Transactions of the American Fisheries Society

ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1002/tafs.10337

FEATURED PAPER

Population Genetics of Brook Trout in the Southern Appalachian Mountains

David C. Kazyak* and **Barbara A. Lubinski**
U.S. Geological Survey, Eastern Ecological Science Center at the Leetown Research Laboratory, 11649 Leetown Road, Kearneysville, West Virginia 25430, USA

Matt A. Kulp
Great Smoky Mountains National Park, 107 Park Headquarters Road, Gatlinburg, Tennessee 37738, USA

Kasey C. Pregler
Department of Fish, Wildlife, and Conservation Biology, Colorado State University, 1474 Campus Delivery, Fort Collins, Colorado 80523, USA

Andrew R. Whiteley
Wildlife Biology Program, Department of Ecosystem and Conservation Sciences, Franke College of Forestry and Conservation, University of Montana, Missoula, Montana 59812, USA

Eric Hallerman
Department of Fish and Wildlife Conservation, Virginia Tech University, 100 Cheatham Hall, Blacksburg, Virginia 24061, USA

Jason A. Coombs
U.S. Fish and Wildlife Service, Northeast Fishery Center, Lamar, Pennsylvania 16848, USA

Yoichiro Kanno
Department of Fish, Wildlife, and Conservation Biology, Colorado State University, 1474 Campus Delivery, Fort Collins, Colorado 80523, USA

Jacob M. Rash
North Carolina Wildlife Resources Commission, 645 Fish Hatchery Road, Marion, North Carolina 28752, USA

Raymond P. Morgan II
University of Maryland Center for Environmental Science, Appalachian Laboratory, 301 Braddock Road, Frostburg, Maryland 21532, USA

Jim Habera
Tennessee Wildlife Resources Agency, 3030 Wildlife Way, Morristown, Tennessee 37814, USA

Jason Henegar
Tennessee Wildlife Resources Agency, 5107 Edmondson Pike, Nashville, Tennessee 37211, USA

T. Casey Weathers[1]
Department of Ecosystem Science and Management, The Pennsylvania State University, 321 Forest Resources Building, University Park, Pennsylvania 16802, USA

Matthew T. Sell
Maryland Department of Natural Resources, Fisheries Service, Inland Fisheries Division, 301 Braddock Road, Frostburg, Maryland 21401, USA

Anthony Rabern
Georgia Department of Natural Resources, Wildlife Resources Division, Fisheries Section, 3695 Highway 197 North, Clarkesville, Georgia 30523, USA

Dan Rankin
South Carolina Department of Natural Resources, 311 Natural Resources Drive, Clemson, South Carolina 29631, USA

Tim L. King[2]
U.S. Geological Survey, Eastern Ecological Science Center at the Leetown Research Laboratory, 11649 Leetown Road, Kearneysville, West Virginia 25430, USA

Abstract

Broad-scale patterns of genetic diversity for Brook Trout *Salvelinus fontinalis* remain poorly understood across their endemic range in the eastern United States. We characterized variation at 12 microsatellite loci in 22,020 Brook Trout among 836 populations from Georgia, USA, to Quebec, Canada, to the western Great Lakes region. Within-population diversity was typically lower in the southern Appalachian Mountains relative to the mid-Atlantic and northeastern regions. Effective population sizes in the southern Appalachians were often very small, with many estimates less than 30 individuals. The population genetics of Brook Trout in the southern Appalachians are far more complex than a conventionally held simple "northern" versus "southern" dichotomy would suggest. Contemporary population genetic variation was consistent with geographic expansion of Brook Trout from Mississippian, mid-Atlantic, and Acadian glacial refugia as well as differentiation among drainages within these broader clades. Genetic variation was pronounced among drainages (57.4% of overall variation occurred among 10-digit hydrologic unit code [HUC10] units or larger units) but was considerable even at fine spatial scales (13% of variation occurred among collections within HUC12 drainage units). Remarkably, 87.2% of individuals were correctly assigned to their collection of origin. While comparisons with fish from existing major hatcheries showed impacts of stocking in some populations, genetic introgression did not overwhelm the signal of broad-scale patterns of population genetic structure. Although our results reveal deep genetic structure in Brook Trout over broad spatial extents, fine-scale population structuring is prevalent across the southern Appalachians. Our findings highlight the distinctiveness and vulnerability of many Brook Trout populations in the southern Appalachians and have important implications for wild Brook Trout management. To facilitate application of our findings by conservation practitioners, we provide an interactive online visualization tool to allow our results to be explored at management-relevant scales.

*Corresponding author: dkazyak@usgs.gov
[1]Present address: U.S. Fish and Wildlife Service, Southwestern Native Aquatic Resources and Recovery Center, Post Office Box 219, Dexter, New Mexico 88230, USA.
[2]Deceased.
Received February 16, 2021; accepted September 30, 2021

Over the course of millennia, the distribution and genetic structure of Brook Trout *Salvelinus fontinalis* have been shaped by a long history of repeated glaciation and recolonization of eastern North America (Andersen and Borns 1994; Power 2002; Pilgrim et al. 2012). Following deglaciation, Brook Trout recolonized much of northeastern North America from unglaciated refugia (Danzmann et al. 1998). As charr, Brook Trout are able to exploit a broad variety of coldwater habitats through considerable life history diversity and adaptation (Power 2002). The current native range of Brook Trout extends from the southern Appalachian Mountains, north to the Canadian Maritimes, and west to the Hudson Bay drainage (MacCrimmon and Campbell 1969). Across this vast area, Brook Trout were found historically in nearly all coldwater habitat types, including streams, rivers, lakes, and nearshore marine environments, providing opportunities for recreational angling and serving as an iconic indicator of high-quality coldwater habitats (Power 1980). However, widespread declines have been documented across their native range, with the most precipitous decline occurring in the southeastern United States (Smith 1833; Larson and Moore 1985; Hudy et al. 2008; Stranko et al. 2008).

In the southern Appalachian Mountains (considered here as the area from Maryland to Georgia), nearly all remaining populations of Brook Trout are found in small, higher-elevation headwater streams. Here, the occurrence of Brook Trout in small, isolated populations makes them vulnerable to local extirpation (King 1937, 1939; Lennon 1967; Guffey et al. 1999). Small populations suffer heightened risk of the deleterious effects of genetic drift and inbreeding depression (Hedrick and Kalinowski 2000; Whitlock 2000). They are also at greater risk of extirpation by stochastic events (Lande 1993), which are known to cause erratic population dynamics in even robust populations of stream-dwelling Brook Trout (Roghair et al. 2002; Kazyak 2015; Kanno et al. 2016, 2017). Typically, these habitats are isolated from one another by impediments to connectivity, such as waterfalls, reaches with exotic competitors, and thermally unsuitable areas (Moore et al. 1986; Aunins et al. 2014; Timm et al. 2016; Weathers et al. 2019). The Eastern Continental Drainage Divide (ECDD) has isolated some populations for millions of years, with marked genetic differentiation observed between nearby sites (Danzmann et al. 1998; Hall et al. 2002; King et al. 2012; Kazyak et al. 2015).

There is little opportunity for natural recolonization of Brook Trout in most streams across the southern Appalachian Mountains. In addition, more than a century of supplementing and restoring trout fisheries with hatchery-raised Brook Trout is thought to have resulted in the introgression of hatchery genotypes of northern origin into endemic southern populations (Hayes et al. 1996; Kazyak et al. 2018; Printz et al. 2018), possibly resulting in a loss of regional diversity and local adaptations (Laikre et al. 2010). Given recent declines and the continued vulnerability of these populations, it is important to understand the current population structure and biogeographic context of Brook Trout in the southern Appalachian Mountains to guide management and conservation efforts.

Previous studies have identified unique characteristics of Brook Trout in the southern Appalachians. Because food availability is a limiting factor in this region (Whitworth and Strange 1983; Cada et al. 1987; Ensign et al. 1990; Kulp and Moore 2005; Romaniszyn et al. 2007), adult fish are typically small (Harris et al. 2021) and the life span seldom exceeds 3 years (Konopacky and Estes 1986; Habera et al. 2001). Wesner et al. (2011) reported that Brook Trout native to the southern Appalachian Mountains and introduced northern-origin Brook Trout differed in terms of survival in the laboratory and diet in a natural stream. Early molecular studies observed putatively fixed differences in the allozymes of creatine phosphokinase (enzyme number 2.7.3.2; IUBMB 1992) between northern and southern populations of Brook Trout, and this was widely adopted as a diagnostic marker (Stoneking et al. 1981; McCracken et al. 1993; Hayes et al. 1996). These studies fostered a widespread perspective that southern Appalachian Brook Trout represent a distinct entity (i.e., "northern" versus "southern" strains), with a sharp transition area near the New River drainage (Figure 1; Palmer and Hallerman 2000; Davis 2008; Printz et al. 2018; S. Guffey, 1998 unpublished report to the Virginia Department of Game and Inland Fisheries on population genetics of Brook Trout in Virginia) and potentially even warranting a taxonomic revision (Stoneking et al. 1981). In their study of mitochondrial haplotypes across the native range of Brook Trout, Danzmann et al. (1998) found that the single population they analyzed from south of the New River had a distinct haplotype that was not observed in 154 other populations in the north. Moreover, it is thought that Brook Trout from the southern Appalachian Mountains may have diverged from their northern form over 1.6 million years ago (Fausch 2008). Based on these studies, management guidelines for southern Appalachian Brook Trout have been developed and implemented (Habera and Moore 2005), but the underlying science has not been reevaluated with more contemporary molecular genetic techniques using a larger number of markers.

The advent of more powerful molecular tools provides an opportunity to review and enhance our understanding of Brook Trout in the southern Appalachian Mountains. The purposes of this article are to (1) characterize the population genetic patterns of Brook Trout across their native range, with an emphasis on those populations in the southern Appalachian Mountains; and (2) in doing so, revisit the biogeography of this species. Our geographic scope is much broader than previous genetic assessments of Brook Trout (e.g., Stoneking et al. 1981; McCracken et

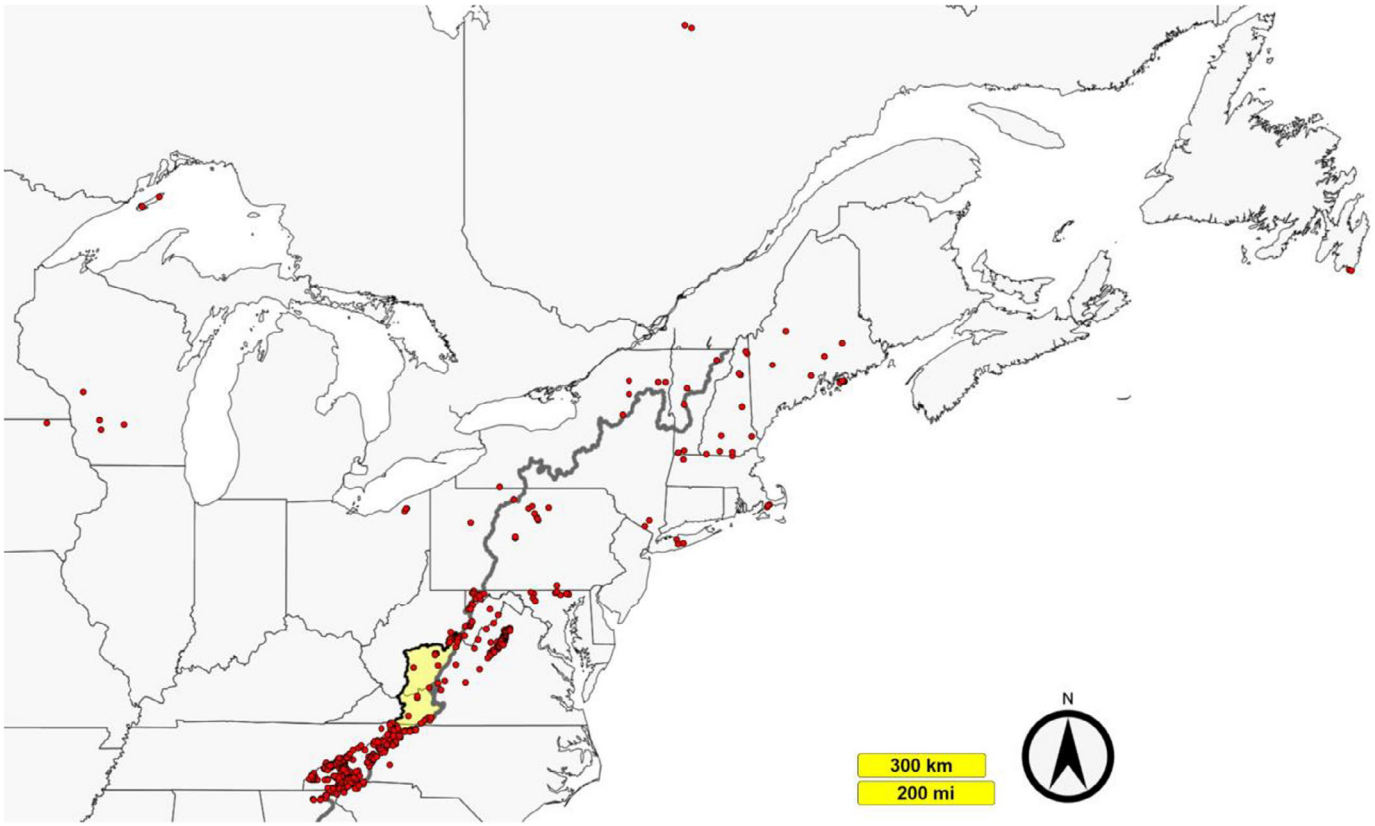

FIGURE 1. Sampling locations (red dots) for 836 collections representing 22,020 wild Brook Trout from across their native range. Geographic coverage extended from Georgia northward to Quebec and from Newfoundland westward to Iowa, representing much of the native range of the species. The Eastern Continental Drainage Divide is shown with a heavy gray line. The New River watershed, which has previously been suggested as a key transition area, is shaded in yellow.

al. 1993; Printz et al. 2018), allowing us to assess the putative genetic break between "northern" and "southern" Brook Trout at the New River drainage and to identify other zones of discontinuity where they occur. This information may help to provide the foundation for ongoing conservation and management activities across the region.

