Characterization of the Genetic Structure among Brook Trout in LeConte Creek, Tennessee

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Abstract: Beginning in the early 1900s, the original range and abundance of brook trout within Great Smoky Mountains National Park (GRSM) was drastically reduced due to landscape alterations resulting from increased logging and introduction of nonnative salmonid species. Consequently, brook trout populations retreated to the headwaters of most streams, resulting in geographic isolation due to waterfalls and eventual genetic differentiation of the fish inhabiting these streams. In an effort to reestablish LeConte Creek's extirpated brook trout population, GRSM fisheries managers collected fish from three streams known to support populations of genetically pure "Southern Appalachian" brook trout (Greenbrier, Cosby and Indian Camp Creeks). Brook trout were collected, pooled, and transplanted into LeConte Creek. Upon successful completion of the stocking effort, managers were interested in determining if all three streams contributed equally to the reestablished population or whether one population was better adapted to the conditions of LeConte Creek than another. Seven years post-stocking, minimally-invasive tissue samples were collected from 50 randomly selected brook trout, genotyped at 10 polymorphic microsatellite loci, and compared to the parental populations. Our preliminary, individual-based analyses suggest the LeConte Creek collection represents three separate, but co-existing, populations as 76% of the sampled fish were found to have parents that originated from either same-stream matings (i.e., positive assortative mating) or from some form of post-reproductive isolating mechanism that selected against individuals resulting from out-crossed matings.

Key words: Salvelinus fontinalis, brook trout, assortative mating, microsatellite DNA, population structure

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Prior to the early 1900s, brook trout (*Salvelinus fontinalis*) range was restricted to bodies of water east of the Saskatchewan and Mississippi rivers and into the cold streams of northeast Iowa, southeast Minnesota, and Canada. Populations thrived in the north within the Hudson Bay and Labrador in Canada and in the south to the French Broad, Savannah, and Tennessee rivers along the Appalachian Mountains (Brandes 1978). This range included habitat between the states of Tennessee and North Carolina in the Great Smoky Mountains National Park (GRSM). Within GRSM, brook trout were the only indigenous salmonid species present (Lohr 1985) and the first trout species monitored in the 1930s (Brandes 1978). Originally, brook trout occupied GRSM waters from 610 m above mean sea level up into stream headwaters (Robinette 1978).

Since 1900, brook trout range has declined approximately 75% (Davis 1994). Brook trout range within the GRSM decreased primarily due to landscape alterations and competition from stocked nonnative salmonid species (Lohr 1985). This decrease in range was partially attributed to the decline in stream health due to extensive logging which continued until 1936 (Lohr 1985). Massive clearcutting of trees on steep slopes, while using splash dams to transport timber downstream, resulted in excessive sedimentation and erosion into the Park's streams (Davis 1994). This affected physical factors of the Park's waterways by introducing large woody debris and increasing the overall complexity of the waterway through scoured stream bottoms (Schlosser 1991). It was shown in Shenandoah National Park that where streams flowed through areas impacted by deforestation, urban construction and agriculture were often unsuitable for trout populations (Roghair et al. 2002).

Decreased brook trout range has also been attributed to increased competitive pressures with the introduction of rainbow (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) into historically brook trout dominated waters. Lennon (1967) stated that brook trout populations would not extend downstream back into the original range due to increased competition with rainbow trout. Unpublished studies suggest an approximate 45% decline in strictly brook trout- inhabited streams between 1950 and 1970 in GRSM, leading to the cessation of brook trout fishing within the park in 1975 (Brandes 1978). Extensive surveys from 1972–1977 (Kelly et al. 1980) showed that brook trout range within the GRSM drastically decreased with a concomitant increase in rainbow trout range (Lohr 1985). Larson and Moore (1985) found that during the approximate 40-year span between previous studies, allopatric brook trout distribution and range decreased from 94.1 km stream distance, whereas sympatric and allopatric rainbow trout range increased by 94.2 km (Lohr 1985). As a result of habitat fragmentation and direct competition from non-native salmonids, brook trout populations in GRSM receded into the isolated stream headwaters, and population numbers decreased (Roghair et al. 2002). Brook trout range within the GRSM is limited to the headwaters 1,066 m above mean sea level (Robinette 1978).

