Chapter 3

Genetic Characteristics of Southern and Northern Brook Trout (*Salvelinus fontinalis*) Populations at the Zone of Contact

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Additional information is available at the end of the chapter

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Abstract

Population genetic evidence suggests differentiation among evolutionarily significant units of southern and northern Appalachian brook trout, with the zone of contact in southwestern Virginia. Before this differentiation was recognized, brook trout of northern origin were stocked throughout the southeastern United States. In order to determine this differentiation, established allozyme markers were used to classify 56 southwest Virginia populations as southern, northern, or introgressed. Variation at 4 polymorphic loci, including the diagnostic creatine kinase (*CK-A2**) locus, indicated that 19 populations were of southern origin, 5 of northern origin, and 32 of mixed genetic origin. Data compiled among genetic studies of brook trout in the southern Appalachians showed that the southern/northern break is sharp, occurring at the New/Roanoke-James watershed divide. New River drainage populations exhibited the southern allele at high frequency, suggesting their historic native character as southern, with presence of northern alleles due to stocking or stream capture events. In conclusion, the present study suggests that management of southern Appalachian brook trout should include: (1) genetically cognizant planning of stocking events, (2) management of populations on a stream-by-stream basis, (3) prioritized conservation of pure southern brook trout populations, and (4) use of southern Appalachian hatchery stocks in restoration efforts.

Keywords: southern Appalachian brook trout, conservation, population genetics, trout management, restoration

1. Introduction

Brook trout, *Salvelinus fontinalis*, is the only salmonid native to the southern Appalachian Mountains, and it is distributed across eastern North America from Canada to Georgia [1].

This species was once abundant in coldwater lakes and streams throughout its range, but environmental disturbances such as deforestation, development, and pollution: and the introduction of non-native rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) have drastically reduced the number and sizes of wild populations [2].

Beginning in the mid-1800s, fishery managers began stocking hatchery-reared brook trout extensively. However, hatchery-reared brook trout often exhibit lower growth, yield, survival, and natural reproduction than locally adapted wild populations [3, 4]. Further, the hybridization of hatchery-derived fish with wild populations can compromise the genetic integrity and fitness of receiving populations by introducing foreign alleles and breaking up locally adapted gene complexes [5, 6]. The stocking of northern-derived hatchery brook trout is of particular concern in the southern part of its range due to significant population genetic differentiation between southern and northern lineages of brook trout. Genetic differences between the two lineages may be large enough to justify distinction at the subspecies level [7, 8]. In addition, screening of allozyme [7–16], mitochondrial DNA [17–19], and microsatellite nuclear DNA [20, 21] markers has uncovered smaller scale genetic variation throughout the geographic range of brook trout. Differentiation at smaller geographic scales may reflect different colonization histories, as well as differential effects of selective and non-selective population genetic processes.

Native southern Appalachian brook trout (SABT) populations share several biological characteristics [22]. Food availability being a limiting factor in these systems, adult fish are typically small (<229 mm total length) and life span seldom exceeds 3 years [23, 24]. Native SABT and introduced northern-lineage brook trout differed in terms of survival in the laboratory and diet in a natural stream [25]. Comparison of external microbial assemblages suggested that SABT exhibit greater ability to inhibit microbial growth in their epidermal mucus than do northern brook trout of hatchery ancestry [26]. Demonstration that SABT are genetically distinct from northern-origin hatchery stocks led management agencies to assess the heritage of populations within their jurisdiction, for example, in the Great Smoky Mountains National Park [8, 13], Tennessee [11], North Carolina [12, 16, 27], and Georgia [10]. Molecular and adaptive differentiation may warrant management of brook trout populations or groups of populations as evolutionary significant units [28], although some of their population genetic differentiation may reflect stocking history.

The zone of contact between the southern and northern lineages of Appalachian brook trout is roughly at the New River watershed [14, 15, 29]. Against the background of decline of the southern form and history of stocking with non-native strains, genetic characterization of brook trout populations at the zone of contact is needed to support informed management decisions and conserve the native form of the species. The objective of this study was to use established allozyme markers to wild Appalachian brook trout populations at the zone of contact in southwest Virginia as southern or northern lineages or introgressed.

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2. Methods

2.1. Sampling

Seventy-eight historic wild brook trout streams from the New, James, Holston, and Yadkin river drainages [30] were sampled by backpack electrofishing. Brook trout tissue samples were collected from 916 individuals from 56 streams (**Table 1**). Sample sizes ranged from 8 to 26 individuals per stream. Fish were anesthetized, and two samples of dorsal muscle tissue (from fish greater than 120 mm TL) were collected non-lethally using an 18-gauge Monopty Biopsy Instrument (C.R. Bard, Inc., Covington, GA) and immediately placed on dry ice. Anesthetized fish were fully revived in fresh water prior to release. A limited number of fish of <120 mm total length were sacrificed to sample streams from which few adults were collected. Samples were stored at −80°C.

