Genetic Origin of Wild Brook Trout Populations in the Upper French Broad River System, North Carolina

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Abstract: Brook trout *(Salvelinus fontinalis)* is the only salmonid native to the southern Appalachian Mountains. The range of brook trout within this region was greatly reduced during the 20th century due to environmental degradation and the introduction of non-native rainbow trout *(Oncorhynchus mykiss)* and brown trout *(Salmo trutta)*. Efforts to supplement trout populations and to repopulate streams in which trout had been extirpated also included stocking of hatchery-reared brook trout, the stocks for which originated from northern populations. Recently, molecular genetic analyses have demonstrated there to be distinct differences between brook trout native to the southern Appalachians and those found north of this region. In the present study, the genetic origin was determined for wild brook trout populations within 37 streams in the upper French Broad River system, Transylvania and Henderson counties, North Carolina. Fish were collected by electroshocking and muscle tissue was obtained non-lethally from each. The tissues were analyzed by cellulose acetate gel protein electrophoresis for creatine kinase and 5 other potentially informative enzymes. In only 6 streams (16%) were the brook trout of the unaltered native Southern Appalachian strain. Seven streams (19%) contained brook trout of solely northern hatchery-derived origin, and 24 streams (65%) contained brook trout of mixed genetic origin. Results indicate that stocking of hatchery brook trout into streams within the French Broad River system has led to widespread establishment of non-native northern strain brook trout or interbreeding between northern strain and Southern Appalachian strain brook trout to produce populations of mixed genetic origin.

Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 55:52–62

The range of brook trout *(Salvelinus fontinalis)* extends from northeastern Canada southward along the Appalachian Mountains to northern Georgia. Within the southern Appalachians of Georgia, Tennessee, South Carolina, North Carolina, and southern Virginia, brook trout is the unique native salmonid species (Lennon 1967, MacCrimmon and Campbell 1969, Stoneking 1981). However, the range of brook trout in the southern Appalachians has been greatly reduced due to environmental

disturbance associated with logging, road construction, frequent fires, and destructive fishing practices. In addition, extensive stream stocking of non-native rainbow trout *(Oncorhynchus mykiss)* and brown trout *(Salmo trutta)* increased competition for food and space, and led to further decline of brook trout in this region. Currently, brook trout are found in only the cleaner, cooler, headwater streams, typically above 900-m elevation (King 1937, Lennon 1967, McCracken et al. 1993).

Stream stocking efforts also included the release of hatchery-reared brook trout. The hatchery stocks of brook trout used in these efforts are derived from northern populations, primarily from Pennsylvania and New Hampshire (Lennon 1967, Kreigler et al. 1995). Fishery managers and fishermen have long suspected phylogenetic differences to exist between hatchery brook trout and brook trout native to the southern Appalachians (Lennon 1967, Stoneking et al. 1981). Guffey (1998) compared coloration and a variety of morphometric measures between wild-caught fish of both types. Although differences in some measures were suggestive of differences between Southern Appalachian and northern brook trout populations, he was unable to identify any characteristics to definitively distinguish between the two. However, studies involving protein electrophoretic analyses (Stoneking et al. 1981, McCracken et al. 1993, Kreigler et al. 1995) and DNA-based analyses (Saidak 1995, Hayes et al. 1996, Danzmann et al. 1998) indicate that distinct genotypic differences do indeed exist between Southern Appalachian and northern brook trout. These differences are indicative of substantial divergence within the species and are of a magnitude consistent with sub-specific differentiation recognized among other salmonids (Stoneking et al. 1981, McCracken et al. 1993, Kreigler et al. 1995, Guffey 1998). To an unknown extent, stream stocking of the non-native northern hatchery strain brook trout has led to interbreeding with indigenous brook trout and the creation of populations of mixed genetic origin in the region or the establishment of purely northern strain populations in cases where the native fish had been extirpated.