Contemporary molecular genetic techniques can identify evolutionary lineages and allow improved understanding of how environmental factors contribute to the magnitude of evolutionary processes acting on a population (Hebert et al. 2000). Microsatellite markers provide information on processes occurring within and among populations such as migration, inbreeding, colonization, and extirpation (King et al. 2006). This information can indicate genetic distinction among populations when undetected or faint morphological differences fail to indicate differences among populations (King et al. 2006). Current genetic methodology also serves as a conservation tool, identifying reproductively-isolated populations which according to the National Park Service (2005) encourage "the delineation of management units allowing assessment of conservation priorities in existing populations." Therefore it is necessary that the level and range of genetic diversity and variation within and among populations be determined if reproductively-isolated populations are to be properly maintained (Jones et al. 1996).

LeConte Creek (LC) drains Mount LeConte, one of the highest mountains in the eastern United States, before flowing into the West Prong of the Little Pigeon River in the city of Gatlinburg, Tennessee. The loss of brook trout in LC led to efforts by GRSM management to reestablish the creek's brook trout population. In 1999, fisheries managers selected three geographically proximal source populations from the same drainage basin, Indian Camp Creek, Greenbrier Creek, and Cosby Creek in Cocke County, Tennessee, from which brook trout were removed and transplanted to LC. Although all three populations of brook trout were from the same drainage as LC, habitat fragmentation (from historic land-use and elevation) caused the populations to retreat to the headwaters of each creek.

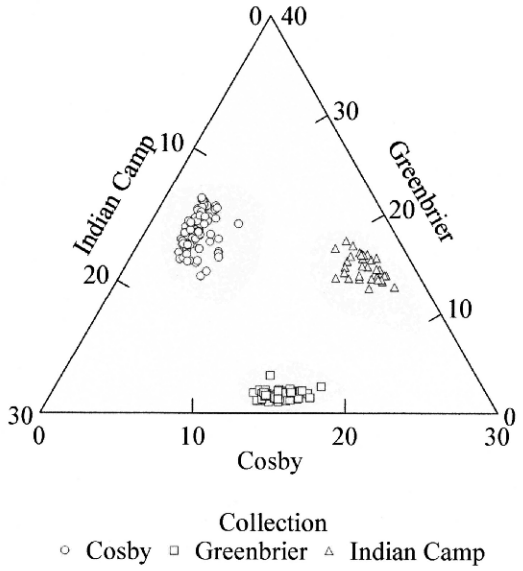

Figure 1. Triangular plot of assignment scores depicting the differentiation among brook trout (*Salvelinus fontinalis*) collected from three source populations (Cosby, Greenbrier, and Indian Camp creeks) used to found a population in LeConte Creek, Great Smoky Mountains National Park, Tennessee. Assignment tests were performed using the program (GENECLASS 2.0). Graph generated from assignment scores using SYSTAT 11.0.

Following successful completion of the stocking effort and the subsequent genetic analysis of the transplanted fish, managers became aware of the high degree of genetic differentiation that existed among the three parental brook trout populations (Figure 1). This led to the primary objective of the study: to describe the genetic composition of the LC population in order to determine whether each population was equally represented or if one parental population may be better adapted to the conditions of LC. Here we describe the characterization of the genetic diversity and variation of LC brook trout.

Methods

Tissue Collections

In September 1999, 320 brook trout were collected, placed into the same hauling trailer, and transferred to LC from Indian Camp Creek (ICC; n = 103), Greenbrier Creek (GC; n = 106) and Cosby Creek (CC; n =111). A total of 168 fish from the three source populations were sampled for establishment of a genotypic baseline: CC (n = 80), GC (n = 55), and ICC (n = 33). Adipose tissue

was taken from each fish and placed into 95% absolute ethanol for preservation. Adipose fin clips (n = 50) were obtained from LC brook trout during summer 2006. All ethanol preserved tissue samples were forwarded to the US Geological Survey, Leetown Science Center, Kearneysville, West Virginia, for DNA extraction.

