

FY2005 NRPP Research

Title: Identification and Adaptive Management of Genetic Diversity among Brook Trout Populations Inhabiting Great Smokey Mountains National Park

JUSTIFICATION AND BENEFITS:

Obtaining and using genetics should be a part of broad-based conservation strategies for fish management in the parks. Even though this study focuses on NPS land, the results will provide a range-wide perspective of brook trout genetic resources. This study will describe the amount and geographic distribution of genetic variation and what remains of native genetic diversity in Great Smokey Mountains National Park (GRSM). These data will augment monitoring programs or establish baselines for genetic conservation to provide for long-term, evolutionary adaptability of populations.

NRPP RESEARCH PROPOSAL

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Funding Status:

Funded:

Unfunded:

RMP Project Numbers: See Cover Page

Abstract:

Native brook trout (*Salvelinus fontinalis*) populations occur in several national parks in the U.S., including ACAD, DEWA, CATO, SHEN, GRSM, and ISRO. This salmonid species inhabits pristine stream environments, and its presence or absence is often perceived as an indicator of ecosystem integrity. Heritable genetic information offers an objective means of depicting management units and provides an evolutionary framework from which to develop and evaluate conservation priorities. Two broad objectives will be addressed by this study. We propose to complete a park-wide baseline study of genetic variation to determine the structure of brook trout populations within the park and to augment allozyme and microsatellite DNA data generated by previous researchers. The second objective will utilize this most fundamental form of biodiversity to explore the adaptability of genetic distinct populations in two separate studies. First, we will compare the reproductive success (i.e., adaptability) of multiple, genetically distinct source populations used for restoration of extirpated populations. The second study will assess the degree of persistent introgression from northern brook trout genes into southern Appalachian brook trout as a direct result of previous stockings. The results of all three studies will be instrumental to sustaining appropriate genetic resources in future brook trout supplementation and restoration programs.

Problem Statement:

Brook trout (*Salvelinus fontinalis*) is the only trout native to the Southern Appalachians. Since the turn of the century, this native trout has lost approximately 75 percent of its range in Great Smoky Mountains National Park (GRSM) (Kelly et al. 1980). Initial range loss (about 50 percent) has been attributed to logging and resultant water quality degradation (King 1937). This activity virtually eliminated brook trout in streams below about 914 m (3,000 ft) in elevation. In turn, residents and loggers became concerned because they had nothing for which to fish. To meet the demand for recreational angling at the time (around 1910), logging companies began stocking both non-native rainbow trout and northern brook trout and continued this activity until the Park was established in 1934. The Park continued to allow the stocking of both species until 1974.

Park staff in the 1930s and 1940s saw no harm in stocking rainbows and believed that as reforestation occurred, brook trout would reclaim lost range (King, personal communication). However, distribution surveys in the 1970s showed this not to be true and that 45 percent of the range exclusively occupied by brook trout had been lost since the mid-1930s (Kelly et al. 1980). The decline in allopatric brook trout range was the direct result of rainbow trout encroachment into previously unstocked brook trout streams (Larson and Moore 1985). Native brook trout had

become restricted to marginal headwater streams above 1,067 m (3,500 ft), characterized by steep gradients and pH that is naturally slightly acidic. Based on the report by Kelly et al. (1980) it was determined that the only places brook trout could not be displaced are in streams above waterfalls where rainbows could not ascend.

Historically, local residents were very vocal about introduced northern strains of brook trout being different from the native brook trout or "speckled trout." Studies in the 1950s showed that physical differences do exist between Southern Appalachian brook trout and hatchery fish. In 1993, a study conducted by the University of Tennessee provided conclusive evidence that Southern Appalachian brook trout are genetically distinct at the subspecies level from northern populations (McCracken et al. 1993). This effort collected brook trout from 47 streams across the Park and demonstrated that brook trout in 36 (64%) were pure Southern Appalachian brook trout. Fish from two streams were northern brook trout and the others were hybrids. The report recommends that everything possible be done to protect Southern Appalachian brook trout.