METHODS

We obtained samples ($n = 22{,}020$) that were collected across the native range of Brook Trout by many agency and academic partners. Among 836 total collections (Figure 1; Supplemental Material 1 available in the online version of this article), 818 collections were taken from wild Brook Trout. We focused primarily on Brook Trout collected in the southern Appalachian Mountains (i.e., Maryland to Georgia; these 718 collections consisted of 17,938 individuals). The northern edge of this focal area corresponds roughly to a key transition area for Brook Trout, near the maximum extent of past glaciation and at a latitude north of which Brook Trout can be found in lower-elevation systems and in a broader diversity of habitats (e.g., lakes, larger rivers, and coastal environments; Batchelor et al. 2019). We included 100 additional genetic collections (comprising 3,294 individuals) from elsewhere in the native range of the species to provide context to the patterns observed in the southern Appalachian Mountains. The remaining 18 collections (comprising 788 individuals) were sampled from captive fish used for production activities. Seventeen hatchery collections represented northern-origin hatchery strains used for conventional stocking (Kazyak et al. 2018). The Tellico collection (Tennessee Wildlife Resources Agency, Tellico Trout Hatchery) is unique in that this facility does not rear domestic stocks but instead propagates the progeny of wild Brook Trout from selected streams in the southern Appalachians to be used in restoration (this collection was omitted from all hatchery analyses but is presented for contrast). Collection protocols varied, but the majority of samples were fin clips that were taken from trout collected in wadeable streams using backpack electrofishing and preserved in 95% ethanol. Sample sizes varied among collections (range = 2–152) but averaged 26 individuals. Most collections represent mixed-age samples drawn from several-hundred

meters of contiguous stream habitat. A subset of samples (12 collections) represents single-cohort samples that focused on age-0 (young-of-the-year) individuals. Young of the year were sampled from approximately three spatially distinct sites, each approximately 100 m in length, within contiguous stream habitat (Pregler et al. 2018).

Extraction of DNA and microsatellite genotyping.— Molecular analyses were performed at the U.S. Geological Survey's (USGS) Eastern Ecological Science Center (EESC), Kearneysville, West Virginia. Genomic DNA was isolated from fish tissue using the Puregene Tissue Kit (Gentra Systems, Minneapolis, Minnesota) or the E-Z 96 Tissue DNA Kit (Omega Bio-Tek, Norcross, Georgia). The DNA concentrations were evaluated using a Tecan SpectraFluor Plus (Tecan Group Ltd., Männedorf, Switzerland), a Nanodrop ND-1000 or 8000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts), or a Qubit fluorometer (Thermo Fisher Scientific). Stock DNA was diluted and normalized prior to PCR.

All samples were screened for 12 microsatellite loci (*SfoB52*, *SfoC24*, *SfoC28,* *SfoC38*, *SfoC79*, *SfoC86*, *SfoC88*, *SfoC113*, *SfoC115*, *SfoC129*, *SfoD75*, and *SfoD91*) designed for Brook Trout (King et al. 2012). Polymerase chain reaction amplification of microsatellite loci was carried out on a thermal cycler (PTC-225 Tetrad [MJ Research, Hercules, California], PTC-200 [MJ Research], or T100 [Bio-Rad, Hercules, California]) using the following procedure: initial denaturing at 94°C for 2 min; 35 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 2 min; and a final extension at 72°C for 10 min. Four multiplexed PCRs were generated to genotype the 12 microsatellite DNA markers. The PCR master-mix composition, thermal cycling parameters, and multiplexing were generally as provided by King et al. (2012); more recent laboratory work had slight changes to PCR composition and fragment analysis multiplexes (Kazyak et al. 2018). The PCR products were combined, diluted, and run in two separate reactions on an ABI 3100 or 3130XL genetic analyzer (Applied Biosystems, Inc., Foster City, California) using an internal size standard (LIZ-500; Applied Biosystems). A positive control sample (of known multilocus genotype) was included on each PCR plate for checking the success of PCR amplifications and for correct binning success in the analysis software. A negative control sample (containing all of the ingredients for PCR amplification except DNA) was included on each PCR plate to check for contamination in the PCR products. GeneMapper or Genotyper fragment analysis software (Applied Biosystems) was used to score, bin, and output the allelic data. All microsatellite scoring was automated and then checked by experienced laboratory personnel. Polymerase chain reaction was performed again on all samples with missing data due to weak or unamplified alleles. The PCR amplifications that had to be repeated were done with single loci and not in a multiplexed PCR. All GeneMapper files were double-checked for scoring errors.

Sibship.— Because family structure can obscure comparisons among populations, we used COLONY version 2.0.5.0 (Jones and Wang 2010) to identify full-sibling families within each collection. Due to the large number of collections, a custom R-script (R Core Team 2015) was used to run COLONY from the Windows command line and to store results. Model parameters included an assumption of male and female polygamy and the absence of inbreeding. Single-cohort samples with numerous siblings from the same family can cause deviations from Hardy–Weinberg (HW) expectations, elevated linkage disequilibrium (LD), and bias in genetic structure analyses (Whiteley et al. 2013; Waples and Anderson 2017). Since 12 of the collections included in our analysis were single-cohort samples, we performed sibship removal following the "yank-2" procedure of Waples and Anderson (2017). When families were identified (pairwise sibship probability > 0.95), full siblings were retained for all estimated family sizes of either one or two. For larger family sizes, we randomly removed siblings until two representatives remained. This sibling-purged data set was used for all analyses of among-population differentiation and diversity (e.g., F'_{ST} and hierarchical analysis of molecular variance [AMOVA]).

Within- and among-population diversity.— We tested each collection for conformance to HW proportions and for LD using Genepop version 4.3 (Raymond and Rousset 1995). Descriptive statistics for each collection were generated using GenAlEx version 6.502 (Peakall and Smouse 2006, 2012). Allelic richness (mean number of alleles per locus N_A), unbiased expected heterozygosity (uH_E), observed heterozygosity (H_O), and a measure of departure from HW proportions (inbreeding coefficient F_{IS}) were calculated for each collection. Rarified allelic richness (A_R) was calculated using HP-Rare version 1.1 (Kalinowski 2005) based on a sample size of 40 genes (20 diploid individuals). This metric was not calculated for collections with fewer than 20 individuals. Single-sample estimates of effective population size (N_e) based on LD were produced with NeEstimator version 2 (Do et al. 2014) using a rare-allele cutoff frequency of 0.02 and jackknifed confidence intervals. We refer to this as an estimate of N_e rather than the effective number of breeders (N_b) because the majority (98.6%) of our collections included samples with mixed cohorts. No estimate of N_e was reported for the single-cohort samples. Measures of allelic fixation (F_{ST}) and differentiation (F'_{ST}; Hedrick 2005) among collections were calculated using the diveRsity package (Keenan et al. 2013) in R.

To assess evidence of genetic drift, we investigated whether there was a negative relationship between genetic

differentiation and genetic diversity metrics by using linear regression models. Rarefied allelic richness, uH_E, and N_e were regressed against mean population-specific F'_{ST} estimates for each population (Coleman et al. 2013). For this analysis, we only used those collections with sample sizes of 20 or more individuals.

To examine the geographic structure of genetic variation, we used a hierarchical AMOVA implemented with the pegas package (Paradis 2010) in R. Five hierarchical levels were considered: collection and 12-, 10-, 8-, and 6-digit hydrologic unit code (HUC12, HUC10, HUC8, and HUC6) units. The HUC units were established by USGS and represent a series of nested units defined by basin topography (Seaber et al. 1987). A small proportion of the sample collections were missing latitude and longitude information. For the purposes of this analysis, those collections were not considered in the AMOVA or assignment tests.

To further assess the uniqueness of each collection, we assessed our ability to assign each individual to its source collection based on genotype data. Assignment testing was conducted using GeneClass2 (Piry et al. 2004) based on the Bayesian approach of Rannala and Mountain (1997). We summarized classification efficiencies (i.e., the percentage of individuals correctly assigned) at different spatial scales (collection, patch, and HUC units). We used patches that were developed by the Eastern Brook Trout Joint Venture (EBTJV; https://easternbrooktrout.org), which are intended to represent contiguous stream habitats that support Brook Trout. Collections that were not located within an existing EBTJV patch or that were missing sampling coordinates were omitted from assignment testing.

Cluster analyses.— We examined population structure with discriminant analysis of principal components (DAPC) using the adegenet package (Jombart 2008) in R. Analyses were performed on the filtered data set (≥20 individuals/collection) that contained 20,220 individuals from 665 collections. We used the "find.clusters" function to detect genetically distinct populations. This function uses *k*-means clustering to decompose the total genetic variance into between- and within-group components. Bayesian information criterion scores were evaluated to assess optimal clustering. Patterns of population clustering were examined using the "dapc" function, which transforms the data using principal components analysis and then performs discriminant analysis on the retained principal components (PCs; Jombart et al. 2010). The number of PCs corresponding to the asymptote in cumulative variance explained ($N = 100$ PCs) was determined visually. We retained all discriminant functions for analysis for each number of clusters examined. The DAPC results were visualized using the "scatter" function, and posterior membership probabilities were used to examine individual genetic similarities to each population cluster. Preliminary analyses indicated that clustering using STRUCTURE (Pritchard et al. 2000) provided results that were largely congruent with DAPC; the STRUCTURE analyses are described in Supplemental Material 3.

To compare overall genetic diversity among the major clusters identified (based on DAPC, $K = 3$ clusters) while standardizing for sampling intensity, we subsampled the overall data set and retained 20 randomly selected individuals from 47 randomly selected collections in each of the three clusters. Using this subsampled data set, we compared the total number of alleles as well as the number of private alleles in each of the three genetic clusters. In addition, we used a hierarchical Shannon diversity analysis (Sherwin 2015; Smouse et al. 2015) to compare levels of genetic diversity among regions. Due to limitations of the GenAlEx implementation of the Shannon diversity analysis, we compared diversity within each of the regions using a smaller number of random samples (20 random individuals from 20 randomly selected populations within each of the three clusters; populations that were assumed to be introgressed in the southern Appalachians were excluded). The hierarchical Shannon diversity analysis was repeated 10 times with independently selected random samples.

RESULTS

Sibship

COLONY identified 17,562 full-sibling families across the 836 collections included in the sibship analysis. Mean family size across all collections was 1.40, with a range of 1 to 84. Eighty-four percent of the identified families contained a single individual. Among the 836 collections, siblings were purged from 12 age-0-only samples containing full-sibling families of three or more individuals. Ultimately, sib-purging reduced our sample size from 22,020 total individuals to 21,998.

Within-Population Diversity

Genotype frequencies generally conformed with HW proportions and showed LD among loci. At a Bonferroni-corrected *P*-criterion of 0.00417 (0.05/12 loci), collections showed a mean of 0.21 loci that deviated from HW proportions. Most collections showed no significant departures; however, four loci in the Greens Creek, North Carolina, collection (sample size = 33) and seven loci in the Flat Creek, North Carolina, collection (sample size = 19) showed significant departures from HW proportions. At a critical Bonferroni-corrected *P*-criterion of 0.00076 (0.05/66 tests per collection) for tests of LD, collections showed a mean of 0.89 significant test results between pairs of loci, with most collections showing no significant results. Thirteen collections (eight from the southern

Appalachians, two from the Shenandoah River drainage, and three northern collections, all small or known to have been stocked) showed 10 or more significant test results (range of sample sizes = 15–152; Supplemental Material 1). Since the majority of tests for departures from HW proportions and LD showed nonsignificance, we concluded that collections behaved as populations and that the respective microsatellite loci segregated independently.

Within-population diversity for Brook Trout populations in the southern Appalachians was lower than for most populations from the northern portion of the range (Figures 2, 3; Supplemental Material 1). The N_A ranged from 1.00 to 9.33 (mean = 3.56) and tended to be lower in the southern portion of the range than in the mid-Atlantic and northeastern portions. Rarified allelic richness ranged from 1.00 to 7.55 (mean = 3.43) and showed a similar geographic trend (Figures 2A, 3). Observed heterozygosities (mean = 0.44; range = 0.00–0.76) were comparable to uH_E (mean = 0.43; range = 0.00–0.73) and tended to be lower in the southern part of the range (Figure 2B). Although some F_{IS} values departed from zero (range = −0.55 to 0.73), the mean F_{IS} of −0.03 gave no indication of widespread departures from random mating across the populations surveyed. Estimated N_e ranged from 1 to over 2,000 (median = 55.1). Effective population sizes of Brook Trout populations in the south were often less than 30 (Figures 2C, 3; 60.3% of populations in this region), which is consistent with observations across much of the species' range and a history of bottlenecks in isolated populations. Notably, one population (Boone Fork Watauga River, North Carolina) exhibited no variation within any of the 12 microsatellite loci despite an apparently robust census population size (Jacob Rash, North Carolina Wildlife Resources Commission, unpublished data).

Genetic variation tended to be higher within domestic hatchery Brook Trout populations than wild populations, particularly compared to populations in the southern Appalachians. Within the 17 domestic hatchery Brook Trout populations, N_A ranged from 3.00 to 6.08 (mean = 4.40), and A_R ranged from 2.81 to 6.08 (mean = 4.10; Supplemental Material 1). Values of H_O (mean = 0.54; range = 0.41–0.70) approximated those of uH_E (mean = 0.53; range = 0.43–0.68). The F_{IS} values were near zero

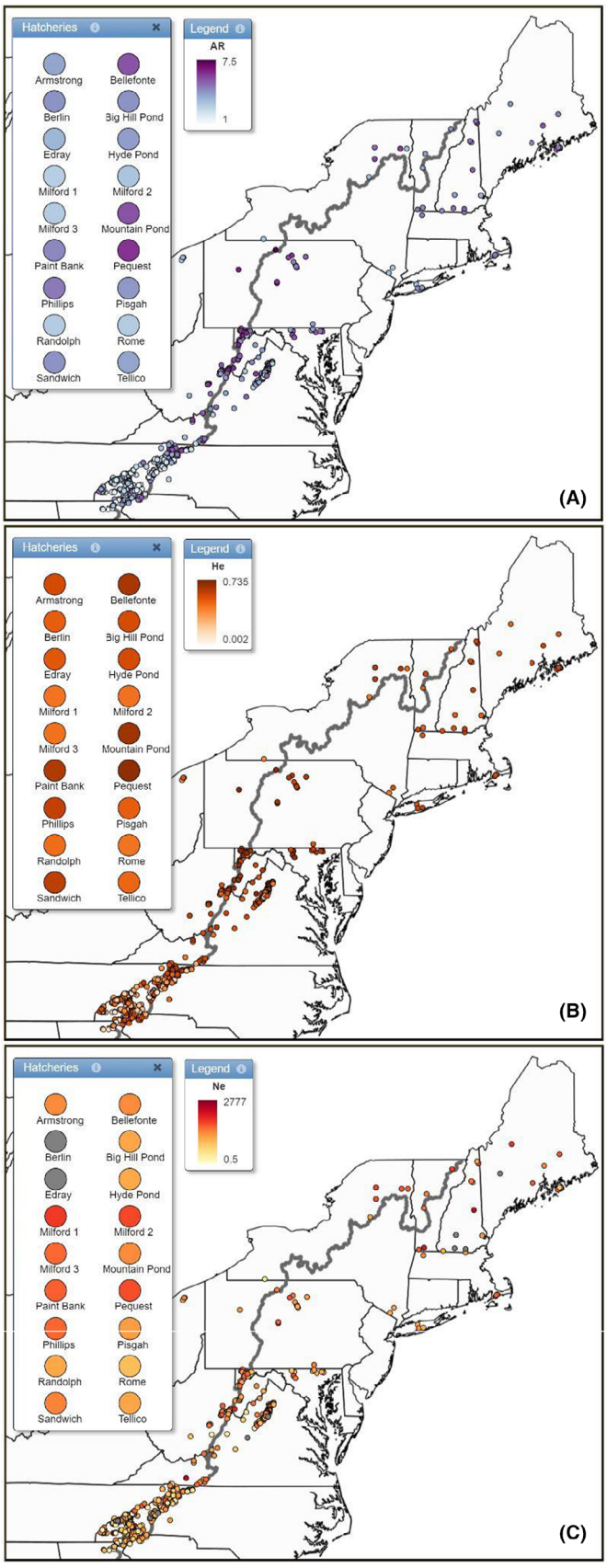

FIGURE 2. Three measures of within-population diversity estimated for wild Brook Trout populations in the eastern United States: **(A)** mean rarefied allelic richness per locus (A_R), **(B)** unbiased expected heterozygosity (H_E), and **(C)** effective population size (N_e). Samples outside of the eastern United States are truncated for visual purposes but were included in the analysis and can be viewed with the interactive online viewer, Brook Trout Explorer (https://bte.ecosheds.org/). The inset panel shows metrics for each of the hatchery collections.