DNA Extraction

Genomic DNA was obtained from tissue samples (in 95% ethanol) using minor modifications of the Puregene DNA extraction kit (Gentra Systems) and resuspended in TE (10 mM Tris, pH 8.0, 1 mM EDTA) (King et al. 2006). DNA integrity was visually determined on 1% agarose gels.

Microsatellite DNA Amplification

Ten microsatellite loci were amplified in the individuals sampled. Five master mixes were utilized for the 10 markers: (A) SfoB52 and SfoD75; (B) SfoD91; (C) SfoC113, SfoC115, SfoC86, SfoC88, and SfoC129; (D) SfoC28; and (E) SfoD100 (TLK; unpublished primers). Forward primers were labeled with one of four fluorescent dyes (*fam*, *vic*, *pet*, or *ned*). Stock reagent concentrations were: 10 mM trisHCl [pH 8.3] buffer, 25 mM $MgCl_2$, 10 mM dNTPs, and 5 mM, *Taq* DNA polymerase. Master mix components, volumes, and concentrations are provided in Table 1. Amplifications were conducted using a PTC-225 Thermal Cycler (MJ Research) using the following program: initial denaturing at 94 C for 2 min, 35 cycles of 94 C for 45 sec, 56 C for 45 sec, 72 C for 2 min, and final extension at 72 C for 10 min.

Fragment Analysis

PCR product was diluted with deionized water and an aliquot added to LIZ-labeled size standard (Applied Biosystems) and deionized Hi-Di formamide. Capillary electrophoresis was executed using Applied Biosystems PRISM 3100 Genetic Analyzer. Genescan 2.1 analysis software was utilized to determine the size of fluorescently labeled DNA fragments (King et al. 2001). Allelic and genotypic data were scored, binned and output using Genotyper

Table 1. Five master mixes used to amplify 10 microsatellite loci in brook trout (*Salvelinus fontinalis*) collections from Great Smoky Mountain National Park. Forward primers are labeled with fluorescent dye (*fam*, *vic*, *pet*, or *ned*). Stock concentrations of reagents were: 5X trisHCl [pH 8.3] PCR buffer, 25 mM $MgCl_2$, 10 mM dNTPs, and 5 units/uL, *Taq* DNA polymerase.

Master mix A		Master mix B		Master mix C		Master mix D		Master mix E	
Reagent concentration	Vol. (*u*L)	Reagent concentration	Vol. (*u*L)	Reagent concentration	Vol. (*u*L)	Reagent concentration	Vol. (*u*L)	Reagent concentration	Vol. (*u*L)
dH20	6.12	dH20	2.71	dH20	3.76	dH20	2.91	dH20	3.43
1X bufer	4.00	1X buffer	2.00	1X buffer	4.00	1X buffer	2.00	1X buffer	2.00
3.75 mM $MgCl_2$	3.00	3.75 mM $MgCl_2$	1.50	3.75 mM $MgCl_2$	3.00	3.75 mM $MgCl_2$	1.50	3.75 mM $MgCl_2$	1.50
0.3175 mM dNTPs	2.54	0.3175 mM dNTPs	1.27	0.3175 mM dNTPs	2.54	0.3175 mM dNTPs	1.27	0.3175 mM dNTPs	1.27
0.18 uM *SfoB52 fam*	0.72	0.23 uM *SfoD91a pet*	0.46	0.15 uM *SfoC113 fam*	0.60	0.18 uM *SfoC28 ned*	0.36	0.05 uM *SfoD100 vic*	0.10
0.18 uM *SfoB52*	0.72	0.23 uM *SfoD91a*	0.46	0.15 uM *SfoC113*	0.60	0.18 uM *SfoC28*	0.36	0.05 uM *SfoD100*	0.10
015 uM *SfoD75 ned*	0.60	0.05 units/uL *Taq*	0.10	0.20 uM *SfoC115 fam*	0.80	0.05 units/uL *Taq*	0.10	0.05 units/uL *Taq*	0.10
0.15 uM *SfoD75*	0.60	DNA template	1.50	0.20 uM *SfoC115*	0.80	DNA template	1.50	DNA template	1.50
0.05 units/uL *Taq*	0.20			0.06 uM *SfoC86 vic*	0.24				
DNA template	1.50			0.06 uM *SfoC86*	0.24				
				0.06 uM *SfoC88 vic*	0.24				
				0.06 uM *SfoC88*	0.24				
				0.15 uM *SfoC129 pet*	0.60				
				0.15 uM *SfoC129*	0.60				
				0.06 units/uL *Taq*	0.24				
				DNA template	1.50				
Total	20.0	Total	10.0	Total	20.0	Total	10.0	Total	10.0

