchery derived populations indicating that hatchery derived populations are genetically distinct.

Significant genetic heterogeneity occurred among all native populations at SAAT-1,2*, GPI-2*, and GPI-1* ($P<0.001$). G-statistics were significant ($P<0.05$) for 96 of 252 pair-wise comparisons among the 22 native populations. Five populations (Brown Gap Creek, Gentry Creek, Leonard Branch, Middle Prong of Gulf Creek, and Bald River) were involved in all significant comparisons. Three of these five populations (Bald River, Middle Prong of Gulf Creek, and Brown Gap Creek) were responsible for most of the genetic differentiation among the native populations.

Among the hybrid populations there was significant genetic differentiation ($P < 0.001$) at all nine polymorphic loci. All pair-wise comparisons were significant ($P < 0.001$) except between Little Stony Creek and Round Knob Branch and between Cook Branch and Henderson Branch.

Populations within watersheds

G-statistic analysis among populations within watersheds were significant ($P < 0.001$) in all but the South Fork of the Holston watershed (Table 3.8). G-statistics were not significant within the Watauga and the French Broad watersheds when only the putative native populations were compared. G-statistics remained highly significant in the predominately hatchery derived and hybrid watersheds (Nolichucky and Tellico) when a single putative native population was excluded.

Transplanted population pairs

Bivens (1984) documented four pairs of populations included in this study with known transplantation histories involving the movement of fish from one stream to another between 1969 and 1983 (Table 3.9). G-statistics were examined for these pairs. Two comparisons were significant at $P < 0.001$ and one at $P < 0.025$. Little Stony Creek and Round Knob Branch were not different.

Table 3.8. Standardized genetic variation for East Tennessee brook trout populations within watersheds. G-test for the null hypothesis that $F_{st}=0$.

Comparison	Number of loci	Average F_{st}	Total G	Total df
Tellico (3 Hatchery, 2 Hybrid 1 Native)	9	0.479	794*	90
Tellico minus BLD (3 Hatchery 2 Hybrid)	9	0.410	593*	72
Watauga River (2 Hatchery, 13 Native)	9	0.329	563*	252
Watauga minus CKB & LST (13 Native)	4	0.329	110	156
Nolichucky (4 Hatchery, 3 Hybrid 1 Native)	10	0.371	988*	126
Nolichucky minus DAV (4 Hatchery, 3 Hybrid)	10	0.309	768*	108
French Broad (1 Hatchery, 2 Native)	5	0.722	414*	34
French Broad minus NOR (2 Native)	2	0.051	5.8	14
S. Fk. Holston (1 Hybrid, 5 Native)	5	0.225	88	80
S. Fk. Holston minus HOH (5 Native)	4	0.299	72	60

* $P<0.001$, all other G-statistics were not significant at $P>0.05$

Table 3.9. Standardized genetic variances for East Tennessee brook trout populations. Comparison of pairs of streams with known transplantation histories. G-test for the null hypothesis that $F_{st}=0$.

Comparison	Transplantation	Number of loci	Average F_{st}	Total G	Total df
Little Stony (LST) and Round Knob Branch (RKB)	60 fish from LST to RKB 1983	2	0.011	2.18	13
Henderson Branch (HEN) and Brookshire Creek (BRO)	65 fish from HEN to BRO 1982	8	0.194	119**	17
Henderson Branch (HEN) and Johnson Blevins Branch (JBB)	37 fish from JBB to HEN 1969	7	0.142	54.8**	16
Brookshire Creek (BRO) and Bald River (BLD)	33 fish from BLD to BRO 1982	6	0.064	30.6*	17

**P<0.001 **P<0.025

Fixation Indexes (F_{is})

F_{is} statistics averaged for populations within the groups of putative native, hybrid, and putative hatchery-derived were all positive (Table 3.10) indicating an overall pattern of heterozygote deficiency in brook trout populations. Average F_{is} values were generally greatest among the hybrid populations and lowest among the native populations. Positive average F_{is} values were calculated for five of the six polymorphic loci over all eight hybrid populations (Table 3.11). One hybrid population, Rocky Fork, had positive F_{is} values at all six polymorphic loci.

Table 3.10. Average fixation indexes (F_{is}) for East Tennessee brook trout populations.

Comparison	F_{is} (N loci)	* F_{is} (N loci)
All populations (38 populations)	+0.161 (10)	+0.132 (9)
Hybrid (8 populations)	+0.236 (6)	+0.150 (5)
Hatchery-derived (8 populations)	+0.113 (7)	+0.137 (6)
Native (22 populations)	+0.024 (2)	+0.024 (2)

* Average F_{is} values calculated without LDH-B1*

Table 3.11. Fixation indexes (F_{is}) at polymorphic loci for eight putative hybrid populations of East Tennessee brook trout

Population	Polymorphic loci						
	<u>CK-A2</u> *	<u>LDH-B1</u> *	<u>sMDH-3,4</u> *	<u>MEP-1</u> *	<u>FH-1,2</u> *	<u>GPI-2</u> *	Average F_{is}
Round Knob Branch	-0.263	-0.043	--	--	--	--	-0.220
Little Stony Creek	-0.154	--	--	--	--	--	-0.154
Brookshire Creek	-0.064	-0.020	--	-0.087	--	+0.272	+0.123
Cook Branch	+0.063	+0.430	-0.143	-0.173	+0.481	-0.081	+0.199
Rocky Fork Creek	+0.147	+0.811*	+0.226	+0.285	+0.380	+0.390	+0.406*
Blockstand Creek	+0.238	+0.625*	+0.464	-0.071	-0.17	-0.001	+0.322*
Henderson Branch	+0.271	+0.433	+0.766	+0.019	-0.133	--	+0.281
Hoot Owl Hollow	--	-0.053	--	--	--	+0.524	+0.221
Average F_{is} (Eight Populations)	+0.086	+0.534	+0.321	-0.031	+0.272	+0.260	+0.236*
Average F_{is} minus BLK & RPK (Six Populations)	+0.015	+0.337	+0.342	-0.107	+0.224	+0.332	+0.137
High F_{is} values due to the fact that <u>LDH-B1*117</u> /100 heterozygotes were not detected							

*High F_{is} values due to the fact that $\frac{LDH-B1*117}{100}$ heterozygotes were not detected.

The hybrid populations were divided into two groups based on the frequency of the CK-A2*78 hatchery allele and the recency of stocking. Group I (Henderson Branch, Rocky Fork, and Blockstand Creek) were stocked in the late 1970's and early to mid 1980's and have the highest proportion of the CK-A2*78 allele ($f=0.41$ to 0.57). Group II (Little Stony Creek, Brookshire Creek, Round Knob Branch, Cook Branch, and Hoot Owl Hollow) had the lowest frequencies of the CK-A2*78 allele ($f=0.0$ to 0.28). There were no records of stockings in Cook Branch and Hoot Owl Hollow. Brookshire Creek was stocked in 1952. Little Stony Creek was stocked in 1984, however, this stocking probably did not contribute any hatchery alleles to the population (see below). The Round Knob Branch population was derived from 60 brook trout from Little Stony Creek.

The average F_{is} ($+0.223$) for the populations from group I (calculated without LDH-B1*) was higher compared to the average F_{is} for the populations from group II ($+0.053$). These values were not significantly different ($P>0.1$) reflecting the large variation of individual loci F_{is} values. However, average individual loci F_{is} values were positive for all 5 loci in group I ($+0.99$ to $+0.645$), whereas F_{is} values for three loci were negative and positive for two loci for group II (-0.160 to $+0.481$).

Allele Frequency Changes in Blockstand Creek and Rocky Fork

Brook trout from Rocky Fork and Blockstand Creek were also collected for genetic analysis by Stoneking et al. (1981) in 1979. Allele frequencies at CK-A2* and GPI-2* were compared between 1979 and 1991 for these populations (Table 3.12 and Figure 3.6). Allele frequencies at GPI-2* and CK-A2* differed significantly between the two collections ($P < 0.001$) in Rocky Fork. In Blockstand Creek allele frequencies at CK-A2* differed significantly ($p < 0.05$) and the frequencies at GPI-2* did not differ. It should be noted that collection sites in 1979 and 1991-2 may be different and allele frequency differences may partially represent within stream differences rather than temporal differences.

Table 3.12. Allele frequencies at two loci in Blockstand Creek and Rocky Fork from 1979 (Stoneking et al. 1981) and 1991-92. Sample size are given in parenthesis.

Locus	Allele	Populations			
		Blockstand		Rocky Fork	
		1979 (23)	1992 (29)	1979 (31)	1991-92 (31)
<u>CK-A2*</u>	* <u>100</u>	0.38	0.53	0.84	0.43
	* <u>78</u>	0.62	0.47	0.16	0.57
<u>GPI-2*</u>	* <u>121</u>	--	--	--	0.05
	* <u>100</u>	0.44	0.48	0.12	0.66
	* <u>70</u>	0.56	0.52	0.88	0.29

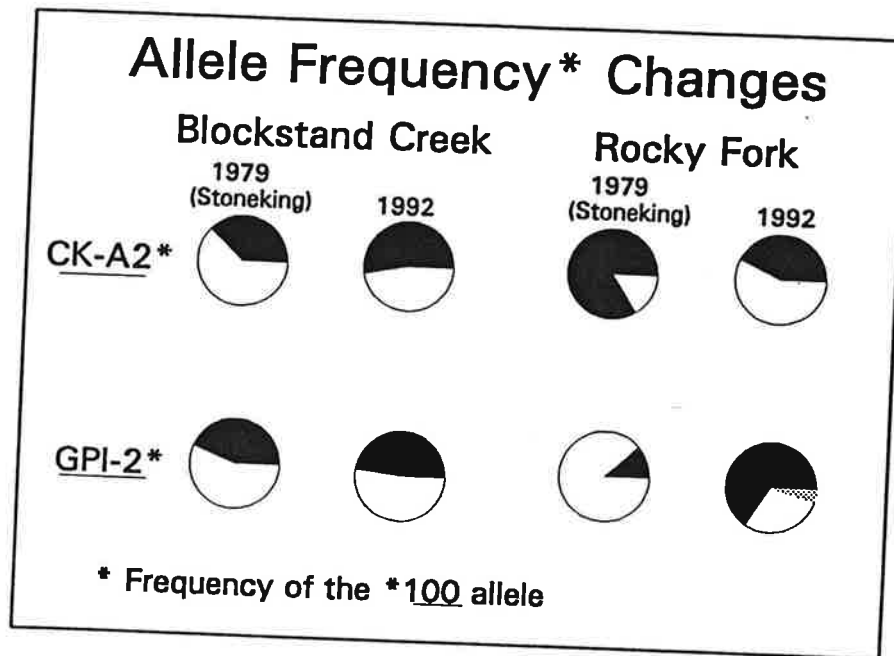


Figure 3.6. Pie diagrams of allele frequencies at CK-A2* and GPI-2* in Blockstand Creek and Rocky Fork in 1979 (Stoneking et al. 1981) and 1991-2. Black shading represents the frequency of the *100 allele.