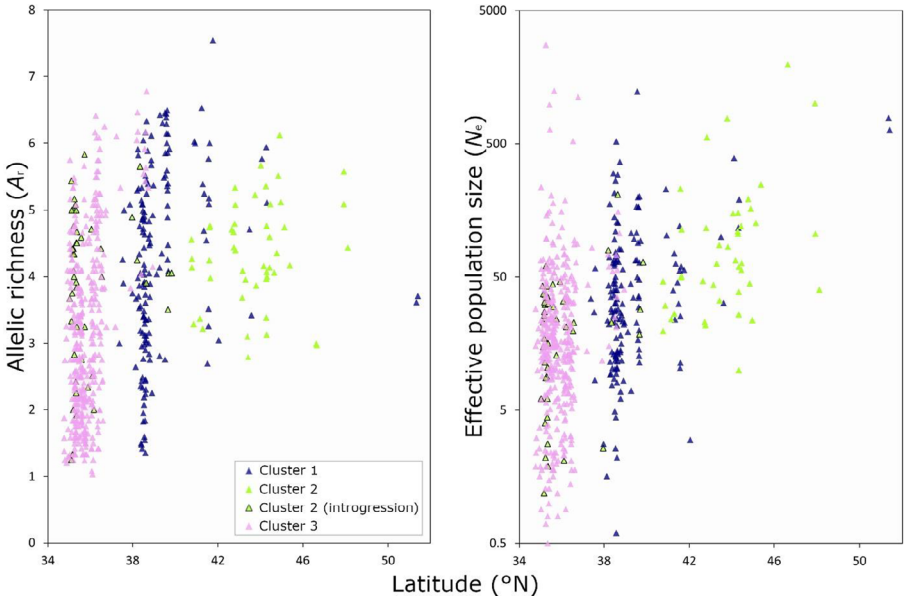

FIGURE 3. Observed variation in allelic richness (A_R) and effective population size (N_e) for Brook Trout across a latitudinal gradient. Points are color coded by clusters that were identified with discriminant analysis of principal components ($K = 3$ clusters) and represent collections with at least 20 samples. For the purposes of this visualization, collections in cluster 2 that were found south of the Maryland–Pennsylvania border were considered to reflect hatchery introgression.

(mean = 0.00; range = −0.09 to 0.08). Values of N_e ranged from 14.2 to 212.7 (median = 57.3).

Results from our genetic analyses of these Brook Trout populations can be seen in an interactive, web-based viewer (Brook Trout Explorer) located at https://bte.ecosheds.org/. The user can select geographic layers (e.g., state outlines), overlay layers (e.g., ECDD, HUC watersheds), data layers (e.g., genetic differentiation metrics, STRUCTURE results, and DAPC results), and histograms and scatter plots of key metrics. Furthermore, the viewer can zoom in to view features of regional interest.

Among-Population Diversity

Brook Trout showed marked differentiation among wild populations in the study range (mean F'_{ST} = 0.746; range = 0.000–0.998). Clear spatial trends were evident in pairwise comparisons of populations within and among the three genetic clusters identified by DAPC ($K = 3$, see *Cluster Analyses* section below; Table 1). Populations within the northern regional genetic cluster were the least differentiated (mean F'_{ST} = 0.478; range = 0.040–0.812). In contrast, populations within the southern regional genetic cluster were differentiated to a much greater extent (mean F'_{ST} = 0.722; range = 0.000–0.998). Comparisons within the mid-Atlantic regional genetic cluster showed intermediate levels of differentiation among populations (mean $F'_{ST} = 0.666$; range = 0.000–0.996). Notably, the average level of differentiation between pairs of populations in the southern genetic cluster was only slightly lower than the average in comparisons between populations in the southern region and those in the mid-Atlantic region or the northern region (mean $F'_{ST} = 0.796$ and 0.793, respectively). The domestic hatchery collections were highly differentiated from nearly all wild collections but were comparatively similar to one another. Additional comparisons may be viewed in Table 1.

Based on our AMOVA, genetic variation was pronounced among drainages (57.4% of overall variation could be explained by differences among HUC10 units or larger units; Table 2), but considerable variation occurred even at fine spatial scales (13.0% of variation reflected differences among populations within HUC12 units). Remarkably, 87.2% of individuals were correctly assigned to their collection of origin (Table 3), even though many collections were taken from geographically proximate locations within the same watersheds. An even greater percentage (94.6%) of Brook Trout were assigned to the correct EBTJV patch. Across broader hydrologic scales, nearly all individuals could be correctly assigned (e.g., 98.2% to the HUC8 level; Table 3).

A comparison of mean population-specific F'_{ST} values with A_R, uH_E, and N_e (Figure 4) provided strong evidence that the pronounced among-population differences are due, in part, to genetic drift. Many N_e estimates were very low—reflecting conditions that may lead to rapid, random changes in allele frequencies and loss of intrapopulation genetic diversity. Linear regression models revealed a significant negative relationship between F'_{ST} and A_R ($P =$

TABLE 1. Pairwise differentiation (F'_{ST}) between Brook Trout populations, summarized within and among the three genetic clusters ($K = 3$) identified by discriminant analysis of principal components (Northern, Mid-latitude, and Southern) and the domestic hatchery collections (Hatchery).

Category	Groups	Pairwise comparisons	Mean F'_{ST}	Minimum F'_{ST}	Maximum F'_{ST}
Wild type	Northern and Northern	1,081	0.478	0.040	0.812
	Mid-latitude and Northern	7,379	0.728	0.201	0.977
	Northern and Southern	17,343	0.793	0.289	0.984
	Mid-latitude and Mid-latitude	12,246	0.666	−0.004	0.996
	Mid-latitude and Southern	57,933	0.796	0.293	0.992
	Southern and Southern	67,896	0.722	−0.010	0.998
Comparisons with introgressed populations	Northern and Northern (Introgression)	1,974	0.537	0.108	0.905
	Mid-latitude and Northern (Introgression)	6,594	0.703	0.179	0.983
	Northern (Introgression) and Southern	15,498	0.764	0.022	0.994
	Northern (Introgression) and Northern (Introgression)	861	0.530	0.007	0.952
Comparisons with domestic lineages	Northern and Hatchery	799	0.924	0.843	0.963
	Mid-latitude and Hatchery	2,669	0.942	0.843	0.998
	Southern and Hatchery	6,273	0.935	0.828	0.981
	Northern (Introgression) and Hatchery	714	0.922	0.840	0.975
	Hatchery and Hatchery	136	0.224	−0.015	0.424

TABLE 2. Hierarchical analysis of molecular variance for 612 populations of wild Brook Trout. Variance at five strata was assessed, including 6-, 8-, 10-, and 12-digit hydrologic unit code (HUC) units (U.S. Geological Survey) and collections of Brook Trout.

Hierarchical level	Sum of squared differences	Variance explained (%)
Among HUC6s	32,939,443	30.1
Among HUC8s within HUC6s	13,642,363	12.5
Among HUC10s within HUC8s	16,172,066	14.8
Among HUC12s within HUC10s	10,459,602	9.6
Among populations within HUC12s	14,244,488	13.0
Among individuals within populations	22,029,914	20.1
Total	109,487,876	100.0

TABLE 3. Proportion of Brook Trout individuals that were correctly assigned to various geographic units with GeneClass2 by using the criterion of Rannala and Mountain (1997). Only collections that fell within an existing Eastern Brook Trout Joint Venture (EBTJV) patch (coverage restricted to the eastern United States) were considered for this analysis (HUC = hydrologic unit code [6, 8, 10, or 12 digits], U.S. Geological Survey).

Assignment unit	Correct	Total	Percentage correct
Collection	14,282	16,371	87.2
EBTJV patch	15,494	16,371	94.6
HUC12	15,729	16,371	96.1
HUC10	15,955	16,371	97.5
HUC8	16,070	16,371	98.2
HUC6	16,122	16,371	98.5

0.03; effect size = −0.21). Populations that were the most distinct (i.e., had the greatest mean F'_{ST}) consistently had very low levels of A_R. Conversely, the populations that were the least distinct were also among those with the greatest levels of A_R observed in this study. There was also a tight negative linear relationship between mean population-specific F'_{ST} and uH_E ($R^2 = 0.76$; $P = 0.02$; effect size = −0.57). Although most estimated values of N_e were small, there was not a significant relationship ($P =$ 0.09) between N_e and mean population-specific F'_{ST}. Overall, these results suggest that populations have lost diversity through genetic drift and that the observed distinctness among populations is likely to have been substantively driven by this process.

Cluster Analyses

In the DAPC, Bayesian information criterion values progressively declined for up to 200 evaluated clusters, providing no clear indication of an optimal K for this data set (Supplemental Material 2). We therefore used the "dapc" function to evaluate a set of clusters that was reasonable based on STRUCTURE results ($K = 2$–7, 10, 15,

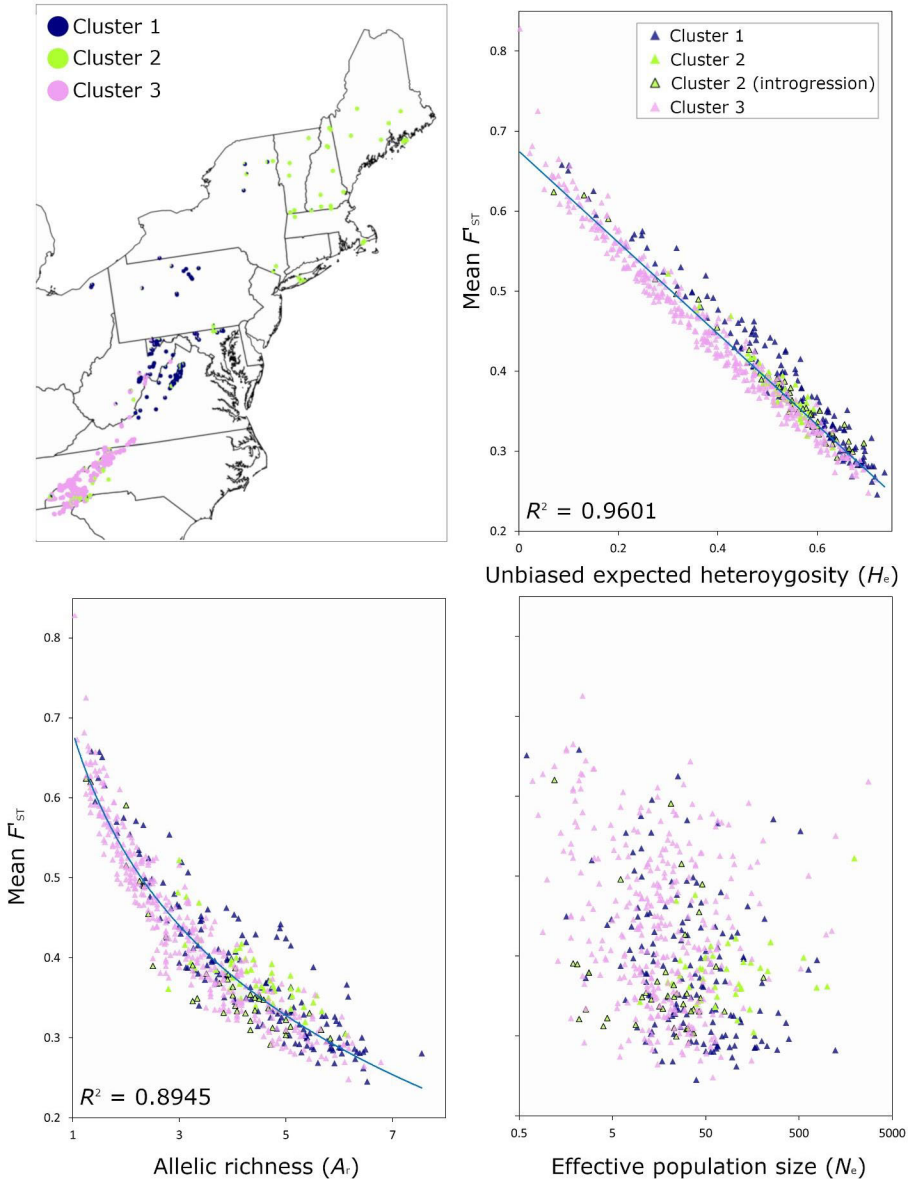

FIGURE 4. Relationships between rarefied allelic richness (A_R), expected heterozygosity (H_E), effective population size (N_e), and mean F'_{ST} for Brook Trout. Points are color coded using clustering results ($K = 3$; the distribution of each cluster is shown on the map) from discriminant analysis of principal components. Samples outside of the eastern United States are truncated from the map for visual purposes but were included in the analysis and can be viewed with the interactive online viewer, Brook Trout Explorer (https://bte.ecosheds.org/). Only collections with at least 20 samples are shown. For the purposes of this visualization in the scatterplots, collections in cluster 2 that were found south of the Maryland–Pennsylvania border were considered to reflect hatchery introgression.