Parkwide water quality monitoring was initiated in 1992 as part of the Inventory and Monitoring program. Data from this program clearly show that stream acidity increases with increasing elevation (Robinson et.al. 2001). Models developed by these authors indicate that if current trends for pH continue that in four years that streams above 1,067m (3,500 ft.) will have a pH of 6.0 and in 15 years the pH will be 5.5 this eliminating brook trout and other aquatic species from these stream segments. Brook trout distribution monitoring in the 1990's has documented headwater range loss in six streams that had brook trout in previous surveys. All losses have been in streams at or above 1,067m and field pH measurements indicate the streams are too acidic to support fish life. These data increase the urgency of restoring stream segments identified in the Park's Fishery Management Plan for this native trout as rapidly as possible to provide a long-term refuge for native brook trout.

GRSM is committed to developing systematic approaches to inventorying composition and monitoring functions and processes of park ecosystems. The historic use of hatchery-reared brook trout for supplemental stocking in almost every watershed of GRSM and the potential negative impacts to the overall health and long-term maintenance of populations resulting from this approach underscore the need to recognize the lineage of each population. Increased interest in restoring native southern Appalachian brook trout to selected streams in GRSM requires a better understanding of the distribution of native, introduced northern strain, and their hybrids. While genetic surveys have been conducted in selected streams within GRSM, numerous streams remain to be typed to major evolutionary lineage (i.e., southern Appalachian, northern). Knowledge from these surveys should be incorporated into all management efforts to identify unique genetic diversity and to prevent the potential deleterious effects associated with introduction and translocation of maladapted fish.

Ecological and evolutionary effects associated with introducing nonnative hatchery strains have been documented for other salmonid species (Ferguson 1990; Allendorf 1991; Evans and Willox 1991; Krueger and May 1991). Potential problems associated with the mixing of genetically divergent stocks of brook trout include the loss of local adaptations, disruption of locally-adapted gene combinations (i.e., outbreeding depression), and the spread of pathogens. Declines in numbers of breeding individuals in populations can result in reduced levels of genetic diversity among wild brook trout populations (e.g., through genetic drift). Moreover, moderate levels of genetic divergence have been documented at local and regional levels, which suggests that extirpation of local populations has likely led to the irretrievable loss of

evolutionarily significant lineages. Relatively large genetic differences can occur over short geographic distances among stream-dwelling brook trout populations (McCracken et al. 1993; Perkins et al. 1993); therefore, transplantations of fish may create undesirable effects.

Surveys of genetic variation among brook trout populations have focused on (1) nuclear DNA variation in the form of protein differences or polymorphisms (i.e., allozymes) and (2) cytoplasmic DNA sequence variation in the rapidly evolving, maternally-inherited mitochondrial (mt) DNA. Allozyme studies have demonstrated that some genetic variation still exists among extant inland brook trout populations (Perkins et al. 1993; Kriegler et al. 1995) and that barriers to gene flow primarily existed among brook trout originating in distinct regional river systems (Stoneking et al. 1981; McCracken et al. 1993). Comparisons of mtDNA diversity between brook trout from putative refugial and recolonization zones indicated that large phylogeographic differences were found between northern and southern populations, that collections outside the zone of glaciation were the most genetically heterogeneous, and that multiple refugia contributed to northern recolonization after glacial retreat (Danzmann et al. 1998). [\[Stan: Insert a couple of sentences summarizing the allozyme results from Guffey et al. for GRSM. Perhaps a sentence summarizing the results from Danzmann et al. 1998\]](#)

