Transactions of the American Fisheries Society 144:480–490, 2015

ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2015.1012300

ARTICLE

Spatial Structure of Morphological and Neutral Genetic Variation in Brook Trout

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Abstract

Brook Trout *Salvelinus fontinalis* exhibit exceptional levels of life history variation, remarkable genetic variability, and fine-scale population structure. In many cases, neighboring populations may be highly differentiated from one another to an extent that is comparable with species-level distinctions in other taxa. Although genetic samples have been collected from hundreds of populations and tens of thousands of individuals, little is known about whether differentiation at neutral markers reflects phenotypic differences among Brook Trout populations. We compared differentiation in morphology and neutral molecular markers among populations from four geographically proximate locations (all within 24 km) to examine how genetic diversity covaries with morphology. We found significant differences among and/or within streams for all three morphological axes examined and identified the source stream of many individuals based on morphology (52.3% classification efficiency). Although molecular and morphological differentiation among streams ranged considerably (mean pairwise F_{ST}: 0.023–0.264; pairwise P_{ST}: 0.000–0.339), the two measures were not significantly correlated. While in some cases morphological characters appear to have diverged to a greater extent than expected by neutral genetic drift, many traits were conserved to a greater extent than were neutral genetic markers. Thus, while Brook Trout exhibit fine-scale spatial patterns in both morphology and neutral genetic diversity, these types of biological variabilities are being structured by different ecological and evolutionary processes. The relative influences of genetic drift versus selection and phenotypic plasticity in shaping morphology appear to vary among populations occupying nearby streams.

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Received February 26, 2014; accepted January 21, 2015

Brook Trout *Salvelinus fontinalis* are a highly variable salmonid species capable of exhibiting remarkable phenotypic and molecular differentiation at extremely small spatial scales. In some cases, genetic differentiation at neutral molecular markers among neighboring populations is comparable with species-level distinctions in other taxa (Near and Keck 2005; Berendzen et al. 2009; Aunins et al. 2015). In Maryland, populations located within a few kilometers of one another, but across the eastern continental drainage divide, have been reproductively isolated for 2.7×10^6 years and are highly divergent (pairwise $F_{ST} = 0.13$–0.40; King et al. 2012; R.P.M. and T.L.K., unpublished data). Even within a local stream network, gene flow is sometimes limited, and isolation can cause genetic differentiation of geographically proximate populations (Letcher et al. 2007; Aunins et al. 2015). Similarly, the diverse suite of life history strategies expressed by Brook Trout within even a single water body can be substantial. Trophic polymorphism has been observed between pelagic and littoral Brook Trout living within the same lake (Bourke et al. 1997). Resident and anadromous Brook Trout may co-occur in many coastal streams (Morinville and Rasmussen 2003; Thériault and Dodson 2003). Growth and longevity also range widely within and among populations (Power 1980; Magnan et al. 2005; Kazyak et al. 2014). Viewed broadly, Brook Trout exhibit considerable diversity in phenotypic and molecular traits that vary across fine spatial scales.

Morphology is one of the most fundamental expressions of phenotype and is often a result of natural selection (Leinonen et al. 2008; Kingsolver et al. 2011). In salmonids, morphological variation has been associated with habitat characteristics (Quinn et al. 2001), feeding behaviors (Malmquist et al. 1992; Woods et al. 2013), migratory strategy (Letcher 2003; Morinville and Rasmussen 2008), and physiological performance (Proulx and Magnan 2002). In addition, morphological differences may enhance reproductive success (Fleming and Gross 1994; Quinn and Foote 1994) or contribute to partial reproductive isolation of sympatric individuals (Dynes et al. 1999). However, not all morphological variability among populations necessarily reflects adaptive genetic differentiation as some aspects of morphology may be selectively neutral or reflect phenotypic plasticity (Meyer 1987; Bourke et al. 1997).

Although genetic samples of Brook Trout have been collected from hundreds of populations and tens of thousands of individuals (King et al. 2012), the extent to which neutral genetic distance between population pairs is mirrored by morphological differences remains poorly understood. An approach to the problem is to test for a correlation between pairwise population differentiation in phenotypic traits and genetic markers (e.g., using a Mantel test), but there are many reasons why a correlation between morphologic and molecular diversity might break down in natural populations, and a lack of correlation says little about the potential ecological and evolutionary processes that are shaping differentiation. A complimentary approach is to compare standardized metrics of population differentiation in quantitative traits such as morphology (Q_{ST}) to that of neutral molecular markers (F_{ST}). When variation in quantitative traits is selectively neutral and determined by additive genetic effects, the expectation from evolutionary theory is for Q_{ST} and F_{ST} to be approximately equal (Whitlock 2008). Thus, when $Q_{ST} > F_{ST}$, population differentiation in traits exceeds that of neutral markers, suggesting there is a history of spatially or temporally divergent selection. Likewise, when $Q_{ST} < F_{ST}$, populations are phenotypically more similar than expected under genetic drift, providing evidence of a history of stabilizing selection that favors the same phenotype across populations. For studies of natural populations measured in situ, a strict partitioning of additive genetic variance in traits is often not possible, and in these instances Q_{ST} is often replaced by its phenotypic proxy, P_{ST} (Leinonen et al. 2006; Brommer 2011). Since the environment commonly influences phenotype, care must be taken in interpreting P_{ST} values (Pujol et al. 2008). However, they still provide a useful metric of differences among populations on the same scale as F_{ST}, and thus give insight into the relative magnitude that traits and neutral markers have diverged among populations (Leinonen et al. 2013).