Mitochondrial DNA

Three restriction enzymes (Dra I, BamH I and Pvu II) have consistently shown at least one site difference between native GRSM brook trout and northern derived hatchery populations (Hayes pers. comm.) (Table 3.13). The size of the brook trout mtDNA molecule has been estimated to be $16,540 \pm 99$ base pairs (Quattro and Morgan 1990). The fragments obtained with the three restriction enzymes are consistent with this estimate (Appendix F).

Table 3.13. MtdNA restriction fragment length polymorphisms for three restriction enzymes diagnostic of hatchery and native GRSM brook trout.

Restriction enzymes	Type of Population	Digestion Profile	Haplotype Designation
DraI	Hatchery	6.2 KB	A
		5.3 KB	
		0.6 KB	
		Total 16.2 KB	
	Native	10.3 KB	B*
		5.3 KB	
		0.6 KB	
		Total 16.2 KB	
BamH I	Hatchery	14.5 KB	A
		2.4 KB	
		Total 16.9 KB	
	Native	9.2 KB	B*
		5.3 KB	
		2.4 KB	
		Total 16.9 KB	
PvuII	Hatchery	8.5 KB	A
		6.7 KB	
		1.3 KB	
		0.8 KB	
	Native	Total 17.3 KB	B*
		8.5 KB	
		5.5 KB	
		1.3 KB	
		0.8 KB	
		0.8 KB	
		Total 17.0 KB	

* Additional haplotypes are present in some GRSM populations with the B haplotype being most common.

The three diagnostic restriction enzymes were used to digest the DNA of all individuals from Blockstand Creek, Henderson Branch, Rocky Fork, and Brookshire Creek, as well as five individuals from Meadow Branch. Two of the enzymes were used to digest a few individuals from the Middle Prong of Gulf Creek, Doe River, North Fork of Stony, and Johnson Blevins Branch populations. Successful hybridizations and auto-radiations were performed for all but the individuals from Brookshire Creek (Table 3.14).

Mitochondrial DNA results were consistent with allozyme data for those populations investigated. The three hybrid populations had both native and hatchery mtDNA haplotypes. The five individuals for Meadow Branch (hatchery derived) had hatchery haplotypes and the 13 individuals from the four putative native populations had native haplotypes.

Table 3.14. Haplotype Frequencies for three restriction enzymes diagnostic of native GRSM brook trout.

Population	Haplotype Frequencies									
	DraI		BamH I				PvuII			
	(N)	A	B	(N)	A	B	(N)	A	B	
Blockstand Creek	(22)	0.77	0.23	(30)	0.73	0.27	(30)	.73	.27	
Henderson Branch	(17)	0.65	0.35	(16)	0.625	0.375	(13)	.54	.46	
Rocky Fork	(20)	0.85	0.15	(28)	0.89	0.11	(28)	.89	.11	
Meadow Branch	(4)	1.00	--	(5)	1.00	--	(5)	1.00	--	
North Fork of Stony	(2)	--	1.00	(2)	--	1.00	--	--	--	
Middle Prong of Gulf Creek	(4)	--	1.00	(4)	--	1.00	--	--	--	
Doe River	(4)	--	1.00	(4)	--	1.00	--	--	--	
Johnson Blevins Branch	(3)	--	1.00	(3)	--	1.00	--	--	--	

Chapter 4

DISCUSSION

Population Origins

Genetic origins

Of the 39 populations examined, 22 consist of putative native brook trout. This proportion (56%) is much higher than expected by many state fisheries biologists (Habera pers. comm.). These results are encouraging because they indicate that a substantial native brook trout resource remains at least in Tennessee and possibly in other southern states.

It should be noted that sampling error is an important consideration in determining the genetic origins of populations. In particular this may be important in regards to genetic origins of native populations. A hypothetical population might have a hatchery allele at a single locus at a frequency of 0.05. If 15 individuals from this population (30 copies of a gene) are surveyed for this diagnostic locus the probability of not detecting this allele would be $(0.95)^{30}=0.21$ (21%). However, this probability drops dramatically when additional diagnostic loci are surveyed and with five loci this probability becomes $(0.95)^{150}$ which is $\ll 0.001$. The determination of population origins in this study has relied on use of several previously determined diagnostic loci (McCracken et al. 1993) as well

as the corroboration of mtDNA data. Even so, because of sampling error populations in this study are placed in putative categories only.

The proportion of putative native populations varied greatly between counties (Figures 4.1-4.6). Only one of the seven populations sampled in Monroe County, where the Tellico fish hatchery is located, is putatively native. Additionally, only one of the eight populations sampled in Greene and Unicoi counties, near to the state fish hatchery in Erwin, is putatively native. In contrast 17 of the 21 populations sampled in Johnson and Carter counties are putative native. Thus it appears that hatchery and hybrid populations may cluster around the locations of hatcheries.

Stocking histories

Many of the hatchery derived populations have a history of being heavily stocked (Table 4.1) and it is likely that many of the hatchery-derived populations were created through the stocking of streams that were either devoid of fish or had extremely low population densities of native brook trout. Meadow Branch was stocked repeatedly and it apparently had only a remnant population of native brook trout in the 1950's. Roaring Branch has a history of fire damage in the watershed and was devoid of fish when it was first stocked in 1955 (Bivens 1984). The West Fork of Dry Creek, Sugar Cove Creek, and Norton Creek were presumably devoid of fish when they were initially stocked.

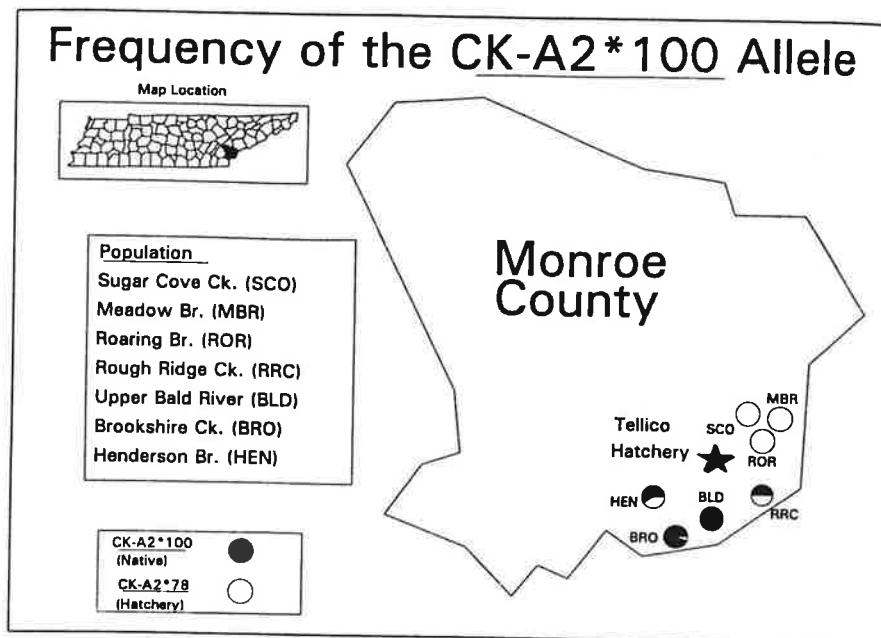


Figure 4.1. Creatine kinase allele frequencies for seven brook trout populations in Monroe County, Tennessee. Black shading represents the frequency of the CK-A2*100 allele.

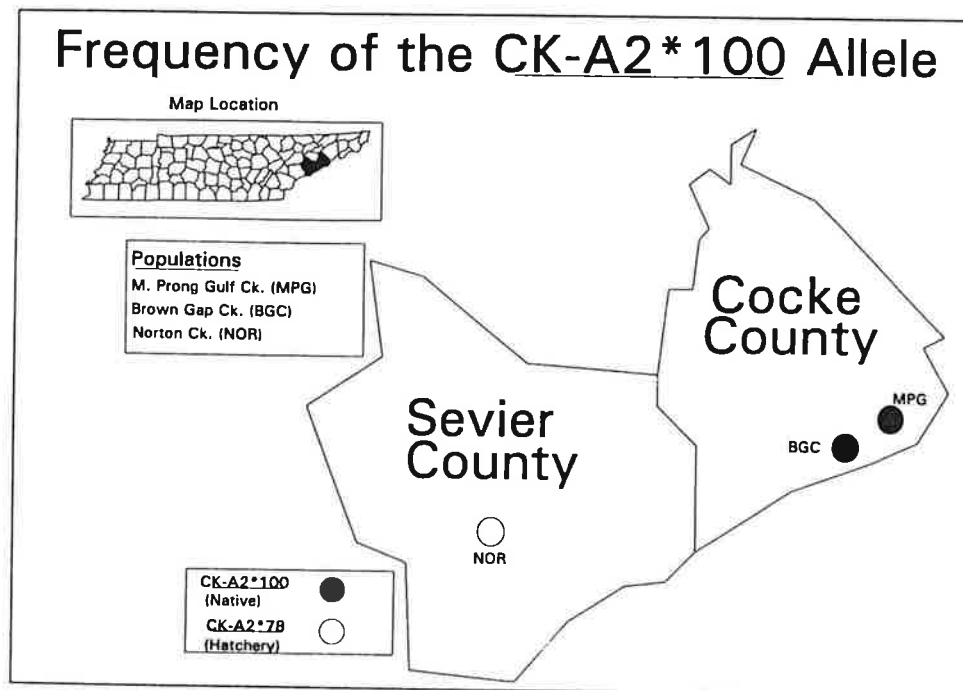


Figure 4.2. Creatine kinase allele frequencies for three Tennessee brook trout populations in Cocke and Sevier counties. Black shading represents the frequency of the CK-A2*100 allele.

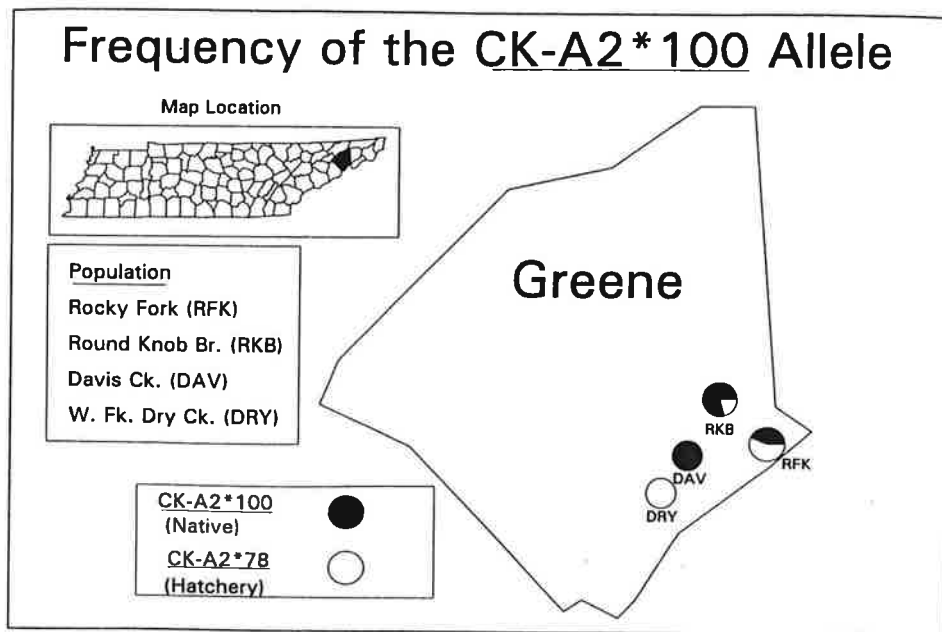


Figure 4.3. Creatine kinase allele frequencies for four brook trout populations in Greene County, Tennessee. Black shading represents the frequency of the CK-A2*100 allele.