Allozyme and mtDNA techniques have identified significant, genetic differentiation on a regional scale among brook trout; however, neither technique has detected sufficient levels of polymorphism to allow fine-scale resolution of local sub-population or metapopulation structure (i.e., isolation or high levels of gene flow). Recent studies have shown that microsatellite DNA variation affords the highest resolution of population structure of any technique brought to bear on fish species to date and suggest that in many instances the population unit functions at a finer-scale than the river (Beacham and Dempson 1998; Garant et al. 2000; Spidle et al. In Review). Following the discovery of microsatellite DNA markers over a decade ago (Tautz 1989), thousands of these loci have been described in eukaryotic organisms, including brook trout (Angers et al. 1995; two viable markers). Microsatellite DNA assays permit determination of the magnitude of genetic variation and diversity at the individual and population levels from the perspective of a more highly mutable set of markers. Due to the tendency for hypervariability and because only small amounts of tissue (often taken nondestructively) are required for genotyping, microsatellite DNA markers have supplanted allozymes in recent years as the genetic markers of choice for many management and biological problems including genetic stock identification, parentage assignment, forensics, and genome mapping (Schlötterer and Pemberton 1994; Jarne and Lagoda 1996, Goldstein and Schlötterer 1999).

The objectives of the proposed study are to: (1) extensively survey microsatellite DNA diversity among selected brook trout populations inhabiting Great Smokey Mountains National Park not surveyed in previous studies (see Table 1) to identify the nature of the population structure found among these streams and compare this structure with that observed throughout the species' range. (2) The second objective will utilize this most fundamental form of biodiversity to explore the adaptability of genetic distinct populations in two separate studies. First, we will compare the reproductive success (i.e., adaptability) of multiple, genetically distinct source populations used for restoration of extirpated populations. The second study will assess the degree of persistent introgression from northern brook trout genes into southern Appalachian brook trout as a direct result of previous stockings. The results of all three studies will be instrumental to sustaining appropriate genetic resources in future brook trout supplementation and restoration programs.

Recommended Project or Activity:

Scientists from GRSM, University of Tennessee (UT), and USGS-Leetown Science Center (LSC) have collaborated to develop this project. GRSM recommends this research to achieve the goal of obtaining the most detailed look available at the ecological and evolutionary relationships among brook trout populations and to gain insight into future management strategies to assist in maximizing the success of supplementation and restoration programs.. Specific research objectives are to: (1) identify previously undetected structure among wild brook trout populations in each of targeted national parks using genetic variation identified from novel microsatellite DNA markers, (2) compare the nature and the magnitude of the genetic variation observed within and among these parks with variation observed throughout the species range, and (3) provide park resource managers with long-term guidance in management of the genetic resources identified. This is anticipated to include explaining the implications of appropriate genetic resources for supplementation and restorative stockings.

Personnel:

USGS-BRD scientists at the LSC involved in planning the proposed research project include three Population Geneticists (two permanent-BRD; one contract post-doctoral fellow-Johnson Controls World Services). Stan Guffey and an unnamed graduate student, University of Tennessee, Knoxville, will participate in all aspects of this research. Allied, they possess considerable experience characterizing salmonid populations using molecular genetic techniques. The genetics component of the LSC-Aquatic Ecology Branch (AEB) will contribute a fully equipped, state-of-the-science molecular genetics facility with two Applied Biosystems automated DNA sequencers (two ABI-3100 Genetic Analyzers; Applied Biosystems) to conduct microsatellite analysis and the computer hardware and software as well as the expertise for appropriate statistical treatment of data.

Tissue Sampling:

Sample collections will be performed by NPS personnel with assistance from UT and USGS-BRD personnel. A collection will consist of 30 to 50 individual brook trout sampled (via adipose fin clipping) from each collection site. BRD personnel at LSC will provide training to all park staff in techniques for collecting and preserving the tissue samples. General information regarding sample collection sites from the park includes: [Insert Table]

Molecular Methods:

UT and LSC staff will extract and quantify DNA from the fin tissue of each animal collected. Genomic DNA will be extracted from fin or other tissue using the Puregene DNA extraction kit (Gentra Systems, Inc., Minneapolis, MN) and resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). DNA concentrations will be determined by fluorescence assay and integrity of the DNA will be visually inspected in 1% agarose gels.