Given the high variability of Brook Trout, we sought to examine differences in their morphology within versus between streams and to extend this inference to genetic data collected previously for these populations. To date, no rigorous studies have compared patterns of morphological variation among Brook Trout populations or attempted to relate these patterns to estimates of neutral genetic differentiation. Our objectives were to determine (1) whether there was a spatial structure in Brook Trout morphology in western Maryland and (2) whether these differences could be used to differentiate among populations. Additionally, we sought to (3) relate observed patterns of phenotypic differentiation to genotypic differentiation within these systems to test the hypothesis that genotypic and phenotypic similarities among populations are correlated.

METHODS

In Garrett County, Maryland, we sampled four streams known to support robust Brook Trout populations distributed over at least 3 km of stream length (Figure 1; Table 1). Two of the streams were located in the Mississippi River basin, while the other two were located across the eastern continental drainage divide in the Potomac River basin. At each stream, Brook Trout were sampled at sites in the upper reaches and lower reaches using backpack electrofishing with a buffer of at least 2.1 km between collections. Because of the linear nature of stream-dwelling populations, we expected this sampling design would allow us to capture the most divergent morphologies expressed within a stream if there was a within-stream structure to morphology. Sampling was conducted in the fall, 2–4 weeks before the observed peak of spawning activity.

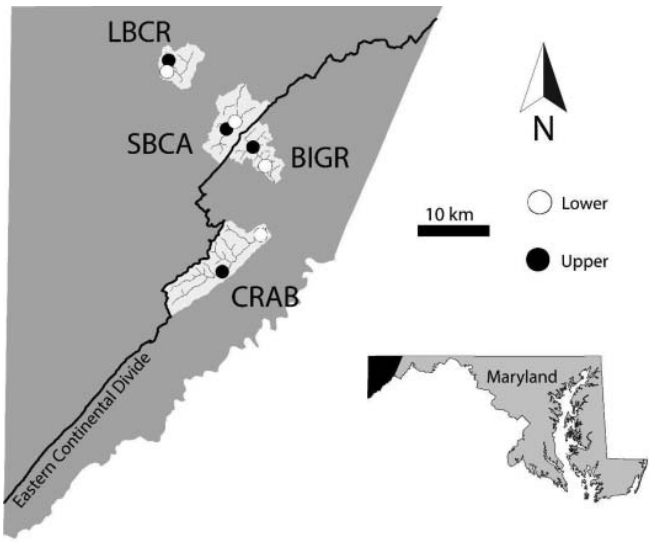

FIGURE 1. The four streams in western Maryland from which Brook Trout were collected. LBCR = Little Bear Creek, SBCA = South Branch of the Casselman River, BIGR = Big Run, CRAB = Crabtree Creek.

Captured Brook Trout were chemically anesthetized (80 mg/L tricaine methanesulfonate buffered with 0.2 mM $NaHCO_3$, pH = 7), and TL was measured and recorded. A profile image of each fish was taken using a digital camera mounted 20 cm above a pale background. In the laboratory, we used ImageJ (version 1.46r) to measure distances between anatomical landmarks (Table 2) established by Janhunen et al. (2009) on each fish. All measurements were corrected for scale by comparing field measurements of TL with laboratory measurements and converted to millimeters.

We used principal components analysis (PCA) to account for correlation among metrics and distill 20 anatomical measures into a smaller set of orthogonal variables that captured most of the variation present in the measurements. Next, we used ANCOVA to compare morphological measurements across streams and sites. For each comparison, we started with a saturated model that included a three-way interaction among stream, site, and fish length. We reduced the model using backwards selection with a critical *P*-value of 0.01.

Next, we conducted a linear discriminant analysis using the MASS package (Venables and Ripley 2002) in program R (R Development Core Team 2012) to determine whether morphological differences among streams could be used to effectively predict the source stream of an unknown individual. This analysis used scores for principal components 2–4 to make jackknifed predictions of individual origin (the first principal component primarily reflected body size). The outputs of the linear discriminant analysis were used to create a classification confusion matrix and evaluate the morphological distinctiveness of populations.

To evaluate the genetic variation among populations at neutral loci, we used 13 microsatellite DNA loci (King et al. 2012) to assign unique multilocus genotypes to ≥30 individuals from each stream. Tissue samples for these individuals had been collected as part of previous surveys in the area. The allelic data generated for all collections was examined using MicroChecker (van Oosterhout et al. 2004) and the microsatellite Toolkit (Park 2001) to determine the presence of null alleles, scoring errors, and/or large allele drop-out.