The North Carolina Wildlife Resource Commission (NCWRC) is currently creating a trout species distribution database for streams in western North Carolina to aid in establishment of appropriate fishery management policies (NCWRC 1989). The NCWRC desires to supplement the data on wild brook trout populations with information regarding the genetic origin of each native Southern Appalachian strain versus purely hatchery-derived northern strain versus mixed genetic origin. Some wild brook trout populations in North Carolina have been sampled and analyzed for genetic origin determination. However, this determination has yet to be made for the majority of brook trout populations, including most of those within the French Broad River system. To help overcome this deficit, 37 streams within this watershed were selected for sampling, and tissues from the brook trout were analyzed to determine the genetic origin of each population.

We are very grateful for the financial support contributed by Appalachian Voice and the Sierra Club of Transylvania County, North Carolina, and to Than Axtell for his efforts in promotion of the project and for help in the field sampling. We also thank the Pisgah Chapter of Trout Unlimited, Hendersonville, North Carolina, for the financial support and the many volunteer hours contributed by the members for the

Table 1. Streams in the upper French Broad River system, grouped within watersheds of the major tributary streams to the river, containing wild brook trout populations that were sampled for genetic origin determination (S = native Southern Appalachian strain, N = northern hatchery-derived strain, M = mixed genetic origin). See Figure 1 for tributary locations.

Major tributary / Stream name	Genetic origin	Major tributary / Stream name	Genetic origin
North Fork Mills River		Buckhorn Creek	M
Spencer Branch	M	Tarkiln Creek (LR)[a]	M
Fletcher Creek	M	Jim Branch	M
Horse Cove	S		
Boby Cove	S	*Cathey's Creek*	
		Charles Creek	M
South Fork Mills River		Cedar Rock Creek	M
Pea Branch	S	Kagle Branch	M
Cantrell Creek	M	Kuykendall Creek	N
Thompson Creek	M	Negro Prong	S
Clawhammer Creek	N	Tarkiln Branch (CC)[a]	M
Davidson River		*Cherryfield Creek*	
Bennett Cove	M	Cherryfield Creek	M
Log Hollow Branch	N	Sawmill Creek	S
Big Bearpen Branch	M		
Cove Creek	S	*North Fork French Broad*	
Long Branch	M	Shoal Creek (NF)[a]	N
Searcy Creek	N	Diamond Creek	M
Grogan Creek	M		
Rockhouse Creek	N	*West Fork French Broad*	
Looking Glass	M	Tributary to Parker Creek	M
		Double Branch	M
Little River		Miser Creek	N
Shoal Creek (LR)[a]	M		
Tom's Creek	M	*East Fork French Broad*	
Tributary to Tom's Creek	M	Murr Creek	M

a. Stream identified by tributary so as to distinguish it from another stream with same name in a different watershed.

field sampling, with special thanks to Dr. J. Frisch. We express our appreciation to J. Borawa of the NCWRC and to A. Rowe and S. Bryan of the U.S. Forest Service (USFS) for their collaboration. We also thank the Nat Greene Federation of Fly Fishermen/Trout Unlimited Chapter for their interest and financial support.

Methods

Wild brook trout were sampled in 37 headwater streams within 9 tributary watersheds of the upper French Broad River system located primarily within the Pisgah National Forest and the DuPont State Forest in Transylvania and Henderson counties, North Carolina (Table 1 and Fig. 1). The upper French Broad River is defined here as the portion of the system upstream of its confluence with the Mills River. Each stream was known, or suspected, to contain a wild population of brook trout based on information provided by the NCWRC, USFS, Appalachian Voice, and the

Pisgah Chapter of Trout Unlimited. The 37 streams are estimated to represent the near totality of those containing wild brook trout populations within the study area. Brook trout in each stream were collected at a single site—generally a stream length of approximately 100 m. However, when a barrier waterfall was located within the portion of the stream containing brook trout, 2 sample sites were identified, 1 site above and one below the waterfall. A waterfall was deemed a potential barrier to upstream migration of fish if it presented a (near) vertical drop greater than 2m high.

