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Coding Gene Single Nucleotide Polymorphism Population Genetics of Nonnative Brook Trout: The Ghost of Introductions Past

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ARTICLE

Coding Gene Single Nucleotide Polymorphism Population Genetics of Nonnative Brook Trout: The Ghost of Introductions Past

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Abstract

Fish have been translocated throughout the world, and introductions often have been executed repeatedly and have used mixtures of different strains from the native range. This history might have contributed to their invasive potential by allowing introduced and invading populations to circumvent expected reductions in genetic diversity from founder effects in a scenario termed the "genetic paradox" of invasions. We characterize patterns of genetic diversity in nonnative Brook Trout *Salvelinus fontinalis*, which have been introduced across the western United States for over a century but have also invaded broadly and pose a primary threat to native trout. We analyzed 155 coding gene single nucleotide polymorphisms (SNPs) in 34 nonnative Brook Trout populations sampled across eight large river systems as well as samples from the only four hatchery strains with documented use in Idaho. We uncovered similar within-population genetic diversity and large effective population sizes in naturalized populations compared with hatchery samples. Naturalized populations also showed substantial genetic structuring (maximum pairwise $F_{ST} = 0.23$) across and even within watersheds and indicated suggestions of admixture in certain regions. Assignment probabilities confirmed two main hatcheries as the origin of most fish collected in the field; however, the four hatcheries were excluded as being the origin for 8% of individuals, mirroring results from clustering analyses and suggesting the influence of an additional unsampled hatchery source or sources. Simulated admixtures of hatchery samples produced genetic patterns similar to those observed in field samples, further supporting an influence of multiple historic hatchery stocks on the contemporary genetic structure of Brook Trout in Idaho. Our study highlights the potential contribution of historic hatchery and introduction practices in creating genetically variable and structured naturalized Brook Trout populations across Idaho, which may have allowed these fish to defy the "genetic paradox" early on in their nonnative history and set the stage for successful establishment and subsequent invasion.

The application of genetic tools to understanding an invasion of nonnative species has increased greatly in recent years, and much attention has been focused on what has been termed the "genetic paradox" of invasions (Roman and Darling 2007); that is, how do introduced species establish and invade when they should have reduced genetic variability from founder effects and therefore high extinction risk and little evolutionary potential? Recent empirical studies of invasions have unraveled this paradox via several mechanisms. First, many studies have found invasive populations often do not have reduced genetic variation compared with native populations (e.g., Blum et al. 2007; Lavergne and Molofsky 2007). This is often attributed to large propagule sizes arising from multiple introductions (Lockwood et al. 2005; Roman 2006; Roman and

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Darling 2007; Dlugosch and Parker 2008; Simberloff 2009). Where introductions involve different strains from across the native range, subsequent admixture of these divergent sources can have beneficial effects on fitness (Sexton et al. 2011) and can cause dramatic shifts in genetic variation, creating novel genotypes and phenotypes on which selection can act in founding populations (Roman 2006; Lavergne and Molofsky 2007). Ultimately, the "catalytic" genetic effects (Ellstrand and Schierenbeck 2000) of these dynamic processes can actually facilitate successful establishment and invasion and make invasive fronts hotspots for unique evolutionary and ecological change (Suarez and Tsutsui 2008; Facon et al. 2008).

While many invasions follow unintentional introductions, nonnative fishes have been introduced purposefully and intensively throughout the world, often with catastrophic consequences for native fishes (Casal 2006; Gozlan et al. 2010). Salmonines (salmon, trout, and char) have been a particular focus of aquaculture and introduction programs to the extent that they are now one of the broadest invaders in the world (Lever 1996; Lowe et al. 2000; Dunham et al. 2004). Salmonine introductions in many cases have spanned decades, if not centuries, and often have been executed repeatedly using mixtures of different strains from the native range (Behnke 1992; Moyle 2002; Helfman 2007; Crawford and Muir 2008). Collectively, these aspects of the history of salmonine introductions may greatly facilitate the invasive potential of these fish.