Unidentified family structure can also be problematic for the detection of hidden population structure, as collections dominated by one or a few families can lead to the false interpretation of genetic differentiation or an entire population being out of Hardy–Weinberg equilibrium (HWE) (Allendorf and Phelps 1981; Ramilo and Wang 2012). To determine whether our collections consisted of a small number of families, we analyzed the collection for the presence of full sibling families using the program COLONY version 2.0 (Wang and Santure 2009). Settings for COLONY analyses included the assumption of male and female polygamy–polyandry, no per-locus genotyping error information, no inbreeding, long run length with the full likelihood analysis method, high likelihood precision, no allele frequency updates, and no sibship prior. Samples were analyzed as offspring without individuals

TABLE 1. Sampling locations, geographic extent, and number of Brook Trout selected for morphometric (Morphology) and genetic analysis (Genetics) at each location. Number of full sibs removed from the genetic analysis are shown in parentheses. Buffer refers to distance between sampling locations.

Stream	Morphology sample date	Reach	Latitude (N)	Longitude (W)	Length of reach (km)	Buffer (km)	Morphology (*n*)	Genetics (*n*)
South Branch	Sep 4, 2012	Lower	39°36′45.02″	79°11′26.01″	1	2.1	30	30 (2)
Casselman River		Upper	39°36′06.36″	79°12′16.63″	0.8		16	
Little Bear Creek	Aug 30, 2012	Lower	39°39′28.15″	79°16′47.40″	0.2	2.5	30	49 (3)
		Upper	39°40′08.05″	79°16′38.84″	0.7		30	
Crabtree Creek	Aug 28, 2012	Lower	39°29′56.91″	79°09′41.99″	0.3	6.5	30	49 (0)
		Upper	39°27′47.25″	79°12′36.08″	0.3		30	
Big Run	Oct 6–8, 2011	Lower	39°33′58.37″	79°09′19.39″	0.5	2.3	26	49 (12)
		Upper	39°35′04.31″	79°10′16.33″	2		30	

TABLE 2. Principal component loadings based on data from Brook Trout collected in four streams. Morphometric (M) variables with loadings of no more than −0.30 or at least +0.30 are shown in bold italics.

Metric	Description	PC_1	PC_2	PC_3	PC_4
Cumulative variance explained (%)		95.49%	96.81%	97.62%	98.30%
Total length		−0.23	0.05	0.07	−0.13
M_1	Crown to snout tip	−0.22	−0.17	***0.40***	−0.13
M_2	Snout tip to anterior pectoral fin	−0.22	***−0.30***	***0.41***	0.03
M_6	Crown to anterior pectoral fin	−0.23	−0.17	0.22	0.02
M_7	Crown to anterior pelvic fin	−0.23	0.06	0.07	−0.09
M_8	Crown to anterior dorsal fin	−0.23	0.09	0.08	−0.02
M_9	Anterior pectoral fin to anterior dorsal fin	−0.23	0.00	0.01	0.02
M_{10}	Total pectoral width	−0.22	−0.06	0.28	−0.06
M_{11}	Anterior pectoral fin to anterior pelvic fin	−0.22	0.20	0.03	−0.23
M_{12}	Anterior dorsal fin to anterior pelvic fin	−0.22	−0.23	−0.08	0.27
M_{13}	Anterior pelvic fin to posterior dorsal fin	−0.22	***−0.31***	−0.27	***0.32***
M_{14}	Anterior pelvic fin to anterior anal fin	−0.22	0.19	−0.18	−0.04
M_{15}	Anterior dorsal fin to posterior dorsal fin	−0.21	***−0.32***	−0.29	***−0.69***
M_{16}	Anterior dorsal fin to anterior anal fin	−0.23	0.04	−0.07	0.01
M_{17}	Posterior dorsal fin to anterior anal fin	−0.23	0.15	−0.05	***0.30***
M_{18}	Posterior dorsal fin to posterior anal fin	−0.22	***0.33***	−0.05	0.14
M_{19}	Posterior dorsal fin to anterior adipose fin	−0.22	***0.60***	0.16	−0.08
M_{20}	Anterior anal fin to anterior adipose fin	−0.23	−0.03	−0.12	0.14
M_{21}	Anterior anal fin to posterior anal fin	−0.22	0.03	***−0.53***	−0.12
M_{22}	Posterior anal fin to anterior adipose fin	−0.22	−0.14	−0.10	***0.31***

being assigned as candidate males or females, as these data were not available for the samples. While the inference of family relationships is weakened in this situation, which has no sex, age, or relationship information, and because there is the assumption of polygamy for both sexes, COLONY is predicted to be more accurate than pairwise estimates of relationships (Wang and Santure 2009). Our sibship analysis in COLONY identified a total of 33 full sibling pairs representing a total of 10 families within the genetics collections. We randomly removed full sibs ($n = 17$) from the data set until only a single representative of each family remained. Removal of excess full sibs from the data reduced the number of individuals available for analysis in each stream by 0.0–24.4%. An additional 265 half-sibling pairs were identified; however, these individuals were included in all subsequent analysis.