Fish were collected by electroshocking with a gasoline powered backpack electroshocker generating 650 volts. Any rainbow trout or brown trout captured were counted and released back into the stream. Brook trout were held in a bucket until,

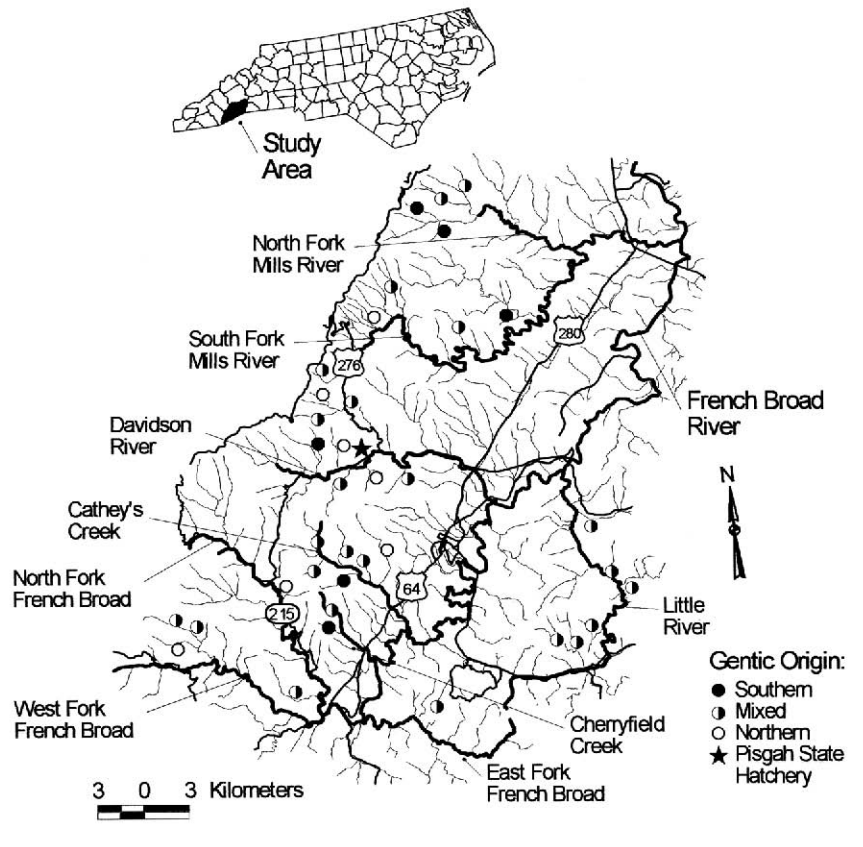

Figure 1. Location of streams within watersheds of the major tributaries to the upper French Broad River containing wild brook trout populations that were sampled for genetic origin determination. Differences between symbols distinguish between genetic origin categories as determined by allozyme analyses: Southern = native Southern Appalachian strain, Northern = northern hatchery-derived strain, Mixed = mixed genetic origin. See Table 1 for stream names.

when possible, the desired number of 20 was obtained. Following anesthesia in a solution of 50 mg/liter clove oil (Anderson et al. 1997, Taylor and Roberts 1999), length was recorded for each fish and 2 non-lethal muscle biopsies were taken using a 14-gauge Bard Monopty Muscle Biopsy Needle (C.R. Bard, Inc., Covington, Ga). The biopsies were collected from the dorsal muscle, 1 sample on either side immediately below the dorsal fin. For each site, therefore, 2 sample sets were obtained, 1 for initial laboratory analysis and 1 for re-analysis if required. The tissue samples were placed in individually labeled microcentrifuge tubes and frozen over dry ice. Following recovery from the anesthesia, the fish were released back into the stream. The tissue samples were brought to Western Carolina University, and stored at –70 C prior to laboratory analysis.

Cellulose acetate gel electrophoresis followed by allozyme analysis for 6 different enzyme loci was performed for a set of tissue samples per site. The enzyme loci included creatine kinase (*CK-A2**), which is considered diagnostic for determining genetic origin of brook trout (McCracken et al. 1993, Kreigler et al. 1995). The other 5 loci included: aspartate aminotransferase (*sAAT-1.2**), glyceraldehyde 3-phosphate dehydrogenase (*G3PDH-1**), malate dehydrogenase (*sMDH-B1.2**), glucose-6-phosphateisomearase (*GPI-B1**), and peptidase (*PEPB**). Southern Appalachian strain populations are typically fixed for a single allele at these loci, whereas northern strain populations are generally polymorphic. Results for these loci are, therefore, potentially useful for corroboration of determinations based on results for *CK-A2**.