20, and 25; see Supplemental Material 3 for a full presentation of STRUCTURE results). At a *K*-value of 2 (Figure 5A; see Supplemental Material 4 for collection-specific DAPC scores), one of the two clusters (shown in blue) was distributed throughout much of the southern portion of the species' range and presumably represents what has been traditionally referred to as southern Appalachian Brook Trout. Contributions from this cluster were distributed not only to the southwest of the New River drainage but also farther north on the west side of the ECDD in West Virginia, with smaller contributions in Pennsylvania, southwestern New York, and Ohio.

We observed additional—and likely biologically meaningful—substructure at higher values of *K*. At a *K*-value of 3 (Figure 5B), a northern cluster of populations (shown in green) was distinguished from a central Appalachian cluster (blue) and a southern Appalachian cluster (pink). Several West Virginia and Blue Ridge Mountain, Virginia,

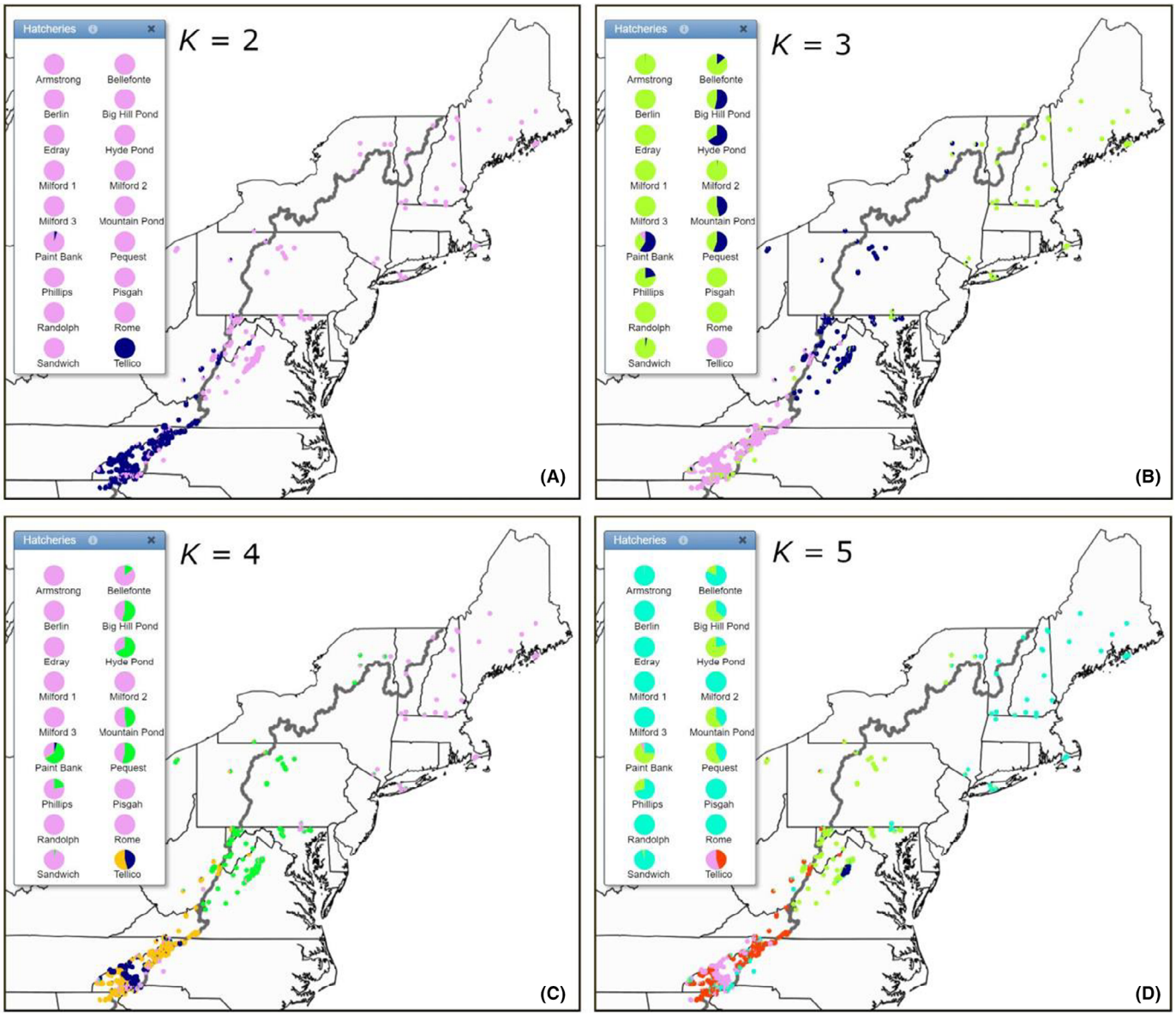

FIGURE 5. Geographic distribution of discriminant analysis of principal components (DAPC)-based population-level assignment to $K = 2$, 3, 4, or 5 clusters of multilocus genotypes in Brook Trout. The Eastern Continental Drainage Divide is shown with a gray line. To observe DAPC-based population assignments at a finer scale or for populations farther north or west, visit the interactive online viewer, Brook Trout Explorer (https://bte.ecosheds.org/), and select the DAPC data layers by using the pull-down menu. Samples outside of the eastern United States are truncated for visual purposes but were included in the analysis and can be viewed with Brook Trout Explorer.

populations clustered with the southern Appalachian cluster. At a *K*-value of 4 (Figure 5C), populations in the Pigeon River watershed of North Carolina were clustered separately from other Brook Trout populations. At a *K*-value of 5 (Figure 5D), a new cluster of 21 populations in central Virginia was identified, primarily on the east side of the Blue Ridge Mountains in the Rapidan and Rappahannock River basins. At higher values of *K*, subdivision became more apparent in the southernmost populations. Additional clusters were added within the southern Appalachian set of populations at *K*-values of 6 and 7. At a *K*-value of 10, the former central Appalachian cluster was divided into two (while maintaining the Virginia Blue Ridge cluster) and southern populations comprised six clusters that tended to fall within HUC8 watersheds (Supplemental Material 4 and Brook Trout Explorer [https://bte.ecosheds.org/]). Further subdivision within the southern Appalachian region occurred at a *K* of 15. At a *K*-value of 20, some geographic structure among the northern populations became apparent. One cluster was located in Maine, New

Hampshire, Vermont, and western Massachusetts. Another cluster occurred in coastal drainages of Maine, New Hampshire, Massachusetts, and coastal New York. Northern New York and Great Lakes populations formed a third cluster in this region (Supplemental Material 4 and Brook Trout Explorer). At a *K*-value of 25, clusters were generally similar to those observed at a *K* of 20 but with subdivision at increasingly finer spatial scales. For example, collections within the Susquehanna River (Pennsylvania) formed a separate cluster at $K = 25$, with cohesion at the HUC6 level; farther to the south, conformity with HUC8 watersheds further increased.

Results of DAPC for hatchery stocks revealed that at a *K*-value of 2, the captive lineages belonged entirely to the cluster associated with populations in northern areas, with a small amount of southern ancestry in the Paint Bank stock (Figure 5A). Only the stock at the Tellico Trout Hatchery, a propagation facility that cultures Brook Trout from the southern Appalachians, was entirely of southern origin (Figure 5A). At a *K*-value of 3, 13 of 17 hatchery stocks were predominantly of northeastern origin, while four were predominantly of mid-Atlantic origin (Figure 5B). At higher levels of *K*, all 17 hatchery stocks showed varying compositions of northeastern and mid-Atlantic ancestry. The Tellico collection showed indications of multiple southern lineages (Figure 5C, D). Within the southern Appalachian Mountains, there was a signature of apparent introgression of the northern Brook Trout lineage into some populations across values of *K* (Figure 5A–D).

A comparison of allelic diversity among the three broad genetic clusters identified with DAPC ($K = 3$; using the subsampled data set to account for sampling intensity) contrasted somewhat with patterns of within-population diversity. The mid-latitude cluster contained the greatest number of alleles ($n = 174$). However, despite generally low levels of allelic diversity within populations, the southern cluster as a whole showed more allelic diversity ($n = 165$) than the northern cluster ($n = 147$). Hierarchical Shannon diversity analysis further indicated that the mid-latitude cluster contained the highest amount of within-region genetic diversity (mean ${}_{S}H[\mathrm{WR}_r] = 0.625$; SD = 0.014), followed by endemic populations in the southern Appalachian Mountains (mean ${}_{S}H[\mathrm{WR}_r] = 0.560$; SD = 0.015) and then by populations in the northern cluster identified by DAPC ($K = 3$; mean ${}_{S}H[\mathrm{WR}_r] = 0.514$; SD = 0.013). Although genetic diversity was low within most individual populations in the southern Appalachians relative to other regions, the region harbors considerable total genetic diversity because of high degrees of differentiation among populations.

DISCUSSION

This study presents results from the largest population genetic survey yet conducted on wild and cultured Brook Trout populations in eastern North America. Although many studies have examined the population genetic structure of this species (e.g., McCracken et al. 1993; Hayes et al. 1996; Danzmann et al. 1998; Kazyak et al. 2016; Printz et al. 2018; Nathan et al. 2019, 2020; Morgan et al. 2021), no previous effort has characterized relationships among populations at such a broad spatial scale with nuclear DNA markers, particularly in the southern Appalachian Mountains. The large number of populations represented in our study allows insights that would not be available with analysis of smaller, more spatially restricted data sets. This underscores the value of collaborative, broad-scale approaches to studying widely distributed taxa. Notably, we made the following observations and inferences: (1) populations in the south tend to have small values of N_e, and genetic drift has been a strong driver of contemporary population structure; (2) relationships among populations across the landscape are complex and more complicated than the simple north–south division that was suggested in earlier studies; and (3) major genetic clusters reflect large-scale dispersal from Pleistocene refugia. Our findings highlight the distinctiveness and vulnerability of many Brook Trout populations in the southern Appalachian Mountains and have important implications for wild Brook Trout management.

Within- and Among-Population Genetic Variation

Genetic variation within native southern Appalachian Brook Trout populations tended to be substantially lower than that within populations at higher latitudes. While low estimates of genetic variation have been reported in isolated high-latitude populations within the native range (Kelson et al. 2015; Bernos and Fraser 2016), the proportion of small and isolated populations with low genetic variation is greater at southern latitudes. This pattern appears to be due to strong genetic drift—an inference supported by our observation that populations with the lowest estimates of genetic variation (in terms of uH_E and A_R) were also the most genetically differentiated. This pattern of genetic distinctiveness owing to genetic drift also has been observed in isolated populations at finer spatial scales than the present study: for example, in isolated populations of salmonids (Whiteley et al. 2010, 2014), an Australian galaxiid (Coleman et al. 2013), and small mammals (Weeks et al. 2016). Small estimates of N_e, often less than 30 in many southern populations that we examined, were consistent with the expectation for strong genetic drift. We are confident that N_e is small in many of these populations, although some of the variation in N_e estimates was likely due to small sample size and, given violation of the assumption of nonoverlapping generations, whether estimates from mixed-age samples were more similar to N_b or N_e (Luikart et al. 2010; Waples and Do 2010).

Given small effective and census sizes, the risk of population extinction is likely to be raised in this large set of isolated populations due to strong genetic drift causing deleterious alleles to either shift to high frequency or become fixed. Low genetic variation is also likely to cause limited adaptive potential. Under similar circumstances, others have argued that continued management of fragmented populations in isolation could increase extinction risk (Weeks et al. 2016). Notably, populations at the edge of a species' range are expected to encounter more frequent demographic bottlenecks, which would further increase the rate of genetic drift (Allendorf 1986; Hampe and Petit 2005) and the frequency of deleterious alleles in the population. Continued erosion of genetic variation is likely to limit future adaptive potential and population resiliency under future environmental conditions. Although we found significant positive correlations between allelic diversity and estimates of N_e, it is worth noting that Weathers et al. (2019) observed no significant correlation between the amount of phenotypic variation within populations and any of the examined measures of genetic diversity or the amount of occupied habitat sampled. However, additional work may be needed to understand the most appropriate scale of Brook Trout management, as there is some evidence to suggest that Brook Trout populations differ in their upper thermal tolerance and capacity for acclimation (Stitt et al. 2014), at least partly due to differences in routine metabolic rates (Hartman 2019). Among-population differences may be attributable, at least in part, to regional differences in bioenergetics, as southern populations have had much longer to develop local adaptations to warmer stream temperatures and restricted energy availability (Whitworth and Strange 1983; Cada et al. 1987; Ensign et al. 1990; Romaniszyn et al. 2007) than northern populations. Collectively, this information suggests that more work is needed to understand the relationship between genetic drift and differentiation as well as adaptive traits in isolated populations within and among geographic regions.

Nearly all Brook Trout populations were significantly genetically differentiated and typically to a great extent. High divergence among populations has been widely reported across the northern portion of the Brook Trout's native range (Angers and Bernatchez 1998; Castric and Bernatchez 2003; Richards et al. 2008; Bruce et al. 2018), but genetic differentiation was even greater across much of the southern Appalachians than has been previously reported. Patterns of strong differentiation may be due, in part, to habitat alteration and competition with introduced Rainbow Trout *Oncorhynchus mykiss* and Brown Trout *Salmo trutta*, which have restricted native Brook Trout to more isolated, higher-elevation habitat patches in the south (Larson and Moore 1985; Hudy et al. 2008).

Despite the limited genetic variation observed within many populations (alpha diversity), most populations in the southern Appalachian Mountains were highly differentiated (beta diversity; Table 1). However, when viewed in aggregate this region contains more genetic diversity than the northern cluster (gamma diversity; see results of hierarchical Shannon diversity analysis). This finding highlights the importance of conserving endemic genetic diversity within the southern region, as populations are often unique and irreplaceable. Moreover, it challenges the notion that Brook Trout in the south are genetically depauperate (Pregler et al. 2018; Weathers et al. 2019). There is, in fact, high genetic diversity here, but it is spread among many populations that have had a long time to diversify and adapt to local conditions.