The microsatellite data were used to compute genetic differentiation (F_{ST}; Weir and Cockerham 1984) between each population pair and run assignment tests using the criterion of Rannala and Mountain (1997) in GeneClass2 (Piry et al. 2004). The Brook Trout used here for DNA extraction and genotyping were based on previous collections from the same streams, and thus were not necessarily the same individuals used in the morphological analysis.

We tested for correlations between morphological and neutral genetic differentiation using Mantel tests (Mantel 1967) implemented in the ade4 package of program R (Dray and Dufour 2007). A Bonferroni-adjusted P-value (0.01) was applied to all test results to minimize the family-wise error rate.

To compare population structure in traits and markers, we used analysis of molecular variance (AMOVA) in Arlequin (Excoffier et al. 2005) to generate locus-by-locus F_{ST} values for each population pair. We generated 95% CIs around each mean pairwise F_{ST} value based on 20,000 bootstraps in Arlequin. Similarly, we calculated pairwise phenotypic differentiation among populations (P_{ST}; Leinonen et al. 2006; Brommer 2011) for each morphological metric to quantify the amount of phenotypic variation attributable to differences among populations. We estimated P_{ST} using a random effects model with REML estimation, specifying stream as the random effect. Variance components from the random effects were then used to estimate P_{ST} according to Brommer (2011), assuming that the proportion of the among and within-population variance in morphological traits due to additive effects (c and h^2, respectively) were both equal to a value of 1. Brommer (2011) has shown that for phenotypic studies of P_{ST} using environmental samples, the ratio c/h^2 is most influential in determining how closely P_{ST} approximates Q_{ST}, and hence the robustness of the inference from comparing P_{ST} to F_{ST}. Therefore, we evaluated our comparisons with a sensitivity analysis across a range of c/h^2 values that represent different assumptions about how much of the within- versus between-population phenotypic variance is based on additive genetics (Brommer 2011). We also used hierarchical modeling (AMOVA and random effects ANOVA)

TABLE 3. Results of ANCOVA (*P*-values) on the first through fifth principal components (PC_1–PC_5). Significant test results ($P < 0.01$) are shown in bold italics. The ANCOVA models with nonsignificant (NS) terms were refit without those terms, unless the main effect was part of a significant interaction.

Principal component	Terms of model						
	Length	Stream	Reach	Length × Stream	Length × Reach	Stream × Reach	Length × Stream × Reach
PC_1	***0.000***	***0.005***	0.322	***0.002***	NS	***0.000***	NS
PC_2	0.012	***0.000***	0.037	NS	***0.004***	NS	NS
PC_3	0.013	***0.000***	***0.000***	NS	***0.000***	NS	NS
PC_4	NS	***0.000***	0.173	NS	NS	***0.000***	NS
PC_5	NS	NS	NS	NS	NS	NS	NS

to partition the observed molecular and morphological diversity into variation among drainages, among streams within drainages, and within streams.

RESULTS

We collected and photographed 220 Brook Trout for morphometric analysis during early fall in 2011 and 2012 (range in length, 108–298 mm TL; Table 1). Sample sizes were close to our target at all locations except at the upper site on the South Branch of the Casselman River, where low abundance prohibited the collection of additional individuals. Several metrics (M_3–M_5 and M_{23}–M_{27}; see Table 2 for descriptions) used by Janhunen et al. (2009) were omitted due to ambiguities in identifying anatomical landmarks on some individuals.

Principal components analysis was effective at distilling the variation present in the morphological measurements into a smaller set of representative metrics (Table 2). The first principal component (PC_1) explained most of the overall variance in the measurements (95.49%) and was highly correlated with TL ($R^2 = 0.991$). It was not further considered because it primarily reflected variation in individual size, and our focus was instead on morphology irrespective of size. The remaining principal components were orthogonal to TL ($R^2 \ll 0.001$). For subsequent analysis, we focused on principal components 2–4, which accounted for 62.3% of the variation remaining in the data set after controlling for the effect of body size (PC_1). Principal component two (PC_2) was dominated by variation in postdorsal proportions, especially with respect to the length of the region between the dorsal and adipose fin. The third principal component (PC_3) predominantly described variation in head structure and anal fin length. Principal component four (PC_4) largely reflected variation in dorsal fin length and postdorsal height.

Using ANCOVA, we found a significant effect of stream or a length by stream interaction for all three principal components examined (PC_2–PC_4), indicating a spatial structuring of Brook Trout morphology among streams. Furthermore, we found a significant length by reach or stream by reach interaction for PC_1–PC_4 (Table 3), suggesting there were fine-scale differences in morphology even within streams. Although morphological relationships among streams and reaches were complex, spatial differences in morphology were found in all three principal components we considered.

