Biochemical Genetics of Brook Trout in Georgia: Management Implications

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Abstract: Twenty-eight populations of brook trout *(Salvelinus fontinalis)* in Georgia were genetically compared using isozymes and their genetic relatedness determined. Eight populations (29%) were classified as southern based on fixation for *CK-A2*122* allele, 2 (7%) populations were classified as northern based on fixation for *CK-A2*100* allele, and the remaining 18 (64%) were northern-southern hybrid populations. All 8 southern populations shared some variant alleles with northern populations. Northern brook trout in Georgia had much greater genetic variation than southern brook trout, and hybrid populations were intermediate. Among the 8 southern populations and hybrid populations that are strongly southern, 2 major genotypes exist based on fixed or large differences at the *sAAT-3** locus. Combinations of migration, non-random mating and selection are occurring, as many loci were not at Hardy-Weinberg equilibrium. Data and historical records indicated that stocking had little genetic impact when established brook trout populations were already present. Among the 8 southern populations, allele frequencies indicate 4 pairs of populations that are highly similar to each other. Logan Creek and Bryant Creek have similar allele frequencies and patterns of genetic variations. Similar allele frequencies and genetic variation patterns were also observed at Emory Branch and North Prong Left, Keener Creek and Gizzard Brand, and Rough Creek and High Shoals Creek.

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Brook trout is native to eastern North America occurring from Ontario to Georgia (King 1937, Jones 1978, Stoneking et al. 1981, McCracken et al. 1993). Brook trout is the only salmonid that is native to the southeastern United States, and is only found in high elevation streams at the southern limit of the Appalachian Mountains

(King 1937, Jones 1978, Stoneking et al. 1981). Brook trout stocks in the southeastern United States have declined in the past century, mainly as a result of extensive human activity including the introduction of non-native rainbow trout *(Oncorhynchus mykiss)* into the region's streams (Jones 1978, McCracken et al. 1993). Georgia brook trout populations are limited to first and second order streams above barrier falls that prevent upstream expansion of naturalized rainbow and brown trout populations. Hatchery stocks originating from northeastern brook trout populations were often used to restore declining populations of brook trout in southeastern streams (Jones 1978). Stocking efforts were so repetitive, especially between 1930 and 1970, that it became impossible to identify the native and transplanted gene pools or their intergrades (McCracken et al. 1993).

Based on limited morphological data, Lennon (1967) first suggested that a separate subspecies or species of brook trout existed in the southern United States, particularly in the isolated headwaters of streams in the Great Smoky Mountains National Park. Harris et al. (1978) conducted an isozyme survey of 35 brook trout populations ranging from Georgia to New York and concluded that there were no differences at the subspecific level between northern and southern populations. Brook trout have tetraploid ancestry, and about 40% of their isozyme loci are duplicated (May et al. 1980), complicating data interpretation. Subsequent studies demonstrated that at certain loci, allele frequencies are significantly different between southern and northern populations (Stoneking et al. 1981, McCracken et al. 1993). Stoneking et al. (1981) studied 5 wild northern and 3 wild southern brook trout populations—Bunches Creek, Blockstan Creek, and Rocky Fork Creek in North Carolina—which may have been privately stocked with hatchery derived brook trout (M. Seehorn, U.S. For. Serv., pers. commun.), and found 4 loci indicating genetic divergence in terms of allele frequency differences. McCracken et al. (1993) examined 9 southern populations of which 5 had putatively had never been stocked with northern brook trout (Bunches Creek, Flat Creek, Starky Creek, Buck Fork, and Eagle Rocks Prong) and 3, which had received northern brook trout (Ledge Creek, Hyatt Creek, and Beach Flats Prong). One southern stream, Meigs Creek, had no brook trout before it was stocked with northern brook trout. McCracken et al. (1993) demonstrated substantial genetic divergence as a result of fixed genetic differences at 1 locus *(CK-A2*)* and 9 additional loci that had allele frequencies significantly different between northern and southern brook trout populations.

These studies concluded that northern and southern brook trout were genetically different, and that probably 2 subspecies existed. Stoneking et al. (1981) data showed Bunches Creek to be the most distinctive of the southern populations. More genetic variation was observed in northern populations than in southern populations (McCracken et al. 1993). McCracken et al. (1993) presented evidence of hybridization between northern brook trout of hatchery origin and the native southern populations. In cases where northern hatchery populations were stocked with southern brook trout, the 2 genotypes readily hybridized. The genetic variation in these mixed populations was intermediate compared to the northern and southern parentals.

In this study, the genetic composition of 28 populations of brook trout from different river basins in northeastern Georgia was analyzed using isozymes. The objectives of this study were to examine the allozyme frequencies in 28 populations of brook trout in Georgia, to determine the extent of northern and southern heritage, and to estimate the genetic relatedness of these populations. This project was funded by the Southeastern Cooperative Project on Fish Genetics.

Methods

Brook trout populations were collected by electrofishing from different locations in northeastern Georgia by fisheries biologists from the Georgia Department of Natural Resources (DNR) and the U.S. Forest Service, Chattahoochee National Forest (USFS). Sample sites were spread across all Georgia river basins known to contain wild brook trout (Fig. 1, Dr. B. Freeman, Univ. Ga., pers. commun.). Streams selected included streams that had been stocked with hatchery raised brook trout 1 or more times in the past, and streams for which no stocking records were found.