3.6 fragment analysis software (Applied Biosystems) (King et al. 2001).

Data Analysis

Observed genotype frequencies were tested for consistency with Hardy-Weinberg and linkage equilibrium expectations using randomization tests implemented by GENEPOP 4.0 (Raymond and Rousset 1995). The Hardy-Weinberg test used the Markov chain randomization test of Guo and Thompson (1992) to estimate exact two-tailed *p* values for each locus in each sample. Tests for linkage equilibrium were conducted using the randomization method of Raymond and Rousset (1995) for all pairs of loci. Bonferroni adjustments (Rice 1989) determined statistical significance for these and all other simultaneous tests. Allele counts, private alleles, average observed (H_O) and expected (H_E) heterozygosities, and estimations of pairwise population differentiation (F_{ST} and R_{ST}) among populations were determined using GenAlEx 6.0 (Peakall and Smouse 2006). F_{ST} assumes allelic diversity results from migration and gene drift, while R_{ST} also measures mutational differences between alleles.

Using GeneClass 2.0 (Piry et al. 2004), maximum likelihood assignment tests were used to determine the likelihood of each source individual's multilocus genotype being found in the collection (CC, GC, or ICC) from which it was sampled and each LC individual's multilocus genotype being found among one of the source populations. To avoid bias introduced by counting the current individual in its population when estimating allelic frequencies, each individual, when tested, was excluded from its population sample (i.e., the "leave one out" procedure). In the event of null frequencies, a constant likelihood of 0.01 was assumed.

Cervus 3.0 was used to conduct a parentage analysis using an offspring's (LC fish's multilocus genotype) data to test against that of candidate parents from the source populations (ICC, GC, and CC). Parentage was assigned to the most likely candidate pair (Kalinowski et al. 2006).

Results

Brook trout from LC (n = 50) were genotyped at 10 microsatellite DNA loci and compared to the allele frequencies and genotypes representative of the source populations: CC (n = 80), GC (n = 55), and ICC (n = 33) (Table 2). Genetic diversity was sufficient at the 10 loci to generate unique multilocus genotypes for each fish from the source populations (CC, GC, and ICC) and the founded population (LC). In general, allelic diversity and variation were lowest in GC (with four monomorphic loci) and highest in LC. Allelic diversity (N_a) averaged 2.9 alleles/locus and ranged from 1.9 (GC) to 3.4 alleles/locus (ICC and LC). The effective number of alleles (N_e) averaged 1.81 alleles/locus and ranged from 1.4 (GC) to 2.27 (LC). Average observed (H_O) and expected (H_E) heterozygosities were similar (36% and 35%, respectively) (Table 2). H_O ranged from 19% (GC) to 47% (LC). Overall F-statistics for each locus suggested little indication of inbreeding (F_{IS} = –0.032) combined with the presence of a high degree of population differentiation (F_{ST} = 0.25).

A high degree of differentiation was also present in the number and frequencies of private alleles. Of the total number of alleles observed (n = 50), 16 (32%) were found exclusively within one population: 5 in CC, 10 in ICC, 1 in LC. No private alleles were observed in GC (Table 3). The frequencies of the private alleles ranged from < 1% (SfoD91 and SfoC88 in CC) to > 24% (SfoD75 in CC).