A suite of 13 species-specific microsatellite DNA markers developed by King et al. (In Review) will be amplified using PCR. Amplification reactions will consist of 100-200 ng genomic DNA, 1X PCR buffer (10mM Tris-HCl, pH 8.3, 50 mM KCl), 2 mM MgCl₂, 0.25 mM dNTPs, 0.5 µM forward and reverse primer, and 1.0 U AmpliTaq® DNA polymerase in a total volume of 10-20

μl. The forward primer will be 5' modified with TET (or NED), FAM, or HEX fluorescent labels. Amplifications will be carried out on one of several PTC-200 Thermal Cycler under the following conditions: initial denaturing at 94 °C for 2 min; 35 cycles of 94 °C denaturing for 30 sec, 50-56 °C annealing (Table 1) for 30 sec, 72 °C extension for 1 min; and a final extension at 72 °C for 10 min. Amplified, labeled products will be subjected to capillary electrophoresis on an ABI Prism™ 3100 Genetic Analyzer. Genescan™ 2.1 Analysis software and Genotyper™ 2.0 Fragment Analysis software will be used to score, bin, and output allelic (and genotypic) data (King et al. 2001).

Statistical Analyses:

T. King (LSC), S. Guffey (UT), and an unnamed student (UT) will jointly perform all data analyses and prepare draft interim (yearly or upon request) and peer-reviewed final reports, summarizing all the findings by _____. Estimates of individual pair-wise genetic distances, using the proportion of shared alleles algorithm, will be calculated using a 32-bit version of Microsat 1.5d (Eric Minch, Stanford University). GENEPOP (v. 3.1) (Raymond and Rousset 1995) will be used to test for conformation to Hardy-Weinberg expectations, genotypic linkage disequilibrium, population differentiation, and to calculate F_{ST} statistics. All probability values will be adjusted for multiple simultaneous table-wide tests using the sequential Bonferroni technique to minimize type-I statistical inference errors (Rice 1989). Arlequin version 2.0 will be used to quantify genetic differentiation among vernal pools within national parks, between national parks, and range-wide by analysis of variance using the AMOVA option (Excoffier et al. 1992). Variance components at three hierarchical levels will be estimated and tested for significance. F statistics will be used to compare genetic structure within and among populations. This analysis will yield information on the number of effective migrants among vernal pools within national parks per generation ($N_e m$). The genetic relatedness among collections will be illustrated with a phenogram generated from the genetic distance matrix using the neighbor-joining (N-J) algorithm. The N-J phenogram with bootstrap estimates (as percentage of 10,000 replications) will be obtained by resampling loci within samples using NJBPOP (JM Cornuet, INRA, Bures-sur-Yvette, France). The significance of the relationship between the genetic distance and geographic distance matrices will be determined from a Mantel (1967) test, as implemented by NTSYS-PC 1.8 (Rohlf 1993). An assignment test (Paetkau et al. 1997) will be used to determine how well each individual's unique multilocus genotype represents the collection from which it came. Genotype likelihood ratio distances (D_{LR}) will be estimated (Paetkau et al. 1997). Observed numbers of correct assignments will be tested for significance from prior probabilities by Fisher's Exact Test (Fisher 1934) as implemented by SYSTAT 8.0 for Windows (SPSS, Chicago, IL).

Additionally, genetic consulting and analysis services will be available throughout the study to the park conducting stocking or translocations. These services could include genotyping of all broodfish used in a stocking program to maximize genetic diversity, minimize inbreeding, or to allow direct assessment of the supplementation or restoration effort through genetic marking.

Schedule:

Year 1: Identification of streams in GRSM to be sampled for brook trout populations, begin non-invasive (adipose-fine) tissue collections, provide training for park staff in collection and preservation of tissue samples, perform DNA extractions, PCR, and begin genotyping brook trout at 13 microsatellite DNA loci. Provide a status report to each participating park at the end of each fiscal year.

Year 2: Continue and complete tissue sampling, complete DNA extractions, and continue PCR and genotyping brook trout at selected microsatellite DNA loci. Provide a status report to each participating park.