Eye, liver, and skeletal muscle tissue were taken from each fish and frozen and

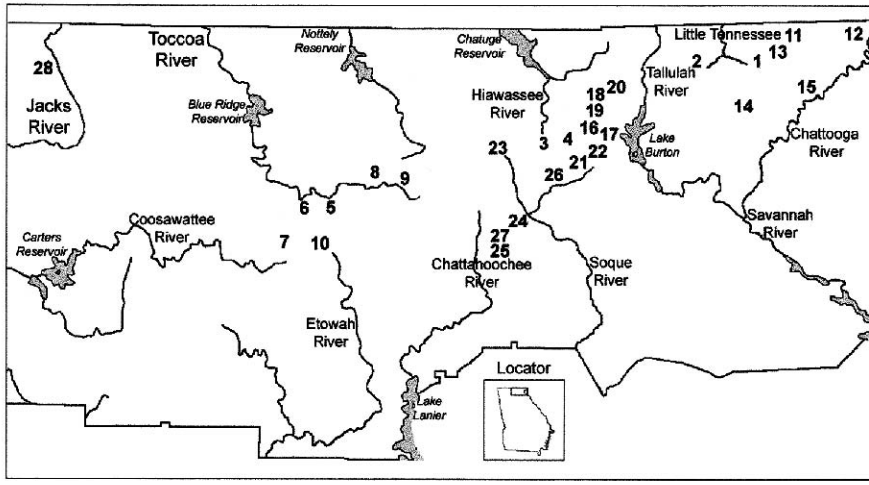

Figure 1. Sampling locations for brook trout, *Salvelinus fontinalis*, in Georgia. 1. Thomas Creek, 2. Keener Creek, 3. High Shoal Creek, 4. Gizzard Branch, 5. Upper L Rock Creek KV, 6. Upper L Rock Creek BF, 7. Lovingood Creek, 8. Bryant Creek, 9. Logan Creek, 10. Long Creek, 11. Emory Branch, 12. Hedden Creek, 13. Holcomb Creek, 14. Finney Creek, 15. Goldmine Creek, 16. Jessie Branch, 17. Hellhole Creek, 18. Firescald Creek, 19. Moccasin Creek, 20. Popcorn Creek, 21. N Prong L Fork Soque River, 22. Goshen Creek, 23.Chattahoochee River, 24. Davis Creek, 25. Winn Branch, 26. York Creek, 27. Dover Creek, and 28.Rough Creek.

transported on dry ice to the Genetics Laboratory of the Department of Fisheries and Allied Aquacultures at Auburn University. Samples were kept at –80 C until analysis. Tissue homogenization, buffers and horizontal starch gel electrophoresis procedures were described in May et al. (1979), Stoneking et al. (1981), McCracken et al (1993), and Norgren et al. (1986).

Nomenclature was based on the system of Stoneking et al. (1981) and Shaklee et al. (1990). Alleles were assigned numbers according to their relative electrophoretic mobility as measured from the gels, with the largest numbers indicating the most migration on the gel. Genetic analysis was performed on 18 enzymes expressed by 36 putative loci (Table 1).

Genotypes, allele frequencies, percentage of loci polymorphic and mean heterozygosities were determined utilizing Biosys-1 (Swofford and Selander 1981). A locus was defined as polymorphic if it had at least 2 alleles. Mean heterozygosity was calculated by averaging the percentage of heterozygous genotypes at all loci for all fish within a population. Genetic relationships among the populations were estimated using Rogers' (1972) genetic similarity (S), and a dendrogram of these rela-

Table 1. Enzymes, locus designations, tissues, and buffers used to examine biochemical genetics of brook trout, *Salvelinus fontinalis*, in Georgia. Enzymes were selected from McCracken et al. (1993). Tissues used were eye (E), liver (L) and muscle (M). Buffer systems used were A = tris citrate (pH 7.1) [Ayala et al. (1973) modified by May et al. (1979)], C = citrate buffer (pH 7.0) [Clayton and Tretiak (1972) modified by May et al. (1979)], M = tris borate (pH 8.7) [Markert and Faulhaber (1965)], R = tris citrate (pH 8.5) [Ridgway et al. (1970)], 4 = tris citrate (pH = 6.5) [Selander et al. (1971)], and S-9 = tris maleic (pH 8.0) [Selander et al. (1971)].

Enzyme	*N* of loci	Enzyme number	Loci	Tissue	Buffer
Aspartate aminotransferase	3	2.6.1.1	*sAAT-1**, *sAAT 2**, *sAAT- 3**	M,E	R
Alcohol dehydrogenase	1	1.1.1.1	*ADH**	L	R
Aldolase	1	4.1.2.13	*ALD**	E	A
Creatine kinase	4	2.7.3.2	*CK-A1**, *CK-A2**, *CK-B**, *CK-C**	M	R
Esterase	2	3.1.1.-	*EST-1**, *EST-2**	M	R
Fumarate hydratase	1	4.2.1.2.	*FH**	M	R
Glycerol-3-phosphate dehydrogenase	1	1.1.1.8	*G3PDH**	M	S-9
Glucuse-6-phosphate isomerase	3	5.3.1.9	*GPI-A**, *GPI-B**, *GPI-C**	M	R
Isocitrate dehydrogenase	1	1.1.1.42	*sIDHP**	M	4
Lactate dehydrogenase	5	1.1.1.27	*LDH- A1**, *LDH-A2**, *LDH-B1**, *LDH-B2**, *LDH-C1**	M,L,E	M
Malate dehydrogenase	4	1.1.1.37	*MDH-A1**, *MDH-A2**, *MDH-B1**, *MDH-B2**	M	A, C
Malic enzyme (NADP+)	3	5.3.1.8	*mMEP-1**, *mMEP-2**, *sMEP-1**	M	C
Mannose-6-phosphate isomerase	1	5.3.1.8	*MPI**	L	R
Octanol dehydrogenase	1	1.1.1.73	*ODH**	L	M
Phosphogluconate dehydrogenase	1	1.1.1.44	*PGDH**	M	R
Phosphoglucomutase	2	5.4.2.2	*PGM**	M	A
General (unidentified) protein	1	no number	*PROT**	M	R
Superoxide dismutase	1	1.15.1.1	*sSOD**	L	R

tionships was generated from the similarity measures. Populations were classified as southern, northern, or hybrid based on analysis of the *CK-A2** locus (McCracken et al. 1993). Populations were tested for genetic differences with contingency chi-square (Swofford and Selander 1981), and a modified *t*-test (Hallerman et al. 1986) and confidence estimates of the nodes of the phylogenetic tree were estimated using Nei's genetic distance, Da (Nei et al.1983). A scale of 1 to 10 was used to show the degree of hybridization between northern and southern populations according to allele frequencies, 1 for southern, 10 for northern, and 2–9 for hybrid classification.