Here, we evaluated contemporary genetic structure in naturalized nonnative populations of Brook Trout *Salvelinus fontinalis* in Idaho. Brook Trout are native to the eastern and midwestern regions of North America but have been introduced across the west for over a century, where they have invaded broadly and pose a primary threat to native trout and other species (see Dunham et al. 2002, 2004; Fausch et al. 2009). In their nonnative range Brook Trout are characterized by rapid maturity and substantial reproductive plasticity, and often have high productivity and densities compared with the native species they are commonly thought to displace (Dunham et al. 2002; McGrath and Lewis 2007; Benjamin and Baxter 2010, 2012). They are highly mobile and thus are excellent upstream dispersers in small mountain streams (Gowan and Fausch 1996; Adams et al. 2000; Peterson and Fausch 2003). In many places in the mountainous west they readily invade downstream as well, and their broad-scale introduction to headwater lakes has allowed them to access otherwise unreachable habitats including headwater refugia for native trout (Adams et al. 2001; Paul and Post 2001). Their association with headwater lakes (Adams et al. 2001), as well as valley bottoms with complex habitats (Benjamin et al. 2007; Wenger et al. 2011a), helps ensure the persistence of stable source populations and has made eradication difficult.

We characterized genetic patterns in this nonnative species through analysis of 155 single nucleotide polymorphisms (SNPs) identified in coding gene regions among 34 samples of Brook Trout populations across eight large river systems in Idaho. To provide insight to the potential origin of genetic diversity observed today we also analyzed samples from the only four hatchery strains documented to have been introduced in Idaho; these strains were of a broad geographic origin from across eastern North America. We integrated our evaluation of population structure and population genetic diversity with assignments of individuals to introduced strains. To our knowledge, our study is the first characterization of broad-scale genetic diversity in Brook Trout in its nonnative range.

METHODS

Field sampling of nonnative Brook Trout populations.—Tissue samples were collected from fish captured by electrofishing in the summer of 2010 from 34 established Brook Trout populations in Idaho (Figure 1). For field collections we enlisted several agency and nongovernmental organization crews who collected samples of Brook Trout in conjunction with other planned field work (see Acknowledgments); thus, our samples do not represent *all* of the distribution of Brook Trout in the state, but we designed collections to achieve reasonably in-depth sampling in each watershed while covering eight of the large river systems in Idaho. Within each river system, Brook Trout were collected from three to four different tributaries or upper mainstem river (each of which we refer to as a "sample"), though in some cases fewer samples were collected (e.g., Priest River, see Table 1; Figure 1) and in one case (Teton River, see Table 1; Figure 1) sampling was more extensive related to a more comprehensive study of the native and nonnative trout in the system. To ensure a collection of a representative sample and prevent biasing genetic information towards family groups (Hansen et al. 1997) sampling within each population was spread out geographically (i.e., collected from multiple stretches of stream or river separated by several hundreds of meters) and care was taken to sample only adult fish. Collectors targeted 35 fish in each population, although in some areas fewer Brook Trout were encountered. Fin clips were collected, desiccated, and stored in paper coin envelopes.

Samples from hatchery Brook Trout historically introduced in Idaho.—In an effort to determine the original (native) source of Brook Trout introduced to Idaho, we reviewed or contacted various sources of information, including Idaho Department of Fish and Game (IDFG) reports and personnel, U.S. Fish Commission reports, various hatchery supervisors and historians, and peer-reviewed and grey literature. Brook Trout were transported and introduced throughout the western United States since the late 1800s. Early distributions occurred via U.S. Fish Commission trains, which shipped numerous fish species tens of thousands of miles each year to various state and federal hatcheries, individual townships, sports clubs, and individual enthusiasts (Smith 1895; Leonard 1979; Crawford and Muir 2008). Private aquaculturists also distributed Brook Trout (Karas 2002), and fish from both sources were subsequently introduced into waters across the west by various sanctioned and unsanctioned means (Pister