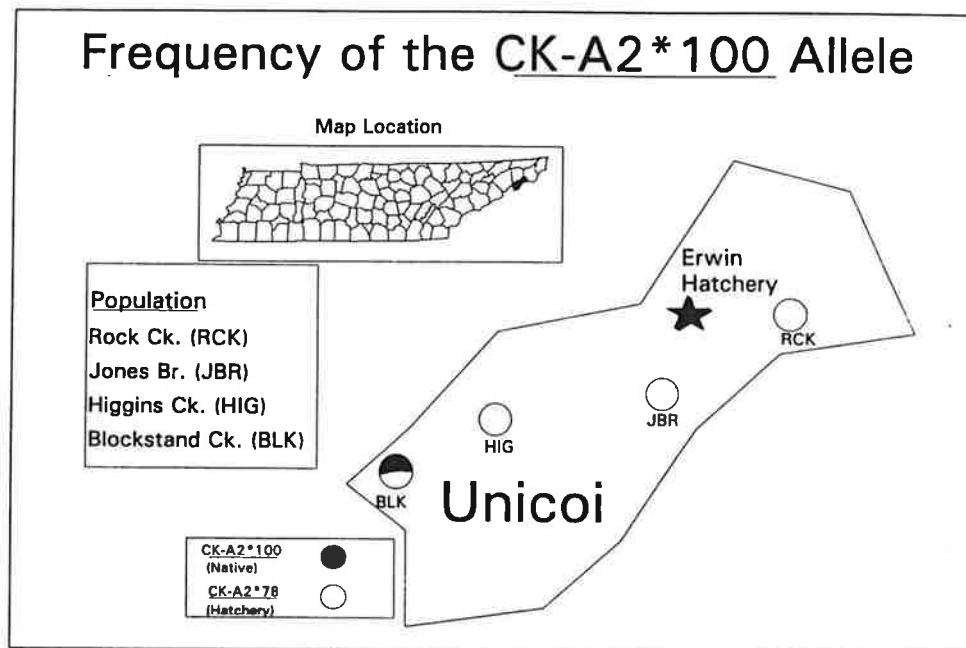


Figure 4.4. Creatine kinase allele frequencies for four brook trout populations in Unicoi county, Tennessee. Black shading represents the frequency of the CK-A2*100 allele.

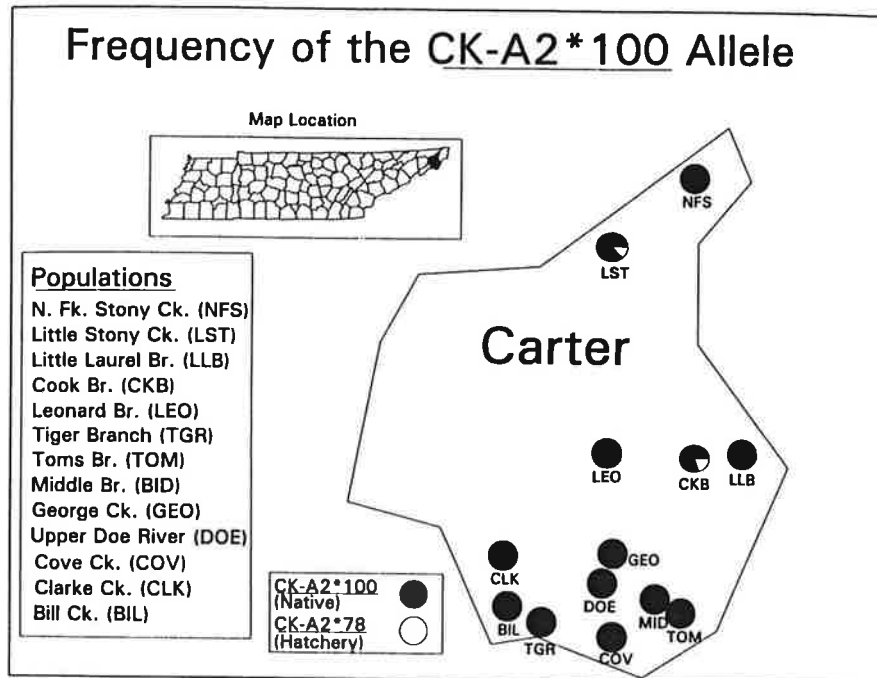


Figure 4.5. Creatine kinase allele frequencies for 13 brook trout populations in Carter county, Tennessee. Black shading represents the frequency of the CK-A2*100 allele.

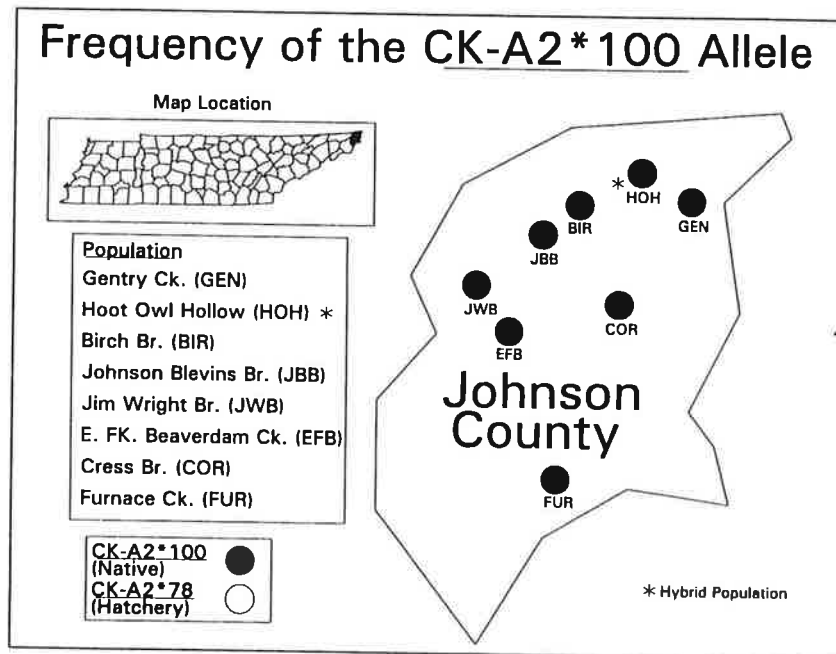


Figure 4.6. Creatine kinase allele frequencies for eight brook trout populations in Johnson County, Tennessee. Black shading represents the frequency of the CK-A2*100 allele.

Table 4.1. Brook trout stocking histories of study streams from the Tellico and Erwin state fish hatcheries based on weekly fish output reports.

Stream	Years Stocked	Number Stocked	Hatchery
Rough Ridge Creek	1952-1962, 1964, 1969, 1970-1973	10528	Tellico
Sugar Cove	1952-1957, 1964-1965, 1968	15,302	Tellico
Meadow Branch	1952-1957, 1967, 1978	9,760	Tellico
Roaring Branch	1955, 1957-1961, 1964	2002	Tellico
Brookshire Creek	1953, 1955	900	Tellico
Henderson Branch	1953, 1955, 1962, 1965, 1967, 1977	1850	Tellico
Davis Creek	1956	50	Tellico
Rock Creek	1952, 1955-1957, 1959-1964, 1980	4075	Erwin
Jones Branch	1951-1953	2150	Erwin
Higgins Creek	1951-1953, 1959, 1962-1965, 1986	5198	Erwin
Rocky Fork	1951-1956, 1960, 1962-1965, 1980, 1981, 1983, 1986-1988	17,374	Erwin
Toms Creek	1951	400	Erwin
Little Stony Creek	1955, 1957, 1959, 1960, 1984	975	Erwin
Birch Branch	1952, 1956, 1957	1225	Erwin
Jennings Creek (Round Knob Branch)	1951, 1964-1965	1250	Erwin
Gentry Creek	1951, 1953, 1955-1959, 1962	2000	Erwin
Dry Creek	1951, 1957, 1958, 1961, 1963-1965	1125	Erwin
Cove Creek	1956, 1960-1963	1300	Erwin
Blockstan Creek	1952, 1980	900	Erwin
Johnson Blevins Branch	1951	200	Erwin

Rock Creek was likely devoid of brook trout in the early 1980's (Bivens 1984). No information about the historic brook trout populations in Jones Branch and Higgins Creek were available.

Many hybrid populations apparently contained substantial native populations before they were stocked. Some of the hybrid populations were heavily stocked (eg. Rough Ridge Creek, Rocky Fork, and Henderson Branch). It is likely that only a relatively small proportion of hatchery fish are able to survive when they were stocked into healthy wild populations (Hulett and Leider 1989). Henderson Branch and Brookshire Creek had healthy native populations in the 1930's (Bivens 1984). Rocky Fork and Blockstand had healthy brook trout populations in the 1950's (Bivens 1984). No information about the historic populations was available for the Cook Branch, Hoot Owl Hollow, and Little Stony Creek populations. Round Knob Branch apparently had only a few non-reproducing Owhi-strain brook trout when it was stocked in 1983 with brook trout from Little Stony Creek (Bivens 1984).

Four populations identified as putative native (Gentry Creek, Davis Creek, Toms Creek, Birch Branch, and Cove Creek) have records of stocking, indicating that genetic origins of populations may not always be reliably inferred from stocking histories. Gentry Creek has a history of relatively heavy stocking. Davis Creek is another putative

native population of particular interest. This stream was thought to be devoid of native fish when it was stocked with Owhi-strain fish in 1978-80. (Bivens 1984). It was believed that the Owhi fish did not survive and reproduce. Presently this stream has a healthy brook trout population which may have been derived from a previously undetected headwater population (Strange and Habera 1993).

Genetic Differentiation Among Populations

Hatchery populations

The genetic differentiation among the hatchery derived populations was high. The three hatchery populations analyzed in the GRSM study (McCracken et al. 1993) had a relatively high genetic identity ($\bar{I} = 0.986 \pm 0.003$). In addition, hatchery populations exhibited lower genetic diversity compared to wild populations in Wisconsin (Krueger and Menzel 1979).

In the present study several hatchery derived populations are distinct. Rock Creek and Norton Creek, for example, are fixed for presumptive hatchery alleles at MEP-1* and sAAT-1,2* respectively. The Jones Branch population also has two alternate alleles at GPI-3* not present in any other hatchery derived population.

One possible explanation for the differentiation among hatchery populations is that the hatchery fish used in stocking may have had high genetic differentiation. Brook trout at the state hatcheries were likely genetically

uniform in any given year. However, hatcheries received eyed eggs from a variety of sources over the years and some streams were stocked repeatedly in several years.

Survivorship of hatchery trout is also highly variable, thus, it is likely that the hatchery fish which actually founded any given population were genetically distinct.

