12-1998

A Population Genetics Study of Southern Appalachian Brook Trout

Stanley Zane Guffey
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Stanley Zane Guffey entitled "A Population Genetics Study of Southern Appalachian Brook Trout." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Gary F. McCracken, Major Professor

We have read this dissertation and recommend its acceptance:

Christine R. Boake, David Etnier, Michael McKinney, Charles R. Parker

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a dissertation written by Stanley Zane Guffey entitled "A Population Genetics Study of Southern Appalachian Brook Trout." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Dr. Gary F. McCracken, Major Professor

We have read this dissertation
And recommend its acceptance:

Dr. Christine R. Boake

Dr. David Etnier

Dr. Michael McKinney

Dr. Charles R. Parker

Accepted for the Council:

Associate Vice Chancellor and
Dean of the Graduate School

A Population Genetics Study of Southern Appalachian Brook Trout

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Stanley Zane Guffey
December, 1998

DEDICATION

I dedicate this small effort to the trout, and to all friends and admirers of wild creatures.

And to my parents, Gladys I. Guffey and Roy F. Guffey, Jr.: common folk of the noblest sort who taught me by example that quality of life is found in love, respect, and humility.

ACKNOWLEDGEMENTS

I give my humblest thanks to the spirit of brook trout dead whose essence is so poorly represented on these pages.

Debt and gratitude to my conspecifics must begin with my friend and major professor Dr.Gary McCracken. Gary is an exceptional teacher, scientist, humanitarian, and friend. It was he who got me into this pleasant mess, who guided, encouraged, tolerated, and ignored me as needed, but never pushed or cajoled, much, even when it might have seemed the last best course of action. Our friendship goes back two decades, and I can only hope that we have four or five more decades of it. Thanks Gary!

It is also customary, if not down right obligatory, to thank the other members of ones graduate committee, and I wish to do the same. But I'm pleased to say that my gratitude to them and respect for them as scientists, teachers, and human beings goes well beyond the perfunctory. I'm very pleased to call each of them my friend as well.

Dr. Charles R. Parker, National Park Service biologist, first brought the question of Southern Appalachian brook trout to Dr. McCracken's and my attention. Chuck supported, assisted, and collaborated in our initial investigations of the molecular genetics of brook trout in Great Smoky Mountains National Park and in our subsequent expanded studies of brook trout in the Park and throughout the Southern Appalachian region. But this is but a small part of Chuck's contribution. Throughout the time, as Congress kept changing the name on his stationary, and as the reorganizations inevitably brought greater and greater administrative irritations his way, Chuck has continued to work on behalf of our understanding of and the conservation of Southern Appalachian biodiversity. Thanks Chuck!

Dr. Christine Boake arrived in the old Zoology Department shortly before I did, she a well respected and enterprising young biologist, me a half-fair carpenter, woods-wanderer, and brand new non-traditional graduate student. She showed me pretty quickly, by example and expectation, that a key element of research and study is extremely hard work and dedication. She reminded me time and again of the old adage, in somewhat different words, and by example, "if something is worth doing, it is worth doing well." Somewhat boldly, and optimistically I think, she selected me to be one of her teaching assistants in genetics, and it is to this fortunate circumstance that I trace the beginnings of my pleasure in teaching. Thanks Chris!

Dr. David Etnier is a legend in his own time, but he seems unaware of it: he is too busy looking at creatures, teaching others how to look, and thoroughly enjoying himself. Ets is naturalist, teacher, and scientist all in one. As anyone of these he would be exceptional, in combination he is extraordinary. If I could achieve in one of these what Ets has achieved in all three, I might be satisfied. My one regret is that I did not set aside the time to absorb more of his knowledge of creatures. I think there is still time. Thanks Ets!

Dr. Michael McKinney came to my committee late in my game. The Graduate School made me do it, a role he is often called upon to fill. But he would have been, should have been, a natural choice from the get go. Mike was the organizer of the first graduate student seminar that I participated in during my overly long graduate tenure. The topic was biological extinction, an area that has come to occupy a great deal of my research and thought. It is to Mike as a conservation biologists that I owe my greatest gratitude. He is the scientist who showed me most clearly that it is not only possible to be

a good scientist and an impassioned activist for nature preservation, but that for some folks like us, it is the only way. Thanks Michael!

Mr. Steve Moore was not on my committee, but is in the work. In addition to being the direct or indirect source of most of my samples, I benefited from and enjoyed numerous long conversations with him about brook trout biology and conservation. As the Great Smoky Mountains National Park fisheries biologist, Steve was working for the preservation of Southern Appalachian brook trout long before we began our genetics work. Steve is the kind of person I want on the public payroll: he takes seriously the mandate to preserve and conserve natural resources and he works harder than most people can imagine to accomplish this. Most of the Park populations examined in our studies were sampled by Steve and his fisheries crews. Steve also provided the contacts for obtaining samples from populations in Maryland, Virginia, North Carolina, and South Carolina. Walking in the woods for miles with Steve, then taking to a stream for a bout of fish shocking were some of the most enjoyable aspects of this work. And Steve is an example, though none is needed, that good 'ol country boys like us can do some good and have fun too. Thanks Steve!

In addition to my extended committee, I had the good fortune of collaborating directly with other fine biologists at the University of Tennessee. Dr. John Hayes (now with Oregon State University) worked on the brook trout project as a post-doctoral associate in Dr. Gary McCracken's laboratory. John introduced "modern" molecular techniques to Gary's laboratory and was a source of considerable insight for my understanding of the application of the techniques to questions in conservation biology.

Dr. Richard Strange of the Department of Forestry, Wildlife, and Fisheries, and Mr. Jim Habera (now with the Tennessee Wildlife Resources Agency), actively participated in our genetics work. They helped secure funding, provided samples, and, most importantly, shared with me their enormous knowledge of Southern Appalachian brook trout.

Many folks, mostly colleagues of Steve Moore and members of his fisheries crews, helped with obtaining fish samples. During the course of my work Steve was ably assisted by Mr. Bart Carter, now with the Tennessee Wildlife Resources Agency, and by Mr. Matt Kulp. In addition to these two, there were at least a hundred seasonal Park employees and Student Conservation Association volunteers who made up the fisheries crews over the years. I also had assistance with sampling from Mr. Lonnie Shull of Appalachian State University and Mr. Rikki Hall of the University of Tennessee. Working with them was an enormous pleasure for me.

Other fisheries biologist from state agencies in the Southern Appalachian region also provided samples, assistance, and insights during the course of my work: Mr. Jim Borawa and Mr. Joe Mickey of the North Carolina Division of Boating and Inland Fisheries; Mr. Dan Rankin of the South Carolina Department of Natural Resources; Mr. Larry Mohn, Mr. Joe Williams, and Mr. Scott Smith of the Virginia Department of Game and Inland Fisheries; and Dr. Ray Morgan of the University of Maryland – Frostburg.

In the laboratory I had the able assistance of Dr. John Hayes, Mr. Frank Kriegler, Ms. Leslie Saidak, Ms. Sylvia Houts, and Mr. Todd Yoakley, not to mention the guidance of Dr. McCracken. Steve Moore also dispatched some of his employees and volunteers to help me from time to time. Steve's folks performed ably and well in the laboratory

environment, even cheerfully, but I always understood their desire to get back to their work in the mountains.

My research was funded by many agencies and organizations. Our initial work was funded by the National Park Service through a contract with Dr. McCracken (1989 to 1991). The National Park Service and the North Carolina Wildlife Resources Commission provided funding for our expanded work from 1992 through 1994. I was also the recipient of Great Smoky Mountains Conservation Association, Carlos Campbell Memorial Research Fellowships in 1992 and 1993. The South Carolina Department of Natural Resources, Trout Unlimited of South Carolina, and the National Forest Service provided funding for my studies of South Carolina brook trout during 1994 and 1995. The Virginia Department of Game and Inland Fisheries, the Trout Committee of the Southeastern Division of the American Fisheries Society, and the Tennessee Wildlife Resources Agency provided funding for my studies of Virginia brook trout during 1996 and 1997. The Tennessee Wildlife Resources Agency, through the Wild Trout Project administered by Dr. Richard Strange and Mr. Jim Habera, also supported two Masters degree students investigating brook trout population genetics in Dr. McCracken's laboratory. Throughout my work I was supported by the University of Tennessee Departments of Zoology, and Ecology and Evolutionary Biology.

The advantage of staying around a place for a very long time, even of over staying one's welcome, is all the fine people you meet and get to know, all the fine friendships you make, all the gratitude that should be acknowledged. The list is long, I can only provide a sampling:

My good friends Michael Burke, Janelle Stueck, Phil Hamlin, Marianne Woodside, Rikki Hall, and Danielle Mahanes who put up with my eccentricities and have always made sure that I had plenty of plumbing, carpentry, grubbing, tilling, roofing, . . . to do. My brother Michael knows I enjoyed it all, but enjoyed the firewood cutting and beer drinking the most.

Graduate student colleagues and friends too numerous to mention, but I will single out a few: Ms. Corey Samuels, Dr. Andy Redfearn, Dr. Phil Nott, Mr. Glenn Gerber, Mr. Jim Minesky, Ms. Rebecca Rivers, Mr. Barron Moody, Ms. Lisa Comeaux, Mr. Chris Paxton, Mr. Chris Skelton, and Mr. Jeff Duncan.

My ex-wife, Joan Elizabeth Meredith, who found that being married to a graduate student was more than she had bargained for. I certainly can't hold that against her.

My other teachers and mentors and friends over the years: The late Dr. A. J. Sharp and the late Dr. James T. Tanner. Dr. Stan Lusby, Dr. Ed Clebsch, Dr. Charles Faulkner, Dr. Maxwell Springer, Dr. Frank McCormick, Dr. Arthur Echternacht, Dr. Ken Monty, Dr. Karen Hughes, Dr. Susan Riechert, Dr. Stuart Pimm, Dr. David Smith, Dr. Eugene Wofford, Dr. Ken McFarland, Dr. M. L. Pan, Dr. Mary Ann Handel, Dr. Steve Handel, Dr. Tom Chen, Dr. Jeffrey Becker, Dr. David Fox, and Dr. Stuart Riggsby. More recent faculty friends and mentors: Dr. Frank Harris, Dr. Patricia Cox, Dr. Mitch Cruzan, and Dr. Massimo Pigliucci.

The support staff in the Division of Biology: In the front office, Ms. Rhonda Mattingly; Mr. Rod Bunn and the Biology Service Facility staff, with special thanks to Mr. David Pratt; Ms. Sandy Coward, Ms. Jerry Duncan, Ms. Marie Gillespie, and the rest of the Biology Business Office staff; the Department of Ecology and Evolutionary

Biology staff: Ms. Phyllis Bice, Ms. Cheryl Lynn, Ms. Anne Mintz, and Ms. Linda McMillan.

Near the end, I wish to thank Ms. Ann Lacava, Thesis/Dissertation Consultant for the University of Tennessee Graduate School. I wish that I had made her acquaintance much earlier; not because I had all that much trouble in preparing my dissertation, but because she is such a delight to visit with. She performs her job with extraordinary skill and attention; more than that, she is a delightfully caring and joyous person. I nominate her for President of the university.

Last but not least, I wish to thank my good friend, companion, colleague and all around buddy, Ms. Carolyn Leigh Wells. I look forward to many more wonderful times in the woods and swamps with Cleigh, exploring the diversity of creatures with whom we share the planet.

ABSTRACT

Observations of anglers and naturalists, and the findings of molecular genetics, indicate that native Southern Appalachian brook trout populations comprise a distinct lineage of *Salvelinus fontinalis*. In this century, the stream mileage inhabited by the region's only native salmonid has been reduced by 70-80%. Attempts to restore declining populations by stocking have eroded the genetic integrity of many remaining native populations through hybridization with hatchery strains. In this study I review the decline and current status of brook trout in the Southern Appalachians (Part I), document the genetic structure of populations in Great Smoky Mountains National Park (Part II), delineate the geographical range and genetic structure of the Southern Appalachian lineage (Part III), and initiate the investigation of morphometric variation among northern and Southern Appalachian lineage populations and their hybrids (Part IV).

Brook trout populations did not recover with Southern Appalachian forests after the depredations of the early decades of this century. Currently, much of their former range is occupied by introduced salmonids, primarily rainbow trout. In Great Smoky Mountains National Park, as elsewhere, attempts to restore declining populations relied extensively on stocking with hatchery strains derived from northeastern populations. Stocking records indicate that only 12 streams in the Park were not stocked with hatchery fish. Eleven of these unstocked streams and an additional 40 streams that had been stocked, were sampled. These samples, representing the majority of known Park populations, plus two hatchery strains and one naturalized hatchery derived population from the Park, were analyzed for variation in 15 proteins encoded by 24 loci. The unstocked samples and the hatchery samples were fixed for different alleles at the CK-A2* locus, and exhibited significant differences in allele frequency at an additional 9 of 10 polymorphic loci. Samples from 28 of the 40 stocked streams were fixed for the diagnostic Southern Appalachian CK-A2* allele, indicating surprisingly low levels of hatchery gene introgression.

Samples from 48 brook trout populations in Maryland, Virginia, North Carolina, and South Carolina were analyzed for variation at the same loci as Park samples to assess

regional genetic structure. Variation at the CK-A2* locus and other variable loci demonstrates the existence of two discrete lineages of evolutionary and probably taxonomic significance. The Southern Appalachian lineage is found in Ohio River and Atlantic drainage streams from the New River southward, and the northern lineage is found in drainages north of the New River. Genetic heterogeneity among Southern Appalachian lineage populations is greater than that among northern lineage populations in my sample and indicates that the Southern Appalachians are a center of brook trout genetic diversity.

Morphometric variation among northern and Southern Appalachian brook trout samples was not as clear as variation observed at allozyme loci. Significant differences between northern, southern, and hybrid samples were most evident in the means of head region morphometrics adjusted for standard length. Means significantly different between northern and southern samples were intermediate in the hybrid samples. Southern Appalachian and hybrid samples also showed significantly lower variance than northern populations in some head region morphometrics. Discriminant analyses of seven truss grid and two standard morphometrics from a subset of the samples analyzed for allozyme variation were unable to distinguish between individuals from northern, Southern Appalachian, and hybrid populations.

TABLE of CONTENTS

CONTENTS (continued)

LIST of TABLES

TABLES (continued)

TABLES (continued)

LIST of FIGURES

LIST of APPENDICES

PART I:
BROOK TROUT in the SOUTHERN APPALACHIANS

Introduction

For many people, trout and clear, free-flowing trout streams are synonymous with mountain wildness. From this perspective preservation of wild trout requires preservation of wildness, and loss of wild trout is indicative of environmental deterioration (Leopold, 1949). Jordan and Evermann (1896) expressed this perception: "The members of this genus [*Salvelinus*] are by far the most active and handsome of the trout, and live in the coldest, cleanest, most secluded waters. No higher praise can be given to a Salmonid than to say it is a charr." The history of brook trout distribution in the Southern Appalachians over the past century supports the reality of this relationship between trout well-being and environmental quality. Since 1900, stream mileage occupied by brook trout in the Southern Appalachians has decreased by about 79%, as a consequence of human activities (Bivens, 1985). While most of this decline occurred prior to 1970 and brook trout populations have fared much better in recent years (Habera and Strange, 1993; Strange and Habera, 1998), land use and fisheries management practices still impact native brook trout. Their current restricted habitat makes the remaining populations highly vulnerable to extinction (Strange, 1979; Platts and Nelson, 1988; Nagel, 1991).

Concerns for the preservation of Southern Appalachian brook trout are heightened by findings from molecular population genetics. Southern Appalachian brook trout are genetically distinct from populations throughout the taxon's main range, and they may warrant recognition as a distinct evolutionary unit (Stoneking et al., 1981; McCracken et al., 1993; Hayes et al., 1996). The molecular studies also show high levels of differentiation among native Southern Appalachian populations (Kriegler et al., 1995;

Saidak, 1995; Hayes et al., 1996), indicating that extinction of populations will also result in the irretrievable loss of genetic diversity.

The objectives of my study are to: 1) evaluate genetic differentiation between native Southern Appalachian brook trout populations and the northern derived hatchery populations used for stocking in the region; 2) determine the extent of hatchery introgression into brook trout populations in Great Smoky Mountains National Park; 3) elucidate the geographical range of the distinct Southern Appalachian brook trout and examine the genetic structure of native populations throughout the Southern Appalachians; 4) initiate analysis of morphological variation among brook trout populations; and 5) provide management and monitoring guidelines for the preservation of variation in native brook trout. I also hope that the results will provide insights and suggest additional research avenues into the diversity and uniqueness of the rich Southern Appalachian biota, and contribute to making the preservation of this biota a continuing priority.

Systematics of *Salvelinus fontinalis*

The brook trout or brook charr *Salvelinus fontinalis* is the only salmonid native to the Southern Appalachians. *Salvelinus fontinalis* is a member of the holarctic teleost family Salmonidae (Order Salmoniformes). All salmonids are freshwater or anadromous, and many species, including brook trout, have some populations that are landlocked and others that are anadromous (Scott and Crossman, 1973; Hendricks, 1980). Their plastic morphological variation, influenced by the physiological requirements of freshwater or

anadromous living, has confounded systematic interpretations in the past (Behnke, 1980; Power, 1980). Salmonid species are polytypic in many aspects of morphology, behavior, and life history, as well as existing in numerous reproductively isolated populations. Consequently, taxonomic evaluation of species and subspecies in Salmonidae is problematic. In fact, the limits of taxonomic nomenclature may preclude formal recognition of the enormous biological diversity in salmonids (Behnke, 1972). Guonther (1866), writing about the genus *Salvelinus,* observed that "no other group of fishes . . . offer so many difficulties to the ichthyologist with regard to the distinction of species . . . as this genus."

These difficulties are further complicated by the propensity of humans to move salmonids about the landscape. The enormous popularity of trouts and salmons as food and game fishes has led to the establishment of hatchery strains of several species for stocking into regions lacking salmonids and into regions with native populations and species. Hybridization between conspecifics or congenerics (Krueger and Menzel, 1979; Ferguson, 1990; Skaala et al., 1990; Evans and Wilcox, 1991; Hindar et al., 1991; Waples, 1991; Carmichael, et al. 1993) confounds species and biogeographical assessments and jeopardizes the goal of conserving genetic diversity among taxa and populations. Competitive interactions between non-hybridizing taxa (Fausch, 1988; Griffith, 1988; DeWald and Wilzbach, 1992) further jeopardizes the conservation of native taxa.

Salvelinus, the charrs, is one of ten salmonid genera. Members of the family are characterized by a single dorsal fin and a posterior adipose fin, a pelvic axillary process,

numerous small cycloid scales embedded in slimy mucous, numerous pyloric caecae, and by the absence of spines in the fins (Rounsefell, 1962; Etnier and Starnes, 1993). The genus *Salvelinus* is further characterized by the white anterior margins of lower fins, red to cream colored spots on the body (Scott and Crossman, 1973), vermiculate markings dorsally (Etnier and Starnes, 1993), and the placement of teeth on the head of the volmer, not on the shaft (Vladykov, 1954). *Salvelinus fontinalis* is more robust than the other charrs, with a deeper body and a larger head (approaching a quarter of the body length), and the tail is square or only slightly forked (Power, 1980). All species in the genus are interfertile and produce fertile offspring (Behnke, 1980).

The eight or nine recognized *Salvelinus* species are grouped into three subgenera: *Salvelinus* (the *S. alpinus* complex, *S. malma*, and *S. confluentus*); *Cristivomer* (*S. namaycush*); and *Baione* (*S. fontinalis*). Behnke (1972; 1980) recognizes a second species of *Baione*, the extinct silver charr *S. agassizi* from Dublin Pond, New Hampshire. He also concurs with Sale (1967) and Quadri (1968) that the aurora charr of three Montreal River tributary lakes should be recognized as a subspecies, *S. fontinalis timagamiensis*. No other subspecies of *S. fontinalis* are currently recognized although Morgan (Ray Morgan, University of Maryland - Frostburg, personal communication) has proposed the existence of an additional three to five subspecies on the basis of mitochondrial DNA sequence variation.

Salvelinus fontinalis lacks much of the morphological differentiation observed in the *S. alpinus* complex and among salmonids generally. The lake trout, *S. namaycush*, shows a similar lack of morphological variability. Behnke (1972) speculated that lake

trout and brook trout diverged from an ancestral *Salvelinus* lineage to fill two salmonid niches: the large lacustrine predator (lake trout) and the smaller, littoral, riverine, and stream dwelling generalist (brook trout). By this line of reasoning the variability observed in many other salmonid species is a function of divergent stocks of a single species occupying different spatial and feeding niches. Indirect support for this hypothesis comes from the observation that the few examples of differentiated stocks of lake trout and of brook trout are from heterogeneous habitats (Behnke, 1980). The siscowet (recognized by some authors as *S. namaycush siscowet* although it is not morphologically differentiated from *S. namaycush*) and the lake trout both occur in Lake Superior, with the siscowet occupying the deeper waters of the lake (Qadri, 1967; Khan and Qadri, 1970). Similarly the aurora charr is found with brook trout in deep headwater lakes of the Montreal River (Henn and Rinkenbach, 1925), and the extinct silver charr inhabited the deep waters of Dublin Pond where brook trout were found in the shallower water (Behnke, 1972).

Extending the hypothesis of a relationship between niche specialization and inter- and intraspecific differentiation might suggest the existence of observable differentiation of brook trout populations across the taxon's broad range. Behnke (1980) recognizes two major life history types among brook trout populations and relates these types to differences in habitat. In the northern part of their range, south to the Great Lakes and northern New England (Figure 1.1)*, brook trout have a longer life span (6 to 7 years on average) and attain a larger size. In the northern range, brook trout also inhabit a wide

* Tables and figures are found at the end of the text of each part.

diversity of habitats including large and small lakes, rivers, streams and the sea. To the south, brook trout have shorter life spans (3-4 years) and are limited to small, low order streams.

There may be a relationship between these life history types and the limits of Wisconsinan glaciation, but existing data cannot distinguish between alternative scenarios (Behnke, 1980). Alternatively, observed life history differences may be a function of post-glaciation colonization by differentiated populations or may be the result of post-glaciation differentiation *in situ*. A third alternative is that observed differences between northern and southern brook trout populations are a selective response to factors of the Holocene landscape or climate (Meisner et al., 1988; Meisner, 1990), or to competition with the more diverse fish faunas in the southern range. In the northern range, brook trout from small streams are smaller and become sexually mature at a younger age than individuals from larger rivers and lakes (Kendall, 1914) as do populations under intensive angling pressure (Cooper et al., 1962; Cooper, 1967; Power, 1980). In the absence of a significant Pleistocene fossil record, resolution of these questions will require detailed comparative ecological, genetic, and morphological investigations.

Although apparently less variable than other species in the subgenus *Salvelinus*, "extreme variability" (Power, 1980) is a hallmark of *Salvelinus fontinalis*. Morphological variability observed among populations from different habitats in the northern range (Kendall, 1914; Wilder, 1952; Vladykov, 1954; Scott and Crossman, 1973) appears to be a plastic response to environmental differences and is often reversible in experimental situations (Wilder, 1952). Other morphological differences also have been noted between

southern and northern populations. Lennon (1967) suggested that native brook trout from the Southern Appalachians have smaller and more numerous red spots than those from the northern part of the range. Haberra and Fraley (1996) confirmed this impression, observing a statistically significant difference between presumed pure Southern Appalachian populations in Tennessee and wild populations of northern hatchery origin. The genetic basis of this character has not been investigated nor have other meristic or morphometric characters been examined. Until such studies are undertaken, the nature of brook trout morphological variation remains unresolved.

History and Status of Brook Trout Populations in the Southern Appalachians

Distribution of brook trout - The native range of brook trout extends from northern Quebec, westward to Manitoba, and roughly east of longitude 85° south to northern Georgia (Figure 1.1; MacCrimmon and Campbell, 1969). Prior to extensive human disturbance the limits of the southern range were abiotically determined, principally limited by maximum summer air temperature and maximum ground water temperature. McCrimmon and Campbell (1969) demonstrated that the southern range of native brook trout is approximately delimited by the 21° C mean July isotherm. However temperature of ground water sources, not air temperature, is the major determinant of brook trout range in the Southern Appalachians (Meisner, 1990). For brook trout, the 15° ground water isotherm occurring at 35-39° N latitude is thought to be the major limit to their distribution in the Southern Appalachians (Power, 1980; Meisner et al., 1988; Meisner, 1990).

The 21° C mean July isotherm and the 15° C ground water isotherm are functions of both latitude and elevation. Calculating 15° C groundwater temperature as approximately 1.5° C plus the mean annual air temperature, Meisner (1990) demonstrates the lower stream boundary for brook trout rises from sea level at 39° N latitude to about 600 meters at 35° N in northeastern Georgia. This corresponds to an estimate of 1° C change in temperature in 188 meters of elevation or 110 kilometers of latitude (Meisner, 1990). In the absence of human impacts, suitable habitat will extend below this groundwater isotherm as a result of aspect shading and shading by riparian vegetation. However, as a consequence of human activities, few Southern Appalachian brook trout populations are presently found near this theoretical boundary (Kelly et al., 1980; Bivens, 1985; Habera and Strange, 1993). In most locations brook trout in the Southern Appalachians are confined to first and second order stream segments above at least 900 meters in elevation (Habera and Strange, 1993; Flebbe, 1994).

Brook Trout Decline in the Southern Appalachians - Logging, road and railroad construction, land clearing for agriculture, over-fishing, and the introduction of non-native salmonids have all contributed to the habitat compression of southern brook trout (Kelly et al., 1980; Bivens, 1985). Timber harvest and clearing for agriculture directly influence the thermal characteristics of streams by removing shading vegetation (Swift and Messer, 1971). These activities, as well as associated road and railroad construction, also can result in stream siltation leading to changes in stream faunas (Hansen, 1971). Although the effects of these changes on brook trout feeding ecology have not been

investigated in the Southern Appalachians, brook trout are visual predators and increased turbidity would likely reduce feeding success. Siltation also negatively impacts brook trout reproduction by smothering eggs in spawning sites and by covering or filling in spawning sites, rendering them unusable. Road construction in the high elevations of Great Smoky Mountains National Park has revealed another source of negative impacts, the exposure of acidic rock strata (Huckabee et al., 1975). Leachate from argillaceous slates, phyllites, and schists of the Anakeesta Formation (King et al., 1968) can reduce Park stream pH from 6.7 to 4.5 or lower, and mobilize heavy metal sulfide constituents of the Anakeesta rocks (Morgan et al., 1976). These inputs can render stream segments sterile (Green, 1975; Huckabee et al., 1975). Even after pH recovery, the resultant metal hydroxide precipitates inhibit benthic macroinvertebrate recolonization (Morgan et al., 1976). Ironically, rainbow trout are more sensitive to low pH than are brook trout (King, 1943; Lennon, 1967; Wood and McDonald, 1987) and this can inhibit rainbow trout encroachment in acidified streams where brook trout are not altogether eliminated (Bivens, 1985).

During the early part of this century, logging and associated activities appear to have been the primary factors responsible for reduction of brook trout range in the Southern Appalachians. Powers (1929) and King (1937; 1942) indicated that brook trout distribution at the time the Park was established in 1934 was limited to areas that had little or no logging. By 1930, extensive logging in Tennessee south of the Park had reduced brook trout populations to a few stream segments in Monroe County and eliminated brook trout from Polk County (Bivens, 1985). Studies of the impact of logging

on native brook trout decline are lacking for most of the Southern Appalachian region; however, it is likely that such studies would confirm the relationship between logging intensity and brook trout decline.

These landscape level impacts have diminished over the past century, at least on public lands. Timber harvest is prohibited on National Park and National Forest wilderness lands. On public and private lands where these activities take place, harvest methods exist, and are often employed, that can minimize impacts on aquatic systems including brook trout populations. However, while many habitats have largely recovered, brook trout have generally not regained range lost due to prior impacts. Indeed, brook trout continued to decline after the end of extensive logging and forest recovery (Seehorn, 1979; Kelly et al., 1980). Widespread introductions of foreign salmonids, brown trout (*Salmo trutta*) from northern Europe and rainbow trout (*Oncorhynchus mykiss*) from the Pacific northwest have been strongly implicated in this continued decline (King, 1937; Seehorn, 1979; Kelly et al., 1980; Bivens, 1985; Bivens et al., 1985; Larson and Moore, 1985; Habera and Strange, 1993). However, recent studies indicate that during the last 20 years no net loss of brook trout range has occurred in Tennessee streams where brook trout and rainbow trout are sympatric (Strange and Habera, 1998).

Great Smoky Mountains National Park - The dimensions of these various impacts on Southern Appalachian brook trout have been most thoroughly examined in Great Smoky Mountains National Park. King (1937) reported that prior to 1900, brook trout were abundant in streams of the Park above 2000 feet (610 meters), with some populations

extending to 1600 feet (490 meters). By the time of his surveys in 1935, brook trout were generally limited to stream segments above 3000 feet (910 meters) where extensive logging had not taken place. The loss of brook trout habitat was estimated at over 160 stream miles (260 kilometers) or 55% of the estimated range in 1900 (Kelly et al., 1980). Surveys in the 1950s indicated brook trout had not regained lost habitat after the end of logging in 1935, but had instead declined an additional 15% (Lennon, 1967). These surveys observed that stream segments previously inhabited by brook trout were now inhabited by rainbow trout (Lennon, 1967) that had been stocked into the lower sections of most Park streams between 1910 and 1930 (Kelly et al., 1980). By the 1970s brook trout occupied about 123 miles (198 kilometers) of Park streams (Kelly et al., 1980), only a slight decline from the 1950s. However the number of stream segments with sympatric brook trout and rainbow trout populations had increased (Kelly et al., 1980). Brown trout invading from stocked stream segments outside the Park had become important by the 1970s as well, with reproducing populations sympatric with rainbow trout in 50 miles of low elevation Park streams (Kelly et al., 1980).

The studies cited above identify two broad categories of impacts, operating more or less sequentially, which negatively impacted brook trout in the Park: logging and associated activities, and stocking with non-native salmonids. As in areas outside the Park, logging directly impacted brook trout streams through removal of shading vegetation and increased surface runoff and siltation (King, 1937). Road, railroad, bridge, and dam construction in support of timber harvest directly impacted brook trout streams as well (King, 1937). Even after the timber had been removed, landscape and stream

recovery were delayed by the frequent fires that swept through slash and remaining vegetation on cut-over lands (King, 1937). Even more direct during this period of destructive exploitation, although perhaps of lesser consequence, were the impacts of destructive fishing practices. Fishing was not regulated and the use of baits, nets, and dynamite insured successful fishing (King, 1937). With the establishment of Great Smoky Mountains National Park in 1936, logging was terminated and some regulation of fishing was instituted, but brook trout populations did not recover with the landscape (Lennon, 1967).

Effects of Rainbow Trout - King (1937) suggested that rainbow trout invaded stream segments from which brook trout had been extirpated as a consequence of rainbow trout's broader habitat tolerances, higher fecundity, and higher growth rate. The thermal maximum and optimum of rainbow trout are higher than those of brook trout (Coutant, 1977; Peterson et al., 1979) and they are generally more tolerant of silt and pollution than are brook trout (King, 1937). Thus, they could successfully occupy recovering habitat prior to brook trout. Presumably this priority of arrival, coupled with higher fecundity and growth rate, inhibited the downstream invasion by brook trout after habitat recovery. Lennon (1967) ascribed the failure of brook trout to recover lost habitat, and the continued loss of habitat to rainbow trout, to consequences of life in small headwater streams. Fish in these populations are smaller than those found in larger downstream segments and fecundity is lower because of the scarcity and small size of spawning sites. Lennon (1967) also believed these small headwater populations had higher incidence of

disease than larger brook trout and rainbow trout populations living in larger lower elevation segments, a factor working to the further demographic detriment of brook trout. Empirical support for most of these hypothesized effects is strong, but experimental evidence for the mechanisms involved in the replacement of brook trout by invading rainbow trout has been more difficult to obtain, and results have been difficult to interpret (Fausch, 1988).

It is well documented that Southern Appalachian brook trout populations south of central Virginia have lost habitat to rainbow trout (Jones, 1978: Kelly et al., 1980; Moore et al., 1984; Bivens et al., 1985; Larson and Moore, 1985; Fleebe, 1994). In Great Smoky Mountains National Park, allopatric brook trout populations above natural barriers which prevent upstream movement of rainbow trout have not declined significantly during the period of monitoring by Park biologists. But in Park streams without barriers, rainbow trout populations have expanded upstream to the detriment of brook trout (Larson and Moore, 1985). In sympatry, there is a negative relationship between the densities of adult brook trout and the densities of rainbow trout suggesting competitive superiority of rainbow trout. A negative relationship also exists between the adult population size of either species and numbers of young of the year of the other species (Larson and Moore, 1985), suggesting either interspecific predation or greater access to spawning sites by the higher density species. Stomach content analysis of both species in allopatry and sympatry has excluded interspecific predation as a significant factor in the displacement of brook trout by rainbow trout (Habera, 1987; Ensign, 1988). Removal experiments in the Park indicate that this biotic exchange is a consequence of interspecific interactions

rather than the occupation by rainbow trout of empty habitat created by declining brook trout populations. Electrofishing to remove rainbow trout in streams where they are sympatric with brook trout results in increases in the biomass of brook trout and the establishment of age structures similar to that of allopatric brook trout populations (Moore et al., 1981; 1984; 1985; 1986). This is achieved even if rainbow trout are not fully eradicated, although without complete removal of rainbows, brook trout recovery is temporary (Moore et al., 1984).

Interactions between brook trout and rainbow trout involve differences in stream microhabitat utilization. In both sympatry and allopatry, brook trout prefer pools in low velocity stream segments near cover while rainbow trout prefer higher velocity riffles and runs (Cunjak and Green, 1983; Lohr, 1985; Lohr and West, 1992; Welsh, 1994). Brook trout allopatric from rainbow trout are largely confined to high gradient headwater stream segments where water temperatures are lower. However high gradient segments are not the preferred habitat of brook trout, but of rainbow trout. The observation that rainbow trout do not displace brook trout in low gradient high elevation streams even in the absence of barriers to upstream movement (Moore et al., 1986) supports the contention that lower water temperature of headwater streams is important for brook trout. At intermediate elevations, rainbow trout encroachment in steep gradient streams exhibits considerable annual variation as a consequence of different habitat tolerances of the two species and of annual variation in stream flow (Nagel, 1991; Larson et al., 1995). This interannual variation suggests that interactions between rainbow trout and brook trout populations are more properly viewed as a dynamic ebb and flow rather than as absolute

displacement of brook trout by rainbow trout (Larson et al., 1995; Strange and Habera, 1998).

Segregation of microhabitat utilization suggests the possibility of stable sympatry in at least some stream segments. Although food utilization by adult brook trout and rainbow trout in the Park overlaps significantly (Lohr, 1985; Habera, 1987; Ensign, 1988), populations of neither species appear to be limited by prey abundance during most of the year (Ensign, 1988). At the same time, populations of both species may be food limited in high elevation, high gradient Park streams (Ensign, 1988). Encroachment of rainbow trout or their removal from sympatry with brook trout does not result in a shift in prey taken by adults of either species (Habera, 1987; Ensign, 1988), suggesting that competitive interactions are not a factor in limiting foraging success of adults of either species.

Long term monitoring studies of one stream in Great Smoky Mountains National Park indicate that zones of sympatry are narrow and spatially and temporally fluctuating (Larson et al., 1995). Monitoring of sympatric brook trout and rainbow trout populations in Tennessee outside the Park also shows temporal variation in the elevation of zones of sympatry (Strange and Habera, 1998). Significantly, Strange and Habera (1998) observed no net loss of brook trout across the 25 sympatric populations examined: downstream expansion of brook trout populations into stream segments previously occupied by allopatric rainbow trout occurred with approximately the same frequency as rainbow trout encroachment upstream displacing allopatric brook trout. Incongruities between the observed displacement of brook trout by rainbow trout prior to 1970 and recent studies

documenting substantial ebb and flow of contiguous allopatric populations of both species underscores the inadequacies of our understanding of the mechanisms involved. Most importantly these observations suggest that interpretations from observations of a limited number of streams and across short time scales may be quite misleading. Resolution of the uncertainties will require renewed focus on biotic factors in brook trout and rainbow trout interactions, and on temporal variation in population distributions and abiotic factors such as rainfall and stream flow.

Numerical displacement (Chapman, 1965) involving some type of interaction at some stage in the life cycle, though incomplete in its formulation, appears to be a component of brook trout and rainbow trout population dynamics in the Southern Appalachians (Fausch, 1988). Competition for spawning sites, which may be limiting (Morgan and Robinette, 1978), is a factor only if sites are defended throughout most of the year or if use by rainbow trout renders them unsuitable for use by brook trout. In the Park, and throughout the Southern Appalachians generally, brook trout spawn in the fall and emerge in early spring. Rainbow trout spawn in the spring and emerge in early summer, and by late summer are larger than the earlier emerging brook trout. Effects of rainbow trout spawning at sites containing emerging brook trout fry have not been investigated. The effects of behavioral interactions between the two species as adults and as juveniles have been investigated primarily in the laboratory (Cunjak and Green, 1983; 1984; 1986), and the results are equivocal (Fausch, 1988; Lohr and West, 1992).

Differences in spawning season and differences in preferred stream microhabitat suggest that seasonal and interannual variation in stream flow may be of considerable

importance in understanding the ebb and flow dynamics of contiguous brook trout and rainbow trout populations. Variation in stream flow may limit or promote reproduction and survival of one species in some years and the other species in other years. Under this hypothesis, numerical superiority of the species with highest recruitment and/or survival would swamp the less abundant species. Displacement of the less abundant species by the more abundant species might involve hypothesized (but as yet undemonstrated) interactions or might be a function of the localized stochastic extinction of the low density species.

Temporal variation in the location of contact zones between contiguous brook trout and rainbow trout populations (Larson et al., 1995; Strange and Habera, 1998) does not eliminate concern for the long term survival of Southern Appalachian brook trout. Processes of displacement of one species by the other are probably similar to those which resulted in the successful establishment of rainbow trout in the first half of the century. Rainbow trout became established in stream segments from which brook trout had been eliminated or in which brook trout population levels had been considerably reduced. There is no evidence that brook trout have reoccupied significant areas of this former habitat (Moore et. al, 1985; Bivens, 1985). The current situation appears to be one of a dynamic draw with brook trout expanding in some streams and some years and rainbow trout expanding in others, with no net loss of brook trout when averaged across the landscape. However, these hopeful observations are tempered by consideration of differences in the recruitment potential of downstream rainbow trout populations and the upstream brook trout populations. Extinction or decline of rainbow trout populations or

population segments opens habitat for occupation by rainbow trout from lower stream segments or from upstream brook trout populations. Habitat opened by extinction or decline of brook trout populations can be occupied by rainbow trout, but migration of brook trout from nearby first order stream segments is unlikely (Nagel, 1991).

Distinctiveness of Southern Appalachian Brook Trout and the Effects of Hatchery Introductions

Stocking History - Brook trout, like most salmonids, have been successfully reared in hatcheries for over a century and have been widely stocked throughout the world (MacCrimmon and Campbell, 1969). Attempts to restore declining populations of brook trout in the Southern Appalachians have involved extensive stocking with hatchery reared or propagated fish (King, 1937; Holloway, 1945; Kelly et al., 1980). In the 1930s federal and state fish hatcheries were established in the region to propagate brook trout and other salmonids of interest to anglers. Stocking was terminated in Great Smoky Mountains National Park in 1975 (Kelly et al., 1980) in keeping with National Park Service policies of managing for native species and strains. In response to concerns that hatchery strains and native populations might be significantly differentiated, brook trout stocking was halted in Tennessee outside the park in 1980 (Kriegler et al., 1995). Other nonnative salmonids are still stocked into Tennessee waters. Brook trout and nonnative Salmonids are currently stocked in Virginia, North Carolina, Georgia, and South Carolina (Flebbe, 1994). However these continued stockings are predominantly part of put-and-take fisheries conducted under management policies of stocking only where wild brook trout

populations are not affected (North Carolina Wildlife Resources Commission, 1989; Tennessee Wildlife Resources Agency, 1990).

Most brook trout stocking in the Southern Appalachians employed domesticated hatchery strains derived from northeastern U. S. populations (Holloway, 1945; Lennon, 1967; Kelly et al., 1980; McCracken et al., 1993; Kriegler et al., 1995). Brook trout were one of the first North American fishes successfully reared in fish hatcheries (Bowen, 1970) and some named strains have been in existence for over 100 years (Bowen, 1970; Kincaid, 1981). Selection under domestication is for characteristics conducive to the hatchery environment, such as high growth rate and tolerance of crowding (Vincent, 1960). Selection for characteristics deemed desirable by anglers, such as catchability and size, has also been imposed (Bowen, 1970). The effects of these introductions of northern derived domesticated strains into native Southern Appalachian brook trout gene pools are of obvious conservation concern, particularly if the source populations are genetically differentiated from indigenous populations.

Stocking with hatchery reared fish was largely unsuccessful in halting the loss of brook trout, with stream mileage occupied by brook trout continuing to decline until at least the mid 1970s. Management efforts over the past 25 years have emphasized stream restoration and construction of barriers against rainbow trout encroachment where feasible, and removal of rainbow trout from sympatric populations above barriers. Work in the Park has demonstrated the effectiveness, as well as the large manpower requirements, of rainbow trout removal (Moore et al., 1986). Removal of rainbow trout from all former or potential brook trout habitat is clearly not feasible, and for many

managers not desirable. However rainbow trout removal from selected streams appears to be the best strategy for establishing brook trout sport fisheries, and as a hedge against demographic extinction of small headwater populations.

The Native Southern Appalachian Brook Trout - The distinctiveness of native Southern Appalachian brook trout is part of the lore of the region (Yuskavitch, 1991; Venters, 1993). Known in the region as speckled trout or speckles, Southern Appalachian brook trout are smaller and have more spots than northern derived hatchery fish. Southern Appalachian anglers also maintain that native speckles are more brightly colored, offer a more exciting fishing experience, and are tastier than stocked hatchery strains. A few fisheries biologists have also suggested the existence of morphological and life history differences between northeastern and Southern Appalachian brook trout that are of potential taxonomic significance and management importance (King, 1942; Holloway, 1945; Lennon, 1967). However, only with the application of molecular population genetics techniques coupled with examination of stocking history has the distinctiveness of the Southern Appalachian brook trout been recognized.

From a preliminary study of allozyme variation in Southern Appalachian brook trout, Brussard and Nielsen (1976) suggested that populations from Great Smoky Mountains National Park might be different from northeastern populations at a level of management concern. A more extensive study examining both allozyme and morphological variation among northern hatchery strains and wild populations from the Park concluded that there was not significant differentiation between the two (Harris et

al., 1978). This second study was contradicted by a third study of allozyme variation which concluded that populations from the southern part of the range might represent a distinct subspecies (Stoneking et al., 1981). Stoneking et al. also pointed out that the Harris et al. study suffered from significant deficiencies in the interpretation of complex allozyme banding patterns. Most importantly, Stoneking et al. emphasized that all three studies were limited by their failure to consider the history of stocking with northern hatchery strains.

McCracken et al. (1993) explicitly tested the hypothesis of genetic differentiation between native populations in the Great Smoky Mountains National Park and northern derived hatchery strains used for stocking in the Park. Following the concerns of Stoneking et al. (1981) we took special effort to sample putative native brook trout from streams with no record of stocking, and with minimal likelihood of undocumented stocking or movement of fish from stocked stream segments. The results of this study were unequivocal. Data from 34 allozyme loci showed a clear distinction between unstocked Park populations and northern derived hatchery strains used in stocking. Unstocked Park populations and northern derived hatchery strains were fixed for alternative alleles at one allozyme locus and showed significant allele frequency heterogeneity at an additional 9 loci. Unstocked populations were monomorphic at most loci. Hatchery strains were polymorphic at these loci with alternative alleles segregating with the same alleles that were fixed in the Park populations. Populations from streams with known history of hatchery stocking onto existing populations had both alleles from

the fixed diagnostic locus segregating, as well as northern alleles from other diagnostic loci.

General Methods

Because of their economic importance, brook trout and other salmonid fishes have been the focus of numerous molecular population genetics studies examining questions of possible management import. Initially these studies focused largely on stock and strain identification (Utter et al., 1976). Elucidation of diagnostic genetic markers readily permitted investigation of population subdivision (Allendorf and Phelps, 1981; Kruger et al., 1989) and of the effects of transplantations on native populations (Krueger and Menzel, 1979; Carmichael et al., 1993). Other research examined genetic variation per se, particularly as this might bear on the presumed health of populations and the conservation of genetic diversity (Morgan and Baker, 1991; Perkins et al., 1993). My research, and the research program of which it is a part, had its inception in these traditions.

Since our initial study (McCracken et al., 1993) we have employed the diagnostic allozyme markers to examine all known brook trout populations in Tennessee and South Carolina, most from Great Smoky Mountains National Park, and a sampling of populations from Maryland, Virginia, and North Carolina. We have also examined mitochondrial DNA variation in native Southern Appalachian populations and northern derived hatchery strains. Some of this work has been published (Kriegler et al., 1995; Hayes et al., 1996), is in graduate theses (Kriegler, 1993; Saidak, 1995; Shull, 1995), and is in reports to management agencies (McCracken and Guffey, 1994; Guffey, 1995;

1997). In this dissertation I present unpublished genetic data from Great Smoky Mountains National Park, South Carolina, Virginia, and Maryland, and I attempt to integrate these results with of all of the data from our investigations of Southern Appalachian brook trout.

The remainder of this dissertation consists of three parts. In Part 2 I present the results of my allozyme analysis of 47 brook trout populations from the Park. These populations plus those reported in McCracken et al. (1993) represent most of the known brook trout populations in the Park. My data extends the findings of McCracken et al. (1993) that native Southern Appalachian brook trout and northern derived hatchery strains are differentiated to a degree that is of conservation importance and potential taxonomic significance. I also document that levels of hatchery gene introgression in Park populations is surprisingly low given the extent of past stocking.

In Part 3 I present the results of my allozyme analysis of brook trout populations from South Carolina, North Carolina, Virginia, and Maryland. I attempt to integrate these data with the data from Part 2, our previous studies, and from other published studies to address the historical biogeography of brook trout in eastern North America. I conclude that native Southern Appalachian and northeastern populations are distinct, discrete evolutionary units (lineages) representing colonizations from separate Pleistocene refugia. I also observe that a substantially greater proportion of lineage heterozygosity is found among Southern Appalachian populations than among northeastern populations. These conclusions and observations have important implications for brook trout management

and conservation, and extend our understanding of biogeographical patterns among eastern North American fishes.

Part 4 begins the investigation of morphological variation among brook trout populations. Morphometric data were used to address overall shape differences and similarities among brook trout populations from different regions, and representing different evolutionary lineages. My data indicate morphological intermediacy of hybrids between northern derived hatchery strains and Southern Appalachian populations, but I was not able to unequivocally demonstrate morphological differences between specimens from Southern Appalachian and northern populations. These equivocal results may reflect the absence of morphological differentiation among populations, or may be a function of the traits examined and the measurement and statistical methods employed. Given the morphological plasticity of salmonids generally, and the landscape level structuring of brook trout populations into numerous, small, reproductively isolated populations, it is difficult to distinguish between these alternative interpretations. Investigation of other morphological traits is an area of potentially fruitful future research for addressing questions of morphological plasticity and morphological variation under different selection regimes, and for investigating relationships among patterns of morphological and molecular evolution.

Literature Cited

Allendorf, F. W., and S. R. Phelps. 1981. Use of allelic frequencies to describe population structure. Can J. Fish. Aquat. Sci. 38:1507-14.

Behnke, R. J. 1972. The systematics of salmonid fishes of recently glaciated lakes. J. Fish. Res. Board Can. 29:639-71.

Behnke, R. J. 1980. A systematic review of the genus *Salvelinus*. Pp. 441-481 *In* E. K. Balon (ed.), Charrs: Salmonid Fishes of the Genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands.

Bivens, R. D. 1985. History and distribution of brook trout in the Appalachian region of Tennessee. Tennessee Wildlife Resources Agency Technical Report No. 85-1. The University of Tennessee, Knoxville.

Bivens, R. D., R. J. Strange, and D. C. Peterson. 1985. Current distribution of the native brook trout in the Appalachian region of Tennessee. J. Tenn. Acad. Sci. 60:101-105.

Bowen, J. T. 1970. A history of fish culture as related to the development of fishery programs. Am. Fish. Soc. Spec. Publ. 7:71-93.