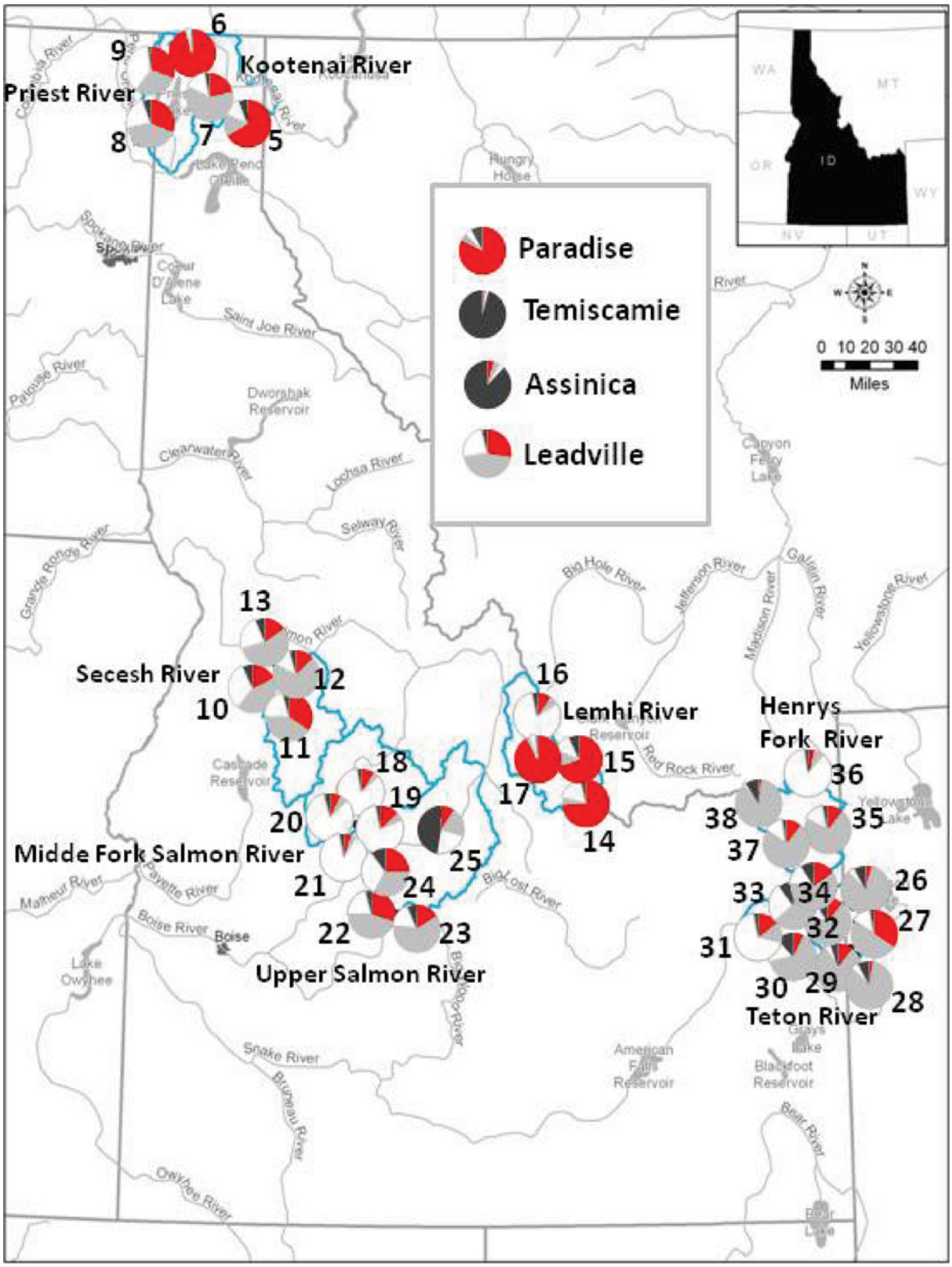

FIGURE 1. The state of Idaho indicating eight watersheds where nonnative Brook Trout were sampled (outlined and named), and sample locations numbered as in Table 1. Each population sample, or hatchery (inset), is represented by a pie chart showing the average proportional ancestry (*Q*) in each of the four STRUCTURE clusters (red, light grey, dark grey, and white) described in the text and in Figure 5. [Figure available online in color.]