2.2. Protein analysis

Genetic analysis was performed using cellulose acetate gel electrophoresis to observe variability at nine loci encoding five polymorphic enzymes: creatine kinase (*CK-A2**), aspartate aminotransferase (*sAAT-1,2**), glycerol-3-phosphate dehydrogenase (*G3PDH**), glucose-6-phosphate isomerase (*GPI-A**, *GPI-B1,2**), and malate dehydrogenase (*sMDH-B1,2**). Muscle tissue was homogenized in 200 μl of 0.09 M tris-HCl (pH 8.0), and subjected to electrophoresis in tris-glycine buffer (pH 7.5 or 8.0) for 45 min, followed by staining for enzyme activity. Electrophoretic conditions and histochemical staining procedures were modified from those described by Hebert and Beaton [31] and Galbreath et al. [16]. Individuals from the Paint Bank Hatchery in Virginia were included in the analysis as a northern reference population because the hatchery is known to culture the northern lineage. The North Carolina Wildlife Resource Commission provided tissue samples from individuals from Charles Creek of the North Toe River drainage, a known SABT population, for use as a reference population.

2.3. Data analysis

Allele frequencies for *CK-A2**, *G3PDH**, *GPI-A**, and *MDH-B1,2** were calculated for all populations using the Excel Microsatellite Toolkit [32]. Allele frequencies could not be calculated for *sAAT-1,2** and *GPI-B1,2** using that program because both enzymes are encoded by isoloci (i.e., duplicated loci with alleles of overlapping mobility). Since genotypes among heterozygous individuals could not be determined with certainty for *sAAT-1,2**, phenotype frequencies were calculated using the program FDASH [33]. The *GPI-B1,2** isoloci contain multiple alleles that could not be assigned to either locus with confidence; hence, they were treated as a single tetraploid locus and allele frequencies were estimated using the program AUTOTET [34]. Initially, allele frequency data from all nine marker loci were used to calculate genetic distance, population differentiation, contingency-table analysis of heterogeneity among populations, and hierarchical cluster analysis using the program BIOSYS-1 [35]. The same statistics then were calculated using only the five marker loci with unambiguous interpretation of allelic expression (i.e., omitting data from *sAAT-1,2** and *GPI-B1,2**), to determine any effect of

		*CK-A2**		*G3PDH**		*GPI-A**			*MDH-B1,2**					
	N	****78***	****100***	****45***	****100***	****87***	****100***	****115***	****100***	****145***	***P***	***A***	***HO***	***HE***
Controls														
Charles Creek, NC	5		1.00		1.00		1.00		1.00					
Paint Bank Hatchery	16	1.00		0.44	0.56		1.00		1.00					
Holston River drainage														
Grassy Branch	12		1.00		1.00		1.00		1.00		0.00	1.0	0.000	0.000
Henshew Branch	20	1.00		0.45	0.55		1.00		1.00		0.25	1.3	0.125	0.127
Parks Creek	10	0.05	0.95		1.00		1.00		1.00		0.25	1.3	0.025	0.025
Pennington Branch	12	0.08	0.92		1.00		1.00		1.00		0.25	1.3	0.042	0.040
Roaring Fork	8	0.56	0.44		1.00	0.69	0.31		1.00		0.50	1.5	0.188	0.246
Sturgill Branch	16	0.19	0.81		1.00		1.00		0.75	0.25	0.50	1.5	0.219	0.175
James River drainage														
Barbours Creek	20	1.00		0.08	0.93		1.00		1.00		0.25	1.3	0.021	0.036
Ewins Run	20	1.00			1.00		1.00		1.00		0.00	1.0	0.000	0.000
Pickles Branch	20	1.00			1.00		1.00		1.00		0.00	1.0	0.000	0.000
New River drainage														
Bear Creek	23	0.02	0.98		1.00		1.00		1.00		0.25	1.3	0.016	0.016
Big Horse Creek	18		1.00		1.00		1.00		1.00		0.25	1.3	0.011	0.011
Big Laurel Creek	11	0.05	0.95	0.09	0.91		1.00		1.00		0.00	1.0	0.000	0.000
Big Reed Island Creek	20	0.08	0.93		1.00		1.00		0.95	0.05	0.50	1.5	0.068	0.066
Bournes Branch	16	0.03	0.97		1.00		1.00		1.00		0.50	1.5	0.063	0.061
Buffalo Branch	16	0.06	0.94		1.00		0.97	0.03	1.00		0.75	1.8	0.125	0.111
Cabin Creek	20	0.05	0.95		1.00		1.00		1.00		0.50	1.5	0.047	0.046
Chestnut Creek	17	0.12	0.88		1.00		1.00		1.00		0.25	1.3	0.000	0.024
Chisholm Creek	12		1.00		1.00		1.00		0.96	0.04	0.25	1.3	0.021	0.021
Crooked Creek	15		1.00		1.00		1.00		1.00		0.25	1.3	0.059	0.053
Ding Branch	26	0.25	0.75	0.02	0.98		1.00		0.94	0.06	0.00	1.0	0.000	0.000