Results and Discussion

Genetic Variation

Genetic composition of 28 brook trout populations from Georgia was analyzed using isozyme markers. Fourteen of the 36 loci scored were polymorphic in these populations (Table 2). Five out of the 14 polymorphic loci (*sAAT-1,2**; *CK-A2**, *GPI-B**, *sMDH-4** and *ODH**) exhibited genetic variation in majority of populations, but the rest showed little variation in all 28 populations (Table 2).

Eight populations were fixed for the diagnostic southern allele *(CK-A2*122)* (designated **100* by McCracken et al.(1993), whereas only 2 populations were fixed for the northern allele *(CK-A2*100)* (designated *78 by McCracken et al.(1993) (Table 2). Brook trout from Logan Creek, Keener Creek, Bryant Creek, Gizzard Branch, and Left Fork Soque River were homozygous at most of the loci analyzed and contained alleles specific for southern populations (Table 3). Although samples from Rough Creek, High Shoals Creek, Keener Creek, Gizzard Branch, and Left Fork Soque River were fixed for the southern diagnostic allele *(CK-A2*122)*, all of them were polymorphic at *sAAT-1, 2** and except High Shoal Creek, all of them contained *sAAT-1,2*100* allele at low frequencies (Table 2). This allele was present at higher frequencies in brook trout populations containing the northern diagnostic allele *CK-A2*100*. Two populations, Rough Creek and High Shoals Creek, had alleles sometimes found in northern populations. However, we considered that the 8 populations fixed for *CK-A2*122* were southern because there is a high likelihood that some alleles occasionally found in the north could also be naturally present and shared by some southern populations at lower frequencies. Alternatively, it is possible that this is the result of low-level introgression between the northern and southern populations.

Brook trout from Hellhole and Goldmine Creeks were fixed for the *CK-A2*100* northern allele, and had alleles specific for northern populations at other loci. Additionally, 3 populations, Firescald, Davis Creek and Jessie Branch, possessed the northern diagnostic allele at frequencies higher than 0.5.

Eighteen of the populations were hybrid, and 5 of these had very low variation at all loci. Six populations had northern diagnostic allele frequencies higher than 0.5. The levels and type of genetic variation are described in Table 4. Gizzard Branch had

Table 2. Allele frequencies for polymorphic loci of brook trout *(Salvelinus fontinalis)* populations from Georgia.

Locus	Allele *(N)*	Logan Creek 20	Bryant Creek 20	Keener Creek 23	Gizzard Branch 20	Emory Branch 18	N. Prong Left 20	Left Rough Creek 13	High Shoals Creek 20	Chatta-hoocee River 18	Long Creek 20	Lovin-good Creek 20	Popcorn Creek 18	L. Rock Creek BF 20	Moc-casin Creek 20
*sAAT-1**	100	0.000	0.000	0.370	0.025	0.278	0.025	0.154	0.600	0.278	0.000	0.125	0.250	0.250	0.350
	118	1.000	1.000	0.630	0.975	0.722	0.975	0.846	0.400	0.722	1.000	0.875	0.750	0.750	0.650
*sAAT-2**	100	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.025	0.028	0.000	0.000	0.000	0.000	0.050
	118	1.000	1.000	1.000	1.000	0.972	1.000	1.000	0.975	0.972	1.000	1.000	1.000	1.000	0.950
	80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*sAAT-3**	100	1.000	1.000	0.000	0.000	0.750	1.000	0.000	0.000	1.000	1.000	0.850	0.000	0.400	0.000
	93	0.000	0.000	1.000	1.000	0.250	0.000	1.000	1.000	0.000	0.000	0.150	1.000	0.600	1.000
*ADH**	100	1.000	1.000	1.000	1.000	1.000	0.800	1.000	1.000	1.000	0.800	1.000	1.000	1.000	1.000
	205	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.000
*CK-A2**	100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.050	0.025	0.025	0.100	0.275
	122	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.800	0.950	0.975	0.975	0.900	0.725
*FH **	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	0.925	0.900	1.000	1.000
	107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.075	0.000	0.000	0.000
	93	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000
*G3PDH**	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	78	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*GPI-A**	100	0.000	0.050	0.000	0.000	0.000	0.000	0.154	0.000	0.132	0.353	0.350	0.000	0.275	0.000
	56	1.000	0.950	1.000	1.000	1.000	1.000	0.846	1.000	0.868	0.647	0.650	1.000	0.725	1.000
	82	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*GPI-B**	100	0.684	0.525	0.000	0.000	0.000	0.000	0.577	0.375	0.211	0.588	0.675	0.026	0.575	0.225
	55	0.316	0.475	1.000	1.000	1.000	1.000	0.423	0.625	0.789	0.412	0.325	0.974	0.425	0.775
	39	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	82	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*LDH-B2**	100	1.000	1.000	1.000	1.000	1.000	1.000	0.769	1.000	0.975	1.000	0.975	1.000	0.900	0.950
	72	0.000	0.000	0.000	0.000	0.000	0.000	0.231	0.000	0.025	0.000	0.025	0.000	0.100	0.050
*MDH-B1**	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000
	74	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000
*MDH-B2**	100	0.975	1.000	1.000	1.000	0.975	0.950	1.000	1.000	0.950	1.000	1.000	0.625	0.950	0.875
	170	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.050	0.125
	74	0.000	0.000	0.000	0.000	0.025	0.050	0.000	0.000	0.025	0.000	0.000	0.375	0.000	0.000
*mMEP-1**	100	1.000	1.000	1.000	0.975	1.000	1.000	1.000	0.800	0.950	1.000	0.925	0.958	0.889	0.950
	0	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.200	0.050	0.000	0.075	0.042	0.111	0.050
*sODH**	100	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.125	0.000	0.000	0.267	0.028	0.300	0.450
	116	1.000	1.000	1.000	1.000	1.000	1.000	0.923	0.875	1.000	1.000	0.733	0.972	0.700	0.550