2001; Rahel 2004). Despite poor historical documentation, we were able to uncover records of what are believed to be the four major sources for Brook Trout introductions in Idaho.

The first record of Brook Trout transport to Idaho was of several shipments from the Leadville National Fish Hatchery (LNFH) in Leadville, Colorado, (outside the native range of Brook Trout) from 1896 to 1905 (Chris Kennedy, LNFH, personal communication). The native origin of LNFH Brook Trout is unclear, as their stocks were developed starting in 1895 from a mixture of fish from 13 various sources; these encompassed fish from naturally reproducing but nonnative populations of unknown origin already established in Colorado at the time, several hatcheries, and fish from a private local aquaculturist that included Brook Trout previously obtained from Wisconsin (LNFH, unpublished documents). Though the hatchery no longer propagates Brook Trout, fish assumedly representative of this original mix (Ed Stege, LNFH, personal communication) persist today in a stream on the LNFH property (Rock Creek) from which we were able to obtain tissue samples. Records from IDFG also document shipments of Brook Trout from Paradise Fish Hatchery in Pennsylvania in 1902, and of two particular strains of Brook Trout, the Assinica and Temiscamie strains, from Brandon Enterprise Fisheries in New York in the early 1980s (Tom Frew and Sharon Clark, IDFG, personal communications). The latter two strains originated from Quebec and were targeted for introduction in Henrys Lake, Idaho because of their large size (Karas 2002). All three stocks are still being propagated by these original private hatcheries, and we were able to obtain samples from each in 2010 (Table 1). Although it would have been interesting to sample fish from the native populations that were used to establish these hatchery sources as well, the murky history of the original sources for the LNFH and even Paradise Fish Hatchery left no basis for designating appropriate "native" populations; similarly, it was not possible to get permission to access remote tribal lands housing Assinica and Temiscamie strains of Brook Trout in Quebec and, in fact, Brandon Enterprise Fisheries is the only known source for these strains. Furthermore, given that Brook Trout introductions occurred intentionally via hatcheries as opposed to through unintentional introductions directly from native sources, the documented hatcheries were the most appropriate representatives of historic genetic variability introduced in Idaho.

Idaho stocking records.—Stocking details for Brook Trout are sparse, but historical records were obtained from IDFG to provide heuristic characterization of stocking characteristics (e.g., propagule sizes and number of stocking events) that may have affected the genetic diversity of our sampled field populations. Official IDFG record keeping began in 1914, although purposeful stocking in Idaho certainly occurred before this (Chris Kennedy, LNFH, and Sharon Clark, IDFG, personal communications). Records from 1914 to 1965 included information only about which creeks were stocked in which years. For eight of our sample sites, basic geographic information (e.g., the county) needed to confirm the location of a creek in the stocking records was missing. In these instances IDFG personnel attributed a location based on stocking associations or other factors; we included these stocking instances despite some uncertainty that they referred to one of our sampled streams (Table 1). Starting in 1965 the numbers of fish stocked were recorded, but this information was relevant for only two of our sites; records of headwater lake introductions were not spatially resolved enough to use here. In most cases there was no information about the hatchery used (i.e., original hatchery or subsequent Idaho hatchery source or sources). Along with the generally unresolved nature of the overall stocking history, this presented a major confounding factor for any investigation relating stocking information with observed genetic characteristics as has been done in other studies (e.g., Bennett et al. 2010). We therefore did not pursue any statistical analyses along these lines but present this information to provide the most comprehensive picture possible of the history of Brook Trout introductions in Idaho.