All populations, except CC at locus SfoC28, conformed to the expectations for Hardy-Weinberg equilibrium (α = 0.05, $P < 0.05$). Similarly, a low level of linkage disequilibrium was observed in the three source populations as only 5 of 45 (11%) pair-wise locus comparisons were statistically significant ($P < 0.05$) in CC. Only a single comparison was significant after Bonferroni correction for multiple tests (α = 0.05, $P < 0.001$). However, significantly more linkage disequilibrium was observed in the founded population (LC) than would be expected by chance as 14 of 45 (31%) pair-wise comparisons were found to be statistically significant ($P < 0.05$). A total of four (9%) comparisons were significant after Bonferroni correction for multiple tests. No linkage disequilibrium was observed in GC or ICC.

A high degree of population level genetic differentiation was observed in all pair-wise comparisons as the average F_{ST} was 0.272. The greatest levels of differentiation were observed between GC and ICC (0.364) and CC and ICC (0.347) and the lowest was between ICC and LC populations (0.156) (Table 4). Pair-wise R_{ST} values averaged 0.186 and were, on average, 1.7 times smaller than corresponding F_{ST} values (Table 4). R_{ST} values ranged from 0.098 (CC and LC) to 0.326 (GC and ICC). F_{ST} to R_{ST} ratio values spanned 0.862 (CC and GC) to 3.083 (CC and LC) (Table 3). All F_{ST} and R_{ST} values were statistically significant (α = 0.05, $P < 0.008$).

Of 320 brook trout originally stocked into LC in 1999, 111 (35%) were from CC, 103 (32%) from ICC, and 106 (33%) from GC. Maximum likelihood assignment tests correctly assigned 100% of the 168 fish genotyped from these source populations (Figure 1). Of the 50 LC brook trout sampled and genotyped for this comparison, one-half (n = 25) were assigned to ICC, 32% (n = 16) to GC and 18% (n = 9) to CC (Figure 2). The percentages of LC individuals with candidate parentage including a fish from CC were lower (CC/CC = 16%, CC/GC = 0%, and CC/ICC = 6%) than

Table 2. Allele frequencies at 10 microsatellite DNA markers, sample sizes ( ), F-statistics, average number of alleles (N_a), average number of effective alleles (N_e), and observed (H_o) and expected (H_e) average heterozygosities for four collections of brook trout from Great Smoky Mountains National Park.

Locus	Allele size	Cosby Cr.	Greenbrier Cr.	Indian Camp Cr.	LeConte Cr.	F-statistics F_{IS}	F-statistics F_{ST}
		(80)	(55)	(33)	(50)		
SfoB52	215	0.125	0.000	0.063	0.020	–0.106	0.047
	227	0.875	1.000	0.938	0.980		
SfoC28	175	0.013	0.000	0.000	0.000	–0.049	0.263
	179	0.127	0.000	0.000	0.040		
	183	0.000	0.000	0.045	0.000		
	185	0.000	0.639	0.000	0.130		
	187	0.715	0.000	0.470	0.230		
	201	0.000	0.000	0.485	0.340		
	205	0.146	0.241	0.000	0.070		
	207	0.000	0.120	0.000	0.190		
SfoC86	110	0.000	0.000	0.015	0.000	–0.011	0.006
	113	0.994	1.000	0.985	0.990		
	119	0.000	0.000	0.000	0.010		
SfoC88	181	0.000	0.000	0.030	0.000	–0.006	0.332
	184	0.000	0.000	0.015	0.000		
	190	0.000	0.000	0.015	0.000		
	193	0.590	0.000	0.000	0.190		
	199	0.160	0.264	0.924	0.470		
	202	0.000	0.000	0.015	0.000		
	211	0.006	0.000	0.000	0.000		
	214	0.244	0.736	0.000	0.340		
SfoC113	130	0.800	0.000	0.121	0.240	0.002	0.521
	136	0.000	1.000	0.864	0.760		
	139	0.200	0.000	0.015	0.000		
SfoC115	235	0.475	0.973	0.212	0.690	–0.107	0.255
	237	0.000	0.027	0.545	0.140		
	239	0.363	0.000	0.242	0.080		
	313	0.163	0.000	0.000	0.090		
SfoC129	230	0.294	0.291	0.000	0.060	–0.096	0.131
	233	0.706	0.709	1.000	0.940		
SfoD75	176	0.244	0.000	0.000	0.000	0.025	0.425
	196	0.000	0.000	0.516	0.230		
	200	0.000	0.000	0.453	0.100		
	212	0.000	1.000	0.016	0.450		
	216	0.138	0.000	0.016	0.010		
	220	0.619	0.000	0.000	0.210		
SfoD91	232	0.188	0.536	0.422	0.480	0.009	0.204
	236	0.006	0.000	0.188	0.010		
	240	0.000	0.155	0.313	0.210		
	252	0.000	0.000	0.016	0.000		
	256	0.013	0.300	0.000	0.040		
	260	0.788	0.009	0.063	0.260		
	264	0.006	0.000	0.000	0.000		
SfoD100	206	0.000	0.000	0.078	0.000	0.018	0.312
	214	0.000	0.000	0.031	0.000		
	218	0.000	0.000	0.063	0.000		
	250	0.231	0.973	0.031	0.370		
	258	0.025	0.000	0.203	0.200		
	262	0.688	0.027	0.594	0.430		
	266	0.056	0.000	0.000	0.000		
Mean						–0.032	0.249
SE						0.017	0.051