Table 2. *(continued)*

Locus	Allele *(N)*	Holcomb Creek 20	Goshen Creek 18	Thomas Creek 23	L. Rock Creek KV 20	Hedden Creek 21	Finney Creek 19	Firescald Creek 19	York Creek 20	Winn Branch 19	Dover Creek 21	Davis Creek 19	Jessie Branch 19	Hell-hole Creek 20	Gold-mine Creek 20
*sAAT-1**	100	0.500	0.528	0.283	0.050	0.643	0.342	0.474	0.575	0.763	0.786	0.842	0.763	1.000	0.750
	118	0.500	0.472	0.717	0.950	0.357	0.658	0.526	0.425	0.237	0.214	0.158	0.237	0.000	0.250
*sAAT-2**	100	0.050	0.278	0.000	0.000	0.167	0.026	0.079	0.100	0.211	0.452	0.289	0.263	0.600	0.425
	118	0.950	0.722	1.000	1.000	0.810	0.974	0.921	0.900	0.789	0.548	0.684	0.737	0.400	0.575
	80	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000
*sAAT-3**	100	0.000	0.000	0.000	0.813	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.425
	93	1.000	1.000	1.000	0.188	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.974	1.000	0.575
*ADH**	100	0.650	1.000	1.000	0.900	1.000	1.000	1.000	0.450	0.500	1.000	1.000	1.000	1.000	1.000
	205	0.350	0.000	0.000	0.100	0.000	0.000	0.000	0.550	0.500	0.000	0.000	0.000	0.000	0.000
*CK-A2**	100	0.250	0.206	0.130	0.275	0.381	0.362	0.553	0.250	0.475	0.690	0.947	0.941	1.000	1.000
	122	0.750	0.794	0.870	0.725	0.619	0.638	0.447	0.750	0.525	0.310	0.053	0.059	0.000	0.000
*FH **	100	1.000	0.921	1.000	1.000	1.000	0.974	0.816	1.000	0.775	1.000	0.895	0.875	1.000	0.816
	107	0.000	0.053	0.000	0.000	0.000	0.000	0.053	0.000	0.225	0.000	0.053	0.031	0.000	0.158
	93	0.000	0.026	0.000	0.000	0.000	0.026	0.132	0.000	0.000	0.000	0.053	0.094	0.000	0.026
*G3PDH**	100	0.900	1.000	1.000	1.000	1.000	1.000	1.000	0.800	1.000	1.000	1.000	1.000	1.000	1.000
	78	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.000	0.000	0.000
*GPI-A**	100	0.100	0.025	0.000	0.325	0.000	0.079	0.079	0.000	0.079	0.048	0.053	0.053	0.000	0.324
	56	0.900	0.975	1.000	0.675	1.000	0.737	0.921	1.000	0.921	0.952	0.947	0.947	1.000	0.676
	82	0.000	0.000	0.000	0.000	0.000	0.184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*GPI-B**	100	0.425	0.275	0.152	0.350	0.071	0.289	0.421	0.028	0.389	0.429	0.605	0.579	0.684	0.559
	55	0.575	0.700	0.848	0.650	0.929	0.526	0.579	0.972	0.611	0.571	0.395	0.421	0.316	0.441
	39	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	82	0.000	0.000	0.000	0.000	0.000	0.184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*LDH-B2**	100	0.950	0.825	1.000	1.000	0.762	0.868	1.000	0.600	0.500	1.000	0.737	1.000	0.150	0.588
	72	0.050	0.175	0.000	0.000	0.238	0.132	0.000	0.400	0.500	0.000	0.263	0.000	0.850	0.412
*MDH-B1**	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	74	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*MDH-B2**	100	0.925	0.900	1.000	0.775	0.976	0.921	0.974	1.000	0.789	0.775	1.000	0.975	0.650	0.800
	170	0.075	0.075	0.000	0.225	0.024	0.079	0.026	0.000	0.026	0.225	0.000	0.025	0.350	0.175
	74	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.184	0.000	0.000	0.000	0.000	0.025
*mMEP-1**	100	0.950	0.625	0.696	1.000	0.675	0.765	1.000	0.975	0.600	1.000	0.895	0.711	0.737	0.868
	0	0.050	0.375	0.304	0.000	0.325	0.235	0.000	0.025	0.400	0.000	0.105	0.289	0.263	0.132
*sODH**	100	0.225	0.184	0.065	0.211	0.095	0.367	0.100	0.000	0.765	0.833	0.000	0.306	1.000	0.882
	116	0.775	0.816	0.935	0.789	0.905	0.633	0.900	1.000	0.235	0.167	1.000	0.694	0.000	0.118

Table 3. Genetic heritage of brook trout, *Salvelinus fontinalis,* populations from Georgia. S = southern, SH = southern dominant hybrid, NH = northern dominant hybrid, H = hybrid, N = northern. A scale characterizes the degree of hybridization between northern and southern populations based on allele frequencies: 1 = southern, 10 = northern, and 2–9 = hybrid.