Population Clustering Results and Natural History

The physiographic setting of much of unglaciated eastern North America has been defined by the geologically and ecologically complex Appalachian Mountains (Soltis et al. 2006). Some features of genetic structure observed in our analyses can be related to the ECDD, to current or past drainage patterns, and to dispersal from glacial refugia. The geographic patterning of genetic clusters was strikingly consistent between the two methods used in this study, although DAPC clusters populations based on allele frequencies, whereas STRUCTURE uses an HW model-based clustering algorithm. That the most fundamental differentiation among Brook Trout populations (at $K = 2$ for both DAPC and STRUCTURE analyses) occurred among southern and other Brook Trout assemblages was not surprising, as this distinction has long been suggested on the basis of coloration, morphology, life history (Lennon 1967; Behnke 1980; Power 1980; Bivens et al. 1985), and allozyme frequencies (Stoneking et al. 1981; McCracken et al. 1993; Printz et al. 2018). Our findings based on microsatellite allele frequencies support the distinctiveness of Brook Trout in the southern Appalachian Mountains, which may be explained, in part, by a zoogeographic boundary along the ECDD. This assemblage of populations likely expanded from one or more Pleistocene glacial refugia in the Mississippi River drainage (Danzmann et al. 1998). Other species showing evidence of genetic discontinuity at the Appalachian Mountains include salamanders (Donovan et al. 2000; Church et al. 2003), turtles (Walker and Avise 1998), and plants (Parks et al. 1994; Sewell et al. 1996; Joly and Bruneau 2004; Mylecraine et al. 2004), suggesting that many elements of the regional fauna and flora expanded from distinct glacial refugia east and west of the Appalachians (Soltis et al. 2006).

At higher latitudes, mid-Appalachian Brook Trout populations on the east side of the ECDD were distinguished from other, northerly populations on both sides

of the divide ($K = 3$ for DAPC). A growing body of evidence suggests that some temperate species survived glacial periods in refugia that were located well north of the Gulf Coast (Soltis et al. 2006). We suggest that the mid-Appalachian Brook Trout populations recolonized the landscape from glacial refugia on the Potomac River, the Susquehanna River, and other eastward-flowing drainages of the mid-Atlantic region. More northerly populations likely found refuge in the Delaware, Hudson, and Connecticut rivers and more northerly coastal rivers, sometimes collectively referred to as an Acadian refugium. Such populations may have entered the Great Lakes watershed through the St. Lawrence River and may have entered the upper Mississippi River system through the Brule Glacial Spillway in Wisconsin into the St. Croix River. As discussed below, the geographic distribution of mitochondrial DNA variation (Danzmann et al. 1998) also supports the hypothesis that contemporary Brook Trout populations expanded from three glacial refugia. We note that the group of populations in the vicinity of the Greenbrier River, West Virginia, clustered with others on the opposite side of the ECDD. These populations are located in an area with multiple documented stream captures (Hocutt et al. 1978), which may have facilitated localized expansion of this lineage into the Mississippi River basin.

At finer spatial scales (e.g., $K \geq 4$ for DAPC), the clustering results appear to reflect a combination of geophysical processes and supplemental stocking. Within the southern Appalachian Mountains, populations within the upper Pigeon River watershed were among the first to split out in the clustering analyses. Among the possible explanations, this may in part reflect the presence of numerous waterfalls posing barriers to upstream migration, and northern-derived hatchery stocks might be poorly adapted to such ecosystems (Galbreath et al. 2001; Kazyak et al. 2018). In Supplemental Material 5, we present a case study of stocking and limited introgression of hatchery stocks into native populations in Great Smoky Mountains National Park.

Another distinct cluster was resolved in the vicinity of Shenandoah National Park. This group of 21 populations (shown in dark blue in Figure 5D [$K = 5$ for DAPC]) occurred mostly but not entirely on the eastern side of the Blue Ridge Mountains of central Virginia. A review of stocking records (David Demarest, Shenandoah National Park, personal communication) suggested that this cluster may partly reflect the result of multiple stocking events both inside and outside of Shenandoah National Park starting in the early 1900s and continuing through at least the 1950s. Therefore, we infer that the genetic composition of populations within this cluster, which straddles the watershed divide, is likely a mixture of natural and anthropogenic origins.

In DAPC models with greater complexity (e.g., $K \geq 7$), clusters of populations, especially in the south, tended to become split more finely among watersheds. The finer-scale variation in the south likely reflects that this region was never glaciated (Hewitt 2000). Greater genetic diversity in unglaciated areas than in deglaciated regions has been observed in Brook Trout (Bernatchez and Danzmann 1993), Walleye *Sander vitreus* (Billington and Hebert 1988; Ward et al. 1989; Billington et al. 1992), Red Shiner *Cyprinella lutrensis* (Richardson and Gold 1995), and European Brown Trout (reviewed by Bernatchez and Wilson 1998).

Correspondence with Mitochondrial DNA Variation

Some authors (Radforth 1944; Mandrak and Crossman 1992) have argued that Brook Trout expanded from one Atlantic upland refugium, while others (Bailey and Smith 1981) have argued that northern Brook Trout also arose from a Mississippian refugium. Our interpretations of microsatellite DNA data led to inferences of past expansion of Brook Trout populations from Mississippian, mid-Atlantic, and Acadian glacial refugia to recolonize the deglaciated North American landscape, with subsequent secondary contact among lineages. Our results supporting the view that Brook Trout populations in the Great Lakes region are the product of mixing of ancestral populations from Mississippian and Acadian refugia (results for these collections can be viewed using Brook Trout Explorer [https://bte.ecosheds.org/]) parallel those reached using mitochondrial DNA (Danzmann et al. 1998). The geographic distribution of the Danzmann et al. (1998) sampling sites was mostly in the northern part of the range, which limits direct comparison of their results with ours. Building upon this work, Hall et al. (2002), examining mitochondrial restriction fragment length polymorphism variation in Brook Trout from 10 stream units in five drainages of Maryland, showed three major assemblages: two on the east of the ECDD and one on the west. Drainage basins nested within the two major drainage basins were the major units of population division, a finding that was convergent with our microsatellite nuclear DNA-based results. Furthermore, the inferences that we reached for Brook Trout by using microsatellite markers parallel those for other salmonids that have been assessed using mitochondrial markers (reviewed by Bernatchez and Wilson 1998). A rangewide study of Brook Trout mitochondrial genomes would help to inform a phylogeographic assessment of the species' natural history, including more direct assessment of expansion from glacial refugia and subsequent secondary contact. Application of a molecular clock to DNA sequence variants would support estimation of times of divergence among lineages, in turn supporting interpretation of natural history events.

Southern Lineage

Previous studies have considered southern Appalachian Brook Trout to be a distinct strain (e.g., Hayes et al. 1996; Galbreath et al. 2001) warranting taxonomic review (e.g., Habera and Moore 2005). We found that patterns of population genetic structure of Brook Trout in the southern Appalachians are far more complex than a simple "northern" versus "southern" dichotomy. We did not find evidence for a crisp genetic break between putative northern and southern lineages at the New River watershed (Printz et al. 2018). Rather, we interpret the southern cluster as the descendants of fish radiating from a Pleistocene refugium in the Mississippi River drainage, which colonized much of North America west of the ECDD, with evidence of dispersal as far north as Pennsylvania and New York. Further, within the geographic distribution of this lineage, we noted a tremendous amount of fine-scale variation. Nearly all populations were genetically distinct, and populations within the same watershed commonly were very divergent. The Atlantic slope populations that clustered with interior basin populations in the southern region likely reflect expansion via past stream capture events. This explanation is supported by geological evidence indicating repeated shifts in the ECDD in this region (Gallen 2018; Johnson 2020).

Despite an extensive history of stocking of domesticated conspecifics, many Brook Trout populations in the southern Appalachians show little evidence of hatchery introgression (Pregler et al. 2018; Printz et al. 2018; present study). Rather, the vast majority of populations retain genetic characteristics distinct from those of hatchery strains. However, a small number of populations were genetically similar to stocked hatchery strains, reflecting high levels of admixture or establishment of the population by hatchery-origin individuals. This finding is consistent with those of Kazyak et al. (2018), who used the same techniques to assess hatchery introgression across Brook Trout populations in North Carolina (those populations are included in the present study), and with previous studies across other portions of the southern native range (e.g., Virginia: Humston et al. 2012, Printz et al. 2018; South Carolina: Pregler et al. 2018).

IMPLICATIONS FOR MANAGEMENT

Our findings pose important implications for management. The American Fisheries Society's Southern Division Trout Committee developed a position statement (Habera and Moore 2005) to advocate management approaches that are suitable for conserving southern Appalachian Brook Trout. After expressing the importance of these fish and promoting comprehensive, region-wide management, the committee's recommendations addressed habitat protection and improvement, population restoration, stocking of hatchery Brook Trout, and angling regulations. Our work constitutes the genetic inventory that was called for in the position statement, and our results can inform management planning and implementation, such as prioritizing protection of habitats supporting native gene pools or selecting source and recipient populations for restoration or enhancement actions. The highest-level goal for genetically based Brook Trout management would be to conserve native genetic variation and to practice population restoration as needed to maintain each population's potential to adapt to environmental change. Ultimately, genetically diverse populations representing endemic lineages are critical to conserving our natural heritage in a changing world (Des Roches et al. 2021; Stange et al. 2021).

In light of our findings, managers may wish to review and update the management actions and guidelines proposed by Habera and Moore (2005). Instead of simply viewing Brook Trout in a "northern" versus "southern" context, our data indicate that substantial genetic differences are widespread among Brook Trout collected from many different regions. Management strategies may be most effective when they consider the substantial amount of fine-scale genetic variation that is characteristic of the species and its evolutionary history.

One such approach would be to classify Brook Trout within the southern Appalachian Mountains as an evolutionarily significant unit (Ryder 1986; Waples 1991; Nielsen and Powers 1995) while recognizing the substantial heterogeneity therein as management units (MUs). A population or assemblage of populations meets the criteria for an evolutionarily significant unit if (1) it has been reproductively isolated for long enough that it contains unique evolutionary combinations that are unlikely to re-evolve on an ecological timeframe and (2) it is ecologically or adaptively distinct—that is, it contains genetic or phenotypic variation that is important for adaptive capacity to changing environmental conditions (Waples 1991). Our work and others' work with selectively neutral microsatellite markers and other groups' efforts using allozyme and mitochondrial DNA markers (Stoneking et al. 1981; McCracken et al. 1993; Danzmann et al. 1998; Guffey et al. 1999; Printz et al. 2018) show that southern Appalachian Brook Trout are reproductively isolated from other conspecific units, even at very small spatial scales. Putatively, adaptive characters exhibited by southern Appalachian Brook Trout would include tolerance of relatively high temperatures, an adaptation that has yet to be assessed for populations across the distribution of the species, and small size and early age at maturity compared to Brook Trout of more northerly origin (Konopacky and Estes 1986; Habera et al. 2001; however, note that some populations of Brook Trout in northern areas also are

adapted for early maturity: Hutchings 1993). Further studies of local adaptation of Brook Trout populations would be critical to strengthen this line of inference.

Management units ideally correspond with populations that are demographically independent from one another (Allendorf and Luikart 2007). Identification of MUs is critical for short-term management, such as managing habitat, setting harvest rates, and monitoring population status. Moritz (1994) suggested that MUs are populations that have substantially divergent allele frequencies at many loci; however, allele frequency differentiation cannot be interpreted directly as evidence for demographic independence (Allendorf and Luikart 2007). Palsboll et al. (2007) proposed that identification of MUs from population genetic data should be based upon the amount of genetic divergence at which populations become demographically independent: that is, MU status would be assigned when the observed estimate of genetic divergence is significantly greater than a predefined threshold value (Ramstad et al. 2004). Until the results of such studies become available, we suggest that managers could use watersheds to delineate provisional MUs, as our results indicate that a considerable amount of genetic variation is associated with watershed structure (Table 2) and these units are likely to be demographically independent. Our suggestion is convergent with those of Habera and Moore (2005) and other authors regarding the use of river subbasins and watersheds as MUs for conserving genetic variation in Brook Trout.

Future Brook Trout translocations will have the goal of either re-establishing extirpated populations (hereafter, "reintroduction") or elevating the probability of persistence of extant populations (hereafter, "genetic rescue"). Population extirpations have occurred in southeastern North America (Hudy et al. 2008), and managers often reintroduce Brook Trout (Pregler et al. 2018). In addition, our study revealed many extant populations with low genetic variation that may be potential candidates for genetic rescue. Genetic rescue focuses on small, isolated populations that may be suffering from the effects of inbreeding, and genetic rescue efforts may increase genetic variation and adaptive potential (Hedrick et al. 2011; Whiteley et al. 2015). Some high-profile studies have shown positive fitness effects after translocations into target populations (e.g., Florida panther *Puma concolor coryi*: Johnson et al. 2010; bighorn sheep *Ovis canadensis*: Hogg et al. 2006, Miller et al. 2012), whereas others have not (e.g., gray wolf *Canis lupus*: Adams et al. 2011; note that this example was based on a single immigrant in a limited habitat). Examples of genetic rescue in fishes include Guppy *Poecilia reticulata* (Zajitschek et al. 2009; Fitzpatrick et al. 2016) and Brook Trout populations in Virginia, where Robinson et al. (2017) found evidence of positive fitness effects through the F_1 generation. Wells et al. (2019) detected little evidence of outbreeding depression in Brook Trout populations in Newfoundland; instead, hybridization effects were mostly neutral (60/66 nonhybrid versus hybrid comparisons), with some support for heterosis (6/66). A growing body of evidence suggests that genetic rescue may be beneficial, at least under certain circumstances (Frankham 2015).

Concerns about outbreeding depression have generally limited more widespread implementation of genetic rescue across all taxa (Ralls et al. 2018; Bell et al. 2019). Outbreeding depression is an important genetic concern for both reintroduction and genetic rescue (Whiteley et al. 2015; Ralls et al. 2018), as it can result in the disruption of locally adapted gene complexes, such as those that are likely found in wild populations of Brook Trout throughout the southern Appalachians. Even single-source reintroductions carry this risk if gene flow out of reintroduced populations to other nearby natural populations occurs after translocation. Our results suggest that donor populations should be chosen from within the same watershed to minimize the probability of outbreeding depression. Therefore, our results extend the recommendations of Habera and Moore (2005), who asserted that donor Brook Trout populations should have known genetic origins and that nonnative Brook Trout donor populations should be avoided. Further, if single sources are preferred for reintroductions, it may be best to choose source populations with high genetic variation from similar environmental conditions to maximize matches in local adaptations (Kazyak et al. 2021). The number of translocated individuals should be sufficient to maintain genetic variation in both source and recipient populations. Malone et al. (2018) provided guidance for the number of individuals that should be targeted to match N_e in source and reestablished populations, along with a quantitative method to combine information based on habitat matching, genetic variation, genetic differentiation, and fish density to find suitable source populations. The 50:500 rule provides additional guidance for a minimal N_e to avoid concerns about inbreeding depression in either the source or recipient population (Jamieson and Allendorf 2012). An N_e below 50 corresponds to an increase in genome-wide homozygosity greater than 1% per generation and can be a warning of negative fitness effects of inbreeding. If there are demographic or genetic concerns about removal of adults from single source populations, multiple sources can be used. Interbreeding among individuals from multiple source populations, assuming a lack of assortative mating within the reintroduced population, will elevate genetic variation but could induce outbreeding depression if interbreeding individuals are too genetically divergent (Huff et al. 2011). Finally, we note that there are additional concerns beyond genetics when moving individuals between populations, such as potential introduction of

harmful parasites or microbes (Ruiz et al. 2017). Given the risks and uncertainty, we suggest that future Brook Trout translocations (reintroductions or genetic rescue) should occur within an adaptive management framework (Robinson et al. 2017), with the goal of achieving a general understanding of the efficacy of these approaches for Brook Trout.