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		*CK-A2**		*G3PDH**		*GPI-A**			*MDH-B1,2**					
	N	****78***	****100***	****45***	****100***	****87***	****100***	****115***	****100***	****145***	***P***	***A***	***HO***	***HE***
East Fork Cove Creek	14	0.11	0.89		1.00		0.93	0.07	1.00		0.75	1.8	0.145	0.133
East Fork Crooked Creek	20	0.03	0.98		1.00		0.98	0.02	1.00		0.50	1.5	0.089	0.084
East Fork Dry Run	20		1.00		1.00		1.00		1.00		0.50	1.5	0.025	0.025
East Fork Little Reed Island	10		1.00		1.00		1.00		1.00		0.00	1.0	0.000	0.000
Elkhorn Creek	10		1.00		1.00		1.00		0.95	0.05	0.25	1.3	0.125	0.097
Fox Creek	20	0.18	0.83		1.00		0.95	0.05	0.88	0.12	0.25	1.3	0.025	0.025
Grassy Creek	9		1.00		1.00		1.00		1.00		0.75	1.8	0.150	0.154
Howell Creek	20	0.05	0.95		1.00		1.00		0.98	0.02	0.00	1.0	0.000	0.000
Laurel Branch	22	0.23	0.77		1.00		1.00		0.98	0.02	0.50	1.5	0.038	0.037
Laurel Creek	10		1.00		1.00		1.00		1.00		0.00	1.0	0.000	0.000
Laurel Creek	20	0.10	0.90		1.00		0.98	0.02	1.00		0.50	1.5	0.063	0.059
Little Indian Creek	19	0.79	0.21		1.00		1.00		0.95	0.05	0.50	1.5	0.125	0.101
Little Snake Creek	8		1.00		1.00		1.00		1.00		0.50	1.5	0.132	0.111
Little Stony Creek	14	0.11	0.89		1.00		0.96	0.04	1.00		0.00	1.0	0.000	0.000
Little Wilson Creek	19	0.21	0.79	0.03	0.97		1.00		0.82	0.18	0.50	1.5	0.071	0.067
Middle Fox Creek	12	0.04	0.96		1.00	0.04	0.96		0.58	0.42	0.00	1.0	0.000	0.000
Mill Creek	17	0.12	0.88		1.00		1.00		0.82	0.18	0.75	1.8	0.184	0.176
NB Elk Creek	14	0.25	0.75		1.00		1.00		1.00		0.75	1.8	0.250	0.168
NF Stony Creek	21	0.02	0.98		1.00		0.98	0.02	1.00		0.50	1.5	0.147	0.128
No Business Creek	20	0.20	0.80	0.03	0.98		1.00		0.90	0.10	0.50	1.5	0.024	0.024
Oldfield Creek	12		1.00		1.00		1.00		1.00		0.75	1.8	0.163	0.141
Opossum Creek	17	0.03	0.97		1.00		1.00		0.72	0.28	0.00	1.0	0.000	0.000
Pearis Thompson Branch	17	1.00		0.15	0.85		1.00		0.91	0.09	0.50	1.5	0.155	0.119

		*CK-A2**		*G3PDH**		*GPI-A**			*MDH-B1,2**					
	N	****78***	****100***	****45***	****100***	****87***	****100***	****115***	****100***	****145***	***P***	***A***	***HO***	***HE***
Ripshin Creek	10	0.15	0.85		1.00		1.00		0.75	0.25	0.50	1.5	0.200	0.166
Roads Creek	11		1.00		1.00		0.95	0.05	1.00		0.25	1.3	0.023	0.023
Snake Creek	20		1.00		1.00		1.00		0.98	0.02	0.50	1.5	0.200	0.166
Standrock Branch	20		1.00		1.00		1.00		1.00		0.25	1.3	0.013	0.013
Stony Creek	20	0.18	0.83	0.03	0.98		1.00		0.95	0.05	0.25	1.3	0.100	0.111
Sulfur Springs Branch	10	0.30	0.70		1.00		1.00		1.00		0.00	1.0	0.000	0.000
Tory Creek	19		1.00		1.00		1.00		1.00		0.00	1.0	0.000	0.000
Upper West Fork Dry Run	10		1.00		1.00		1.00		1.00		0.00	1.0	0.000	0.000
West Fork Dry Run	19		1.00		1.00		1.00		1.00		0.25	1.3	0.063	0.057
Whitetop Creek	12	0.13	0.88		1.00		1.00		1.00		0.00	1.0	0.000	0.000
West Fork Furnace Creek	17	0.12	0.88		1.00		1.00		0.97	0.03	0.50	1.5	0.044	0.068
Yadkin River drainage														
Pauls Creek	20		1.00		1.00		1.00		1.00		0.00	1.0	0.000	0.000
South Fork Stewarts Creek	24		1.00		1.00		1.00		1.00		0.00	1.0	0.000	0.000

Charles Creek, a known southern-strain population, was included as a southern-strain reference group. Individuals from Paint Bank Hatchery, which cultures the northern strain, were included as a northern-strain reference group. Abbreviations: number of individuals analyzed (N), proportion of polymorphic loci (*P*), mean number of alleles per locus (*A*), expected heterozygosity (H_o), and observed heterozygosity (H_e).

Table 1. Allele frequencies and genetic diversity at four polymorphic loci (*CK-A2**, *G3PDH**, *GPI-A**, *sMDH-B1,2**) in wild brook trout populations in 56 southwest Virginia streams, grouped by drainage.

omitting these data from analysis. Similar conclusions were drawn from analysis of both data sets. Here, we report results based on analysis of the reduced dataset only.