Protocols for the electrophoresis and allozyme analyses followed those described by Hebert and Beaton (1993), McCracken et al. (1993), Moore (1997), Guffey et al. (1998), and Galbreath et al. (2001). Briefly, 1 set of tissue samples per stream was removed from the freezer and thawed. While thawing, 12 µl of grinding buffer were added to each microcentrifuge tube and the tissue homogenized with a spatula. The tubes were centrifuged for thirty seconds at 16,750xG, then 8 µl of supernatant was pipetted into individual wells of a Super Z-12 Sample Well Plate—10 samples per plate, 2 plates per sample set. Supernatant of a sample from a brook trout of known genotype was included in an eleventh well on each plate for comparative purposes. A small amount of solution from the wells was then transferred onto a Titan III cellulose acetate membrane with a Super Z-12 Applicator. The membranes were presoaked in Tris-glycine buffer adjusted to either pH 7.5 or 8.0. The membranes were placed in a Zip Zone electrophoresis chamber, and electrophoresed at 270 volts for a fixed period ranging from 25 to 40 minutes, depending on the enzyme. During the final 5 minutes, 5 ml aliquots of staining solution were prepared for each pair of membranes, followed by an addition of approximately 0.8 ml of 0.8% agarose at 65 C (except in the case of analysis for *sAAT-1.2** when no agarose was added). The staining solution was then poured over the membranes. After development of banding patterns was complete, staining was halted by the addition of a fixative. The gels were then scored, photographed, and stored in a photo album for future reference.

Table 2. Allele frequencies for populations of brook trout determined to be native Southern Appalachian strain. The number of fish used to calculate a frequency is 20 unless otherwise indicated by a number in parentheses.

Enzyme/Allele	Horse Cove[a]	Boby Cove	Negro Prong[a]	Pea Branch	Cove Creek	Sawmil Creek[a]
*sAAT-1.2** [b]	(18)	(9)	(40)	(4)		(24)
**118/118/118/118*	1.00		1.00	1.00	1.00	1.00
**118,*100* heterozygotes		1.00				
**100/100/100/100*						
*PEPB**	(18)	(9)	(40)	(4)		(24)
**100*	1.00	1.00	1.00	1.00	1.00	1.00
**86*						
*G3PDH-1**	(18)	(8)	(40)	(4)		(24)
**100*	1.00	1.00	1.00	1.00	1.00	1.00
**43*						
*sMDH B1.2**		(18)	(9)	(40)	(4)	(24)
**145*						
**100*	1.00	1.00	1.00	1.00	1.00	1.00
*GPI-B2**	(18)	(9)	(40)	(4)		(24)
**100*						
**70*	1.00	1.00	1.00	1.00	1.00	1.00
*CK-A2**	(18)	(9)	(40)	(4)		(24)
**100*	1.00	1.00	1.00	1.00	1.00	1.00
**78*						

a. Indicates frequencies calculated from the pooled data of 2 sample sets collected above and below a waterfall.

b. Frequencies of homozygous and heterozygous fish. Heterozygote genotypes among fish could not be determined with certainty for *sAAT-1.2**.

Results

Allele frequencies were calculated for the six loci within each sample set, with the exception of *sAAT-1,2**. The 2 isoloci for *sAAT–1,2** are variable for the same alleles; however, resolution of the banding pattern was insufficient to distinguish between the 3 possible heterozygotic forms (*118/100/100/100, *118/118/100/100, and *118/118/118/100). Therefore, instead of allele frequencies, frequencies of homozygotes and heterozygotes were calculated for this enzyme. The frequency data are presented for the stream populations in Tables 2, 3, 4a–4c, grouped according to their genetic origin determination.