	Genetic Heritage									
	Southern				Hybrid					Northern
Population Name	1	2	3	4	5	6	7	8	9	10
Logan Creek	S1									
Bryant Creek	S1									
Keener Creek	S1									
Gizzard Branch	S1									
Emory Branch	S1									
North Prong L. Fork Soque R.	S1									
Rough Creek	S1									
High Shoals Creek	S1									
Long Creek			SH3							
Popcorn Creek			SH3							
Lovingood Creek			SH3							
Little Rock Creek BF section			SH3							
Thomas Creek			SH3							
Chattahoochee River				H4						
Moccasin Creek					H5					
Holcomb Creek					H5					
Goshen Creek					H5					
Little Rock Creek KV section						H6				
Hedden Creek						H6				
Finney Creek						H6				
Firescald Creek						H6				
York Creek						H6				
Winn Branch							NH7			
Dover Creek							NH7			
Davis Creek								NH8		
Jessie Branch								NH8		
Hellhole Creek										N10
Goldmine Creek										N10

the least amount of genetic variation in regards to mean number of alleles/locus (1.1), percentage of loci polymorphic (5.6), and mean heterozygosity (0.003). The other populations identified as southern also had low variability with those designated as the most pure having the least genetic variability. The Goldmine Creek population which was designated as one of only 2 pure northern populations had the highest levels of genetic variability. Again, those populations that appeared to be closer to the northern genotype had the greatest amount of genetic variability, with southern populations having the least genetic variation.

The northern brook trout strain made a major contribution to overall genetic variability in the populations sampled. The north-south cline of genetic variability seen for these brook trout is the opposite of what is observed for largemouth bass,

Table 4. Genetic variability at 36 loci for brook trout, *Salvelinus fontinalis*, populations in Georgia (standard deviations in parenthesis).

Population	Mean sample size per locus	Mean *N* of alleles per locus	Percentage of loci polymorphic[a]	Mean heterozygosity	
				Direct-count	HdyWbg expected[b]
Thomas Creek	23.0 (.0)	1.1 (.1)	13.9	.047 (.022)	.041 (.019)
Rough Creek	12.9 (.1)	1.1 (.1)	13.9	.045 (.026)	.044 (.020)
L Rock Crk Kv	19.6 (.3)	1.2 (.1)	22.2	.068 (.026)	.073 (.025)
L Rock Crk Bf	19.9 (.1)	1.3 (.1)	25.0	.083 (.032)	.080 (.026)
Moccasin Crk	20.0 (.0)	1.2 (.1)	22.2	.065 (.028)	.063 (.024)
High Shoal Crk	20.0 (.0)	1.1 (.1)	13.9	.046 (.027)	.044 (.021)
Dover Creek	20.9 (.0)	1.2 (.1)	19.4	.073 (.030)	.070 (.026)
Holcomb Creek	20.0 (.0)	1.3 (.1)	30.6	.090 (.029)	.084 (.026)
Hedden Creek	20.9 (.0)	1.3 (.1)	22.2	.060 (.023)	.068 (.025)
Keener Creek	23.0 (.0)	1.0 (.0)	2.8	.011 (.011)	.013 (.013)
Lovingood Creek	19.7 (.2)	1.3 (.1)	25.0	.066 (.028)	.061 (.022)
Finney Creek	18.9 (.3)	1.3 (.1)	27.8	.074 (.026)	.092 (.030)
Hellhole Creek	19.6 (.2)	1.1 (.1)	13.9	.060 (.029)	.057 (.025)
Bryant Creek	19.8 (.1)	1.1 (.0)	5.6	.029 (.026)	.017 (.014)
Firescald Creek	18.7 (.1)	1.3 (.1)	22.2	.076 (.034)	.066 (.025)
Gizzard Branch	19.8 (.1)	1.1 (.0)	5.6	.003 (.002)	.003 (.002)
Davis Creek	18.5 (.2)	1.3 (.1)	25.0	.067 (.029)	.066 (.023)
Logan Creek	19.7 (.2)	1.1 (.0)	5.6	.019 (.018)	.014 (.012)
Chatta River	18.8 (.4)	1.3 (.1)	27.8	.051 (.019)	.048 (.018)
Emory Branch	19.2 (.2)	1.1 (.1)	11.1	.015 (.012)	.025 (.016)
Goldmine Creek	19.1 (.2)	1.3 (.1)	27.8	.123 (.039)	.110 (.031)
Goshen Creek	19.1 (.2)	1.4 (.1)	27.8	.069 (.023)	.088 (.027)

(table continues)

Table 4. (continued).

Population	Mean sample size per locus	Mean *N* of alleles per locus	Percentage of loci polymorphic[a]	Mean heterozygosity	
				Direct-count	HdyWbg expected[b]
Jessie Branch	19.1 (.2)	1.3 (.1)	30.6	.089 (.033)	.078 (.025)
Long Creek	19.3 (.2)	1.1 (.1)	11.1	.039 (.027)	.039 (.021)
N Prong L Fork	19.8 (.1)	1.1 (.0)	8.3	.004 (.003)	.013 (.009)
Popcorn Creek	18.1 (.4)	1.2 (.1)	19.4	.039 (.020)	.036 (.018)
Winn Branch	19.1 (.2)	1.3 (.1)	30.6	.105 (.032)	.124 (.033)
York Creek	19.5 (.1)	1.2 (.1)	22.2	.063 (.028)	.070 (.026)

a. A locus is considered polymorphic if more than 1 allele was detected.

b. Unbiased estimate (see Nei 1978).