Initial characterization of the genetic origin of each population was based on allele frequencies at the diagnostic *CK-A2** locus. Allele frequencies at the other markers were compared to those observed in northern and SABT populations characterized in previous studies [7–16]. Individual heterozygosity and polymorphism were calculated across five loci to assess levels of genetic diversity within each population [32]. Arlequin [36] was used to test for departures from Hardy-Weinberg equilibrium and to perform analysis of molecular variance (AMOVA) to characterize the distribution of the genetic diversity within and among populations and river basins. Cluster analysis using the unweighted pair-group with arithmetic averaging algorithm (UPGMA, [37]) was performed using BIOSYS-1 [35], and a dendrogram was built based on Nei's unbiased genetic distance [38].

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Allele frequency data from previous studies of brook trout population genetics were compiled and combined with the results from this study to gain a better understanding of the geographic distribution of SABT in Virginia, as well as the genetic composition of brook trout populations throughout the Appalachian portions of the native range.

3. Results

Of 56 wild brook trout populations from 4 major river drainages analyzed in this study, 19 were fixed for the diagnostic *CK-A2**100 allele, and were designated as pure SABT populations (**Table 1**). Five populations fixed for the *CK-A2**78 allele were designated as northern, and 32 populations exhibiting variation at the *CK-A2** locus were designated as introgressed. The three James watershed populations exhibited alleles characteristic of northern-form brook trout. Populations in other watersheds were characterized as southern ($n = 19$), northern ($n = 2$), or introgressed ($n = 32$).

Only the Cabin Creek population (New River drainage, Grayson County) deviated significantly ($p < 0.05$) from Hardy-Weinberg equilibrium at the *CK-A2** locus. No other deviations from Hardy-Weinberg equilibrium were detected, indicating that the respective populations were in reasonable conformance with assumptions underlying the model. The proportions of polymorphic loci (P), the mean number of alleles per locus (A), and mean heterozygosities (H) for each population are listed in **Table 1**. Observed mean P and H_0 values were lowest in the putative southern populations ($P = 0.05$, $H_0 = 0.004$; **Table 2**). The introgressed populations exhibited the highest means for metrics of genetic variability ($P = 0.48$, $H_0 = 0.099$), and the northern populations exhibited intermediate means ($P = 0.20$, $H_0 = 0.053$). Grouped by drainage, Yadkin River populations had the lowest means ($P = 0$, $H_0 = 0$), followed by James River

Group	*N*	*P*	*A*	*Ho*	*He*
Holston River drainage	6	0.29	1.3	0.100	0.102
James River drainage	3	0.08	1.1	0.007	0.012
New River drainage	45	0.34	1.4	0.064	0.058
Yadkin River drainage	2	0.00	1.0	0.000	0.000
Southern lineage	19	0.05	1.1	0.004	0.004
Northern lineage	5	0.20	1.2	0.053	0.036
Introgressed	32	0.48	1.5	0.099	0.091
Atlantic Ocean drainages	5	0.05	1.1	0.004	0.007
Gulf of Mexico drainages	51	0.33	1.4	0.068	0.063

Based on analysis at four polymorphic allozyme loci (*CK-A2*, G3PDH*, GPI-A*, sMDH-B1,2**). Abbreviations: number of populations per group (*N*), proportion of polymorphic loci (*P*), mean number of alleles per locus (*A*), expected heterozygosity (*Ho*), and observed heterozygosity (*He*).

Table 2. Genetic diversity of brook trout populations, variously grouped by drainage, lineage, and geographic location relative to the eastern continental divide.

(P = 0.08, H_0 = 0.007), New River (P = 0.34, H_0 = 0.064), and Holston River (P = 0.29, H_0 = 0.100) populations. Atlantic-slope populations exhibited lower mean percent polymorphic loci and heterozygosity values (P = 0.05, H_0 = 0.004) than Gulf of Mexico drainage populations (P = 0.33, H_0 = 0.068). Analysis of molecular variance showed that approximately 34% of the total genetic diversity resulted from variation within populations, 18% among populations within drainages, and 48% among drainages. Most of the total limiting variance was attributed to the *CK-A2** locus, meaning that most of the variance that we measured with allozyme markers was due to differentiation among northern and southern lineages of the species.

There was no apparent pattern regarding where populations characterized as southern, northern, or introgressed were located geographically within the New, Holston, Yadkin, and James drainages (**Figure 1**). Cluster analysis of unbiased genetic distances [38] among all populations showed that all populations of northern origin or with a high frequency of the *CK-A2**78 allele clustered together; these included populations from the James River drainage (Barbours Creek, Ewin Run, and Pickles Branch), the Holston drainage (Henshew Creek), the New River drainage (Pearis Thompson and Little Indian Creek), and Paint Bank Hatchery. The Roaring Fork population in the Holston drainage had a high frequency of the northern allele, but did not cluster closely with the other northern populations due to a high frequency of a rare allele at the *GPI-A** locus. Cluster analysis of unbiased genetic distances [38] among populations showed no geographic patterns of genetic variation among the populations of putative southern Appalachian origin.