Genetic origin was initially determined for each population based on results for *CK-A2**: 100% *CK-A2**100 = native Southern Appalachian strain, 100% *CK-A2**78 = northern hatchery-derived strain, and the presence of both alleles = mixed origin. Then, allele frequencies for the 5 additional loci were compared to those observed for pure Southern Appalachian and pure northern strain populations by Kreigler et al. (1997), Guffey (1998); and Galbreath et al. (2001). Concordance of the allele fre-

Table 3. Allele frequencies for populations of brook trout determined to be solely nothern hatchery-derived origin. The number of fish used to calculate a frequency is 20 unless otherwise indicated by a number in parentheses.

Enzyme/Allele	Clawhammer Creek	Log Hollow Branch	Searcy Creek	Rockhouse Creek	Kuykendall Creek	Shoal Creek (NF)	Miser Creek[a]
*sAAT-1.2**[b]	(3)		(10)			(9)	(7)
**118/118/118/118*	0.33				0.12		
**118,*100* heterozygotes	0.33		0.70		0.18	1.00	1.00
**100/100/100/100*	0.33	1.00	0.30	1.00	0.70		
*PEPB**	(3)		(8)			(9)	(7)
**100*						0.33	
**86*	1.00	1.00	1.00	1.00	1.00	0.67	1.00
*G3PDH-1**	(3)		(7)			(9)	(7)
**100*	0.83	1.00	0.93	1.00	0.26	0.94	1.00
**43*	0.17		0.07		0.74	0.06	
*sMDH B1.2**	(3)		(10)			(9)	(7)
**145*	0.50		0.10				
**100*	0.50	1.00	0.90	1.00	1.00	1.00	1.00
*GPI-B2**	(3)		(8)			(9)	(7)
**100*	0.83	1.00	0.94	1.00	1.00	0.50	1.00
**70*	0.17		0.06			0.50	
*CK-A2**	(3)		(10)			(9)	(7)
**100*							
**78*	1.00	1.00	1.00	1.00	1.00	1.00	1.00

a. Indicates frequencies calculated from the pooled data of 2 sample sets collected above and below a waterfall.

b. Frequencies of homozygous and heterozygous fish. Heterozygote genotypes among fish could not be determined with ertainty for *sAAT-1.2**.

quencies to the range of frequencies observed in these previous studies was considered as corroboration of the *CK-A2** based determinations. In all cases where a stream was sampled above and below a potential barrier falls, genetic origin determinations were similar and the stream was considered to contain a single population.

Discussion

Of the 37 streams sampled in this study, only 6 (16%) found within 5 tributary watersheds contained populations of unaltered native Southern Appalachian strain brook trout (Table 2). Seven other streams (19%) found within 5 tributary watersheds were determined to contain brook trout of solely northern hatchery-derived genetic origin (Table 3), and 24 streams (65%) contained brook trout of mixed genetic origin (Tables 4a–4c). The latter included Buckhorn Creek and Tarkiln Creek (LR), both very small in size and from which only 4 and 2 brook trout, respectively, were collected. Analyses for *CK-A2** indicated that these fish were homozygous for the *100 allele, and the populations presumptively Southern Appalachian strain. However, a

Table 4a. Allele frequencies for populations of brook trout determined to be of mixed genetic origin. The number of fish used to calculate a frequency is 20 unless otherwise indicated by a number in parentheses.

Enzyme/Allele	Spencer Branch	Fletcher Creek	Cantrell Creek	Thompson Creek	Bennett Cove	Big Bearpen Branch	Long Branch	Grogan Creek[a]
*sAAT-1.2**[b]			(10)	(4)				(39)
**118/118/118/118*		0.20			0.20		0.10	0.21
**118,*100* heterozygotes		0.80	1.00	0.75	0.80		0.90	0.76
**100/100/100/100*				0.25				0.03
*PEPB**			(10)	(4)				(39)
**100*	0.70	0.75	0.75	0.63	0.35	0.63	0.65	0.71
**86*	0.30	0.25	0.25	0.37	0.65	0.37	0.35	0.29
*G3PDH-1**			(7)	(4)			(19)	(39)
**100*	1.00	0.90	1.00	0.87	0.97	1.00	0.82	1.00
**43*		0.10		0.13	0.03		0.18	
*sMDH B1.2**			(10)	(4)				(39)
**145*	0.08	0.30	0.10		0.05		0.03	0.37
**100*	0.92	0.70	0.90	1.00	0.95	1.00	0.97	0.63
*GPI-B2**			(10)	(4)				(39)
**100*	0.05	0.08	0.40	0.63	0.72	0.28	0.23	0.69
**70*	0.95	0.92	0.60	0.37	0.28	0.72	0.77	0.31
*CK-A2**			(7)	(4)				(39)
**100*	0.98	0.90	0.79	0.63	0.27	0.95	0.75	0.50
**78*	0.02	0.10	0.21	0.37	0.73	0.05	0.25	0.50