Genetic drift may also have contributed to the differentiation among hatchery-derived populations. Significant migration is not likely to occur between adjacent streams. Stream brook trout are relatively sedentary (Whitworth and Strange 1983) and adjacent streams are often physically isolated by barriers. In addition, a small number of individuals may be exchanged and still allow for significant genetic differentiation (Allendorf and Phelps 1981). For example, two hatchery-derived populations, Meadow Branch and its tributary Roaring Branch, are significantly differentiated probably because these streams have different stocking histories and they are isolated by a barrier.

Native populations

Most of the variation among the 22 putative native populations can be attributed to differences between three populations from the French Broad and Tellico watersheds (Bald River, Middle Prong of Gulf Creek and Brown Gap Creek) and among the 19 native populations from the Watauga, Nolichucky, and Holston River watersheds. Bald River,

Middle Prong of Gulf Creek, and Brown Gap Creek are fixed for GPI-2*70 while the other putative native populations, except for Gentry Creek, are fixed for GPI-2*100. Gentry Creek also lacks GPI-2*70 but has both GPI-2*100 and GPI-2*40. Most other native GRSM populations that have been examined are also fixed for GPI-2*70. (Guffey pers. comm.) Stoneking et al. (1981) determined that Bunches creek was fixed for GPI-2*70. It may be that populations south of the French Broad River are fixed for the GPI-2*70 allele. In addition, the five individuals from Muncy Creek in Pennsylvania were fixed for GPI-2*100. The presumptive native East Tennessee populations from Greene, Carter, and Johnson counties group together with the northern populations at this locus.

The sAAT-1,2* locus was not previously reported as diagnostic between hatchery and native southern Appalachian populations. The allele frequency patterns at these loci, however, suggest that they are diagnostic. Most native populations (except Brown Gap Creek, Middle Prong of Gulf Creek, and Bald River) had a very low frequency of sAAT-1,2*100 compared to hatchery populations. In fact, the allele frequency distribution suggests that one locus in the sAAT-1,2* isolocus pair may be fixed among the native populations. No more than two copies of the sAAT-1,2*100 allele were found in any of the putative native populations, with the exception of a single individual from Brown Gap

Creek which had three copies of sAAT-1,2*100. All eight hatchery-derived populations, however, had more than two copies of sAAT-1,2*100 in more than one individual.

The information at sAAT-1,2* indicates that there is a distinct genetic difference between native populations south and north of the French Broad River. Most of the native populations north of the French Broad are fixed for four copies of sAAT-1,2*118. The sAAT-1,2*100 allele appeared at a relatively high frequency ($f > 0.3$) in only the three native populations south of the French Broad. Two other native populations north of the French Broad River (Johnson Blevins Branch and Davis Creek) had sAAT-1,2 100* at a rare frequency ($f < 0.05$).

The MEP-3* locus was not used in the analysis because heterozygous individuals were not detected. However apparent allele frequencies at this locus provide additional evidence for the differentiation among native populations (Appendix G). All of the native populations except the two in the Gulf Fork of Big Creek watershed (Brown Gap Creek and Middle Prong of Gulf Creek) were fixed for the presumptive MEP-3*100 allele or polymorphic for the presumptive MEP-3*100 and MEP-3*105 alleles. The population from Middle Prong of Gulf Creek was fixed for a presumptive MEP-3*95 and the Brown Gap Creek population had a high frequency of this allele. Some of the hatchery populations were polymorphic for the presumptive MEP-3*95 and MEP-3*100 alleles.

Transplanted pairs

Transplantation histories are known for four pairs of populations (Table 3.9). The populations in all but one pair were genetically distinct from each other. The only pair of transplanted populations which was not significantly different was Little Stony Creek and Round Knob Branch. Little Stony Creek was used to stock Round Knob Branch in 1983 when Round Knob Branch was known to have had a low density of brook trout (Bivens 1984). Little Stony Creek was stocked subsequently in 1984 and the lack of differentiation between Brown Gap Creek and Little Stony Creek suggests that very few hatchery brook trout successfully reproduced after this stocking.

Consequences of Stocking Hatchery Brook Trout

As in other native salmonid species, ecological and genetic impacts of previous stockings have threatened native southern Appalachian brook trout (Martin et al. 1992, Waples, 1991, Evans and Willox, 1991). Although hatchery brook trout are not currently stocked in Tennessee the potentially negative consequences of past stocking must be recognized for future management, and considered in any remedial management programs.

Brook trout have been raised in hatcheries for well over 100 years. Several characteristics have been selected for such as faster growth, more egg production, earlier spawning, and increased disease resistance (Vincent 1962,

Moyle 1969). This same selective breeding has resulted in fish that are less able to survive under natural conditions. Hatchery brook trout placed in the wild are more susceptible to angling, exhibit lower stamina, and suffer higher natural mortality compared to wild fish (Flick and Webster 1962).

Repeated and heavy stocking increases the likelihood of hybridization (Hulett and Leider 1989), while causing strong behavioral interactions between wild and hatchery fish. Heavy stockings depress standing crops of wild trout (Vincent 1984, Hulett and Leider 1989, Petrosky and Bjornn 1984) and could alter the ecological environment within a stream so as to place unique selective pressures on native fish (Krueger and Menzel 1979). Thus, the introduction of hatchery trout into wild populations may reduce the fitness of wild populations, and potentially obliterate native gene pools (Ferguson 1990).

Potential directional selection and non-random mating

Positive F_{is} values indicate heterozygote deficiency and potential directional selection as well as possible non-random mating between native and hatchery fish. The average F_{is} values were highest among the hybrid populations with the greatest proportion of the CK-A2* hatchery allele. The highest average F_{is} value among hybrid populations was observed for the Rocky Fork population which has the greatest proportion of hatchery genes and is the most recently stocked.

Non-random mating and differential survival of native genotypes may be occurring in the hybrid populations. Wild trout generally have higher reproductive success and survival than hatchery trout (Reisenbichler and McIntyre 1977, Flick and Webster 1962) and selection may be acting against hatchery genes (Campton et al. 1991). These factors may explain the higher average F_{is} values among the hybrid populations in this study.

The three hybrid populations with the highest proportion of CK-A2*78 (Henderson Branch, Blockstand Creek, and Rocky Fork) were analyzed with the three restriction enzymes diagnostic of hatchery and native GRSM populations. These populations all had a higher frequency of the hatchery mtDNA polymorphism than the frequency of CK-A2*78. In contrast hybrid populations in the GRSM which have not been recently stocked all had a lower frequency of the hatchery mtDNA polymorphism than the frequency of the CK-A2*78 (Hayes pers. comm.).

Disparity between mtDNA haplotype and nuclear gene frequencies is not uncommon (Harrison 1989) and has been documented for subspecies of cutthroat trout (Forbes and Allendorf 1991). Differential introgression of nuclear versus mtDNA gene markers could be a result of differential mate choice. It is possible that hatchery female brook trout are more successful at reproducing than male hatchery brook trout. A few large females could produce a large

number of young. Because mtDNA is maternally inherited a large year class from hatchery females could carry a disproportionate amount of hatchery mtDNA into subsequent generations. This would explain the relatively higher proportion of hatchery mtDNA compared to nuclear genes in recently stocked populations.

The allele frequency changes in Blockstand and Rocky Fork coupled with their stocking histories may also support the hypothesis of directional selection or non-random mate choice. This data has limited value because allele frequency changes from year to year may be relatively large in salmonid populations (Waples 1991). The frequency of CK-A2*78 in Rocky Fork in 1991 was over twice that in 1979. In addition, the GPI-2*100 frequency was five times higher in 1991 than in 1979. These results suggest that hatchery genes have been introduced since 1979. Again, Rocky Fork is known to have been heavily stocked as recently as 1988.

The frequency of the hatchery allele at CK-A2* in Blockstand Creek was nearly twice as high in 1979 as it was in 1992 which could be a result of selection against hatchery alleles or non-random mating. However, the allelic distribution at GPI-2* did not change significantly suggesting the changes in CK-A2* alleles may have been due largely to genetic drift. A stocking in Blockstand just prior to sampling in 1979 would explain this data. Stoneking et al. (1981) speculated that a hatchery

introduction could have contaminated the gene pool of this stream. The only documented records of brook trout stockings in Blockstand Creek indicate they occurred in 1952 and 1980. An unrecorded stocking of Blockstand in 1979, just prior to Stoneking's sampling cannot be ruled out.

Management Implications

Tennessee's wild brook trout resource has been the focus of active management during the past 25 years. State fisheries managers are committed to expanding the current distribution and improving the habitat of brook trout. Many management decisions, however, await the results of the ongoing genetic surveys of brook trout populations in the state. There is considerable concern over the genetic integrity of brook trout populations in East Tennessee and preservation of brook trout native to the region is a priority.

Several issues need to be resolved before specific management decisions can be made. Many southeastern states have justifiably adopted a "wait and see" philosophy with regards to brook trout genetics management. Genetic effects, however, cannot be ignored in implementing a successful management program. Tennessee has a fairly aggressive management regime for brook trout and management decisions should take into account several issues related to brook trout genetics.

Subspecific differentiation

The results from this study are consistent with the study by McCracken et al. (1993) and Stoneking et al. (1981) which suggest that differences between northern and northern derived hatchery populations, and native southern populations are on the order of those seen between subspecies. Morgan and Baker (1991) also noted large genetic differences among native brook trout populations in Maryland. These authors suggest that there are at least four other distinct taxa of brook trout. There is evidence that native brook trout in Canada are highly polytypic, and a separate subspecies, the Aurora trout also may exist.

Subspecific differentiation of southern Appalachian brook trout could mean special management action, especially if the subspecies was determined to be at risk. Classifying a distinct brook trout taxa, however, requires that genetic data be coupled with morphological and life history information (Utter 1981) and this is unlikely to occur in the near future. It has been suggested that brook trout north of the New River Basin in Virginia may be distinct. Sampling native populations along the entire length of the Appalachians would help resolve this issue.

Stocking of native brook trout

Stocking waters devoid of fish and restocking streams from which rainbow trout have been removed are important to expanding the range of brook trout. Managers in Tennessee

are interested in stocking "native" strains of brook trout. TWRA is now attempting to rear native strains of brook trout and the US Fish and Wildlife Service also has expressed an interest in this endeavor. Several issues need to be considered before a stocking program for native southern Appalachian brook trout is initiated.

From a management perspective, a major goal might be to maintain a healthy hatchery population of native southeastern brook trout. Hulett and Leider (1989) describe two separate effects which need to be taken into account in starting and maintaining a native hatchery population. First is the "hatchery effect". Hatchery fish should be bred for traits necessary for survival in the wild. Hatchery conditions should simulate natural conditions (i.e. water chemistry, cover, natural water temperature fluctuations, etc.) as closely as possible. TWRA fish managers have noted that native brook trout reared in the Tellico brook trout hatchery grow slower, eat less, and have lower survivorship than the populations of hatchery derived wild trout from Meadow Branch (Bivens pers. comm.) Effectively simulating natural conditions within the hatchery may partially eliminate selection for undesirable traits.

The second effect which needs to be considered is the "transplant effect". Locally adapted populations of anadromous salmonids appear to survive and reproduce better

than transplanted populations (Hulett and Leider 1984, Reisenbichler 1984). Genetic adaptations may afford local populations selective advantages. This effect should certainly be taken into account in developing a native southern "stock". Wild brook trout are very polytypic. Trout native to a specific watershed are likely to be genetically adapted to specific environmental conditions within those streams. Populations of trout transplanted from distant watersheds may not exhibit the same survivorship as transplantations from populations within the same or from adjacent watersheds.