Since morphological differences in the ANCOVA analyses were at least partially based on reach, we structured our linear discriminant analysis to classify individuals to specific reaches within streams. Although our approach was unable to account for length by stream or length by reach interactions, we were able to correctly classify 52.3% of individuals to their stream of origin for the four streams and 35.9% of individuals to their within-stream reach across the eight possible reaches (Table 4).

Based on analysis of our 13 microsatellite loci, we found considerable genetic differences between some of the Brook Trout populations in the study. Mean pairwise F_{ST} values ranged from 0.023 to 0.264. Mean pairwise F_{ST} values for streams within the same drainage (0.023–0.100) were always lower than for stream comparisons across the eastern continental drainage divide (0.141–0.264). Pairwise locus-by-locus F_{ST} values also varied widely, ranging from 0.000 to 0.654 (Figure 2). A genetic assignment test found that Brook Trout from the four study streams could be effectively distinguished, as 100% were assigned to the correct drainage and 93.8% were assigned to the correct stream (Table 5). Where misclassifications occurred (9 of 160 assignments), they were always between Big Run and Crabtree Creek, which exhibited the lowest genetic divergence in the study ($F_{ST} = 0.023$).

Population differentiation based on morphology was also highly variable, and pairwise P_{ST} values ranged from 0.000 to 0.339. However, unlike the F_{ST} values that were higher between streams from different drainages, there was no consistent relationship between P_{ST} values and drainage (Figure 2). Although considerable variability was observed in pairwise P_{ST} and F_{ST} values, Mantel tests failed to detect a significant relationship between morphological and molecular divergence for any of the principal components considered based on six pairwise comparisons and a Bonferroni-adjusted critical

TABLE 4. Confusion matrix comparing the assigned sources of individual Brook Trout based on morphology with their true origin. Correct assignments are shown in bold. Assignments within the correct drainage are shown in italics. See Figure 1 for definitions of abbreviations; Upper = upper reach, Lower = lower reach, n = number of fish.

		Assignment								Percentage (%) assigned to correct reach	Percentage (%) assigned to correct stream	Percentage (%) assigned to correct drainage
Source	n	BIGR Upper	BIGR Lower	CRAB Upper	CRAB Lower	LBCR Upper	LBCR Lower	SBCA Upper	SBCA Lower			
BIGR Upper	30	***10***	*6*	*4*	*5*	3	2	0	0	33.3	53.3	83.3
BIGR Lower	24	*6*	***8***	*3*	*2*	3	2	0	0	33.3	58.3	79.2
CRAB Upper	30	*1*	*2*	***10***	*3*	6	3	0	5	33.3	43.3	53.3
CRAB Lower	30	*4*	*3*	*2*	***11***	4	6	0	0	36.7	43.3	66.7
LBCR Upper	30	6	1	3	5	***5***	*6*	*0*	*4*	16.7	36.7	50.0
LBCR Lower	30	1	0	7	5	*1*	***14***	*0*	*2*	46.7	50.0	56.7
SBCA Upper	16	0	0	5	0	*0*	*1*	***0***	*10*	0.0	62.5	68.8
SBCA Lower	30	0	0	4	0	*0*	*3*	*2*	***21***	70.0	76.7	86.7

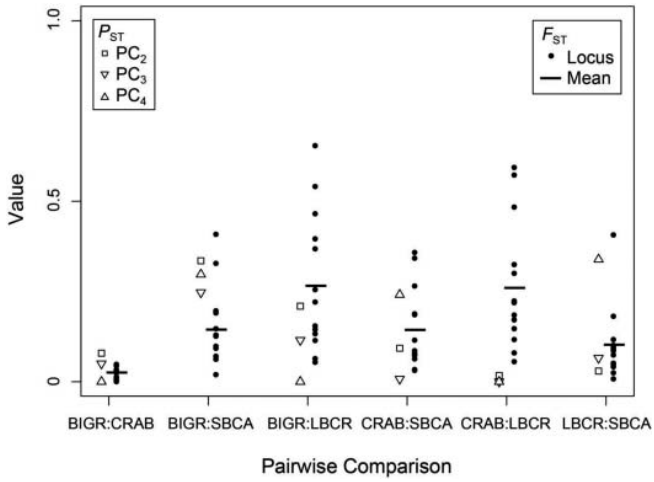

FIGURE 2. Comparison of pairwise P_{ST} and F_{ST} values based on three principal components representing morphology and 13 microsatellite markers for Brook Trout in four streams in western Maryland. See Figure 1 for definitions of abbreviations.

P-value (0.01; Table 6; Figure 3). A sensitivity analysis indicated that morphology characterized by PC_2 through PC_4 were often less variable among sampling locations than were neutral genetic markers, regardless of our assumption of the ratio of c to h^2 (Figure 4). Conversely, there were some population pairs where P_{ST} was greater than neutral genetic differentiation when viewed across a range of c to h^2 ratios (e.g., Big Run versus South Branch of the Casselman River).