Micropterus salmoides (Philipp et al. 1983). It appears that as upper or lower temperature limits are reached within a geographic range, populations become more homozygous for their isozyme loci. Alternatively, this phenomenon could be a result of isolation, small population sizes and random genetic drift for these populations on the fringes of the geographic range.

Stocking and Hybridization

Besides its diagnostic value, allozyme variation at the *CK-A2** locus can also be used to evaluate the degree of hybridization or mixing between northern and southern populations (Stoneking et al. 1981). Eighteen of 28 populations had both *CK-A2*122* and *CK-A2*100* alleles, indicating a mixing of southern and northern brook trout genomes. There are several possible origins of the observed differences in Georgia brook trout genotypes. The original distribution of brook trout in Georgia is unknown due to widespread changes in land use and the introduction of hatchery-reared rainbow, brown, and brook trout prior to the time when accurate records were kept. Historically, brook trout may have been absent from many streams that now contain wild reproducing populations. Known stockings of hatchery-reared brook trout came from at least 5 different federal and state hatcheries (Table 5). It is reasonable to assume that many of these hatchery fish were of northern origin, but at least 1 hatchery, the Walhalla National Fish Hatchery in Walhalla, South Carolina, is known to have obtained wild fish to use for brood stock from streams that now contain fish identified as pure southern strain. According to hatchery personnel, they had poor success with attempts to raise pure wild fish, but crosses between the wild fish and the existing hatchery stock did well in the hatchery, and these hybrid fish were used

Table 5. Georgia brook trout, *Salvelinus fontinalis*, grouped by cluster (Rogers Similarity). Genetic type (southern, northern, or hybrid), watershed, and known stocking history are shown.

Stream name	Cluster	Genetic type	Major watershed	Sub watershed	Stocking
Hellhole	1	N10	Savannah	Tallulah	No
Goldmine	1	N10	Savannah	Chattooga	Yes
Jessie	1	NH8	Savannah	Tallulah	Yes
Dover	1	NH7	Chattahoochee		No
Davis	1	NH7	Chattahoochee		No
Winn	1	NH7	Chattahoochee		No
York	1	H6	Chattahoochee		No
Chattahoochee River	2	H4	Chattahoochee		Yes
Lovingood	2	SH3	Toccoa		No
Long	2	SH3	Toccoa		Yes
L. Rock	2	H6, SH3	Toccoa		No
Logan	2	S1	Toccoa		No
N. P. W.F. Soque	2	S1	Chattahoochee	Soque	No
Bryant	2	S1	Toccoa		No
Emory	2	S1	Savannah	Chattooga	No
Goshen	3	H5	Chattahoochee	Soque	No
Holcomb	3	H5	Savannah	Chattooga	Yes
Moccasin NF	3	H5	Savannah	Tallulah	No
Finney	3	H6	Savannah	Chattooga	No
Hedden	3	H6	Savannah	Chattooga	No
Firescald	3	H6	Savannah	Tallulah	No
Thomas	4	SH3	Tennessee	Little Tenn.	No
Popcorn	4	SH3	Savannah	Tallulah	Yes
Rough	4	S1	Alabama	Jacks	No
High Shoals	4	S1	Tennessee	Hiawassee	Yes
Gizzard	4	S1	Tennessee	Hiawassee	No
Keener	4	S1	Tennessee	Little Tenn.	Yes

for stocking purposes for many years. In addition to fish stocked from hatcheries, wild fish were transferred from stream to stream in buckets by early U.S. Forest Service personnel (Monte Seehorn, pers. commun.). There is no doubt that early settlers moved fish from stream to stream as well, and private efforts by groups of early anglers to improve fishing included stocking and the transfer of fish from stream to stream.

Based on the available information, it is apparent that most of the existing brook trout populations could have developed in any one or more of the following ways. The stream in question may have contained no brook trout prior to stocking, and the present population is the direct result of these stockings. This situation may be more common than it first appears since both old and recent sampling records indicate some streams, such as Goldmine Creek, existed that had no fish of any kind. Usually these are streams in very steep areas, above a barrier falls that would prohibit the movement of fishes from below. Any of the 3 types of brook trout (northern, southern, or hybrid) could have originated in this way, since we know that northern and

hybrid types were stocked freely in the region, and most likely some movement of native southern stocks occurred as well.

Streams may have had an existing southern population that was hybridized by the stocking of northern or hybrid types. Streams may have originally had an existing southern population and were subsequently stocked with fish of northern or hybrid ancestry, but no genetic contamination occurred, and the populations remained relatively unchanged. This possibility appears to be likely for both Keener and High Shoals creeks, where the current brook trout populations are classified as S1, and are assumed to be of pure southern ancestry. However, both of these streams were extensively stocked during the 1960s (Table 5) with fish from several different state and federal hatcheries, yet these fish appear to have made little or no contribution to the current gene pool. This is further evidence to support the premise that stocking of wild conspecifics usually does not result or results in minimal genetic impact, and draws into question the usefulness of many stocking programs. Stocking and movement of fish must have been widespread, even prior to the late 1950s when record keeping began. No stocking records exist for Hellhole, Davis, Dover, Winn, York, Little Rock, or Finney creeks (Table 4), but they all contain either northern or hybrid populations that could have arisen by no other means.

The frequency of northern allele *(CK-A2*100)* in the 18 hybrid populations varied (Table 2). Most of these hybrid populations had this allele at a lower frequency (0.025 to 0.475), whereas 4 hybrid populations had a considerably higher frequency of *CK-A2*100* allele (0.553 to 0.947). Many of these hybrid populations may have been established by stocking streams devoid of brook trout with F1 northern-southern hybrids. If that is the case, there appears to be a general trend in these hybrid populations of an allele frequency shift in favor of the southern *CKA2** allele, possibly indicating a selective advantage for this allele in the Georgia environment. If these populations were established through introduction of northern fish followed by introgression, the preponderance of the southern allele could again be a result of selection or from the introduction of small numbers of northern progenitors resulting in the low gene frequency for the northern allele.