Transplant populations should come from within the same watershed if possible to assure that the source population gene pool was as similar to the historic population as possible. Native populations were found in all of the major watersheds in this survey, although only one native population (Bald River headwaters) was found in the Tellico watershed and one (Davis Creek) in the Nolichucky watershed. Other populations in these watersheds remain to be sampled.

A complete genetic survey should be performed on any potential source populations before it is used for transplantation or for establishing a hatchery population. This would allow for periodic genetic monitoring of stocked streams for random effects which may be detrimental over multiple generations.

Management of hatchery and hybrid populations

Several issues remain to be resolved with regards to management of hybrid and hatchery populations. For example there may be differences in productivity (standing crop, density, etc.) between hatchery, hybrid, and native populations. Quantitative data can be used to test for these differences (Habera pers. comm.).

The hypothesis that selection favors native brook genotypes needs to be investigated further. This is potentially an important management issue if hatchery-derived populations are to be replaced eventually with native fish. While it may not be practical to remove hatchery-derived populations and replace them with native populations, stocking of native trout into hatchery derived populations may be reasonable. Any management plan which includes stocking would need to consider the ecological and genetic consequences. Long-term genetic and ecological studies of hybrid, hatchery, and native populations may be necessary to resolve this issue.

Stream-by-stream management

Krueger and Menzel (1979) have suggested that brook trout streams might be managed on a stream-by-stream basis and possibly in smaller units within streams. Brook trout are relatively sedentary and it is possible that significant structuring occurs along the length of a stream. Higher elevation headwater streams may have greater selective

forces favoring the native genotype compared to lower elevation streams (Guffey pers. comm.). Genetic monitoring of brook trout populations for allele frequency changes and gene flow should be considered.

Conclusion

The results presented here are important to future management of native Tennessee brook trout. There appears to be a number of healthy native brook trout populations in Tennessee. Unfortunately several populations have been replaced by hatchery brook trout or have hybridized with them. However, fisheries managers in the state have an opportunity to protect many purely native populations of Tennessee brook trout. This prospect is appealing to trout anglers as well as fisheries biologists, and preservation of these unique gene pools should certainly be emphasized.

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APPENDIXES

Appendix A

Starch gel preparation and running protocol

Gel making protocol

1. Make buffers.
2. Into a 1 liter flask put 30 grams of electrostarch and 17 grams of Sigma Starch.
3. Add 400 ml of gel buffer to the starches. Many of the gel buffers are dilutions of tray buffers.
4. Mix by swirling the flask until all starch is suspended in the buffer.
5. While swirling, heat starch solution over a bunsen burner to boiling. Vigorous swirling is necessary to prevent starch from sticking to the flask and burning.
6. Continue to boil the mixture while swirling until it will boil when removed from the flame. It takes about 30 to 60 seconds of boiling to reach this point.
7. Immediately de-gas the gel by placing it under vacuum for 50 seconds. Release vacuum slowly to prevent bubbles.
8. Pour the entire starch gel slowly into a clean, dry gel tray.
9. Remove any flecks of dirt and bubbles with a pasteur pipet.
10. Allow gel to cool about 1 hour, then wrap it in plastic wrap. Avoid air bubbles at this step, since they tend to dry out the gel.
11. Allow the gel to polymerize overnight at room temperature.

Gel running protocol

Starting a run

1. Remove prepared samples from freezer and thaw on ice.
2. Soak 1 dit (3 by 9 mm Whatman 3MM chromatography paper) in each sample. No more than 20 samples should be on each gel.
3. Blot excess liquid from dits on 3 MM paper.
4. When all dits are blotted, fold back plastic wrap to expose 1/3 of the gel on the narrow axis. Slit the gel along the narrow axis using the gel guide. This slit should a) go completely to the bottom of the gel tray; b) be straight across the gel; c) be perpendicular to the bottom of the gel tray.
5. Leave a 1 cm gap at the left edge, then begin inserting dits left to right. Dits should a) go into the slit perfectly straight; b) touch the bottom of tray; c) be spaced so that they are 3 to 4 mm apart.
6. Gently pat the gel on both sides of the slit to ensure good gel contact with all dits and to remove air bubbles.
7. Examine the gel from the bottom to see that dit line is straight and evenly spaced, and to be sure air bubbles are removed.
8. Place plastic wrap over slit so that it overlaps by 1 cm.
9. Place gel on top of trays between the guides so that slit end is toward the anode. (Buffer wicks should be in place).
10. Align anodal wick exactly with slit. Pat into place to insure good contact with gel. Cover wick with plastic.
11. Expose 1/3 of cathodal end of gel by folding plastic back.
12. Align cathodal wick so that the edge is exactly 10 cm from the anodal wick all across gel. Cover with plastic.
13. Place glass plate on top of gel.
14. Place tray of loosely packed ice onto glass plate.
15. Turn voltage to appropriate setting.

Appendix A

During a run

1. Be sure that gel remains cold by changing ice frequently.
2. On some buffer systems the voltage tends to creep upward. Be sure to watch voltage and milliamperage limits.

Ending a run

1. Shut off power supply.
2. Remove ice, glass plate and plastic wrap from the gel set.
3. Trim the gel as follows. a) Trim about 1/2 cm off each long axis. b) Trim top of gel down to wick line. c) Trim bottom of gel 1/2 way to the slit from origin. d) Notch upper left and lower left corners of gel to mark sample orientation.
4. Place gel with outer surface up onto slicing tray and pat it down to get rid of air bubbles.
5. Slice bottom slice as follows. a) Be sure wire goes into gel evenly all the way across the gel. b) Watch gel while slicing so that no air bubbles cause uneven cutting. c) Pull slicer firmly and continuously through the gel.
6. Flip entire gel over.
7. Carefully pick up slice, supporting it along edge, then place it into staining box with inner most surface up.
8. Flip gel over onto slicing tray and do next slice exactly as the first.
9. Slice remaining gel half into 2 slices. Place each into staining boxes. Total of four slices all with inner most surfaces up.
10. Proceed to stain gels.

Appendix A

Enzyme staining recipes

Enzyme	Stain Buffer	Add just prior to staining
Supernatant aspartate aminotransferase	50 ml 0.2 molar Tris-HCl (pH 8.0) 50 mg L-aspartic acid 100 mg alpha-ketoglutaric acid Incubate gel in stain for overnight at 37 degrees farenheit.	0.5 mg of pyridoxal-5'-phosphate 100 mg of fast blue BB salt
Creatine kinase *Agar overlay	25 ml of 0.2 molar Tris-HCl (pH 8.0) 900 mg of Glucose 1.1 ml of 0.1 molar Magnesium Chloride 0.05 mg of Hexokinase 50 mg of Phosphocreatine 100 mg of Adenosine Di-phosphate	1.75 ml NADP 0.6 ml PMS 0.6 ml MTT
Agar Solution: 25 ml distilled H ₂ O 500 mg agar	Heat agar solution to boiling and monitor temperature. Add stain solution to agar when agar has cooled to 60 degrees farenheit and swirl gently. Pour agar stain over the sliced gel. Allow the agar to set before placing in the incubator for approximately 30 to 45 minutes before fixing.	
Dihydroamide dehydrogenase	50 ml of 0.025 molar Tris-HCl (pH 8.5)	5 mg of 2,6 dichlorophenol-idophenol 10 mg of NADH Incubate gel for 30 minutes at 37 degrees farenheit then add 0.75 ml of MTT around the edges of the gel. Incubate the gel for at least one more hour and allow it to set overnight before scoring.

Appendix A

Enzyme staining recipes

Enzyme	Stain Buffer	Add just prior to staining
Esterase pH 6.0	50 ml Sodium Phosphate (pH 6.0)	1.5 ml alpha naphthyl proprionate
		1.5 ml of beta naphthyl proprionate
		25 mg Fast Garnet Salt
Fumerate hydrase	50 ml of 0.2 molar Tris-HCl (pH 8.0)	2.0 ml of NAD
	50 ml of Fumaric Acid	0.5 ml of NBT
	2.5 ml of MDH stock	0.5 ml of PMS
	Incubate for at least one hour at 37 degrees farenheit.	
General protein	20 grams of Napthol blue black in 1 liter of fixer (5:5:1 of methanol, water and acetic acid)	Stain with 40 milliliters
	Allow to sit for at least one hour. Pour off the protein stain and re-use. Rinse thouroughly with water and destain repeatedly with fixer until gel can be scored.	
L-Lactate dehydrogenase	40 ml of 0.2 molar Tris-HCl (pH 8.0)	1.3 ml of NAD
	9 ml of 0.5 molor L-lithium lactate	0.3 ml of NBT
		0.5 ml of PMS
		0.5 ml of MTT
Incubate gel in stain for at least one hour at 37 degrees farenheit before fixing.		

Appendix A

Enzyme staining recipes

Enzyme	Stain Buffer	Add just prior to staining
Malate dehydrogenase	40 ml of 0.2 molar Tris-HCl (pH 8.0)	2.5 ml NBT
	6 ml of MDH stock	2.5 ml NAD
		0.6 ml PMS
	Incubate gel for at least one hour at 37 degrees farenheit before fixing.	
Malic Enzyme	40 ml of distilled water	1.0 ml of NADP
	5 ml of 0.5 molar Tris-HCl (pH 7.0)	2.0 ml of NBT
	0.1 molar Magnesium Chloride	0.1 ml of PMS
	5.0 ml of MDH stock	
	Incubate gel in stain for at least 2 hours at 37 degrees farenheit before fixing. Gel may be stained overnight at room temperature.	
Peptidase	Weighed out into a clean flask and placed in freezer:	50 ml 0.2 M Tris-HCl (pH 8.0)
	20 mg dipeptidase (L-Phenylalanyl-L leucine	0.5 ml of 0.25 M Manganese Chloride
	20 mg tripeptidase (L-Leucylglycl-glycine)	
	20 mg peroxidase	
	10 mg O-dianisidine di HCl	
	10 mg snake venom	

Appendix A

Enzyme staining recipes

Enzyme	Stain Buffer	Add just prior to staining
Glucose-6-phosphate isomerase	15 ml of distilled water	0.6 ml NADP
*Agar overlay	10 ml of fructose-6-phosphate	0.1 ml PMS
	0.3 ml of 0.1 molar Magnesium Chloride	1.2 ml MTT
	0.3 ml of G-6-PDH	
Agar Solution:	Add stain solution to agar when it has cooled to 60 degrees farenheit and swirl gently. Pour agar stain over gel and allow it to set before incubating. Gel may be fixed in as little as 20 minutes.	
25 ml Tris-HCl (pH 8.0)		
500 mg agar	Incubate stain for at least one hour at 37 degrees farenheit before fixing.	

Electrode and Gel Buffer Recipes

Buffer	Recipe (one liter)
RSL Electrode	2.52 grams LiOH 18.55 grams H ₃ BO ₃
RSL gel	3.63 grams of Tris 0.105 grams citric acid Gel: 1 ml electrode buffer in 99 ml gel buffer
CT Electrode	8.40 grams of citric acid To pH 6.1 with N-(3-aminopropyl)-morpholine.
CT gel	Gel: 1 ml electrode buffer in 9 ml distilled water.