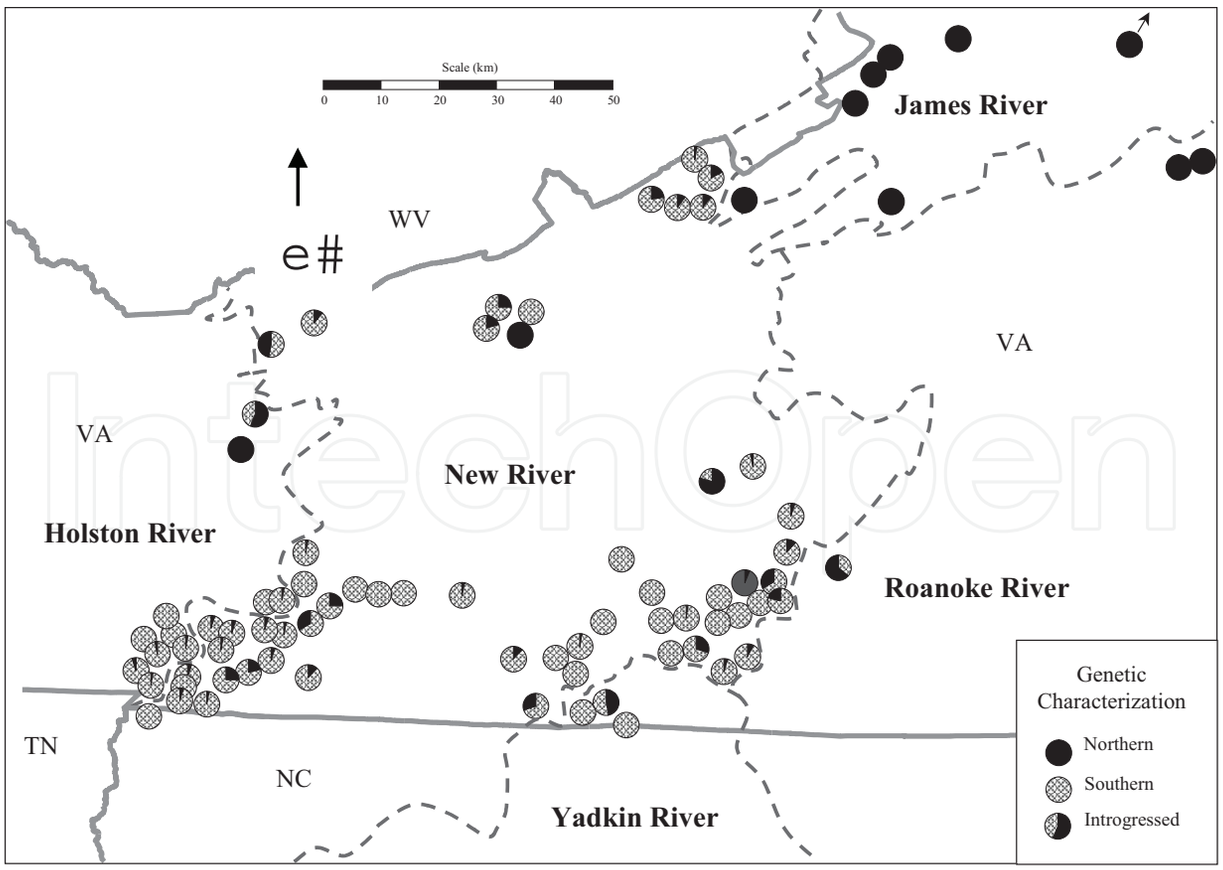

Figure 1. Genetic characterization at the *CK-A2** locus for 83 wild brook trout populations in southwest Virginia, including 56 populations characterized in this study and 27 populations characterized previously.

4. Discussion

4.1. Decline of brook trout

We sampled 78 streams that historically contained brook trout populations, but found the species in only 56 of them [30]. The range of brook trout is shrinking [39] for several reasons, including habitat alteration, overexploitation, competition with introduced rainbow trout (*O. mykiss*) and brown trout (*S. trutta*) and more recently, climate change.

4.2. Duplicated isozyme loci in brook trout

Certain allozyme markers posed complications to interpretation of underlying genotype. Brook trout show a high incidence of duplicated enzyme loci due to the tetraploid ancestry of salmonids [40]. Duplicated loci (termed isoloci) are genetically independent, but exhibit alleles of similar electrophoretic mobility that cannot be unambiguously assigned to either locus. Three of the five enzymes that we screened were encoded by isoloci (i.e., *MDH-B1,2**, *sAAT-1,2**, and *GPI-B1,2**). Ambiguous interpretation of the banding patterns of two of these isoloci, *sAAT-1,2** and *GPI-B1,2**, led us to eliminate them from statistical analysis [30]. Precise estimation of genetic diversity and differentiation metrics require data from many loci [41, 42]. Information from only four markers clearly limited the power of statistical analysis of genetic differentiation, especially with small sample sizes for some of the populations [43]. Genotypic data from more markers likely would reveal genetic differentiation not detected with only four loci. Ongoing screening of additional, more highly polymorphic markers, such as microsatellite DNA markers, will increase the ability to quantify population genetic differentiation.

4.3. Geographic distribution of SABT in southwest Virginia

Based on fixation for the diagnostic allele at the *CK-A2** locus and allele frequency differences at three other marker loci, 34% (n = 19) of the brook trout populations analyzed in this study were of southern Appalachian origin, 9% (n = 5) were of northern origin, and 57% (n = 32) were of mixed genetic origin (**Tables 1** and **2**). The level of certainty for precise characterization of a population is directly related to sample size. That is, any population observed to be fixed for the common allele actually may harbor the alternate allele at a low, undetected frequency. For example, with a sample size (s) of 20, our likelihood (p) of detecting an allele with a frequency (p_a) of 5% is 36% (i.e., $p = (1-p_a)\, s = 0.9520$, [44]). Therefore, there is a non-zero likelihood that some populations characterized as "pure" southern Appalachian are of mixed genetic origin. Similarly, sample size also affects estimation of within-population diversity statistics such as P and H_0. Sampling of a limited number of populations in a watershed also would affect estimates of between-population genetic variability.