We partitioned the observed variance in morphology and microsatellites into three levels: variation among drainages, among streams within drainages, and within streams (Table 7). Most of the observed variation (≥71.2%) was attributed to differences among individuals within streams. For molecular markers and for PC_2 and PC_3, differences among drainages (>10% of the observed variation) were stronger than differences among streams (<10% of the observed variation). Conversely, for PC_4, there were strong differences among streams (25.8% of the observed variation) but only weak differences between drainages (1.5% of the observed variation).

TABLE 6. Mantel test results on the second through fourth principal components (PC_2–PC_4).

Principle component	Mean P_{ST}	Mantel *P*-value	Correlation (r)
PC_2	0.127	0.375	0.127
PC_3	0.081	0.456	−0.012
PC_4	0.146	0.959	−0.337

DISCUSSION

Morphological divergence is well documented among Brook Trout with different life history tactics (Bourke et al. 1997; Morinville and Rasmussen 2008), but little is known about the morphological diversity of stream-resident Brook Trout in different locations. Our study is the first to document morphological differences among streams or between locations within a single stream for stream-resident Brook Trout. Although previous studies have documented high levels of neutral genetic divergence among Brook Trout populations (King et al. 2012; Aunins et al. 2015), our project is the first to compare neutral genetic divergence and phenotypic divergence. These results add to our understanding of morphological variability and adaptation in Brook Trout.

We found clear evidence for spatial structuring of morphological variation in Brook Trout populations in close proximity. For all three of the principal components considered, stream was an important determinant of individual morphology (Table 3). Morphological differences were also detected between reaches on the same stream, but there were no clear patterns in characters between upstream and downstream reaches. Collectively, these morphological differences were sufficient to effectively identify many individuals to their collection locality (52.3% to stream, 35.9% to reach; Table 4). While we had some success in identifying individuals to their source, it is likely that the observed interactions between size and location (based on the ANCOVA) confounded our classification efforts. Regardless, it is clear that there are differences in Brook Trout morphology among streams and reaches.

Despite the observed spatial structuring of morphological variation, we found no consistent relationship between measures of morphological and neutral genetic divergence among

TABLE 5. Confusion matrix comparing the assigned sources of individual fish based on 13 microsatellite markers with their true origin. Assignments within the correct drainage are shown in italics. See Figure 1 for definitions of abbreviations.

Source	n	Assignment				Percentage (%) assigned to correct stream	Percentage (%) assigned to correct drainage
		BIGR	CRAB	LBCR	SBCA		
BIGR	37	***33***	*4*	0	0	89.2	100.0
CRAB	49	*5*	***44***	0	0	89.8	100.0
LBCR	46	0	0	***46***	*0*	100.0	100.0
SBCA	28	0	0	*0*	*28*	100.0	100.0

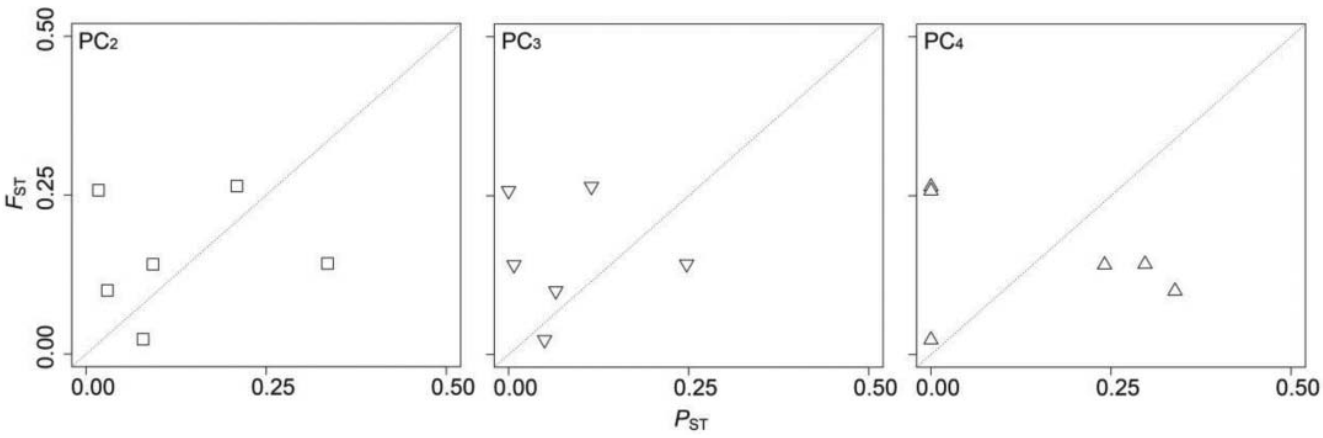

FIGURE 3. Relationships between mean pairwise F_{ST} values and P_{ST} values derived from the second through fourth principal components (PC_2–PC_4) of morphology. The diagonal line represents a 1:1 relationship between the two measures of differentiation.

populations (P_{ST} and F_{ST}, respectively). Although the power of our Mantel test was probably very low (Legendre and Fortin 2010), we documented a relatively strong neutral genetic structure among populations that was not consistently reflected in morphology. Fish collected in Big Run and Crabtree Creek (both Atlantic slope) exhibited greater morphological differentiation (along PC_2) than neutral genetic differentiation (Figure 4). Conversely, some collections that were morphologically similar were found to be highly divergent genetically (e.g., Crabtree Creek and Little Bear Creek). Our data show that in some cases Brook Trout populations isolated for 2.7×10^6 years (R.P.M. and T.L.K., unpublished data) are more convergent in morphology than in-network streams.