Captively reared individuals could serve as the source for either reintroduction or genetic rescue efforts. However, caution is warranted when using captive fish for this purpose because recent studies indicate that hatchery stocks propagated from wild broodfish have lower fitness than wild fish (Araki et al. 2008; Christie et al. 2012a; Evans et al. 2015); lower reproductive success (Theriault et al. 2011; Christie et al. 2012a); decreased allelic richness, higher LD, and higher levels of genetic drift (Christie et al. 2012b); and often very unequal contributions among individual broodstock (Beirão et al. 2019). Additionally, Le Luyer et al. (2017) identified epigenetic modifications induced by captive rearing as a potential explanation for reduced fitness in hatchery-reared salmon, suggesting a mechanism for transgenerational inheritance of these deleterious effects on gene expression. Due to these concerns, we view the use of hatchery-reared individuals as less preferable than the use of wild individuals for translocation purposes. However, if it is necessary to use hatchery individuals, the use of local genetic source stocks (Olson et al. 2004; Cooper et al. 2010; Fisch et al. 2015; Trushenski et al. 2015) should minimize outbreeding depression risks for reintroductions or genetic rescue attempts. Ongoing work at the Tennessee Aquarium and Conservation Institute and the Tellico Trout Hatchery supports the case that propagation of southern Appalachian Brook Trout is a viable technique (Johnson 2016). To support reintroductions, a model of habitat variables determining the suitability of streams for Brook Trout restoration has been developed (Romines 2017). Habera et al. (2001) reported the restoration of Brook Trout in 17 Tennessee streams, including extension of their distribution in Sevier County by outplanting the progeny of wild Brook Trout propagated at the Tellico Trout Hatchery.

Caveats and Limitations

Although the present study is based on an unusually large genetic data set, we faced several limitations that could be addressed in future work. First, many of our collections comprised fewer samples than are generally recommended. This reflects sampling of many marginal populations with limited numbers of individuals as well as the reuse of tissue samples that were collected for other purposes. We addressed this issue by restricting much of our analysis to collections with at least 20 individuals. Although sample sizes of at least 25–30 (Hale et al. 2012) have been recommended to provide a reasonable likelihood of observing rare alleles or haplotypes, it can still be worthwhile to report genetic metrics for marginal populations with smaller sample sizes (Pruett and Winker 2008). Our sampling intensity also varied among collections and among regions. Uneven sampling is associated with a greater propensity to identify subdivision in more heavily sampled units using STRUCTURE (Peuchmaille 2016; but note that the simulations in that study used far lower levels of differentiation among populations than was generally observed within our study). However, the impacts of uneven sampling on DAPC have not been explored (Miller et al. 2020). Given that our sampling effort was more intense within the southern Appalachian Mountains, we may have had greater power to resolve structure within this region. Further sampling in northern areas may shed more light on the lineages present in that part of the range of Brook Trout. However, we note that our general findings were consistent among different analytical approaches and with hypotheses associated with glacial history. The high levels of differentiation observed in many areas likely moderated any impacts of uneven sampling. There were also differences in the length of stream from which the samples were collected. While most collections included multiple cohorts, some collections were restricted to only age-0 individuals. Future population genetics studies of Brook Trout would benefit from the adoption of consistent sampling guidelines that effectively support their goals, with target sample sizes based on guidelines for the class of marker that will be used. To obtain the best possible genetic characterization of a population, it should ideally be sampled along the entire length of its habitat patch and samples should include members of all cohorts present.

Future Directions for Studies of Genomics and Local Adaptation

We screened variations in microsatellite DNA, which are regarded as indicative of selectively neutral population genetic processes. Such markers are well suited for detecting the signatures of demographic events such as population expansions and contractions, gene flow, and introgression from hatchery-derived Brook Trout. Patterns of microsatellite variation are not, however, indicative of adaptive genetic variation within and between populations of Brook Trout. Fraser et al. (2014) examined coding-gene polymorphisms associated with various biological functions in fragmented Newfoundland Brook Trout populations of varying sizes and found that fragmentation affects natural selection and that population size affects adaptive changes and population differentiation. Ferchaud et al. (2020) identified genomic regions associated with anadromy in Canadian Brook Trout as well as an overrepresentation of transposable elements associated with environmental variables, suggesting the importance of

transposable elements in adaptation. They also observed considerable accumulation of maladaptive mutations, which they associated with genetic drift. Wood et al. (2015) observed that population size was only weakly related to quantitative genetic variation and expression of 15 traits across nine Brook Trout populations, although large studies would be needed to reach strong conclusions. Brook Trout body size, shape, and coloration differences were most frequently and directly linked to habitat variation and operational sex ratio rather than to population size (Zastavniouk et al. 2017), suggesting that selection may overcome drift at small population sizes and that selection may be acting more strongly on females than on males. Taken together, these studies provide fresh insight into the role of genetic variation in adaptation and population resilience; however, there is still much to learn to enhance management outcomes.

Investigation of adaptive genetic variation has not yet been extended to Brook Trout populations across the range of the species. While the genetic basis of adaptation in Brook Trout remains largely unknown, further understanding of adaptive genetic variation would inform management of populations to conserve their long-term adaptive potential. Future research may utilize next-generation genomics technologies to further investigate how the adaptive potential of Brook Trout varies among populations and to identify putatively resilient populations and management practices that optimize the evolutionary potential for the species. The development of a standardized single-nucleotide polymorphism panel that is suitable for reduced representation sequencing would allow for rangewide marker comparisons in a manner similar to that presented here for microsatellites.

ACKNOWLEDGMENTS

We would like to express our sincere gratitude to the many technicians, collaborators, personnel, and volunteers that helped collect or coordinate the tissue samples used in this study: Brendan Annett (Waquoit Bay National Estuarine Research Reserve), William Ardren (U.S. Fish and Wildlife Service [USFWS], Abernathy Fish Technology Center), Jim Atkinson (National Park Service [NPS], Shenandoah National Park), Aaron Aunins (USGS, EESC), Andy Burt (Ohio Department of Natural Resources), Larry Claggett (Wisconsin Department of Natural Resources), Joe Clark (USGS, Southern Appalachian Field Branch at the University of Tennessee), Bruce Connery (NPS, Acadia National Park), Scott Cornett (New York State Department of Environmental Conservation [NYSDEC]), David Demarest (NPS, Shenandoah National Park), Mike Eackles (USGS, EESC), Robert Fawcett (New Hampshire Fish and Game Department), Dylan Fraser (University of Laval), Merry Gallagher (Maine Department of Inland Fisheries and Wildlife), Frank Getchell (Trout Unlimited), Pat Hamilton (New Jersey Division of Fisheries and Wildlife), Shannon Julian (USGS, Leetown Science Center), Bill Kalishek (Iowa Department of Natural Resources), Rich Kirn (Vermont Fish and Wildlife Department), Matt Kline (University of Maryland Center for Environmental Science [UMCES], Appalachian Laboratory), Mike LaVoie (Cherokee Fisheries and Wildlife Management), Lori Maloney (Tioga County Conservation District), David Manski (NPS, Acadia National Park), Pat Mazik (West Virginia University), Roger McPherson (Clarion University of Pennsylvania), Gonzalo Mendez (U.S. Forest Service [USFS] at the University of Massachusetts Amherst), Steve Moore (NPS, Great Smoky Mountains National Park), Keith Nislow (USFS at the University of Massachusetts Amherst), Jack Oelfke (NPS, Isle Royale National Park), Tom Oldham (West Virginia Department of Natural Resources), Todd Petty (West Virginia University), Richard Preall (NYSDEC), Joanne Printz (Virginia Polytechnic Institute and State University), Andrew Roach (NPS, Catoctin Mountain Park), Dan Sinopoli (Burnt Hills-Ballston Lake High School, New York), Jay Stauffer (Pennsylvania State University), Wendylee Stott (USGS, Great Lakes Science Center), John Switzer (USGS, Leetown Science Center), Dianne Timmins (New Hampshire Fish and Game Department), Chris van Maaren (NYSDEC), James Voigt (NPS, Catoctin Mountain Park), Dave Weller (USGS, EESC), Derek Wiley (UMCES, Appalachian Laboratory), Joe Williams (Virginia Department of Game and Inland Fisheries), Alastair Wilson (University of Guelph), Jeb Wofford (NPS, Shenandoah National Park), and Colleen Callahan Young (USGS, EESC). (The affiliation of the individual at the time of fish collection is indicated in parentheses next to the name and may not be the current affiliation of that individual.) We apologize to anyone we may have inadvertently missed who helped during the many years this study has been ongoing. We thank Mike Eackles and Robin Johnson (USGS, EESC) for help with laboratory work. Shannon Julian and Colleen Callahan Young (both formerly at the USGS, Leetown Science Center) were also instrumental with laboratory work and data collection. We appreciate the numerous helpful conversations we have had with others as we developed the manuscript, especially our dialogues with members of the Trout Technical Committee, American Fisheries Society Southern Division. David Demarest (NPS, Shenandoah National Park) and Larry Kallemeyn (USFWS, National Archives) provided useful information on historical Brook Trout stocking in the region. We thank Shannon L. White (USGS, EESC) for her thorough and helpful assistance with revisions. We have included the late Tim King as a coauthor, as he played a critical role in the design and execution of the project; without his vision and ambition, this effort would not have been possible. The participation of E.M.H. was supported in part by the Virginia Agricultural Experiment Station under the Hatch Program of the U.S. Department of

Agriculture's National Institute of Food and Agriculture. Funding was provided by the North Carolina Wildlife Resources Commission to USGS to support the genotyping and analysis of many of the samples. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. There is no conflict of interest declared in this article.

ORCID

David C. Kazyak https://orcid.org/0000-0001-9860-4045
Barbara A. Lubinski https://orcid.org/0000-0003-3568-2569
Kasey C. Pregler https://orcid.org/0000-0002-0664-9594
Andrew R. Whiteley https://orcid.org/0000-0002-8159-6381
Eric Hallerman https://orcid.org/0000-0002-5839-858X
Yoichiro Kanno https://orcid.org/0000-0001-8452-5100

REFERENCES

Adams, J. R., L. M. Vucetich, P. W. Hedrick, R. O. Peterson, and J. A. Vucetich. 2011. Genomic sweep and potential genetic rescue during limiting environmental conditions in an isolated wolf population. Proceedings of the Royal Society B: Biological Sciences 278:3336–3344.

Allendorf, F. W. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology 5:181–190.

Allendorf, F. W., and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Publishing, Malden, Massachusetts.

Andersen, B. G., and H. W. Borns Jr. 1994. The Ice Age world. Scandinavian University Press, Oslo, Norway.

Angers, B., and L. Bernatchez. 1998. Combined use of SMM and non-SMM methods to infer fine structure and evolutionary history of closely related Brook Charr (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. Molecular Biology and Evolution 15:143–159.

Araki, H., B. A. Berejikian, M. J. Ford, and M. S. Blouin. 2008. Fitness of hatchery-reared salmonids in the wild. Evolutionary Applications 1:342–355.

Aunins, A. W., J. T. Petty, T. L. King, M. Schilz, and P. M. Mazik. 2014. River mainstem thermal refuges influence population structuring within an Appalachian Brook Trout population. Conservation Genetics 16:15–29.

Bailey, R. M., and G. R. Smith. 1981. Origin and geography of the fish fauna of the Laurentian Great Lakes basin. Canadian Journal of Fisheries and Aquatic Sciences 38:1539–1561.

Batchelor, C. L., M. Margold, M. Krapp, D. K. Murton, A. S. Dalton, P. L. Gibbard, C. R. Stokes, J. B. Murton, and A. Manica. 2019. The configuration of Northern Hemisphere ice sheets through the Quaternary. Nature Communications [online serial] 10:article 3713.

Behnke, R. J. 1980. A systematic review of the genus *Salvelinus*. Pages 441–481 *in* E. K. Balon, editor. Charrs: salmonid fishes of the genus *Salvelinus*. Dr W. Junk, The Hague, The Netherlands.

Beirão, J., T. B. Egeland, C. F. Purchase, and J. T. Nordeide. 2019. Fish sperm competition in hatcheries and between wild and hatchery origin fish in nature. Theriogenology 133:201–209.

Bell, D. A., Z. L. Robinson, W. C. Funk, S. W. Fitzpatrick, F. W. Allendorf, D. A. Tallmon, and A. R. Whiteley. 2019. The exciting potential and remaining uncertainties of genetic rescue. Trends in Ecology and Evolution 34:1070–1079.

Bernatchez, L., and R. G. Danzmann. 1993. Congruence in control-region sequences and restriction site variation in mitochondrial DNA of Brook Char (*Salvelinus fontinalis* Mitchill). Molecular Biology and Evolution 10:1002–1014.

Bernatchez, L., and C. C. Wilson. 1998. Comparative phylogeography of Nearctic and Palearctic fishes. Molecular Ecology 7:431–452.

Bernos, T. A., and D. J. Fraser. 2016. Spatiotemporal relationship between adult census size and genetic population size across a wide population size gradient. Molecular Ecology 25:4472–4487.

Billington, N., R. J. Barrette, and P. D. N. Hebert. 1992. Management implications of mitochondrial DNA variation in Walleye stocks. North American Journal of Fisheries Management 12:276–284.

Billington, N., and P. D. N. Hebert. 1988. Mitochondrial DNA variation in Great Lakes Walleye (*Stizostedion vitreum*) populations. Canadian Journal of Fisheries and Aquatic Sciences 45:643–654.

Bivens, R. D., R. J. Strange, and D. C. Peterson. 1985. Current distribution of the native Brook Trout in the Appalachian region of Tennessee. Journal of the Tennessee Academy of Science 60:101–105.

Bruce, S. A., M. P. Hare, M. W. Mitchell, and J. Wright. 2018. Confirmation of a unique and genetically diverse 'heritage' strain of Brook Trout (*Salvelinus fontinalis*) in a remote Adirondack watershed. Conservation Genetics 19:71–83.

Cada, G. F., J. M. Loar, and M. J. Sale. 1987. Evidence of food limitation of Rainbow and Brown trout in southern Appalachian soft-water streams. Transactions of the American Fisheries Society 116:692–702.