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

Carmichael, G. J., J. N. Hanson, M. E. Schmidt, and D. C. Morizot. 1993. Introgression among Apache, cutthroat and rainbow trout in Arizona. Trans. Am. Fish. Soc.122:121-130.

Chapman, D. W. 1965. Food and space as regulators of salmonid populations in streams. Am. Nat. 100:131-138.

Cooper, E. L., J. A. Boccardy, and J. K. Anderson. 1962. Growth rate of brook trout at different population densities in a small infertile stream. Prog. Fish-Cult. 24:74-80.

Cooper, E. L. 1967. Growth and longevity of brook trout (*Salvelinus fontinalis*) in populations subjected to light exploitation. Trans. Am. Fish. Soc. 96:383-86.

Coutant, C. C. 1977. Compilation of temperature preference data. J. Fish. Res. Board Can. 34:739-745.

Cunjak, R. A., and J. M. Green. 1983. Habitat utilization by brook char (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) in Newfoundland streams. Can. J. Zool. 61:1214-1219.

Cunjak, R. A., and J. M. Green. 1984. Species dominance by brook trout and rainbow trout in a simulated stream environment. Trans. Am. Fish. Soc. 113:737-743.

Cunjak, R. A., and J. M. Green. 1986. Influence of water temperature on behavioral interactions between juvenile brook char, *Salvelinus fontinalis*, and rainbow trout, *Salmo gairdneri*. Can. J. Zool. 64:1288-1291.

DeWald, L., and M. A. Wilzbach. 1992. Interactions between native brook and hatchery brown trout: effects on habitat use, feeding, and growth. Trans. Am. Fish. Soc. 121: 287- 296.

Ensign, W. E. 1988. The importance of competition for food resources in the interaction between brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). Unpublished Masters thesis, University of Tennessee, Knoxville.

Etnier, D. A., and W. C. Starnes. 1993. The fishes of Tennessee. The University of Tennessee Press, Knoxville.

Evans, D. O., and C. C. Wilcox. 1991. Loss of exploited, indigenous populations of lake trout, *Salvelinus namaycush*, by stocking of non-native stocks. Can. J. Fish. Aquat. Sci. 68(Sup. 1):134-147.

Fausch, K. D. 1988. Tests of competition between native and introduced salmonids in streams: What have we learned? Can. J. Fish. Aquat. Sci. 45:2238-2246.

Ferguson, M. M. 1990. The genetic impact of introduced fishes on native species. Can. J. Zool. 68:1053-1057.

Flebbe, P. A. 1994. A regional view of the margin: Salmonid abundance and distribution in the Southern Appalachian Mountains of North Carolina and Virginia. Trans. Am. Fish. Soc. 123:657-667.

Green, R. L. 1975. Benthic macroinvertebrate communities in Great Smoky Mountains National Park streams influenced by acid drainage. unpublished Masters thesis. Tennessee Technological University, Cookeville.

Griffith, J. S. 1988. Review of competition between cutthroat trout and other salmonids. Am. Fish. Soc. Symp. 4:134-140.

Guffey, S. Z. 1995. Allozyme genetics of South Carolina brook trout. Unpublished report submitted to the South Carolina Department of Natural Resources, Clemson.

Guffey, S. Z. 1997. Population genetics of brook trout in Virginia. Unpublished report submitted to the Virginia Department of Game and Inland Fisheries, Richmond.

Guonther, A. 1866. Catalogue of fishes of the British Museum. Vol. 6. London.

Habera, J. W. 1987. Effects of rainbow trout removal on trout populations and food habits in Great Smoky Mountains National Park. Unpublished Masters thesis, University of Tennessee, Knoxville.

Habera, J. W., and S. J. Fraley. 1996. Variation in red spot number among native Southern Appalachian and hatchery derived brook trout populations. *In* Strange, R. J., and J. W. Habera. 1996. Wild trout project: 1995 Annual report. Tennessee Wildlife Resources Agency and the University of Tennessee. Pub. No. R11-2217-39-001-96.

Habera, J. W., and R. J. Strange. 1993. Wild trout resources and management in the Southern Appalachian Mountains. Fisheries 18:6-13.

Hansen, E. A. 1971. Sediment in a Michigan trout stream: Its source, movement and some effects on fish habitat. U.S. Dept. Agric., Forest Service Research Paper NC-59.

Harris, J., R. D. Estes, M. Van Den Avyle, W. F. Brandes, E. Shipp, and D. Y. White. 1978. Systematics of the brook trout *Salvelinus fontinalis* (Mitchill) in the Great Smoky Mountains National Park. Part I: Electrophoresis; Part II: Meristics; Final Report to the National Park Service, Tennessee Cooperative Fishery Unit, Cookeville, Tennessee. Unpublished report submitted to Great Smoky Mountains National Park, Gatlinburg, Tennessee.

Hayes, J. P., S. Z. Guffey, F. J. Kriegler, G. F. McCracken, and C. R. Parker. 1996. The genetic diversity of native, stocked, and hybrid populations of brook trout in the Southern Appalachians. Conserv. Biol. 10:1403-1412.

Hendricks, M. L. 1980. *Salvelinus fontinalis* (Mitchill), brook trout. P. 114 *In* Lee, D. S., C. R. Gilbert, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. R. Stauffer, Jr. (eds.), Atlas of North American Freshwater Fishes. North Caroiina State Museum of Natural History, Raleigh.

Henn, A. W., and W. H. Rinkenbach. 1925. Description of the aurora trout (*Salvelinus timagamiensis*), a new species from Ontario. Ann. Carnegie Mus. 16:131-41.

Hindar, K., N. Ryman, and F. Utter. 1991. Genetic effects of cultured fish on natural fish populations. Can. J. Fish. Aquat. Sci. 48:945-57.

Holloway, A. D. 1945. Report on the fisheries of the Great Smoky Mountains National Park.Unpublished internal report to Great Smoky Mountains National Park, Gatlinburg, Tennessee.

Huckabee, J. W., C. P. Goodyear, and R. D. Jones. 1975. Acid rock in the Great Smokys: Unanticipated impact on aquatic biota of road construction in regions of sulfide mineralization. Trans. Am. Fish. Soc. 104:677-684.

Jones, R. D. 1978. Regional distribution trends of the trout resource. Pp. 1-10 *In* Harshbarger, T. J. (ed.), Southeastern Trout Resource: Ecology and Management Symposium Proceedings. U. S. Forest Service, Southeastern Forest Experiment Station, Asheville, North Carolina.

Jordan, D. S., and B. W. Evermann. 1896-1900. The fishes of North and Middle America: A descriptive catalogue of the species of fish-like vertebrates found in the waters of North America, north of the Isthmus of Panama. Bull. U. S. Nat.Mus. 47:1-3313.

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

Kendall, W. C. 1914. The fishes of New England. The salmon family, Part I. The trout or charrs. Mem. Boston Soc. Nat. Hist. 8:1-103.

Khan, N. Y., and S. U. Qadri. 1970. Morphological differences in Lake Superior lake charr. J. Fish. Res. Board Can. 27:161-67.

Kincaid, H. L. 1981. Trout strain registry. U. S. Fish and Wildlife Service, National Fisheries Center - Leetown / 81-1. Kearneysville, West Virginia.

King, J. E. 1943. Survival time of trout in relation to occurrence. Am. Midl. Natur. 29:624-42.

King, P. B., R. B. Neuman, and J. B. Hadley. 1968. Geology of the Great Smoky Mountains National Park, Tennessee and North Carolina. U. S. Geological Survey Professional Paper 587.

King, W. 1937. Notes on the distribution of native speckled trout and rainbow trout in the streams of Great Smoky Mountains National Park. J. Tenn. Acad. Sci. 12:351-361.

King, W. 1942. Trout management studies at Great Smoky Mountains National Park. J. Wild. Manag. 6:147-161.

Kriegler, F. J. 1993. The genetics of native and stocked brook trout populations in east Tennessee. Unpublished Master's thesis. University of Tennessee, Knoxville.

Kriegler, F. J., G. F. McCracken, J. W. Habera, and R. J. Strange. 1995. Genetic characterization of Tennessee brook trout populations and associated management implications. N. Am. J. Fish. Manag. 15:804-813.

Krueger, C. C., and B. W. Menzel. 1979. Effects of stocking on genetics of wild brook trout populations. Trans. Am. Fish. Soc. 108:277-287.

Krueger, C. C., J. E. Marsden, H. L. Kincaid, and B. May. 1989. Genetic differentiation among lake trout strains stocked into Lake Ontario. Trans. Am. Fish. Soc. 118:317-330.

Larson, G. L. and S. E. Moore. 1985. Encroachment of exotic rainbow trout into stream populations of native brook trout in the Southern Appalachian Mountains. Trans. Am. Fish. Soc. 114:195-203.

Larson, G. L., S. E. Moore, and B. Carter. 1995. Ebb and flow of encroachment by nonnative rainbow trout in a small stream in the Southern Appalachians. Trans. Am. Fish. Soc. 124:613-622.

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

Leopold, A. 1949. A sand county almanac, and sketches here and there. Oxford University Press, New York.

Lohr, S. C. 1985. Comparison of microhabitat selection and diet of brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) in a Great Smoky Mountains National Park stream. Unpublished Masters Thesis, Western Carolina University, Cullowhee, N.C.

Lohr, S. C., and J. L. West. 1992. Microhabitat selection by brook and rainbow trout in a Southern Appalachian stream. Trans. Am. Fish. Soc. 121:729-736.

MacCrimmon, H. R., and J. S. Campbell. 1969. World distribution of brook trout, *Salvelinus fontinalis*. J. Fish. Res. Board Can. 26:1699-1725.

McCracken, G. F., C. R. Parker, and S. Z. Guffey. 1993. Genetic differentiation and hybridization between stocked hatchery and native brook trout in Great Smoky Mountains National Park. Trans. Am. Fish. Soc. 122:533-542.

McCracken, G. F., and S. Z. Guffey. 1994. Population genetics and distribution of brook trout in Great Smoky Mountains National Park. Unpublished report submitted to Great Smoky Mountains National Park, Gatlinburg, TN.

Meisner, J. D., J. S. Rosenfeld, and H. A. Regier. 1988. The role of groundwater in the impact of climate warming on stream salmonines. Fisheries 13:2-8.

Meisner, J. D. 1990. Effect of climatic warming on the southern margins of the native range of brook trout, *Salvelinus fontinalis*. Can. J. Fish. Aquat. Sci. 47:1065-1070.

Moore, S. E., G. L. Larson, and B. Ridley. 1984. A summary of changing standing crops of native brook trout in response to removal of sympatric rainbow trout in Great Smoky Mountains National Park. J. Tenn. Acad. Sci. 59:76-77.

Moore, S. E., B. L. Ridley, and G. L. Larson. 1981. Changes in standing crop of brook trout concurrent with removal of exotic trout species. Great Smoky Mountains National Park. Research and Resources Management Report 37.Uplands Field Research Laboratory, Great Smoky Mountains National Park, Gatlinburg, Tennessee.

Moore, S. E., B. L. Ridley, and G. L. Larson. 1985. Dispersal of brook trout in rehabilitated streams in Great Smoky Mountains National Park. J. Tenn. Acad. Sci. 60:1-4.

Moore, S. E., B. L. Ridley, and G. L. Larson. 1986. Population control of exotic rainbow trout in streams of a natural area park. Environ. Mgmt. 10:215-219.

Morgan, E. L., and J. R. Robinette. 1978. Life history study of brook trout *Salvelinus fontinalis* (Mitchill). Great Smoky Mountains National Park. Gatlinburg, Tennessee.

Morgan, E. L., M. H. Hoff, and W. F. Trumpf. 1976. Anakeesta leachate studies and brook trout investigations. Tennessee Technological University Environmental Biology Research Center Report Series No. 8. Cookeville.

Morgan, R. P., and B. M. Baker. 1991. Development of genetic inventories for Maryland game fish: brook trout. Final draft report. Appalachian Environmental Laboratory Center for Environmental and Estuarine Studies, University of Maryland, Frostburg.

Nagel, J. W. 1991. Is the decline of brook trout in the Southern Appalachians resulting from competitive exclusion and/or extinction due to habitat fragmentation? J. Tenn. Acad. Sci. 66:141-143.

North Carolina Wildlife Resources Commission. 1989. Casting the Future of Trout in North Carolina: A plan for management of North Carolina's trout resources. Division of Boating and Inland Fisheries, North Carolina Wildlife Resources Commission, Raleigh.

Perkins, D. L., C. C. Kruger, and B. May. 1993. Heritage brook trout in northeastern USA: Genetic variability within and among populations. Trans Am. Fish Soc. 122:515-532.

Peterson, R. H., A. M. Sutterlin, and J. D. Metcalfe. 1979. Temperature preference of several species of *Salmo* and *Salvelinus* and some of their hybrids. J. Fish. Res. Board Can.36:1137-1140.

Platts, W. S., and R. L. Nelson. Fluctuations in trout populations and their implications for land-use evaluation. N. Am. J. Fish. Manag. 8:333-345.

Power, G. 1980. The brook charr (*Salvelinus fontinalis*). Pp. 797-840 *In* E. K. Balon (ed.), Charrs: Salmonid Fishes of the Genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands.

Powers, E. B. 1929. Freshwater studies I. The relative temperature, oxygen content, alkali reserve, the carbon dioxide tension and pH of the water of certain streams at different altitudes in the Smoky Mountains National Park. Ecology 10:97-111.

Qadri. S. U. 1967. Morphological comparisons of three populations of lake charr, *Cristivomer namaycush*, from Ontario and Manitoba. J. Fish. Res. Board Can. 24:1407-1411.

Qadri, S. U. 1968. Morphology and taxonomy of the aurora charr, *Salvelinus fontinalis timagamiensis*. Bul. Nat. Mus. Can. 237:1-18.

Rounsefell, G. A. 1962. Relationships among North American Salmonidae. Fish. Bull. U. S. Fish and Wildlife Service 62:235-70.

Saidak, L. R. 1995. Brook trout (*Salvelinus fontinalis*) in Tennessee: An ecological genetic study using allozyme electrophoresis and random amplified polymorphic DNA. Unpublished Masters thesis. University of Tennessee, Knoxville.

Sale, P. F. 1967. A re-examination of the taxonomic position of the aurora trout. Can. J. Zool. 45:213-225.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 184. Ottawa.

Seehorn, M. E. 1979. Status of brook trout in the southeast. Pp. 16-17 *In* R. D. Estes, T. Harshbarger, and G. D. Pardue (eds.). Proceedings of the brook trout workshop. U. S. Forest Service, Southeastern Forest and Range Experiment Station, Asheville, North Carolina.

Shull, L. N. 1995. An allozyme analysis of brook trout (*Salvelinus fontinalis*) from headwater streams of the Blue Ridge Parkway. Unpublished Masters thesis. Appalachian State University, Boone, North Carolina.

Skaala, O., G. Dahle, K. E. Jorstad, and G. Naevdal. 1990. Interactions between natural and farmed fish populations: information from genetic markers. J. Fish. Biol. 36:449-460.

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

Strange, R. J. 1979. Our native trout of the south: will it survive? Trout 20:10-13.

Strange, R. J., and J. W. Habera. 1998. No net loss of brook trout distribution in areas of sympatry with rainbow trout in Tennessee streams. Trans. Am. Fish. Soc., *in press*.

Swift, L. W., Jr., and J. B. Messer. 1971. Forest cutting raises temperatures of small streams in the Southern Appalachians. J. Soil Water Conserv. 26:111-116.
Tennessee Wildlife Resources Agency. 1990. A draft strategic wildlife resources management plan for entering the 21st century. Tennessee Wildlife Resources Agency, Nashville.

Utter, F. M., F. W. Allendorf, and B. May. 1976. The use of protein variation in the management of salmonid populations. Pp. 373-384 *In* Transactions of the 41st North American Wildlife Natural Resource Conference. Wildlife Management Institute, Washington, D. C.

Venters, V. 1993. In search of speckles. Wildlife in North Carolina. July:11-15.

Vincent, R. E. 1960. Some influences of domestication upon three stocks of brook trout (*Salvelinus fontinalis* Mitchill). Trans. Am. Fish. Soc. 89:35-52

Vladykov, V. D. 1954. Taxonomic characters of the eastern North American charrs (*Salvelinus* and *Cristivomer*). J. Fish. Res. Board Can. 11:904-932.

Waples, R. S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the pacific northwest. Can. J. Fish. Aquat. Sci. 48(Sup. 1):124-133.

Welsh, T. L. 1994. Interactive dominance: Chinook salmon and eastern brook trout (*Oncorhynchus tshawytscha*, *Salvelinus fontinalis*). Unpublished PhD. thesis. University of Idaho, Moscow.

Wilder, D. G. 1952. A comparative study of anadromous and freshwater populations of brook trout (*Salvelinus fontinalis*, Mitchill). J. Fish. Res. Board Can. 9:169-203.

Wood, C. M., and D. G. McDonald. 1987. The physiology of acid/aluminum stress in trout. Pp. 399-410 *In* Witters, H., and O. Vanderborght, eds. Ecophysiology of acid stress in aquatic organisms. Antwerp.

Yuskavitch, J. A. 1991. Saved by the barriers. Trout. Summer:18-27.

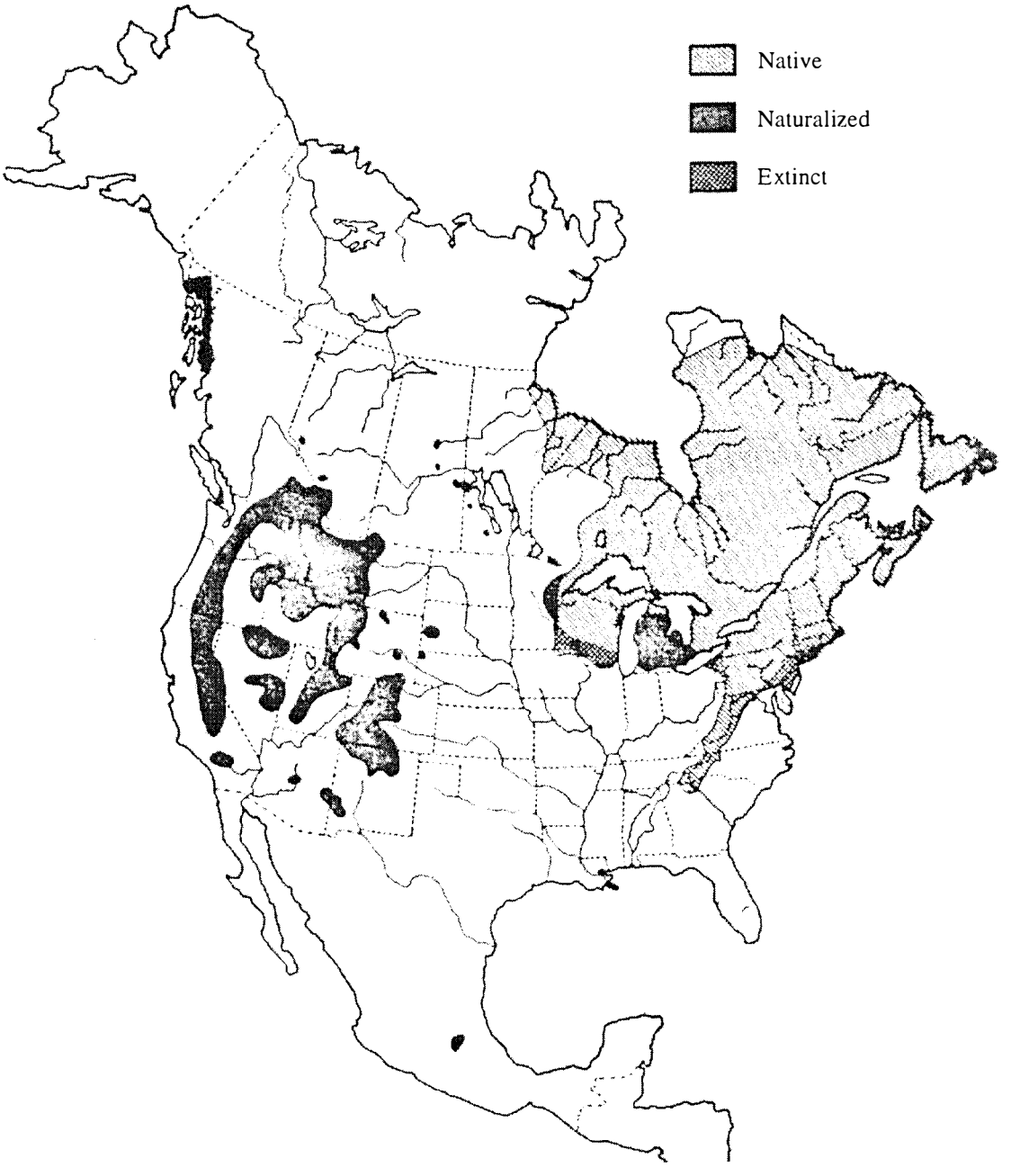


Figure 1.1. Brook trout range in North America. From MacCrimmon, H. R., and J. S. Campbell. 1969. World distribution of brook trout, *Salvelinus fontinalis*. J. Fish. Res. Board Can. 26:1699-1725.

PART II:
ALLOZYME GENETICS of BROOK TROUT in GREAT SMOKY MOUNTAINS NATIONAL PARK

Introduction

Between 1900 and the establishment of Great Smoky Mountains National Park in 1934, brook trout range in the Park area declined an estimated 60 - 70% (Lennon, 1967). Attempts to curb this decline and expand the range to its historical limits included establishment of fishing regulations and stocking with hatchery reared brook trout (King, 1942). Stocking of brook trout in the Park began in 1937. From 1937 through 1939 about 200,000 hatchery reared native Southern Appalachian brook trout were stocked annually into Park streams (King, 1942). Heavy stocking continued from 1940 through 1947 using hatchery strains derived from northeastern populations. Evidence from this experience indicated that northern derived hatchery strains used in stocking were less hardy than native populations and less likely to become established (Holloway, 1945). Extensive stocking efforts was reduced after 1947 but continued, employing northern derived hatchery strains, until 1975. Over the period 1940 - 1975 more than 800,000 fertilized eggs, fry, fingerlings, and adults from northern derived hatchery strains were stocked into at least 76 Park streams (McCracken et al, 1993). Only 12 streams in the Park have no record of stocking (Table 2.1). The effects of these introductions of northern derived domesticated strains into native Southern Appalachian brook trout gene pools are of obvious conservation concern, particularly if the source populations are genetically differentiated from indigenous populations.

In an unpublished report to the Park, Brussard and Nielsen (1976) concluded on the basis of allozyme variation that Southern Appalachian brook trout populations might differ from northeastern populations at the level of species or subspecies. Stoneking et al.

(1981) reached a similar conclusion from their observation of significant allele frequency differences between three southern and four northern populations at 4 of 39 allozyme loci, and estimated a mean genetic similarity of I = 0.890 between the two regions. Stoneking et al. (1981) also suggested that stocking of northern hatchery strains in the Southern Appalachians might be a confounding factor in their study.

McCracken et al. (1993) considered stocking history to examine genetic differences between native brook trout populations and the northern derived hatchery strains used for stocking in the Park. Five presumed native Southern Appalachian populations with no record of stocking with hatchery fish were sampled as well as three other Park streams that had documented stocking onto existing populations. Northern derived hatchery strains were represented by two strains used in stocking in the region (Pisgah and Owhi strains) and by a wild reproducing population from a Park stream, Meigs Creek, which was devoid of brook trout prior to stocking. McCracken et al. (1993) observed that unstocked populations and hatchery strains were fixed for different alleles at one locus (CK-A2*) and had significant allele frequency differences at an additional 9 of 16 polymorphic loci that contained alternative alleles of presumed northern ancestry. They estimated a mean genetic similarity (Nei, 1972) of I = 0.906 between unstocked and northern hatchery strains, comparable in magnitude to the estimate of Stoneking et al. (1981). The effects of hybridization were evident in the three populations where hatchery strains had been stocked onto extant populations. Homozygotes and heterozygotes were observed for both CK-A2* alleles and the alternative "northern" alleles were present at several other loci in the three stocked populations.

Here I report protein electrophoretic studies on the genetic structure of 47 brook trout populations from the Park (Table 2.2). Four of the populations studied were also sampled by McCracken et al. (1993), and one, Bunches Creek, was studied by McCracken et al. (1993) and by Stoneking et al. (1981). The total of 52 distinct populations sampled in this study and by McCracken et al. (1993) are thought to represent most of the known brook trout populations in the Park (Steve Moore, Great Smoky Mountains National Park, personal communication). I also examined two additional hatchery strains derived from northeastern populations, the EdRay strain and the Rome strain. These strains are known to have been stocked in the region, if not specifically in the Park. Examination of the diagnostic loci documented by Stoneking et al. (1981) and McCracken et al. (1993), and other diagnostic loci that are first reported here, allow me to evaluate the extent of hatchery strain introgression into native gene pools throughout the Park.

The extensive population coverage also allows me to investigate differentiation among native populations in the Park's major watersheds. The 209,376 hectare Great Smoky Mountains National Park is drained by four rivers, of unequal order, tributary to the Tennessee River (Figure 2.1). The Little Tennessee River and the Pigeon River originate in North Carolina east of the Park and skirt the southern and northern boundaries of the Park respectively. The Little River, which drains a portion of the southwestern part of the Park in Tennessee, is of lower order than the other two but is a direct tributary of the Tennessee River. Northeast of the Little River drainage area on the Tennessee side of the Park, streams flow into the Little Pigeon River. Both the Little

Pigeon River and the Pigeon River are tributary to the French Broad River which joins with the Holston River above Knoxville to form the Tennessee River. In this analysis I treat streams in the Little Pigeon and Pigeon Rivers as a single hydrological unit.

Methods

Collections. – Between 1992 and 1994, 47 wild brook trout populations in the Park were sampled by electrofishing. Two hatchery strains were sampled by dip netting from hatchery raceways. Only age 1 and older fish were collected. In the field, fish were euthanized with MS-222 (100mg/liter) after capture. Eyes, liver, and a skeletal muscle tissue sample were removed and immediately frozen in liquid nitrogen. Upon return to the laboratory tissues were stored at -80^{O} C prior to and after processing.

Sample size (Table 2.2) varied according to population densities observed in the field and according to long term data on population density. Because most streams lack long term census data, most samples were limited to 10 specimens to minimize possible negative effects on population viability. Three populations are represented by fewer specimens because of extremely low population densities observed at the time of sampling. Sample sizes from eight streams are larger than 10 specimens, and three of these streams with large brook trout populations were sampled on three separate occasions. Multiple samples were obtained from the same general stream locality in each of the three years of sampling. The two hatchery samples consist of 25 specimens each.

McCracken et al. (1993) examined five of the 12 streams in the Park with no record of stocking. Here I examine populations from six of the other seven unstocked

streams (Table 2.1). The remaining unstocked stream was not sampled because of low population density. The Meigs Creek population in the Park is a naturalized population derived from the Pisgah hatchery strain. Meigs Creek was devoid of brook trout prior to stocking (McCracken et al., 1993; John Boaz, Fish and Wildlife Associates, Inc., personal communication). The EdRay strain was collected at the state fish hatchery in Pisgah, North Carolina, and the Rome strain at the state hatchery in Marion, Virginia.

Protein electrophoresis. - Horizontal starch gel electrophoresis was used to examine all samples for variation in 15 muscle proteins encoded by 24 gene loci (Table 2.3). Electrophoresis of tissue extracts followed the procedures of Selander et al. (1971) and McCracken and Wilkinson (1988). Buffer systems for resolving brook trout allozymes are after May et al. (1979) and Stoneking et al (1981). Loci and alleles were designated as recommended by Shaklee et al. (1990). By convention, the most common mobility product was designated as the 100 allele. Lower frequency allelic products at a locus were identified by their mobility relative to the common allele. The practice of designating the common allele as the 100 mobility variant can lead to some confusion in comparing studies of the same taxon where the common allele at a locus differs between studies. For example, the CK-A2* 100* allele that is fixed or at high frequency in Southern Appalachian populations (McCracken et al., 1993) is synonymous with the 122* allele observed by Stoneking et al. (1981). Appendix 2.1 addresses the synonymy of the brook trout allozyme alleles reported in various previous studies.

Data analysis - Genetic variation within populations is assessed as percent polymorphic loci and heterozygosity. Observed and expected heterozygosities were calculated for each polymorphic locus in each population sample. The duplicated sAAT-1,2* locus and the duplicated sMDH-B1,2* locus were each treated as single loci in these analyses. Observed genotype frequencies were evaluated for conformance to Hardy-Weinberg expected frequencies with the fixation index F_{IS} and the G-test (Levene, 1949; Nei, 1977; Sokal and Rohlf, 1981). The average fixation index F_{IS} for all populations and subsets of populations was tested for significant difference from zero by the t-test a $p < 0.01$. The duplicated locus sAAT-1,2* was not included in these analyses because both loci are polymorphic in some populations and alleles could not be assigned to a specific locus.

Sample sizes and heterozygosities of most samples were not sufficient to attempt assessment of possible linkage disequilibrium. All pairs of polymorphic loci in samples with more than ten specimens were evaluated for conformity to expected two locus genotypes by Chi-square contingency analysis. Possible linkage disequilibrium was evaluated in these samples using the procedure of Hill (1974). Samples from populations sampled three times were evaluated independently and as a pooled sample. Association between CK-A2* 78* allele frequency and the frequency of alternative alleles at other loci was evaluated for samples polymorphic at CK-A2* and for all samples using the Pearson correlation coefficient.

Genetic heterogeneity among populations was evaluated with G-tests of allele frequencies (Sokal and Rohlf, 1981), standardized genetic variances (F_{ST}; Wright, 1978; Nei, 1977), genetic identity coefficients (Nei, 1972; 1978), and gene diversity analysis

(Nei, 1973; Chackraborty, 1980; Perkins et al., 1993). Variation at the duplicated sAAT-1,2* locus was partitioned equally between the two loci for purposes of these analyses (Krueger et al., 1989). Test statistics and coefficients were calculated for each pairwise comparison between and among all populations, groups of populations within watersheds, and populations classified as Park or hatchery. Multiple samples from the same population were pooled for these analyses. Heterogeneity among samples was evaluated for the three populations that had been sampled on three occasions. Rare alleles were pooled where appropriate in the contingency table analyses (Sokal and Rohlf, 1981). G-test critical values were adjusted to account for the increase in type I error associated with multiple independent tests of the null hypothesis (Cooper, 1968). The adjusted 0.01 alpha value for these tests was 0.001 using Sidak's multiplicative inequality (Sokal and Rohlf, 1981). G-values with more than 100 degrees of freedom were evaluated using Fisher's normal approximation (Fisher, 1953). Standardized genetic variances were estimated for loci polymorphic among all populations and among subsets of populations. Sampling variances of F_{ST} estimates were calculated following Workman and Niswander (1970).

For the gene diversity analysis total heterozygosity (H_T) of Park populations was partitioned into three components: average heterozygosity within samples ($\overline{H}_S$), among samples within river drainages ($D_{SW} = H_W - \overline{H}_S$), and among river drainages ($D_{WT} = H_T - H_W$), where H_W is the average heterozygosity of river drainages (watersheds). The three components of total heterozygosity ($\overline{H}_S$, D_{SW}, and D_{WT}) were divided by H_T to determine their relative contribution to total heterozygosity. Genetic data analysis was performed with the "Genes in Populations" microcomputer program by B. May, C. C. Krueger, and

W. Eng of Cornell University. Other statistical analyses were performed using the SAS System (SAS Institute, 1985).

Results

Genetic variation within samples - Ten of the 22 (45%) loci were polymorphic with alternative alleles at a frequency greater than 0.05 (Table 2.3; Appendix 2.2). Three additional loci carried rare alleles in one sample each. Thirty seven samples were polymorphic at one or more loci. Seventeen samples from the Park were identically monomorphic across all loci, and one sample was monomorphic at all loci but fixed for the DDH* 117* allele that was at lower frequencies in four other Park populations. Average polymorphism across all polymorphic loci (frequency of alternative alleles greater than 0) was 11.7% (Table 2.4). Average polymorphism of Park samples across all polymorphic loci was 9.4% (range: 0.0% - 36.4%). The Meigs Creek samples were polymorphic at an average of 30.3% of the loci examined (range: 22.7% - 36.4%). The EdRay and Rome hatchery strain samples were polymorphic at 31.8% and 18.2% of the loci respectively.

Mean expected heterozygosity ($\overline{H}_S$) was 0.053 for all samples (range: 0.0 – 0.143), 0.032 (range: 0.0 - 0.143) for the samples from the Park , and 0.096 (range: 0.070 - 0.130; Table 2.4) for the hatchery and Meigs Creek samples. Four of 89 observed genotype frequencies exhibited deviations from Hardy-Weinberg expectation by the G-test (Table 2.5). This is the number that would be expected by chance alone at the 5% level. These deviations were found in different populations and included heterozygote

deficiencies at three loci (GPI-B2*, LDH-B1*, and PEPB*) and a heterozygote excess at one locus (G3PDH-1*). Average fixation indices (F_{IS}) were not significantly different from zero in any of the subsets examined.

Samples from 35 Park populations, including samples from the seven unstocked streams, were fixed for the CK-A2* 100* allele (Appendix 2.2), and fifteen samples from eleven populations carried the 100* allele at high frequency (average frequency of the 100* allele: 0.79; range: 0.50 - 0.95; Table 2.6). The Meigs Creek samples and the hatchery strain samples were fixed for the CK-A2* 78* allele. The Dunn Creek population from the Park carried an 83* allele at the CK-A2* locus at a frequency of 0.16, in addition to the 100* and 78* alleles. The 83* allele has not been reported in previous studies of brook trout allozyme variation. Following McCracken et al. (1993), Park populations fixed for the CK-A2* 100* allele are designated "native" populations, and those polymorphic at the CK-A2* locus are designated "hybrid." The EdRay and Rome hatchery strains and the hatchery derived Meigs Creek population are referred to collectively as hatchery strains.

Variation at PEPB*, which has not been previously reported, exhibited a pattern similar to and largely concordant with variation at CK-A2* (Table 2.6). Six of the seven unstocked populations were fixed for the PEPB* 100* allele (Table 2.7). The putatively unstocked McGinty Branch population (MGB) was polymorphic for PEPB* with the 68* allele at a frequency of 0.05. The two hatchery strain samples were fixed for the 68* allele. The samples from the Meigs Creek population were polymorphic with the 68* allele at a high frequency (average frequency of the 68* allele: 0.83; range: 0.77 - 0.86;

Table 2.7). Overall, 37 populations from the Park were fixed for the PEPB* 100* allele and 13 samples from 11 populations carried the 100* allele at high frequency (average frequency of the 100* allele: 0.78; range: 0.30 - 0.97). Ten of the Park samples polymorphic at PEPB* were also polymorphic at the CK-A2*.

Unstocked Park populations and hatchery samples also had different common alleles at AAT-1,2* and GPI-B2* (Tables 2.6, 2.7). The AAT-1,2* 118* allele was fixed in 29 Park samples and was the common allele in 15 samples (average frequency of the AAT-1,2* 118* allele: 0.78). The AAT-1,2* 100* allele was the high frequency allele in the hatchery derived Meigs Creek samples and in the hatchery samples (average frequency of the AAT-1,2* 100* allele: 0.83). The highest frequency of the AAT-1,2* 100* allele among Park populations was observed in the Taywa Creek samples (average frequency: 0.64). The Taywa Creek samples also had the highest CK-A2* 78* allele frequency among Park populations (average frequency, 0.47; Appendix 2.2).

Thirty five Park samples were fixed for the GPI-B2* 70* allele and the other 11 carried the 70* allele at high frequency (average frequency of the GPI-B2* 70* allele: 0.75; range: 0.50 - 0.99). The GPI-B2* 70* allele was not observed in the hatchery or Meigs Creek samples. Two of the Meigs Creek samples and the Rome hatchery strain were fixed for the GPI-B1* 100* allele and one Meigs Creek sample and the EdRay hatchery strain sample carried the 100* allele at high frequency and the 40* allele at low frequency (Table 2.7).

Alleles at four other loci that were polymorphic in two or more samples (Appendix 2.2) showed patterns of variation that appear to reflect strain or stocking

history but which are not diagnostic between hatchery strains and Park populations. Unstocked streams were fixed for the 100* alleles at G3PDH-1*, LDH-B1*, MDH-B1,2* and sMEP-1*. These loci were polymorphic in the hatchery samples and in few Park populations that had been stocked, but the 100* allele was the common allele in all samples.

Low frequency polymorphisms at DDH* and PROT-1* also exhibited variation among samples indicating allelic differences between native populations and hatchery strains. One Park sample was fixed for the DDH* 117* allele, one carried the 117* allele at a frequency of 0.80, and three samples had the 117* allele at low frequency (average frequency, 0.27). All other Park samples and the hatchery samples were fixed for the DDH* 100* allele. Polymorphism at PROT-1* was observed in two of the Meigs Creek samples and the EdRay hatchery strain, and in three Park samples that were also polymorphic at the CK-A2* locus. The average frequency of the PROT-1* -130* allele in these six samples was 0.04 (range: 0.02 - 0.06).

Three loci were polymorphic with a single heterozygous individual in one sample each. I observed the GPI-B1* 150* allele in the Chasteen Creek sample and the LDH-A2* 44* allele in a Meigs Creek sample. The Dunn Creek sample had an 84* mobility allele at the MDH-A2* locus. Variation at the brook trout MDH-A2* locus has not been observed previously.

Association between alleles – The observed two-locus genotype frequencies for all pairs of loci were not significantly different from those expected with random assortment of

alleles. Statistically significant linkage disequilibrium was not detected between any pair of loci. However, in samples that were polymorphic for CK-A2*, the frequency of the CK-A2* 78* allele was significantly correlated with the frequencies of PEPB* 68*, LDH-B1* 67*, AAT-1/2* 100*, and sMEP-1* 63* alleles (Table 2.8). The frequency of the CK-A2* 78* allele was not significantly correlated with low frequency alleles at the MDH-B1,2*, GPI-B2*, G3PD-1*, and PROT-1* loci in samples polymorphic at CK-A2*. However, across all samples the CK-A2* 78* allele frequency was significantly correlated with low frequency alleles at all loci examined except MDH-B1,2* (Table 2.8)

Genetic variation among samples - Significant allele frequency heterogeneity was observed among all samples and all subsets of samples. The number of loci showing significant heterogeneity ranged from nine of the ten polymorphic loci among all samples, and among the subset of Park samples fixed for the CK-A2* 100* plus hatchery, to five of 10 loci for the subset of Park samples fixed for the CK-A2* 100* allele (Table 2.9). No significant heterogeneity was observed among the three Hyatt Creek samples or among the three Taywa Creek samples (Table 2.10). The LDH-B1* locus exhibited significant allele frequency heterogeneity among the three Meigs Creek samples. Across all polymorphic loci, allele frequency heterogeneity among the three Meigs Creek samples was not significant.

The average standardized genetic variance (F_{ST}) for all populations was F_{ST} = 0.542 (Table 2.9). The highest F_{ST}, 0.659, was for the subset Park native plus hatchery. The lowest F_{ST}, 0.113, was observed for the hatchery subset. Because of the large total

sample sizes, sampling variances of F_{ST} estimates were small, 0.001 for all populations and all subsets of populations except the subsets hatchery and Park hybrid. Sampling variances for these subsets were 0.005 and 0.002 respectively. Average F_{ST} values for polymorphic loci were significantly different from zero for all sample subsets examined.

The total average heterozygosity (H_T) in all Park samples was 0.050 (Table 2.4). Within sample variation ($\overline{H}_S / H_T = 1 - F_{ST}$) accounted for 64% of the total gene diversity among Park samples. Variation among river drainages (D_{RT} / H_T) accounted for 16% of the total, and variation among samples within watersheds (D_{SR} / H_T) accounted for the remaining 20% of total gene diversity (Table 2.11). The total average heterozygosity of Park samples fixed for the CK-A2* 100* allele (i.e., native populations), was $H_T = 0.025$. Partitioning of gene diversity in this subset was similar to that observed for the set of all Park samples: 56% was due to variation within samples, 12% was due to variation among watersheds, and 32% was due to variation among samples within watersheds (Table 2.11).

The average normalized genetic identity (Nei's I) among all samples was 0.950 (range: 0.718 - 1.0; Table 2.12). Among all Park populations the average genetic identity was 0.985 (range: 0.859 - 1.0). The highest genetic identity was observed among the subset Park samples fixed for the CK-A2* 100* allele (natives), $\bar{I} = 0.988$ (range: 0.925 - 1.0). Genetic identities among the population subsets Park samples polymorphic at CK-A2* (hybrids, $\bar{I} = 0.981$; range: 0.922 - 1.0), hatchery ($\bar{I} = 0.978$; range: 0.973 - 0.986), and Park natives plus Park hybrids ($\bar{I} = 0.973$; range: 0.859 - 1.0) were similar. The lowest genetic identity was observed among the subset Park natives plus hatchery ($\bar{I}$ =

0.778; range: 0.718 - 0.832; Table 2.12). The normalized genetic identities among multiple samples from the same population (Table 2.13) were, 0.997 (HYC), 0.991 (TAY), and 0.993 (MEG). The matrix of normalized genetic identities for all pairwise comparisons is provided in Appendix 2.3.

Discussion

Identification of marker alleles - This study corroborates and extends our earlier findings (McCracken et al., 1993) that native (unstocked) Park brook trout populations and northern derived hatchery strains differ at a number of loci. As in earlier studies (Stoneking et al., 1981; McCracken et al., 1993), the samples from all unstocked streams were fixed for the CK-A2* 100* allele (Table 2.7). Also concordant with earlier studies, the naturalized Meigs Creek samples and the hatchery strain samples were fixed for the CK-A2* 78* allele. Stoneking et al. (1981) also observed that the 78* allele (designated the 100* allele) was fixed in their samples from New York and Pennsylvania. All unstocked, naturalized, and hatchery samples that were examined by Perkins et al. (1993) from New York and Pennsylvania were also fixed for the same slower allele. I conclude that presence of the CK-A2* 78* allele in Park populations is evidence of introgression with northern derived hatchery strains and that the frequencies of the northern allele can provide an index of the relative intensities of hatchery introgression.

The frequencies of the common alleles at PEPB*, AAT-1,2* and GPI-B2* also differed between native brook trout populations in the Park and northern derived hatchery strains, but the differences did not involve fixation of alternative alleles in the two

groups. The presence of the PEPB* 68* allele and the GPI-B2* 100* allele in some Park populations appears to suggest introgression with hatchery strains, but the presence of these alleles at a low frequency in some unstocked populations cannot be excluded. The hatchery derived Meigs Creek population and the reportedly unstocked McGinty Branch population are polymorphic for PEPB*, and I cannot determine if these polymorphisms are consequences of recent hybridization or reflect low frequency polymorphism in source populations. The relatively high frequency of the GPI-B2* 100* allele segregating in the McGinty Branch population suggests that this reportedly unstocked population may have in fact received undocumented stocking.

It seems likely that the high frequency of the AAT-1,2* 100* allele in Park populations is indicative of introgression from hatchery strains, but it also appears that some unstocked native populations carry the AAT-1,2* 100* allele at low frequency. Correlation between CK-A2* 78* allele frequencies and the frequencies of alternative alleles at G3PDH-1*, LDH-B1*, sMEP-1*, and PROT-1* suggests a hatchery origin of these alleles as well, but low frequency polymorphism in native Park populations cannot be excluded.

Polymorphism and heterozygosity - My data corroborate the observations of McCracken et al. (1993) and Kriegler et al. (1995) that unstocked Southern Appalachian populations show relatively low average heterozygosity and polymorphism. Both these measures of genetic variability within populations were highest for the hatchery strains, lowest for the native populations, and intermediate for the hybrid populations. Unstocked northern

populations also have higher heterozygosity and polymorphism than southern populations (Perkins et al., 1993). The low variability found in native Southern Appalachian populations is an anticipated consequence of genetic drift and inbreeding in small populations. Whether this is due to recent population constrictions or to earlier population bottlenecks can not be determined from my data. Historic records of brook trout distribution in the Park area indicate that prior to declines early in the 20th century, populations inhabited contiguous first, second, and third order stream segments of a watershed. These potentially or actually interbreeding populations were reproductively isolated from numerous other populations in other high order watershed networks. This physiographically subdivided population structure likely resulted in random loss and retention of different alleles in different subpopulations. Population constrictions and habitat loss in this century reduced effective population sizes of the remaining populations and would have accelerated the loss of heterozygosity and the chance retention and loss of alleles in subpopulations.

Genetic differentiation - My estimates of genetic similarity (Nei's *I*) indicate substantial genetic divergence between brook trout populations from Great Smoky Mountains National Park and hatchery strains used in stocking. The average genetic identity between hatchery strains and samples fixed for the CK-A2* 100 allele ($\bar{I}$ = 0.778) was the lowest observed among all subsets and across all samples (Table 2.12). This estimate is in contrast to the high average identity observed between all Park samples ($\bar{I}$ = 0.985) and between all hatchery samples ($\bar{I}$ = 0.978). These estimates of genetic identity may be

usefully compared to estimates obtained from other studies of allozyme variation in brook trout. Examining a larger number of loci, Perkins et al. (1993) obtained estimates of I ranging from 0.910 to 0.999 in comparisons between 28 samples of wild and hatchery brook trout in New York and Pennsylvania. In an earlier study Stoneking et al. (1981) obtained a mean I of 0.973 between populations from New York and Pennsylvania, a mean I of 0.955 between populations from Tennessee and North Carolina, and a mean I of 0.890 between the northern and southern populations. These are similar in magnitude to our earlier estimate of a mean similarity of 0.995 between unstocked populations from the Park and of a mean similarity of 0.906 between these unstocked populations and three hatchery strains (McCracken et al., 1993). Our current estimate of a mean genetic similarity of 0.778 is clearly at the low end of observed genetic similarity between brook trout populations. This extremely low value results in part from the calculation of I from a subset of loci that were selected in this study because they are informative.

Our assessment of differentiation among brook trout populations at allozyme loci does not directly address taxonomy within *Salvelinus fontinalis*, and we recognize the limitations of making taxonomic assessments on the basis of biochemical data (Frost and Hillis, 1990; Dowling et al., 1992). However it is clear from our data that populations from Great Smoky Mountains National Park and northern derived hatchery strains are significantly differentiated. This points to the existence of two evolutionarily significant units within the taxon, an observation that is of considerable biogeographical interest and importance to the conservation of biodiversity.

Genetic structure among populations within Great Smoky Mountains National Park - The heterogeneity among brook trout populations in the Park, as evidenced by the high standardized genetic variance, indicates that these populations cannot be treated as a homogeneous unit. Across all Park samples, 36% of the gene diversity as measured by F_{ST} is due to variation among samples (Table 2.11). Partitioning of gene diversity using heterozygosity estimates shows even higher variation among populations monomorphic for the CK-A2* 100* allele. In these presumably native populations, an estimated 44% of the gene diversity is due to variation among samples.

Brook trout populations in the Park are reproductively isolated as a consequence of historic habitat distribution and recent range constrictions. Population estimates for most Park populations are lacking, but it is likely that effective population sizes for most are less than 100 (Steve Moore, personal communication). Estimates of brook trout range in the Park in 1900 suggest that the effective population sizes of historic populations were probably much larger. Genetic heterogeneity among populations is an expected consequence of genetic drift in small, reproductively isolated populations. Heterogeneity among watersheds and river drainages reflects more temporally distant founder events and gene flow restrictions relative to the location of populations along stream order networks. I estimate that 12% - 16% of the current total gene diversity among Park populations is due to variation among river drainages. The probable consequences of recent population constrictions are both a decrease in total gene diversity due to loss of alleles by genetic drift, and a decrease in the proportion of heterozygosity due to differentiation among river drainages.

Hybridization - Eleven of 47 Park populations sampled in this study gave evidence at the CK-A2* locus of hybridization with hatchery strains. Samples from seven populations were monomorphic for the CK-A2* 100* allele but carried suspected hatchery alleles at one or more other loci (G3PDH-1*, GPI-B2*, LDH-B1*, sMEP-1*, PEPB*). Unfortunately the hatchery origin of these alleles cannot be established with certainty, nor can the absence of CK-A2* 78* alleles definitively preclude hatchery introgression. However, the relatively small number of samples (23.4%) containing the diagnostic CK-A2* 78* allele is surprising given the intensity of documented stocking and the small number of streams with no record of stocking. This observation of intense stocking, and the low sample size from most populations, encourages the suspicion that 23.4% (or 38.3% if I include populations with other suspected hatchery alleles) is an underestimate of the number of brook trout populations in the Park that carry hatchery genes. This is likely to be the case, but the only clear conclusion that can be drawn from the allozyme data is that a relatively low percentage of Park populations carry high frequencies of hatchery genes.