Hardy-Weinberg Equilibrium

Several loci in various populations were not at Hardy-Weinberg equilibrium indicating that in these populations either non-random mating, migration, selection, mutation or combinations of these factors were occurring. Obviously, migration as perpetuated by man has occurred, but this alone cannot explain the results of the Hardy-Weinberg results. *GPI-B** was the locus most commonly not at equilibrium with excessive numbers of heterozygotes found in Rough Creek, both samplings of Logan Creek, Lovingood, Rock BF, Bryant, Dover, Davis, and Goldmine creeks, and Jessie Branch, and excess homozygotes only found in Rock KV. Excess heterozygotes were found at *GPI-A** for Rock KV, Long, and Lovingood creeks, but excess homozygotes were found at Finney Creek. Excess homozygotes were found for *ADH** in Long, Prong, Winn, and York creeks. Rough, Moccasin, and Hellhole

creeks had excess homozygotes for *LDH-C**. Davis and Long creeks and Jessie Branch had excess homozygotes for *CK-B**, but Firescald Creek had excess heterozygotes. More homozygotes than expected were observed for Moccasin and Goldmine creeks for *sAAT-1,2**, Davis and Winn creeks for *FH-1,2**, Rock BK, Moccasin and High Shoals creeks for *mMEP-1**; and Finney Creek for *ODH**. Goshen Creek had both more than expected homozygotes and heterozygotes for *GPI-B** because this population had 3 alleles for this locus.

Different types of loci, when they were not at equilibrium tended to favor either homozygotes or heterozygotes. The dehydrogenases most commonly had excess homozygotes while the other types of enzymes tended to have excess heterozygotes. The unequal distribution and type of disequilibrium among loci cannot be explained by migration and hybridization alone, as the disequilibrium should have been more widespread among all loci if migration was the only cause of the unexpected allele frequencies.

Natural populations can be subject to different forms of natural selection. The preponderance of heterozygotes at some loci could be a result of recent crossing of northern and southern strains of brook trout; however, if that were the case there should not be so many additional loci that have an excess of homozygotes. Different selective forces may be acting at different loci. Stabilizing selection could increase the number of heterozygotes, directional selection would favor one of the homozygous genotypes and disruptive selection would cause an excess of both homozygous genotypes.

Genetic Similarity

A cluster analysis of genetic similarity was constructed (Rogers et al. 1972) and the resulting dendrogram is shown in Fig. 2. Calculations using the 36 loci analyzed for all 28 populations resulted in the formation of more than 1 cluster ($P = 0.05$). All northern and strongly northern-hybrid populations formed a cluster because of northern allele frequencies, especially at *CK-A2*100*. Hellhole Creek and Goldmine Creek are the only 2 populations which appear to be pure northern. Hellhole Creek branches separately and is a very unique population in this cluster because it contains *LDH-A1*72*, and *sMDH-4*170* alleles at higher frequencies compared to rest of the samples. If natural gene banking were employed, this population is different and would be kept distinct from Goldmine Creek because of large differences at *sAAT-3**, *LDH-C**, and many other loci. Of the remaining 3 northern-like populations, Jessie Branch is very similar to Davis Creek, and Goldmine Creek and Dover Creek are similar to each other. Winn Branch and York Creek populations, which are hybrid populations that are strongly northern, formed a separate cluster because these populations had higher frequencies of *sAAT-1,2*100* and *ADH*205* alleles.

A third cluster, formed by streams containing strongly hybridized populations, included North Moccasin, Finney, Holcomb, Firescald, Hedden, and Goshen Creeks. All populations in these streams were classified or either H5 or H6.

Little Rock Creek KV, Little Rock Creek BF, Lovingood Creek, Bryant Creek,

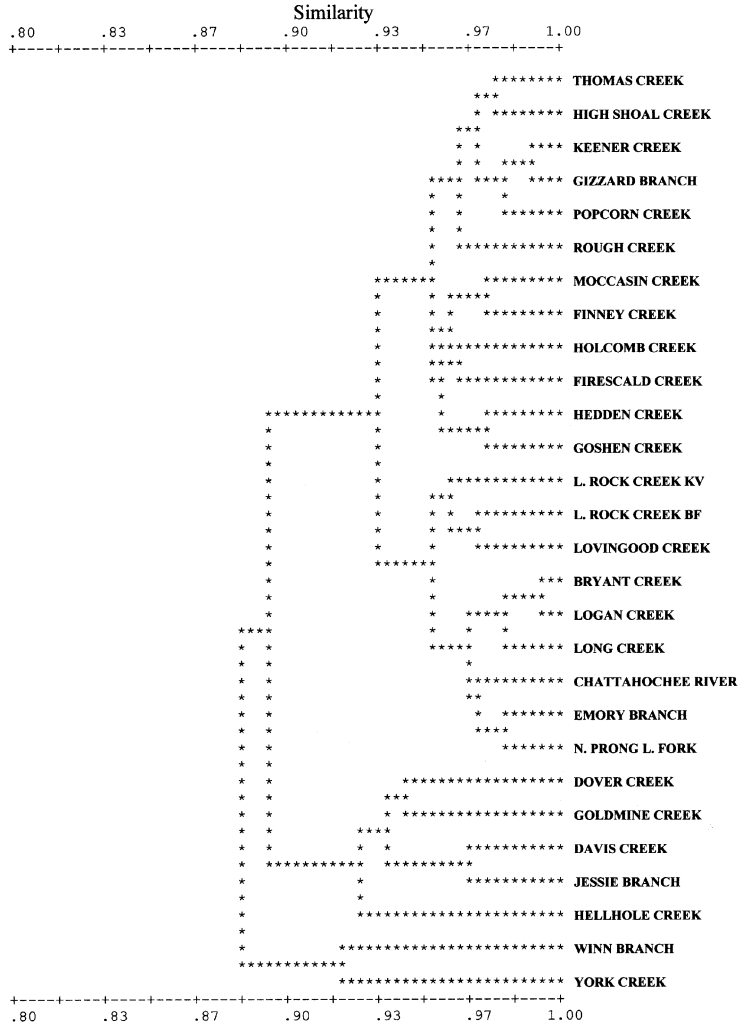

Figure 2. Dendrogram showing the genetic relatedness (Rogers similarity, Rogers 1972) of brook trout, *Salvelinus fontinalis*, populations from Georgia.