Of the six populations from the Holston drainage, four were of mixed genetic origin, with the southern allele at frequencies ranging from 0.44 to 0.95. The Grassy Branch population was characterized as southern Appalachian, and the Henshew Branch population was characterized as pure northern. Results from earlier genetic studies [8, 11, 14] and its geographic location suggest that the Holston River historically contained the southern Appalachian lineage, so the presence of the northern allele is likely due to stocking.

The Yadkin (upper Pee Dee) River is an Atlantic-slope watershed. Despite the common presumption that Atlantic-slope drainages would contain native northern-form brook trout [8, 12, 15], two pure southern Appalachian populations (Pauls Creek, South Fork Stewarts Creek) were found in the Yadkin drainage. Although no early sampling efforts are known from the upper Pee Dee in Virginia [45], the section of the river that flows through North Carolina was excluded from the range of brook trout originally described by Smith [46]. However, several stream capture events have been inferred in this region, suggesting that these populations are descendants of brook trout captured from the New River [45]. Inspection of stocking records showed that both Pauls Creek and South Fork Stewarts Creek were stocked in the recent past, implying that the "native" southern strain persisted despite stocking.

Earlier genetic study [14] and geographic location suggest that the James River historically contained northern-form brook trout. Three populations from the James River screened in this study were characterized as northern form. This finding leaves little doubt that the New River is the boundary between northern and southern Appalachian brook trout populations.

In this study, 16 populations from the New River drainage (36%) were characterized as southern Appalachian brook trout. No geographic patterns of genetic variation were observed among the populations of putative pure southern origin. Interestingly, two of these "pure southern" populations (Crooked Creek and West Fork Dry Run) were stocked in the recent past with northern-derived hatchery fish. Crooked Creek is a "put-and-take" fishing area, and 5000 brook trout are stocked annually, yet it maintained an apparently pure southern population. Sixty-three percent of the populations from the New River drainage were of mixed origin, with the southern allele at frequencies ranging from 0.21 to 0.98. Although stocking records are limited, only two of these (Howell Creek and Little Indian Creek) are known to have been stocked with northern-derived hatchery fish. Only one population (Pearis Thompson Branch) in the New River was characterized as pure northern.

In addition to the 56 populations characterized in this study, we compiled data from all known genetic studies of brook trout populations in southwest Virginia [12, 14, 15]. Forty-seven percent (n = 39) of all 83 populations characterized in southwest Virginia were of mixed genetic origin (**Table 3**); however, many of these introgressed populations were largely southern. In addition, the "pure" southern populations (n = 26) that remain provide opportunities for restoration of southern Appalachian brook trout in Virginia.

4.4. Range-wide geographic distribution and genetic affinity of New River brook trout populations

With the zone of contact between the northern and southern forms lying roughly at the New River watershed, it is unknown whether the New River historically contained the pure southern Appalachian form, or whether it was a zone of intergradation among southern and northern Appalachian lineages. Interpreting data across this study and the three studies noted above [12, 14, 15], the New River drainage contains 20 pure southern populations, suggesting that the presence of northern alleles could be due to either stocking or stream capture events. However, a large proportion (64%) of populations from the New

Stream	River drainage	County	*N*	% Southern allele	Source
Green Cove Creek	Holston	Washington	19	95	[15]
Grindstone Branch	Holston	Smyth	16	97	[14]
Houndshell Branch	Holston	Smyth	12	100	[14]
Jerry Creek	Holston	Smyth	11	100	[14]
Little Laurel Creek	Holston	Smyth	16	100	[14]
Johns Creek	James	Giles	23	0	[14]
Shawvers Run	James	Giles	23	0	[14]
Spy Run	James	Augusta	21	0	[14]
Valley Branch	James	Craig	15	0	[14]
Burks Fork	New	Floyd	15	67	[15]
Cox Branch	New	Tazewell	15	53	[15]
Dry Creek	New	Smyth	24	100	[14]
Hanks/EF Chestnut Creek	New	Grayson	10	70	[14]
Helton Creek	New	Grayson	21	79	[15]
Jerry Creek	New	Grayson	15	67	[15]
Killinger Creek	New	Smyth	12	88	[14]
Laurel Branch	New	Floyd	15	97	[14]
Laurel Fork	New	Floyd	7	79	[12]
Lewis Fork	New	Grayson	21	79	[15]
Middle Fork Helton	New	Grayson	20	100	[14]
NF Elk Creek	New	Grayson	19	100	[14]
NP Buckhorn Creek	New	Carroll	25	100	[14]
Wilburn Branch	New	Grayson	21	75	[15]
Big Stony Creek	Roanoke	Bedford	10	0	[12]
Little Stony Creek	Roanoke	Bedford	6	0	[12]
Rock Castle Creek	Roanoke	Patrick	25	36	[14]
Turkey Creek	Yadkin	Carroll	15	47	[15]

N = number of individuals per sample.