The low frequencies of diagnostic hatchery alleles in Park populations suggest low levels of introgression. Only two Park populations, Taywa Creek and Indian Flats Branch, carried the CK-A2* 78* allele at a frequency of 0.35 or greater (Appendix 2.2).. The average frequency of the CK-A2* 78* allele in the other hybrid populations was 0.10. The Taywa Creek and Indian Flats Branch populations also had the putative hatchery PEPB* 68* allele at the highest frequency among Park populations. The absence

of gametic phase disequilibrium between CK-A2* and PEPB* alleles in hybrid populations suggests that these populations are randomly mating hybrid swarms. Random association of alleles at loci diagnostic between native populations and hatchery strains also suggests that selection against these alleles or against alleles at linked loci is weak or is not occurring. However, the low levels of detectable introgression may reflect past selection against hatchery or hybrid genotypes in stocked populations. Support for this hypothesis comes from an observed correlation between the recency but not the intensity of stocking, and levels of mtDNA and allozyme introgression in populations from Tennessee outside the Park (Kriegler et al., 1995; Hayes et al., 1996). Long term genetic monitoring of hybrid populations are required to determine if hatchery alleles decline in hybrid populations over time, and determination of the relative roles of selection and drift in the dynamics of hybrid gene pools will require comparative habitat studies and studies of the relative fitness of different genotypes in wild populations. Holloway's (1945) contention that stocking with hatchery strains was ineffective in stemming the decline of brook trout in the Park because hatchery fish were not sufficiently hardy to become established or were rapidly fished out, remains the most parsimonious explanation for the low levels of hatchery introgression in Park brook trout gene pools.

Sample size and sampling bias - The small sample sizes from most populations impose limitations on interpretation of the data. Small sample sizes are expected to underestimate polymorphism through failure to encounter rare or low frequency alleles (Archie et al., 1989; Sjogren and Wyoni, 1994). Failure to detect low frequency alleles is an especially

important concern in studies of gene flow (Slatkin, 1985), including studies such as this one examining levels of hybridization under secondary contact. Finite allele model simulations indicate that in randomly mating populations with more than 80 individuals, sample sizes of 25 - 30 are necessary for a 95% probability of detecting alleles with a frequency of 0.05 (Sjogren and Wyoni, 1994). Few of the samples in this study are this large. Although population sizes of most Park brook trout populations have not been estimated, samples of 10 individuals are most certainly inadequate to detect rare and low frequency alleles with statistical confidence. Estimates of allele frequencies and single locus heterozygosity are also distorted in small samples. For example, frequencies of 0.0, 0.05, 0.10, 0.15, etc. are inevitable estimates from samples of 10 individuals (Garcia-Main and Pla, 1996).

The effects of sample size on estimates of average heterozygosity and genetic identity are not as severe. The number of loci examined has a substantially greater impact on these calculations than sample size (German and Renzi, 1979). However, Lewontin's (1974) admonition that "... on the order of 100 randomly chosen loci..." are required to obtain estimates of heterozygosity and polymorphism with acceptably low variances is superseded by advances in techniques for examining genomic level variation and in our understanding of genomic complexity. Nei's (1978) suggestion that "...ideally more than 50 loci should be used to obtain a reliable estimate of average heterozygosity for the total genome" explicitly addresses the problem posed in interpretations of allozyme variation: to what extent is electrophoretically and histochemically detectable protein variation representative of genomic level variation? While electrophoretically detectable variation

of proteins is probably a biased sampling of genomic variation (Gillespie and Kojima, 1968; Leigh Brown and Langley, 1979), allozyme analyses have clear demonstrable utility where marker alleles are used to investigate gene flow, hybridization, and geographical variation (Avise, 1994). Questions of sample size and number of loci then become questions specific to the research problem, and interpretations beyond these must be confined to levels of detectable protein variation rather than levels of genome wide variation.

On average, samples of 8 - 12 individuals examined at 20 - 30 arbitrarily selected allozyme loci yield estimates of allozyme heterozygosity within 1% of estimates obtained with samples of 20 -30 individuals sampled at the same loci (Gorman and Renzi, 1979). This suggests that interpretations based on allozyme heterozygosity estimates (genetic variance, genetic identity, and hierarchical gene diversity) can be made with some fair level of confidence. Detection of low frequency alleles is another matter.

Many of the proteins sampled in my study were selected for their potential informative value in examining hybridization and differentiation, and therefore are not an arbitrary sample. That is, I specifically examined loci known or suspected to be highly variable in brook trout populations. Therefore, levels of allozyme heterozygosity and average genetic variance are overestimated, and genetic identity is underestimated. However, underestimates of genetic identities would not bias the relative magnitude of genetic identities between the population subsets examined.

Management Implications - Brook trout populations in the Park and elsewhere in the Southern Appalachians have declined substantial since 1900. The molecular genetic data indicate that stocking with hatchery brook trout contributed little to the persistence of brook trout in the Park, and did not promote downstream expansion. An important and positive consequence of the limited effectiveness of hatchery stockings is that many native brook trout gene pools have not been severely modified through introgression of hatchery genes. About 75% of Park populations have no evidence of hatchery genes in my samples, and most of the hybrid samples have hatchery genes at low frequency. As discussed above, the small sample sizes from most populations are likely to make this an underestimate of the number of populations carrying hatchery genes. However, the conclusion that levels of hatchery introgression are low is unaffected. For purposes of management emphasizing native gene pool conservation, populations with no evidence of hatchery introgression should be treated as native Southern Appalachian populations.

Evidence of relatively low levels of hatchery introgression in Park populations in no way argues for a role of hatchery strains in fisheries management in the Park. Consistent with National Park Service policy (National Park Service, 1988) to manage for native taxa and strains, stocking with hatchery strains should not be considered an option. Management efforts should instead focus on maintenance of habitat quality, removal of rainbow trout from selected streams to permit establishment of lower elevation brook trout populations, and establishment of native brook trout populations in streams from which brook trout have been extirpated. Because there is significant heterogeneity among native populations in the Park, establishment of new populations in the Park should be

through stock transfer or hatchery propagation of native populations from adjacent watersheds within river drainages. The allozyme data presented in this paper should be employed in making such decisions.

The goal of re-establishing a brook trout sport fishery in the Park should also be informed by the allozyme data. Fishery regulations such as size and take limits are primarily instituted in response to and for control of target population demographics. Predicated on the assumption that some populations have greater native biodiversity value than others, the genetic data may be of use in suggesting populations to open to sport fishing. For example, opening the hatchery derived Meigs Creek population to fishing would help meet public desire for a brook trout fishery and might be employed as a means of eradicating the population. With the hatchery population eradicated or reduced, Meigs Creek would become a candidate for introductions of native brook trout from other populations in the Little River watershed. Similarly, the hybrid population in Taywa Creek could probably support an active regulated fishery, but even in the event of a decline induced by overfishing, the loss would be of little consequence in terms of loss of native biodiversity and would open a location for stocking with local native populations.

Literature cited

Archie, J. W., C. Simon, and A. Martin. 1989. Small sample size does decrease the stability of dendrograms calculated from allozyme frequency data. Evolution 43:678-683.

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman & Hall, NY.

Bivens, R. D. 1985. History and distribution of brook trout in the Appalachian region of Tennessee. Tennessee Wildlife Resources Agency Technical Report No. 85-1. The University of Tennessee, Knoxville.

Bivens, R. D., R. J. Strange, and D. C. Peterson. 1985. Current distribution of the native brook trout in the Appalachian region of Tennessee. J. Tenn. Acad. Sci. 60:101-105.

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

Chakraborty, R. 1980. Gene diversity analysis in nested subdivided populations. Genetics 96:721-726.

Clayton, J. W., and D. N. Tretiak. 1972. Amine-citrate buffers and pH control in starch gel electrophoresis. J. Fish. Res. Board Can. 29:1169-1172.

Cooper, D. W. 1968. The significance level in multiple tests made simultaneously. Heredity 23:614-617.

Dowling, T. E., B. D. Demarais, W. L. Minckley, M. E. Douglas, and P. C. Marsh.1992. Use of genetic characters in conservation biology. Conserv. Biol. 6:7-8.

Fisher, R. A. 1954. Statistical methods for research workers. 12th ed. Oliver and Boyd, Edinburgh.

Frost, D. R., and D. M. Hillis. 1990. Species in concept and practice: herpetological applications. Herpetologica 46:97-104.

Flebbe, P. A. 1994. A regional view of the margin: Salmonid abundance and distribution in the Southern Appalachian Mountains of North Carolina and Virginia. Trans. Am. Fish. Soc. 123:657-667.

Garcia-Marin, J. L., and C. Pla. 1996. Origins and relationships of native populations of *Salmo trutta* (brown trout) in Spain. Heredity 77:313-323.

Gillespie, J. H. and K. Kojima. 1968. The degree of polymorphism in enzymes involved in energy production compared to that in nonspecific enzymes in two *D. ananassae* populations. Proc. Natl. Acad. Sci. USA 61:582-85.

Gorman, G. C., and J. Renzi, Jr. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies; effects of sample size. Copeia 1979:242-249.

Habera, J. W., and R. J. Strange. 1993. Wild trout resources and management in the Southern Appalachian Mountains. Fisheries 18:6-13.

Hill, W. G. 1974. Estimation of linkage disequilibrium in randomly mating populations. Heredity 33:229-239.

Holloway, A. D. 1945. Report on the fisheries of the Great Smoky Mountains National Park. Unpublished internal report to Great Smoky Mountains National Park, Gatlinburg, Tennessee.

Jones, R. D. 1978. Regional distribution trends of the trout resource. Pp. 1-10 *In* Harshbarger, T. J. (ed.), Southeastern Trout Resource: Ecology and Management Symposium Proceedings. U. S. Forest Service, Southeastern Forest Experiment Station, Asheville, North Carolina.

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

Kincaid, H. L. 1981. Trout strain registry. U.S. Fish and Wildlife Service, National Fisheries Center - Leetown / 81-1, Kearneysville, West Virginia.

King, W. 1937. Notes on the distribution of native speckled trout and rainbow trout in the streams of Great Smoky Mountains National Park. J. Tenn. Acad. Sci. 12:351-361.

King, W. 1942. Trout management studies at Great Smoky Mountains National Park. J. Wild. Manag. 6:147-61.

Kriegler, F. J., G. F. McCracken, J. W. Habera, and R. J. Strange. 1995. Genetic characterization of Tennessee brook trout populations and associated management implications. N. Am. J. Fish. Manag. 15:804-813.

Krueger, C. C., J. E. Marsden, H. L. Kincaid, and B. May. 1989. Genetic differentiation among lake trout strains stocked into Lake Ontario. Trans. Am. Fish. Soc.118:317-330.

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

Leary, R. F., F. W. Allendorf, S. R. Phelps, and K. L. Knudsen. 1987. Genetic divergence and identification of seven cutthroat subspecies and rainbow trout. Trans. Am. Fish. Soc. 116:580-587.

Leigh Brown, A. J. and C. H. Langley. 1979. Reevaluation of level of genic heterozygosity in natural populations of *Drosophila melanogaster* by two-dimensional electrophoresis. Proc. Natl. Acad. Sci. USA 76:2381-2384.

Levene, H. 1949. On a matching problem arising in statistics. Annals Mathematical Stat. 20:91-94.

Lewontin, R. C., 1974. The genetic basis of evolutionary change. Columbia University Press, New York.

McCracken, G. F., C. R. Parker, and S. Z. Guffey. 1993. Genetic differentiation and hybridization between stocked hatchery and native brook trout in Great Smoky Mountains National Park. Trans. Am. Fish. Soc. 122:533-542.

McCracken, G. F., and G. S. Wilkinson. 1988. Allozyme techniques and kinship assessment in bats. Pp. 141-156 *In* T. H. Kunz (ed.), Ecological and behavioral methods for the study of bats.

May, B., J. E. Wright, and M. Stoneking. 1979. Joint segregation of biochemical loci in Salmonidae: results from experiments with *Salvelinus* and review of the literature on other species. J. Fish. Res. Board Can. 36:1114-1128.

National Park Service. 1988. Management policies. National Park Service, Washington.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.

------. 1973. Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA 70:3321-3323.

------. 1977. F-statistics and analysis of gene diversity in subdivided populations. Ann. Human Genetics 41:225-233.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 41:225-233.

Perkins, D. L., C. C. Kruger, and B. May. 1993. Heritage brook trout in northeastern USA: Genetic variability within and among populations. Trans Am. Fish Soc. 122:515-532.

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

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus* I. Variation in the old field mouse (*Peromyscus polionotus*). Univ. Texas Studies in Genetics 6:49-90.

Shaklee, J. B., F. W. Allendorf, D. C. Moritz, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Trans. Am. Fish. Soc. 119:2-15.

Sjogren, P., and P.-I. Wyoni. 1994. Conservation genetics and detection of rare alleles in finite populations. Conserv. Biol. 8:267-270.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evol. 39:53-65.

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

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

Workman, P. L., and J. D. Niswander. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Human Genetics 22:24-49.

Wright, S. 1978. Evolution and the genetics of populations: 4 variability within and among natural populations. University of Chicago Press.

Table 2.1. Streams of Great Smoky Mountains National Park with no record of stocking with hatchery brook trout.

Stream	Reference
Little Tennessee River	
Bunches Creek	Stoneking et al., 1981 McCracken et al., 1993 this study
Flat Creek	McCracken et al., 1993
upper Raven Fork	this study
Enloe Creek	this study
Little River	
Sams Creek	this study
Starkey Creek	McCracken et al., 1993
Silers Creek	this study
Grouse Creek	not sampled
Pigeon River	
Buck Fork	McCracken et al., 1993
Eagle Rocks Prong	McCracken et al., 1993
Lost Bottoms Creek	this study
McGinty Branch	this study

Table 2.2: Brook trout populations sampled in Great Smoky Mountains National Park by drainage, sample abbreviation, and sample size (n).

Sample	Sample Abbreviation	n
Little Tennessee River		
Aden Creek	ADN	9
Balsam Corner Creek	BCC	10
Beech Flats Prong	BFP	15
Bunches Creek	BUN	38
Chasteen Creek	CHS	10
Defeat Creek	DFC	10
Desolation Creek	DSC	10
Enloe Creek	ENL	10
Hazel Creek	HAZ	10
Huggins Creek	HUG	10
Hyatt Creek	HYC2	28
	HYC3	10
	HYC4	23
Jack Bradley Creek	JBC	10
Keeyuga Creek	KEE	10
Kanati Fork	KNF	10
Peruvian Branch	PBR	10
Proctor Creek	PRC	10
Raven Fork	RAV	10
Straight Fork	STF	18
Steel Trap Creek	STL	10
Taywa Creek	TAY1	20
	TAY2	10
	TAY3	27
Walker Branch	WAC	10
French Broad River		
Andy Branch	ANB	10
Beech Creek	BCK	10
Big Creek	BGC	5
Cooks Creek	CCR	10
Camel Hump Creek	CHC	10
Conrad Branch	CON	10
Correll Branch	COR	10
Dunn Creek	DUN	34
Gunter Fork	GFK	10

Table 2.2 continued.

Sample	Sample Abbreviation	n
French Broad River (continued)		
Indian Camp Creek	ICC	39
Little Greenbriar Creek	LGB	20
Lost Bottoms Creek	LBC	10
McGinty Branch	MGB	10
Pretty Hollow Creek	PHC	10
Rock Creek	RCR	26
Road Prong	RPG	10
Woody Creek	WDY	7
Little River		
Indian Flats Branch	IFB	10
Lynn Camp Prong	LCP	10
Marks Creek	MRC	10
Meigs Post Creek	MPC	10
Rough Fork	RGH	10
Sams Creek	SAM	8
Silers Creek	SIL	14
Sweet Creek	SWE	10
Hatchery Strains		
Meigs Creek	MEG2	18
	MEG3	11
	MEG4	11
Rome	ROM	26
Ed Ray	EDY	25

Table 2.3. Enzymes, locus designations, proportion of samples polymorphic (%P), number of alleles, tissue sources and electrophoresis buffer systems used in the study. Enzyme numbers follow the reccomendations of IUBNC (1984). Locus nomenclature follows Shaklee et al. (1990). Tissues used were skeletal muscle (M) and eye (E). Electrophoresis buffers were morpholine-citrate, pH 6.1 (C) after Clayton and Tretiak (1972) as modified by May et al. (1979), and discontinuous lithium-borate (R) after Ridgeway et al. (1970).

Enzyme or other protein	Enzyme number	Locus	%P	Number of alleles	Tissue	Buffer system
Aspartate aminotransferase	2.6.1.1	sAAT-1, 2*	47	3	M	R
Creatine kinase	2.7.3.2	CK-A1*	0	1	M	R
		CK-A2*	27	3	M	R
Dihydrolipoamide dehydrogenase	1.8.1.4	DDH-3*	9	2	M	C
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH-1*	15	2	M	R
Glucose-6-phospate isomerase	5.3.1.9	GPI-A*	0	1	M	R
		GPI-B1*	2	2	M	R
		GPI-B2*	31	3	M	R
L-Lactate dehydrogenase	1.1.1.27	LDH-A1*	0	1	M	R
		LDH-A2*	2	2	M	R
		LDH-B1*	24	3	M, E	R
		LDH-B2*	0	1	M, E	R

Table 2.3 continued.

Enzyme or other protein	Enzyme number	Locus	%P	Number of alleles	Tissue	Buffer system
Malate dehydrogenase	1.1.1.37	sMDH-A1*	0	1	M	C
		sMDH-A2*	2	2	M	C
		sMDH-B1,2*	22	2	M	C
Malic enzyme ($NADP^+$)	1.1.1.40	sMEP-1*	31	2	M	C
Peptidase-B[a]	3.4._._	PEPB*	29	2	M	C
Peptidase-S[b]	3.4._._	PEPS*	0	1	M	R
General (unidentified) protein	No number	PROT-1*	11	2	M	R
		PROT-2*	0	1	M	R
		PROT-3*	0	1	M	R
		PROT-4*	0	1	M	R

[a] Peptidase-B resolved on leu-gly-gly substrate

[b] Peptidase-S resolved on leucyl-alanine substrate

Table 2.4. Genetic variability within samples: Summary statistics. N is the number of samples; average H_S is the average expected heterozygosity of samples in the subset; H_T is the expected heterozygosity of pooled samples; P is the average proportion of loci polymorphic in the samples (frequency of the common allele less than 1.0).

	All samples	All Park samples	Samples fixed for CK-A2* 100*	Samples polymorphic at CK-A2*	Hatchery samples
N	55	50	35	15	5
Average H_S	0.053	0.032	0.014	0.074	0.096
(Standard error)	(.015)	(0.010)	(0.005)	(0.005)	(0.030)
range	0 - 0.143	0 - 0.143	0 - 0.044	0.008 - 0.143	0 .070 - 0.130
H_T	0.111	0.050	0.025	0.096	0.109
(Standard error)	(0.031)	(0.015)	(0.009)	(0.030)	(0.035)
Average P	0.117	0.099	0.031	0.242	0.291
range	0 - 0.364	0 - 0.364	0 - 0.227	0.045 - 0.364	0.174 - 0.364

Table 2.5. Deviations from Hardy-Weinberg expectation.

Sample - Locus Genotypes	Observed	Expected
BGC - GPI-B2*		
100*/100*	2	0.8
70*/100*	0	2.4
70*/70*	3	1.8
IFB - PEPB*		
100*/100*	4	2
68*/100*	1	5
68*/68*	5	3
MEG4 - G3PD*		
100*/100*	1	2.5
78*/100*	8	5
78*/78*	1	2.5
TAY3 - LDH-B1*		
100*/100*	14	10.7
67*/100*	6	12.6
67*/67*	7	3.7

Table 2.6. Average allele frequencies of CK-A2*, PEPB*, AAT-1,2* and GPI-B2* in unstocked populations, samples fixed for the CK-A2* 100* allele, samples polymorphic at CK-A2*, and hatchery strains.

Population subset	CK-A2*		PEPB*		AAT-1,2*			GPI-B2*		
	100*	78*	100*	68*	100*	80*	118*	100*	40*	70*
Unstocked	1.0	-	0.99	0.01	0.05	-	0.95	0.05	-	0.95
Fixed CK-A2*	1.0	-	0.99	0.01	0.06	-	0.94	0.04	-	0.96
Polymorphic at CK-A2*	0.79	0.21	0.81	0.19	0.26	-	0.74	0.17	-	0.83
Hatchery	-	1.0	0.06	0.94	0.78	0.10	0.12	0.89	0.11	-

Table 2.7. Allele frequencies of unstocked populations and hatchery strains at CK-A2*, PEPB*, AAT-1,2* and GPI-B2*.

Population	CK-A2*		PEPB*		AAT-1,2*			GPI-B2*		
	100*	78*	100*	68*	100*	80*	118*	100*	40*	70*
Unstocked										
BUN	1.0	-	1.0	-	0.22	-	0.78	-	-	1.0
ENL	1.0	-	1.0	-	-	-	1.0	-	-	1.0
RAV	1.0	-	1.0	-	-	-	1.0	-	-	1.0
SAM	1.0	-	1.0	-	-	-	1.0	-	-	1.0
SIL	1.0	-	1.0	-	-	-	1.0	-	-	1.0
LBC	1.0	-	1.0	-	-		1.0	-	-	1.0
MGB	1.0	-	0.95	0.05	0.10	-	0.90	0.35	-	0.65
Hatchery										
MEG	-	1.0	0.17	0.83	0.91	-	0.09	0.99	0.01	-
EDY	-	1.0	-	1.0	0.70	0.10	0.20	0.68	0.32	-
ROM	-	1.0	-	1.0	0.73	0.19	0.08	1.0	-	-

Table 2.8. Correlation of allele frequencies with CK-A2* 78* allele frequency.
(* p[r = 0] < 0.05; ** p[r = 0] < 0.01)

Locus - allele	Samples polymorphic at CK-A2* Pearsons r	All samples Pearsons r
PEPB* 68*	0.884 **	0.962 **
LDH-B1* 67*	0.733 **	0.815 **
AAT-1,2* 100*	0.595 **	0.856 **
sMEP-1* 63*	0.376 *	0.750 **
GPI-B2* 100*	0.339	0.878 **
G3PD* 78*	0.058	0.797 **
PROT-1* -130*	0.318	0.554 **
MDH-B1,2* 120*	-0.036	0.166

Table 2.9. Genetic heterogeneity among sample subsets: G-test statistics, number of loci exhibiting significant allele frequency heterogeneity, genetic variance (F_{ST}), and sampling variance of F_{ST} estimate.

Sample subset (N)	Total G	Total df	Number of loci showing significant heterogeneity	F_{ST}	Sampling variance
All samples (49)	7010.6	1222	9	0.542	0.001
Great Smoky Mountains National Park samples (46)	3173.3	968	8	0.363	0.001
Park samples fixed for CK-A2* 100* (35)	1259.2	510	5	0.453	0.001
Park samples polymorphic at CK-A2* (11)	1097.5	220	7	0.223	0.002
Hatchery samples (3)	218.2	40	7	0.113	0.005
Park samples fixed for CK-A2* 100* plus Hatchery samples (38)	5817.8	814	9	0.659	0.001

Table 2.10. Genetic heterogeneity among multiple samples from Hyatt Creek (HYC), Taywa Creek (TAY), and Meigs Creek (MEG): G-test statistics, number of loci showing significant allele frequency heterogeneity, and genetic variance (F_{ST}).

Population	Total G	Total df	Number of loci showing significant heterogeneity	F_{ST}
HYC	16.6	22	0	0.022
TAY	29.3	28	0	0.037
MEG	29.6	36	1	0.045

Table 2.11. Hierarchical partitioning of heterozygosity among all samples and among all native samples from Great Smoky Mountains National Park. H_T is total heterozygosity of pooled samples, $\overline{H}_S$ is the average expected heterozygosity of samples, H_R is the average of total heterozygosities of samples pooled by river drainage (Little Tennessee, Pigeon, and Little Rivers). $D_{RT} = H_T - H_R$, $D_{SR} = H_R - \overline{H}_S$. Each diversity measure (D) is divided by H_T to asses its relative contribution to overall heterozygosity. (The naturalized hatchery Meigs Creek samples were excluded).

All samples from Great Smoky Mountains National Park

$\overline{H}_S$: 0.032		$\overline{H}_S / H_T$: 0.64
H_T: 0.050	D_{RT}: 0.008	D_{RT} / H_T: 0.16
H_R: 0.042	D_{SR}: 0.010	D_{SR} / H_T: 0.20

All native samples from Great Smoky Mountains National Park

$\overline{H}_S$: 0.014		$\overline{H}_S / H_T$: 0.56
H_T: 0.025	D_{RT}: 0.003	D_{RT} / H_T: 0.12
H_R: 0.022	D_{SR}: 0.008	D_{SR} / H_T: 0.32

Table 2.12. Average normalized genetic identities (Nei's *I*) across all samples and selected subsets of samples.

Sample	Number of Comparisons	Average *I*	Range of *I*'s
All samples	1485	0.950	0.718 - 1.0
Great Smoky Mountains National Park samples	1225	0.985	0.859 - 1.0
Park samples fixed for CK-A2* 100*	595	0.988	0.925 - 1.0
Park samples polymorphic at CK-A2*	55	0.981	0.922 - 1.0
Hatchery	3	0.978	0.973 - 0.986
Park samples fixed for CK-A2* 100* plus Park samples polymorphic at CK-A2*	385	0.973	0.859 - 1.0
Park samples fixed for CK-A2* 100* plus Hatchery samples	105	0.778	0.718 - 0.832

Table 2.13. Normalized genetic identities (Nei's *I*) of multiple samples from Hyatt Creek (HYC), Taywa Creek (TAY), and Meigs Creek (MEG).

Population	Average *I*	Range of *I*'s
HYC	0.997	0.996 - 0.999
TAY	0.991	0.988 - 0.996
MEG	0.993	0.988 - 0.999

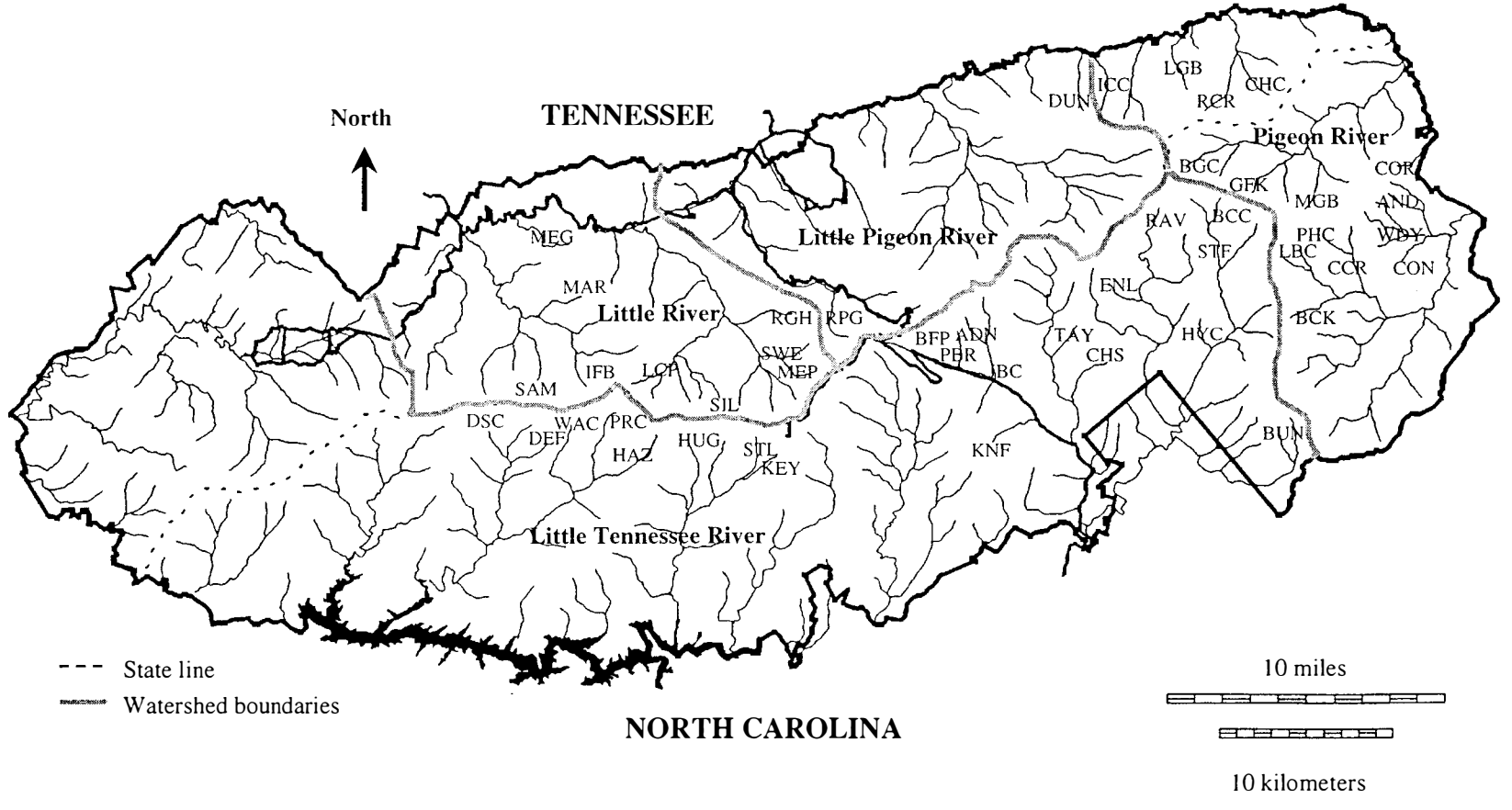


Figure 2.1. Brook trout sampling localities in Great Smoky Mountains National Park. First order streams are not shown.

Appendix 2.1: Synonymy of allozyme allele designations.

This study		McCracken et al. (1993)	Stoneking et al. (1981)	Perkins et al. (1993)
Locus	allele	Synonymy	Synonymy	Synonymy
AAT-1,2*	100*	-	-	100*
	80*	-	-	80*
	118*	-	-	118*
CK-A1*	100*	100*	CPK-1 100	CK-1* 100*
CK-A2*	100*	100*	CPK-2 122	-
	78*	78*	100	CK-2* 100*
	83*	-	-	-
DDH-3*	100*	DDH* 100*	DIA 100	100*
	117*	117*	89 (?)	85* (?)
G3PDH-1*	100*	100*	AGP-1 100	100*
	78*	43*	67	78*
GPI-A*	100*	-	PGI-3 100	100*
GPI-B1*	100*	-	PGI-1 100	100*
	150*	-	150	150*
GPI-B2*	100*	-	PGI-2 100	100*
	70*	-	70	-
	40*	-	39	39*

Appendix 2.1 continued.

This study		McCracken et al. (1993)	Stoneking et al. (1981)		Perkins et al. (1993)
Locus	allele	Synonymy	Synonymy		Synonymy
LDH-A1*	100*	100*	LDH-1	100	100*
LDH-A2*	100*	100*	LDH-2	100	100*
	44*	-		-	44*
LDH-B1*	100*	100*	LDH-3	100	100*
	67*	67*		72	72*
	86*	-		86	86*
LDH-B2*	100*	100*	LDH-4	100	100*
sMDH-A1*	100*	100*	MDH-1	100	100*
sMDH-A2*	100*	100*	MDH-2	100	100*
	84*	-		-	-
sMDH-B1,2*	100*	100*	MDH-3,4	100	100*
	120*	145*		120	120*
sMEP-1*	100*	100*	ME-1	100	100*
	63*	88*		0 (?)	0* (?)

Appendix 2.1 continued.

This study		McCracken et al. (1993)	Stoneking et al. (1981)	Perkins et al. (1993)
Locus	allele	Synonymy	Synonymy	Synonymy
PEPB*	100*	-	-	-
	68*	-	-	-
PEPS*	100*	PEPA-2* 100*	-	100*
PROT-1*	-100*	-100*	CMP 100	100*
	-130*	-130*	-	-
PROT-2*	100*	100*	-	100*
PROT-3*	100*	-	-	100*
PROT-4*	100*	-	-	100*

Appendix 2.2: Allele frequencies of loci polymorphic in two or more samples, sample size (N), expected heterozygosity (H_S), observed heterozygosity (H_O), and proportion of loci polymorphic (P) for all samples.

Sample	CK-A2*			AAT-1,2*			DDH*	
	100*	83*	78*	100*	80*	118*	100*	117*
Little Tennessee River								
ADN	1.0	-	-	0.36	-	0.64	1.0	-
BCC	1.0	-	-	0.18	-	0.82	1.0	-
BFP	0.87	-	0.13	0.12	-	0.88	1.0	-
BUN	1.0	-	-	0.22	-	0.78	0.92	0.08
CHS	0.95	-	0.05	0.23	-	0.77	1.0	-
DSC	1.0	-	-	0.30	-	0.70	1.0	-
HAZ	1.0	-	-	0.05	-	0.95	1.0	-
HYC2	0.93	-	0.07	0.31	-	0.69	1.0	-
HYC3	0.80	-	0.20	0.30	-	0.70	1.0	-
HYC4	0.93	-	0.07	0.40	-	0.60	1.0	-
JBC	1.0	-	-	0.52	-	0.48	1.0	-
KNF	1.0	-	-	0.48	-	0.52	1.0	-
PBR	0.90	-	0.10	0.13	-	0.87	1.0	-
STF	0.94	-	0.06	0.18	-	0.82	1.0	-
TAY1	0.55	-	0.45	0.72	-	0.28	1.0	-
TAY2	0.55	-	0.45	0.50	-	0.50	1.0	-
TAY3	0.50	-	0.50	0.70	-	0.30	1.0	-
DFC, ENL, HUG, KEE, PRC, RAV, STL, WAC	1.0	-	-	-	-	1.0	1.0	-
French Broad River								
BGC	1.0	-	-	-	-	1.0	1.0	-
CHC	1.0	-	-	-	-	1.0	1.0	-
COR	1.0	-	-	-	-	1.0	-	1.0
DUN	0.71	0.16	0.13	0.03	-	0.97	1.0	-
GFK	0.90	-	0.10			1.0	1.0	-
ICC	1.0	-	-	0.03	-	0.97	1.0	-
LBC	1.0	-	-	-	-	1.0	0.20	0.80
LGB	1.0	-	-	-	-	1.0	1.0	-
MGB	1.0	-	-	0.10	-	0.90	1.0	-
RCR	0.82	-	0.18	-	-	1.0	1.0	-
RPG	1.0	-	-	-	-	1.0	1.0	-

Appendix 2.2 continued.

Sample	CK-A2*			AAT-1,2*			DDH*	
	100*	83*	78*	100*	80*	118*	100*	117*
French Broad River (continued)								
WDY	0.86	-	0.14	-	-	1.0	0.71	0.29
ANB, BCK, CCR,CON, PHC	1.0	-	-	-	-	1.0	1.0	-
Little River								
IFB	0.65	-	0.35	0.35	-	0.65	1.0	-
LCP	1.0	-	-	-	-	1.0	1.0	-
RGH	1.0	-	-	-	-	1.0	0.55	0.45
MRC, MPC, SAM, SIL, SWE	1.0	-	-	-	-	1.0	1.0	-
Hatchery								
MEG2	-	-	1.0	0.94	-	0.06	1.0	-
MEG3	-	-	1.0	0.87	-	0.13	1.0	-
MEG4	-	-	1.0	0.93	-	0.07	1.0	-
EDY	-	-	1.0	0.70	0.10	0.20	1.0	-
ROM	-	-	1.0	0.73	0.19	0.08	1.0	-

Appendix 2.2 extended.

Sample	G3PDH-1*		GPI-B2*			LDH-B1*		
	100*	78*	100*	70*	40*	100*	86*	67*
Little Tennessee River								
ADN	1.0	-	-	1.0	-	1.0	-	-
BCC	1.0	-	-	1.0	-	0.90	-	0.10
BFP	1.0	-	0.13	0.87	-	0.87	-	0.13
BUN	1.0	-	-	1.0	-	1.0	-	-
CHS	1.0	-	0.15	0.85	-	1.0	-	-
DSC	1.0	-	-	1.0	-	1.0	-	-
HAZ	1.0	-	-	1.0	-	1.0	-	-
HYC2	1.0	-	0.48	0.52	-	0.96	-	0.04
HYC3	1.0	-	0.25	0.75	-	1.0	-	-
HYC4	1.0	-	0.41	0.59	-	0.93	-	0.07
JBC	1.0	-	-	1.0	-	1.0	-	-
KNF	1.0	-	-	1.0	-	1.0	-	-
PBR	1.0	-	0.05	0.95	-	0.90	-	0.10
STF	0.97	0.03	0.03	0.97	-	1.0	-	-
TAY1	1.0	-	0.25	0.75	-	0.67	-	0.33
TAY2	1.0	-	0.30	0.70	-	0.85	-	0.15
TAY3	1.0	-	0.50	0.50	-	0.63	-	0.37
DFC, ENL, HUG, KEE, PRC, RAV, STL, WAC	1.0	-	-	1.0	-	1.0	-	-
French Broad River								
BGC	1.0	-	0.40	0.60	-	1.0	-	-
CHC	1.0	-	-	1.0	-	1.0	-	-
COR	1.0	-	-	1.0	-	1.0	-	-
DUN	1.0	-	0.04	0.96	-	1.0	-	-
GFK	1.0	-	-	1.0	-	1.0	-	-
ICC	1.0	-	0.01	0.99	-	0.94	-	0.06
LBC	1.0	-	-	1.0	-	1.0	-	-
LGB	1.0	-	-	1.0	-	1.0	-	-
MGB	1.0	-	0.35	0.65	-	1.0	-	-
RCR	1.0	-	-	1.0	-	1.0	-	-
RPG	0.85	0.15	0.45	0.55	-	1.0	-	-

Appendix 2.2 continued.

Sample	G3PDH-1*		GPI-B2*			LDH-B1*		
	100*	78*	100*	70*	40*	100*	86*	67*
French Broad River (continued)								
WDY	1.0	-	-	1.0	-	1.0	-	-
ANB, BCK, CCR, CON, PHC	1.0	-	-	1.0	-	1.0	-	-
Little River								
IFB	0.90	0.10	-	1.0	-	1.0	-	-
LCP	1.0	-	-	1.0	-	1.0	-	-
RGH	1.0	-	-	1.0	-	1.0	-	-
MRC, MPC, SAM, SIL SWE	1.0	-	-	1.0	-	1.0	-	-
Hatchery								
MEG2	0.71	0.29	0.97	-	0.03	0.69	-	0.31
MEG3	0.70	0.30	1.0	-	-	0.59	-	0.41
MEG4	0.50	0.50	1.0	-	-	1.0	-	-
EDY	0.56	0.44	0.68	-	0.32	0.40	-	0.60
ROM	0.96	0.04	1.0	-	-	0.46	0.10	0.44

Appendix 2.2 extended.

Sample	MDH-B1,2*		sMEP-1*		PEPB*		PROT-1*	
	100*	120*	100*	63*	100*	68*	-100*	-130*
Little Tennessee River								
ADN	1.0	-	1.0	-	1.0	-	1.0	-
BCC	1.0	-	1.0	-	1.0	-	1.0	-
BFP	0.93	0.07	1.0	-	0.97	0.03	1.0	-
BUN	1.0	-	1.0	-	1.0	-	1.0	-
CHS	1.0	-	0.60	0.40	0.95	0.05	1.0	-
DSC	1.0	-	1.0	-	1.0	-	1.0	-
HAZ	1.0	-	1.0	-	1.0	-	1.0	-
HYC2	0.86	0.14	1.0	-	0.86	0.14	1.0	-
HYC3	0.80	0.20	1.0	-	1.0	-	1.0	-
HYC4	0.85	0.15	1.0	-	0.91	0.09	1.0	-
JBC	1.0	-	1.0	-	1.0	-	1.0	-
KNF	1.0	-	1.0	-	1.0	-	1.0	-
PBR	1.0	-	1.0	-	0.85	0.15	1.0	-
STF	0.89	0.11	0.94	0.06	0.97	0.03	0.97	0.03
TAY1	0.92	0.08	0.82	0.18	0.30	0.70	1.0	-
TAY2	1.0	-	0.75	0.25	0.60	0.40	0.95	0.05
TAY3	0.92	0.08	0.75	0.25	0.39	0.61	1.0	-
DFC, ENL, HUG, KEE, PRC, RAV, STL, WAC	1.0	-	1.0	-	1.0	-	1.0	-
French Broad River								
BGC	1.0	-	1.0	-	1.0	-	1.0	-
CHC	1.0	-	0.60	0.40	1.0	-	1.0	-
COR	1.0	-	1.0	-	1.0	-	1.0	-
DUN	1.0	-	0.88	0.12	0.97	0.03	0.96	0.04
GFK	1.0	-	1.0	-	1.0	-	1.0	-
ICC	1.0	-	0.91	0.09	0.91	0.09	1.0	-
LBC	1.0	-	1.0	-	1.0	-	1.0	-
LGB	0.15	0.85	1.0	-	1.0	-	1.0	-
MGB	1.0	-	1.0	-	0.95	0.05	1.0	-
RCR	1.0	-	0.98	0.02	1.0	-	1.0	-
RPG	1.0	-	0.95	0.05	1.0	-	1.0	-

Appendix 2.2 continued.

Sample	MDH-B1,2*		sMEP-1*		PEPB*		PROT-1*	
	100*	120*	100*	63*	100*	68*	-100*	-130*
French Broad River (continued)								
WDY	1.0	-	1.0	-	1.0	-	1.0	-
ANB, BCK, CCR, CON, PHC	1.0	-	1.0	-	1.0	-	1.0	-
Little River								
IFB	1.0	-	0.75	0.25	0.45	0.55	1.0	-
LCP	1.0	-	0.70	0.30	1.0	-	1.0	-
RGH	1.0	-	1.0	-	1.0	-	1.0	-
MRC, MPC, SAM, SIL, SWE	1.0	-	1.0	-	1.0	-	1.0	-
Hatchery								
MEG2	0.89	0.11	0.72	0.28	0.17	0.83	0.94	0.06
MEG3	0.82	0.18	0.75	0.25	0.23	0.77	0.95	0.05
MEG4	0.94	0.06	0.33	0.67	0.14	0.86	1.0	-
EDY	0.86	0.14	0.72	0.28	-	1.0	0.98	0.02
ROM	1.0	-	0.25	0.75	-	1.0	1.0	-

Appendix 2.2 extended.

Sample	N	H_O	SE (H_O)	H_S	SE (H_S)	P
Little Tennessee River						
ADN	9	0.063	0.044	0.040	0.028	0.045
BCC	10	0.039	0.022	0.033	0.019	0.091
BFP	15	0.064	0.022	0.056	0.020	0.273
BUN	38	0.045	0.027	0.036	0.021	0.091
CHS	10	0.074	0.030	0.075	0.029	0.227
DSC	10	0.043	0.031	0.037	0.025	0.045
HAZ	10	0.009	0.006	0.008	0.006	0.045
HYC2	28	0.088	0.033	0.089	0.034	0.273
HYC3	10	0.083	0.037	0.081	0.033	0.182
HYC4	23	0.096	0.036	0.092	0.035	0.273
JBC	10	0.048	0.033	0.043	0.030	0.045
KNF	10	0.065	0.045	0.043	0.030	0.045
PBR	10	0.048	0.021	0.050	0.019	0.227
STF	18	0.060	0.023	0.053	0.019	0.364
TAY1	20	0.098	0.035	0.128	0.040	0.318
TAY2	10	0.183	0.066	0.136	0.043	0.318
TAY3	27	0.141	0.045	0.143	0.044	0.318
DFC, ENL, HUG, KEE, PRC, RAV, STL, WAC	10	0	-	0	-	0
French Broad River						
BGC	5	0.000	0.000	0.021	0.021	0.045
CHC	10	0.009	0.009	0.021	0.021	0.045
COR	10	0	-	0	-	0
DUN	34	0.045	0.022	0.045	0.021	0.273
GFK	10	0.009	0.009	0.008	0.008	0.045
ICC	39	0.028	0.012	0.026	0.011	0.227
LBC	10	0.009	0.009	0.014	0.014	0.045
LGB	20	0.013	0.013	0.011	0.011	0.045
MGB	10	0.039	0.017	0.044	0.022	0.136
RCR	26	0.015	0.012	0.016	0.013	0.091
RPG	10	0.030	0.018	0.037	0.024	0.136

Appendix 2.2 continued.

Sample	N	H_O	SE (H_O)	H_S	SE (H_S)	P
French Broad River (continued)						
WDY	7	0.025	0.017	0.028	0.020	0.091
ANB, BCK, CCR, CON, PHC	10	0	-	0	-	0
Little River						
IFB	10	0.100	0.042	0.105	0.039	0.227
LCP	10	0.017	0.017	0.018	0.018	0.045
RGH	10	0.022	0.022	0.022	0.022	0.045
MRC, MPC, SAM, SIL, SWE	10, 10 8, 14 10	0	-	0	-	0
Hatchery						
MEG2	18	0.084	0.027	0.091	0.031	0.364
MEG3	11	0.096	0.031	0.107	0.035	0.318
MEG4	11	0.087	0.044	0.070	0.030	0.227
EDY	25	0.136	0.045	0.130	0.042	0.318
ROM	26	0.097	0.045	0.082	0.037	0.182

Appendix 2.3. Matrix of normalized genetic identities (Nei's *I*) between all samples. (The 18 samples that were identically monomorphic are represented by SIL).

	LGB	BUN	RCR	STF	ICC	SIL	MEG2	MEG3	MEG4
LGB									
BUN	0.964								
RCR	0.967	0.994							
STF	0.972	0.999	0.996						
ICC	0.967	0.996	0.998	0.997					
SIL	0.969	0.996	0.999	0.996	0.999				
MEG2	0.740	0.795	0.780	0.782	0.783	0.766			
MEG3	0.751	0.798	0.786	0.807	0.789	0.773	0.999		
MEG4	0.736	0.795	0.780	0.782	0.782	0.767	0.993	0.988	
DUN	0.964	0.993	0.998	0.995	0.997	0.996	0.795	0.802	0.796
HYC2	0.957	0.986	0.979	0.988	0.981	0.980	0.853	0.857	0.852
HYC3	0.969	0.993	0.988	0.995	0.987	0.986	0.838	0.843	0.837
HYC4	0.955	0.987	0.976	0.988	0.979	0.977	0.856	0.860	0.854
BFP	0.969	0.996	0.997	0.998	0.997	0.997	0.807	0.814	0.782
ADN	0.956	0.998	0.987	0.996	0.989	0.989	0.811	0.814	0.811
BCC	0.965	0.999	0.995	0.999	0.997	0.997	0.791	0.797	0.789
BGC	0.961	0.988	0.992	0.990	0.992	0.993	0.792	0.799	0.794
CHC	0.961	0.988	0.992	0.990	0.993	0.993	0.769	0.776	0.769
CHS	0.954	0.991	0.987	0.992	0.989	0.987	0.818	0.823	0.818
COR	0.925	0.959	0.955	0.952	0.955	0.957	0.720	0.726	0.722
DSC	0.959	0.999	0.991	0.998	0.993	0.992	0.805	0.808	0.805
GFK	0.967	0.995	1.000	0.996	0.999	1.000	0.773	0.780	0.775
HAZ	0.969	0.997	0.998	0.998	0.999	1.000	0.773	0.779	0.774
IFB	0.933	0.975	0.971	0.978	0.974	0.967	0.877	0.877	0.879
JBC	0.943	0.991	0.974	0.988	0.977	0.975	0.826	0.826	0.826
KNF	0.947	0.994	0.978	0.991	0.981	0.980	0.822	0.823	0.822
LBC	0.940	0.972	0.970	0.967	0.970	0.972	0.735	0.741	0.736
LCP	0.964	0.991	0.995	0.994	0.997	0.996	0.780	0.787	0.779
MGB	0.961	0.993	0.992	0.994	0.994	0.994	0.808	0.814	0.808
PBR	0.965	0.997	0.997	0.998	0.998	0.997	0.806	0.811	0.804
RGH	0.959	0.990	0.990	0.987	0.990	0.991	0.754	0.760	0.755
RPG	0.958	0.985	0.989	0.987	0.990	0.990	0.799	0.807	0.782
WDY	0.964	0.993	0.996	0.992	0.994	0.996	0.770	0.776	0.772
TAY1	0.882	0.934	0.917	0.935	0.922	0.911	0.934	0.933	0.924
TAY2	0.919	0.967	0.959	0.970	0.962	0.954	0.917	0.919	0.912
TAY3	0.877	0.927	0.912	0.930	0.918	0.906	0.953	0.953	0.953
EDY	0.764	0.810	0.802	0.817	0.804	0.788	0.975	0.975	0.976
ROM	0.729	0.787	0.777	0.795	0.782	0.763	0.989	0.989	0.989

Appendix 2.3 continued.