	CC		GC		ICC		LC		Overall	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
[a]N_a	3.00	0.39	1.90	0.31	3.40	0.50	3.40	0.48	2.93	0.23
[b]N_e	1.78	0.15	1.40	0.17	1.81	0.24	2.27	0.35	1.81	0.13
[c]H_o	0.38	0.05	0.19	0.07	0.38	0.10	0.47	0.10	0.36	0.04
[d]H_e	0.40	0.06	0.20	0.08	0.35	0.08	0.44	0.09	0.35	0.04

a. N_a = Number of different alleles
b. N_e = Number of effective alleles = 1 / (sum pi^2)
c. H_o = Observed heterozygosity = Number of heterozygotes / *n*
d. He = Expected heterozygosity = 1 – sum pi^2
Where pi is the frequency of the ith allele for the population and sum pi^2 is the sum of the squared population allele frequencies.

Table 3. Private alleles and their frequency for 10 microsatellite DNA loci surveyed in brook trout from Crosby Creek (CC), Indian Camp Creek (ICC), and LeConte Creek (LC) of Great Smoky Mountains National Park.

Population	Locus	allele (bp)	Frequency
CC	D75	176	0.244
CC	D91	264	0.006
CC	D100	266	0.056
CC	C28	175	0.013
CC	C88	211	0.006
ICC	D91	252	0.016
ICC	D100	206	0.078
ICC	D100	214	0.031
ICC	D100	218	0.063
ICC	C28	183	0.045
ICC	C86	110	0.015
ICC	C88	181	0.030
ICC	C88	184	0.015
ICC	C88	190	0.015
ICC	C88	202	0.015
LC	C86	119	0.010

Table 4. F_{ST} and R_{ST} values and F_{ST}/R_{ST} ratios indicating the degree of genetic differentiation at 10 microsatellite DNA loci compared among Crosby Creek (CC), Indian Camp Creek (ICC), Greenbrier Creek (GC), and LeConte Creek (LC) of Great Smoky Mountains National Park.

Pop 1	Pop 2	F_{ST}	R_{ST}	F_{ST}/R_{ST}
CC	GC	0.208	0.241	0.862
CC	ICC	0.347	0.207	1.677
GC	ICC	0.364	0.326	1.117
CC	LC	0.303	0.098	3.084
GC	LC	0.256	0.127	2.013
ICC	LC	0.156	0.116	1.336
Mean		0.272	0.186	1.681