Appendix B

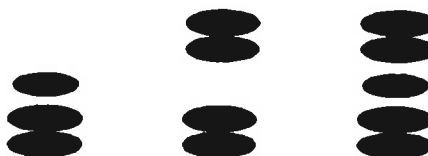
Aspartate Amino Transferase Phenotypes Brook Trout Muscle



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Genotype	*118	*118	*100	*118	*118
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Creatine Kinase Phenotypes Brook Trout Muscle



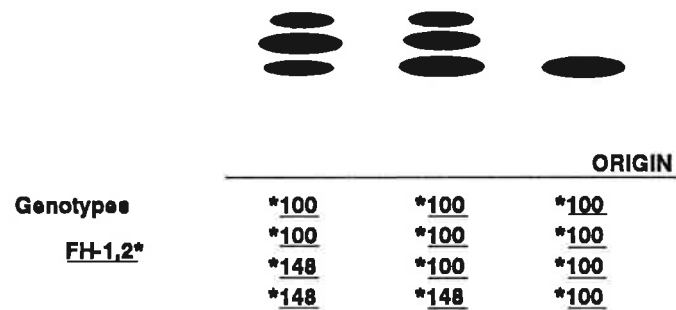
Locus

ORIGIN

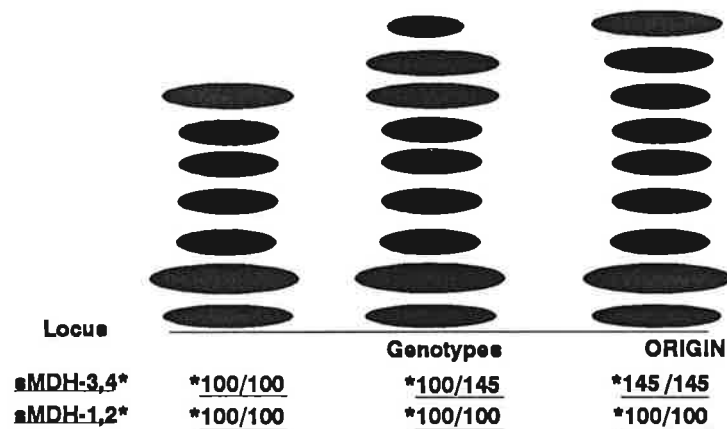
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CK-A1*	*100/100	*100/100	*100/100

Appendix B

Fumerate hydrase Phenotypes Brook Trout Muscle

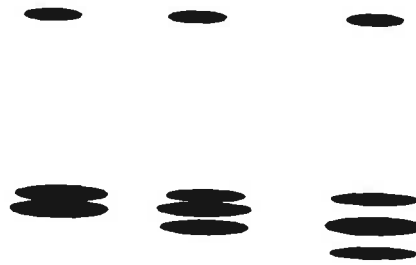


Malate Dehydrogenase Phenotypes Brook Trout Muscle



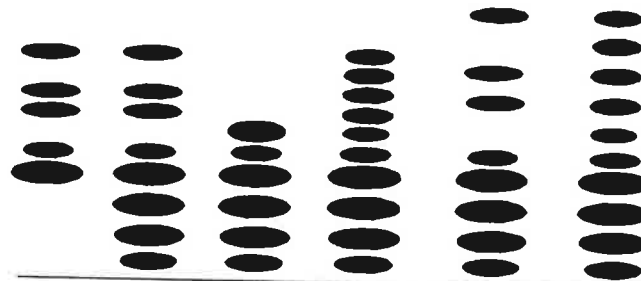
Appendix B

Malic Enzyme Phenotypes Brook Trout Muscle



Locus	Genotypes			ORIGIN
ME-1*	<u>*100/100</u>	<u>*100/88</u>	<u>*88/88</u>	
ME-2*	<u>*100/100</u>	<u>*100/100</u>	<u>*100/100</u>	
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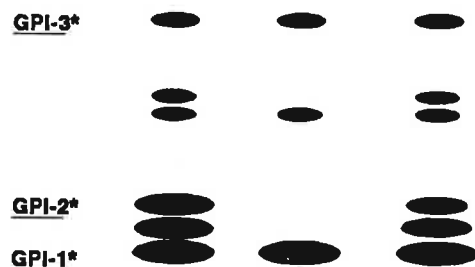
L-lactate Dehydrogenase Phenotypes Brook Trout Muscle



Locus	Genotypes						ORIGIN
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LDH-A1,2*	NULL	<u>*100/100</u>	<u>*100/100</u>	<u>*100/100</u>	<u>*100/100</u>	<u>*100/100</u>	

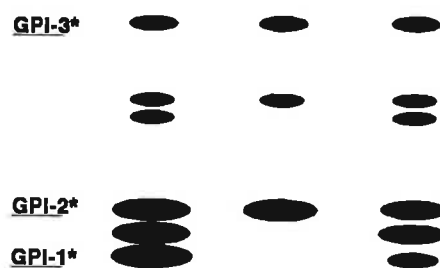
Appendix B

Glucose-6-Phosphate Isomerase Phenotypes Brook Trout Muscle



Locus	ORIGIN		
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GPI-2*	<u>*100/100</u>	<u>*70/70</u>	<u>*100/70</u>
GPI-3*	<u>*100/100</u>	<u>*100/100</u>	<u>*100/100</u>
Genotype			

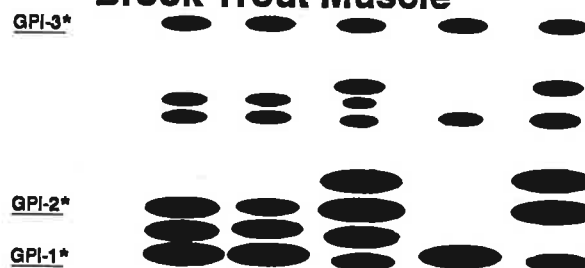
Glucose-6-Phosphate Isomerase Phenotypes Brook Trout Muscle



Locus	ORIGIN		
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GPI-2*	<u>*100/100</u>	<u>*100/100</u>	<u>*100/100</u>
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Genotype			

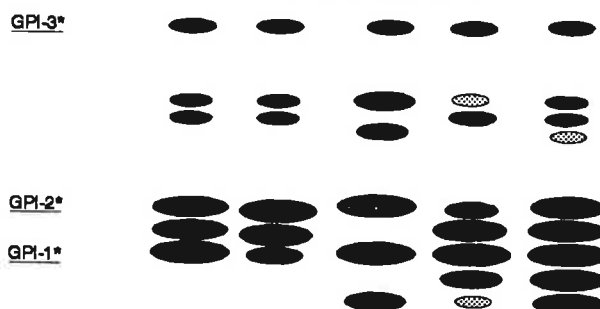
Appendix B

Glucose-6-Phosphate Isomerase Phenotypes Brook Trout Muscle



Locus	ORIGIN				
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GPI-2*	*100/100	*100/70	*115/100	*70/70	*115/70
GPI-3*	*100/100	*100/100	*100/100	*100/100	*100/100
Genotype					

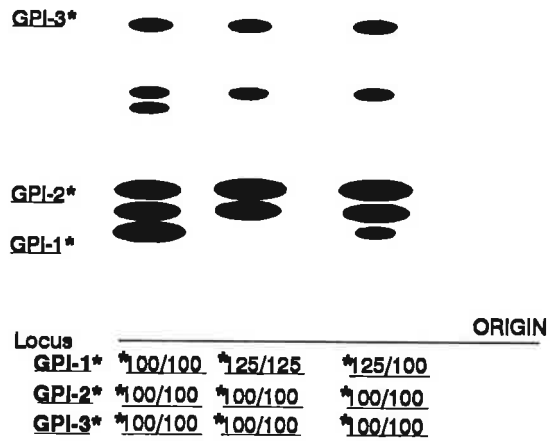
Glucose-6-Phosphate Isomerase Phenotypes Brook Trout Muscle



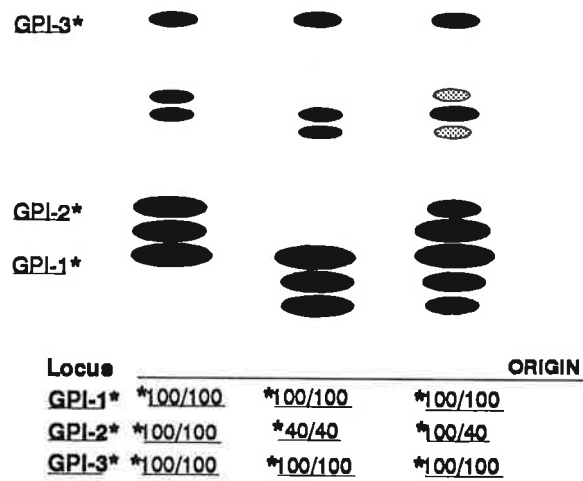
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GPI-2*	*100/100	*100/100	*40/40	*40/100	*40/100
GPI-3*	*100/100	*100/100	*100/100	*100/100	*100/100

Appendix B

Glucose-6-Phosphate Isomerase Phenotypes Brook Trout Muscle

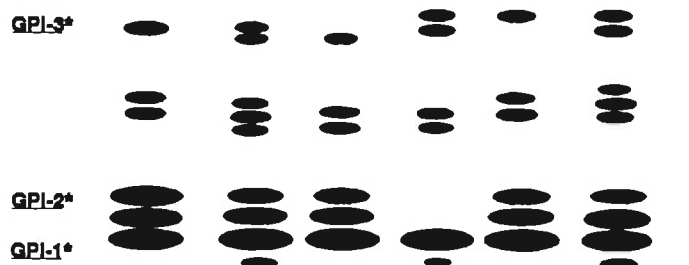


Glucose-6-Phosphate Isomerase Phenotypes Brook Trout Muscle



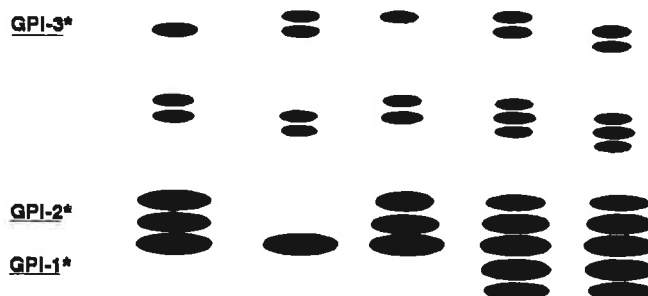
Appendix B

Glucose-6-Phosphate Isomerase Phenotypes Brook Trout Muscle



Locus	ORIGIN					
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<u>GPI-2*</u>	<u>*100/100</u>	<u>*100/70</u>	<u>*100/70</u>	<u>*70/70</u>	<u>*100/70</u>	<u>*100/70</u>
<u>GPI-3*</u>	<u>*100/100</u>	<u>*100/95</u>	<u>*95/95</u>	<u>*105/100</u>	<u>*105/105</u>	<u>*105/100</u>