Overall, many aspects of morphology appear to be conserved relative to neutral genetic drift. We observed greater variability in pairwise comparisons of microsatellite markers compared with principal component scores of morphology (Figure 2). Additionally, our assignment test based on molecular markers was more accurate than its morphological analog, further demonstrating greater structure in neutral genetics than in morphology. However, in some cases we found evidence of local adaptation for the aspects of morphology we considered.

TABLE 7. Relative importance of variation among drainages, among streams within drainages, and within streams in structuring molecular and morphological diversity in four populations of Brook Trout in western Maryland.

Measure of differentiation	Percentage (%) of variation		
	Among drainages	Among streams	Within streams
F_{ST}	16.8	4.7	78.5
P_{ST}			
PC_2	20.2	8.6	71.2
PC_3	11.3	9.3	79.3
PC_4	1.5	25.8	72.7

Previous studies on salmonids have reported broad variation in P_{ST} and Q_{ST} values, ranging from essentially from zero to nearly one (Rogers et al. 2002; Perry et al. 2005; Jensen et al. 2008; Lin et al. 2008). Our results generally contrast the patterns of greater local adaptation than those of neutral genetic drift reported in previous studies in salmonids (Jensen et al. 2008; Lin et al. 2008; Fraser et al. 2011). However, most of these studies focused on characteristics other than morphology, such as early life history traits or behavior. These traits may be more prone to selection than morphology, which may be restricted by trophic or hydrodynamic limitations. Thus, the aspects of morphology we examined may be inherently constrained compared with other traits.

We detected morphological differences between Brook Trout among reaches on individual streams. We propose four hypotheses that can explain spatial patterns in Brook Trout morphology within a single stream: (1) phenotypic plasticity, (2) multiple discrete populations within a stream, (3) behavioral segregation linked to morphology, or (4) a complex interaction of these factors. Environmental conditions are known to contribute to morphological variability in fishes (Meyer 1987). However, in our study the close geographic proximity of the sample locations and similar channel size and habitats imply similar instream environments were experienced by Brook Trout in each stream. Nonetheless, fine-scale environmental differences likely contributed to morphological variation among the groups.

Genetic structuring within a stream could lead to spatial variation if morphology is heritable and Brook Trout movement is minimal. Unfortunately, our genetic samples were not collected in a manner that allowed us to determine whether individuals at the upper and lower reaches within a stream represented a single population. It is possible that the morphological variation observed in this study represents ongoing