	DUN	HYC2	HYC3	HYC4	BFP	ADN	BCC	BGC	CHC
HYC2	0.980								
HYC3	0.988	0.995							
HYC4	0.977	0.999	0.996						
BFP	0.996	0.989	0.994	0.988					
ADN	0.986	0.987	0.993	0.990	0.992				
BCC	0.994	0.986	0.992	0.986	0.998	0.996			
BCK	0.992	0.972	0.979	0.969	0.990	0.982	0.991		
BGC	0.991	0.989	0.987	0.984	0.994	0.981	0.990		
CHC	0.991	0.971	0.978	0.969	0.989	0.981	0.990	0.986	
CHS	0.988	0.985	0.988	0.985	0.990	0.990	0.991	0.985	0.994
COR	0.952	0.934	0.941	0.931	0.952	0.945	0.953	0.949	0.949
DSC	0.990	0.987	0.994	0.990	0.994	1.000	0.998	0.985	0.985
GFK	0.998	0.979	0.987	0.976	0.997	0.988	0.996	0.992	0.992
HAZ	0.996	0.982	0.988	0.980	0.997	0.991	0.998	0.993	0.993
IFB	0.975	0.972	0.976	0.972	0.975	0.977	0.974	0.959	0.964
JBC	0.973	0.983	0.989	0.989	0.982	0.998	0.988	0.969	0.969
KNF	0.978	0.984	0.991	0.990	0.985	0.999	0.991	0.972	0.972
LBC	0.967	0.950	0.957	0.946	0.967	0.959	0.969	0.965	0.965
LCP	0.995	0.975	0.981	0.972	0.992	0.984	0.993	0.989	0.994
MGB	0.991	0.994	0.992	0.990	0.996	0.988	0.994	0.999	0.986
PBR	0.996	0.987	0.991	0.985	0.999	0.993	0.998	0.991	0.989
RGH	0.987	0.969	0.976	0.967	0.987	0.979	0.988	0.984	0.984
RPG	0.988	0.988	0.985	0.982	0.991	0.978	0.986	0.999	0.983
WDY	0.994	0.975	0.983	0.972	0.993	0.983	0.992	0.988	0.988
TAY1	0.922	0.953	0.948	0.958	0.935	0.946	0.932	0.912	0.906
TAY2	0.965	0.980	0.979	0.982	0.970	0.973	0.967	0.957	0.950
TAY3	0.919	0.957	0.948	0.959	0.933	0.939	0.927	0.916	0.901
EDY	0.812	0.863	0.848	0.864	0.829	0.822	0.811	0.810	0.783
ROM	0.793	0.845	0.827	0.845	0.804	0.801	0.787	0.790	0.767

Appendix 2.3 continued.

	CHS	COR	DSC	GFK	HAZ	IFB	JBC	KNF	LBC
COR	0.943								
DSC	0.991	0.947							
GFK	0.987	0.956	0.992						
HAZ	0.989	0.956	0.995	0.999					
IFB	0.974	0.921	0.977	0.969	0.970				
JBC	0.983	0.931	0.995	0.975	0.980	0.974			
KNF	0.985	0.936	0.997	0.979	0.984	0.975	1.000		
LBC	0.958	0.998	0.964	0.971	0.971	0.937	0.946	0.951	
LCP	0.988	0.952	0.988	0.996	0.996	0.969	0.971	0.975	0.967
MGB	0.989	0.949	0.991	0.993	0.994	0.967	0.977	0.981	0.965
PBR	0.990	0.952	0.995	0.997	0.998	0.981	0.983	0.987	0.967
RGH	0.978	0.987	0.983	0.991	0.991	0.957	0.967	0.970	0.995
RPG	0.983	0.946	0.981	0.989	0.990	0.958	0.965	0.969	0.962
WDY	0.983	0.976	0.987	0.996	0.995	0.967	0.970	0.975	0.987
TAY1	0.936	0.865	0.942	0.915	0.918	0.975	0.954	0.952	0.881
TAY2	0.971	0.908	0.972	0.957	0.958	0.990	0.975	0.975	0.880
TAY3	0.934	0.859	0.934	0.909	0.912	0.967	0.946	0.945	0.875
EDY	0.824	0.742	0.818	0.796	0.794	0.890	0.832	0.829	0.757
ROM	0.812	0.718	0.796	0.771	0.770	0.874	0.812	0.809	0.732

	LCP	MGB	PBR	RGH	RPG	WDY	TAY1	TAY2	TAY3	EDY
MGB	0.989									
PBR	0.993	0.995								
RGH	0.987	0.984	0.987							
RPG	0.987	0.997	0.988	0.981						
WDY	0.991	0.989	0.993	0.998	0.985					
TAY1	0.911	0.929	0.940	0.900	0.909	0.910				
TAY2	0.956	0.967	0.972	0.944	0.955	0.954	0.988			
TAY3	0.908	0.931	0.934	0.895	0.915	0.906	0.996	0.989		
EDY	0.790	0.825	0.829	0.776	0.816	0.793	0.945	0.920	0.957	
ROM	0.777	0.805	0.805	0.751	0.794	0.768	0.932	0.913	0.951	0.973

Part III:
ALLOZYME ASSESSMENT of REGIONAL VARIATION AMONG BROOK TROUT POPULATIONS

Introduction

Most of the contemporary range of brook trout (MacCrimmon and Campbell, 1969) was covered by the Laurentide ice sheet during the Wisconsinan glaciation. However, landscapes south of New York, including the Southern Appalachian Mountains, were unglaciated throughout this period (Andrews, 1987). Within both the formerly glaciated and unglaciated parts of its current range, brook trout occur within drainage basins and watersheds in numerous populations (Behnke, 1980; Power, 1980) that may have been reproductively isolated for thousands of years. Elucidation of the biogeographical affinities and levels of genetic differentiation of populations in these different physiographic settings is important to our understanding of post-glacial faunal assembly and the geographic-genetic structure of populations.

Efforts to conserve brook trout also require an understanding of biogeographical history and genetic differentiation. As a consequence of human activities, brook trout have declined throughout much of their native range and have been extirpated from some areas (Powers, 1980). Attempts to augment or restore populations have relied extensively on stocking with hatchery strains without reference to the geographical origin or genetic divergence of the strains (Ferguson, 1989; Phillip et al., 1993). If a goal of conservation is the preservation of maximum genetic diversity, the identification of populations for augmentation or protection, and for reintroduction into former habitats, requires information on intraspecific phylogeny.

Molecular population genetics provides useful tools for studying intraspecific phylogeny, historical biogeography, and hybridization (Avise, 1994), and molecular

markers have been applied extensively in studies of salmonids, including brook trout. Allendorf and Ryman (1987) noted that molecular studies of population structure in salmonids observe a consistent pattern of differentiation into genetically discrete populations. Genetic differentiation among Southern Appalachian brook trout populations and northern derived hatchery strains, and hybridization between the two, has been examined in several studies (McCracken et al., 1993; Kriegler et al., 1995; Hayes et al., 1996; Kriegler, 1994; Saidak, 1995), including in the preceding chapter. Other workers have observed genetic differentiation among populations in New York and Pennsylvania (Perkins et al., 1993), Maryland (Morgan and Baker, 1991), and Georgia (Dunham et al., 1994). Stoneking et al.(1981) have been the only workers to directly address questions of molecular differentiation among wild northern and southern populations; but their study was limited by the small number of populations examined (two from New York, three from Pennsylvania, two from Tennessee, and one from Great Smoky Mountains National Park, North Carolina), and most importantly by the absence of populations between Pennsylvania and North Carolina / Tennessee.

In this paper I report the results of my allozyme analysis of 37 brook trout populations from Maryland, Virginia, and northwestern North Carolina and of 11 populations from the extreme southern part of the brook trout range in South Carolina. I evaluate the data from these populations with reference to data from Great Smoky Mountains National Park and the previously published studies. This broader geographical coverage permits exploration of brook trout intraspecific phylogeny, historical

biogeography, and genetic differentiation throughout a substantial part of the taxon's native range in the United States (Part I: Figure 1.1).

Studies of the biogeography of other eastern North American fishes reveal patterns that are relevant to my investigations of differentiation among brook trout populations. At the broadest scales, contemporary fish faunas of eastern North America reflect continental drainage patterns and glacial history (Mayden, 1988; Sheldon, 1988; Briggs, 1986; Miller, 1965; Robison, 1986; Gilbert, 1976). These contemporary faunas may have been assembled by postglacial colonization from different drainage sources, may represent vicariants in different unglaciated drainages, or both (Mayden, 1988; Wiley and Mayden, 1985). With respect to post glacial colonization of brook trout, Bailey and Smith (1981) and Perkins et al. (1993) have hypothesized two Pleistocene refugia as sources of northeastern populations. On the basis of current distributions and allozyme variation they postulate a Mississippi River refugium as the source of populations currently within that drainage, and a coastal Atlantic refugium as the source of Atlantic drainage populations, including those in the St. Lawrence River basin. Allozyme differences between Southern Appalachian brook trout and northern derived hatchery strains (Hayes et al., 1996; Kriegler et al., 1995; McCracken et al., 1993; Part I, this study) suggest that a third refugium was the source of native Southern Appalachian populations.

Biogeographical patterns of other fishes and other organisms also support the possibility of different sources of brook trout in Southern Appalachian and northeastern Mississippi basin drainages. Differences among fish assemblages in the lower Ohio

River (including the Tennessee River) and upper Ohio River, and the distinctiveness of the New River fauna, have been recognized (Mayden, 1988; Burr and Page, 1986; Hocutt et al., 1978; Hocutt et al., 1986). Within the upper Ohio River clade, Mayden (1988) also identified an upper Kanawaha River (New River) fauna distinct from that of the other rivers in the clade. Earlier studies had also identified the New River as a major faunal break for fishes and aquatic insects in the central Appalachians (Jenkins et al., 1971). These observations point to the possible significance of the New River in the biogeography and intraspecific phylogeny of brook trout.

Sampling of brook trout populations in Maryland, Virginia, North Carolina, and South Carolina allowed me to investigate differentiation among populations from the major drainage regions (northern Atlantic, upper Ohio River, New River, lower Ohio River [Tennessee River], and southern Atlantic) indicated by earlier analyses of species assemblages (Table 3.1). Twenty eight populations from Maryland, Virginia, and North Carolina, and 11 from South Carolina were sampled from Atlantic drainages, and one Maryland population is from the upper Ohio drainage. Fourteen populations from Virginia and North Carolina were from the New River drainage (upper Kanawaha River). Five populations from Virginia and North Carolina were from the upper Tennessee River (lower Ohio) drainage. Phylogenetic and geographic patterns emerging from this analysis provide an empirical basis for the conservation of brook trout genetic diversity, and contribute to our understanding of the post-glacial assembly of aquatic faunas in eastern North America.

Methods

Collections. - Age 1 year and older brook trout were collected from the 48 wild populations by electrofishing. Populations in Virginia were sampled in April and August, 1994. Populations in North Carolina were sampled in the spring and summer of 1993. The Maryland samples were collected by Dr. Ray Morgan (University of Maryland-Frostburg) in the summer of 1993. South Carolina samples were collected by Mr. Dan Rankin (South Carolina Department of Natural Resources) during the summer of 1994. Locations, stream names, and stream codes for populations sampled in this study are given in Table 3.1.

After capture, fish were euthanized with MS-222 (100mg/liter). Eyes, liver, and a skeletal muscle tissue sample were dissected in the field from fish from Virginia and North Carolina and immediately frozen in liquid nitrogen. The Maryland fish were frozen whole in liquid nitrogen and shipped frozen on dry ice to our laboratory for dissection. Samples from South Carolina were transported whole on ice to the South Carolina Department of Natural Resources laboratory in Clemson, South Carolina. Immediately upon arrival at the Clemson laboratory, specimens were dissected and the tissue samples were stored at -80^{O} C prior to shipping to us on dry ice. In our laboratory, tissues were stored at -80^{O} C prior to and after processing.

Sample sizes ranged from 10 to 26 according to population densities observed in the field (Table 3.1). The wide range of sample sizes was deemed preferable to attempting to obtain equal samples at the risk of unnecessarily limiting sample sizes from large populations, or possibly having a negative impact on small populations. Most brook

trout populations in northern Virginia and Maryland are larger than those to the south (Fleebe, 1994), and my sample sizes reflect this pattern.

The stocking history of most wild populations is not known and where records do exist they are incomplete. Populations sampled in Virginia were selected on the basis of their having no record of stocking, and the judgment of fisheries personnel that they probably had not been stocked. The three Maryland populations are also believed not to have been stocked with hatchery fish or by stock transfer (Morgan and Baker, 1991). Collections from South Carolina and North Carolina were made without reference to stocking history. North Carolina has more brook trout populations than any other southeastern state (Fleebe, 1994), and the 12 samples represent a small fraction of the populations in that state. The 11 South Carolina populations sampled represent all but one of the known populations from that state. The remaining known population from South Carolina was not sampled because only three fish were observed while electrofishing. Most South Carolina populations except Slicking Creek (SL) were thought to have been stocked with fish from the Valhalla hatchery (Dan Rankin, personal communication).

Protein electrophoresis - Horizontal starch gel electrophoresis was used to examine all samples for variation at the same allozyme loci discussed in Part II. Table 3.2 lists the enzymes and loci examined, the percentage of populations polymorphic at each locus, and the number of alleles observed at each locus.

Data analysis - Allozyme variation within and among brook trout populations was evaluated by the same procedures discussed in Part II. Population genetics parameters were estimated for individual samples, all samples, and for appropriate subsets of samples. Three hatchery strains and four of the presumed native populations from Great Smoky Mountains National Park with the largest sample sizes (BUN, LGB, ICC, and SIL, see Part II) were included in some analyses, as indicated in the text. Gene diversity analysis of populations (Chakraborty, 1980: Perkins et al., 1993) from the entire region was extended by partitioning diversity into a regional component (north of the New River, and from the New River south) and drainage basin component (northern Atlantic, upper Ohio River, New River, lower Ohio River, and southern Atlantic), in addition to named river and within sample components. Unweighted paired group mean cluster analysis (UPGMA; Sneath and Sokal, 1973) was used to evaluate estimates of Nei's (1972) index of genetic similarity, and to provide an illustration of relationships between populations.

Results

Variation at the CK-A2 locus – Eleven of the 12 samples from Maryland and Virginia north of the New River were fixed for the CK-A2* 78* allele identified as fixed in Ohio River and Atlantic drainage populations from New York and Pennsylvania (Perkins et al., 1993), and in northern hatchery strains (McCracken et al., 1993; Part II). One population north of the New River, Rock Castle Creek (RCC, Roanoke River drainage, Virginia) had the CK-A2* 78* allele at a frequency of 0.64. Sixteen of the 36 samples from the New

River southward, and from southern Atlantic drainage streams east and south of the New River (Yadkin, Catawba, Saluda, and Savannah rivers) were fixed for the CK-A2* 100* allele identified as diagnostic of unstocked Southern Appalachian populations (McCracken et al., 1993; Part II). Nineteen of the samples from the southern region were polymorphic at the CK-A2* locus, segregating for both the CK-A2* 100* and 78* alleles. Average frequency of the CK-A2* 100* allele in these samples was 0.64 (range: 0.18 - 0.97). Following McCracken et al. (1993), I designate samples fixed for the CK-A2* 100* allele as native Southern Appalachian, samples fixed for the 78* allele as northern, and samples polymorphic at the CK-A2* locus as native-hatchery hybrids. One southern sample, Matthews Creek (MC, South Carolina), was fixed for the CK-A2* 78* allele, suggesting that it may be a naturalized hatchery derived population. The Matthews Creek population was thought to be of hatchery origin prior to the allozyme analysis (Dan Rankin, personal communication)

Polymorphism and heterozygosity – Including variation at the CK-A2* locus, 11 of the 22 allozyme loci examined were polymorphic in two or more samples with alternative alleles at a frequency of >0.05 and >0.01 (Appendix 3.1). Average polymorphism across all samples was 0.187 (range: 0.0 - 0.409) (Table 3.3). Two population samples, Jerrys Creek (JCT, lower Ohio River drainage, Virginia) and Slicking Creek (SC, southern Atlantic drainage, South Carolina) were monomorphic at all loci, and fixed for the same allele at all loci except GPI-B2*.

Average polymorphism (Table 3.3) was lowest in samples from the New River southward that were fixed for the CK-A2* 100* allele (0.085) and highest in the southern samples polymorphic at CK-A2* (0.273). Average polymorphism in samples from northern populations fixed for the CK-A2* 78* allele was 0.174.

Average heterozygosity, $\overline{H}_S$, across all samples was 0.055 (range 0.0 - 0.143). Average heterozygosities across subsets of samples demonstrated the same relationship as that observed for average polymorphism (Table 3.3). Average heterozygosity was lowest in samples fixed for the CK-A2* 100 allele (0.015), highest in samples polymorphic at CK-A2* (0.077), and intermediate in northern samples fixed for the CK-A2* 78 allele (0.049). Six of 172 observed genotype frequencies (3.5%) exhibited deviations from Hardy-Weinberg expectation by the G-test (Table 3.4). At least six deviations would be expected at random from 172 comparisons at $p = 0.05$. The six observed deviations were from six different population samples, all from South Carolina. Five of the deviations were in hybrid samples, and included heterozygote deficiencies at three loci (CK-A2*, GPI-B2*, and sMEP-1*) and heterozygote excesses at two loci (CK-A2* and MDH-B-1,2*). The presumed hatchery derived Matthews Creek sample exhibited an excess of heterozygotes at the LDH-B1* locus. Average fixation indices (F_{IS}) were not significantly different from zero across all samples or any subset of samples examined.

Across all samples, variation at four loci (CK-A2*, PEPB*, AAT-1,2*, and GPI-B2*) accounted for 74% of the total heterozygosity (H_T) and 59% of the average heterozygosity of samples ($\overline{H}_S$). Three loci (LDH-B1*, MDH-B1,2*, and sMEP-1*)

accounted for an additional 20% of total heterozygosity and 32% of the average heterozygosity of samples (Table 3.5).

Variation at other loci – In addition to variation at the CK-A2* locus, populations north of the New River and populations from the New River southward have different common alleles at the PEPB*, AAT-1,2*, and GPI-B2*loci (Table 3.6). I observed three alleles segregating at the PEPB* locus. Six of the 12 northern samples were fixed for the PEPB* 68* allele. The 68* allele was also the highest frequency allele in all other samples north of the New River except the Little Bear Creek population (LBM, Ohio River drainage, Maryland) and the Dry River population (VLR, Potomac River, Virginia), where the 100* allele was the common allele. The average frequency of the 68* allele in all northern samples was 0.79 (range: 0.16 - 1.0); excluding LBM and VLR the average frequency of the 68* allele was 0.90 (range: 0.54 - 1.0). The PEPB* 100* allele was fixed in 17 of the 36 samples from the New River southward, and was the highest frequency allele in 14 samples polymorphic at PEPB*. Average frequency of the 100* allele in southern population samples polymorphic at PEPB* was 0.72 (range: 0.19 - 0.96). Across all southern samples the average frequency of the PEPB* 100* allele was 0.85 (range: 0.0 - 1.0). The 68* allele was at high frequency in the presumed hatchery derived MC samples and in three hybrid populations, and was fixed in the hybrid Widows Creek sample (WC, Yadkin River, North Carolina). Fourteen of the 19 southern samples polymorphic at the PEPB* locus were also polymorphic at the CK-A2* locus, and 13 samples fixed for the PEPB* 100* allele were also fixed for the CK-A2* 100* allele. Average frequency of the

PEPB* 100* allele in hybrid populations was 0.74 (range: 0.0 - 1.0). Across native southern Appalachian samples the average frequency of the PEPB* 100* allele was 0.96 (range: 0.64 - 1.0). Correlations between frequencies of the CK-A2* 78* allele and the PEPB* 68* allele were significant across the subsets of all samples, all southern samples, and all hybrid samples (Table 3.7).

At the duplicated AAT-1,2* locus the 100* allele was the high frequency allele in all samples north of the New River except Little Bear Creek (LBM, upper Ohio drainage) from Maryland and the hybrid RCC population from Virginia (Table 3.6). Three samples from northern Virginia were fixed for the 100* allele. Across all samples north of the New River the average frequency of the AAT-1,2* 100* allele was 0.79 (range: 0.34 - 1.0). Excluding LBM and RCC the average frequency was 0.88 (range: 0.61 - 1.0). Thirty four samples from the New River southward, including the presumed hatchery derived Matthews Creek population from South Carolina, carried the AAT-1,2* 118* allele at high frequency (average: 0.88; range: 0.51 - 1.0). Sixteen southern samples were fixed for the 118* allele. The two southern samples with high frequency of the AAT-1,2* 100* allele were polymorphic at the CK-A2* locus. Across all hybrid southern populations the average frequency of the AAT-1,2* 118* allele was 0.74 (range: 0.21 - 1.0). Correlations between frequencies of the CK-A2* 78* allele and the AAT-1,2* 100* allele were significant across all samples and across all southern samples, but not across the subset of hybrid samples (Table 3.7).

Seven of the 12 samples north of the New River were fixed for the GPI-B2* 100* allele, and another four carried the 100* allele at high frequency (Table 3.6). Average

frequency of the 100* allele across all northern samples except LBM was 0.95 (range 0.66 - 1.0). The LBM sample had the GPI-B2* 40* allele at a frequency of 0.88, segregating with the 100* allele. From the New River south, including the southern Atlantic drainage populations, the allelic distributions at GPI-B2* are more complex. involving the 40*, 70*, and 100* alleles. Nine of the 20 samples from the New River and Atlantic draining rivers east of the New were fixed for the GPI-B2* 40* allele, and eight had the 40* allele at high frequency. Average frequency of the 40* allele across the 20 New River samples was 0.89 (range: 0.20 - 1.0). The 100* allele was the only alternative GPI-B2* allele observed in these samples. The 40* allele was also the low frequency variant in the two samples from the Atlantic drainage river adjacent to the New, the Roanoke River. Three of the five samples from the upper Tennessee River drainage were fixed for the GPI-B2* 100* allele and one had the 100* allele at high frequency (0.91). The other upper Tennessee drainage samples had the GPI-B2* 40* allele at high frequency, segregating with the 100* allele. Exclusive of the FCM population from Maryland, the GPI-B2* 70* allele was only seen in the South Carolina populations. The two native southern Appalachian samples from South Carolina were fixed for the 70* allele, and three of the hybrid samples had the 70* allele at high frequency (average: 0.68). South Carolina hybrid populations carried GPI-B2* 40*, 70*, and 100* alleles in various combinations. Frequency of the GPI-B2* 100* allele was significantly correlated with frequency of the CK-A2* 78* allele across all samples but not for the subsets of all southern samples and hybrid populations. I observed a significant negative correlation

between GPI-B2* 40* allele frequencies and CK-A2* 78* allele frequencies across all samples but not for the southern and hybrid subsets (Table 3.7).

Variation at the sMEP-1* locus also exhibited differences between populations north of the New River and populations from the New River southward, but the 100* allele is the common allele in both regions and the 63* variant was the only other allele observed (Appendix 3.1). All samples from north of the New River were polymorphic with both sMEP-1* alleles segregating. Average frequency of the 63* allele in northern populations was 0.41 (range: 0.10 - 0.75). Fifteen southern samples were polymorphic at the sMEP-1* locus with the 63* at an average frequency of 0.24 (range: 0.02 - 0.97). Twelve of the 15 southern samples polymorphic at sMEP-1* were also polymorphic at the CK-A2* locus. Correlations between frequencies of the CK-A2* 78* allele and the sMEP-1* 63* allele were significant across all samples and across all southern samples (Table 3.7).

Variation at G3PDH-1*, GPI-B1*, GPI-A*, LDH-A2*, LDH-B1*, and MDH-B1,2* involved low frequency alternative alleles with no apparent geographical patterns. However, the frequency of the G3PDH-1* 78* allele was significantly correlated with the CK-A2* 78* allele frequency across all southern samples and across all hybrid samples. The frequencies of the LDH-B1* 67* allele and the CK-A2* 78 allele were significantly correlated only across the subset of southern samples (Table 3.7).

Genetic variance – I observed significant allele frequency heterogeneity among all samples and all subsets of samples examined (Table 3.8). The greatest number of loci

showing significant heterogeneity (10 of the 11 polymorphic loci) was among the subset of all samples fixed for alternative alleles at the CK-A2* locus (Table 3.8). Across all samples nine loci showed significant heterogeneity. The four loci exhibiting significant allele frequency heterogeneity among the northern Atlantic drainage samples was the lowest of all sample subsets examined (Table 3.8).

The average standardized genetic variance (F_{ST}) for all samples was 0.587 (Table 3.8). The highest average F_{ST} was 0.774 in the subset of samples fixed for the alternative CK-A2* alleles (n = 28). The lowest average F_{ST}'s were among the subset of New River samples fixed for the CK-A2* 100* allele, F_{ST} = 0.159 (n = 8), and the subset of northern Atlantic drainage samples fixed for the CK-A2* 78* allele, F_{ST} = 0.211 (n = 10). Sampling variances of F_{ST} estimates (Workman and Niswander, 1970) ranged from 0.0006 for all samples to 0.003 for the subset of New River native samples. The relative contribution of polymorphic loci to total genetic variance of all presumed unstocked populations, unstocked southern and northern populations, and hybrid populations is detailed in Appendix 3.2.

Across all samples 59% of total heterozygosity was due to differentiation among populations ($F_{ST} = 1 - \overline{H}_S / H_T$; Tables 3.3, 3.8). In the subset of all presumed unstocked (native) northern and Southern Appalachian samples, 77% of the total heterozygosity was due to differentiation among populations, and among hybrid samples 33% was due to differentiation among populations. Among native northern samples 35%, and among native southern 62% of genetic diversity was due to differentiation among populations.

Evaluated on a regional basis, including samples of four native populations from Great Smoky Mountains National Park, 79% of the total genetic variation in unstocked samples is due to differentiation among populations ($1 - \overline{H}_S/H_T$; Table 3.9; Appendix 3.3). Hierarchical gene diversity analysis (Chakraborty, 1980: Perkins et al., 1993) partitions this 79% as follows: 52% is due to differences among populations north of the New River and populations from the New River southward (regions), 4% is due to differentiation among drainage basins within regions, 13% is due to differentiation among river drainages within basins, and 10% is due to differentiation among populations within river drainages (Table 3.9). Components of the hierarchical analysis of gene diversity are detailed in Table 3.9 and Appendix 3.3

Among all northern samples fixed for the CK-A2* 78* allele, 65% of the total heterozygosity is shared among samples (Table 3.9). Differentiation among upper Ohio drainage (LBM) and northern Atlantic drainage populations accounts for 10% of the total heterozygosity among northern samples, 21% is due to differentiation among rivers within the Atlantic basin, and 4% is due to differentiation among populations within rivers (Table 3.9). This is likely an underestimate of the degree of differentiation between upper Ohio and northern Atlantic drainage populations because the former is represented by only a single sample. Among northern Atlantic drainage samples, 22% of total heterozygosity is due to differences among river drainages (Table 3.9). Differentiation among river drainages accounts for all of the differentiation among populations, with none due to differentiation among populations within river drainages.

Differentiation among populations accounted for 62% of total heterozygosity among Southern Appalachian samples fixed for the CK-A2* 100* allele, and 33% of the total heterozygosity among hybrid samples. Among the presumed unstocked Southern Appalachian brook trout populations, including four samples from Great Smoky Mountains National Park, 68% of total heterozygosity is due to differentiation among populations, partitioned as follows: 7% is due differentiation among basins (New River, lower Ohio River, and Atlantic), 40% is due to differentiation among river drainages within basins, and 21% is due to differentiation of populations within river drainages (Table 3.9).

Genetic identity - The average normalized genetic identity (Nei's I) of all samples was 0.922 (range: 0.755 - 1.0; Table 3.10). Among the 12 samples north of the New River the average normalized genetic identity was 0.963 (range: 0.875 - 0.999). Among the 35 samples from the New River south, the average I was 0.953 (range 0.841 - 1.0). The highest average genetic identities were observed among southern samples fixed for the CK-A2* 100* allele, I = 0.973, and among northern samples fixed for the CK-A2* 78* allele, excluding LBM, I = 0.986. The lowest normalized genetic identity was observed among the subsets of samples fixed for the alternative CK-A2* alleles, I = 0.814 (range: 0.769 - 0.947; Table 3.10). Although it was fixed for the CK-A2* 78* allele, the LBM sample from the Ohio drainage in western Maryland showed greater genetic similarity to southern populations than to northern populations. The average normalized genetic identity of LBM with the other northern samples fixed for the CK-A2* 78* allele was

0.900 (range: 0.875 - 0.945). The average normalized genetic identity of LBM with samples fixed for the CK-A2* 100* allele was 0.923 (range: 0.870 - 0.947). The matrix of normalized genetic identities for all pairwise comparisons among the 48 samples is provided in Appendix 3.4.

Cluster analysis (UPGMA) of normalized genetic identities among samples from presumably unstocked populations pooled by drainage including four samples from Great Smoky Mountains National Park (Part II), also demonstrates this regional structure (Figure 3.2). One group consists of Atlantic drainage populations (Potomac, Rappahannock, James, and Roanoke rivers), naturalized hatchery derived populations (MEG, and MC), and hatchery strains (EDY, and ROM) fixed for the CK-A2* 78* allele. The other group consists of New River, Tennessee River (upper Tennessee and Great Smoky Mountains National Park), and southern Atlantic drainage (Yadkin and Saluda/Savannah) populations. The Ohio drainage LBM population which is fixed for the CK-A2* 78* allele, clusters with this latter group. The two groups are differentiated at I = 0.819. The LBM population and the southern populations are differentiated at I = 0.923.

Cluster analysis of samples fixed for the CK-A2* 78* allele, including two hatchery strains (EDY and ROM) and the hatchery derived Meigs Creek population (MEG) discussed in Part II, identified two groups: the LBM population and all other populations and hatchery strains (Figure 3.3). The two groups are differentiated at I = 0.899.

Discussion

Genetic structure among regions - Genetic variation among brook trout populations from Maryland to South Carolina is structured hierarchically on the basis of region and drainage basin. Regionally a northern group and a Southern Appalachian group are most clearly distinguished on the basis of fixed differences at the CK-A2* locus. This regional structure is also shown by the distribution of common alleles at the PEPB*, AAT-1,2*, and GPI-B2* loci (Table 3.6). Over all populations sampled, approximately 46% of the total gene diversity (H_T) is due to variation among these regions.

Genetic identity estimates indicate substantial differentiation between northern and Southern Appalachian populations. The average identity of 0.814 between unstocked northern and Southern Appalachian populations is substantially lower than the range of 0.975 to 0.995 observed between three subspecies of cutthroat trout, *Oncorhynchus clarki*, lacking fixed allozyme differences, and are within the range observed between three cutthroat trout subspecies with two or more diagnostic loci, 0.743 to 0.928 (Leary et al., 1987). Leary et al. (1987) concluded that the three highly differentiated cutthroat subspecies should be treated as distinct species. Examining 60 allozyme loci to assess genetic relationships among *Salvelinus* sp., Crane et al. (1994) obtained estimates for pairs of species that are also higher than those observed between northern and Southern Appalachian brook trout populations. In their UPGMA inference of phylogenetic relationships using Nei's index, *S. alpinus* and *S. malma* were sister taxa differentiated at I = 0.879. *S. confluentus* and *S. pluvius* were sister taxa differentiated at I = 0.844. The two sister clades were differentiated at I = 0.805.

My analysis involved loci that were selected because they are variable, and this influences the magnitude of the genetic identities obtained. However, northern and Southern Appalachian brook trout lineages are differentiated to a degree that is of considerable management importance, and perhaps indicative of deep evolutionary divergence. Traditional taxonomic methods have evidently failed to recognize this level of differentiation. Given the molecular data it is evident that a taxonomic assessment of *Salvelinus fontinalis sensu lato* is in order. This identification of divergent northern and southern lineages should be the starting point for this assessment.

Genetic structure among populations – Average observed heterozygosity and polymorphism are higher in northern populations than unstocked Southern Appalachian populations. A higher proportion of total heterozygosity is shared among northern populations than is shared among Southern Appalachian populations, with 65% of total genetic variance in northern samples, and 78% of the total variance among northern Atlantic drainage samples shared among samples. This is comparable to the 63% within-sample variation observed by Perkins et al. (1993) in northern Atlantic and Allegheny drainage populations from New York and Pennsylvania. Among Southern Appalachian populations fixed for the CK-A2* allele, including four populations from Great Smoky Mountains National Park, 32% of the total heterozygosity is shared among samples.

Within the northern region genetic structure among river drainages is reflected in variation at PEPB*, AAT-1,2*, and GPI-B2*. Variation at these loci also distinguishes between northern Ohio River drainage populations and northern Atlantic drainage

populations. Average frequencies of AAT-1,2* and GPI-B2* alleles in Atlantic drainage populations from Maryland and Virginia north of the New River are similar to those observed by Perkins et al. (1993) in populations from the Delaware, Hudson, and St. Lawrence River drainages in New York (Table 3.11). Allele frequencies of these loci in the Ohio drainage LBM population differ substantially from the Atlantic drainage populations but are similar to those observed by Perkins et al. (1993) in Allegheny River (upper Ohio River) drainage populations from New York and Pennsylvania (Table 3.11).

The relatively low differentiation among northern Atlantic drainage populations observed in my study and by Perkins et al. (1993) may reflect low levels of differentiation since post-Pleistocene colonization, or may represent the homogenizing effects of extensive stocking with hatchery strains. If northern Atlantic populations are derived from small founder populations from a single Pleistocene refugium (Perkins et el., 1993; Smith, 1981), the former hypothesis appears most likely. However, the effects of stocking on the genetic structure of northern populations is difficult to assess because hatchery strains are derived from northern populations and share diagnostic alleles with northern populations. Clarification of the extent of differentiation among northern populations and of the effects of stocking with hatchery strains will require the examination of other genomic markers.

Elucidation of genetic structure among Southern Appalachian populations is less obscured by the effects of stocking because diagnostic markers allow the identification of native populations. UPGMA cluster analysis of my samples revealed three biogeographical subdivisions of Southern Appalachian brook trout (Figure 3.4). These

subdivisions are identified primarily on the basis of different fixed or high frequency common alleles at the GPI-B2* locus (Table 3.11). New and Yadkin River populations carry the GPI-B2* 40* allele at high frequencies. Similarities among allele frequencies of New River populations and populations in the Atlantic drainage Yadkin River suggests that the latter are derived from New River populations, either by stream capture or undocumented stock transfer. Populations from upper Tennessee River tributaries, the Holston, Watauga, and Nolichucky Rivers, are fixed for the GPI-B2* 100* allele or carry the allele at high frequency (Kriegler, 1993; Kriegler et al. 1995; Saidak, 1995). South of the Nolichucky River, unstocked populations in the Pigeon, Little, and Little Tennessee River drainages are fixed for the GPI-B2* 70* allele or carry the allele at high frequency (Part II; Kriegler, 1993; Kriegler et al. 1995; Saidak, 1995). Atlantic drainage South Carolina populations identified as Southern Appalachian are also fixed for the GPI-B2* 70* allele. Southern Appalachian populations in South Carolina probably originated from undocumented stock transfer from the Tennessee River basin or from headwater capture of Tennessee River streams by Atlantic drainage streams.

Northern Ohio River populations - My data demonstrate that the LBM population and northern Ohio drainage Allegheny River populations examined by Perkins et al. (1993) are more similar to Southern Appalachian populations than they are to more geographically proximate northern Atlantic drainage populations (Table 3.11, Figure 3.2). The data support Perkins et al.'s hypothesis of separate Pleistocene refugia for northern populations in the Ohio drainage and in Atlantic Ocean drainages (Perkins et al., 1993).

The relationships between populations in the northern Ohio River drainage, the New River drainage, and the Tennessee River drainage are problematic. Molecular genetic similarities among populations in these drainages indicate a more recent common ancestry for all Ohio drainage (northern Ohio, Allegheny, and Tennessee) populations than for northern Atlantic and Ohio drainage populations. This observation supports Mayden's (1988) assessment of the biogeographical relationships among eastern North American fish faunas. However, the northern Ohio River populations are fixed for the same CK-A2* allele as the northern Atlantic populations that provides the basis for distinguishing northern and Southern Appalachian lineages.

Hypotheses for the relationships among northern Ohio, northern Atlantic, and lower Ohio brook trout populations must account for the fixed differences between northern and Southern Appalachian populations at the CK-A2* locus, and for the greater overall genetic similarity of northern Ohio to lower Ohio populations than to northern Atlantic populations. Several hypotheses are possible. If the hypothesis of separate Wisconsinan refugia for northern Ohio and northern Atlantic populations (Perkins et al., 1993) is correct, the greater genetic similarity among Ohio River populations reflects a more recent pre-Wisconsinan geographical isolation of northern and lower Ohio populations than of Ohio River and Atlantic drainage populations. Fixation of northern Ohio and lower Ohio populations for different CK-A2* alleles would have occurred after the geographical isolation of the two Ohio River lineages, by genetic drift or selection for the different CK-A2* alleles. Alternatively, northern Ohio populations may be derived from the same Wisconsinan glaciation refugium as Southern Appalachian populations

fixed for the CK-A2* 100* allele. Introgression of the CK-A2* 78 allele into descendent northern Ohio populations would thus be a consequence of post-glaciation hybridization with northern Atlantic populations. Fixation of the CK-A2* 78 allele in these northern Ohio populations might be a consequence of genetic drift, selection for the CK-A2* 78* allele, or selection for northern lineage alleles linked to the CK-A2* locus. A third hypothesis is that northern Ohio populations are derived from northern Atlantic populations fixed for the CK-A2* 78* allele, and that their greater genetic similarity with lower Ohio populations is a consequence of post-Wisconsinan hybridization with populations from the lower Ohio (Southern Appalachian) refugium. Although my allozyme data do not permit clear selection among these alternatives, the greater overall genetic similarity between northern Ohio and lower Ohio populations than between northern Ohio and northern Atlantic populations argues for a more recent common ancestry for Ohio River populations than Ohio and Atlantic drainage populations. This is also consistent with the observed differences between fish assemblages in Atlantic and Ohio River drainages (Mayden, 1988).

Management and conservation implications – Between New York and the Southern Appalachians there are at least two major biogeographically discrete, evolutionarily significant units of *Salvelinus fontinalis*. At the broadest scale, conservation of brook trout genetic diversity requires that these biogeographical lineages be managed as distinct entities. In particular, brook trout from one lineage should not be stocked into the range of another lineage. In addition, heterogeneity among populations within these two lineages

is largely structured by watershed. If preservation of brook trout genetic diversity is a management goal, this finer scale structure must be considered as well.

The fixed CK-A2* markers permits the identification of hatchery strain – Southern Appalachian hybrid populations and the identification of Southern Appalachian populations with little or no hatchery introgression. Given the 60 – 70% decline of brook trout range in the Southern Appalachians during this century (Bivens, 1985) and the extent of stocking with northern derived hatchery strains (Kriegler et al., 1995), the number of extant populations with no evidence of hatchery introgression is perhaps surprising. Approximately 50% of the approximately 100 populations in Tennessee are identified as native Southern Appalachian by diagnostic allozyme markers (Guffey et al., 1998; Strange and Habera, 1995). In Great Smoky Mountains National Park, 35 of 47 populations were identified as native by the same criteria (Part II).

Many of the populations identified as native Southern Appalachian are found in streams known to have been stocked with hatchery strains. Some of these populations may very well contain hatchery genes that were not detected by the allozyme assays. However, if these are cryptic hybrid populations, the levels of hatchery introgression in them is evidently low. There are several possible reasons why hatchery genes may not have been established in Southern Appalachian populations. Holloway (1945) suggested that hatchery strains were less hardy than native Southern Appalachian populations, and, additionally, that stocked fish were rapidly removed by anglers. The heaviest stocking was usually at low elevation sites away from most high elevation stream segments. These low elevation sites had road access for stock trucks and easy access for anglers (Monty

Seehorn, United States Forest Service, personal communication). Alternatively, the failure of northern genotypes to become established in most Southern Appalachian streams may reflect adaptive differences between the two lineages rather than hatchery conditioning or angler rapaciousness. The hypothesis of adaptation of northern and Southern Appalachian genotypes to different environments is intuitively appealing, if difficult to test. Whatever the causes of hatchery strains failing to become established in many streams, the continuation or renewal of stocking with hatchery strains has no place in the future management of Southern Appalachian brook trout. Instead, native genetic diversity should become the focus of brook trout management in the region.

The improved situation for Southern Appalachian brook trout over the past three decades (Part I; Strange and Habera, 1998) may be short lived. As demands for timber wood fiber continue (Nolt et al., 1997; SAMAB, 1996), and if predictions of global warming materialize (Meisner, 1990), the survival of Southern Appalachian brook trout may require a new level of intensive management. The Southern Appalachians are a center of diversity for brook trout, and as such are of obvious biodiversity conservation concern. In contrast to northern lineage populations, the greatest proportion of genetic diversity in southern lineage populations is found among rather than within populations. Therefore, the loss of individual Southern Appalachian populations has a greater likelihood of resulting in a loss of local, regional, and taxon wide genetic diversity. Population surveys coupled with the genetic data suggest a number of strategies for the management of genetic diversity among these populations.

Native brook trout should be reintroduced in watersheds where brook trout have been extirpated. Reintroductions can provide a hedge against the extinction of locally adapted or differentiated populations, and meet the demands of anglers for trout fishing opportunities. In order to maintain genetic diversity and heterogeneity among populations, reintroductions and augmentation of declining populations should rely on geographically proximate native populations from the same river drainage. Because of the heterogeneity among populations from different watersheds, the establishment of Southern Appalachian hatchery strains for these stockings should not be considered. Streams with native populations should be regularly monitored to assess the status and distribution of salmonid populations and the quality of habitats. Because of the genetic structure among Southern Appalachian populations and because the stream mileage occupied by native populations has declined by almost 80% during this century (Habera and Strange, 1993; Bivens, 1984), indications of native brook trout decline in individual streams should be a source of concern and an impetus to ameliorative actions.

Finally, management of Southern Appalachian brook trout should incorporate a strong educational and public relations effort emphasizing the value of native diversity and the significance of the region's only native salmonid. Southern Appalachian brook trout, and Southern Appalachian biodiversity generally, will only be preserved if there is public desire to do so. Educational efforts should focus on the uniqueness of the native Southern Appalachian biota, and promote the region's only native salmonid as a symbol of wild biodiversity, and as an indicator of environmental quality. Fisheries management

should focus on promoting the unique aesthetic and recreational value of native brook trout and on increasing native trout angling opportunities.

Literature Cited

Allendorf, F. W., and N. Ryman. 1987. Genetic management of hatchery stocks. Pp. 141-160 *In* Ryman, N., and F. Utter. eds. Population genetics and fisheries management. Univ. of Washington Press, Seattle.

Andrews, J.T. 1987. The late Wisconsin glaciation and deglaciation of the Laurentide ice sheet. Pp. 13-37 *In* Ruddiman, W. F. and H. E. Wright, Jr., eds., The geology of North America, Vol K-3: North America and adjacent oceans during the last deglaciation. Geological Society of America, Boulder, CO.

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman & Hall, NY.

Bailey, R. M., and G. R. Smith. 1981. Origin and geography of the fish fauna of the Laurentian Great Lakes basin. Can. J. Fish. Aquat. Sci. 38:1539-1561.

Behnke, R. J. 1980. A systematic review of the genus *Salvelinus*. Pp. 441-481 *In* E. K. Balon (ed.), Charrs: Salmonid Fishes of the Genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands.

Bivens, R. D. 1985. History and distribution of brook trout in the Appalachian region of Tennessee. Tennessee Wildlife Resources Agency Technical Report No. 85-1. The University of Tennessee, Knoxville.

Briggs, J. C. 1986. Introduction to the zoogeography of North American freshwater fishes. Pp. 1-16 *In* Hocutt, C. H., and E. O. Wiley, eds., The zoogeography of North American freshwater fishes. John Wiley and Sons, New York.

Burr, B. M., and L. M. Page. 1986. Zoogeography of fishes of the lower Ohio-upper Mississippi basin. Pp. 287-324 *In* Hocutt, C. H., and E. O. Wiley, eds., The zoogeography of North American freshwater fishes. John Wiley and Sons, New York.

Chakraborty, R. 1980. Gene diversity analysis in nested subdivided populations. Genetics 96:721-726.

Clayton, J. W., and D. N. Tretiak. 1972. Amine-citrate buffers and pH control in starch gel electrophoresis. J. Fish. Res. Board Can. 29:1169-1172.

Crane, P. A., L. W. Seeb, and J. E. Seeb. 1994. Genetic relationships among *Salvelinus* species inferred from allozyme data. Can. J. Fish. Aquat. Sci. 51 (Suppl. 1): 182-197.

Dunham, R. A., J. DiBona, L. Robison, K. Norgren, J. Durniak, and M. Spencer. 1994. Biochemical genetics of brook trout, *Salvelinus fontinalis*, in Georgia. Unpublished report submitted to the Georgia Department of Natural Resources, Social Circle, GA.

Ferguson, M. M. 1989. The genetic impact of introduced fishes on native species. Can. J. Zool. 68:1053-1057.

Flebbe, P. A. 1994. A regional view of the margin: Salmonid abundance and distribution in the southern Appalachian Mountains of North Carolina and Virginia. Trans. Am. Fish. Soc. 123:657-667.

Gilbert, C. R. 1976. Composition and derivation of the North American freshwater fish fauna. Fla. Sci. 39:104-111.

Guffey, S. Z., G. F. McCracken, S. E. Moore, and C. R. Parker. 1998. Management of isolated populations: native Southern Appalachian brook trout. *In* J. Peine, ed., Ecosystem management for sustainability: principles and practices illustrated by the Southern Appalachian Region. CRC Press, Boca Raton, FL. (in press).

Habera, J. W., and R. J. Strange. 1993. Wild trout resources and management in the Southern Appalachians. Fisheries 18:6-13.

Hayes, J. P., S. Z. Guffey, F. J. Kriegler, G. F. McCracken, and C. R. Parker. 1996. The genetic diversity of native, stocked, and hybrid populations of brook trout in the southern Appalachians. Conserv. Biol. 10:1403-1412.

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

Hocutt, C. H., R. E. Jenkins, and J. R. Stauffer, Jr. 1986. Zoogeography of the fishes of the central Appalachians and central Atlantic coastal plain. Pp. 161-211 *In* Hocutt, C. H., and E. O. Wiley, eds., The zoogeography of North American freshwater fishes. John Wiley and Sons, New York.

Jenkins, R. E., E. A. Lochner, and F. J. Schwartz. 1971. Fishes of the central Appalachian drainages: Their distribution and dispersal. Pp. 43-117 *In* Holt, P. C., ed. The distributional history of the biota of the Southern Appalachians. Part III: Vertebrates. Research Division Monograph 4. Virginia Polytechnic Institute and State University. Blacksburg, Virginia.

Kriegler, F. J., G. F. McCracken, J. W. Habera, and R. J. Strange. 1995. Genetic characterization of Tennessee brook trout populations and associated management implications. N. Am. J. Fish. Mgmt. 15:804-813.

Kriegler, F. J. 1993. The genetics of native and stocked brook trout populations in east Tennessee. Unpublished Master's thesis. University of Tennessee, Knoxville.

Leary, R.F., F.W. Allendorf, S.R. Phelps, and K.L. Knudsen. 1987. Genetic divergence and identification of seven cutthroat subspecies and rainbow trout. Trans. Am. Fish. Soc. 116:580-587.

McCracken, G. F., C. R. Parker, and S. Z. Guffey. 1993. Genetic differentiation and hybridization between stocked hatchery and native brook trout in Great Smoky Mountains National Park. Trans. Am. Fish. Soc. 122:533-542.

MacCrimmon, H. R., and J. S. Campbell. 1969. World distribution of brook trout, *Salvelinus fontinalis*. J. Fish. Res. Board Can. 26:1699-1725.

May, B., J. E. Wright, and M. Stoneking. 1979. Joint segregation of biochemical loci in Salmonidae: results from experiments with *Salvelinus* and review of the literature on other species. J. Fish. Res. Board Can. 36:1114-1128.