a. Indicates frequencies calculated from the pooled data of 2 sample sets collected above and below a waterfall.

b. Frequencies of homozygous and heterozygous fish. Heterozygote genotypes among fish could not be determined with ertainty for *sAAT-1.2**.

relatively high incidence of *sMDH-B1,2*145* (0.38) in Buckhorn Creek and of *PEPB*86* (0.25) and *GPI-B2*100* (0.50) in Tarkiln Creek (LR) was recorded. These alleles are absent or rare in Southern Appalachian strain populations (McCracken et al. 1993, Kreigler et al. 1995, Guffey 1998, Galbreath et al. 2001). It was therefore inferred that these populations are most likely of mixed genetic origin, and that the *CK-A2*78* allele was not observed due to small sample size.

The low percentage (16%) of streams containing unaltered Southern Appalachian strain brook trout in the upper French Broad River system is in sharp contrast to the percentage of Southern Appalachian populations (65%) reported by Galbreath et al. (2001) for streams in the adjacent Pigeon River system, Haywood County, North Carolina, and to percentages reported by Guffey (1998) for streams in the Great Smoky Mountains National Park (76%) and by Kreigler et al. (1995) for streams in Tennessee outside the Park (58%).

The NCWRC has relatively complete written records of brook trout stream stocking activities conducted by their agency in North Carolina beginning in the early 1940s. Within these archives, 1 or more stocking events with hatchery-reared

Table 4b. Allele frequencies for populations of brook trout determined to be of mixed genetic origin. The number of fish used to calculate a frequency is 20 unless otherwise indicated by a number in parentheses.

Enzyme/Allele	Looking Glass	Shoal Creek (LR)	Tom's Creek	Unnamed tributary to Tom's Creek[a]	Buckhorn Creek	Terklin Creek (LR)	Jim Branch	Charles Creek
*sAAT-1.2**[b]				(10)	(4)	(2)	(10)	
**118/118/118/118*	0.40	1.00	0.98	1.00	1.00	1.00	1.00	
**118,*100* heterozygotes	0.60		0.02					0.90
**100/100/100/100*								0.10
*PEPB**	(19)			(9)	(4)	(2)	(10)	
**100*	0.39	0.53	0.65	0.83	1.00	0.75	0.35	0.21
**86*	0.61	0.47	0.35	0.17		0.25	0.65	0.79
*G3PDH-1**	(19)			(9)	(4)	(2)	(10)	
**100*	0.95	1.00	1.00	1.00	1.00	1.00	0.55	0.93
**43*	0.05						0.45	0.07
*sMDH B1.2**	(10)			(10)	(4)	(2)	(10)	
**145*		0.95	0.13	0.10	0.38			0.35
**100*	1.00	0.05	0.87	0.90	0.62	1.00	1.00	0.65
*GPI-B2**	(19)			(10)	(4)	(2)	(10)	
**100*	0.82	0.63	0.53	0.40		0.50	0.65	0.66
**70*	0.18	0.37	0.47	0.60	1.00	0.50	0.35	0.34
*CK-A2**				(9)	(4)	(2)	(10)	
**100*	0.33	0.88	0.55	0.50	1.00	1.00	0.25	0.25
**78*	0.67	0.12	0.45	0.50			0.75	0.75

a. Indicates frequencies calculated from the pooled data of 2 sample sets collected above and below a waterfall.

b. Frequencies of homozygous and heterozygous fish. Heterozygote genotypes among fish could not be determined with ertainty for *sAAT-1.2**.