Year 3: Complete genotyping of brook trout at microsatellite DNA loci, complete data analysis, submit final report, and present pertinent findings and management implications to each participating park.

Budget:

Activity	Year 1 FY2002		Year 2 FY2003		Year 3 FY2004	
	NRPP	BRD In Kind	NRPP	BRD In Kind	NRPP	BRD In Kind
Salaries -						
T. King Geneticist (PI-LSC) 20%	0.0	0.0	0.0	0.0	0.0	0.0
M. Curtis Geneticist (PI-GLSC) 20%	0.0	0.0	0.0	0.0	0.0	0.0
A. Spidle, Post-Doc (Co-PI-LSC) 25%	0.0	0.0	0.0	0.0	0.0	0.0
S. Julian, Biol. Tech. (LSC) 60%	0.0	0.0	0.0	0.0	0.0	0.0
TBA, Biol. Tech. (GLSC) 60%	0.0	0.0	0.0	0.0	0.0	0.0
Expendables/Supplies -						
DNA extract., PCR, LSC	0.0	0.0	0.0	0.0	0.0	0.0
& fragment analysis GLSC	0.0	0.0	0.0	0.0	0.0	0.0
Travel -						
LSC	0.0	0.0	0.0	0.0	0.0	0.0
GLSC	0.0	0.0	0.0	0.0	0.0	0.0
Equipment - LSC will contribute all required equipment	0.0	0.0	0.0	0.0	0.0	0.0
Totals (\$)	00.0	0.00	0.00	00.0	00.0	00.0

Total amount requested from NRPP: **\$000.0**

Subtotal: Contributed in-kind – BRD (PI salaries): \$106.2

Subtotal: Contributed in-kind – NPS (staff salaries, support): \$ 14.0

GRSM	biologist	\$ 5.0
GRSM	1-6 staff for 60 hrs	1.2
GRSM	biologist for 1 week	3.0
UT	Professor for 1 week	1.5
UT	MS student for 2 weeks	2.3
	biotech for 2 weeks	1.0

Total amount In-Kind: \$120.2

Total Project Cost: \$380.1

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RANKING CRITERIA

1. Significance of the Resource or Issue to the Park

The brook trout is the only native salmonid in GRSM. GRSM emphasized that genetic diversity is the fundamental component of biodiversity, as is true in all parks. This project is among the top ranked resource management needs at GRSM (?). Essential genetic baseline data for the management for GRSM populations will be established by this project. Restoration and enhancement of native species requires understanding the partitioning of genetic variation among different drainages and regions. This research will shed light on the differential adaptability of genetically distinct fish and will allow park personnel to evaluate the level of introgression of non-native genes into the native southern Appalachian brook trout

2. Severity of Resource Threat, Problem, or Need

Previous work showing genetically structured populations indicates that extirpation of local populations has likely led to the irretrievable loss of evolutionarily significant brook trout lineages. The increasing acidification of headwater streams threatens the persistence of brook trout populations in GRSM and other parks. Defining geographic variation of genetic diversity is needed to alert management of priorities in the face of extensive resource degradation. Microsatellites can ascertain whether areas hold genetically unique material or overlap other regions having more common alleles. It is important that GRSM address management questions by defining the genetic relationships of populations within and among different streams and answer whether most of the genetic diversity is distributed between or within populations.

3. Problem Definition and Information Base

It is well known that native populations are spatially structured into local breeding assemblages and effective migration among these subpopulations affects local population dynamics, including population reestablishment following extirpation. Gene flow is the integrative element in maintaining the ecological and evolutionary integrity of metapopulation dynamics. Effective conservation programs require clearly definable units of management. Therefore, heritable genetic information offers an objective means of depicting management units and providing an evolutionary framework from which to develop and evaluate conservation priorities. Allozyme and mtDNA techniques have identified significant genetic differentiation on a regional scale among brook trout; however, neither technique has detected sufficient polymorphism to allow fine-scale resolution of local sub-population or metapopulation structure. Microsatellite DNA assays will provide a clear determination of the magnitude of genetic variation and diversity at the individual and population levels from the perspective of a more highly mutable set of markers. This study will identify unique genetic differences that can be monitored to prevent deleterious effects, including those associated with introductions and translocation programs.