Mayden, R. L. 1988. Vicariance biogeography, parsimony, and evolution in North American freshwater fishes. Syst. Zool. 37:329-355.

Meisner, J. D. 1990. Effect of climatic warming on the southern margins of the native range of brook trout, *Salvelinus fontinalis*. Can. J. Fish. Aquat. Sci. 47:1065-1070.

Miller, R. R. 1965. Quaternary freshwater fishes of North America. Pp. 569-581 *In* Wright, H. E., Jr., and D. G. Frey, eds. The Quaternary of the United States. Princeton University Press, Princeton, NJ.

Morgan, R. P., II, and B. M. Baker. 1991. Development of genetic inventories for Maryland game fish: brook trout. Unpublished report submitted to the Maryland Department of Natural Resources, Annapolis.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.

Nolt, J., A. L. Bradley, M. Knapp, D. E. Lampard, and J. Scherch. 1997. What have we done? Earth Knows Publications, Washburn, TN.

Perkins, D. L., C. C. Kruger, and B. May. 1993. Heritage brook trout in northeastern USA: Genetic variability within and among populations. Trans. Am. Fish Soc. 122: 515-32.

Philipp, D. P., J. M. Epifanio, and M. J. Jennings. 1993. Point/Counterpoint: Conservation genetics and current stocking practices - are they compatible? Fisheries 18:14-16.

Power, G. 1980. The brook charr (*Salvelinus fontinalis*). Pp. 797-840 *In* E. K. Balon (ed.), Charrs: Salmonid Fishes of the Genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands.

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

Robison, H. W. 1986. Zoogeography implications of the Mississippi River basin. Pp. 267- 285 *In* Hocutt, C. H., and E. O. Wiley, eds., The zoogeography of North American freshwater fishes. John Wiley and Sons, New York.

Saidak, L. R. 1995. Brook trout (*Salvelinus fontinalis*) in Tennessee: An ecological genetic study using allozyme electrophoresis and random amplified polymorphic DNA. Unpublished Master's thesis. University of Tennessee, Knoxville.

SAMAB. 1996. Southern Appalachian assessment social/cultural/economic technical report. Southern Appalachian Man and the Biosphere Program, Gatlinburg, TN.

Shaklee, J. B., F. W. Allendorf, D. C. Moritz, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Trans. Am. Fish. Soc. 119:2-15.

Sheldon, A. L. 1988. Conservation of stream fishes: patterns of diversity, rarity, and risk. Conserv. Biol. 2:149-156.

Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. W. H. Freeman, San Francisco.

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

Wiley, E. O., and R. L. Mayden. 1985. Species and speciation in phylogenetic systematics, with examples from the North American fish fauna. Ann. Missouri Bot. Gard. 72:596-635.

Workman, P. L., and J. D. Niswander. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Human Genetics 22:24-49.

Table 3.1. Brook trout populations sampled from Maryland, Virginia, North Carolina and South Carolina, by drainage and state, sample abbreviation, and sample size (n).

Sample	Sample Abbreviation	n	Map location (**Figure 3.1**)
Upper Ohio River			
Little Bear Creek (MD)	LBM	25	1
Northern Atlantic Ocean			
Potomac River			
Fishing Creek (MD)	FCM	25	2
Poplar Lick Run (MD)	PLC	25	3
Jeremeys Run (VA)	JRS	25	4
Dry River (VA)	VLR	20	5
Rappahannock River			
Piney River (VA)	PBS	25	6
James River			
Spy Run (VA)	SPY	21	7
Johns Creek (VA)	JCJ	23	8
Shawvers Run (VA)	SRJ	23	9
Valley Branch (VA)	VBN	15	10
Roanoke River			
Big Stoney Creek (VA)	BSR	23	11
Rock Castle Creek (VA)	RCC	24	12
Southern Atlantic Ocean			
Yadkin River			
North Fork Stewarts Creek (VA)	NFS	10	13
Pauls Creek (VA)	PCY	26	14
Ramey Creek (NC)	RAM	24	15
Saddle Mountain Creek (NC)	UTS	25	16
Widows Creek (NC)	WC	25	17
Catawba River			
New Years Creek (NC)	NYC	23	18
New River			
Dry Creek (VA)	DCN	24	19
Hanks Creek (VA)	HCN	10	20
Middle Fork Helton Creek (VA)	HCW	20	21

Table 3.1 continued.

Sample	Sample Abbreviation	n	Map location (Figure 3.1)
New River (continued)			
Killinger Creek (VA)	KCN	12	22
Laurel Branch (VA)	LBN	15	23
North Prong Buckhorn Creek (VA)	NBN	25	24
North Fork Elk Creek (VA)	NFE	19	25
Darnell Creek (NC)	DC	25	26
Elk Creek (NC)	EC	20	27
Little Phoenix Creek (NC)	LPC	20	28
Long Hope Creek (NC)	LHC	25	29
Middle Fork New River (NC)	MFN	24	30
Three Top Creek (NC)	TTC	25	31
Big Piney C reek (NC)	UTP	20	32
Lower Ohio River(Tennessee River)			
Grindstone Branch (VA)	GCT	16	33
Houndshell Branch (VA)	HBT	12	34
Jerrys Creek (VA)	JCT	11	35
Little Laurel Creek (VA)	LLT	16	36
Pond Creek (NC)	PC	25	37
Southern Atlantic			
Saluda River			
Falls Creek (SC)	FC	20	38
Head Foremost Creek (SC)	HF	20	39
Matthews Creek (SC)	MC	21	40
Slicking Creek (SC)	SC	10	41
Savannah River			
Bad Creek (SC)	BC	20	42
Crane Creek (SC)	CC	18	43
Emory Creek (SC)	EMC	19	44
Indian Camp Creek (SC)	IC	20	45
Ira Branch (SC)	IR	17	46
Jacks Creek (SC)	JC	22	47
Pig Pen Creek (SC)	PP	20	48

Table 3.2. Enzymes, locus designations, proportion of samples polymorphic (%P), number of alleles, tissue sources and electrophoresis buffer systems used in the study. Enzyme numbers follow the reccomendations of IUBNC (1984). Locus nomenclature follows Shaklee et al. (1990). Tissues used were skeletal muscle (M) and eye (E). Electrophoresis buffers were morpholine-citrate, pH 6.1 (C) after Clayton and Tretiak (1972) as modified by May et al. (1979), and discontinuous lithium-borate (R) after Ridgeway et al. (1970).

Enzyme or other protein	Enzyme number	Locus	%P	Number of alleles	Tissue	Buffer system
Aspartate aminotransferase	2.6.1.1	sAAT-1, 2*	60	3	M	R
Creatine kinase	2.7.3.2	CK-A1*	0	1	M	R
		CK-A2*	42	2	M	R
Dihydrolipoamide dehydrogenase	1.8.1.4	DDH-3*	0	0	M	C
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH-1*	19	2	M	R
Glucose-6-phospate isomerase	5.3.1.9	GPI-A*	8	2	M	R
		GPI-B1*	13	4	M	R
		GPI-B2*	56	4	M	R
L-Lactate dehydrogenase	1.1.1.27	LDH-A1*	0	1	M	R
		LDH-A2*	17	2	M	R
		LDH-B1*	42	2	M, E	R
		LDH-B2*	0	1	M, E	R
Malate dehydrogenase	1.1.1.37	sMDH-A1*	0	1	M	C
		sMDH-A2*	1	1	M	C
		sMDH-B1,2*	48	2	M	C

Table 3.2 continued.

Enzyme or other protein	Enzyme number	Locus	%P	Number of alleles	Tissue	Buffer system
Malic enzyme ($NADP^+$)	1.1.1.40	sMEP-1*	56	2	M	C
Peptidase-B[a]	3.4._._	PEPB*	50	3	M	C
Peptidase-S[b]	3.4._._	PEPS*	0	1	M	R
General (unidentified) protein	No number	PROT-1*	0	1	M	R
		PROT-2*	0	1	M	R
		PROT-3*	0	1	M	R
		PROT-4*	0	1	M	R

[a] Peptidase-B resolved on leu-gly-gly substrate

[b] Peptidase-S resolved on leucyl-alanine substrate

Table 3.3: Genetic variation within samples: Summary statistics. N is the number of samples in the subset; average H_S is the average expected heterozygosity of samples; H_T is the expected heterozygosity of pooled samples; H_S/H_T is a measure of the over all contribution of sample heterozygosity to total pooled heterozygosity; average P is the average proportion of loci polymorphic in the samples.

	All samples	Northern fixed CK-A2* 78*	Southern fixed CK-A2* 100*	Polymorphic CK-A2*
N	48	11	16	20
Average H_S	0.055	0.049	0.015	0.087
(standard error)	(0.015)	(0.020)	(0.005)	(0.025)
range	0.0 - 0.143	0.020 - 0.078	0.0 - 0.069	0.018 - 0.143
H_T	0.133	0.075	0.039	0.130
(standard error)	(0.041)	(0.028)	(0.021)	(0.040)
Average H_S/H_T	0.414	0.653	0.385	0.669
Average P	0.187	0.174	0.085	0.273
range	0.0 - 0.409	0.091 - 0.273	0.0 - 0.273	0.091 - 0.409

Table 3.4. Deviations from Hardy-Weinberg expectation.

Sample - Locus Genotypes	Observed	Expected
BC - CK-A2*		
100*/100*	11	8.4
78*/100*	4	9.1
78*/78*	5	2.5
PP - CK-A2*		
100*/100*	0	1.5
78*/100	11	8
78*/78*	9	10.5
EMC - MDH-B1,2*		
100*/100*	2	4.3
100*/120*	14	9.5
120*/120*	3	5.3
HF - GPI-B2*		
100*/100*	2	0.2
70*/100*	0	3.6
70*/70*	18	16.2
IC - sMEP-1*		
100*/100*	9	5.5
63*/100*	3	10
63*/63*	8	4.5
MC - LDH-B1*		
100*/100*	0	2.3
67*/100*	14	9.3
67*/67*	7	9.3

Table 3.5. Average single locus heterozygosities of all samples. H_T is the expected heterozygosity of pooled samples; H_S is the average expected heterozygosity of all samples; ratios of single locus heterozygosity to the sum of heterozygosities provide a measure of each locus' contribution to over all heterozygosity; H_S/H_T is a measure of the over all contribution of sample heterozygosity to total pooled heterozygosity.

Locus	H_T	$H_T/\partial H_T$	H_S	$H_S/\partial H_S$	H_S/H_T
CK-A2*	0.482	0.183	0.134	0.125	0.278
PEPB*	0.449	0.171	0.146	0.136	0.325
AAT-1,2*	0.431	0.164	0.190	0.177	0.441
GPI-B2*	0.588	0.224	0.168	0.157	0.286
LDH-B1*	0.102	0.039	0.073	0.068	0.716
MDH-B1,2*	0.132	0.050	0.093	0.087	0.705
sMEP-1*	0.304	0.116	0.179	0.167	0.589
G3PDH-1*	0.033	0.013	0.030	0.028	0.909
GPI-B1*	0.065	0.025	0.021	0.020	0.323
GPI-A*	0.018	0.007	0.016	0.015	0.889
LDH-A2*	0.026	0.010	0.023	0.021	0.885

Table 3.6. Average frequency of CK-A2*, PEPB*, AAT-1,2*, and GPI-B2* alleles in samples from north of the New River and in samples from the New River southward. Rare alternate alleles are not included. (The PEPB* 82* and the GPI-B-2* 115* allele were each found in one sample, and the AAT-1,2* 80* allele was found at a frequency of less than 0.05 in three samples).

Population subset	n	CK-A2*		PEPB*		AAT-1,2*		GPI-B2*		
		100*	78*	100*	68*	100*	118*	100*	40*	70*
North of the New River	12	0.03	0.97	0.18	0.79	0.80	0.20	0.88	0.10	0.01
Northern, fixed for CK-A2* 78*	11	-	1.0	0.15	0.81	0.83	0.17	0.90	0.08	0.01
New River southward	35	0.81	0.19	0.85	0.15	0.14	0.86	0.31	0.55	0.14
Southern, fixed for CK-A2* 100*	16	1.0	-	0.96	0.04	0.01	0.99	0.18	0.69	0.13
Hybrid	20	0.63	0.37	0.74	0.26	0.26	0.74	0.45	0.41	0.14

Table 3.7. Correlation of allele frequencies with CK-A2* 78* allele frequency.
(* p[r = 0] < 0.05; ** p[r = 0] < 0.01)

Locus - allele	All samples Pearsons *r*	All southern samples Pearsons *r*	Hybrids Pearsons *r*
PEPB* - 68*	0.834**	0.679**	0.617**
AAT-1,2* - 100*	0.812**	0.524**	0.194
sMEP-1* - 63*	0.664**	0.380*	0.163
GPI-B2* - 100*	0.630**	0.324	0.170
GPI-B2* - 40*	-0.533**	-0.332	-0.229
G3PDH-1* - 78*	0.279	0.662**	0.571*
LDH-B1* - 67*	0.290	0.458**	0.368
GPI-B2* - 70*	-0.157	0.075	0.107
MDH-B1,2* - 120*	-0.050	0.209	0.055

Table 3.8. Genetic heterogeneity among samples: Genetic variance (F_{ST}), standard error (SE) of variance estimate, G-test statistics, and number of loci showing significant allele frequency heterogeneity.

Population (n)	Total G	Total df	Number of loci showing significant heterogeneity $p < 0.05$	$p < 0.01$	F_{ST}	$SE(F_{ST})$
All populations (48)	11,400	1222	9	9	0.587	0.001
All northern fixed CK-A2* 78* (11)	1,220	210	5	5	0.345	0.002
Atlantic drainage fixed CK-A2* 78* (10)	653	189	4	4	0.211	0.003
All southern fixed CK-A2* 100* (16)	1324	270	7	6	0.627	0.002
New River drainage fixed CK-A2* 100* (8)	314	98	5	5	0.159	0.003
Hybrid (20)	2661	475	8	8	0.330	0.002
Fixed for alternative alleles at CK-A2* (27)	8407	594	10	10	0.774	0.001

Table 3.9. Hierarchical partitioning of heterozygosity among all unstocked samples (including samples from four Great Smoky Mountains National Park populations), and subsets of populations. H_T is total heterozygosity of pooled samples, $\overline{H}_S$ is the average expected heterozygosity of samples, H_R is the average of total heterozygosities of samples pooled by region (north of the New River; New River southward), H_D is the average of total heterozygosities of samples pooled by drainage (northern Atlantic, northern Ohio, New and southern Ohio, and southern Atlantic), and H_W is the average of total heterozygosities of samples pooled by watershed (river). $D_{RT} = H_T - H_R$, $D_{DR} = H_R - H_D$, $D_{WD} = H_D - H_W$, and $D_{SW} = H_W - \overline{H}_S$. Each diversity measure (D) is divided by H_T to asses its relative contribution to overall heterozygosity. $F_{ST} = 1 - \overline{H}_S / H_T$ is the proportion of heterozygosity due to differentiation among populations.

All samples fixed at CK-A2*		
$\overline{H}_S$: 0.027		$\overline{H}_S / H_T$: 0.21
H_T: 0.128	D_{RT}: 0.067	D_{RT} / H_T: 0.52
H_R: 0.061	D_{DR}: 0.005	D_{DR} / H_T: 0.04
H_D: 0.056	D_{WD}: 0.016	D_{WD} / H_T: 0.13
H_W: 0.040	D_{SW}: 0.013	D_{SW} / H_T: 0.10

All native southern samples (fixed CK-A2* 100*)		
$\overline{H}_S$: 0.015		$\overline{H}_S / H_T$: 0.32
H_T: 0.047	D_{DT}: 0.003	D_{DT} / H_T: 0.07
H_D: 0.044	D_{WD}: 0.019	D_{WD} / H_T: 0.40
H_W: 0.25	D_{SW}: 0.010	D_{SW} / H_T: 0.21

All northern samples fixed CK-A2* 78*		
$\overline{H}_S$: 0.049		$\overline{H}_S / H_T$: 0.65
H_T: 0.075	D_{DT}: 0.007	D_{DT} / H_T: 0.09
H_D: 0.068	D_{WD}: 0.016	D_{WD} / H_T: 0.21
H_W: 0.052	D_{SW}: 0.003	D_{SW} / H_T: 0.04

Northern Atlantic samples fixed CK-A2* 78*		
$\overline{H}_S$: 0.046		$\overline{H}_S / H_T$: 0.78
H_T: 0.059	D_{WT}: 0.013	D_{WT} / H_T: 0.22
H_W: 0.046	D_{SW}: 0	D_{SW} / H_T: 0

Table 3.10. Average normalized genetic identities (Nei's *I*) across all samples and selected subsets of samples.

Sample	Number of Comparisons	Average *I*	Range of *I*'s
All	1128	0.922	0.755 - 1.0
Northern	66	0.963	0.875 - 0.999
Northern fixed CK-A2* 78*	55	0.969	0.875 - 0.999
Atlantic drainage fixed CK-A2* 78*	45	0.986	0.964 - 0.999
Southern	595	0.953	0.841 - 1.0
Fixed CK-A2* 100*	120	0.973	0.923 - 1.0
Hybrid	190	0.950	0.883 - 0.998
Fixed CK-A2* 78* plus fixed CK-A2* 100*	192	0.814	0.755 - 0.947
Fixed CK-A2* 78* plus hybrid	220	0.887	0.769 - 0.988
Fixed CK-A2* 100* plus hybrid	320	0.944	0.841 - 0.999

Table 3.11. Average frequency of CK-A2*, PEPB*, AAT-1,2*, and GPI-B2* alleles in selected native northern and southern populations. Rare alleles are not included in this table.

Population subset	n	CK-A2*		PEPB*		AAT-1,2*		GPI-B2*		
		100*	78*	100*	68*	100*	118*	100*	40*	70*
Atlantic drainage fixed CK-A2* 78*	10	-	1.0	0.08	0.92	0.88	0.12	0.98	-	0.01
Delaware River drainage[1]	5	-	1.0	no data		0.96	0.04	1.0	-	-
Hudson River drainage[1]	4	-	1.0	no data		0.76	0.23	0.98	0.02	-
St. Lawrence River drainage[1]	10	-	1.0	no data		0.81	0.19	0.98	0.02	-
LBC - Maryland (Allegheny River drainage)	1	-	1.0	0.84	0.16	0.34	0.66	0.12	0.88	-
Allegheny River drainage[1]	4	-	1.0	no data		0.53	0.47	0.20	0.80	-
New River drainage	8	1.0	-	0.94	0.06	0.02	0.98	0.03	0.97	-
Yadkin River drainage	3	1.0	-	1.0	-	-	1.0	0.17	0.83	-
Upper Tennessee River drainage (Watauga and Holston)	3	1.0	-	0.96	0.04	0.01	0.99	0.71	0.29	-
Savannah and Saluda River drainages	2	1.0	-	1.0	-	-	1.0	-	-	1.0

Table 3.11 continued.

Population subset	n	CK-A2*		PEPB*		AAT-1,2*		GPI-B2*		
		100*	78*	100*	68*	100*	118*	100*	40*	70*
Great Smoky Mountains[2] National Park - unstocked	7	1.0	-	0.99	0.01	0.05	0.95	0.05	-	0.95
Holston River drainage[3]	5	1.0	-	no data		0.01	0.99	0.88	0.12	-
Watauga River drainage[3]	3	1.0	-	no data		0.01	0.99	1.0	-	-
Nolichucky River drainage[3]	1	1.0	-	no data		0.03	0.97	1.0	-	-
French Broad River drainage[3]	2	1.0	-	no data		0.27	0.73	-	-	1.0
Tellico River drainage[3]	1	1.0	-	no data		0.16	0.83	-	-	1.0

[1] Data from Perkins et al., 1993
[2] Data from Chapter II
[3] Data from Kriegler et al., 1995

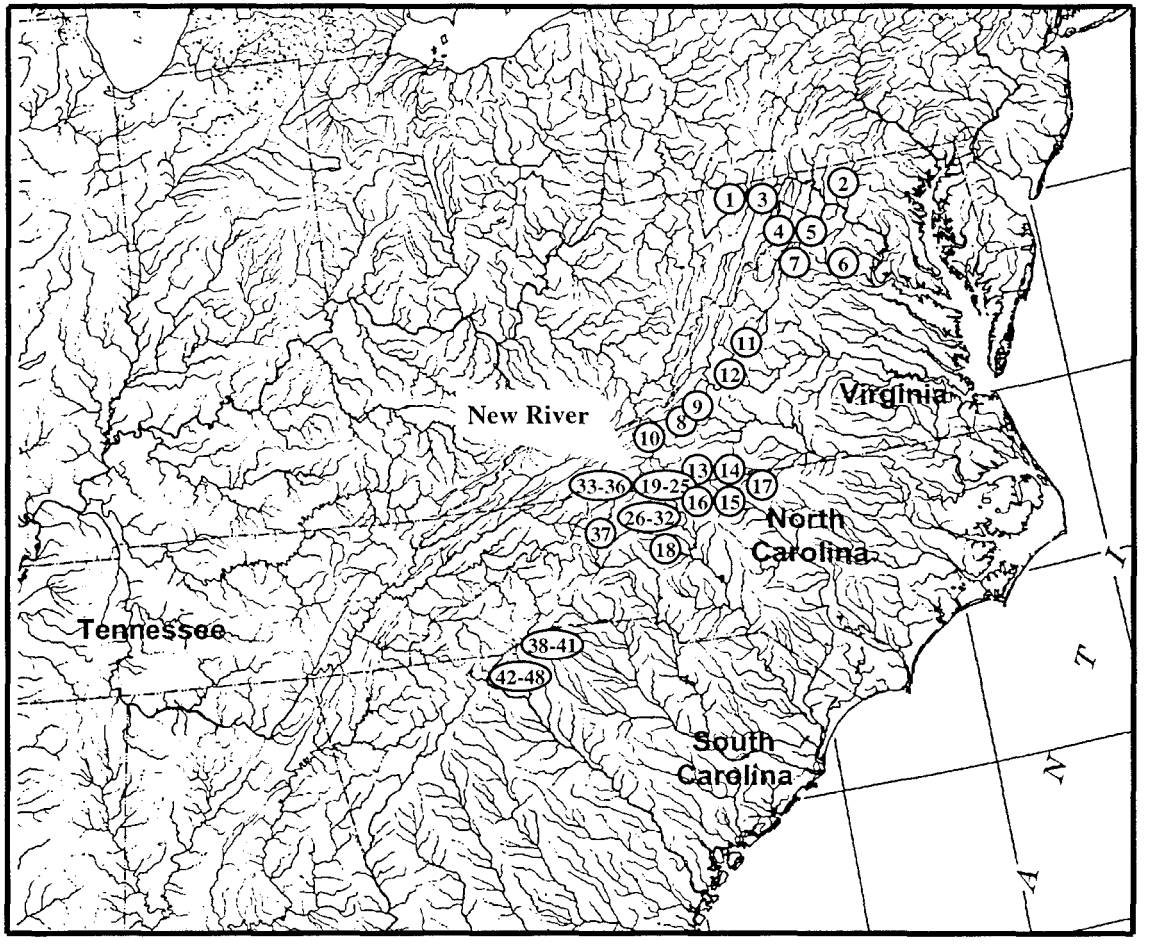


Figure 3.1. Approximate brook trout sampling locations in Maryland. Virginia, North Carolina, and South Carolina. Stream codes are indexed in **Table 3.1.**

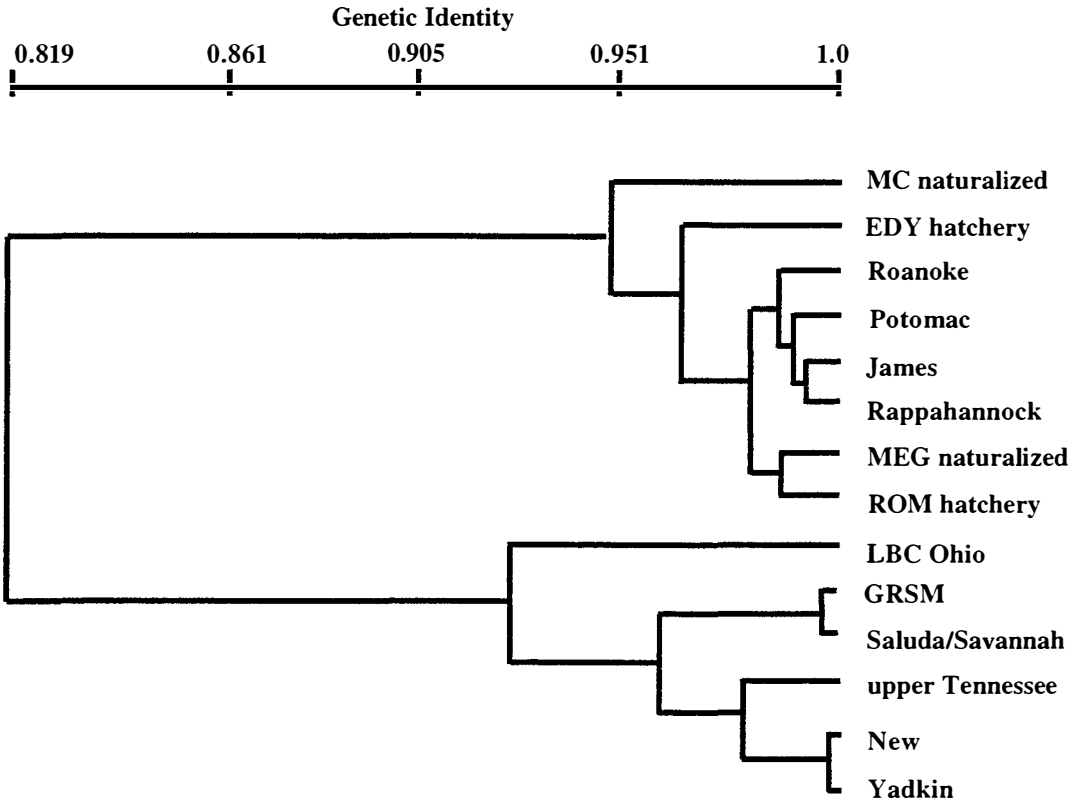


Figure 3.2. UPGMA dendrogram of unstocked populations pooled by river drainage, hatchery strains, and naturalized hatchery populations using Nei's index of genetic identity.

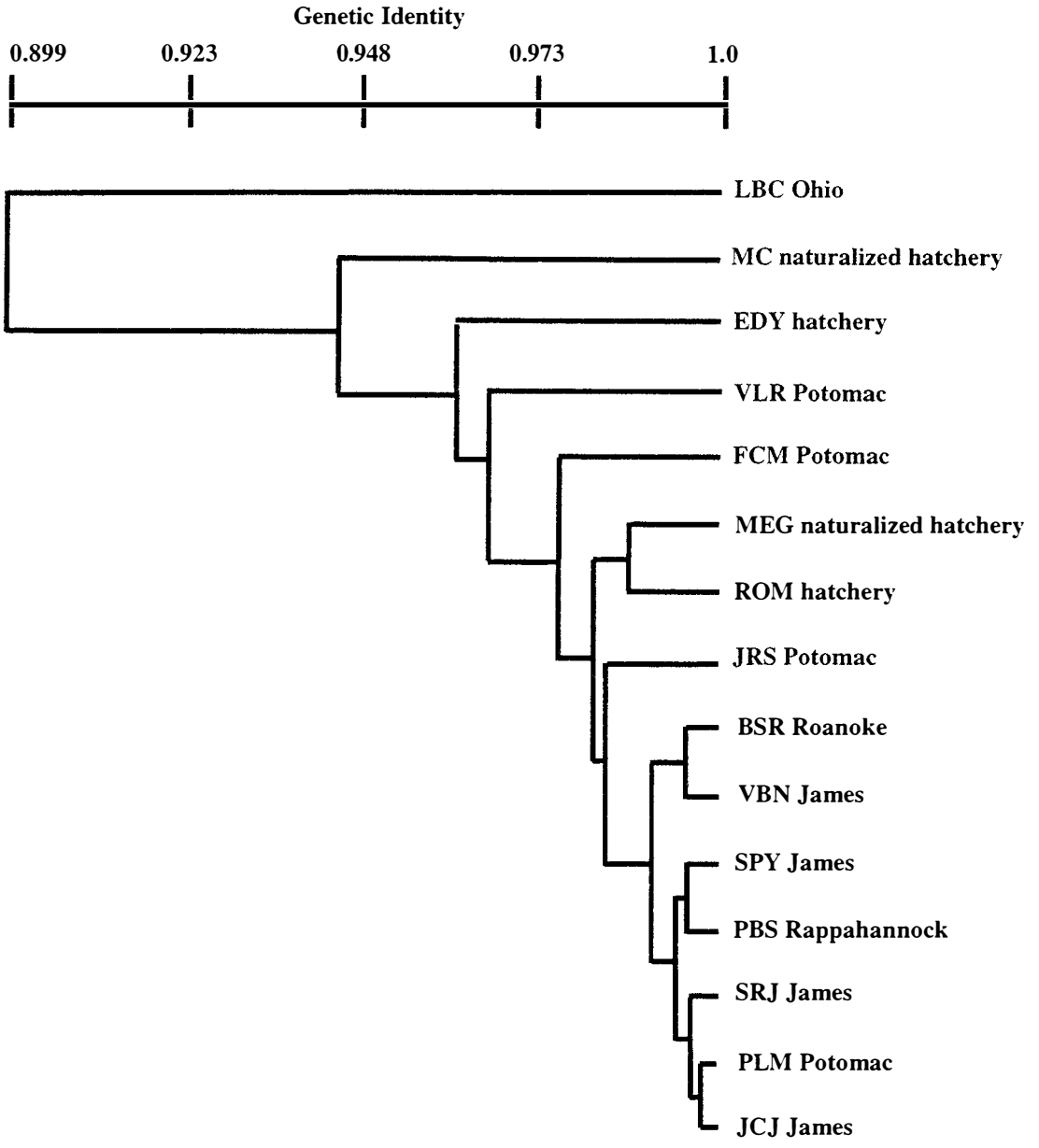
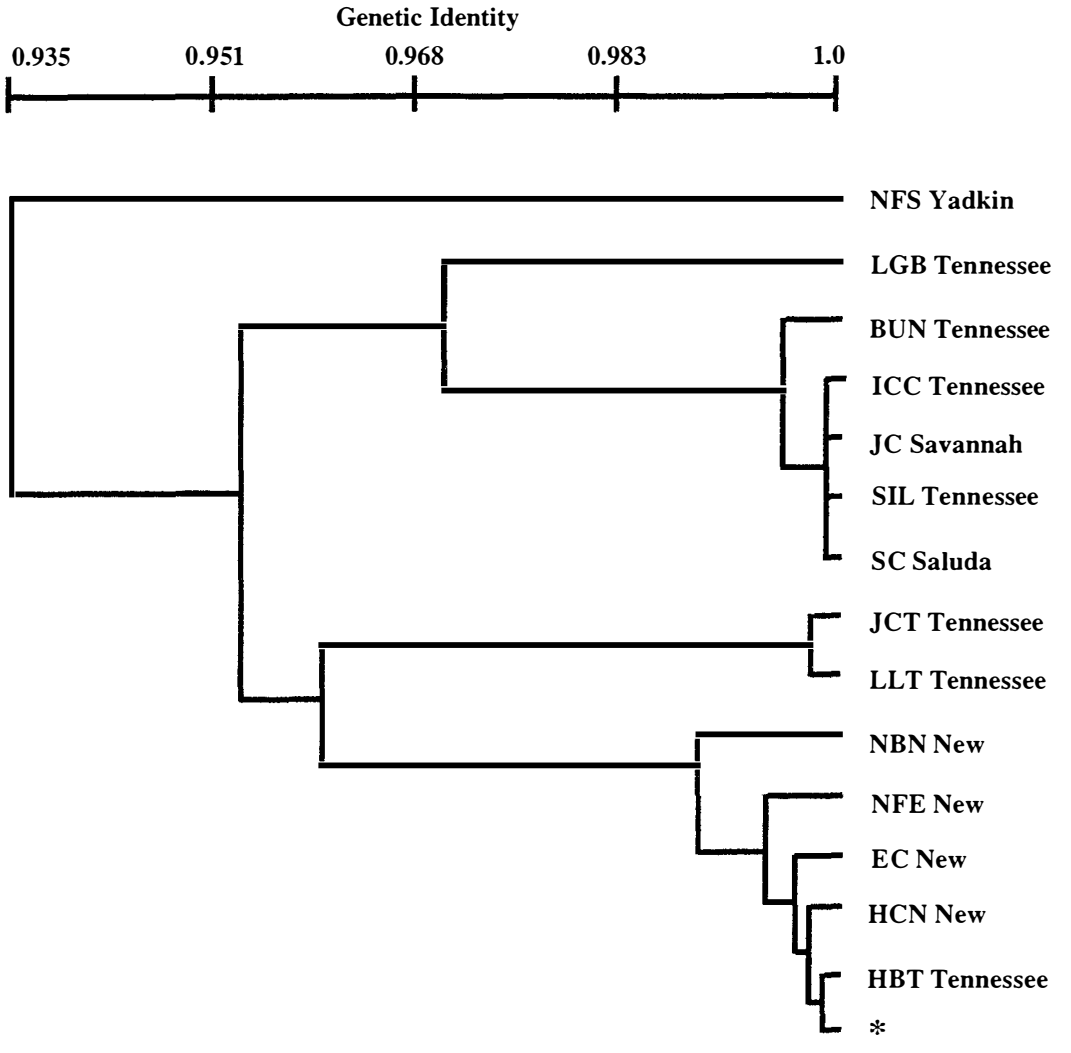


Figure 3.3. UPGMA dendrogram of northern populations and hatchery strains using Nei's index of genetic identity.



* TTC, DCN, LHC, DC (New); UTS, PCY (Yadkin)

Figure 3.4. UPGMA dendrogram of unstocked Southern Appalachian populations using Nei's index of genetic identity.

Appendix 3.1. Allele frequencies of polymorphic loci, sample size (N), expected heterozygosity (H_S), observed heterozygosity (H_O), and proportion of loci polymorphic (P)for all samples.

Sample	CK-A2*		AAT-1,2*			G3PDH-1*	
	100*	78*	100*	80*	118*	100*	78*
Ohio River							
LBM (MD)	-	1.0	0.34	-	0.68	1.0	-
Potomac River							
FCM (MD)	-	1.0	0.89	-	0.11	1.0	-
PLC (MD)	-	1.0	1.0	-	-	1.0	-
JRS (VA)	-	1.0	0.61	-	0.39	1.0	-
VLR (VA	-	1.0	0.70	0.03	0.27	1.0	-
Rappahannock River							
PBS (VA)	-	1.0	0.78	-	0.22	1.0	-
James River							
SPY (VA)	-	1.0	0.88	-	0.12	1.0	-
JCJ (VA)	-	1.0	0.99	-	0.01	1.0	-
SRJ (VA)	-	1.0	0.97	-	0.03	0.86	0.14
VBN (VA)	-	1.0	1.0	-	-	1.0	-
Roanoke River							
BSR (VA)	-	1.0	1.0	-	-	1.0	-
RCC (VA)	0.36	0.64	0.43	-	0.57	1.0	-
Yadkin River							
NFS (VA)	1.0	-	-	-	1.0	1.0	-
PCY (VA)	1.0	-	-	-	1.0	1.0	-
RAM (NC)	0.84	0.16	0.07	-	0.93	0.94	0.06
UTS (NC)	1.0	-	-	-	1.0	0.98	0.02
WC (NC)	0.18	0.82	0.48	0.04	0.48	0.84	0.16
Catawba River							
NYC (NC)	0.83	0.17	0.21	-	0.79	1.0	-
New River							
DCN (VA)	1.0	-	-	-	1.0	1.0	-
HCN (VA)	0.70	0.30	0.15	-	0.85	1.0	-
HCW (VA)	1.0	-	-	-	1.0	1.0	-
KCN (VA)	0.87	0.13	0.02	-	0.98	1.0	-

Appendix 3.1 continued.

Sample	CK-A2*		AAT-1,2*			G3PDH-1*	
	100*	78*	100*	80*	118*	100*	78*
New River (continued)							
LBN (VA)	0.97	0.03	0.73	-	0.27	1.0	-
NBN (VA)	1.0	-	0.10	-	0.90	1.0	-
NFE (VA)	1.0	-	-	-	1.0	1.0	-
DC (NC)	1.0	-	-	-	1.0	1.0	-
EC (NC)	1.0	-	0.09	-	0.91	1.0	-
LHC (NC)	1.0	-	-	-	1.0	1.0	-
LPC (NC)	0.86	0.14	0.19	-	0.81	1.0	-
MFN (NC)	0.90	0.10	0.05	-	0.95	1.0	-
TTC (NC)	1.0	-	-	-	1.0	1.0	-
UTP (NC)	0.52	0.48	0.40	-	0.60	0.92	0.08
Tennessee River							
PC (NC)	0.80	0.20	0.18	-	0.82	1.0	-
GCT (VA)	0.97	0.03	-	-	1.0	1.0	-
HBT (VA)	1.0	-	0.02	-	0.98	1.0	-
JCT (VA)	1.0	-	-	-	1.0	1.0	-
LLT (VA)	1.0	-	-	-	1.0	1.0	-
Saluda River							
FC (SC)	0.80	0.20	0.20	-	0.80	0.92	0.08
HF (SC)	0.82	0.18	0.39	-	0.61	1.0	-
MC (SC)	-	1.0	0.35	-	0.65	1.0	-
SC (SC)	1.0	-	-	-	1.0	1.0	-
Savannah River							
BC (SC)	0.65	0.35	0.38	-	0.62	1.0	-
CC (SC)	0.42	0.58	0.46	0.03	0.51	0.92	0.08
EMC (SC)	0.37	0.63	0 46	-	0.54	0.92	0.08
IC (SC)	0.18	0.82	0.41	-	0.59	1.0	-
IR (SC)	0.27	0.73	-	-	1.0	1.0	-
JC (SC)	1.0	-	-	-	1.0	1.0	-
PP (SC)	0.28	0.72	-	-	1.0	0.87	0.13

Appendix 3.1 extended.

Sample	GPI-B1*				GPI-B2*			
	100*	36*	125*	150*	100*	40*	70*	115*
Ohio River								
LBM (MD)	1.0	-	-	-	0.12	0.88	-	-
Potomac River								
FCM (MD)	0.79	0.21	-	-	0.94	-	0.06	-
PLC (MD)	0.94	0.06	-	-	1.0	-	-	-
JRS (VA)	1.0	-	-	-	1.0	-	-	-
VLR (VA)	0.97	0.03	-	-	1.0	-	-	-
Rappahannock River								
PBS (VA)	1.0	-	-	-	1.0	-	-	-
James River								
SPY (VA)	1.0	-	-	-	0.90	-	-	0.10
JCJ (VA)	1.0	-	-	-	1.0	-	-	-
SRJ (VA)	1.0	-	-	-	1.0	-	-	-
VBN (VA)	1.0	-	-	-	1.0	-	-	-
Roanoke River								
BSR (VA)	1.0	-	-	-	0.98	0.02	-	-
RCC (VA)	1.0	-	-	-	0.66	0.34	-	-
Yadkin River								
NFS (VA)	-	1.0	-	-	0.50	0.50	-	-
PCY (VA)	1.0	-	-	-	-	1.0	-	-
RAM (NC)	0.98	-	-	0.02	0.08	0.92	-	-
UTS (NC)	1.0	-	-	-	-	1.0	-	-
WC (NC)	1.0	-	-	-	0.54	0.46	-	-
Catawba River								
NYC (NC)	1.0	-	-	-	0.14	0.86	-	-
New River								
DCN (VA)	1.0	-	-	-	-	1.0	-	-
HCN (VA)	1.0	-	-	-	0.25	0.75	-	-
HCW (VA)	1.0	-	-	-	-	1.0	-	-
KCN (VA)	1.0	-	-	-	-	1.0	-	-

Appendix 3.1 continued.

Sample	GPI-B1*				GPI-B2*			
	100*	36*	125*	150*	100*	40*	70*	115*
New River (continued)								
LBN (VA)	1.0	-	-	-	0.80	0.20	-	-
NBN (VA)	1.0	-	-	-	0.18	0.82	-	-
NFE (VA)	1.0	-	-	-	-	1.0	-	-
DC (NC)	1.0	-	-	-	0.02	0.98	-	-
EC (NC)	1.0	-	-	-	-	1.0	-	-
LHC (NC)	1.0	-	-	-	-	1.0	-	-
LPC (NC)	1.0	-	-	-	0.24	0.76	-	-
MFN (NC)	1.0	-	-	-	-	1.0	-	-
TTC (NC)	1.0	-	-	-	0.06	0.94	-	-
UTP (NC)	0.97	-	-	0.03	0.48	0.52	-	-
Tennessee River								
PC (NC)	1.0	-	-	-	1.0	-	-	-
GCT (VA)	1.0	-	-	-	0.91	0.09	-	-
HBT (VA)	1.0	-	-	-	0.13	0.87	-	-
JCT (VA)	1.0	-	-	-	1.0	-	-	-
LLT (VA)	1.0	-	-	-	1.0	-	-	-
Saluda River								
FC (SC)	1.0	-	-	-	0.38	-	0.62	-
HF (SC)	1.0	-	-	-	0.10	-	0.90	-
MC (SC)	0.79	-	0.21	-	0.83	-	0.17	-
SC (SC)	1.0	-	-	-	-	-	1.0	-
Savannah River								
BC (SC)	1.0	-	-	-	0.72	0.28	-	-
CC (SC)	1.0	-	-	-	0.89	-	0.11	-
EMC (SC)	1.0	-	-	-	0.47	-	0.53	-
IC (SC)	1.0	-	-	-	0.92	0.03	0.05	-
IR (SC)	1.0	-	-	-	-	0.68	0.32	-
JC (SC)	1.0	-	-	-	-	-	1.0	-
PP (SC)	1.0	-	-	-	0.37	0.40	0.23	-

Appendix 3.1 extended.

Sample	GPI-A*			LDH-A2*		LDH-B1*	
	100*	95*	105*	100*	50*	100*	67*
Ohio River							
LBM (MD)	1.0	-	-	1.0	-	0.90	0.10
Potomac River							
FCM (MD)	1.0	-	-	1.0	-	1.0	-
PLC (MD)	1.0	-	-	1.0	-	1.0	-
JRS (VA)	1.0	-	-	0.94	0.06	0.96	0.04
VLR (VA)	1.0	-	-	1.0	-	1.0	-
Rappahannock River							
PBS (VA)	1.0	-	-	1.0	-	1.0	-
James River							
SPY (VA)	1.0	-	-	0.98	0.02	1.0	-
JCJ (VA)	1.0	-	-	1.0	-	1.0	-
SRJ (VA)	1.0	-	-	1.0	-	0.93	0.07
VBN (VA)	1.0	-	-	1.0	-	0.90	0.10
Roanoke River							
BSR (VA)	1.0	-	-	1.0	-	0.98	0.02
RCC (VA)	1.0	-	-	0.96	0.04	1.0	-
Yadkin River							
NFS (VA)	1.0	-	-	1.0	-	1.0	-
PCY (VA)	1.0	-	-	1.0	-	1.0	-
RAM (NC)	1.0	-	-	1.0	-	0.74	0.26
UTS (NC)	1.0	-	-	1.0	-	1.0	-
WC (NC)	1.0	-	-	1.0	-	0.54	0.46
Catawba River							
NYC (NC)	1.0	-	-	0.91	0.09	1.0	-
New River							
DCN (VA)	1.0	-	-	1.0	-	1.0	-
HCN (VA)	0.95	-	0.05	1.0	-	0.95	0.05
HCW (VA)	1.0	-	-	0.82	0.18	1.0	-
KCN (VA)	1.0	-	-	1.0	-	1.0	-

Appendix 3.1 continued.

Sample	GPI-A*			LDH-A2*		LDH-B1*	
	100*	95*	105*	100*	50*	100*	67*
New River (continued)							
LBN (VA)	1.0	-	-	1.0	-	1.0	-
NBN (VA)	0.76	-	0.24	1.0	-	0.96	0.04
NFE (VA)	1.0	-	-	0.97	0.03	1.0	-
DC (NC)	1.0	-	-	1.0	-	1.0	-
EC (NC)	1.0	-	-	1.0	-	0.90	0.10
LHC (NC)	1.0	-	-	1.0	-	1.0	-
LPC (NC)	1.0	-	-	1.0	-	1.0	-
MFN (NC)	1.0	-	-	0.85	0.15	1.0	-
TTC (NC)	0.98	-	0.02	1.0	-	1.0	-
UTP (NC)	1.0	-	-	1.0	-	0.85	0.15
Tennessee River							
PC (NC)	1.0	-	-	1.0	-	0.96	0.04
GCT (VA)	1.0	-	-	1.0	-	1.0	-
HBT (VA)	1.0	-	-	1.0	-	1.0	-
JCT (VA)	1.0	-	-	1.0	-	1.0	-
LLT (VA)	1.0	-	-	0.94	0.06	0.94	0.06
Saluda River							
FC (SC)	1.0	-	-	1.0	-	0.92	0.08
HF (SC)	1.0	-	-	1.0	-	1.0	-
MC (SC)	1.0	-	-	1.0	-	0.33	0.67
SC (SC)	1.0	-	-	1.0	-	1.0	-
Savannah River							
BC (SC)	1.0	-	-	1.0	-	0.87	0.13
CC (SC)	1.0	-	-	1.0	-	0.92	0.08
EMC (SC)	1.0	-	-	1.0	-	0.95	0.05
IC (SC)	0.92	-	0.08	1.0	-	0.95	0.05
IR (SC)	1.0	-	-	1.0	-	1.0	-
JC (SC)	1.0	-	-	1.0	-	0.95	0.05
PP (SC)	1.0	-	-	1.0	-	1.0	-

Appendix 3.1 extended.

Sample	MDH-B1,2*		sMEP-1*		PEPB*		
	100*	120*	100*	63*	100*	82*	68*
Ohio River							
LBM (MD)	0.98	0.02	0.90	0.10	0.84	-	0.16
Potomac River							
FCM (MD)	1.0	-	0.82	0.18	0.04	0.42	0.54
PLC (MD)	1.0	-	0.36	0.64	0.06	-	0.94
JRS (VA)	1.0	-	0.68	0.32	-	-	1.0
VLR (VA)	1.0	-	0.57	0.43	0.72	-	0.28
Rappahannock River							
PBS (VA)	1.0	-	0.30	0.70	-	-	1.0
James River							
SPY (VA)	1.0	-	0.41	0.59	0.02	-	0.98
JCJ (VA)	1.0	-	0.50	0.50	-	-	1.0
SRJ (VA)	0.93	0.07	0.25	0.75	-	-	1.0
VBN (VA)	1.0	-	0.63	0.37	-	-	1.0
Roanoke River							
BSR (VA)	0.96	0.43	0.83	0.17	-	-	1.0
RCC (VA)	0.26	0.74	0.78	0.22	0.44	-	0.56
Yadkin River							
NFS (VA)	1.0	-	1.0	-	1.0	-	-
PCY (VA)	1.0	-	1.0	-	1.0	-	-
RAM (NC)	0.94	0.06	1.0	-	0.78	-	0.22
UTS (NC)	1.0	-	1.0	-	1.0	-	-
WC (NC)	0.92	0.08	0.92	0.08	-	-	1.0
Catawba River							
NYC (NC)	0.89	0.11	0.96	0.04	0.96	-	0.04
New River							
DCN (VA)	1.0	-	0.98	0.02	1.0	-	-
NCN (VA)	0.85	0.15	0.95	0.05	1.0	-	-
HCW (VA)	1.0	-	1.0	-	1.0	-	-
KCN (VA)	0.92	0.08	1.0	-	0.92	-	0.08

Appendix 3.1 continued.