Laboratory procedures.—Brook Trout DNA was extracted at the Nevada Genomics Center (http://www.ag.unr.edu/Genomics/) using the Qiagen DNA Blood and Tissue Kit per manufacturer's instructions up to the elution step, which was performed using 50 μL of 0.01 M ultrapure tris-HCL (pH 8.0). The extracted DNA was quantified using a fluorescent nucleic acid stain (PicoGreen) and read on a Labsystems Fluoroskan Ascent fluorescence plate reader. Extractions were shipped to the McGill University and Génome Québec Innovation Centre (http://www.gqinnovationcentre.com) for SNP amplification and genotyping.

SNP genotyping.—All SNP markers used in this study were developed from RNA (cDNA) sequences, validated, and used in another study to build a genetic map and identify quantitative trait loci for phenotypic traits of aquaculture interest in Brook Trout (detailed in Sauvage et al. 2012). No population investigated in this study was used for SNP development, thus equalizing the effect of any ascertainment bias, for instance, in terms of differences in allelic diversity among populations analyzed here. For validation, cDNA sequences were assembled into contigs and polymorphic positions were identified using CLC Genomic Workbench version 3.7 (CLC Bio, Aarhus, Denmark) (see Sauvage et al. 2012 for details). From many thousands of putative SNPs, a subset ($n \approx 1{,}000$) was selected for validation using the following four steps (Sauvage et al. 2012). First, a pair of PCR primer pairs was designed for each SNP of interest to generate an amplicon of 250–400 bp. Amplicons of over 400 bp were removed to avoid the amplification of intronic regions. Second, both strands of the selected amplicons were sequenced in order to confirm real polymorphism and discard false positives. Third, a genotyping assay was designed for the remaining SNPs using the iPLEX Gold protocol for the Sequenom MassARRAY (Sequenom, San Diego, California). Loci that did not fulfill the Sequenom assay technical requirements were removed. Loci that satisfied all requirements were multiplexed in panels of 28–32 markers on the

TABLE 1. Characteristics of nonnative Brook Trout from four hatcheries and populations sampled across Idaho. Shown are hatchery or watershed origin, sample name and number on Figure 1 (Fig), abbreviated codes (Code), the number of individuals genotyped (N), genetic diversity (H_E), allelic richness (R_S), the number of times a site was recorded as stocked (Stock), and the range of dates over which stocking is known to have occurred. NR = no record of stocking, NA = not applicable.