Logan Creek, Long Creek, Chattahoochee River, Emory Branch, and Left Fork Soque River form a cluster. Based on CK allele frequencies, Logan Creek, Emory Branch, Left Fork Soque River, and Bryant Creek would be pure southern. This cluster contained populations with strongly southern allele frequencies. In this cluster, there are 2 major types of southern brook trout characterized by a high frequency of the *sAAT-3*100* allele.

The remaining populations, mostly the hybrids and some strongly southern, formed a fourth major cluster. This represents the second form of southern brook trout characterized by fixation of the *sAAT-3*93* allele. Keener Creek and Logan Creek fell into separate clusters because of alternate allele presence at *sAAT-1,2*100* locus. Within this group Keener Creek, Gizzard Creek, Rough Creek and High Shoals Creek are southern, although Rough Creek and High Shoals Creek have greater frequencies of alleles sometimes found in northern populations.

Two forms of southern brook trout exist in Georgia and should be managed as distinct units until the significance of the difference is ascertained. Keener, Gizzard Branch, Rough and High Shoals Creeks are fixed for the *sAAT-3*93* allele while Logan Creek, Bryant Creek, and Left Fork Soque River are fixed for the *sAAT-3*100* allele, and Emory Branch has a high frequency of the latter allele.

Management Implications

The genetic composition and origins of brook trout populations in the Southeastern United States are important for management and conservation purposes. Allozyme analyses from the previous and current studies showed that southern brook trout populations are genetically distinct from northern and hatchery stocks of northern ancestry. A large amount of genetic variability exits in Georgia brook trout populations and several distinct potential management units exist. The relative performance of these different genetic groups should be studied and correlated to their ancestry and isozyme genotypes. However, initial observations indicate that the best brook trout fishing streams are evenly distributed among southern, northern, and hybrid populations, and that under the current circumstances environmental factors such as stream fertility are much more important than genetic composition for establishment of quality populations.

Eight (29%) of the 28 populations analyzed were classified as southern Appalachian, 2 (7%) were classified as northern and the remaining 18 (64%) were hybrid (Tables 2, 3). This data and that of Kriegler et al. (1995), Dunham et al. (unpubl.), Habera et al. (unpubl.), and Galbreath et al. (unpubl.) have found similar percentages of southern, northern, and hybrid populations in North Carolina and Tennessee.

Population differences were large as F(IT) and F(ST) were 0.455 and 0.474, respectively. These values indicate the existence of distinctive lines and differences among watersheds. In contrast, F(IS) was very near zero, –0.035, indicative of random breeding within populations. However, when the F(IS) values are examined for individual loci, many deviate substantially from zero in either the positive or negative direction and average near zero. For some loci distinct lines or families appear to exist within populations, but for other loci there appears to be heterozygote advantage. The distribution of the F(IT) and F(ST) for individual loci support the conclusion of the overall values for these statistics that distinct population differences exist. Significant differences were found among populations ($P = 0.05$).

Present distribution patterns enable managers to better understand the current

distribution of brook trout genotypes, but the determination of historic distribution patterns in Georgia remains highly problematic. With that in mind, it would be naive to attempt to "restore" southern populations to their former range, since there is no accurate way to determine what that range may have been in many cases.

However, logical management options exist. The southern strain of brook trout is widely distributed across the southeast, the habitat of the vast majority of these populations is generally well protected on federal lands, and the danger of the extirpation of the strain appears to be quite small. Because of the relatively short life span and early age of sexual maturity for brook trout in Georgia, it is unlikely that angling could ever pose a threat to population viability. Even with that in mind, managers should be aware of any potential threats, physical or genetic, to populations classified as S1 or S2, and take steps to minimize these threats. Since widespread stocking of trout into brook trout waters has not taken place for many years, the primary threats to existing brook trout populations in Georgia are habitat loss due to acid rain and climate change due to global warming. Some unauthorized transfer of fish by anglers may still occur, but haphazard transfers by state and federal management agencies are no longer done.

Management agencies do have an interest in re-establishing brook trout in a few selected streams where other trout species, particularly rainbows, may have extirpated previously existing brook trout populations. When a management agency considers the movement or stocking of brook trout in Georgia streams, the following recommendations should be considered.

Where available, southern fish from the same drainage would be preferred for the re-establishment of extirpated brook trout populations. Fish with a high degree of southern characteristics from a nearby drainage would be an acceptable alternative, if northern or northern hybrid types were the only brook trout available within the target drainage. The stocking of any brook trout on top of existing southern populations would normally be considered inappropriate.

In regards to northern and hybrid populations which are not native or natural to Georgia, more than 1 option or philosophy exists. One philosophy would be that no special genetic management concerns are needed for streams containing northern or hybrid populations since the present genetic composition of these populations were established artificially and thus do not represent legitimate examples of natural genetic diversity. This simplifies management, makes it more feasible, and more cost effective. Although artificial, some of these populations could serve as genetic resources or gene banks for future utilization by Georgia or other states as the knowledge base increases for the relationship between genotype and performance.

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