Sample	MDH-B1,2*		sMEP-1*		PEPB*		
	100*	120*	100*	63*	100*	82*	68*
New River (continued)							
LBN (VA)	0.87	0.13	0.67	0.33	0.93	-	0.07
NBN (VA)	0.98	0.02	1.0	-	0.64	-	0.36
NFE (VA)	0.66	0.34	1.0	-	1.0	-	-
DC (NC)	1.0	-	1.0	-	1.0	-	-
EC (NC)	0.92	0.08	0.95	0.05	0.85	-	0.15
LHC (NC)	0.98	0.02	1.0	-	1.0	-	-
LPC (NC)	0.95	0.05	0.86	0.14	0.90	-	0.10
MFN (NC)	0.83	0.17	1.0	-	1.0	-	-
TTC (NC)	0.94	0.06	1.0	-	1.0	-	-
UTP (NC)	0.97	0.03	0.79	0.21	0.47	-	0.53
Tennessee River							
PC (NC)	0.80	0.20	0.92	0.08	0.78	-	0.22
GCT (VA)	0.94	0.06	1.0	-	0.97	-	0.03
HBT (VA)	1.0	-	1.0	-	1.0	-	-
JCT (VA)	1.0	-	1.0	-	1.0	-	-
LLT (VA)	0.87	0.13	1.0	-	0.87	-	0.13
Saluda River							
FC (SC)	1.0	-	0.80	0.20	0.90	-	0.10
HF (SC)	1.0	-	1.0	-	1.0	-	-
MC (SC)	1.0	-	0.33	0.67	0.19	-	0.81
SC (SC)	1.0	-	1.0	-	1.0	-	-
Savannah River							
BC (SC)	1.0	-	0.03	0.97	0.50	-	0.50
CC (SC)	0.75	0.25	1.0	-	0.19	-	0.81
EMC (SC)	0.47	0.53	0.79	0.21	0.89	-	0.11
IC (SC)	1.0	-	0.52	0.48	0.60	-	0.40
IR (SC)	1.0	-	1.0	-	1.0	-	-
JC (SC)	1.0	-	1.0	-	1.0	-	-
PP (SC)	1.0	-	0.87	0.13	0.60	-	0.40

Appendix 3.1 extended.

Sample	n	H_O	SE (H_O)	H_S	SE (H_S)	P
Ohio River						
LBM (MD)	25	0.071	0.028	0.077	0.033	0.273
Potomac River						
FCM (MD)	25	0.067	0.027	0.072	0.030	0.227
PLC (MD)	25	0.028	0.018	0.030	0.021	0.136
JRS (VA)	25	0.066	0.032	0.069	0.033	0.182
VLR (VA)	20	0.093	0.043	0.078	0.035	0.182
Rappahannock River						
PBS (VA)	25	0.057	0.032	0.048	0.027	0.091
James River						
SPY (VA)	21	0.056	0.027	0.051	0.024	0.227
JCJ (VA)	23	0.019	0.017	0.024	0.022	0.091
SRJ (VA)	22	0.043	0.021	0.043	0.020	0.227
VBN (VA)	15	0.035	0.027	0.028	0.021	0.091
Roanoke River						
BSR (VA)	23	0.019	0.012	0.020	0.013	0.182
RCC (VA)	25	0.146	0.049	0.139	0.044	0.318
Yadkin River						
NFS (VA)	10	0.017	0.017	0.022	0.022	0.091
PCY (VA)	26	0	-	0	-	0
RAM (NC)	25	0.077	0.025	0.073	0.024	0.364
UTS (NC)	25	0.002	0.002	0.002	0.002	0.045
WC (NC)	25	0.142	0.053	0.123	0.042	0.318
Catawba River						
NYC (NC)	23	0.079	0.028	0.074	0.024	0.318
New River						
DCN (VA)	24	0.002	0.002	0.002	0.002	0.045
NCN (VA)	10	0.100	0.037	0.080	0.028	0.318
HCW (VA)	20	0.011	0.011	0.013	0.013	0.045
KCN (VA)	12	0.022	0.011	0.026	0.013	0.182

Appendix 3.1 continued.

Sample	n	H_O	SE (H_O)	H_S	SE (H_S)	P
New River (continued)						
LBN (VA)	15	0.081	0.031	0.086	0.032	0.273
NBN (VA)	25	0.066	0.026	0.069	0.028	0.273
NFE (VA)	19	0.023	0.021	0.022	0.020	0.091
DC (NC)	25	0.002	0.002	0.002	0.002	0.045
ECN (NC)	20	0.048	0.018	0.043	0.016	0.227
LHC (NC)	25	0.002	0.002	0.002	0.002	0.045
LPC (NC)	21	0.091	0.033	0.075	0.026	0.273
MFN (NC)	24	0.042	0.019	0.040	0.018	0.182
TTC (NC)	25	0.012	0.007	0.012	0.007	0.136
UTP (NC)	20	0.145	0.044	0.143	0.043	0.409
Tennessee River						
PC (NC)	25	0.088	0.031	0.078	0.027	0.273
GCT (VA)	16	0.014	0.007	0.018	0.009	0.182
HBT (VA)	12	0.014	0.011	0.013	0.010	0.091
JCT (VA)	11	0	-	0	-	0
LLT (VA)	16	0.027	0.014	0.029	0.014	0.182
Saluda River						
FC (SC)	20	0.107	0.034	0.096	0.031	0.318
HF (SC)	20	0.074	0.042	0.062	0.031	0.136
MC (SC)	21	0.130	0.045	0.118	0.039	0.273
SC (SC)	10	0	-	0	-	0
Savannah River						
BC (SC)	20	0.111	0.043	0.111	0.040	0.273
CC (SC)	18	0.164	0.052	0.140	0.042	0.318
EMC (SC)	19	0.178	0.058	0.140	0.043	0.364
IC (SC)	20	0.113	0.042	0.113	0.039	0.318
IR (SC)	17	0.026	0.018	0.036	0.025	0.091
JC (SC)	22	0.004	0.004	0.004	0.004	0.045
PP (SC)	20	0.107	0.048	0.085	0.038	0.227

Appendix 3.2 Heterozygosities of presumed native (unstocked) and hybrid populations. H_T is the expected heterozygosity of pooled samples; average H_S is the average expected heterozygosity of samples; H_S/H_T is a measure of the over all contribution of sample heterozygosity to total pooled heterozygosity.

Locus	Fixed CK-A2*			Northern fixed CK-A2* 78*			Fixed CK-A2* 100*			Hybrids		
	H_T	H_S	H_S/H_T	H_T	H_S	H_S/H_T	H_T	H_S	H_S/H_T	H_T	H_S	H_S/H_T
CK-A2*	0.483	0	0	-	-	-	-	-	-	0.467	0.321	0.687
PEPB*	0.476	0.085	0.179	0.322	0.123	0.382	0.076	0.058	0.763	0.383	0.221	0.577
AAT-1,2*	0.454	0.095	0.209	0.280	0.199	0.711	0.026	0.024	0.923	0.390	0.306	0.785
GPI-B2*	0.570	0.063	0.111	0.176	0.049	0.278	0.469	0.073	0.156	0.609	0.304	0.499
LDH-B1*	0.042	0.040	0.952	0.058	0.055	0.948	0.031	0.029	0.935	0.126	0.101	0.802
MDH-B1,2*	0.056	0.046	0.821	0.024	0.023	0.958	0.077	0.062	0.805	0.229	0.160	0.699
sMEP-1*	0.293	0.168	0.573	0.491	0.399	0.813	0.009	0.008	0.889	0.289	0.182	0.630
G3PDH-1*	0.012	0.010	0.833	0.024	0.021	0.875	0.002	0.002	1.0	0.064	0.059	0.922
GPI-B1*	0.091	0.018	0.198	0.052	0.045	0.865	0.117	0	0	0.009	0.009	1.0
GPI-A*	0.019	0.015	0.789	0	0	-	0.032	0.025	0.781	0.017	0.016	0.941
LDH-A2*	0.025	0.023	0.920	0.015	0.014	0.933	0.032	0.029	0.906	0.028	0.025	0.893
Average	0.115	0.026	0.226	0.075	0.049	0.653	0.040	0.014	0.350	0.130	0.087	0.669
Standard error	0.042	0.009	-	0.028	0.020	-	0.021	0.005	-	0.040	0.025	-

Appendix 3.3. Heterozygosities of sample subsets. N is the number of samples; H_T is the expected heterozygosity of pooled samples; average H_S is the average expected heterozygosity of samples.

Samples	N	H_T	Average H_S
All	48	0.133	0.055
All natives (with Great Smoky Mountains National Park)	31	0.128	0.027
Hybrids	20	0.130	0.087
Northern, fixed CK-A2* 78*	11	0.075	0.049
Northern Atlantic, fixed CK-A2* 78*	10	0.059	0.046
Potomac River	4	0.078	0.062
James River	4	0.039	0.036
Southern Appalachian	35	0.095	0.053
Southern Appalachian, fixed CK-A2* 100*	16	0.039	0.015
Southern Appalachian, fixed CK-A2* 100* (with Great Smoky Mountains National Park)	20	0.047	0.015
Ohio River natives (with GRSM)	16	0.056	0.022
Southern Atlantic	16	0.122	0.067
Southern Atlantic, fixed CK-A2* 100*	5	0.040	0.005
Tennessee River, fixed CK-A2* 100* (with Great Smoky Mountains National Park)	7	0.041	0.016
New River	14	0.062	0.045
New River, fixed CK-A2* 100*	8	0.024	0.020
Yadkin River, fixed CK-A2* 100*	3	0.032	0.008
Saluda and Savannah Rivers, fixed CK-A2* 100*	2	0.002	0.002

Appendix 3.4. Matrix of normalized genetic identities (Nei's *I*) between all samples.

	EC	DC	LPC	WC	PC	NYC	LHC	UTP	RAM
EC									
DC	0.998								
LPC	0.993	0.992							
WC	0.897	0.877	0.913						
PC	0.950	0.948	0.973	0.921					
NYC	0.995	0.994	0.998	0.908	0.963				
LHC	0.998	1.000	0.991	0.876	0.946	0.994			
UTP	0.962	0.951	0.978	0.974	0.971	0.972	0.950		
RAM	0.997	0.993	0.991	0.919	0.955	0.993	0.993	0.970	
UTS	0.998	1.000	0.991	0.876	0.946	0.994	1.000	0.950	0.993
MFN	0.996	0.997	0.991	0.883	0.948	0.996	0.997	0.954	0.993
TTC	0.998	1.000	0.993	0.878	0.952	0.994	1.000	0.952	0.993
PLM	0.790	0.768	0.838	0.936	0.868	0.823	0.766	0.922	0.804
FCM	0.824	0.808	0.868	0.950	0.898	0.858	0.807	0.939	0.840
LBM	0.947	0.943	0.961	0.946	0.929	0.965	0.943	0.973	0.958
BSR	0.804	0.783	0.847	0.956	0.880	0.836	0.781	0.931	0.820
RCC	0.919	0.904	0.941	0.951	0.959	0.938	0.905	0.969	0.927
VBN	0.798	0.775	0.842	0.953	0.875	0.829	0.773	0.929	0.815
LBN	0.928	0.919	0.957	0.903	0.964	0.947	0.918	0.962	0.923
PCY	0.998	1.000	0.991	0.876	0.946	0.994	1.000	0.950	0.993
JCJ	0.795	0.773	0.841	0.946	0.873	0.826	0.771	0.926	0.810
SRJ	0.783	0.760	0.830	0.936	0.862	0.814	0.757	0.919	0.799
HCN	0.991	0.991	0.997	0.913	0.970	0.998	0.991	0.974	0.992
HBN	0.993	0.990	0.991	0.914	0.961	0.989	0.990	0.970	0.992
NFS	0.942	0.946	0.948	0.841	0.934	0.943	0.945	0.914	0.940
HBT	0.997	0.999	0.994	0.882	0.957	0.995	0.999	0.956	0.993
JCT	0.953	0.958	0.972	0.880	0.992	0.961	0.956	0.948	0.955
VLR	0.864	0.855	0.910	0.939	0.935	0.899	0.853	0.957	0.876
JRS	0.845	0.829	0.884	0.973	0.919	0.871	0.827	0.957	0.864
SPY	0.810	0.789	0.854	0.949	0.882	0.839	0.787	0.935	0.824
DCN	0.998	1.000	0.992	0.876	0.946	0.994	1.000	0.951	0.993
KCN	0.998	0.999	0.993	0.892	0.950	0.996	0.999	0.959	0.996
NFE	0.995	0.995	0.987	0.871	0.946	0.991	0.995	0.945	0.989
GCT	0.962	0.966	0.977	0.888	0.993	0.969	0.964	0.954	0.964
HCW	0.996	0.999	0.990	0.874	0.945	0.993	0.999	0.948	0.992
LLT	0.954	0.956	0.970	0.890	0.994	0.960	0.954	0.952	0.957
PBS	0.812	0.792	0.857	0.948	0.889	0.840	0.791	0.937	0.827
IC	0.904	0.897	0.942	0.952	0.965	0.928	0.895	0.977	0.917
SL	0.953	0.957	0.959	0.855	0.946	0.955	0.956	0.926	0.951
BC	0.916	0.904	0.943	0.921	0.947	0.926	0.902	0.967	0.919
CC	0.899	0.883	0.931	0.969	0.961	0.916	0.882	0.978	0.911

Appendix 3.4 continued.

	EC	DC	LPC	WC	PC	NYC	LHC	UTP	RAM
EMC	0.921	0.914	0.949	0.925	0.963	0.946	0.914	0.962	0.929
FC	0.960	0.959	0.977	0.908	0.978	0.969	0.958	0.967	0.963
HF	0.946	0.946	0.963	0.890	0.953	0.960	0.945	0.950	0.946
IR	0.969	0.971	0.974	0.908	0.944	0.977	0.971	0.958	0.975
JC	0.953	0.957	0.959	0.856	0.946	0.954	0.956	0.926	0.952
MC	0.850	0.833	0.881	0.960	0.908	0.864	0.831	0.946	0.876
PP	0.958	0.957	0.971	0.945	0.966	0.967	0.956	0.978	0.969

	UTS	MFN	TTC	PLM	FCM	LBM	BSR	RCC	VBN
MFN	0.997								
TTC	1.000	0.998							
PLM	0.766	0.777	0.770						
FCM	0.807	0.817	0.811	0.980					
LBM	0.943	0.950	0.942	0.882	0.912				
BSR	0.780	0.793	0.785	0.990	0.988	0.894			
RCC	0.903	0.919	0.909	0.918	0.931	0.945	0.928		
VBN	0.773	0.784	0.776	0.996	0.986	0.889	0.998	0.922	
LBN	0.917	0.921	0.921	0.908	0.919	0.916	0.908	0.942	0.908
PCY	1.000	0.997	1.000	0.766	0.807	0.943	0.781	0.903	0.773
JCJ	0.771	0.782	0.775	0.999	0.984	0.886	0.995	0.921	0.999
SRJ	0.758	0.769	0.762	0.998	0.972	0.875	0.984	0.917	0.992
HCN	0.990	0.993	0.992	0.827	0.864	0.970	0.840	0.945	0.834
HBN	0.990	0.987	0.991	0.812	0.846	0.943	0.829	0.927	0.822
NFS	0.945	0.941	0.947	0.758	0.811	0.890	0.766	0.874	0.760
HBT	0.999	0.997	1.000	0.779	0.819	0.944	0.793	0.911	0.786
JCT	0.956	0.953	0.961	0.811	0.849	0.908	0.823	0.919	0.817
VLL	0.853	0.863	0.857	0.969	0.975	0.945	0.966	0.948	0.968
JRS	0.827	0.836	0.831	0.981	0.980	0.921	0.985	0.949	0.986
SPY	0.787	0.798	0.791	0.998	0.981	0.898	0.990	0.930	0.995
DCN	1.000	0.997	1.000	0.767	0.807	0.943	0.781	0.903	0.773
KCN	0.999	0.998	0.999	0.783	0.823	0.954	0.799	0.919	0.791
NFE	0.995	0.997	0.996	0.758	0.799	0.936	0.775	0.919	0.766
GCT	0.964	0.961	0.969	0.813	0.852	0.917	0.827	0.927	0.820
HCW	0.999	0.998	0.998	0.763	0.804	0.940	0.778	0.901	0.770
LLT	0.954	0.953	0.960	0.816	0.853	0.907	0.830	0.930	0.824
PBS	0.791	0.800	0.795	0.995	0.975	0.897	0.983	0.931	0.990
IC	0.895	0.901	0.899	0.951	0.958	0.955	0.946	0.962	0.948
SL	0.956	0.953	0.959	0.766	0.810	0.902	0.780	0.887	0.773

Appendix 3.4 continued.

	UTS	MFN	TTC	PLM	FCM	LBM	BSR	RCC	VBN
BC	0.902	0.904	0.906	0.926	0.909	0.919	0.900	0.939	0.912
CC	0.882	0.889	0.887	0.959	0.960	0.929	0.960	0.977	0.961
EMC	0.913	0.926	0.919	0.899	0.923	0.950	0.906	0.976	0.902
FC	0.958	0.957	0.961	0.851	0.881	0.937	0.856	0.933	0.855
HF	0.945	0.946	0.946	0.839	0.876	0.929	0.853	0.916	0.846
IR	0.971	0.974	0.971	0.814	0.856	0.980	0.828	0.924	0.820
JC	0.956	0.952	0.959	0.766	0.809	0.902	0.780	0.887	0.773
MC	0.831	0.837	0.834	0.936	0.930	0.915	0.926	0.924	0.936
PP	0.956	0.958	0.958	0.864	0.895	0.973	0.875	0.949	0.870

	LBN	PCY	JCJ	SRJ	HCN	NBN	NFS	HBT	JCT
PCY	0.918								
JCJ	0.908	0.771							
SRJ	0.900	0.758	0.996						
HCN	0.946	0.991	0.831	0.820					
NBN	0.931	0.990	0.819	0.806	0.987				
NFS	0.895	0.946	0.757	0.744	0.945	0.941			
HBT	0.928	0.999	0.783	0.770	0.993	0.991	0.949		
JCT	0.945	0.957	0.815	0.803	0.968	0.961	0.946	0.967	
VLR	0.950	0.853	0.969	0.964	0.909	0.875	0.842	0.865	0.899
JRS	0.910	0.827	0.985	0.978	0.879	0.871	0.814	0.839	0.872
SPY	0.910	0.787	0.998	0.997	0.845	0.833	0.771	0.799	0.828
DCN	0.918	1.000	0.772	0.759	0.991	0.990	0.945	0.999	0.956
KCN	0.919	0.999	0.789	0.776	0.994	0.991	0.943	0.998	0.955
NFE	0.915	0.995	0.763	0.751	0.989	0.984	0.939	0.994	0.951
GCT	0.946	0.964	0.818	0.806	0.974	0.968	0.948	0.972	0.999
HCN	0.915	0.999	0.768	0.755	0.989	0.988	0.944	0.998	0.955
LLT	0.944	0.954	0.821	0.810	0.968	0.963	0.943	0.965	0.998
PBS	0.907	0.791	0.994	0.995	0.847	0.836	0.776	0.803	0.835
IC	0.951	0.895	0.950	0.947	0.939	0.918	0.879	0.906	0.937
SL	0.908	0.957	0.771	0.758	0.957	0.953	0.923	0.961	0.957
BC	0.952	0.903	0.919	0.929	0.930	0.923	0.876	0.910	0.923
CC	0.949	0.882	0.962	0.960	0.923	0.918	0.865	0.892	0.923
EMC	0.957	0.913	0.900	0.895	0.955	0.923	0.887	0.921	0.935
FC	0.955	0.958	0.853	0.845	0.973	0.963	0.931	0.966	0.975
HF	0.950	0.945	0.843	0.828	0.960	0.947	0.912	0.951	0.949
IR	0.899	0.971	0.818	0.805	0.984	0.962	0.922	0.972	0.942
JC	0.908	0.956	0.770	0.757	0.957	0.953	0.923	0.961	0.956

Appendix 3.4 continued.

	LBN	PCY	JCJ	SRJ	HCN	NBN	NFS	HBT	JCT
MC	0.882	0.831	0.935	0.941	0.879	0.865	0.823	0.841	0.869
PP	0.911	0.956	0.869	0.861	0.975	0.964	0.920	0.960	0.955

	VLR	JRS	SPY	DCN	KCN	NFE	GCT	HCN	LLT
JRS	0.973								
SPY	0.972	0.989							
DCN	0.854	0.828	0.788						
KCN	0.866	0.845	0.805	0.999					
NFE	0.846	0.820	0.780	0.995	0.996				
GCT	0.900	0.875	0.831	0.964	0.963	0.960			
HCW	0.850	0.825	0.785	0.999	0.997	0.994	0.962		
LLT	0.898	0.880	0.834	0.954	0.954	0.952	0.999	0.953	
PBS	0.971	0.990	0.998	0.791	0.808	0.783	0.839	0.787	0.842
IC	0.988	0.976	0.961	0.896	0.906	0.888	0.939	0.892	0.938
SL	0.853	0.827	0.787	0.956	0.955	0.951	0.960	0.955	0.954
BC	0.948	0.938	0.935	0.905	0.908	0.896	0.927	0.900	0.926
CC	0.969	0.984	0.969	0.882	0.895	0.882	0.927	0.879	0.932
EMC	0.957	0.925	0.908	0.913	0.924	0.923	0.941	0.910	0.939
FC	0.922	0.896	0.867	0.958	0.959	0.951	0.977	0.956	0.974
HF	0.908	0.875	0.852	0.945	0.946	0.939	0.952	0.943	0.946
IR	0.903	0.876	0.835	0.971	0.978	0.966	0.949	0.969	0.939
JC	0.853	0.827	0.787	0.956	0.954	0.950	0.960	0.955	0.954
MC	0.946	0.964	0.947	0.832	0.846	0.823	0.874	0.829	0.878
PP	0.930	0.928	0.886	0.956	0.965	0.949	0.961	0.954	0.956

	PBS	IC	SL	BC	CC	EMC	FC	HF	IR
IC	0.966								
SL	0.791	0.896							
BC	0.946	0.972	0.890						
CC	0.973	0.984	0.887	0.969					
EMC	0.908	0.965	0.938	0.934	0.960				
FC	0.870	0.953	0.986	0.945	0.943	0.970			
HF	0.849	0.928	0.985	0.910	0.919	0.967	0.990		
IR	0.839	0.934	0.956	0.905	0.906	0.947	0.965	0.954	
JC	0.790	0.896	1.000	0.890	0.887	0.938	0.987	0.985	0.956

Appendix 3.4 continued.

	PBS	IC	SL	BC	CC	EMC	FC	HF	IR
MC	0.957	0.967	0.839	0.950	0.964	0.915	0.902	0.866	0.883
PP	0.893	0.965	0.948	0.938	0.951	0.954	0.970	0.946	0.986

	JC	MC
MC	0.841	
PP	0.947	0.929

PART IV:
MORPHOMETRIC VARIATION AMONG SOUTHERN APPALACHIAN, NORTHERN, and HYBRID BROOK TROUT POPULATIONS

Introduction

Brook trout, and salmonids generally, are polytypic in many aspects of morphology, behavior, and life history (Jones, 1947; Behnke, 1980; Power 1980; Scott and Crossman, 1973). This phenotypic variability is distributed among numerous geographically isolated populations that inhabit a diversity of stream and lake habitats (Power, 1980). As a consequence of their phenotypic variability, the diversity of their habitats, and the disjunct distribution of their populations, most students of salmonid taxonomy have necessarily practiced a philosophy of broad species inclusiveness (Behnke, 1980).

Though they are top predators throughout their range, brook trout populations can occupy distinctly different niches. In the northern part of their range, anadromous and land-locked populations inhabit a diversity of stream and lake habitats (Scott and Crossman, 1973). In lake and river habitats northern brook trout are often sympatric with lake trout, *S. namaycush*, where brook trout generally occupy the littoral spatial and feeding niches and lake trout occupy deeper waters (Behnke, 1980; Cunjak and Green, 1983). Distinct brook trout populations may occupy these separate niches in some deep northern lakes (Behnke, 1972; 1980). South of about 37° latitude, brook trout are confined to low order high elevation streams (MacCrimmon and Campbell, 1969). In the Southern Appalachians brook trout are the only native salmonid and are the solitary top predator in headwater habitats.

Habitat and niche differences, and correlated morphological and life history differences, have formed the basis of previous taxonomic assessments of *Salvelinus*

fontinalis, sensu lato. While various workers have ascribed taxonomic significance to the differences of anadromous and freshwater populations (MacCrimmon and Campbell, 1969; Rounsefell, 1962; Vladykov, 1954; Kendall, 1914), most of the variation observed among northern populations appears to be a plastic response to unspecified environmental variation (Behnke, 1980; Wilder, 1952). Currently only one subspecies of brook trout is recognized, the aurora charr *S. fontinalis timagamiensis* from three deep lakes tributary to the Montreal River (Behnke, 1980; Quadri, 1968; Sale, 1967). Behnke (1980) also recognizes a distinct species within the otherwise monotypic subgenus *Baione*, the extinct silver charr *S. agassizi* from Dublin Pond, New Hampshire.

A number of fisheries biologists also have suggested that morphological, ecological, and life history differences between northern and Southern Appalachian brook trout populations may be of taxonomic significance. Holloway (1945) specifically noted that northern derived hatchery strains were not adapted to the Southern Appalachian stream environment, and King (1947) and Lennon (1967) suggested that life history differences between northern and southern populations, particularly longevity and age at sexual maturity, might be indicative of significant divergence. Behnke (1980) also recognized different life history patterns for land-locked northern and southern populations but did not ascribe any formal taxonomic significance to the differences.

Southern Appalachian anglers have long held that the native brook trout differs morphologically from northern forms (Venters, 1993; Yuskavitch, 1991). Lennon (1967) agreed with this perception and indicated that native Southern Appalachian brook trout had larger heads, were more brightly colored, and had more red spots than northern brook

trout. Although he suggested that these supposed differences might be indicative of taxonomic differences, he did not provide any quantitative data to support his assertion. In an unpublished study, Habera and Fraley (1996) observed a statistically significant difference in spot number between presumed native Southern Appalachian populations and naturalized northern derived hatchery strains. However, the genetic nature of the character was not explored, and it is not known if the differences apply to wild northern populations.

The most recent suggestions of phylogenetically, hence taxonomically, significant differentiation among brook trout populations have come from molecular genetics. Stoneking et al. (1981) suggested that allozyme differences between northern and Southern Appalachian populations were comparable to differences observed between recognized species and subspecies in other taxa. McCracken et al. (1993), Hayes et al. (1996), and Kriegler et al. (1996) confirmed the extent of differentiation between Southern Appalachian populations and northern derived hatchery strains at allozyme and mitochondrial DNA loci, but did not directly address questions of formal taxonomy. As yet these molecular data and the earlier suggestions of phenotypic and life history differences have not inspired taxonomists to undertake a systematic assessment of brook trout.

More extensive application of the tools of molecular population genetics, coupled with considerations of Pleistocene biogeography (Chapters II & III), provides worthwhile information for assessing brook trout phylogeny. Although the appropriateness of molecular, especially allozyme, criteria for taxonomic assessment is questioned by some

workers (Dowling et al., 1992; Frost and Hillis, 1990), molecular population genetics is well suited to the task of generating phylogenetic hypotheses that can be tested with phenotypic and ecological, as well as other molecular data (Baum, 1992). Even the tentative identification of evolutionary lineages permits the investigation of evolutionary change within lineages and the further testing and refinement of phylogenetic hypotheses.

Molecular variation among native Southern Appalachian and northern brook trout (including northern derived hatchery strains) indicates the existence of at least two evolutionary lineages within the taxon (Chapters II & III). Populations of the northern lineage inhabit Atlantic drainage streams and lakes north of the New River. Southern Appalachian populations are found in headwater streams of the New and Tennessee River drainages and in Atlantic drainages east and south of the New River. Populations in upper Ohio drainage streams of Maryland, West Virginia, Pennsylvania, and New York may represent a third lineage, but the existing data do not permit selection among alternative hypotheses. With the possible exception of the upper Ohio River populations, the range of the two major lineages do not overlap. Natural hybridization probably only occurs through stream capture in the New River – Atlantic divide.

Given the extensive use of northern derived hatchery strains in the management of brook trout throughout their range (Kriegler et al., 1995; Bowen, 1970; Lennon, 1967), the results of our genetic studies investigating hybridization in the Southern Appalachians are surprising. By the allozyme criteria, a high percentage of the populations in Great Smoky Mountains National Park (39 of 52 populations sampled; Part II; McCracken et al., 1993), and in Tennessee outside the Park (48 of 95 populations sampled; Kriegler et

al., 1995; Saidak, 1995), were native Southern Appalachian. One population in the Park, and 14 outside the Park are naturalized hatchery populations, and the remaining populations, 31% of the total, are native Southern Appalachian – hatchery strain hybrids.

The apparent failure of northern derived hatchery strains to hybridize more extensively with Southern Appalachian populations, or of introgressed hatchery genes to persist in hybrid populations, could be a consequence of selection within hatcheries that has resulted in the loss of characteristics necessary for survival and reproduction in the wild. Alternatively, northern derived hatchery strains may be little differentiated from their wild northern ancestors, and northern lineages may have different adaptive characteristics than southern lineages. Under this latter hypothesis the genetic divergence of northern and southern populations involves differentiation of adaptive characteristics in addition to the presumed neutral differentiation of allozyme loci.

General biogeographical concordance between the allozyme and life history data, and suggestions of adaptive differentiation, lend considerable support for the existence of distinct Southern Appalachian and northern Atlantic drainage brook trout lineages. Based on the molecular data, and to some extent on experience with native and hatchery derived populations, fisheries biologists in the Southern Appalachians now recognize the distinctiveness of Southern Appalachian brook trout (Kriegler et al., 1995). While recognition of distinct biogeographical lineages, or evolutionarily significant units, within the taxon, on the basis of molecular data, may be sufficient and appropriate for most management objectives (Baum, 1992), failure to recognize morphological, behavioral,

and adaptive differentiation, neglects some of the more interesting and potentially informative evolutionary questions.

Here I report the results of my initial, limited, studies of morphological variation among brook trout populations from northern and Southern Appalachian lineages and their hybrids. Studies of this type will provide the data for evaluating the taxonomic significance of these lineages, and for investigating patterns of phenotypic variation and evolution.

Methods

Samples - I examined 236 individuals from 14 wild populations and two hatchery strains (Table 4.1). The wild populations included five native Southern Appalachian populations, six Southern Appalachian – northern derived hatchery hybrids, two wild northern populations, and one naturalized hatchery derived population. The native Southern Appalachian, hybrid, and naturalized hatchery samples are from Great Smoky Mountains National Park (Chapter II). The wild northern samples are from populations in central Virginia and western Maryland (Chapter III). The northern derived hatchery strains were obtained from the Pisgah fish hatchery in Pisgah, North Carolina.

I grouped the samples in two classifications for the analyses. The class TYPE has five groups corresponding to: (1) native Southern Appalachian, (2) hybrid, (3) hatchery, (4) naturalized hatchery derived, and (5) wild northern populations. The classification LINEAGE has three levels corresponding to (1) native Southern Appalachian, (2) hybrid, and (3) northern (wild northern + hatchery + naturalized hatchery) populations. Sample

sizes were unequal for samples and for all classification groups (Table 4.1). My LINEAGE classification was informed by the molecular genetic data indicating that Southern Appalachian and northern populations are from distinct evolutionary lineages. My rationale for further subdividing the northern lineage into wild, hatchery, and naturalized hatchery TYPES was to investigate the range of variability of wild northern populations and their derivative hatchery strains.

Morphometrics - I examined external morphometric variation (Pimentel, 1979) using three standard ichthyological measurements (Andersen and Gutreuter, 1983) and seven metrics forming a simple truss grid (Strauss and Bookstein, 1982). These measures allowed me to investigate variation in overall body shape and to test the general hypotheses that variation in body shape is associated with the different habitats of northern and Southern Appalachian lineages. Previous theoretical and empirical studies indicate that a regionally unbiased truss network provides more information about overall body shape variation than do traditional morphometrics (Bookstein et al., 1985), and that truss grids reveal more information about local body proportions than traditional methods (Winans, 1984). I did not examine variation in meristic characters, which are known to be highly plastic in northern brook trout populations (Behnke, 1980). The standard and truss grid measurements investigated are listed and described in Table 4.2. The data array is reproduced in Appendix 4.1.

Individuals were measured fresh, shortly after collection, following euthanasia with MS-222. All sampled individuals were one year or older. Most samples were

measured prior to dissection of tissue samples for genetic analysis. All specimens were measured on the left side with the skeletal muscle sample for genetic analysis taken from the right side. Sex was not determined, as brook trout exhibit sexual dimorphism primarily in the breeding season (King, 1937) and all samples were collected in spring or summer prior to the development of male nuptual characters. Total length and standard length were taken with a ruler and recorded in millimeters. The other measurements were made with a digital caliper and recorded to the nearest tenth of a millimeter. Angles between morphometrics were calculated trigonometrically from triangle dimensions.

Statistical analysis - I employed both exploratory and hypothesis testing approaches in my statistical analyses (Table 4.3). Exploratory analysis allowed me to evaluate specific characters for hypothesis testing. Exploratory analysis also allowed me to evaluate the appropriateness of the morphometrics examined, and to speculate about possible morphometric approaches that might be useful in future studies of brook trout morphological variation. The majority of my statistical analyses are exploratory; therefore I primarily organize my presentation of results and my discussion around the particular techniques rather than around tests of my specific hypotheses. Results bearing on my two hypotheses are perused in the discussion.

The specific hypotheses tested were: 1). that differences in body shape exist between northern and Southern Appalachian lineages, and 2). that the variance of morphometric characters is higher in hybrid populations. The first hypothesis addresses differentiation of body form that may be associated with adaptation to the different

habitats of northern and Southern Appalachian populations. Morphological variation associated with habitat differences have been observed in brook trout (Power, 1980) and in other salmonid species (Flemming et al., 1994; Swain et al., 1991; Swain and Holtby, 1989). The second hypothesis addresses the morphological effects of hybridization among individuals belonging to distinct lineages. Leary et al. (1983; 1985) observed substantial developmental instability in interspecific salmonid hybrids, and hypothesized that this was a result of the disruption of coadapted gene complexes. If the observed molecular differentiation of northern and Southern Appalachian brook trout populations (Chapter III; Stoneking et al., 1981) is indicative of genome wide differentiation, developmental instability is predicted for their hybrids. Similarly, if the two lineages are morphologically differentiated, I would expect to observe higher variance of morphometric characters in their hybrid populations. The statistical methods and significance tests employed are listed in Table 4.3. Statistical analyses were performed using the SAS System (SAS Institute, 1985).

I investigated three methods to reduce the effects on individual metrics of overall size differences in the fish. These three methods of adjusting for size are appropriate if the allometric coefficients are constant among fish of different age / size classes. However, I did not undertake the longitudinal studies necessary to investigate the validity of this assumption. The simplest method of adjusting for size differences involved constructing ratios of morphometric variables to standard length. The derived variables were then $\log_e$ transformed to homogenize variances (Marcus, 1990). Statistical properties of ratios may limit the validity of significance tests on these derived variables

(Atchley et al., 1976;), but ratios are widely used in studies of morphological variation in fishes (Marcus, 1990; Keenlyne et al., 1994). A second, more statistically robust method, involved calculating the least squares residuals of $\log_e$ measurement variables regressed on $\log_e$ standard lengths (Reist, 1986; Kelsch, 1995). The transformed variable is the residual of each variable from regressions of all observations (fish). Regressions were also calculated for observations pooled by TYPE and LINEAGE to test for differences in allometric coefficients between classification groups. The third source of size adjusted variables was provided by the angles of the three triangles formed by metrics between shared landmarks (Table 4.2). Means and variances of the ratio, residual, and angle variables are given in Appendix 4.2.

Association between variables was evaluated using Pearson's correlation coefficient and Hoeffding's measure of dependence, D (Hoeffding, 1948). Multivariate and univariate analysis of variance, Mahalanobis distances, and Wilcoxon rank sum scores were used to examine differences between TYPE and LINEAGE classifications means for the three sets of derived variables ($\log_e$ ratios, residuals, and angles). Equality of univariate variances among groups was evaluated with Levene's test (Levene, 1960). Equality of mutivariate variances was evaluated with the multivariate extension of Levene's test (Manly, 1986). Differences among bivariate allometric coefficients (log-log regression slopes) and tests of the hypothesis that regression slopes are 1.0 were tested with the F-test.

Multivariate discrimination of shape was explored using cluster analysis, canonical discriminant analysis, principal components analysis, and principal factor

analysis (Manly, 1986) of the three derived variable sets. Discrimination was explored using all variables in a variable set, and subsets of variables identified as potentially informative by analysis of variance. Three clustering procedures were investigated: Ward's (1963) minimum variance method, average linkage (Sokal and Michener, 1958), and centroid hierarchical analysis (Sokal and Michner, 1958). Variation in overall body shape was also evaluated using sheared principle components analysis (Humphries et al., 1981; Strauss and Bookstein, 1982; Rohlf and Bookstein, 1987). Sheared principle components analysis corrects for differences in overall body size by translating conventional principle components values from raw data to 0.0 mean, and regressing these on the group size components. The first principal component accounts for the overall size factor and subsequent components load on variables reflecting shape differences.

Results

Association between variables – The ten morphometrics were all highly correlated, demonstrating overall body size dependence (Table 4.4). The average correlation (Pearson's R) between standard length and the other metrics was 0.961 (range: 0.897 – 0.989). Correlations among metrics excluding body length ranged from 0.853 to 0.981. Snout length (NL) had the lowest overall correlation with the other metrics, ranging from 0.853 to 0.948 (Table 4.4). All correlations and all measures of association (Hoeffding's D) were significant at $p < 0.01$.

Means – Multivariate TYPE and LINEAGE effects were significant at $p < 0.01$ for $\log_e$ ratios, regression residuals, and angles (Table 4.5). I observed significant differences between the means of samples by TYPE for six of the nine $\log_e$ ratio variables using the F-test and the Kruskal-Wallace test (Table 4.5). The means of five $\log_e$ ratio variables were significantly different between samples classified by LINEAGE with both the parametric and nonparametric tests. Between TYPES, means of six regression residuals were significantly different by the F-test, and seven were significantly different by the Kruskal-Wallace test. Three regression residuals of samples classified by LINEAGE were significantly different using both the F-test and the Kruskal-Wallace test. Six angle means were significantly different between TYPES and LINEAGES by the F-test, and seven were significantly different by the Kruskal-Wallace test. The number of significant pairwise differences in each of the three variable sets was greater than expectation at $p = 0.05$ under the null hypothesis of no differences between classification groups (Table 4.6). Pairwise comparisons between variable means that were significantly different by TYPE are given in Table 4.7.

Variation in the means between samples within LINEAGES showed about as much variation as was observed between LINEAGES. Eight of the log ratio variables, seven of the regression residuals, and four of the angles were significantly different between Southern Appalachian samples. Between northern samples the means of eight log ratio variables, six regression residuals, and eight angles were significantly different. All of the angles, six of the log ratios, and five of the regression residuals were significantly different between hybrid population samples. The number of significant

pairwise differences between samples within lineages was higher than expectation under than the null hypothesis at $p = 0.05$ for all variable sets (Table 4.6).

Variance – Multivariate variances of log ratios and of regression residuals were significantly different at $p < 0.01$ between samples classified according to TYPE and between samples classified according to LINEAGE. Multivariate angle variances were not significantly different at $p < 0.01$ between TYPES or between LINEAGES. Variance of one log ratio was significantly different between TYPES, and four were significantly different between LINEAGES. Variances of three regression residuals were significantly different between TYPES and four were significantly different between LINEAGES.

I observed significant differences in the variances among groups in six of 180 paired tests where samples were classified by TYPE (10 paired tests of 9 variables in each of the variable sets of regression residuals and log ratios). At least this many differences would be expected at random at $p = 0.05$, if all parameter values did not differ significantly. For individual variable sets the maximum number of significantly different variances was three, which is also less than random expectation at $p = 0.05$. Between LINEAGES, eight of 54 paired tests showed significant differences in variance, a number greater than expected at $p = 0.05$.

Six of the eight significant paired differences between LINEAGES were among metrics from the head region. Variances of the $\log_e$ head length (HL) ratios and of the snout length (NL) regression residuals of the Southern Appalachian and hybrid samples were significantly lower than those observed in the pooled northern samples. Variance of

the head length regression residual was significantly lower in the hybrid sample than the pooled northern sample, and the snout to pelvic (SV) fin residual was significantly lower in the hybrid sample than the Southern Appalachian sample. In the body region, variances of the $\log_e$ pelvic fin to adipose fin (VA) ratio and the VA residual were significantly higher in the Southern Appalachian samples and the northern LINEAGE samples.

Allometric coefficients - Allometric coefficients were significantly different from 1.0 in 13 of the 45 tests of samples classified by TYPE, and nine of 27 tests of samples classified by LINEAGE (Table 4.8). Eight of the deviations from isometry in TYPES, and seven in LINEAGES involved the four head region metrics (HL, NL, SV, SD). Native Southern Appalachian samples showed significant positive allometry for the four head region morphometrics. Hybrid and naturalized hatchery samples also had significantly positive allometry for snout length. The snout to pelvic fin metric demonstrated negative allometry in the naturalized hatchery, wild northern, and pooled northern samples. The head length allometric coefficent was also significantly less than 1.0 in all pooled samples and the pooled northern samples All other head region metrics for samples analyzed by TYPE and LINEAGE were isometric with respect to standard length.

The four head region morphometrics (HL, NL, SD, SV) showed the greatest variability between allometric coefficients of samples classified by TYPE (Table 4.9). Coefficients for the head region morphometrics of Southern Appalachian samples were significantly larger than the coefficients for all other TYPES. Hybrid sample HL, NL, and

SV coefficients were also significantly larger than those of the other TYPES except for the Southern Appalachian populations.

Mahalanobis distances – Mahalanobis distances were significantly different ($p < 0.01$) between all TYPES for all log ratios and for the subset of log ratio variables identified as significantly different by analysis of variance (Table 4.10). Southern Appalachian and hybrid samples were not significantly different ($p < 0.05$) from northern samples for the set of all angles. For the subset of angles identified by analysis of variance, the Southern Appalachian samples were not significantly different from the northern samples, and the hybrid samples were not significantly different from the naturalized hatchery sample. Hybrid samples were not significantly different from the naturalized hatchery sample for all regression residuals or the subset of residuals identified by analysis of variance. All Mahalanobis distances between LINEAGES for all variables in each of the three variable sets and for the subsets identified by analysis of variance were significantly different at $p < 0.01$ (Table 4.11).

Multivariate discrimination – The multivariate discrimination techniques did not identify groupings consistent with TYPE or LINEAGE classifications. The average linkage and centroid hierarchical clustering algorithms returned only one cluster for each variable set. Ward's minimum distance clustering procedure identified more than one cluster in all variable sets, but none of the clusters were consistent with TYPE or LINEAGE classifications. Bivariate plots of cannonical coefficients, principal components, and

principal factors did not discriminate between samples classified by TYPE or by LINEAGE.

Ward's minimum distance clustering procedure identified three major clusters in the full $\log_e$ ratio and regression residual variable sets, and four major clusters in the angles variable set analyzed by TYPE (Table 4.12). Ward's procedure identified two major clusters in each of the three variable sets with samples classified by LINEAGE (Table 4.13). For the full angle variable set the procedure placed all but two Southern Appalachian fish in a single cluster. However that cluster contained the majority of hybrid and northern LINEAGE specimens as well. Cluster analyses of subsets of variables identified by analysis of variance mirrored the results obtained with the full variable sets.

Canonical discriminant analysis of each of the three full variable sets by TYPE calculated three canonical variables having non-zero correlations with the original variables (Appendix 4.3). Log_e ratio variables associated with the length of the fish (HL, NL, SV, SD, VC, and DA) were negatively correlated with the first canonical variable (Appendix 4.4). The residual variables for these morphometrics were all positive (Appendix 4.4). These are the same variables identified as significantly different between types by analysis of variance. The remaining variables, associated with the depth of the fish, were of opposite correlation with the first canonical variable. The second and third canonical correlations of these variable sets and the angle variable canonical correlations were not interpretable. Both canonical variables calculated for the three variable sets classified by LINEAGE had significant non-zero correlations with the original variables

(Appendix 4.3). I could not interpret the canonical correlations with the variables in the analyses by LINEAGE.

Principle component analysis of the full $\log_e$ and of the full regression residual variable sets calculated nine eigenvalues of the correlation matrices. Five eigenvalues accounted for about 79% of the variation in both variable sets; the first eigenvalue accounted for only about 27% of the total variation in both (Appendix 4.3). The first principle component had large positive loadings for the four variables associated with the head of the fish (HL, NL, SV, SD) and small positive or negative loadings for the remaining variables (Appendix 4.5). The second principle component had large positive loadings for VA and VC, and the third component had large positive loadings for DV and AC. There were six eigenvalues of the angles correlation matrix. (Appendix 4.3). I could not interpret the factor pattern of the angle variables.

Three eigenvalues of the reduced correlation matrices account for all of the variance in the full $\log_e$ ratio and full regression residual variable sets (Appendix 4.3). Kaiser's measure of sampling adequacy was acceptable for the HL, NL, SV, SD, and VA variables in the two variable sets, but was unacceptable for the other four variables (Table 4.14). However, the overall measure of sampling adequacy for both variable sets (0.58) indicated that there are too few variables for the factor model to be appropriate. The correlation matrix of the full angle variable set was singular, thus Kaiser's measure of sampling adequacy is undefined. Six factors account for all of the variance in the angles variable set (Appendix 4.3). Because there were clearly too few variables for the number of factors identified in any of the variable sets, no further common factor manipulations

were undertaken. However, the variable loadings do provide useful information for designing future morphometric sampling strategies.

Sheared principal components analysis – For the full data set of raw variables, the first sheared principal component (the size component) accounted for 91% of the total variation and the first four principal components account for 98% of the total variation (Appendix 4.3). As expected, variable loadings on the first principal component are similar in magnitude, reflecting the high covariance between size dependent morphometrics (Appendix 4.6). The second sheared component had a large negative loading on the dorsal fin to adipose fin metric, and the third component had a large negative loading on snout length (Appendix 4.6). Eigenvalues of the total covariance matrix of the subset of raw variables identified as potentially informative showed a pattern similar to the full data set. Bivariate plots of the second and third sheared principal components did not discriminate between samples classified by TYPE or by LINEAGE. This was not surprising given the small percentage of variation accounted for by the second and third principal components.

Discussion

Morphometric differences between groups – The morphometric analyses detected significant differences in body proportions and shape between northern, Southern Appalachian, and hybrid brook trout, and between samples grouped according to TYPE. However, there were also significant differences between populations within the same

TYPE and LINEAGE categories. Although Mahalanobis distances were significantly different from zero between most groups, multivariate discrimination failed to group samples by LINEAGE or TYPE. This failure was probably due to the variation among samples within categories. Common factor analysis also indicated that there were too few variables for the number of factors, and the sheared principal components analysis demonstrated that about 90% of the variance in my data set was associated with the length of the fish. These observations of data insufficiency apply to all of the multivariate discrimination techniques, and is another reason why the techniques failed to discriminate between groups.

Differences between Southern Appalachian and northern brook trout are most evident in morphometrics of the head region (HL, NL, SD, SV, and the SD-SV angle: A2; Table 4.7). Lennon (1967) also noted that Southern Appalachian and northern brook trout differ in head dimensions. However it was Lennon's impression that native Southern Appalachian brook trout have larger heads than northern brook trout. I observed significantly larger mean HL/SL ratios in Southern Appalachian samples than in the northern derived hatchery samples, but the mean HL/SL and mean NL/SL of Southern Appalachian, hybrid, hatchery, and naturalized hatchery samples are all significantly smaller than the wild northern samples (Table 4.7; Appendix 4.2). Several studies of conspecific salmonid populations (Kinnison et al., 1998; Beacham and Murray, 1987), including studies of brook trout (Behnke, 1980; Cooper et al., 1962), have observed larger body size and relatively larger heads in fish from large streams versus small streams. In the Southern Appalachians, brook trout inhabit small high elevation, high gradient first

and second order streams, while populations in the north, including those sampled in my study, are found in larger, higher order stream segments. Thus my results are consistent with the earlier studies.

Differences in the availability of prey may correlate with the observed head size differences. In Southern Appalachian streams that have not been stocked with non-native salmonids, brook trout are the sole top predator. The typical high elevation habitats of Southern Appalachian brook trout have low productivity (Kulp, 1994) with only a few other sympatric fish species at low densities (Bivens, 1985). Stomach content analysis shows that brook trout in these habitats eat fishes, including smaller conspecifics, salamanders, crawfish, and aquatic insects, but terrestrial insects make up the bulk of the diet during most of the year (Lohr and West, 1992; Ensign, 1988; Habera, 1987). In contrast, northern brook trout eat a greater diversity of larger prey species (Power, 1980). Romanov (1984) has observed that significant morphological differences between the skulls of masu salmon (*Oncorhynchus masou*) correlate with particle size of food consumed by juveniles. He attributed this difference to plastic changes in both the musculature and the supporting bone structure of the head region. The significantly smaller heads of wild Southern Appalachian compared to wild northern brook trout may be a similar plastic developmental response to prey size. The significantly smaller heads of hatchery trout compared to wild conspecifics is also a widely observed trend in other salmonids (Flemming et al., 1994; Swain et al., 1991; Taylor, 1986). Most of the observed head size difference between conspecifics in hatchery and wild environments

appears to be influenced by food size (Swain et al., 1991) but not by food quantity or water temperature (Currens et al., 1989; Swain et al., 1991).