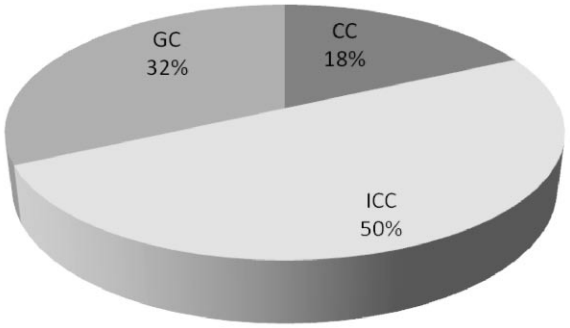

Figure 2. Pie chart depicting the assignment of LeConte Creek brook trout to each of three source populations (CC, Cosby Creek; GC, Greenbrier Creek; ICC, Indian Camp Creek).

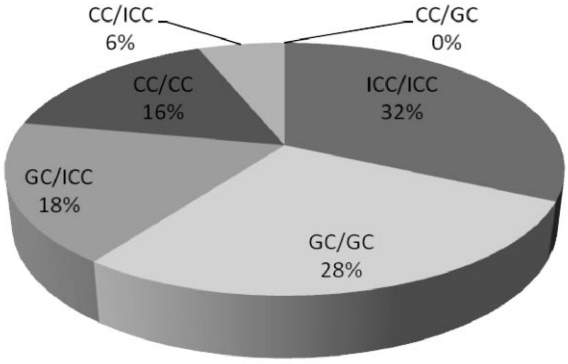

Figure 3. Percentage of LeConte Creek population assigned to each parental lineage combination (i.e., both parents from the same source population) (CC, Cosby Creek; GC, Greenbrier Creek; ICC, Indian Camp Creek).

lineage pairs involving GC and ICC individuals (GC/GC = 28%, GC/ICC = 18%, and ICC/ICC = 32%) (Figure 3). Interestingly, parentage assignment results suggested that the majority (76%) of LC brook trout were not the offspring of randomly mating pairs of fish but involved two individuals from the same source population (ICC/ICC = 32%, GC/GC = 28%, and CC/CC = 16%).

Discussion

This study represents the first survey of microsatellite DNA variation in native southern Appalachian brook trout populations. The findings suggest the pattern of genetic variation observed among the three source populations is consistent with that identified previously in surveys of allozyme (McCracken et al. 1993, Kriegler et al. 1995) and mtDNA restriction fragment length polymorphism (Hayes et al. 1996), although the level of within population resolution (e.g., unique multilocus genotypes) and within drainage differentiation are greater than were previously possible with other genetic markers. In their entirety, these research findings suggest deep genetic discontinuities within the Pigeon-French Broad River system's watershed that could indicate adaptive significance, reflect vicariant geographic events, and/or indicate severe and repeated stochastic events that have facilitated gene drift.

The three source populations of brook trout compared in the present study, although geographically proximal and representing the same drainage sub-basin, have apparently experienced longstanding reproductive isolation illustrated by the accretion of considerable genetic differentiation at 10 microsatellite DNA loci. The magnitude of the observed differentiation was substantial and supported by highly significant findings for every statistical comparison. The strong structuring of nuclear multilocus genotypes among the three source populations warrants consideration as distinct management units.

Previous studies of genetic diversity using microsatellite markers in salmonid species report numbers as low as 1.9 alleles per locus in populations of bull trout (*Salvelinus confluentus*) in the northwestern United States (Costello et al. 2003) to numbers as high as 14.8 alleles per locus in brown trout (*Salmo trutta*) in Norway (Sonstebo et al. 2007). The results from the present study, 2.9 alleles per locus, indicate a moderate level of genetic variation within the source CC, GC, and ICC populations. In addition, there is close agreement between observed and expected heterozygosities (H_O and H_E, respectively) for all microsatellite markers (Table 2), indicating no significant level of inbreeding present at each locus within each population.