Glucose-6-Phosphate Isomerase Phenotypes Brook Trout Muscle



Locus	ORIGIN				
<u>GPI-1*</u>	<u>*100/100</u>	<u>*100/100</u>	<u>*100/100</u>	<u>*100/100</u>	<u>*100/100</u>
<u>GPI-2*</u>	<u>*100/100</u>	<u>70/70</u>	<u>*100/70</u>	<u>*100/40</u>	<u>*100/40</u>
<u>GPI-3*</u>	<u>*100/100</u>	<u>*105/100</u>	<u>*105/105</u>	<u>*105/100</u>	<u>*100/95</u>

Appendix C

Raw Allozyme Data

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Appendix C

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Appendix D

Total DNA preparation from brook trout liver

1. Pulverize approximately 0.10 grams of liver tissue with a mortar and pestal in liquid nitrogen.
2. Homogenize the powdered liver with 1 ml of lifton buffer.
3. Incubate at room temperature for ten to fifteen minutes.
4. Add 150 micro liters of Potassium acetate, mix well and put on ice for at least 20 minutes.
5. Spin at 14000 RPM for 20 minutes.
6. Transfer the supernatant to a clean 2 milliter microcentrifuge tube and add 0.5 milliliters of chloroform and 0.5 milliliters of phenol. Mix well and allow to sit at room temperature for ten minutes.
7. Spin at 14000 RPM for 15 minutes.
8. Transfer the upper phase (chloroform) to a clean microcentrifuge tube and discard the lower phase (phenol).
9. Add .5 ml of chloroform, mix well and let sit for 5 minutes. Mix again and spin 5 minutes at 14000 RPM.
10. Transfer upper phase to a clean tube.
11. Add 0.1 volume of 3 molar Sodium Acetate.
12. Add 2 volumes of absolute Ethyl alchohol.
13. Let sit at -80 degrees celsius for at least twenty minutes.
14. Spin at 14000 RPM for 20 minutes.
15. Discard supernatant.
16. Add 1 milliliter of 75% Ethyl alchohol.
17. Spin at 14000 RPM for 20 minutes
18. Remove all supernatant (alchohol).
19. Air dry until completely dry.
20. Resuspend DNA pellet in 100 to 250 micro litters of TE.

Appendix D

East Tennessee brook trout populations from which total DNA was
extracted from liver tissue

Population	Number	Individuals
Rocky Fork	30	1 to 16, 18-31
Blockstand Creek	29	1 to 29
Brookshire Creek	25	1 to 25
Henderson Branch	17	1 to 17
Meadow Branch	5	1, 2, 3, 4, 5
Jones Branch	3	4, 16, 18
Brown Gap Creek	3	1, 2, 3
Jim Wright Branch	4	2, 4, 9, 15
Middle Prong of Gulf Creek	4	1, 2, 4, 10
North Fork of Stony Creek	3	6, 8, 18
Doe River	4	1, 2, 3, 4
Johnson Blevins Branch	4	1, 2, 3, 4
Round Knob Branch	4	1, 2, 3, 5
Davis Creek	4	1, 2, 3, 4
Tiger Creek	3	1, 2, 3
Gentry Creek	3	1, 2, 3
Rough Ridge Creek	1	1

Appendix E

Brook trout stockings out of the Buffalo Springs State Hatchery. Based on Weekly Fish Distribution Reports.

Stream	County	Years Stocked	Number Stocked
Buffalo Creek	Grainger	1979	342 fingerlings
*Unspecified Streams	Greene Cherokee National Forest	1979, 1980, 1982, 1985	11,477 fingerling
*Unspecified Streams	Monroe (Tellico WMA)	1985	4000 fingerling
*Private Streams	Polk	1979, 1984	900 fingerlings
Blue Mill Creek	Cocke	1986	300 fingerlings

Appendix E

Stocking History of Study streams out of the Tellico Fish Hatchery in Monroe County. Based on Weekly Fish Distribution Reports.

Stream	County	Years Stocked	Number Stocked
Rough Ridge Creek	Monroe	1952-1962, 1964, 1969, 1970-1973	10528
Meadow Branch	Monroe	1952-1957, 1967, 1978	9760
Sugar Cove	Monroe	1952-1962, 1964-1965, 1968	15302
Brookshire Creek	Monroe	1953, 1955	900
Sycamore	Monroe	1952-1956, 1967, 1978	4525
Bald River	Monroe	1952-1958, 1964-1965, 1967-1969, 1975-1977	15,225
North River	Monroe	1955-1958, 1964-1965, 1967-1969, 1988	52,250
Double Camp	Monroe	1952-1961, 1965, 1967, 1969, 1971	9,800
Citico	Monroe	1952, 1953, 1970, 1972, 1983	8,859
Five Poplar	Monroe	1963	300
Tellico	Monroe	1953, 1963, 1968, 1970, 1972, 1978-1981, 1983	29,296
Roaring Branch	Monroe	1955, 1957-1961, 1964	2002
Laurel Branch	Monroe	1965	550
Mill Branch	Monroe	1952-1956, 1959-1961	1400
Eagle Cove Branch	Monroe	1955	600
Crowder Branch	Monroe	1959-1962	1400
Flint Creek	Monroe	1953, 1955	275
Big Oak Cove	Monroe	1955	350
Grassy Branch	Monroe	1955	400

Appendix E

Stocking History of Study streams out of the Tellico Fish Hatchery in Monroe County. Based on Weekly Fish Distribution Reports.

Stream	County	Years Stocked	Number Stocked
Falls Branch	Monroe	1955, 1958	900
Ike Camp	Monroe	1955	300
McNabb Branch	Monroe	1955, 1964, 1965	850
Big Cove Creek	Monroe	1955	500
Hemlock Creek	Monroe	1964, 1965	100
Little Tennessee	Monroe	1972, 1973	774
Johns Creek	Monroe	1973	375
Jakes Best	Monroe	1958	148
Panther Creek	Monroe	1955	300
Green Cove Pond	Monroe	1964, 1965, 1967, 1968	8160
Ball Play Creek	Monroe	1956	600
Turkey Creek	Monroe	1956	100
Cave Creek	Monroe	1956	600
Davis Branch	Monroe	1956	50
Henderson Branch	Monroe	1953, 1955, 1962, 1965, 1967, 1977	1850

Appendix E

Stocking History of Study streams out of the Erwin State Fish Hatchery in Unicoi County.
Based on Weekly Fish Distribution Reports.

Stream	County	Years Stocked	Number Stocked
Rock Creek	Unicoi	1952, 1955-1957, 1959-1964, 1980*	4075
Jones Branch	Unicoi	1951-1953	2150
Higgins Creek	Unicoi	1951-1953, 1959, 1962-1965, 1986	5198
Rocky Fork	Unicoi	1951-1956, 1960, 1962-1965, 1980*, 1981*, 1983, 1986	17374
Coffee Ridge Creek	Unicoi	1951	775
Birch log creek	Unicoi	1959	40
Watauga	Carter	1950-1952	12,676
Beaverdam	Johnson	1951-1955, 1959-1960, 1963-1964, 1983	10,698
Toms Creek	Johnson	1951	400
Little Creek	Cocke	1957	150
Ramsey Brach	Washington	1951	100
Laural Fork Creek	Carter	1951-1953, 1960-1962, 1964, 1983	6750
Little Stoney	Carter	1955, 1957, 1959, 1960, 1984	975
Little Tennessee	Monroe/ Blount	1951, 1952	39370
Five Poplar	Unicoi	1952	500
Clark Creek	Unicoi	1952-1953, 1955-1956, 1962-1965, 1986	5201
Clinch River	Anderson	1952	2477

Appendix E

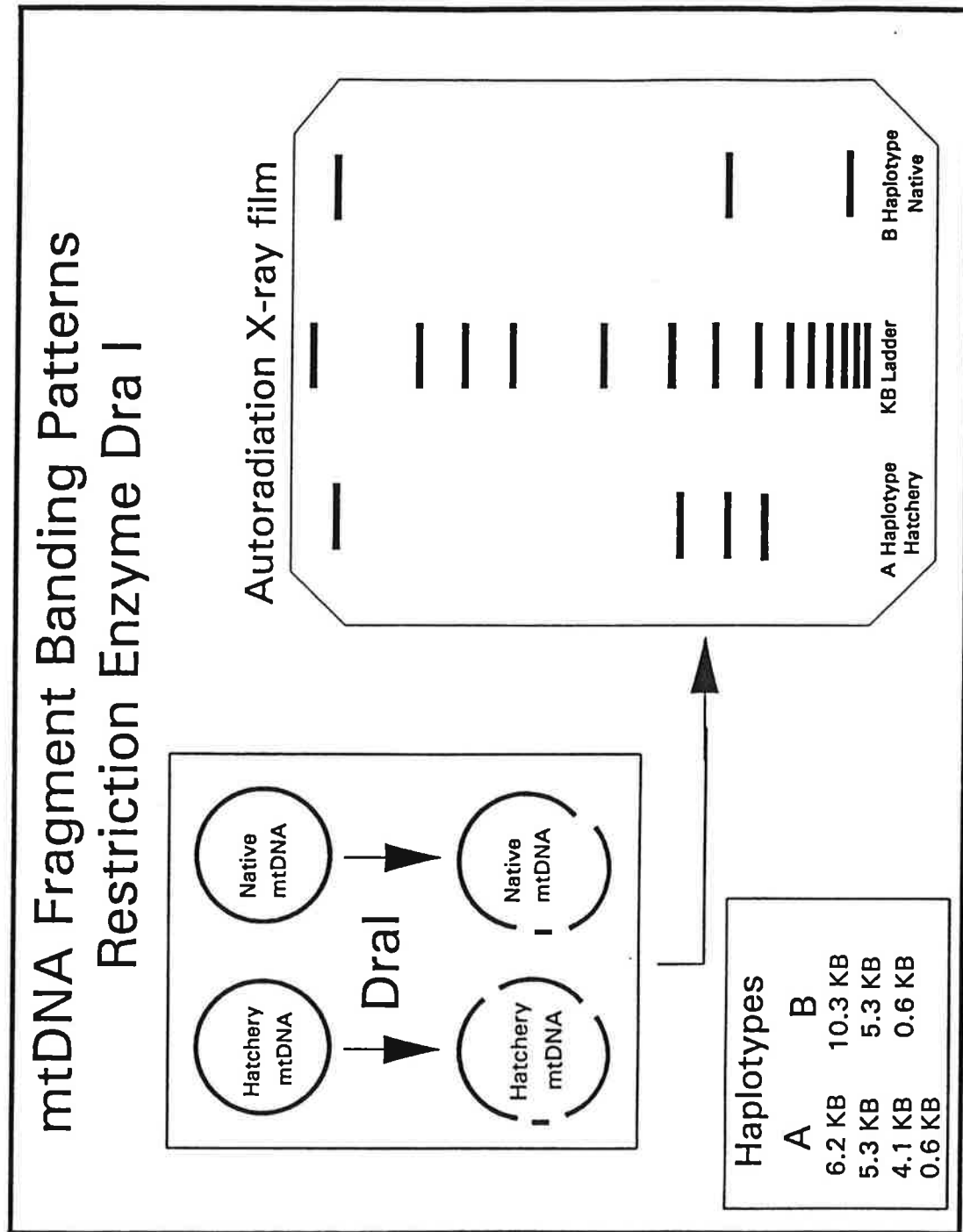
Stocking History of Study streams out of the Erwin State Fish Hatchery in Unicoi County.
Based on Weekly Fish Distribution Reports.