Table 3. Genetic characterization at the *CK-A2** locus for southwest Virginia brook trout populations not sampled in this study, compiled from both published and unpublished data sources.

River are of mixed genetic origin, suggesting either that hatchery fish persisted in the New watershed or that the New River is a zone of natural intergradation. To gain a better understanding of the geographic distribution of southern Appalachian brook trout, we compiled

allele frequency data from all known genetic studies of brook trout populations throughout the native range (**Table 4**). Frequencies of the *CK-A2**100 (i.e., southern) allele were weighted based on sample size and averaged across all populations in each river drainage. **Figure 2** shows the frequency of the southern allele in each of the major river drainages from which data were collected.

River drainage	State	Position[1]	# of streams	# of individuals	% Southern[2]	Source(s)
Susquehanna	PA/MD	East	4	145	0	[7, 9]
Ohio	MD	West	3	110	0	[9]
Gunpowder	MD	East	1	40	0	[9]
Patapsco	MD	East	1	40	0	[9]
Potomac	MD/VA	East	6	190	0	[9, 14]
James	VA	East	7	142	0	[14, current]
Rappahannock	VA	East	1	25	0	[14]
Roanoke	VA	East	3	41	22	[12, 14]
New	VA/NC	West	101	1999	85	[14, 15, current]
Yadkin	VA/NC	East	37	691	58	[8, 12, 15, current]
Holston	VA/TN	West	24	320	91	[8, 11, 14, current]
Nolichucky	NC/TN	West	51	1058	64	[7, 8, 11]
French Broad	NC/TN	West	80	1281	73	[8, 11, 16]
Little Tennessee	NC/TN	West	49	886	82	[8, 13]
Watauga	NC/TN	West	44	691	88	[8, 11]
Broad	NC	East	3	41	29	[8, 11]
Hiwassee	NC	West	6	146	76	[8, 11]
Cheoah	NC	West	10	210	80	[8, 11]
Little	TN	West	8	90	80	[8, 11]
Tellico	TN	West	5	64	42	[11]
Savannah	NC/GA	East	27	533	63	[10, 16]
Chattahoochee	GA	West	1	21	31	[10]
Tennessee	GA	West	7	145	93	[10]
Coosa	GA	West	1	12	100	[10]

[1]Relative to eastern continental divide.

[2]Allele frequency based on number of individuals analyzed per stream and averaged across all populations in each drainage.

Table 4. Genetic characterization of brook trout populations in regional river drainages, based on frequency of the diagnostic *CK-A2*100* allele using data gathered from all available published and unpublished studies.

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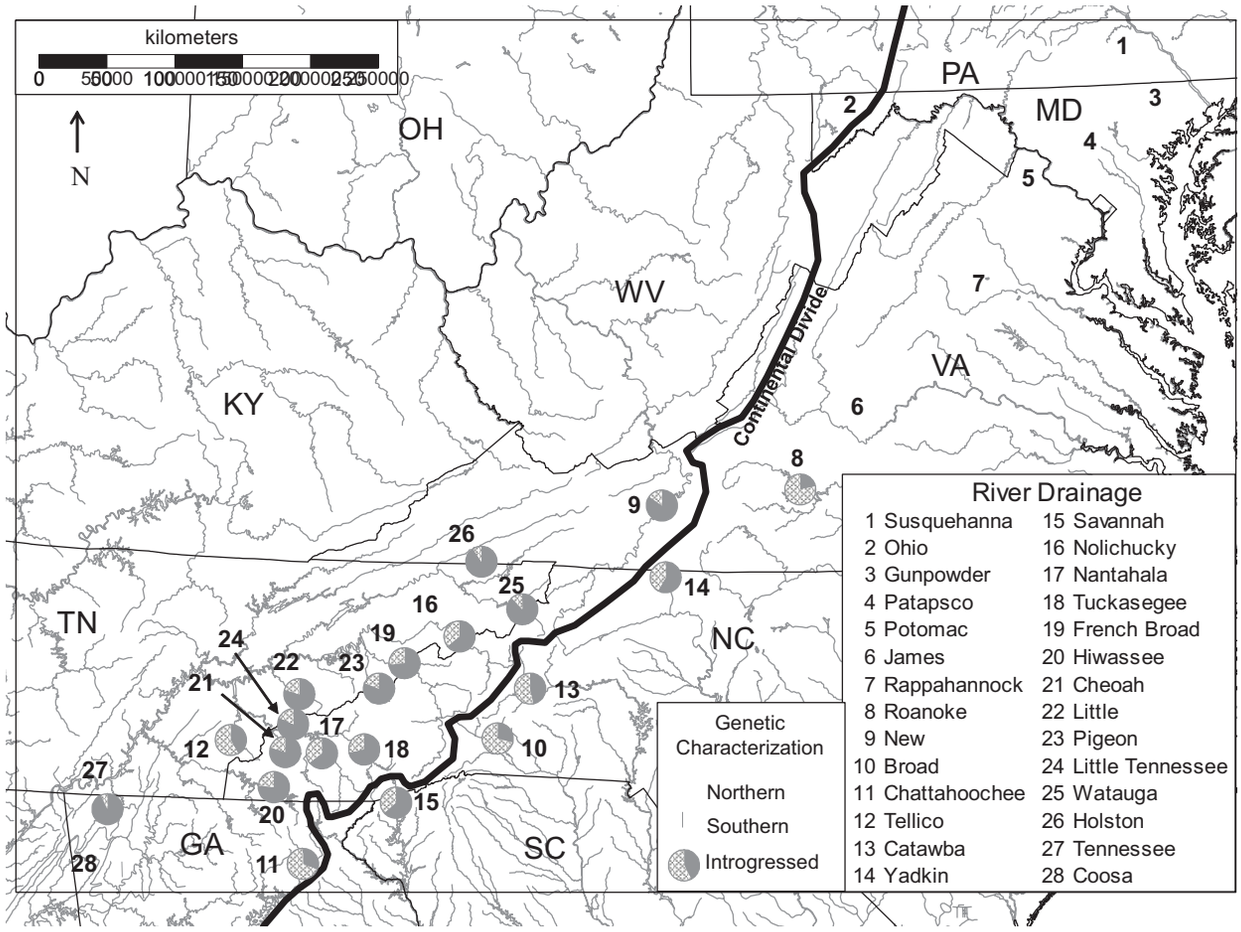