brook trout are recorded for only 2 of the 37 streams sampled in this project—Shoal Creek (NF; northern hatchery-derived) and Cathey's Creek (mixed genetic origin), yet the great majority showed presence of northern strain alleles. In contrast, NCWRC records indicate stocking events for a relatively large portion of Pigeon River streams, yet most of these populations were of unaltered Southern Appalachian origin (Galbreath et al. 2001). Kreigler et al. (1995) also noted that stocking records were not a reliable predictor of genetic origin of wild brook trout populations. Tennessee Wildlife Resources Agency records indicate stocking events (some of them recurring over several years) for 6 of the 22 streams determined in their study to contain unaltered Southern Appalachian populations, and stocking records were lacking for 3 of the 16 streams observed to contain mixed or solely northern hatchery-derived populations (Kreigler et al. 1995). It is known that widespread and unrecorded hatchery stocking of streams in North Carolina was conducted by federal, state, and private agencies prior to the 1940s, and escapement of northern hatchery-strain brook trout from commercial trout farms has also occurred in an undocumented manner (J. Borawa, NCWRC, pers. commun.). At least in the French Broad River system, it ap-

Table 4c. Allele frequencies for populations of brook trout determined to be of mixed genetic origin. The number of fish used to calculate a frequency is 20 unless otherwise indicated by a number in parentheses.

Enzyme/Allele	Cedar Rock Creek	Kagle Branch	Tarkiln Branch	Cherryfield Creek[a]	Diamond Creek[a]	Unnamed Tributary to Parker Creek[a]	Double Branch[a]	Murr Creek
*sAAT-1,2**[b]				(21)	(40)	(24)	(26)	
**118/118/118/118*	0.30	0.60	0.05	0.24		0.96	0.27	0.05
**118,*100* heterozygotes	0.50	0.25	0.90	0.76	0.83	0.04	0.58	0.85
**100/100/100/100*	0.20	0.15	0.05		0.17		0.15	0.10
*PEPB**				(21)	(40)	(24)	(26)	
**100*	0.45	0.65	0.43	0.48	0.51	0.60	0.63	0.70
**86*	0.55	0.35	0.57	0.52	0.49	0.40	0.37	0.30
*G3PDH-1**				(21)	(40)	(24)	(26)	
**100*	0.90	1.00	0.97	1.00	0.90	1.00	1.00	1.00
**43*	0.10		0.03		0.10			
*sMDH B1.2**				(21)	(40)	(24)	(26)	
**145*	0.25	0.05	0.18	0.19	0.05			
**100*	0.75	0.95	0.82	0.81	0.95	1.00	1.00	1.00
*GPI-B2**				(21)	(40)	(24)	(19)	
**100*	0.55	0.20	0.65	0.55	0.31		0.19	0.55
**70*	0.45	0.80	0.35	0.45	0.69	1.00	0.81	0.45
*CK-A2**			(17)	(21)	(40)	(24)	(26)	(19)
**100*	0.43	0.53	0.47	0.31	0.63	0.73	0.44	0.89
**78*	0.57	0.47	0.53	0.69	0.37	0.27	0.56	0.11

a. Indicates frequencies calculated from the pooled data of 2 sample sets collected above and below a waterfall.

b. Frequencies of homozygous and heterozygous fish. Heterozygote genotypes among fish could not be determined with ertainty for *sAAT-1.2**.

pears likely that these non-NCWRC stockings were the primary source of the northern strain alleles observed in the present study.

The high degree of establishment and interbreeding of northern strain hatchery brook trout observed in streams within the upper French Broad River system, contrasts to lower levels observed in other southern Appalachian watersheds. Whether or not "success" of hatchery stocking in this system can be attributed to prior reduction or extirpation of the native brook trout populations due to relative severity of environmental disturbances to the streams, or to a much higher frequency of unrecorded stocking events cannot be determined with certainty.

Information from this study has been communicated to the NCWRC and included in their growing database on distribution of wild trout populations in North Carolina. Identification of the populations of genetically unaltered Southern Appalachian brook trout is of importance in planning for eventual brook trout restoration or enhancement activities in North Carolina (NCWRC 1989, J. Borawa, NCWRC, pers. commun.).

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