Allometric relationships - The significant positive allometry of the four head region morphometrics in Southern Appalachian samples but not in the northern or northern derived hatchery samples (Table 4.8) may also represent a plastic developmental response to habitat differences. Alternatively it may be a function of differences in age and size at maturity. In many salmonid species, including brook trout, populations in smaller streams and at lower latitudes have slower growth rates and attain sexual maturity at a smaller size and younger age than populations in larger streams and at higher latitudes (Kinnison et al., 1998; Beacham and Murray, 1987; Healey, 1987; Thorpe et al., 1983). If head region allometric relationships are not uniform during growth, differences in these parameters between the larger northern brook trout and the smaller Southern Appalachian brook trout are expected. In chinook salmon (*Onchorhynchus tshawytscha*) head region allometries differed for different age/size classes but not between experimental groups of the same age class receiving different quantities of food (Currens et al., 1989), suggesting high heritability within age classes. If head region allometries in brook trout have similar age class heritabilities, allometric differences between northern and southern samples may reflect differences in the ages of individuals in the samples rather than differences in growth allometries.

In the Southern Appalachians, brook trout reach sexual maturity in the second year and rarely live beyond three years (Kulp, 1994; Behnke, 1980). In the North, sexual

maturity is reached after the second year and longevity can exceed six years (Behnke, 1980; Power, 1980). The largest individuals in my Southern Appalachian samples have standard lengths well below the maximum standard length of three year old Southern Appalachian fish examined by Kulp (1994), and the average standard length of my Southern Appalachian samples is significantly smaller at $p < 0.05$ than that of my wild northern samples. It seems likely that my northern samples contain individuals older than three years and have a higher proportion of three year and older fish than my Southern Appalachian samples. Therefore the observed differences in allometric coefficients may be a function of different age classes in the samples rather than of different allometries in northern and Southern Appalachian populations.

Both the hybrid samples and the naturalized hatchery sample from Great Smoky Mountains National Park have positive allometry for snout length, NL (Table 4.8), which is consistent with the hypothesis of differences in age specific allometries. However I cannot exclude the alternative hypotheses of a plastic response to available prey size or selection for positive allometry in Southern Appalachian habitats, or both. Southern Appalachian fish show positive allometry for several other traits including head length (HL) and snout to dorsal fin length (SD). The isometry of HL and SD in hybrid, hatchery, naturalized hatchery, and wild northern samples suggests heritable differences between northern and Southern Appalachian lineages in some head region allometries.

The low variance of head region morphometrics also provides indirect support for the hypothesis they are under natural selection in Southern Appalachian habitats. Six of the eight significant pairwise differences in variances between LINEAGE involved head

region morphometrics. In particular, variances of head length ratios and snout length residuals were significantly lower in Southern Appalachian and hybrid samples than in northern samples, and hybrid head length residuals had significantly lower variance than northern samples. Low variance in these characters is expected if head region morphology or developmental trajectory is under strong selection in Southern Appalachian environments (Falconer, 1989; Fisher, 1930).

Hybrid morphology – Interspecific hybrids and hybrids between genetically distinct populations of conspecifics may be phenotypically intermediate between parental populations, may resemble one parental population, or may exhibit phenotypes different from both parental types. Studies of interspecific and intraspecific hybridization in salmonid taxa have observed all three outcomes in different taxa examined for different phenotypic traits (Hedenskog, 1997). Intermediacy in F_1's is more frequently observed in morphometric traits than in meristic traits (Jones, 1947). This is likely a function of the significantly higher heritabilities of meristic traits than morphometric traits that are consistently observed in quantitative genetic studies of salmonids (Beacham, 1990; Gjerde and Schaeffer, 1989; Simon and Noble, 1968). Intermediacy of quantitative traits in F1 hybrids is indicative of additive genetic variation, while deviations from intermediacy indicates nonadditive gene interactions. Several hypotheses have been proposed to account for deviations from intermediacy in hybrid fishes, including the effects of genetic dominance (Simon and Noble, 1968), modifier genes (Ross and Cavender, 1981), and/or alteration of developmental rate by the disruption of coadapted

gene complexes (Leary et al., 1985; 1983). Deviation from intermediacy of morphometric traits toward the phenotype of a wild parental would also be expected in hybrid swarms if the other parental type was hatchery derived and the traits are selected in the wild, or if the traits are developmentally plastic.

In my study the failure of the multivariate techniques to discriminate between parental morphologies makes it potentially difficult to distinguish between the alternatives of morphological intermediacy and similarity to one parental morphology in hybrid populations. However, it is reasonably clear that hybrid populations of Southern Appalachian and northern derived hatchery strains do not have phenotypes that are beyond the range of variation found in either parental type. Variances of derived morphometric variables in hybrids were either not significantly different from the other TYPES, or in the case of three head region morphometrics, were significantly lower than one of the parental morphologies. Head length (ratios and regression residuals) and snout length (regression residuals) variances were significantly lower in hybrid samples than in the pooled northern samples, and snout to pelvic fin length (residual) variance was significantly lower in hybrid samples than in native Southern Appalachian samples.

Morphological intermediacy of hybrids rather than similarity to one parental lineage is indicated by univariate comparisons of the morphometrics. Means of five morphometric ratios (HL, SD, VC, DV, and AC) and of all six angles showing significant differences between TYPES, were significantly different between hybrid samples and Southern Appalachian and / or hatchery samples (Table 4.7), and hybrid sample means of four of the five significantly different ratios, and all of the angles were intermediate

between Southern Appalachian and hatchery sample means (Appendix 4.2). The mean head length ratio of hybrids was significantly larger than the hatchery samples, but not the Southern Appalachian samples, at $p < 0.05$. This is consistent with the observations discussed above that hatchery reared fish have smaller heads than wild conspecifics. Differences between morphometric traits in Southern Appalachian and hatchery brook trout, and the intermediacy of morphometric traits in their hybrids, would indicate an additive genetic component for the traits if hybrids were F_1's. However, molecular genetic analysis (Part II) indicates that hybrid populations are hybrid swarms, suggesting that intermediacy and low variance are a function of selection and/or plastic response in Southern Appalachian streams.

Taxonomic implications and suggestions for further research – My study demonstrates morphometric differences between Southern Appalachian and northern brook trout lineages that are probably due in part to differences in alleles at additive loci. However, morphological differences between brook trout populations also are evidently due to plastic responses to characteristics of different habitats and feeding niches, and perhaps to differences in age / size specific allometries. Disentanglement of these components of observed morphological variation will require more carefully designed investigations of wild populations and, most especially, experimental crosses of different genotypes under controlled conditions. The components of brook trout morphological phenotype are simply too complex to be comprehensibly evaluated by any other approach.

The head region of brook trout appears to be the most potentially informative area for investigating the factors of genetics and environment interacting through development. The head region appears to be one of the most developmentally constrained regions of salmonid growth and also one of the most plastic in juvenile ontogeny in response to environmental differences. For these reasons I suggest that future research to identify both taxonomically useful morphological characteristics and evolutionarily interesting phenomena should focus particularly on the head region. The small number of head region morphometrics that I examined primarily evaluated head length. However, head length is only one component of head region morphology, and perhaps not the most informative. It seems likely that other morphometrics in the head region would reveal other significant differences between groups that would permit the exploration of the components of variation.

My inability to coherently identify differences between groups in trunk and tail region morphometrics does not imply that significant detectable differences do not exist. The appropriate interpretation is that I examined too few morphometrics to detect and interpret variation in these areas of the body. However, because the trunk region appears to be the most environmentally responsive body region in salmonids (Currens et al., 1989), the problems of disentangling the components of variation are likely to be even greater than those encountered in the head region.

Literature Cited

Andenberg, M. R. 1973. Cluster analysis for applications. Academic Press, NY.

Andersen, R. O., and S. J. Gutreuter. 1983. Length, weight, and associated structural indices. Pp. 283-300, *In* Nielsen, L. A., and D. L. Johnson, eds. Fisheries Techniques. American Fisheries Society, Bethesda, MD.

Atchley, W. R., C. T. Gaskins, and D. Anderson. 1976. Statistical properties of ratios. I. Empirical results. Syst. Zool. 25:137-148.

Baum, D. 1992. Phylogenetic species concepts. Trends Ecol. Evol. 7:1-2.

Beacham, T. D. 1990. A genetic analysis of meristic and morphometric variation in chum salmon (*Onchorhynchus keta*) at three different temperatures. Can. J. Zool. 68:225-229.

Beacham, T. D., and G. B. Murray. 1987. Adaptive variation in body size, age, morphology, egg size, and developmental biology of chum salmon (*Oncorhynchus keta*) in British Columbia. Can J. Fish. Aquat. Sci. 44:244-261.

Behnke, R. J. 1972. The systematics of salmonid fishes of recently glaciated lakes. J. Fish. Res. Board Can. 29:639-671.

Behnke, R. J. 1980. A systematic review of the genus *Salvelinus*. Pp. 441-481 *In* E. K. Balon (ed.), Charrs: Salmonid Fishes of the Genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands.

Bivens, R. D. 1985. History and distribution of brook trout in the Appalachian region of Tennessee. Tennessee Wildlife Resources Agency Technical Report No. 85-1. The University of Tennessee, Knoxville.

Blum, J. R., J. Kiefer, M. Rosenblatt. 1961. Distribution free tests of independence based on the sample distribution function. Ann. Math. Stat. 32:485-498.

Bookstein, F. L., B. Chernoff, R. Elder, J. Humphries, G. Smith, and R. Strauss. 1985. Morphometrics in evolutionary biology. The Academy of Natural Sciences of Philadelphia, Special Pub. No. 15.

Bowen, J. T. 1970. A history of fish culture as related to the development of fishery programs. Am. Fish. Soc. Spec. Publ. 7:71-93.

Cock, A. G. 1966. Genetical aspects of metrical growth and form in animals. Quart. Rev. Biol. 41:131-190.

Conover, W. J. 1980. Practical nonparametric statistics. John Wiley & Sons, New York.

Cooper, E. L. 1967. Growth and longevity of brook trout (*Salvelinus fontinalis*) in populations subjected to light exploitation. Trans. Am. Fish. Soc. 96:383-386.

Cunjak, R. A., and J. M. Green. 1983. Habitat utilization by brook char (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) in Newfoundland streams. Can. J. Zool. 61:1214-1219.

Currens, K. P., C. S. Sharpe, R. Hjort, C. B. Schreck, and H. W. Li. 1989. Effects of different feeding regimes on the morphometrics of chinook salmon (*Onchorhynchus tshawytscha*) and rainbow trout (*O. mykiss*). Copeia. 1989:689-695.

Dowling, T. E., B. D. Demarais, W. L. Minckley, M. E. Douglas, and P. C. Marsh. 1992. Use of genetic characters in conservation biology. Conserv. Biol. 6:7-8.

Ensign, W. E. 1988. The importance of competition for food resources in the interaction between brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). Unpublished Masters thesis, University of Tennessee, Knoxville.

Falconer, D. S. 1989. Introduction to quantitative genetics. Longman, London.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford Univ. Press. (2^{nd} edition, 1958, Dover Books, NY).

-------. 1942. The design of experiments. Oliver & Boyd, Edinburgh.

Flemming, I. A., B. Jonsson, and M. R. Gross. 1994. Phenotypic divergence of sea-ranched, farmed, and wild salmon. Can. J. Fish. Aquat. Sci. 51:2808-2824.

Freund, R. J., and R. C. Littell. 1981. SAS for linear models: A guide to the ANOVA and GLM procedures. SAS Institute, Cary, NC.

Frost, D. R., and D. M. Hillis. 1990. Species in concept and practice: herpetalogical applications. Herpetologica 46:97-104.

Gjerde, B., and L. R. Schaeffer. 1989. Body traits in rainbow trout. II. Estimates of heritabilities and of phenotypic and genetic correlations. Aquacult. 80:25-44.

Gorsuch, R. L. 1974. Factor analysis. W. B. Saunders Co., Philadelphia.

Habera, J. W. 1987. Effects of rainbow trout removal on trout populations and food habits in Great Smoky Mountains National Park. Unpublished Masters thesis, University of Tennessee, Knoxville.

Habera, J. W., and S. J. Fraley. 1996. Variation in red spot number among native Southern Appalachian and hatchery derived brook trout populations. *In* Strange, R. J., and J. W. Habera. 1996. Wild trout project: 1995 Annual report. Tennessee Wildlife Resources Agency and the University of Tennessee. Pub. No. R11-2217-39-001-96.

Hayes, J. P., S. Z. Guffey, F. J. Kriegler, G. F. McCracken, and C. R. Parker. 1996. The genetic diversity of native, stocked, and hybrid populations of brook trout in the southern Appalachians. Conserv. Biol. 10:1403-1412.

Healey, M. C. 1987. The adaptive significance of age and size at maturity in female sockeye salmon (*Oncorhynchus nerka*) Can J. Fish. Aquat. Sci. Special Pub. 96:110-117.

Hedenskog, M., E. Petersson, T. Jarvi, and M. Khamis. 1997. Morphological comparison of natural produced Atlantic salmon (*Salmo salar* L), anadromous brown trout (*S. trutta* L), and their hybrids. Nordic J. Freshwater Res. 73:35-43.

Hoeffding, W. 1948. A non-parametric test of independence. Annals Math. Stat. 19:546-557.

Holloway, A. D. 1945. Report on the fisheries of the Great Smoky Mountains National Park. Unpublished internal report to Great Smoky Mountains National Park, Gatlinburg, Tennessee.

Hotelling, H. 1933. Analysis of complex statistical variables into principal components. J. Educ. Psych. 24: 417-441; 498-520.

Humphries, J. M., F. L. Bookstein, B. Chernoff, G. R. Smith, R. L. Elder, and S. G. Poss. 1981. Multivariate discrimination by shape in relation to size. Syst. Zool. 30:291-308.

Huxley, J. S. 1932. Problems of relative growth. MacVeagh, London. (second edition, Dover Books, NY).

Jones, J. W. 1947. Salmon and trout hybrids. Prc. Zool. Soc. London 1947:708-715.

Keenlyne, K. D., C. J. Henry, A. Tews, and P. Clancey. 1994. Morphometric comparisons of upper Missouri River sturgeons. Trans. Am. Fish. Soc. 123:779-785.

Kelsch, S. W. 1995. Patterns of morphometric variation in the channel and headwater catfishes. Trans. Am. Fish. Soc. 124:272-279.

Kendall, W. C. 1914. The fishes of New England. The salmon family, Part I. The trout or charrs. Mem. Boston Soc. Nat. Hist. 8:1-103.

King, W. 1937. Notes on the distribution of native speckled and rainbow trout in streams at Great Smoky Mountains National Park. J. Tenn. Acad. Sci. 12:351-361.

King, W. 1942. Trout management studies at Great Smoky Mountains National Park. J. Wild. Manag. 6:147-161.

Kinnison, M., M. Unwin, N. Boustead, and Thomas Quinn. 1998. Population-specific variation in body dimensions of adult chinook salmon (*Oncorhynchus tshawytscha*) from New Zealand and their source population, 90 years after introduction. Can. J. Fish. Aquat. Sci. 55:554-563.

Kriegler, F. J., G. F. McCracken, J. W. Habera, and R. J. Strange. 1995. Genetic characterization of Tennessee brook trout populations and associated management implications. N. Am. J. Fish. Mgmt. 15:804-813.

Kulp, M. A. 1994. A comparative ecological study of two strains of brook trout in Great Smoky Mountains National Park. Unpublished Masters thesis, Tennessee Technological University, Cookeville.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1983. Consistently high meristic counts in natural hybrids between brook trout and bull trout. Syst. Zool. 32:369-376.

-------. 1985. Developmental instability and high meristic counts in interspecific hybrids of salmonid fishes. Evol. 39:1318-1326.

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

Levene, H. 1960. Robust tests for equality of variance. Pp. 278-293 *In* I. Olkin, S. G. Ghurye, W. Hoeffding, W. G. Madow, and H. B. Mann, eds. Contributions to prbability and statistics. Stanford University Press, CA.

Littell, R. C. ., R. F. Freund, and P. C. Spector. 1991. SAS system for linear models. SAS Institute. Cary, NC.

Lohr, S. C., and J. L. West. 1992. Microhabitat selection by brook and rainbow trout in a Southern Appalachian stream. Trans. Am. Fish. Soc. 121:729-736.

MacCrimmon, H. R., and J. S. Campbell. 1969. World distribution of brook trout, *Salvelinus fontinalis*. J. Fish. Res. Board Can. 26:1699-1725.

McCracken, G. F., C. R. Parker, and S. Z. Guffey. 1993. Genetic differentiation and hybridization between stocked hatchery and native brook trout in Great Smoky Mountains National Park. Trans. Am. Fish. Soc. 122:533-542.

Manly, B. F. J. 1986. Multivariate statistical methods: A primer. Chapman and Hall, London.

Marcus, L. F. 1990. Traditional morphometrics. Pp. 77-122 *In* Rohlf, F. J., and F. L. Bookstein, eds. Proceedings of the Michigan morphometrics workshop. Special Publication Number 2, The University of Michigan Museum of Zoology, Ann Arbor.

Morrison, D. F. 1976. Multivariate ststistical methods. McGraw-Hill, NY.

Pearson, K. 1901. On lines and planes of closest fit to systems of points in space. Philo. Mag. 6:559-572.

Penrose, L. W. 1953. Distance, size, and shape. Eugenics 18:337-43.

Pimentel, R. A. 1979. Morphometrics: the multivariate analysis of biological data. Kendall – Hunt, Dubuque, Iowa.

Power, G. 1980. The brook charr (*Salvelinus fontinalis*). Pp. 797-840 *In* E. K. Balon (ed.), Charrs: Salmonid Fishes of the Genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands.

Qadri, S. U. 1968. Morphology and taxonomy of the aurora charr, *Salvelinus fontinalis timagamiensis*. Bull. Nat. Mus. Can. 237:1-18.

Rao, C. R. 1973. Linear statistical inference. John Wiley & Sons, NY.

Reist, J. D. 1986. An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. Can. J. Zool. 64:1363-68.

Rohlf, F. J., and F. L. Bookstein. 1987. A comment on shearing as a method for size correction. Syst. Zool. 36:356-367.

Romanov, N. S. 1984. Effects of culture conditions on skull morphology in smolts of the masu salmon, *Oncorhynchus masou* (Brevoort). Aquacul. 41:147-153.

Ross, M. R., and T. M. Cavender. 1981. Morphological analyses of four experimental intergenic cyprinid hybrid crosses. Copeia 1981:377-387.

Rounsefell, G. A. 1962. Relationships among North American Salmonidae. Fish. Bul. U. S. Fish and Wildlife Service 62:235-270.

Saidak, L. R. 1995. Brook trout (*Salvelinus fontinalis*) in Tennessee; An ecological genetic study using allozyme electrophoresis and random amplified polymorphic DNA. Unpublished Masters Thesis, The University of Tennessee, Knoxville.

Sale, P. F. 1967. A re-examination of the taxonomic position of the aurora trout. Can. J. Zool. 45:213-225.

SAS Institute. 1985. SAS users guide. Version 5. SAS Institute, Inc. Cary, NC.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 184. Ottawa.

Simon, R. C., and R. E. Noble. 1968. Hybridization in *Onchorhynchus* (Salmonidae). I. Variability and inheritance in artificial crosses of chum and pink salmon. Trans. Am. Fish. Soc. 97:109-118.

Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. Freeman, San Francisco.

Sokal, R. R., and C. D. Michener. 1958. A ststistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull. 38:1409-1438.

Spearman, C. 1904. 'General intelligence,' objectively determined and measured. Am. J. Psych. 15:201-93.

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

Strauss, R. E., and F. L. Bookstein. 1982. The truss: body form reconstruction in morphometrics. Syst. Zool. 31:113-135.

Swain, D. P., and B. Holtby. 1989. Differences in morphology and behavior between juvenile coho salmon (*Onchorhynchus kisutch*) rearing in a lake or in it's tributary stream. Can. J. Fish. Aquat. Sci. 46:1406-1414.

Swain, D. P., B. E. Riddell, and C. B. Murray. 1991. Morphological differences between hatchery and wild populations of coho salmon (*Onchorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 47:566-571.

Taylor, E. B. 1986. Differences in morphology between wild and hatchery populations of juvenile coho salmon. Prog. Fish. Cult. 48:171-176.

Thorpe, J. E., R. I. G. Morgan, C. Talbot, and M. S. Miles. 1983. Inheritance of developmental rates in Atlantic salmon *Salmo salar* L. Aquacult. 33:119-128.

Vincent, R. E. 1960. Some influences of domestication upon three stocks of brook trout (*Salvelinus fontinalis* Mitchill). Trans. Am. Fish. Soc. 89:35-52

Vladykov, V. D. 1954. Taxonomic characters of the eastern North American charrs (*Salvelinus* and *Cristivomer*). J. Fish. Res. Board Can. 11:904-932.

Ward, J. H. 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Soc. 58:236-244.

Wilder, D. G. 1952. A comparative study of anadromous and freshwater populations of brook trout (*Salvelinus fontinalis*, Mitchill). J. Fish. Res. Board Can. 9:169-203.

Wilkins, N. P., H. P. Courtney, E. Gosling, A. Linnane, C. Jordan, and A. Curatalo. 1994. Morphometric and meristic characters in salmon, *Salmo salar* L, trout, *Salmo trutta* L, and their hybrids. Aquacult. Fish. Mgmt. 25:505-518.

Winans, G. A. 1984. Multivariate morphometric variability in Pacific salmon: technical demonstration. Can. J. Fish. Aquat. Sci. 41:115-1159.

Yuskavitch, J. A. 1991. Saved by the barriers. Trout. Summer:18-27.

Table 4.1. Source, sample classification, and sample size of brook trout populations examined for morphometric variation.

Sample	Source	Classification	n
Aden Creek	Great Smoky Mountains National Park (GRSM)	Southern Appalachian	10
Sams Creek	GRSM	Southern Appalachian	13
Silers Creek	GRSM	Southern Appalachian	4
Starkey Creek	GRSM	Southern Appalachian	12
Steel Trap Creek	GRSM	Southern Appalachian	10
Beech Flats Prong	GRSM	Hybrid	15
Hyatt Creek	GRSM	Hybrid	28
Ledge Creek	GRSM	Hybrid	18
Road Prong	GRSM	Hybrid	9
Straight Fork	GRSM	Hybrid	8
Tawya Creek	GRSM	Hybrid	22
Armstrong strain	Pisgah Hatchery (NC)	Hatchery	25
EdRay strain	Pisgah Hatchery (NC)	Hatchery	7
Meigs Creek	GRSM	Naturalized hatchery	18
Fishing Creek	Potomac drainage, MD	Wild northern	23
Spy Run	James drainage, VA	Wild northern	14

Table 4.2. Brook trout morphometrics in the data set.

Morphometric	Landmarks
Standard length (SL)	tip of snout to distal margin of caudal peduncle
Head length (HL)	tip of snout to distal margin of operculum
Snout length (NL)	tip of snout to proximal margin of eye
Snout to pelvic fin (SV)	tip of snout to proximal origin of pelvic fin
Snout to dorsal fin (SD)	tip of snout to proximal origin of dorsal fin
Pelvic fin to adipose fin (VA)	proximal origin of pelvic fin to proximal origin of adipose fin
Pelvic fin to caudal fin (VC)	proximal origin of pelvic fin to proximal ventral terminus of caudal fin
Dorsal fin to adipose fin (DA)	proximal origin of dorsal fin to proximal origin of adipose fin
Dorsal fin to pelvic fin (DV)	proximal origin of dorsal fin to proximal origin of pelvic fin
Adipose fin to caudal fin (AC)	proximal origin of adipose fin to proximal ventral terminus of caudal fin

Triangle angles formed by morphometrics between common landmarks:

A1: angle of SV and DV
A2: angle of SD and SV
A3: angle of SD and DV

B1: angle of DV and AV
B2: angle of DA and DV
B3: angle of DV and DA

C1: angle of VA and VC
C2: angle of VA and AC
C3: angle of AC and VC

Table 4.3. Statistical methods and significance tests employed.

	Procedure	Significance tests	References
Univariate association:	Pearson's correlation coefficient Hoeffding's measure of dependence		Hoeffding, 1948; Blum et al., 1961
Univariate means:	One-way analysis of variance	F-test Hochberg's GT2 test	Fisher, 1942 Freund and Littell, 1981
	Wilcoxon rank scores	Kruskal – Wallis test	Conover, 1980
Univariate variances:	Levene's test	F-test Hochberg's GT2 test	Levene, 1960
Multivariate means:	Multivariate analysis of variance Mahalanobis distance	F-test (Wilks' Lambda) F-test	Littell et al., 1991 Penrose, 1953; Manly, 1986
Multivariate variances:	Levene's test	F-test (Wilks' Lambda)	Manly, 1986
Allometric coefficients:		F-test	Huxley, 1932; Cock, 1966

Table 4.3 (continued).

Parameter	Procedure	Significance tests	References
Multivariate distribution: (discrimination)	Cluster analysis	none used	Anderberg, 1973; Sneath and Sokal, 1973
	Minimum variance		Ward, 1963
	Average linkage		Sokal and Michner, 1958
	Centroid hierarchical analysis		Sokal and Michner, 1958
	Canonical discriminant analysis	none used	Rao, 1973
	Principal factor analysis	none used	Spearman, 1904; Gorsuch, 1974
	Principal components analysis	none used	Pearson, 1901; Hotelling, 1933; Morrison, 1976
	Sheared principal components	none used	Humphries et al., 1981; Strauss and Bookstein, 1982; Rohlf and Bookstein, 1987

Table 4.4. Pearson correlation coefficients for the ten brook trout morphometrics.

	SL	HL	NL	SV	SD	VA	VC	DA	DV
HL	0.96								
NL	0.90	0.95							
SV	0.99	0.96	0.91						
SD	0.98	0.97	0.92	0.98					
VA	0.99	0.94	0.88	0.97	0.97				
VC	0.95	0.91	0.86	0.94	0.94	0.95			
DA	0.97	0.93	0.87	0.95	0.95	0.97	0.93		
DV	0.95	0.92	0.86	0.95	0.94	0.95	0.89	0.92	
AC	0.96	0.92	0.85	0.96	0.95	0.94	0.92	0.93	0.94

Table 4.5. Analysis of variance showing significant TYPE and LINEAGE effects for all sets of derived variables.

Variable set	Class	Multivariate p(F) (Wilks' Lambda)	Variables showing different means (* p<0.05; **p<0.01) ANOVA (F-test)	Wilcoxon Scores (Kruskal-Wallis test)
Log_e ratios	Type	0.0001	HL**, NL**, SD**, VC**, DV**, AC**	HL**, NL**, SD**, VC**, DV**, AC**
Log_e ratios	Lineage	0.0001	HL**, SD*, VC**, DV**, AC**	HL*, SD*, VC**, DV**, AC*
Angles	Type	0.0001	A2**, B1**, B3**, C1**, C2**, C3**	A2**, B1**, B2*, B3**, C1**, C2**, C3**
Angles	Lineage	0.0001	A2**, B1**, B3*, C1**, C2**, C3**	A2**, A3*, B1**, B3**, C1**, C2**, C3**
Residuals	Type	0.0001	HL**, NL**, SD**, VC**, DV**, AC**	HL**, NL**, SD**, VC**, DA**, DV**, AC**
Residuals	Lineage	0.0001	VC**, DV**, AC**	VC**, DV**, AC**

Table 4.6. Number of pairwise comparisons, number of comparisons with significantly different means at $p < 0.05$ by Hochberg's GT2 procedure., and number of expected significant differences under the null hypothesis of no differences among any samples at $p = 0.05$. Comparisons are by LINEAGE classification, TYPE classification, and by sample for the Southern Appalachian, hybrid, and northern lineages.

Variable set	Classification subset	Number of pairwise comparisons	Number significantly different	Number expected at p = 0.05
Log_e ratios:	Lineage	27	9	2
	Type	90	26	5
	Hybrid	135	19	7
	Northern	90	28	5
	Southern Appalachian	90	24	5
Angles:	Lineage	27	13	2
	Type	90	27	5
	Hybrid	135	29	7
	Northern	90	27	5
	Southern Appalachian	90	7	5
Regression residuals:	Lineage	27	6	2
	Type	90	23	5
	Hybrid	135	22	7
	Northern	90	23	5
	Southern Appalachian	90	18	5

Table 4.7. Pairwise comparisons between samples grouped by TYPE for variable means identified as significantly different at $p < 0.05$ by the F-test. Differences significant at $p < 0.05$ by Hochbergs GT2 multiple comparison method are indicated by "*." Comparisons that were not significantly different are indicated by "ns." (TYPE designations are abbreviated as follows: SA = Southern Appalachian; HY = hybrid; HA = hatchery; NH = naturalized hatchery; WN = wild northern.).

$Log_e(HL/SL)$	SA	HY	HA	NH
HY	ns.			
HA	*	*		
NH	ns.	ns.	ns.	
WN	*	*	*	*

$Log_e(NL/SL)$	SA	HY	HA	NH
HY	ns.			
HA	ns.	ns.		
NH	ns.	ns.	ns.	
WN	*	*	*	*

$Log_e(SD/SL)$	SA	HY	HA	NH
HY	ns.			
HA	*	*		
NH	ns.	ns.	ns.	
WN	ns.	ns.	*	ns.

$Log_e(VC/SL)$	SA	HY	HA	NH
HY	*			
HA	*	ns.		
NH	ns.	ns.	ns.	
WN	ns.	ns.	ns.	ns.

$Log_e(DV/SL)$	SA	HY	HA	NH
HY	*			
HA	*	*		
NH	*	ns.	*	
WN	ns.	ns.	*	*

$Log_e(AC/SL)$	SA	HY	HA	NH
HY	*			
HA	ns.	*		
NH	ns.	ns.	*	
WN	ns.	*	*	ns.

A2	SA	HY	HA	NH
HY	ns.			
HA	*	*		
NH	ns.	ns.	*	
WN	ns.	ns.	*	ns.

B1	SA	HY	HA	NH
HY	ns.			
HA	*	*		
NH	*	ns.	ns.	
WN	ns.	ns.	*	ns.

B3	SA	HY	HA	NH
HY	ns.			
HA	*	*		
NH	ns.	ns.	*	
WN	ns.	ns.	ns.	*

C1	SA	HY	HA	NH
HY	*			
HA	*	*		
NH	*	ns.	ns.	
WN	ns.	ns.	*	ns.

Table 4.7 (continued).

C2

	SA	HY	HA	NH
HY	*			
HA	*	*		
NH	*	ns.	ns	
WN	ns.	ns.	*	ns.

C3

	SA	HY	HA	NH
HY	*			
HA	*	*		
NH	ns.	ns.	*	
WN	ns.	ns.	*	ns.

HL regression residual

	SA	HY	HA	NH
HY	ns.			
HA	*	*		
NH	ns.	ns.	ns.	
WN	*	*	*	*

NL regression residual

	SA	HY	HA	NH
HY	ns.			
HA	*	*		
NH	ns.	ns.	ns.	
WN	*	*	*	*

SD regression residual

	SA	HY	HA	NH
HY	ns.			
HA	*	ns		
NH	ns.	ns.	ns.	
WN	ns.	ns.	ns.	ns.

VC regression residual

	SA	HY	HA	NH
HY	*			
HA	*	ns.		
NH	ns.	ns.	ns.	
WN	ns.	ns.	ns.	ns.

DV regression residual

	SA	HY	HA	NH
HY	*			
HA	*	*		
NH	ns.	ns.	ns.	
WN	ns.	ns.	*	ns.

AC regression residual

	SA	HY	HA	NH
HY	*			
HA	ns.	*		
NH	ns.	ns.	*	
WN	ns.	*	ns.	ns.

Table 4.8. Allometric coefficients of $\log_e$ morphometric regressed on $\log_e$ standard length.
(Coefficients significantly different from 1: * p < 0.05; ** p <0.01). TYPE classifications are Southern Appalachian, hybrid. hatchery, naturalized hatchery, and wild northern; LINEAGE classifications are Southern Appalachian, hybrid, and all northern. The sample size, n, is the number of individuals in the sample.

Morphometric	Pooled	Southern Appalachian	Hybrid	Hatchery	Naturalized Hatchery	Wild Northern	All Northern
n	246	49	110	32	18	37	87
Head length	0.939**	1.173**	1.039	0.904	0.943	1.014	0.885**
Snout length	1.137**	1.619**	1.337**	1.111	1.205**	1.025	0.974
Snout to pelvic fin	0.972**	1.156**	0.999	0.970	0.939**	0.932**	0.944**
Snout to dorsal fin	0.990	1.144**	0.996	0.953	0.999	1.029	0.981
Pelvic to adipose	1.014	0.806**	0.971	0.986	1.043*	1.067**	1.053**
Pelvic to caudal	0.963	0.908	0.954	0.841	0.968	1.084**	0.962
Dorsal to adipose	1.066	0.902	1.194	1.028	1.028	1.060	1.001
Dorsal to pelvic	1.065**	1.125	0.989	0.963	0.981	1.022	1.070*
Adipose to caudal	1.000	1.101	0.949	1.037	0.889*	0.966	0.970

Table 4.9. Pairwise comparisons between allometric coefficients by TYPE for nine morphometrics. Differences significant at $p < 0.05$ by the F-test are indicated by "*." Comparisons that were not significantly different are indicated by "ns." (TYPE designations are abbreviated as follows: SA = Southern Appalachian; HY = hybrid; HA = hatchery; NH = naturalized hatchery; WN = wild northern.)

Head Length (HL)

	SA	HY	HA	NH
HY	*			
HA	*	*		
NH	*	*	ns.	
WN	*	*	ns.	*

Snout length (NL)

	SA	HY	HA	NH
HY	*			
HA	*	*		
NH	*	*	ns.	
WN	*	*	ns.	*

Snout to Pelvic fin (SV)

	SA	HY	HA	NH
HY	*			
HA	*	*		
NH	*	*	*	
WN	*	*	ns.	ns.

Snout to Dorsal fin (SD)

	SA	HY	HA	NH
HY	*			
HA	*	*		
NH	*	ns.	ns.	
WN	*	ns.	*	ns.

Pelvic fin to Adipose fin (VA)

	SA	HY	HA	NH
HY	*			
HA	*	ns.		
NH	*	*	*	
WN	*	*	*	ns.

Pelvic fin to Caudal fin (VC)

	SA	HY	HA	NH
HY	ns.			
HA	ns.	*		
NH	*	ns.	*	
WN	*	*	*	*

Dorsal fin to Adipose fin (DA)

	SA	HY	HA	NH
HY	*			
HA	*	*		
NH	*	*	ns.	
WN	*	*	ns.	ns.

Dorsal fin to Pelvic fin (DV)

	SA	HY	HA	NH
HY	*			
HA	ns.	ns.		
NH	*	ns.	ns.	
WN	ns.	ns.	ns.	ns.

Adipose fin to Caudal fin (AC)

	SA	HY	HA	NH
HY	*			
HA	ns.	*		
NH	*	ns.	*	
WN	*	ns.	ns.	ns.

Table 4.10. Mahalanobis distances between samples classified by TYPE (bottom), and probability > Mahalanobis distance by the F-test (top). TYPE designations are abbreviated as follows: SA = Southern Appalachian; HY = hybrid; HA = hatchery; NH = naturalized hatchery; WN = wild northern..

All $\log_e$ ratios:

	SA	HY	HA	NH	WN
SA		0.0001	0.0001	0.0001	0.0001
HY	1.8493		0.0001	0.0005	0.0001
HA	9.7234	9.0564		0.0001	0.0001
NH	3.8849	2.1165	4.1170		0.0001
WN	1.8128	2.1140	12.5064	5.3729	

HL, NL, SD, VC, DV, AC $\log_e$ ratios:

	SA	HY	HA	NH	WN
SA		0.0001	0.0001	0.0001	0.0001
HY	1.7370		0.0001	0.0001	0.0001
HA	9.2960	8.4503		0.0001	0.0001
NH	3.7246	2.1024	3.5278		0.0001
WN	1.7795	1.8997	12.1064	5.1303	

All angles:

	SA	HY	HA	NH	WN
SA		0.0001	0.0001	0.0001	0.3125
HY	1.1141		0.0001	0.8102	0.0910
HA	6.6189	3.9626		0.0005	0.0001
NH	2.1626	0.3570	2.8086		0.0337
WN	0.5194	0.5874	4.7901	1.5901	

Angles A2, B1, B3, C1, C2, C3:

	SA	HY	HA	NH	WN
SA		0.0001	0.0001	0.0014	0.1287
HY	0.9404		0.0001	0.6018	0.0330
HA	5.9993	3.8255		0.0001	0.0001
NH	1.7501	0.3062	2.7877		0.0144
WN	0.4867	0.5318	4.4226	1.3773	

Table 4.10 (continued).

All regression residuals:

	SA	HY	HA	NH	WN
SA		0.0001	0.0001	0.0001	0.0001
HY	1.8141		0.0001	0.1903	0.0001
HA	5.8198	4.2292		0.0003	0.0001
NH	3.1035	0.8557	2.9829		0.0001
WN	1.9443	2.1800	8.1441	4.3277	

HL, NL, SD, VC, DV, AC regression residuals:

	SA	HY	HA	NH	WN
SA		0.0001	0.0001	0.0001	0.0001
HY	1.7381		0.0001	0.0645	0.0001
HA	5.6189	4.0683		0.0001	0.0001
NH	2.8877	0.8120	2.6775		0.0001
WN	1.8372	1.9131	7.7910	3.8318	

Table 4.11. Mahalanobis distances (bottom) between samples classified by LINEAGE, and probability > Mahalanobis distance by the F-test (top). LINEAGE designations are abbreviated as follows: SA = Southern Appalachian; HY = hybrid; PN = pooled northern (hatchery + naturalized hatchery + wild northern).

All $\log_e$ ratios:

	SA	HY	PN
SA		0.0001	0.001
HY	1.8207		0.001
PN	1.8886	1.5069	

HL, SD, VC, DV, AC $\log_e$ ratios:

	SA	HY	PN
SA		0.0001	0.001
HY	1.7204		0.001
PN	1.8461	1.3616	

All angles:

	SA	HY	PN
SA		0.0004	0.0001
HY	1.0142		0.0045
PN	1.5843	0.5538	

A2, B1, B3, C1, C2, C3 angles:

	SA	HY	PN
SA		0.0002	0.0001
HY	0.8748		0.0005
PN	1.4117	0.5521	

All regression residuals:

	SA	HY	PN
SA		0.0001	0.0001
HY	1.7565		0.0004
PN	1.5831	0.7196	

VC, DV, AC regression residuals:

	SA	HY	PN
SA		0.0001	0.0001
HY	1.2405		0.0004
PN	1.0133	0.4073	

Table 4.12. Clustering of individuals classified by TYPE using Ward's minimum variance method. TYPE designations are abbreviated as follows: SA = Southern Appalachian; HY = hybrid; HA = hatchery; NH = naturalized hatchery; WN = wild northern.. The number of clusters returned by the clustering algorithm was specified *a priori* as the number of TYPES.

Log_e Ratios:

	TYPE				
	SA	HY	HA	NH	WN
Cluster					
1	24	39	11	9	26
2	19	41	1	4	9
3	6	17	19	5	2
4	0	1	1	0	0
5	0	1	0	0	0

Angles:

	TYPE				
	SA	HY	HA	NH	WN
Cluster					
1	18	44	6	8	21
2	11	27	6	2	9
3	2	17	19	6	1
4	18	10	0	2	6
5	0	1	1	0	0

Regression residuals:

	TYPE				
	SA	HY	HA	NH	WN
Cluster					
1	20	29	0	4	23
2	25	46	22	8	9
3	4	22	9	6	5
4	0	1	1	0	2
5	0	1	0	0	0

Table 4.13. Clustering of individuals classified by LINEAGE using Ward's minimum variance method. LINEAGE designations are abbreviated as follows: SA = Southern Appalachian; HY = hybrid; PN = pooled northern (hatchery + naturalized hatchery + wild northern). The number of clusters returned by the clustering algorithm was specified *a priori* as the number of LINEAGES.

Log_e Ratios:

	LINEAGE		
	SA	HY	PN
Cluster			
1	24	39	46
2	25	59	41
3	0	1	0

Angles:

	LINEAGE		
	SA	HY	PN
Cluster			
1	47	81	60
2	2	17	26
3	0	1	1

Regression residuals:

	LINEAGE		
	SA	HY	PN
Cluster			
1	24	51	47
2	25	47	40
3	0	1	0

Table 4.14. Kaiser's measure of sampling adequacy of variables for the factor model. (MSA: the reduction of partial correlations by the factor model relative to the original correlations between variables). MSA's below 0.5 are considered inadequate for the model.

	Variable	Kaiser's MSA
Log ratios:	log(HL/SL)	0.700
	log(NL/SL)	0.743
	log(SV/SL)	0.756
	log(SD/SL)	0.752
	log(VA/SL)	0.313
	log(VC/SL)	0.271
	log(DA/SL)	0.322
	log(DV/SL)	0.374
	log(AC/SL)	0.287
Residuals:	Variable	Kaiser's MSA
	RHL	0.671
	RNL	0.681
	RSV	0.724
	RSD	0.784
	RVA	0.321
	RVC	0.301
	RDA	0.318
	RDV	0.387
	RAC	0.292

Appendix 4.1. Brook trout morphometrics data array. Variable codes are described at the end of the array.

ID POP TL SL HL NL WT SV SD VA VC DA DV AC T GT

1 1 . 142 39.0 10.3 . 77.5 71.6 48.5 67.5 46.8 38.7 33.3 2 2
2 1 . 163 42.5 11.9 . 87.3 83.0 54.9 73.4 51.2 38.8 34.5 2 2
3 1 . 156 41.3 9.7 . 81.2 75.1 51.0 70.9 52.0 38.7 33.3 2 2
4 1 . 99 25.6 5.6 . 50.4 46.7 31.3 41.5 31.4 24.4 23.0 2 2
5 1 . 121 32.4 6.4 . 64.1 56.6 38.2 53.9 41.8 27.0 27.6 2 2
6 1 . 119 33.0 8.1 . 64.3 57.3 40.0 55.6 39.0 29.8 26.9 2 2
7 1 . 126 30.4 7.9 . 65.7 58.8 42.0 55.6 44.9 27.1 23.9 2 2
8 2 165 140 39.7 11.9 34 80.3 71.3 40.0 62.6 43.4 27.5 28.3 1 1
9 2 190 160 43 12.6 58 90.9 81.9 50.6 75.0 51.6 35.0 36.5 1 1
10 2 148 125 36 9.8 27 61.6 66.5 38 52.5 39.5 27 26 1 1
11 2 173 145 42 11 38 87.5 79 46 62.5 46 37 34 1 1
12 2 161 138 37.5 10 34 78.5 68.5 42 65.5 43.5 31 32 1 1
13 2 140 123 31.5 8.5 23 66.5 57 39 56 43 26 28 1 1
14 2 160 143 39 11 34 77.5 69.5 43 67.5 46 30 33 1 1
15 2 155 135 34 9.5 31 72.5 63 43 63.5 45 29.5 32 1 1
16 2 178 160 42.5 10 47 87.5 76 48 71 50.5 37 37 1 1
17 2 150 131 34.5 9.5 29 71 65 39.5 56.5 42 27.5 30 1 1
18 2 148 130 34 8.5 24 71.5 66 38 56.5 41 28 30 1 1
19 2 131 115 27 8 20 59.5 53 36.5 54 37.5 26 26.5 1 1
20 3 155 137 37.7 8.8 35.5 74.6 65.8 44.1 57.4 46.6 32.2 26.7 2 2
21 3 137 120 29.5 6.9 22.5 61.8 54.5 40.0 51.0 42.4 25.9 20.6 2 2
22 3 137 118 30.5 7.5 25 64.5 55.8 38.2 48.4 38.7 28.3 23.8 2 2
23 3 139 120 32.6 8.0 23 62.4 56.4 40.0 53.4 39.6 26.9 23.1 2 2
24 3 98 82 21.9 4.9 10 44.3 38.3 28 37.8 26.9 20.8 17.7 2 2
25 3 141 122 31.9 7.4 28.5 67.6 59.2 39.8 50.8 39.9 30.2 24.0 2 2
26 3 156 136 33.6 8.0 32 67.4 64.9 44.1 61.7 47.2 27.9 28.8 2 2
27 3 133 114 31.4 6.8 23 59.6 57 36.7 48.9 39.3 26.5 22.8 2 2
28 3 100 85 22.6 5.5 11.5 46.3 43 28.9 37 28.3 21.2 17.6 2 2
29 3 97 82 21.2 4.7 9 44.6 38.2 28.2 35.6 2 16.90 16.9 2 2
30 3 90 76 19.9 4.1 7.5 41.5 36.2 25.7 33.7 24.5 18 15.8 2 2
31 3 158 137 36.9 10.7 36 71.9 65.1 46.6 57.5 46.6 33 27 2 2
32 3 171 154 42.5 11.3 64 83.6 73.6 49.3 63.1 48.2 39.5 30.8 2 2
33 3 154 135 35.8 8.1 31 73.2 67.3 39.2 57.9 40.2 25.3 28.7 2 2
34 3 184 165 47 11.5 63 87 76.3 52.7 70 54.3 40.5 33.5 2 2
35 3 97 82 22.1 4 8 43.8 39 25.5 37.6 27.7 18.2 20.1 2 2
36 3 95 82 19.3 3.6 8 42.5 36.6 25.7 37.8 27.2 16.2 18.8 2 2
37 3 94 81 20.4 3.9 8 42.2 37 25.8 35.9 26 17.9 18.5 2 2
38 4 152 131 35.3 9.4 37 74.3 62.9 43.2 55.4 42.5 30.7 25.2 2 2
39 4 135 117 31.1 8.3 22 63 57.1 37.4 52.3 38.1 24.5 23.4 2 2

Appendix 4.1 (continued).