Parentage analyses suggest the presence of morphological, behavioral, ecological, and/or evolutionary differences unique to each population, persuading individuals to mate with those similar to themselves or the presence of post-reproductive isolating mechanisms. Parentage analyses indicated that 76% of the sampled LC fish were produced from matings between parents from the same stream of origin: 32% from ICC/ICC, 28% from GC/GC, and 16% from CC/CC parental matings (Figure 3). This is a much higher overall percentage (76%) than would be expected if random mating were occurring; however, it is noteworthy that only 16% of the LC offspring were from CC/CC matings, which is much lower than the ICC/ICC (32%) and GC/GC (28%) populations and closer to the expected ratio. The distribution of sampled LC offspring (Figure 3) do show that, in some cases, fish from different streams of origin are mating and producing viable offspring, with 18% of the LC sampled individuals derived from GC/ICC parents and 6% from CC/ICC parents. However, it is notable that, of the 50 LC fish sampled, none were identified from CC/GC parental origin. These results suggest the presence of either positive assortative mating or the presence of some type of post-reproductive isolating mechanism(s).

Positive assortative mating can result from nonrandom mating occurring between phenotypically, behaviorally, or ecologically similar individuals (in this case, individuals from the same source creek). Data indicate that each source population is genetically distinct; however, it is undetermined if there are subtle phenotypic or morphological differences associated with the genetic differences existing between these populations. Post-reproductive isolating mechanisms can be behavioral, physiological, structural, or ecological differences that negatively impact the fitness of introgressed individuals. In the case of post-reproductive isolation, the individuals from the source creek population whose habitat most closely resembled LC would be most successful at adapting to the unique ecological factors of the stream, and offspring from these matings would exhibit a reproductive fitness over other offspring. In fact, previous laboratory studies exposing brook trout to various levels of salinity prior to migration into seawater found that those brook trout previously exposed to a saline environment were already acclimated to the saline and therefore had a greater likelihood of survival (Lenormand et al. 2004).

Either of these processes could explain the high percentage of offspring from matings between parents of the same source stream. However, it is not possible at this time to determine which of them is primarily responsible for the observed phenomenon. If positive assortative mating is responsible for the observed genetic distribution of the LC individuals, then three distinct populations of brook trout exist within LC and individuals are mating preferentially with others from the same source (CC/CC, GC/GC, and ICC/ICC). If post-reproductive isolating mechanisms are responsible for the observed distribution, then mating between individuals is random, but the offspring of certain mating pairs are ill-suited for adaptation to the LC environment and suffer a selective disadvantage and a concomitant decrease in their representation within the stream.

Subsequent genetic, ecological, and behavior analyses of LC and the founding populations of brook trout are underway to better understand the mechanisms resulting in this phenomenon, regardless whether positive assortative mating or post-reproductive isolation mechanisms are primarily responsible for the observed distribution of genotypes. In addition, knowing which source population(s) is (are) best adaptable to the new environment and which source population(s) is (are) most likely to interbreed will allow resource stewards to make informed decisions regarding brook trout and stream management strategies, both within the GRSM and other regions.

Management Implications

The U.S. National Park Service is committed to developing systematic approaches to inventorying composition and monitoring functions and processes of park ecosystems. Increased interest in restoring and enhancing brook trout populations in GRSM requires a better understanding of the partitioning of genetic variation within and among different drainages and the effects of past supplementation or restoration efforts. Timber harvest and the associated road and rail construction, clearing for agriculture, over-harvest, and the introduction of nonnative salmonids have all contributed to habitat compression of brook trout into the upper headwaters of GRSM (Guffey et al. 1998) resulting in what functionally appears to be veritable sky islands. Moreover, the preliminary, individual-based analyses conducted in this study suggest the recently established LeConte Creek population represents three separate, but co-existing, populations as 76% of the sampled fish were found to have parents that originated from either same-stream matings (i.e., positive assortative mating) or from some form of post-reproductive isolating mechanism that has selected against individuals resulting from out-crossed matings. This suggests that if reestablishment of a population is the primary objective, fisheries managers in GRSM should choose a single source population that contains sufficient numbers to found a new population. This will maximize the probabilities of success by avoiding competition among discrete populations within the stream segment and conserve resources. While some genetic surveys have been conducted, monitoring should be incorporated into all management efforts to identify unique genetic diversity and to prevent the potential deleterious effects associated with introductions and translocation programs.

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