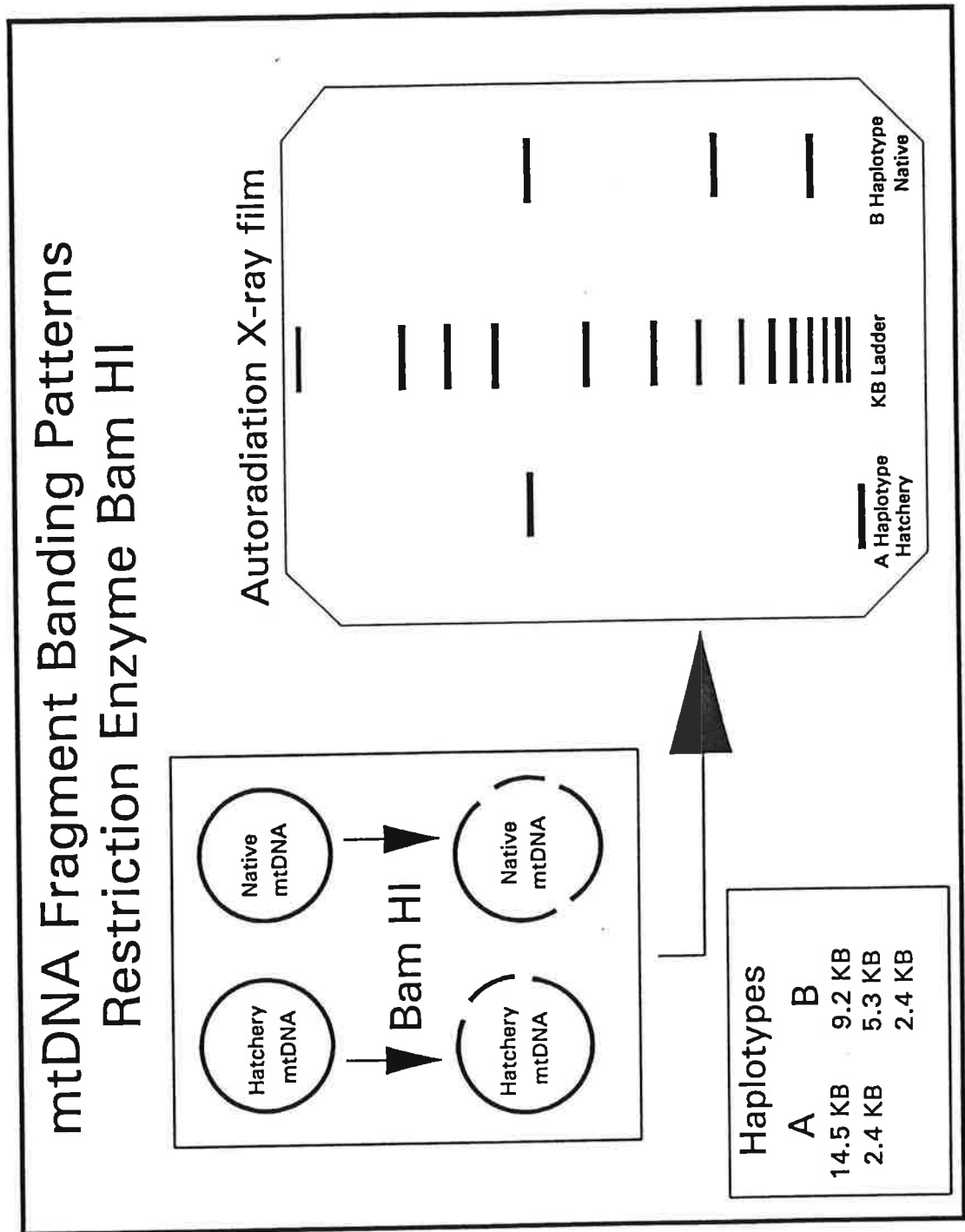
Stream	County	Years Stocked	Number Stocked
Cassi Creek	Unicoi	1952	600
Birch Branch	Johnson	1952, 1956, 1957	1225
Painter Creek	Washington	1952	100
South Fork of Holston	Sullivan	1952	10,642
Flint Creek	Unicoi	1952	650
Roaring fork	Sevier	1952, 1956-1957	425
Broad Branch	Unicoi	1952	500
Big Stoney Creek	Carter	1952, 1961	925
Blockstan Creek	Unicoi	1952, 1980*	2500
Ft Davis Creek	Unicoi	1952, 1980*	1500
Sill Creek	Unicoi	1952, 1980*	1000
California Creek	Unicoi	1952, 1980*	1500
Lower Holston	Jefferson	1952	4902
Shell Creek	Carter	1951, 1952	2100
Big Cove Creek	Carter	1951, 1952	900
Burch Creek	Carter	1952	500
Little Cove Creek	Carter	1952	500
Jennings Creek	Greene	1951, 1964-1965	1250
Jacobs Creek	Johnson	1951, 1954-1959, 1962	3405

Appendix E

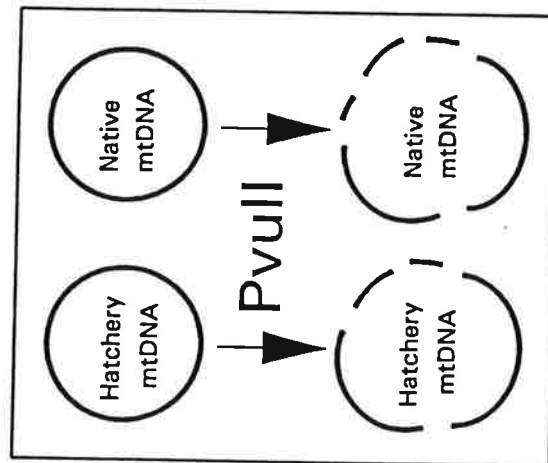
Stocking History of Study streams out of the Erwin State Fish Hatchery in Unicoi County.
Based on Weekly Fish Distribution Reports.

Stream	County	Years Stocked	Number Stocked
Blevins Branch	Johnson	1951	200
Flat Creek	Sevier	1951	150
Mill Creek	Sevier	1951	150
Horse Creek	Greene	1951, 1957, 1959, 1962, 1964, 1986	1087
Spivey Creek	Unicoi	1951, 1955, 1957-1959, 1961, 1962, 1986	1750
Little Pigeon	Sevier	1951	250
Laurel Bloomer Creek	Johnson	1951, 1953, 1957	650
Gentry Creek	Johnson	1951, 1953, 1955-1959, 1962	2000
Dry Creek	Greene	1951, 1957, 1958, 1961, 1963-1965	1125
Fagall Branch	Johnson	1956, 1957	225
Chalk Branch	Johnson	1956, 1957	225
Cove Creek	Carter	1956, 1960-1963	1300
Paint Creek	Unicoi	1957-1959, 1960-1962, 1983, 1986	4025
South Indian Creek	Unicoi	1961, 1984	6402
North Indian Creek	Unicoi	1961, 1984, 1986	7002
Doe River	Carter	1961, 1986	100
Camp Creek	Greene	1961	475
Wilbur Lake	Carter	1962	18728
Granny Lewis Creek	Greene	1963, 1980*, 1984, 1986	3250

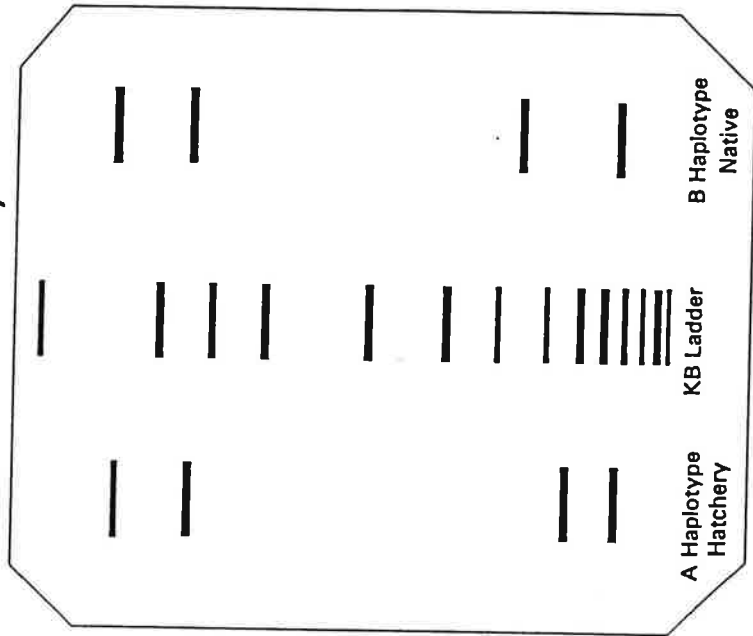




mtDNA Fragment Banding Patterns Restriction Enzyme Pvu II



Autoradiation X-ray film



Haplotypes

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6.7 KB	5.5 KB
1.3 KB	1.3 KB
0.8 KB	2(0.8 KB)

Appendix G

**Presumptive allele frequencies* at MEP-3* for 38 East Tennessee
brook trout populations**

<u>Population</u>	<u>Allele</u>			<u>Population</u>	<u>Allele</u>		
	<u>*95</u>	<u>*100</u>	<u>*105</u>		<u>*95</u>	<u>*100</u>	<u>*105</u>
NFS	--	0.94	0.06	BRO	--	1.0	--
RCK	--	1.0	--	COR	--	1.0	--
JBR	--	0.86	0.14	SCO	--	1.0	--
MPG	1.0	--	--	ROR	0.44	0.56	--
MBR	--	1.0	--	DAV	--	0.53	0.47
BGC	0.81	0.19	--	BLD	--	0.88	0.12
GEO	--	0.44	0.56	RKB	--	1.0	--
MID	--	1.0	--	CLK	--	1.0	--
COV	--	1.0	--	GEN	--	1.0	--
BIR	--	0.94	0.06	LST	--	0.20	0.80
HEN	--	1.0	--	HOH	--	0.90	0.10
JBB	--	0.32	0.68	DRY	--	1.0	--
BIL	--	0.69	0.31	FUR	--	1.0	--
RFK	--	1.0	--	EBD	--	1.0	--
DOE	--	1.0	--	LLB	--	1.0	--
TGR	--	0.82	0.18	HIG	--	1.0	--
TOM	--	1.0	--	NOR	--	0.83	0.17
JWB	--	1.0	--	LEO	--	1.0	--
BLK	--	0.90	0.10	CKB	--	1.0	--

*Only homozygous individuals could be scored for this loci.

VITA

Frank Joseph Kriégler III was born in Santa Monica, California and was raised in Ann Arbor, Michigan. He graduated from Ann Arbor Huron High School in June 1984. From 1984 to 1989 he attended the University of Michigan and graduated with a B.S. in biology and a secondary education teaching certificate. He worked for two years as an environmental and biology teacher before enrolling in graduate school at the University of Tennessee, Knoxville. He received his M.S. in Wildlife and Fisheries Science in May 1993.