Castric, V., and L. Bernatchez. 2003. The rise and fall of isolation by distance in the anadromous Brook Charr (*Salvelinus fontinalis* Mitchill). Genetics 163:983–996.

Christie, M. R., M. L. Marine, R. A. French, and M. S. Blouin. 2012a. Genetic adaptation to captivity can occur in a single generation. Proceedings of the National Academy of Sciences of the USA 109:238–242.

Christie, M. R., M. L. Marine, R. A. French, R. S. Waples, and M. S. Blouin. 2012b. Effective size of a wild salmonid population is greatly reduced by hatchery supplementation. Heredity 109:254–260.

Church, S. A., J. M. Kraus, J. C. Mitchell, D. R. Church, and D. R. Taylor. 2003. Evidence for multiple Pleistocene refugia in the postglacial expansion of the eastern tiger salamander, *Ambystoma tigrinum tigrinum*. Evolution 57:372–383.

Coleman, R. A., A. R. Weeks, and A. A. Hoffmann. 2013. Balancing genetic uniqueness and genetic variation in determining conservation and translocation strategies: a comprehensive case study of threatened Dwarf Galaxias, *Galaxiella pusilla* (Mack) (Pisces: Galaxiidae). Molecular Ecology 22:1820–1835.

Cooper, A. M., L. M. Miller, and A. R. Kapuscinski. 2010. Conservation of population structure and genetic diversity under captive breeding of remnant coaster Brook Trout (*Salvelinus fontinalis*) populations. Conservation Genetics 11:1087–1093.

Danzmann, R. G., R. P. Morgan II, M. W. Jones, L. Bernatchez, and P. E. Ihssen. 1998. A major sextet of mitochondrial DNA phylogenetic assemblages extant in eastern North American Brook Trout (*Salvelinus fontinalis*): distribution and postglacial dispersal patterns. Canadian Journal of Zoology 76:1300–1318.

Davis, J. E. 2008. Geographic distribution of southern- and northern-form Brook Trout populations in southwestern Virginia. Master's thesis. Virginia Polytechnic Institute and State University, Blacksburg.

Des Roches, S., L. H. Pendleton, B. Shapiro, and E. P. Palkovacs. 2021. Conserving intraspecific variation for nature's contributions to people. Nature Ecology and Evolution 5:574–58.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillet, and J. R. Ovenden. 2014. NeEstimator v2: re-implementation of software for

the estimation of contemporary effective population size (N_e) from genetic data. Molecular Ecology Resources 14:209–214.

Donovan, M. F., R. D. Semlitsch, and E. F. Routman. 2000. Biogeography of the southeastern United States: a comparison of salamander phylogeographic studies. Evolution 54:1449–1456.

Ensign, W. E., R. J. Strange, and S. E. Moore. 1990. Summer food limitation reduces Brook and Rainbow Trout biomass in a southern Appalachian stream. Transactions of the American Fisheries Society 119:894–901.

Evans, M. L., M. A. Johnson, D. Jacobson, J. Wang, M. Hogansen, and K. G. O'Malley. 2015. Evaluating a multi-generational reintroduction program for threatened salmon using genetic parentage analysis. Canadian Journal of Fisheries and Aquatic Sciences 73:844–852.

Fausch, K. D. 2008. A paradox of trout invasions in North America. Biological Invasions 10:685–701.

Ferchaud, A. L., M. Leitwein, M. Laporte, D. Boivin-Delisle, B. Bougas, C. Hernandez, E. Normandeau, I. Thibault, and L. Bernatchez. 2020. Adaptive and maladaptive genetic diversity in small populations: insights from the Brook Charr (*Salvelinus fontinalis*) case study. Molecular Ecology 29:3429–3445.

Fitzpatrick, S. W., J. C. Gerberich, L. M. Angeloni, L. L. Bailey, E. M. Broder, J. Torres-Dowdall, C. A. Handelsman, A. Lopez-Sepulcre, D. N. Reznick, C. K. Ghalambor, and W. C. Funk. 2016. Gene flow from an adaptively divergent source causes rescue through genetic and demographic factors in two wild populations of Trinidadian Guppies. Evolutionary Applications 9:879–891.

Fisch, K. M., C. C. Kozfkay, J. A. Ivy, O. A. Ryder, and R. S. Waples. 2015. Fish hatchery genetic management techniques: integrating theory with implementation. North American Journal of Aquaculture 77:343–357.

Frankham, R. 2015. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Molecular Ecology 24:2610–2618.

Fraser, D. J., P. V. Debes, L. Bernatchez, and J. A. Hutchings. 2014. Population size, habitat fragmentation, and the nature of adaptive variation in a stream fish. Proceedings of the Royal Society B: Biological Sciences 281:20140370

Galbreath, P. F., N. D. Adams, S. Z. Guffey, C. J. Moore, and J. L. West. 2001. Persistence of native southern Appalachian Brook Trout populations in the Pigeon River system, North Carolina. North American Journal of Fisheries Management 21:927–934.

Gallen, S. F. 2018. Lithologic controls on landscape dynamics and aquatic species evolution in post-orogenic mountains. Earth and Planetary Science Letters 493:150–160.

Guffey, S. Z., G. F. McCracken, S. E. Moore, and C. R. Parker. 1999. Management of isolated populations: southern strain Brook Trout. Pages 247–266 *in* J. D. Peine, editor. Ecosystem management for sustainability: principles and practices illustrated by a regional biosphere reserve cooperative. CRC Press, Boca Raton, Florida.

Habera, J., and S. Moore. 2005. Managing southern Appalachian Brook Trout: a position statement. Fisheries 30(7):10–20.

Habera, J. W., R. J. Strange, and R. D. Bivens. 2001. A revised outlook for Tennessee's Brook Trout. Journal of the Tennessee Academy of Science 76:68–73.

Hale, M. L., T. M. Burg, and T. E. Steeves. 2012. Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLOS (Public Library of Science) ONE [online serial] 7(9):e45170.

Hall, M. R., R. P. Morgan II, and R. G. Danzmann. 2002. Mitochondrial DNA analysis of mid-Atlantic populations of Brook Trout: the zone of contact for major historical lineages. Transactions of the American Fisheries Society 131:1140–1151.

Hampe, A., and R. J. Petit. 2005. Conserving biodiversity under climate change: the rear edge matters. Ecology Letters 8:461–467.

Harris, A. C., R. D. Hanks, J. M. Rash, D. W. Goodfred, and Y. Kanno. 2021. Standard weight equation for Brook Trout in southern Appalachian Mountain streams. Journal of Fish and Wildlife Management 12:183–189.

Hartman, C. M. 2019. Thermal performance of growth at consumption maximum (*C*-max) and routine metabolic rate (RMR) in Brook Trout *Salvelinus fontinalis* from four populations in central Appalachia. Master's thesis. West Virginia University, Morgantown.

Hayes, J. P., S. Z. Guffey, F. J. Kriegler, G. F. McCracken, and C. R. Parker. 1996. The genetic diversity of native, stocked, and hybrid populations of Brook Trout in the southern Appalachians. Conservation Biology 10:1403–1412.

Hedrick, P. W. 2005. A standardized genetic differentiation measure. Evolution 59:1633–1638.

Hedrick, P. W., J. R. Adams, and J. A. Vucetich. 2011. Reevaluating and broadening the definition of genetic rescue. Conservation Biology 25:1069–1070.

Hedrick, P. W., and S. T. Kalinowski. 2000. Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics 31:139–162.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913.

Hocutt, C. H., R. F. Denoncourt, and J. R. Stauffer. 1978. Fishes of the Greenbrier River, West Virginia, with drainage history of the central Appalachians. Journal of Biogeography 5:59–80.

Hogg, J. T., S. H. Forbes, B. M. Steele, and G. Luikart. 2006. Genetic rescue of an insular population of large mammals. Proceedings of the Royal Society B: Biological Sciences 273:1491–1499.

Hudy, M., T. Theiling, N. Gillespie, and E. P. Smith. 2008. Distribution, status, and land use characteristics of subwatersheds within the native range of Brook Trout in the eastern United States. North American Journal of Fisheries Management 28:1069–1085.

Huff, D. D., L. M. Miller, C. J. Chizinski, and B. Vondracek. 2011. Mixed-source reintroductions lead to outbreeding depression in second-generation descendants of a native North American fish. Molecular Ecology 20:4246–4258.

Humston, R., K. A. Bezold, N. D. Adkins, R. J. Elsey, J. Huss, B. A. Meekins, P. R. Cabe, and T. L. King. 2012. Consequences of stocking headwater impoundments on native populations of Brook Trout in tributaries. North American Journal of Fisheries Management 32:100–108.

Hutchings, J. A. 1993. Adaptive life histories effected by age-specific survival and growth rate. Ecology 74:673–684.

IUBMB (International Union of Biochemistry and Molecular Biology). 1992. Enzyme nomenclature 1992. Academic Press, San Diego, California.

Jamieson, I. G., and F. W. Allendorf. 2012. How does the 50/500 rule apply to MVPs? Trends in Ecology and Evolution 27:578–584.

Johnson, B. 2020. Stream capture and the geomorphic evolution of the Linville Gorge in the southern Appalachians, USA. Geomorphology 368:107360.

Johnson, T. C. III. 2016. Assessment of southern Appalachian Brook Trout propagation for restoring Tennessee populations. Master's thesis. Tennessee Technological University, Cookeville.

Johnson, W. E., D. P. Onorato, M. E. Roelke, E. D. Land, M. Cunningham, R. C. Belden, R. McBride, D. Jansen, M. Lotz, D. Shindle, and J. Howard. 2010. Genetic restoration of the Florida panther. Science 329:1641–1645.

Joly, S., and A. Bruneau. 2004. Evolution of triploidy in *Apios americana* (Leguminosae) revealed by genealogical analysis of the histone *H3-D* gene. Evolution 58:284–295.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405.

Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC (BioMed Central) Genetics [online serial] 11:article 94.

Jones, O. R., and J. Wang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551–555.

Kalinowski, S. T. 2005. HP-Rare: a computer program for performing rarefaction on measures of allelic diversity. Molecular Ecology Notes 5:187–189.

Kanno, Y., M. A. Kulp, S. E. Moore, and G. D. Grossman. 2017. Native Brook Trout and invasive Rainbow Trout respond differently to seasonal weather variation: spawning timing matters. Freshwater Biology 62:868–879.

Kanno, Y., K. C. Pregler, N. P. Hitt, B. H. Letcher, D. J. Hocking, and J. E. B. Wofford. 2016. Seasonal temperature and precipitation regulate Brook Trout young-of-the-year abundance and population dynamics. Freshwater Biology 61:88–99.

Kazyak, D. C. 2015. Conservation and management of Brook Trout in western Maryland. Doctoral dissertation. University of Maryland, College Park.

Kazyak, D. C., R. H. Hilderbrand, S. R. Keller, M. C. Colaw, A. E. Holloway, R. P. Morgan II, and T. L. King. 2015. Spatial structure of morphological and neutral genetic variation in Brook Trout. Transactions of the American Fisheries Society 144:480–490.

Kazyak, D. C., R. H. Hilderbrand, T. L. King, S. R. Keller, and V. E. Chhatre. 2016. Hiding in plain sight: a case for cryptic metapopulations in Brook Trout (*Salvelinus fontinalis*). PLOS (Public Library of Science) ONE [online serial] 11(1):e0146295.

Kazyak, D. C., B. A. Lubinski, J. M. Rash, T. C. Johnson, and T. L. King. 2021. Development of baseline genetic information to support the conservation and management of wild Brook Trout in North Carolina. North American Journal of Fisheries Management 41:626–638.

Kazyak, D. C., J. Rash, B. A. Lubinski, and T. L. King. 2018. Assessing the impact of stocking northern origin hatchery Brook Trout on the genetics of wild populations in North Carolina. Conservation Genetics 19:207–219.

Keenan, K., P. McGinnity, T. F. Cross, W. W. Crozier, and P. A. Prodöhl. 2013. diveRsity: an R package for the estimation and exploration of population genetics parameters and their associated errors. Methods in Ecology and Evolution 4:782–788.

Kelson, S. J., A. R. Kapuscinski, D. Timmins, and W. R. Ardren. 2015. Fine-scale genetic structure of Brook Trout in a dendritic stream network. Conservation Genetics 16:31–42.

King, T. L., B. A. Lubinski, M. K. Burnham-Curtis, W. Stott, and R. P. Morgan II. 2012. Tools for the management and conservation of genetic diversity in Brook Trout (*Salvelinus fontinalis*): tri- and tetranucleotide microsatellite markers for the assessment of genetic diversity, phylogeography, and historical demographics. Conservation Genetics Resources 4:539–543.

King, W. 1937. Notes on the distribution of native Speckled and Rainbow trout in the streams of Great Smoky Mountains National Park. Journal of the Tennessee Academy of Science 12:351–361.

King, W. 1939. A program for the management of fish resources in Great Smoky Mountains National Park. Transactions of the American Fisheries Society 68:86–95.

Konopacky, R. C., and R. D. Estes. 1986. Age and growth of Brook Trout in southern Appalachian streams. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 40:227–236.

Kulp, M. A., and S. E. Moore. 2005. A case history in fishing regulations in Great Smoky Mountains National Park: 1934–2004. North American Journal of Fisheries Management 25:510–524.

Laikre, L., M. K. Schwartz, R. S. Waples, N. Ryman, and the GeM Working Group. 2010. Compromising genetic diversity in the wild: unmonitored large-scale release of plants and animals. Trends in Ecology and Evolution 25:520–529.

Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. American Naturalist 142:911–927.

Larson, G. L., and S. E. Moore. 1985. Encroachment of exotic Rainbow Trout into stream populations of native Brook Trout in the southern Appalachian Mountains. Transactions of the American Fisheries Society 114:195–203.

Le Luyer, J., M. Laporte, T. D. Beacham, K. H. Kaukinen, R. E. Withler, J. S. Leong, E. B. Rondeau, B. F. Koop, and L. Bernatchez. 2017. Parallel epigenetic modifications induced by hatchery rearing in a Pacific salmon. Proceedings of the National Academy of Sciences of the USA 114:12964–12969.

Lennon, R. E. 1967. Brook Trout of the Great Smoky Mountains National Park. U.S. Bureau of Sport Fish, Technical Paper 15, Washington, D.C.

Luikart, G., N. Ryman, D. A. Tallmon, M. K. Schwartz, and F. W. Allendorf. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. Conservation Genetics 11:355–373.

MacCrimmon, H. R., and J. S. Campbell. 1969. World distribution of Brook Trout, *Salvelinus fontinalis*. Journal of the Fisheries Research Board of Canada 26:1699–1725.