4. Feasibility

The specific study objectives are straightforward. The researchers are highly experienced in using molecular genetic techniques to characterize salmonid populations. The genetics component of the LSC-Aquatic Ecology Branch (AEB) is a fully equipped, molecular genetics facility capable of conducting large-scale microsatellite DNA analyses. The Principal Investigators have developed a suite of polymorphic microsatellite DNA markers from brook

trout and these markers have been screened in over 3000 fish collected from throughout the species' range. The PI's have the necessary hardware, software, and extensive expertise to conduct the appropriate statistical treatment of data. The statistical analyses are well established. Given the ample availability of markers and the available team of technicians experienced in high-throughput genotyping, this project should proceed smoothly once samples are collected and follow the time line to project completion. Whenever possible, sample collection in the park will be arranged to coincide with routine sampling efforts.

5. Problem resolution

This study will describe the biodiversity of GRSM brook trout populations at their most basic genetic level (i.e., unique multilocus genotypes). Quantification of subpopulation structure will guide management decisions to protect important source populations in each park and will identify important isolated regions of brook trout biodiversity for conservation. Quantification of metapopulation structure will also reveal the presence of barriers to gene flow, which might otherwise go undetected. The technology will also be available to evaluate and ultimately determine the success of supplemental stocking and translocation programs should the park choose to use the multilocus genotypes as genetic markers.

6. Transferability

The park-wide assessment proposed here is needed to address the increasing concern over the status of brook trout in National Parks and throughout their range in North America. The proposed project will contribute directly to advancement of science-based brook trout management at the state and national levels. Data on metapopulation structure from specific parks may be used with a GIS to model brook trout population structure in other parks and other portions of the species' range. The results of this study are likely to have indirect, yet profound, effects on the management of other organisms inhabiting similar niches in the aquatic ecosystems. All DNA from this study will be preserved and made available for use by other researchers using other DNA-based procedures. Following the final report, results will be published in peer-reviewed journals, and presented at professional and public meetings by the PI's as appropriate. Data suitable for dissemination as metadata will be prepared in compliance with the FGDC standards for DG Metadata and the NBIIB profile.

7. Cost Effectiveness

This study will require intensive laboratory analyses to produce multilocus genotypes for each fish sampled. The experience of the USGS-BRD LSC staff in conducting similar microsatellite DNA surveys for Atlantic salmon, Atlantic sturgeon, and brook trout over the past few years provides a solid basis for efficient operation and accurate budget estimates. All DNA from this study will be archived and made available for use by other researchers using other DNA-based procedures; this will provide extensive cost savings for future studies. The greatest expense in this type of analysis is the development of polymorphic markers. Since this phase has already been funded and completed by USGS-BRD, this project will be relatively inexpensive considering the depth and extent of data generated. Considerable savings also occur because reagents are already in hand sufficient for the analysis of several samples.

8. Project Support

More than 30% of the project costs will be contributed from the USGS staff, NPS project support, and UT staff. It is quite likely that other interested parties (e.g., States of TN and NC,

Trout Unlimited) will provide field support in addition to what is specified in the budget. Most of the funding requested is to provide graduate student salaries. The remaining funding will cover expendable supplies and travel costs over the three (?) years of the project. Three population geneticists from the LSC and UT are involved in planning the proposed research, and collectively, they possess considerable experience characterizing salmonid populations using molecular genetic techniques. The Aquatic Ecology Branch-LSC will engage a fully equipped molecular genetics facility with two Applied Biosystems automated DNA sequencers to conduct microsatellite analysis. The computer hardware and software as well as the expertise for appropriate statistical treatment of data are readily available at the Center.