Figure 2. Genetic characterization of brook trout populations in major river drainages, based on the *CK-A2** locus, using data compiled from all known genetic studies of brook trout populations throughout the native range. See **Table 4** for details.

All river drainages north of the New River were characterized as pure northern, with the exception of the Roanoke River drainage that contained a single population with a low frequency of the southern allele, likely due to the transfer of individuals from another location or stream capture. The frequency of the southern allele in river drainages south of the New River ranges from 29% in the Broad River of North Carolina to 100% in the Coosa River of Georgia. Genetic characterization of individuals from 111 populations in the New River drainage showed an 85% frequency of the southern-form allele. **Figure 2** shows that the south/north break is sharp and that this break occurs at the New/Roanoke-James watershed divide. This weakens the hypothesis that the New River is a zone of natural intergradation between the southern and northern forms of brook trout, and supports the hypothesis that the presence of northern alleles is due to either stocking or stream capture. However, it is important to qualify this inference by noting that genetic characterization is based on variation at a single locus. Ongoing screening of New River populations using microsatellite DNA markers will provide further insights into patterns of population genetic differentiation, shedding light on the native character of New River brook trout populations. In particular, microsatellite variation may clarify whether northern alleles observed in populations examined are characteristic of particular hatchery stocks or of native regional variation.

4.5. Management implications

Brook trout is the only salmonid native to the southern Appalachian region. The American Fisheries Society Southern Division Trout Committee developed a position statement [22] expressing the importance of SABT and presenting recommendations for conservation-oriented management of this regional resource. Our results contribute to the recommended completion of genetic inventory of critical populations using non-lethal sampling methods. In this context, we frame the management implications for management of SABT populations.

Results from this and other studies demonstrate that stocking of non-native genotypes poses long-term genetic impacts and interferes with efforts to conserve southern Appalachian brook trout. Although negative effects of stocking have become well known, some fisheries management agencies maintain imprecise stocking records. Further, hatchery personnel often substitute one brook trout stock for another based on availability. We recommend that all stocking and transfers of brook trout be well planned with cognizance of genetic conservation objectives and thoroughly and accurately documented.

Management units—that is, populations that are demographically independent of one another—may be defined functionally as populations that have substantially divergent allele frequencies at many loci [47]. We had but limited ability to estimate levels of genetic diversity and differentiation among regional brook trout populations using allozyme markers. The results of ongoing screening of microsatellite DNA markers will be used to quantify differentiation among native populations, providing the basis for defining defensible management units. Results to date support the view that southern Appalachian brook trout populations should be managed on a stream-by-stream basis.

Those populations characterized as pure SABT should be given conservation priority. The stocking and transfer of non-native genotypes into these populations should be prohibited. Harvest should be allowed only in those populations that are demographically able to sustain themselves. We recommend that introgressed populations that contain less than 5% admixture from northern-strain brook trout be treated as 'pure' southern. However, we caution that the level of introgression in these populations may be higher than allozyme frequencies suggest; hence, individuals from these streams should not be transferred into streams that contain pure SABT populations. Hatchery brook trout should be stocked only into those streams that contain pure northern-strain populations and those with greater than 5% admixture.

We caution that any negative consequences of stocking also would apply to native northern-strain populations (i.e., in the James and Roanoke river drainages). Allozyme markers do not provide enough resolution to differentiate between native northern and hatchery populations, and so we recommend that all brook trout populations should be screened and characterized using microsatellite or single nucleotide polymorphism markers. Until we know more about the genetic composition of these populations, it may be wise to stock only infertile triploid brook trout [48].

Southern Appalachian brook trout hatchery stocks are being established in conservation-oriented hatchery programs ([49], https://brooktrouthatchery.wordpress.com/, http://archive.

knoxnews.com/news/aquarium-helping-to-restore-native-trout-ep-510367109-355447741.html). SABT can be stocked to re-establish populations in streams where they have been extirpated. Also, while we do not recommend eradicating non-native or introgressed populations in watersheds where brook trout are native, we recommend stocking southern-strain hatchery fish into these populations to shift allele frequencies toward those of native populations. Progress in re-establishing native brook trout populations should be monitored using genetic markers every few generations.

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