Malone, E. W., J. S. Perkin, B. M. Leckie, M. A. Kulp, C. R. Hurt, and D. M. Walker. 2018. Which species, how many, and from where: integrating habitat suitability, population genomics, and abundance estimates into species reintroduction planning. Global Change Biology 24:3729–3748.

Mandrak, N. E., and E. J. Crossman. 1992. Postglacial dispersal of freshwater fishes into Ontario. Canadian Journal of Zoology 70:2247–2259.

McCracken, G. F., C. R. Parker, and S. Z. Guffey. 1993. Genetic differentiation and hybridization between stocked hatchery and native Brook Trout in Great Smoky Mountains National Park. Transactions of the American Fisheries Society 122:533–542.

Miller, J. M., C. I. Cullingham, and R. M. Peery. 2020. The influence of a priori grouping on inference of genetic clusters: simulation study and literature review of the DAPC method. Heredity 125:269–280.

Miller, J. M., J. Poissant, J. T. Hogg, and D. W. Coltman. 2012. Genomic consequences of genetic rescue in an insular population of bighorn sheep (*Ovis canadensis*). Molecular Ecology 21:1583–1596.

Moore, S. E., G. L. Larson, and B. Ridley. 1986. Population control of exotic Rainbow Trout in streams of a natural area park. Environmental Management 10:215–219.

Morgan, R. P., D. C. Kazyak, T. L. King, B. A. Lubinski, M. T. Sell, A. A. Heft, and J. W. Jones. 2021. Genetic structure of Maryland Brook Trout populations: management implications for a threatened species. North American Journal of Fisheries Management 41:1097–1119.

Moritz, C. 1994. Defining 'evolutionarily significant units' for conservation. Trends in Ecology and Evolution 9:373–375.

Mylecraine, K. A., J. E. Kuser, P. E. Smouse, and G. L. Zimmermann. 2004. Geographic allozyme variation in Atlantic white-cedar, *Chamaecyparis thyoides* (Cupressaceae). Canadian Journal of Forest Research 34:2443–2454.

Nathan, L. R., Y. Kanno, B. H. Letcher, A. B. Welsh, A. R. Whiteley, and J. C. Vokoun. 2020. Evaluation of genetic structuring within GIS-derived Brook Trout management units. Transactions of the American Fisheries Society 149:681–694.

Nathan, L. R., A. B. Welsh, and J. C. Vokoun. 2019. Watershed-level Brook Trout genetic structuring: evaluation and application of riverscape genetics models. Freshwater Biology 64:405–420.

Nielsen, J. L., and G. A. Powers, editors. 1995. Evolution and the aquatic ecosystem: defining unique units in population conservation. American Fisheries Society, Symposium 17, Bethesda, Maryland.

Olson, D. E., B. Spateholts, M. Paiya, and D. E. Campton. 2004. Salmon hatcheries for the 21st century: a model at Warm Springs National Fish Hatchery. Pages 585–602 *in* M. J. Nickum, P. M. Mazik, J. G. Nickum, and D. D. MacKinlay, editors. Propagated fish in resource management. American Fisheries Society, Symposium 44, Bethesda, Maryland.

Palmer, G. C., and E. M. Hallerman. 2000. Genetic characterization of southwest Virginia Brook Trout populations. Project Completion Report to the Virginia Department of Game and Inland Fisheries, Richmond.

Palsbøll, P. J., M. Berube, and F. W. Allendorf. 2007. Identification of management units using population genetic data. Trends in Ecology and Evolution 22:11–16.

Paradis, E. 2010. pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics 26:419–420.

Parks, C. R., J. F. Wendel, M. M. Sewell, and Y. L. Qiu. 1994. The significance of allozyme variation and introgression in the *Liriodendron tulipifera* complex (Magnoliaceae). American Journal of Botany 81:878–889.

Peakall, R., and P. E. Smouse. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539.

Pilgrim, B. L., R. C. Perry, J. L. Barron, and H. D. Marshall. 2012. Nucleotide variation in the mitochondrial genome provides evidence for dual routes of postglacial recolonization and genetic recombination in the northeastern Brook Trout (*Salvelinus fontinalis*). Genetics and Molecular Research 11:3466–3481.

Piry, S., A. Alapetite, J.-M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GeneClass2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity 95:536–539.

Power, G. 1980. The Brook Charr, *Salvelinus fontinalis*. Pages 141–203 *in* E. K. Balon, editor. Charrs: salmonid fishes of the genus *Salvelinus*. Dr. W. Junk, The Hague, The Netherlands.

Power, G. 2002. Charrs, glaciation, and seasonal ice. Environmental Biology of Fishes 64:17–35.

Pregler, K. C., Y. Kanno, D. Rankin, J. A. Coombs, and A. R. Whiteley. 2018. Characterizing genetic integrity of rear-edge trout populations in the southern Appalachians. Conservation Genetics 19:1487–1503.

Printz, J. E., J. Williams, and E. M. Hallerman. 2018. Genetic characterization of Brook Trout (*Salvelinus fontinalis*) populations at the zone of contact between southern and northern Appalachian lineages. Pages 55–73 *in* S. Ray, editor. Biological resources of water. InTech, Rijeka, Croatia.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Pruett, C. L., and K. Winker. 2008. The effects of sample size on population genetic diversity estimates in song sparrows *Melospiza melodia*. Journal of Avian Biology 39:252–256.

Puechmaille, S. J. 2016. The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Molecular Ecology Resources 16:608–627.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Radforth, I. 1944. Some considerations on the distribution of fishes in Ontario. Royal Ontario Museum of Paleontology Contributions 25:1–116.

Ralls, K., J. D. Ballou, M. R. Dudash, M. D. B. Eldridge, C. B. Fenster, R. C. Lacy, P. Sunnucks, and R. Frankham. 2018. Call for a paradigm shift in the genetic management of populations. Conservation Letters [online serial] 11:e12412.

Ramstad, K. M., C. A. Woody, G. K. Sage, and F. W. Allendorf. 2004. Founding events influence genetic population structure of Sockeye Salmon (*Oncorhynchus nerka*) in Lake Clark, Alaska. Molecular Ecology 13:277–290.

Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences of the USA 94:9197–9221.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Heredity 86:248–249.

Richards, A. L., T. L. King, B. A. Lubinski, S. E. Moore, M. Kulp, and L. S. Webb. 2008. Characterization of the genetic structure among Brook Trout in LeConte Creek, Tennessee. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 62:195–202.

Richardson, L. R., and J. R. Gold. 1995. Evolution of the *Cyprinella lutrensis* species-complex. II. Systematics and biogeography of the Edwards Plateau Shiner, *Cyprinella lepida*. Copeia 1995:28–37.

Robinson, Z. L., J. A. Coombs, M. Hudy, K. H. Nislow, B. H. Letcher, and A. R. Whiteley. 2017. Experimental test of genetic rescue in isolated populations of Brook Trout. Molecular Ecology 26:4418–4433.

Roghair, C. N., C. A. Dolloff, and M. K. Underwood. 2002. Response of a Brook Trout population and instream habitat to a catastrophic flood and debris flow. Transactions of the American Fisheries Society 131:718–730.

Romaniszyn, E. D., J. J. Hutchens Jr., and J. B. Wallace. 2007. Aquatic and terrestrial invertebrate drift in southern Appalachian Mountain streams: implications for trout food resources. Freshwater Biology 52:1–11.

Romines, C. G. 2017. A predictive model for Brook Trout restoration in the Cherokee National Forest. Master's thesis. University of Tennessee, Knoxville.

Ruiz, C. F., J. M. Rash, D. A. Besler, J. R. Roberts, M. B. Warren, C. R. Arias, and S. A. Bullard. 2017. Exotic "gill lice" species (Copepoda: Lernaeopodidae: *Salmincola* spp.) infect Rainbow Trout (*Oncorhynchus mykiss*) and Brook Trout (*Salvelinus fontinalis*) in the southeastern United States. Journal of Parasitology 103:377–389.

Ryder, O. 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution 1:9–10.

Seaber, P. R., F. P. Kapinos, and G. L. Knapp. 1987. Hydrologic unit maps. U.S. Geological Survey Water-Supply Paper 2294.

Sewell, M. M., C. R. Parks, and M. W. Chase. 1996. Intraspecific chloroplast DNA variation and biogeography of North American *Liriodendron* L. (Magnoliaceae). Evolution 50:1147–1154.

Sherwin, W. B. 2015. Genes are information, so information theory is coming to the aid of evolutionary biology. Molecular Ecology Resources 15:1259–1261.

Smith, J. V. C. 1833. Natural history of the fishes of Massachusetts, embracing a practical essay on angling. Allen and Ticknor, Boston.

Smouse, P. E., M. R. Whitehead, and R. Peakall. 2015. An informational diversity framework, illustrated with sexually deceptive orchids in early stages of speciation. Molecular Ecology Resources 15:1375–1384.

Soltis, D. E., A. B. Morris, J. S. McLachlan, P. S. Manos, and P. S. Soltis. 2006. Comparative phylogeography of unglaciated eastern North America. Molecular Ecology 15:4621–4293.

Stange, M., R. D. H. Barrett, and A. P. Hendry. 2021. The importance of genomic variation for biodiversity, ecosystems and people. Nature Reviews Genetics 22:89–105.

Stitt, B. C., G. Burness, K. A. Burgomaster, S. Currie, J. L. McDermid, and C. C. Wilson. 2014. Intraspecific variation in thermal tolerance and acclimation capacity in Brook Trout (*Salvelinus fontinalis*):

physiological implications for climate change. Physiological and Biochemical Zoology 87:15–29.

Stoneking, M., D. J. Wagner, and A. C. Hildebrand. 1981. Genetic evidence suggesting subspecific differences between northern and southern populations of Brook Trout (*Salvelinus fontinalis*). Copeia 1981:810–819.

Stranko, S. A., R. H. Hilderbrand, R. P. Morgan II, M. W. Staley, A. J. Becker, A. Roseberry-Lincoln, E. S. Perry, and P. T. Jacobson. 2008. Brook Trout declines with land cover and temperature changes in Maryland. North American Journal of Fisheries Management 28:1223–1232.

Theriault, V., G. R. Moyer, L. S. Jackson, M. S. Blouin, and M. A. Banks. 2011. Reduced reproductive success of hatchery Coho Salmon in the wild: insights into most likely mechanisms. Molecular Ecology 20:1860–1869.

Timm, A., E. Hallerman, C. A. Dolloff, M. Hudy, and R. Kolka. 2016. Identification of a barrier height threshold where Brook Trout population genetic diversity, differentiation, and relatedness are affected. Environmental Biology of Fishes 99:195–208.

Trushenski, J. T., H. L. Blankenship, J. D. Bowker, T. A. Flagg, J. A. Hesse, K. M. Leber, D. D. MacKinlay, D. J. Maynard, C. M. Moffitt, V. A. Mudrak, and K. T. Scribner. 2015. Introduction to a special section: hatcheries and management of aquatic resources (HaMAR)—considerations for use of hatcheries and hatchery-origin fish. North American Journal of Aquaculture 77:327–342.

Walker, D., and J. C. Avise. 1998. Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. Annual Review of Ecology and Systematics 29:23–58.

Waples, R. S. 1991. Pacific salmon, *Oncorhynchu*s spp., and the definition of 'species' under the Endangered Species Act. Marine Fisheries Review 53:11–22.

Waples, R. S., and E. C. Anderson. 2017. Purging putative siblings from population genetic data sets: a cautionary view. Molecular Ecology 26:1211–1224.

Waples, R. S., and C. Do. 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evolutionary Applications 3:244–262.

Ward, R. D., N. Billington, and P. D. N. Hebert. 1989. Comparison of allozyme and mitochondrial variation in populations of Walleye, *Stizostedion vitreum*. Canadian Journal of Fisheries and Aquatic Sciences 46:2074–2084.

Weathers, T. C., D. C. Kazyak, J. R. Stauffer Jr., M. A. Kulp, S. E. Moore, T. L. King, and J. E. Carlson. 2019. Neutral genetic and phenotypic variation within and among isolated headwater populations of Brook Trout. Transactions of the American Fisheries Society 148:58–72.

Weeks, A. R., J. Stoklosa, and A. A. Hoffmann. 2016. Conservation of genetic uniqueness of populations may increase extinction likelihood of endangered species: the case of Australian mammals. Frontiers in Zoology [online serial] 13:article 31.

Wells, Z. R., T. A. Bernos, M. C. Yates, and D. J. Fraser. 2019. Genetic rescue insights from population- and family-level hybridization effects in Brook Trout. Conservation Genetics 20:851–863.

Wesner, J. S., J. W. Cornelison, C. D. Dankmeyer, P. F. Galbreath, and T. H. Martin. 2011. Growth, pH tolerance, survival, and diet of introduced northern-strain and native southern-strain Appalachian Brook Trout. Transactions of the American Fisheries Society 140:37–44.

Whiteley, A. R., J. A. Coombs, M. Hudy, Z. Robinson, A. R. Colton, K. H. Nislow, and B. H. Letcher. 2013. Fragmentation and patch size shape genetic structure of Brook Trout populations. Canadian Journal of Fisheries and Aquatic Sciences 70:678–688.

Whiteley, A. R., J. A. Coombs, B. H. Letcher, and K. H. Nislow. 2014. Simulation and empirical analysis of novel sibship-based genetic determination of fish passage. Canadian Journal of Fisheries and Aquatic Sciences 71:1667–1679.

Whiteley, A. R., S. W. Fitzpatrick, W. C. Funk, and D. A. Tallmon. 2015. Genetic rescue to the rescue. Trends in Ecology and Evolution 30:42–49.

Whiteley, A. R., K. Hastings, J. K. Wenburg, C. A. Frissell, J. C. Martin, and F. W. Allendorf. 2010. Genetic variation and effective population size in isolated populations of Coastal Cutthroat Trout. Conservation Genetics 11:1929–1943.

Whitlock, M. C. 2000. Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. Evolution 54:1855–1861.

Whitworth, W. E., and R. J. Strange. 1983. Growth and production of sympatric Brook and Rainbow trout in an Appalachian stream. Transactions of the American Fisheries Society 112:469–475.

Wood, J. L., D. Tezel, D. Joyal, and D. J. Fraser. 2015. Population size is weakly related to quantitative genetic variation and trait differentiation in a stream fish. Evolution 69:2303–2318.

Zajitschek, S. R., F. Zajitschek, and R. C. Brooks. 2009. Demographic costs of inbreeding revealed by sex-specific genetic rescue effects. BMC (BioMed Central) Evolutionary Biology [online serial] 9:article 289.

Zastavniouk, C., L. K. Weir, and D. J. Fraser. 2017. The evolutionary consequences of habitat fragmentation: body morphology and coloration differentiation among Brook Trout populations of varying size. Ecology and Evolution 7:6850–6862.

SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.