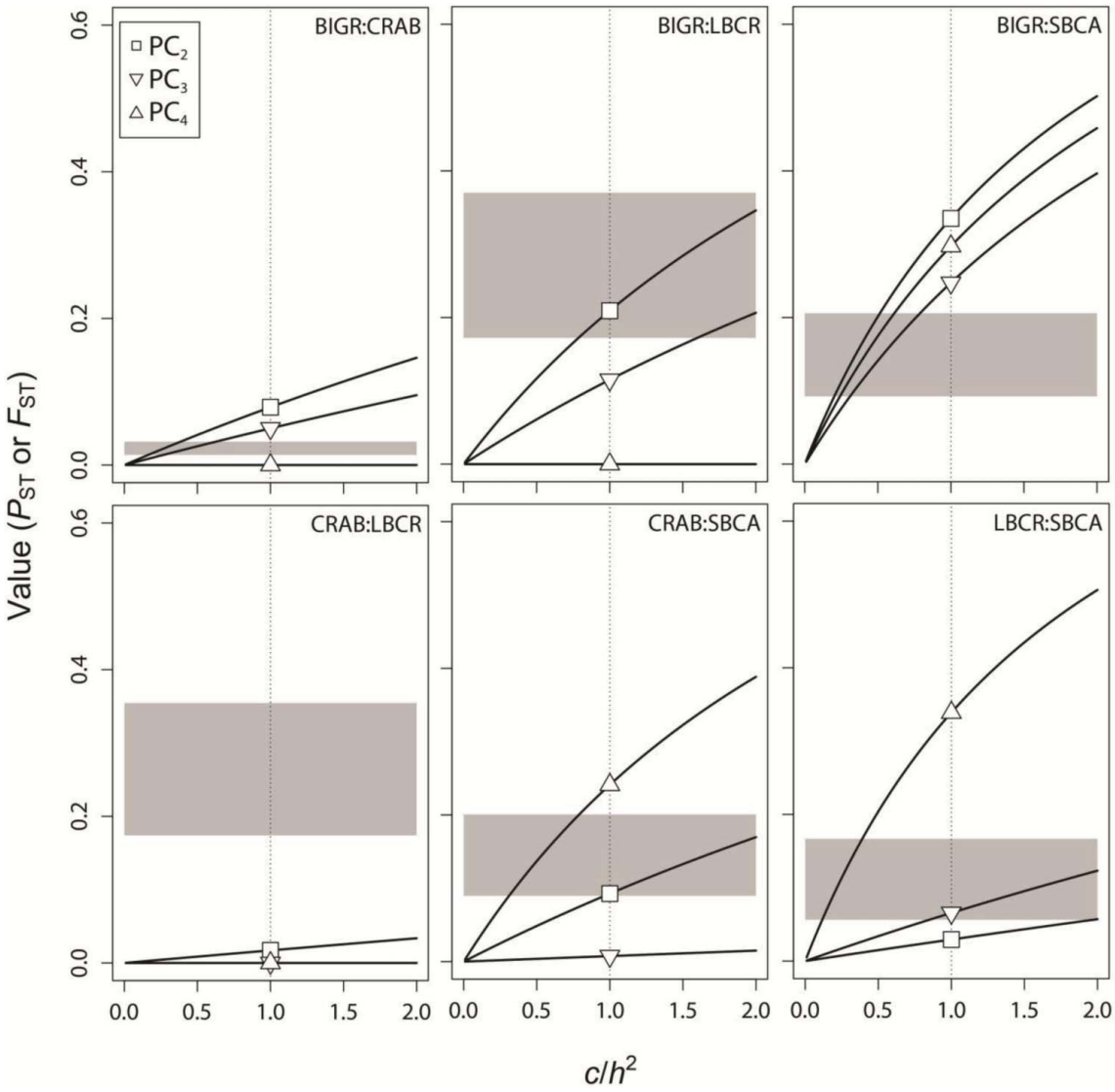

FIGURE 4. Results of a sensitivity analysis comparing F_{ST} (95% CI of the mean shown in gray) and P_{ST} (solid black lines with identifying symbol at $c/h^2 = 1$) estimates across a range of c/h^2 values. The vertical gray dotted line at $c/h^2 = 1$ represents the null assumption for calculating P_{ST} values (Brommer 2011). Each panel represents a pairwise population comparison. See Figure 1 for definitions of abbreviations.

divergent selection pressures that are causing fine-scale genetic segregation and the presence of multiple, discrete populations within a single stream. Similarly, three geographically proximate strains of Brook Trout introduced into a common stream exhibited positive assortative mating or postreproductive isolation, suggesting that phenotypic differences or postreproductive isolation can maintain distinct populations within a single stream (Richards et al. 2008). Hudy et al. (2010) found that family groups tended to be spatially aggregated within a stream, which also suggests that fine-scale genetic differences could contribute to spatial structuring in Brook Trout morphology.

If Brook Trout within each stream represent a single panmictic population with consistent environmental influences, then life history variation must be causing morphologically distinct fish to become spatially segregated. Behavioral segregation of distinct morphotypes in Brook Trout populations has been documented, and Morinville and Rasmussen (2008) found that stream-resident and anadromous Brook Trout were morphologically distinct. Links between morphology and migratory behavior have also been documented in several other fishes (Taylor and McPhail 1986; Taylor and Foote 1991). Under such a scenario, individuals with a migrant morphology might aggregate in the downstream reaches of a

stream, as migratory Brook Trout tend to seasonally occupy the lower reaches of a watershed (Curry et al. 2002; Petty et al. 2005).

The timing of sampling efforts may have influenced the observed patterns in morphology. Due to logistical constraints, Big Run was sampled later in the fall than were the other sites. Although morphological changes associated with maturity are known to occur, we expect that most of these changes would occur prior to an individual reaching sexual maturity and persist thereafter. Based on the size structure of samples, we expect that most individuals were already adults and would not exhibit marked morphological changes around reproduction, especially with regards to the measurements we collected that focused largely on bony landmarks. However, if morphology was influenced by the timing of our sampling efforts, we would expect that morphology would be less variable between sites than we what described. Consequently, morphology may actually be conserved to an even greater extent relative to neutral genetic markers.

In summary, we documented clear morphological differences among Brook Trout collected from four streams in close proximity to each other and even between fish collected in different reaches on the same streams. While morphology varied among streams and drainages, neutral genetic divergence was also very high and sometimes exceeded the magnitude of morphological differences. Thus, for some aspects of Brook Trout morphology, neutral divergence among populations may be proceeding to a greater degree than morphological divergence.

ACKNOWLEDGMENTS

Matt Kline, Matt Hall, and Wes Wagner assisted with the collection of fish used in this study. Barb Lubinski of the Leetown Science Center conducted the laboratory portion of the microsatellite DNA analysis presented in this manuscript. Matt Sell and Alan Klotz of the Maryland Department of Natural Resources Fisheries Service assisted with site selection and project planning. This paper represents scientific series contribution 5008 from the University of Maryland Center for Environmental Science, Appalachian Laboratory.

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