ID POP TL SL HL NL WT SV SD VA VC DA DV AC T GT

40 4 132 114 29.8 8.7 20 61.3 54.1 37.5 49 38.4 24.1 20.3 2 2
41 4 123 105 26.3 6.9 17 53.5 49.3 37.6 50 35.7 22.9 20.6 2 2
42 4 112 97 24.1 4.9 14 53.3 45.1 32.9 40.5 32.9 21 19.1 2 2
43 4 121 113 27.8 6.1 18 54 50.4 34.3 49.1 35.3 22.9 22.5 2 2
44 4 137 119 31.3 9 24 64.2 57.2 40 56 42.4 27.4 24.3 2 2
45 4 159 135 34 8.9 32 71.9 66.7 45.5 64.1 42.6 27.6 27.1 2 2
46 4 108 91 26.1 6.4 13 47.6 45.2 31.9 41.6 30.3 22.9 18.7 2 2
47 4 119 101 27.1 6.7 17 53.8 48.6 35.1 48.2 32.6 23.3 21.5 2 2
48 4 146 123 32.8 8.2 27 68.2 60.9 40.8 53.1 41.4 26.4 23.9 2 2
49 4 179 155 43.2 13.6 47 85.4 77.6 51.8 69.3 50.8 34.6 28.3 2 2
50 4 124 108 28.1 7.3 19 59 51.6 34.3 48.1 34.8 24.6 23.1 2 2
51 4 136 119 32.7 8.6 23 64.5 58.4 38.9 53.2 40.5 27.2 22.6 2 2
52 4 135 117 33.6 8.3 30 63.9 58.7 39.7 47.9 38.4 31.9 25.4 2 2
53 5 131 115 29.2 6.4 . 57.3 54.1 42 56.5 37.7 26.7 26.2 1 1
54 5 138 123 31.2 7.6 . 65.1 58.6 39.6 58.1 37.8 28.8 26.5 1 1
55 5 158 139 34.6 8.4 . 71.3 68.3 46.7 66.7 45.2 29.3 28.7 1 1
56 5 152 130 35.7 8.4 . 71.6 66.3 41.0 62.1 42.6 31.7 29.8 1 1
57 5 142 122 34.3 6.9 . 59.5 59.8 41.0 61.9 43.0 25.0 25.5 1 1
58 5 160 140 37.7 8.2 . 68.8 61.7 46.8 64.1 49.9 35.5 30.8 1 1
59 5 135 117 28.8 6.3 . 62.1 55.6 42.0 56.3 39.0 25.8 23.9 1 1
60 5 161 143 37.0 9.8 . 72.1 67.2 45.3 65.7 46.6 27.8 30.5 1 1
61 5 102 90 22.6 4.2 . 44.6 40.6 29.0 44.0 30.2 20.5 20.0 1 1
62 5 112 98 23.9 5.2 . 50.6 44.6 31.8 45.3 34.6 20.6 20.7 1 1
63 6 175 156 41.1 10.5 . 82.6 72.2 51.2 69.2 50.6 33.8 33.7 2 2
64 6 180 156 40.9 9.9 . 81.9 74.5 49.5 66.9 52.1 27.8 33.2 2 2
65 6 169 149 39.8 8.4 . 81.4 67.8 44.5 66.2 47.4 29.3 31.8 2 2
66 6 125 111 28.7 8.3 . 57.5 48.8 31.5 52.0 43.3 22.6 28.0 2 2
67 6 122 105 27.7 7.0 . 56.5 49.2 30.8 46.4 34.8 21.5 23.5 2 2
68 6 117 102 25.9 6.8 . 54.7 46.9 30.0 44.9 34.3 23.4 23.9 2 2
69 6 154 134 35.0 9.1 . 67.7 66.0 43.9 62.3 44.3 27.9 27.8 2 2
70 6 124 108 27.2 6.5 . 56.7 47.7 33.9 51.4 37.1 26.2 25.5 2 2
71 6 184 163 43.7 11.9 . 85.9 75.1 51.7 73.4 54.9 37.0 36.3 2 2
72 7 213 199 44.6 11.4 108 100.0 92.4 64.8 91.0 67.6 45.7 46.8 3 3
73 7 213 193 45.8 13.7 122 104.5 88.9 64.6 84.5 65.8 52.7 45.4 3 3
74 7 199 184 48.6 12.7 92 97.3 84.5 60.2 78.3 64.4 46.7 40.8 3 3
75 7 192 175 40.2 9.9 81 92.4 83.4 57.0 77.4 58.6 42.4 37.3 3 3
76 7 207 188 42.7 10.6 100 98.5 84.4 61.5 82.3 63.6 46.1 42.5 3 3
77 7 209 190 51.7 13.6 131 106.2 88.4 66.6 86.8 62.8 58.1 41.6 3 3
78 7 203 186 45.9 10.4 101 98.4 83.9 61.4 79.3 59.0 49.4 39.4 3 3
79 7 202 188 50.0 13.4 116 101.4 91.1 67.0 83.1 67.2 55.8 42.5 3 3

Appendix 4.1 (continued).

ID POP TL SL HL NL WT SV SD VA VC DA DV AC T GT

80 7 222 204 50.9 11.0 119 108.3 94.3 66.0 89.4 62.7 48.6 46.5 3 3
81 7 193 177 44.1 10.9 81 77.3 91.0 58.8 78.4 59.2 40.1 37.6 3 3
82 7 215 202 46.9 11.8 117 104.3 87.1 65.2 39.0 65.5 46.3 47.3 3 3
83 7 202 189 41.8 10.6 105 99.1 83.7 64.9 84.2 62.2 45.5 40.5 3 3
84 7 168 154 35.4 8.8 50 83.7 69.5 47.0 65.6 52.2 39.4 37.2 3 3
85 7 157 138 33.6 8.1 50 76.1 64.1 49.5 59.9 44.4 34.0 30.8 3 3
86 7 196 180 47.1 14.1 90 101.5 85.0 61.1 77.4 56.6 47.1 40.2 3 3
87 7 221 203 46.8 13.4 135 108.2 91.0 65.6 88.9 65.1 49.7 46.6 3 3
88 7 174 159 39.2 9.5 62 81.6 71.3 51.8 69.9 52.3 39.6 37.8 3 3
89 7 187 170 45.1 13.6 83 89.9 83.2 57.1 71.6 56.1 44.1 35.7 3 3
90 7 174 161 37.0 8.7 69.2 82.9 70.6 54.9 73.6 54.9 40.3 38.9 3 3
91 7 156 141 36.0 9.7 44 75.8 68.2 47.0 59.5 46.9 36.6 30.0 3 3
92 7 166 151 40.4 10.6 52 79.7 70.4 50.4 62.8 49.7 40.0 33.2 3 3
93 7 164 149 35.1 8.4 54 78.1 68.8 50.4 69.0 45.2 38.1 35.4 3 3
94 7 141 131 30.8 7.4 40 73.8 61.9 44.6 57.6 40.6 36.9 28.9 3 3
95 7 168 154 36.6 8.6 54 79.6 71.6 52.8 68.7 49.2 35.7 33.7 3 3
96 7 156 138 36.7 8.7 40.1 73.2 68.0 42.0 54.4 43.3 35.8 32.3 3 3
97 8 154 138 35.1 8.3 . 69.0 59.0 49.7 69.2 51.3 27.3 29.2 1 1
98 8 118 104 28.2 6.9 . 51.8 49.2 37.3 51.8 34.8 21.6 21.5 1 1
99 8 146 127 36.4 7.9 . 68.5 64.0 42.0 56.5 45.9 24.3 24.2 1 1
100 8 139 124 31.7 7.8 . 60.3 57.8 40.9 55.4 44.7 25.1 25.8 1 1
101 8 173 153 44.0 11.0 . 82.0 73.0 51.3 74.8 50.5 35.2 32.8 1 1
102 8 150 136 37.2 9.8 . 68.4 65.2 45.0 59.6 49.0 30.1 27.8 1 1
103 8 135 123 32.0 7.3 . 63.7 56.0 40.8 56.0 44.8 28.0 23.7 1 1
104 8 139 122 32.3 7.6 . 65.5 60.3 45.0 58.9 45.8 27.4 26.6 1 1
105 8 117 105 26.0 6.0 . 51.6 46.0 36.5 50.4 37.7 21.7 22.5 1 1
106 8 119 106 28.8 6.2 . 55.1 46.4 35.2 48.0 38.6 19.6 20.4 1 1
107 9 150 131 33.9 7.0 . 68.1 63.4 45.8 60.9 49.5 32.8 26.4 5 3
108 9 129 115 30.2 6.4 . 61.8 52.3 38.6 51.1 39.9 27.7 22.9 5 3
109 9 184 164 40.6 10.6 . 83.2 77.2 56.4 80.1 59.4 36.6 30.4 5 3
110 9 201 175 49.6 14.8 . 89.2 88.7 59.4 84.9 65.6 38.2 39.2 5 3
111 9 165 146 45.6 13.3 . 80.3 73.2 47.1 63.5 46.1 40.3 31.3 5 3
112 9 204 182 48.8 11.6 . 89.8 89.3 59.0 81.4 70.7 49.4 41.8 5 3
113 9 113 99 25.2 6.1 . 55.8 47.4 32.0 44.5 37.4 20.2 21.4 5 3
114 9 206 184 51.2 12.5 . 100.5 94.4 61.2 81.6 59.2 46.0 41.5 5 3
115 9 205 185 52.4 14.3 . 96.3 88.8 64.1 87.8 63.9 39.2 41.9 5 3
116 9 195 174 46.7 11.1 . 87.0 79.7 58.7 84.0 60.8 36.9 34.8 5 3
117 9 128 115 30.1 6.8 . 58.4 57.8 38.0 53.6 39.9 24.6 24.1 5 3
118 9 105 93 24.7 5.9 . 50.1 46.1 29.4 40.1 30.4 21.2 19.1 5 3
119 9 105 93 22.9 5.9 . 49.1 42.0 30.6 37.9 35.4 18.9 19.2 5 3

Appendix 4.1 (continued).

ID POP TL SL HL NL WT SV SD VA VC DA DV AC T GT

120 9 104 91 22.5 5.5 . 47.1 41.2 29.5 41.8 33.0 24.2 19.0 5 3
121 10 159 141 37.8 8.2 39.5 73.9 65.9 47.7 63.2 50.5 31.6 27.7 2 2
122 10 125 109 29.0 6.2 22 57.3 52.1 33.5 47.2 34.6 24.5 22.9 2 2
123 10 108 96 23.8 4.8 14 50.4 44.2 30.1 40.6 32.4 21.5 20.6 2 2
124 10 128 112 27.8 6.0 21 58.6 51.5 36.5 47.6 36.9 24.0 23.1 2 2
125 10 181 162 45.4 11.8 73 88.8 77.3 53.7 68.6 53.5 43.1 34.5 2 2
126 10 97 86 22.5 4.1 10 43.7 40.2 27.5 37.4 27.9 19.1 18.4 2 2
127 10 104 93 23.4 4.7 13 47.1 43.6 30.1 40.6 31.0 20.7 19.8 2 2
128 10 120 107 27.1 6.1 19 54.8 48.3 31.9 44.5 36.6 24.9 21.5 2 2
129 11 237 218 50.3 13.2 163 113.8 101.2 70.1 95.7 70.4 55.4 52.2 3 3
130 11 210 189 40.9 9.3 94 99.5 84.6 64.0 87.6 58.9 42.7 43.2 3 3
131 11 159 145 32.5 7.1 43 73.5 65.3 47.0 62.4 48.2 31.9 31.6 3 3
132 11 189 171 41.4 9.7 83 87.1 80.4 57.6 72.6 57.3 40.8 35.7 3 3
133 11 160 144 38.3 8.6 46 71.8 63.9 48.5 63.8 50.0 35.3 32.7 3 3
134 11 177 161 36.1 7.4 59.8 81.6 68.0 54.2 74.7 55.2 36.1 35.8 3 3
135 11 197 180 40.5 9.8 80.9 88.1 79.0 63.0 87.6 59.5 40.8 40.7 3 3
136 12 191 172 43.6 10.5 64 87.1 75.7 57.3 75.9 61.2 37.7 34.4 2 2
137 12 145 130 33.7 8.2 30.2 68 58 40.3 55.3 43.1 29.2 29.5 2 2
138 12 154 137 36.9 9.9 38 73.3 63.9 42.5 29.2 49.1 30.7 27.5 2 2
139 12 118 107 . . 16 52.7 46.7 33.7 43.7 37.6 22.6 19.8 2 2
140 12 152 136 32.6 7.3 28 69.1 59.2 39.9 58.0 44.1 26.7 29.6 2 2
141 12 156 139 40.7 10.9 45 75.6 66.1 44.3 55.8 44.9 35.9 27.2 2 2
142 12 137 122 31.6 6.4 26.6 65.8 55.1 39.8 50.6 41.6 26.2 26.8 2 2
143 12 133 117 31.5 7.6 34 62.1 53.7 38.4 49.3 38.9 24.0 22.7 2 2
144 12 169 151 39.3 10.2 39 77.1 64.5 46.7 66.4 51.2 30.3 32.2 2 2
145 12 137 122 30.1 7.5 26 64.3 57.0 39.4 52.1 43.4 25.8 24.5 2 2
146 12 115 101 25.1 6.1 13 53.0 42.9 33.3 45.5 37.5 20.3 22.7 2 2
147 12 116 103 25.8 6.0 16 54.4 45.5 33.0 46.0 35.4 22.8 21.6 2 2
148 12 121 107 27.9 6.7 18.6 57.4 50.8 34.3 47.6 34.7 23.2 21.5 2 2
149 12 119 106 27.0 5.6 16 57.0 47.5 33.5 46.2 35.5 23.3 21.2 2 2
150 12 129 116 30.4 7.1 21.3 62.3 51.7 37.7 52.8 40.1 25.0 25.5 2 2
151 13 147 128 32 7.5 26 68.0 60.5 40.5 58.0 43.0 25.5 29.0 1 1
152 13 155 135 36 9 40 73.5 67 44 61 46 34 31 1 1
153 13 130 115 27.5 6.5 20 59.5 53.5 38 52 40 26 26 1 1
154 13 120 102 25 5 16 53.5 49 33.5 48 33 21.5 22 1 1
155 13 155 138 33.5 9 35 74 66.5 44.5 58.5 47.5 31 31 1 1
156 13 179 158 41 11.5 58 84 75.5 50 72.5 52 37.5 36 1 1
157 13 152 135 32 8.5 29 69 63 44 60 45 28 28 1 1
158 13 137 120 32.5 9 23 66 59.5 38 55 39 27.5 28.5 1 1
159 13 153 135 33.5 8 32 71.5 63.5 44 62 47 29.5 28 1 1

Appendix 4.1 (continued).

ID POP TL SL HL NL WT SV SD VA VC DA DV AC T GT

160 13 148 130 33 9 29 70 61 41.5 60 45.5 29 28.5 1 1
161 13 139 122 29 7 25 63.5 57.5 40 57 41 25.5 27 1 1
162 13 215 17 13 1 137 119 28 7 20 63.5 56 37 58 39 24 27 1 1
163 13 134 118 28 7.5 21 64 55 38.5 56.5 39.5 26 27 1 1
164 14 136 122 32.8 9.7 27 67.2 57.2 39.9 53.1 41.5 27.6 27.5 5 3
165 14 167 148 40 12.4 40 77.3 68.8 47.3 68.3 50.6 30.5 32.5 5 3
166 14 125 112 32.4 8.9 20 59.9 54.8 34.7 49.9 36.2 25.4 25.7 5 3
167 14 147 133 34.2 9.7 31 72.6 61 41.9 57.6 42.6 28.4 30.1 5 3
168 14 182 166 44.4 13.3 62.3 87.5 76.4 55 76.1 59.4 36.1 36.1 5 3
169 14 104 93 25.6 7.3 12 50.4 44.2 29.9 42.5 30.6 21.2 21.3 5 3
170 14 111 97 28.7 8.2 14 51.8 47.4 33.2 42.7 34.9 23.4 20.7 5 3
171 14 113 100 27.3 7.6 14 53.9 47.7 33.5 46.2 34.7 22.4 21.5 5 3
172 14 152 138 37.9 10.8 36.8 74.1 65.4 45.9 59.4 48.3 29.1 27.3 5 3
173 14 168 149 41.6 12.8 46.1 76.5 70.5 48.5 69.8 51.8 32.5 33 5 3
174 14 110 98 26.9 6.9 13 51.2 47.2 33 47.6 32 22.1 21.9 5 3
175 14 101 87 25.5 6.6 10.5 48.6 42.2 28.2 38.6 28.8 20.3 19.8 5 3
176 14 187 171 47.2 11.2 69 88.1 80.7 56.1 77.8 60 37.2 34.3 5 3
177 14 114 100 28.0 8.5 15.6 54.3 46.7 30.8 43.9 33.0 22.5 22.2 5 3
178 14 127 112 32.1 8.9 21 57.7 56.1 35.8 53.3 35.3 24.8 26.0 5 3
179 14 112 99 26.7 6.7 15 55.6 46.2 29.7 41.5 32.3 21.5 23.6 5 3
180 14 122 111 28.1 7.4 19 58.7 50.8 34.7 51.6 36.8 23.5 24.2 5 3
181 14 147 131 36.8 9.9 26 71 61.2 40.6 60.4 41.2 26.1 29.1 5 3
182 14 112 101 29.2 8.7 15 55.6 49.1 30.9 43.2 34 22.7 23 5 3
183 14 124 109 30.4 7.9 18 58.7 50.6 34.5 46.6 36.2 24.2 24.6 5 3
184 14 95 82 22.1 6.2 8 43.7 37.5 25.8 34.8 26.1 17.6 18 5 3
185 14 130 118 35.9 7.8 22 61.6 55.9 38.7 52.2 39.3 25.1 23.7 5 3
186 14 122 109 30.7 8.1 18 60.2 50.8 32.3 44.5 36.3 22.4 23.1 5 3
187 15 292 263 58.2 15.6 209 129.1 114.6 88.8 117 85.2 55.2 51.1 4 3
188 15 161 142 33.3 7.5 41 72.9 65.4 46.9 62.3 50 32.2 29.3 4 3
189 15 281 246 62.3 18.3 206 128.9 116.6 82 107.2 82.5 53.9 47.8 4 3
190 15 167 147 39.4 10.4 48.8 82.2 71.2 47.6 63 47.3 38.3 21.6 4 3
191 15 152 135 32 6.4 36 70.3 61.6 44 59.4 44.1 30.3 27.7 4 3
192 15 182 159 39.9 10.6 57 82 76.3 53.2 65.8 54 37.2 32.4 4 3
193 15 195 168 44.8 11.4 72.2 89 81.7 54.9 70.4 56.6 41.9 36.2 4 3
194 15 197 173 46.8 13.4 77 91.3 83.4 56.1 72.9 56.5 42.5 37.4 4 3
195 15 282 247 58.2 18.6 212 128 108.1 88 114.1 82.3 56 49.9 4 3
196 15 272 243 61.7 17.8 213 126.4 123 78.1 100.8 74.5 55.2 52.3 4 3
197 15 246 223 52.4 17.5 154 116.3 98.9 73.5 89.9 73.6 56.3 43.2 4 3
198 15 152 132 36.2 9.4 37 71.4 68.4 45.5 56.3 42.9 33.1 26.2 4 3

Appendix 4.1 (continued).

ID POP TL SL HL NL WT SV SD VA VC DA DV AC T GT

199 15 222 201 49.1 12.5 99 104.8 92 67.3 85.2 73.5 44.4 38.1 4 3
200 15 101 89 22.3 4.8 10 49.4 39.9 29.3 38 28.7 19.5 20.8 4 3
201 15 102 88 23.2 4.9 10 47.4 39.6 28.3 38.8 26.8 19.8 20.8 4 3
202 15 86 76 18 3.9 6 41.2 34.4 22.4 32.6 24.4 16.2 16.8 4 3
203 15 86 75 19.6 4.7 7 40.9 35.7 23.8 35.3 24 18 16.3 4 3
204 15 75 63 17.3 3.6 5 36.1 29.5 20.9 28.8 20.6 15.9 14.5 4 3
205 4 119 103 26.5 6.8 16 55.1 49.3 33 43.4 34.6 22.1 20.1 2 2
206 4 139 118 33.8 9.3 28 64.8 57.3 43.1 57.1 39.7 30.5 26 2 2
207 4 121 103 29 7 17 56.2 50 35 46.2 33.6 23.9 19.8 2 2
208 4 130 111 29.2 6.9 21 59.6 57.2 35.3 46.9 35.6 24.6 21.5 2 2
209 4 122 104 26.6 6.2 17.5 54.4 50.4 34.4 46.5 34.9 24.7 21.4 2 2
210 4 115 101 28.4 6.5 15 54.9 47.3 33.5 44.4 32.3 20.7 19.6 2 2
211 4 127 109 28.7 6.9 18 59 53.7 38.4 49.2 35.7 23.1 21.2 2 2
212 4 112 92 25 5.5 12 49.9 47.7 32.1 42.9 33.5 19.4 17.5 2 2
213 4 155 134 36.1 9.9 31 72.9 65.5 45.8 58.6 44.2 29.2 26.5 2 2
214 4 127 109 29 7.3 18 57.2 52.6 35.4 47.5 36 22.8 21.1 2 2
215 4 119 103 25.3 6.2 14 53.9 49.1 36 47.8 35.6 20.5 21.2 2 2
216 4 80 67 18.7 4.1 5.5 37.4 33.4 22.2 29.5 22.7 16.2 14.6 2 2
217 4 127 111 29.8 6.3 19.5 58.3 53.7 37.8 48 38.3 25.5 20.4 2 2
218 1 166 149 40.4 10.8 52.4 79.9 70 50.4 67.4 52.8 36.1 34.2 2 2
219 1 185 163 48.7 13.7 74.5 92.7 83.1 54.1 72.6 53.6 42.4 34.8 2 2
220 1 197 179 45.8 10.9 73.7 97.3 83.6 60.3 77.5 60.6 38 35.9 2 2
221 1 183 167 41.9 10.6 63.5 89.4 73.8 53.4 70.3 56.7 37.2 33.9 2 2
222 1 102 89 25.7 6.1 12.8 48.7 45.7 29.6 36.9 30.8 21.9 18.5 2 2
223 1 206 181 52.8 14.3 85.2 96.9 84 57.8 78.2 62.2 42.1 37.4 2 2
224 1 166 146 43 11.8 46 79.5 72.1 48.3 63.6 45.8 36.2 30.1 2 2
225 1 102 90 24.6 5.9 12.8 50.6 42.9 32.2 39.3 30.9 22.1 18.4 2 2
226 1 112 97 26.2 5.8 14.7 52.2 45.1 33.7 44.1 33.8 24.3 21.4 2 2
227 1 114 100 27.1 5.5 16.5 53.8 47.8 36.2 44.4 34.4 24.5 20.9 2 2
228 1 115 102 29.3 5.9 20.1 55.4 49 34.5 45.1 33.2 27.2 25.5 2 2
229 1 105 91 25.4 4.8 13.4 50.4 42.2 30.7 37.5 32.3 25.5 21 2 2
230 1 114 102 27.3 5.3 15.5 52.9 45.7 34.1 45.3 33.3 23.5 22.6 2 2
231 1 . 140 39.5 9.3 . 75.6 67.3 47.9 63.2 44.7 35.3 31.7 2 2
232 1 . 106 26.1 6.6 . 57.5 46.8 34.3 47.7 38.8 24.1 21.5 2 2
233 16 163 142 39 9.4 35 77.7 70.4 44.1 63.4 46.6 30.5 28.8 1 1
234 16 147 130 32.5 7.8 27 71.1 60.6 39.4 54.5 41.2 25.3 25.6 1 1
235 16 135 120 29.5 7.40 25 63.2 57.7 39.7 53.3 39.6 26.2 23.9 1 1
236 16 126 110 28.6 7.6 18 59.5 52.6 44.1 47.8 35.3 23.2 21.9 1 1

Appendix 4.1 (continued).

Key to data array coding:

ID = specimen number
POP = population number:
- 1 = Taywa Creek (hybrid)
- 2 = Starkey Creek (Southern Appalachian)
- 3 = Ledge Creek (hybrid)
- 4 = Hyatt Creek (hybrid)
- 5 = Aden Creek (Southern Appalachian)
- 6 = Road Prong (hybrid)
- 7 = Armstrong hatchery strain
- 8 = Steel Trap Creek (Southern Appalachian)
- 9 = Spy Run (northern)
- 10 = Straight Fork (hybrid)
- 11 = EdRay hatchery strain
- 12 = Beech Flats Prong (hybrid)
- 13 = Sams Creek (Southern Appalachian)
- 14 = Fishing Creek (northern)
- 15 = Meigs Creek (naturalized hatchery)
- 16 = Silers Creek (Southern Appalachian)

TL = total length (mm)
SL = standard length (mm)
HL = head length (mm)
NL = snout length (mm)
WT = weight (g)
SV = snout to pelvic fin (mm)
SD = snout to dorsal fin (mm)
VA = pelvic fin to adipose fin (mm)
VC = pelvic fin to caudal fin (mm)
DA = dorsal fin to adipose fin (mm)
DV = dorsal fin to pelvic fin (mm)
AC = adipose fin to caudal fin (mm)
T = population TYPE:
- 1 = Southern Appalachian
- 2 = hybrid
- 3 = hatchery
- 4 = naturalized hatchery
- 5 = northern

GT = intraspecific LINEAGE:
- 1 = Southern Appalachian
- 2 = hybrid
- 3 = northern (wild northern + hatchery + naturalized hatchery)

Appendix 4.2. Univariate statistics of ratios, angles, and regression residuals for all samples and subsets of samples.

HL/SL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.26112	.00029718	0.26218	0.095927	0.21640	0.31233
Southern	0.25951	.00022973	0.25874	0.054873	0.23478	0.28966
Hybrid	0.26501	.00017261	0.26474	0.063407	0.23537	0.29877
Hatchery	0.24226	.00027464	0.23748	0.055703	0.21640	0.27211
Naturalized	0.25179	.00025710	0.25210	0.053310	0.22129	0.27460
Northern	0.27369	.00023028	0.27449	0.066092	0.24624	0.31233
All northern	0.25760	.00045225	0.26167	0.095927	0.21640	0.31233

NL/SL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.064907	.000086415	0.063559	0.047193	0.043902	0.091096
Southern	0.064518	.000063897	0.062963	0.038333	0.046667	0.085000
Hybrid	0.063682	.000073074	0.063303	0.043839	0.043902	0.087742
Hatchery	0.060603	.000067295	0.057851	0.034037	0.045963	0.080000
Naturalized	0.064324	.000096201	0.064667	0.031068	0.047407	0.078475
Northern	0.072703	.000090770	0.074312	0.037661	0.053435	0.091096
All northern	0.066519	.00011175	0.066010	0.045133	0.045963	0.091096

SV/SL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.53041	.00038207	0.53145	0.16673	0.43672	0.60345
Southern	0.52860	.00059565	0.53125	0.11716	0.48629	0.60345
Hybrid	0.53279	.00024551	0.53591	0.09084	0.47788	0.56871
Hatchery	0.52403	.00056551	0.52713	0.12717	0.43672	0.56389
Naturalized	0.53099	.00037688	0.52586	0.08214	0.49087	0.57302
Northern	0.53161	.00030181	0.53402	0.07023	0.49341	0.56364
All northern	0.52869	.00041753	0.52800	0.13629	0.43672	0.57302

SD/SL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.47237	.00043148	0.47151	0.12247	0.42236	0.54483
Southern	0.47834	.00052286	0.47521	0.11729	0.42754	0.54483
Hybrid	0.47254	.00040344	0.47466	0.09373	0.42475	0.51848
Hatchery	0.46103	.00036604	0.46200	0.09176	0.42236	0.51412
Naturalized	0.46765	.00051899	0.46441	0.08244	0.43574	0.51818
Northern	0.47612	.00026122	0.47373	0.06143	0.45161	0.51304
All northern	0.46881	.00038966	0.46667	0.09582	0.42236	0.51818

Appendix 4.2 (continued).

VA/SL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.32753	.00025160	0.32715	0.11713	0.28378	0.40091
Southern	0.32593	.00043476	0.32449	0.11519	0.28571	0.40091
Hybrid	0.32726	.00023914	0.32791	0.08147	0.28378	0.36525
Hatchery	0.33331	.00014876	0.33424	0.05435	0.30435	0.35870
Naturalized	0.32878	.00015553	0.32940	0.06154	0.29474	0.35628
Northern	0.32473	.00016127	0.32418	0.05329	0.29633	0.34962
All northern	0.32873	.00016658	0.32903	0.06396	0.29474	0.35870

VC/SL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.44336	.00093571	0.44363	0.31431	0.19307	0.50738
Southern	0.46118	.0004565	0.45926	0.08815	0.41923	0.50738
Hybrid	0.43769	.0008299	0.44025	0.27076	0.21314	0.48390
Hatchery	0.43199	.0021988	0.43861	0.29360	0.19307	0.48667
Naturalized	0.43317	.0003087	0.42876	0.06753	0.40314	0.47067
Northern	0.44989	.0004457	0.44725	0.08089	0.40753	0.48841
All northern	0.43985	.0011159	0.44000	0.29535	0.19307	0.48841

DA/SL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.33397	.00066928	0.33278	0.36570	0.024390	0.39009
Southern	0.33596	.0002972	0.33333	0.06809	0.30732	0.37541
Hybrid	0.33232	.0011661	0.33333	0.36570	0.02439	0.39009
Hatchery	0.32909	.0001658	0.33026	0.05409	0.30336	0.35745
Naturalized	0.32881	.0002064	0.32663	0.06113	0.30455	0.36567
Northern	0.34253	.0004031	0.34016	0.07396	0.31450	0.38846
All northern	0.33475	.0003146	0.33153	0.08511	0.30336	0.38846

DV/SL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.22833	.00043463	0.22470	0.12758	0.17821	0.30579
Southern	0.21751	.00024764	0.21833	0.07027	0.18491	0.25517
Hybrid	0.22716	.00039843	0.22410	0.10201	0.17821	0.28022
Hatchery	0.25007	.00041043	0.24772	0.08579	0.22000	0.30579
Naturalized	0.23319	.00023634	0.22696	0.05066	0.20989	0.26054
Northern	0.22465	.00033729	0.22143	0.07679	0.19924	0.27603
All northern	0.23577	.00046653	0.22796	0.10655	0.19924	0.30579

Appendix 4.2 (continued).

AC/SL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.21365	.00022976	0.21369	0.10531	0.14694	0.25225
Southern	0.21695	.00016593	0.21803	0.046949	0.19055	0.23750
Hybrid	0.20855	.00022632	0.20644	0.080586	0.17167	0.25225
Hatchery	0.22494	.00009427	0.22470	0.032843	0.20877	0.24161
Naturalized	0.20667	.00042118	0.20576	0.089425	0.14694	0.23636
Northern	0.21669	.00014192	0.21951	0.053018	0.18537	0.23838
All northern	0.21765	.00022202	0.21959	0.094676	0.14694	0.24161

Angle A1

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	91.8473	43.1876	92.0318	51.0625	57.5707	108.633
Southern	91.1010	41.3699	91.1487	38.1170	67.8024	105.919
Hybrid	92.6483	33.2445	92.3895	27.2397	81.3935	108.633
Hatchery	89.9884	54.0420	90.7767	40.9961	57.5707	98.567
Naturalized	92.6540	53.5413	92.6379	26.8264	80.6802	107.507
Northern	91.8863	57.5787	93.4383	29.8714	74.5471	104.419
All northern	91.3471	55.3361	91.4662	49.9359	57.5707	107.507

Angle A2

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	23.1373	5.13857	22.9431	12.2418	18.0296	30.2714
Southern	22.0784	3.50910	22.0799	10.2122	18.0490	28.2613
Hybrid	22.9062	4.19068	22.9787	9.6732	18.0296	27.7028
Hatchery	25.7917	3.07720	25.4368	7.2777	22.9936	30.2714
Naturalized	23.5794	2.66867	23.4216	6.2035	20.1844	26.3879
Northern	22.6533	5.15426	22.3702	10.4452	18.7811	29.2263
All northern	23.9992	5.80618	23.7632	11.4903	18.7811	30.2714

Angle A3

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	65.0154	33.3162	64.6596	46.7850	51.9429	98.7279
Southern	66.8206	32.4822	66.3089	34.5212	55.8229	90.3441
Hybrid	64.4455	23.5058	64.1673	24.7159	51.9429	76.6588
Hatchery	64.2199	53.8381	63.0989	40.9732	57.7547	98.7279
Naturalized	63.7666	40.0390	63.2761	23.7110	52.3090	76.0200
Northern	65.4605	37.3781	64.1915	23.5263	55.5067	79.0330
All northern	64.6537	43.4829	63.6557	46.4189	52.3090	98.7279

Appendix 4.2 (continued).

Angle B1

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	40.2230	16.5975	40.0265	23.0068	30.2532	53.2600
Southern	38.3025	13.6662	38.5848	17.0704	30.3579	47.4283
Hybrid	39.9977	14.0115	39.7400	17.9264	30.2532	48.1796
Hatchery	44.3077	13.9216	44.0714	14.7384	38.5216	53.2600
Naturalized	41.5190	10.3707	41.3054	11.1769	36.4265	47.6034
Northern	39.2060	12.7407	39.5785	18.9795	32.2472	51.2266
All northern	41.5610	17.5951	41.0047	21.0129	32.2472	53.2600

Angle B2

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	61.9432	17.6051	61.5001	46.5645	40.7136	87.2781
Southern	62.3336	25.7511	60.8379	29.5741	57.7040	87.2781
Hybrid	61.8971	17.6387	61.9467	30.7834	40.7136	71.4970
Hatchery	62.7199	12.6065	62.3468	15.7224	54.5947	70.3172
Naturalized	63.1571	11.5066	62.5305	12.0306	57.7148	69.7454
Northern	60.2872	11.4880	60.4626	15.6033	50.6439	66.2472
All northern	61.7758	13.3121	61.4902	19.6733	50.6439	70.3172

Angle B3

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	77.8338	32.1967	77.7082	47.8817	61.1515	109.033
Southern	79.3639	26.6775	79.9471	28.9956	61.1515	90.147
Hybrid	78.1051	34.3125	77.9296	39.9808	69.0524	109.033
Hatchery	72.9724	15.8022	72.8226	13.9975	64.9699	78.967
Naturalized	75.3239	16.5698	74.4441	14.8100	70.1985	85.009
Northern	80.5068	23.9805	80.2945	24.8206	68.8352	93.656
All northern	76.6632	30.8099	76.7453	28.6859	64.9699	93.656

Angle C1

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	108.730	104.294	108.834	99.4951	36.3560	135.851
Southern	115.260	70.113	115.209	49.9950	85.8561	135.851
Hybrid	107.830	83.101	108.233	78.9380	42.9885	121.927
Hatchery	100.151	157.169	101.895	77.1855	36.3560	113.542
Naturalized	106.227	69.343	105.931	29.7656	97.2049	126.971
Northern	111.150	55.838	110.805	35.9479	96.4211	132.369
All northern	106.086	117.880	105.655	96.0129	36.3560	132.369

Appendix 4.2 (continued).

Angle C2

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	40.4806	45.9518	40.5153	64.0749	25.0767	89.1515
Southern	36.2335	31.6176	35.7460	36.0334	25.0767	61.1100
Hybrid	41.4446	41.1171	41.1617	60.2830	28.3059	88.5889
Hatchery	45.1056	74.1387	43.8639	51.5029	37.6486	89.1515
Naturalized	42.5355	23.9540	42.6804	17.7170	31.7330	49.4500
Northern	38.4998	17.2905	39.5115	20.0853	28.6114	48.6966
All northern	41.7645	47.5610	41.3937	60.5401	28.6114	89.1515

Angle C3

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	30.7897	16.3249	30.6115	35.4728	19.0197	54.4925
Southern	28.5061	12.1499	28.4997	17.1399	19.0722	36.2122
Hybrid	30.7252	11.2177	30.4295	24.1619	24.2607	48.4226
Hatchery	34.7438	18.1943	34.2902	25.6826	28.8099	54.4925
Naturalized	31.2376	17.9658	31.9715	18.1080	19.1382	37.2462
Northern	30.3503	14.0594	30.4064	18.7087	19.0197	37.7284
All northern	32.1499	20.0663	32.5869	35.4728	19.0197	54.4925

Residual HL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.0	.0041891	.0047475	0.35116	-0.16186	0.18931
Southern	-0.006244	.0037635	-0.011347	0.22418	-0.11066	0.11352
Hybrid	0.010151	.0028168	0.011117	0.28047	-0.12882	0.15166
Hatchery	-0.057449	.0044405	-0.074506	0.22937	-0.16186	0.06751
Naturalized	-0.028340	.0034444	-0.026961	0.18315	-0.12720	0.05595
Northern	0.044582	.0033784	0.048401	0.26529	-0.07598	0.18931
All northern	-0.008034	.0058817	-0.000315	0.35116	-0.16186	0.18931

Residual NL

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.0	0.019252	.00032847	0.69746	-0.36632	0.33114
Southern	-0.00202	0.013116	-0.01683	0.53918	-0.27156	0.26762
Hybrid	-0.00522	0.014907	-0.00559	0.60533	-0.31989	0.28544
Hatchery	-0.10638	0.017202	-0.13733	0.54675	-0.36632	0.18044
Naturalized	-0.02760	0.016536	0.01235	0.45701	-0.31128	0.14573
Northern	0.12207	0.018614	0.14248	0.51861	-0.18747	0.33114
All northern	0.0070769	0.027992	0.017432	0.69746	-0.36632	0.33114

Appendix 4.2 (continued).

Residual SV

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.0	.0013390	.0050694	0.31781	-0.18462	0.13319
Southern	-.0040184	.0022555	.0022930	0.22021	-0.08702	0.13319
Hybrid	0.0021598	.0009143	.0077573	0.18422	-0.10706	0.07716
Hatchery	-.0044184	.0021931	.0016283	0.25603	-0.18462	0.07141
Naturalized	0.0047123	.0007784	.0048658	0.11495	-0.05671	0.05824
Northern	0.0010133	.0008984	.0017029	0.11613	-0.06181	0.05432
All northern	-.0002193	.0013328	.0028513	0.25603	-0.18462	0.07141

Residual SD

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.0	.0019158	-.00046301	0.25358	-0.10869	0.14489
Southern	0.012314	.0023001	0.008707	0.24292	-0.09802	0.14489
Hybrid	-0.000482	.0018184	0.003792	0.19847	-0.10762	0.09085
Hatchery	-0.021338	.0016794	-0.018147	0.19754	-0.10869	0.08885
Naturalized	-0.008943	.0023355	-0.019041	0.16651	-0.07268	0.09383
Northern	0.007800	.0011752	0.003943	0.13424	-0.04711	0.08713
All northern	-0.006381	.0017301	-0.011210	0.20252	-0.10869	0.09383

Residual VA

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.0	.0023201	-.00083355	0.34565	-0.14019	0.20546
Southern	-0.005524	.0039533	-0.004496	0.34215	-0.13669	0.20546
Hybrid	0.000546	.0023209	0.000599	0.25152	-0.14019	0.11133
Hatchery	0.013950	.0013659	0.017540	0.16430	-0.07331	0.09100
Naturalized	0.002490	.0012634	0.003838	0.17297	-0.09697	0.07600
Northern	-0.007437	.0014439	-0.004307	0.16277	-0.09667	0.06610
All northern	0.0024832	.0014378	0.0036458	0.18796	-0.09697	0.09100

Residual VC

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.0	.0073242	.0058584	0.94736	-0.81112	0.13624
Southern	0.041147	0.002066	0.040384	0.18846	-0.05222	0.13624
Hybrid	-0.016067	0.006768	-0.009200	0.81435	-0.72674	0.08761
Hatchery	-0.020841	0.022472	0.006411	0.92022	-0.81112	0.10910
Naturalized	-0.016090	0.001401	-0.025246	0.13998	-0.07119	0.06879
Northern	0.014784	0.002668	0.016992	0.20226	-0.09305	0.10921
All northern	-0.004707	.0097817	0.003065	0.92033	-0.81112	0.10921

Appendix 4.2 (continued).

Residual DA

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.0	0.031043	.0033115	2.75235	-2.57945	0.17291
Southern	0.013545	0.002839	0.002847	0.20067	-0.07232	0.12836
Hybrid	-0.008360	0.069450	0.013081	2.75235	-2.57945	0.17291
Hatchery	-0.026087	0.001550	-0.021739	0.15634	-0.10535	0.05099
Naturalized	-0.016562	0.001994	-0.019177	0.18868	-0.11932	0.06936
Northern	0.035275	0.003124	0.024714	0.21646	-0.05647	0.15999
All northern	0.001980	0.003103	-0.000825	0.27930	-0.11932	0.15999

Residual DV

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.0	.0077593	-0.011556	0.52716	-0.25670	0.27046
Southern	-0.046454	.0049723	-0.049976	0.30175	-0.19470	0.10705
Hybrid	0.001119	.0078761	-0.001023	0.48763	-0.25670	0.23093
Hatchery	0.073178	.0063976	0.075830	0.31172	-0.04126	0.27046
Naturalized	0.014756	.0057079	0.001998	0.27716	-0.12698	0.15017
Northern	-0.011971	.0062007	-0.028947	0.31897	-0.13380	0.18516
All northern	0.024878	.0075037	0.008911	0.40426	-0.13380	0.27046

Residual AC

Type	Mean	Variance	Median	Range	Minimum	Maximum
All	0.0	.0052610	.0027891	0.54035	-0.37171	0.16864
Southern	0.016170	0.003610	0.022875	0.22023	-0.11184	0.10839
Hybrid	-0.024145	0.005097	-0.031725	0.38486	-0.21622	0.16864
Hatchery	0.053230	0.001861	0.053069	0.14609	-0.02045	0.12564
Naturalized	-0.035718	0.011445	-0.035026	0.47523	-0.37171	0.10352
Northern	0.015181	0.003138	0.029542	0.25143	-0.13937	0.11206
All northern	0.018645	.0053156	0.030059	0.49735	-0.37171	0.12564

Appendix 4.3. Eigenvalues and cumulative proportion for the multivariate discrimination procedures.

Procedure (Reference matrix) Variable set	Associated eigenvalue Cumulative proportion 1	2	3	4
Canonical discriminant (INV(E)*H)				
all log ratios (LINEAGE)	1.196	0.312	0.148	0.045
	0.704	0.887	0.974	1.0
all residuals (LINEAGE)	0.692	0.264	0.148	0.031
	0.610	0.842	0.972	1.0
all angles (LINEAGE)	0.608	0.135	0.038	0.002
	0.776	0.949	0.998	1.0
all log ratios (TYPE)	0.301	0.247		
	0.549	1.0		
all residuals (TYPE)	0.254	0.140		
	0.645	1.0		
all angles (TYPE)	0.214	0.088		
	0.710	1.0		

Procedure (Reference matrix) Variable set	Associated eigenvalue Cumulative proportion 1	2	3	4	5	6
Principal components (total correlation)						
all log ratios	2.400	1.342	1.237	1.082	1.021	0.633
	0.267	0.416	0.553	0.673	0.787	0.857
all residuals	2.496	1.350	1.254	1.092	0.994	0.616
	0.277	0.427	0.567	0.688	0.798	0.867
all angles	3.546	2.460	1.398	1.220	0.265	0.111
	0.394	0.667	0.823	0.958	0.988	1.0

Appendix 4.3 (continued).

Procedure (Reference matrix) Variable set	Associated eigenvalue Cumulative proportion 1	2	3	4	5	6
Principal factors (Reduced correlation)						
all log ratios	1.779	0.594	0.502	0.252	0.106	-0.097
	0.738	0.984	1.192	1.297	1.341	1.301
all residuals	1.945	0.602	0.544	0.266	0.071	-0.061
	0.728	0.953	1.157	1.256	1.283	1.260
all angles	3.546	2.460	1.398	1.220	0.265	0.111
	0.394	0.667	0.823	0.958	0.988	1.0

Procedure (Reference matrix) Variable set	Associated eigenvalue Cumulative proportion 1	2	3	4	5	6
Sheared principal components (total covariance)						
morphometrics	0.130	0.006	0.003	0.002	0.001	0.001
	0.910	0.949	0.971	0.982	0.983	0.984

Appendix 4.4. Total canonical structure (canonical correlations) of brook trout morphometric variable sets.

	Variable	CAN1	CAN2	CAN3
All log ratios: (lineage)				
	log(HL/SL)	-0.700	-0.119	0.486
	log(NL/SL)	-0.333	0.284	0.630
	log(SV/SL)	-0.170	-0.218	0.065
	log(SD/SL)	-0.336	0.142	-0.152
	log(VA/SL)	0.216	-0.067	0.011
	log(VC/SL)	-0.250	0.243	-0.261
	log(DA/SL)	-0.048	0.170	0.096
	log(DV/SL)	0.571	-0.185	0.440
	log(AC/SL)	0.270	0.640	0.173

	Variable	CAN1	CAN2	CAN3
All residuals: (lineage)				
	RHL	0.638	-0.332	0.352
	RNL	0.640	-0.093	0.515
	RSV	0.029	-0.197	0.021
	RSD	0.356	0.071	-0.189
	RVA	-0.208	-0.023	0.037
	RVC	0.283	0.431	-0.202
	RDA	0.155	0.083	0.088
	RDV	-0.544	-0.132	0.504
	RAC	-0.175	0.749	0.409

	Variable	CAN1	CAN2
All angles: (lineage)			
	A1	-0.107	0.345
	A2	0.808	-0.096
	A3	-0.194	-0.354
	B1	0.731	0.061
	B2	0.130	0.091
	B3	-0.620	-0.111
	C1	-0.688	-0.364
	C2	0.610	0.515
	C3	0.717	0.057

Appendix 4.4 (continued).

All log ratios: (type)	Variable	CAN1	CAN2
	log(HL/SL)	-0.428	-0.075
	log(NL/SL)	0.257	-0.041
	log(SV/SL)	-0.226	-0.115
	log(SD/SL)	-0.169	0.329
	log(VA/SL)	0.078	-0.143
	log(VC/SL)	0.058	0.550
	log(DA/SL)	0.131	0.079
	log(DV/SL)	0.345	-0.630
	log(AC/SL)	0.544	0.221

All residuals: (type)	Variable	CAN1	CAN2
	RHL	-0.158	-0.325
	RNL	-0.001	0.115
	RSV	-0.150	-0.078
	RSD	0.282	-0.265
	RVA	-0.127	0.072
	RVC	0.558	0.030
	RDA	0.099	0.058
	RDV	-0.549	0.467
	RAC	0.345	0.664

All angles: (type)	Variable	CAN1	CAN2
	A1	0.036	0.347
	A2	0.738	-0.300
	A3	-0.330	-0.276
	B1	0.696	-0.180
	B2	-0.116	-0.031
	B3	-0.414	0.152
	C1	-0.784	-0.240
	C2	0.714	0.404
	C3	0.784	-0.069

Appendix 4.5. Principal components factor pattern for the brook trout morphometrics.

All log ratios:

Variable	Factor 1	Factor2	Factor3
log(HL/SL)	0.756	-0.011	-0.120
log(NL/SL)	0.666	-0.066	-0.085
log(SV/SL)	0.509	-0.145	0.191
log(SD/SL)	0.653	0.088	-0.093
log(VA/SL)	-0.011	0.623	0.032
log(VC/SL)	-0.028	0.230	-0.096
log(DA/SL)	-0.026	0.55	-0.017
log(DV/SL)	0.269	0.254	0.501
log(AC/SL)	-0.065	-0.228	0.417

All residuals:

Variable	Factor 1	Factor2	Factor3
RHL	0.794	0.005	-0.121
RNL	0.749	-0.120	-0.132
RSV	0.513	-0.113	0.256
RSD	0.637	0.099	-0.084
RVA	-0.011	0.617	-0.027
RVC	-0.035	0.243	-0.122
RDA	-0.018	0.037	-0.046
RDV	0.281	0.300	0.493
RAC	-0.063	-0.185	0.422

All angles:

Variable	Factor 1	Factor2	Factor3
A1	-0.277	-0.850	-0.322
A2	0.725	0.384	0.260
A3	0.031	0.815	0.263
B1	0.741	0.233	0.143
B2	0.192	0.333	-0.888
B3	-0.673	-0.414	0.554
C1	-0.828	0.450	-0.046
C2	0.785	-0.389	-0.045
C3	0.777	-0.484	0.192

Appendix 4.6. Eigenvectors (factor pattern) of the total covariance matrix of raw morphometrics by sheared principal components analysis.

Variable	PC1	PC2	PC3	PC4
SL	0.306	0.056	0.155	0.033
HL	0.293	0.137	-0.181	-0.030
NL	0.362	0.294	-0.791	0.058
SV	0.300	0.107	0.069	-0.080
SD	0.306	0.110	0.021	0.025
VA	0.311	0.061	0.232	0.039
VC	0.298	0.043	0.274	0.757
DA	0.342	-0.921	-0.183	-0.027
DV	0.332	0.100	0.251	-0.639
AC	0.307	0.066	0.296	-0.070

VITAE

Stanley Zane Guffey was born in Colorado Springs, Colorado. After brief sojourns in Virginia and Japan, he mostly grew up in Texas and Tennessee. He earned a Bachelor of Arts degree with high honors from the University of Tennessee, with a concentration in Anthropology, and minors in Religious Studies, Asian Studies, and Biology. His original intent was to study biochemistry and what has come to be called molecular biology, but the diversity available at the university was too much for him. As an undergraduate he supported himself and paid his tuition working as an archaeology field hand and as a carpenter. After obtaining his degree he continued working as a carpenter independently and with the University of Tennessee Biology Service Facility. He also dabbled in graduate studies in ecology and did credible research for the Tennessee Heritage Program on the ecology and demography of ginseng. Growing weary of his position with the university, he left that employ and walked from Georgia to Maine on the Appalachian Trail. Afterwards, when his money ran out, he resumed work as an independent carpenter. On a trip to Texas with his friend Dr. Gary McCracken a plan was hatched for him to become a doctoral student of Dr. McCracken's. One result of that plan is his dissertation and degree. Another result was his discovery of a delight in teaching. During his overly long graduate tenure he served as a graduate teaching assistant in a wide variety of biology courses; he also had the opportunity to teach introductory biology for non-biology majors, and to develop and teach an upper level conservation biology course. Additionally, he taught in the University of Tennessee Math and Science Regional Center and the University of Tennessee Academy for Teachers of Science and

Math. He was also active in about any non-paying university service activity that he could find, working with the Great Smoky Mountains Wildflower Pilgrimage, the Tennessee Science Olympiad, and judging science fair and undergraduate research events. Most recently he was a founding participant in the University of Tennessee Darwin Day Celebration. After graduation he plans to carry on as usual with his teaching, research, environmental activism, and carpentry. His research interests include biodiversity preservation, environmental history, Southern Appalachian biogeography, speciation, and the history, philosophy, and social context of science.