Watershed or origin	Sample name	Fig	Code	N	H_E	R_s	Stock	Date
Paradise Hatchery	Paradise	NA	PAR	27	0.28	1.71	NA	NA
Brandon Enterprise Hatchery	Temiscamie	NA	TEMSC	35	0.27	1.66	NA	NA
Brandon Enterprise Hatchery	Assinica	NA	ASSIN	35	0.29	1.72	NA	NA
Leadville National Fish Hatchery	Leadville	NA	LEAD	38	0.29	1.74	NA	NA
Kootenai River	Boulder Creek	5	BLD	20	0.30	1.78	14	1916–1940
	Cow Creek	6	COW	20	0.23	1.58	12	1914–1953
	Myrtle Creek	7	MRT	20	0.29	1.71	5	1914–1933
Priest River	Lamb Creek	8	LMB	20	0.32	1.80	15	1930–1968
	Jackson Creek	9	JCKS	22	0.29	1.73	0	NR
Secesh River	Summit Creek	10	SUMM	35	0.33	1.80	0	NR
	Lick Creek	11	LICK	35	0.29	1.74	3	1945–1972
	Grouse Creek	12	GRSE	35	0.32	1.80	1	1946
	Lake Creek	13	LAKE	35	0.33	1.80	6	1931–1937
Lemhi River	Eighteenmile Creek	14	EIGHT	35	0.24	1.63	0	NR
	Upper Lemhi River	15	UPLEM	35	0.30	1.75	0	NR
	Kenney Creek	16	KENN	35	0.24	1.61	0	NR
	Big Springs Creek	17	BGSPR	35	0.24	1.62	0	NR
Middle Fork Salmon River	Knapp Creek	18	MFK	35	0.30	1.74	0	NR
	Marsh Creek	19	MFM	35	0.30	1.73	2	1914
	Beaver Creek	20	MFB	35	0.28	1.68	1	1922
	Capehorn Creek	21	CPHN	35	0.29	1.72	0	NR
Upper Salmon River	Smiley Creek	22	USS	35	0.32	1.76	6	1922–1955
	Upper Mainstem Salmon River	23	USMS	35	0.33	1.80	0	NR
	Valley Creek	24	USV	35	0.31	1.75	2	1940–1951
	Garden Creek	25	USG	35	0.32	1.78	2	1948–1951
	North Leigh Creek	26	NLE	35	0.34	1.81	0	NR
Teton River	Teton Creek	27	TET	31	0.31	1.77	0	NR
	Game Creek	28	GAM	10	0.30	1.74	11	1948–1965
	Moose Creek	29	MOO	35	0.33	1.79	11	1947–1965
	Trail Creek	30	TRA	34	0.34	1.82	13	1947–1965
	Mahogany Creek	31	MAH	28	0.30	1.73	15	1948–1965
	Horseshoe Creek	32	HOR	14	0.34	1.83	16	1947–1965
	Packsaddle Creek	33	PAC	35	0.32	1.78	14	1947–1965
	Main-stem Teton River	34	TETMS	29	0.33	1.81	1	1921
Henrys Fork River	Warm Springs Creek	35	WMSP	35	0.32	1.78	0	NR
	Targhee Creek	36	TARG	35	0.29	1.72	0	NR
	Squirrel Creek	37	SQRL	34	0.28	1.70	2	1923–1925
	East Dry Creek	38	EDRY	32	0.31	1.72	0	NR

MassARRAY platform (Sequenom) according to the manufacturer's instructions, at the Génome Québec Innovation Centre. Here, individuals were genotyped using 187 markers out of the 280 validated SNPs by Sauvage et al. (2012) using the iPLEX Gold assays on the MassARRAY platform according to the manufacturer's instructions at the Génome Québec Innovation Centre.

Genetic analyses.—We tested for linkage disequilibrium among locus pairs using an adaptation of the program LINKDOS (Garnier-Gere and Dillmann 1992) implemented in the program Genetix version 4.05 (Belkhir et al. 2004). Potentially linked marker pairs were identified based both on statistical significance ($P < 0.0001$, the minimum P-value reported by Genetix, but a conservative value given the number of tests

performed) and high pairwise correlation ($r > 0.5$; see Kaeuffer et al. 2007).

Single nucleotide polymorphism markers have become increasingly popular in ecological and evolutionary studies, particularly because large marker sets can enable evaluation of genetic patterns based on marker neutrality as well as exploration of markers that are potentially under selection (Morin et al. 2004). Tests for F_{ST} outliers are a common method for separating these two classes of markers. In evaluating patterns of pairwise F_{ST} values across all markers, one can identify "candidate" loci that show extreme differentiation among populations and are potentially under selection (Luikart et al. 2003; Storz 2005; Nosil et al. 2009). Further analyses based on logistic regression can then be used to investigate relationships between environmental factors and patterns of differentiation and bolster inferences of possible selective forces on local populations (Joost et al. 2007; Pariset et al. 2009; Narum et al. 2010; Nunes et al. 2011).

Originally, we hoped to use such an approach to build on previous work demonstrating influences of temperature, winter flows, and several landscape features on the distribution of nonnative Brook Trout in the west (Wenger et al. 2011b) by relating allelic patterns in candidate loci (i.e., those showing extreme differentiation) to these environmental factors. As a first step, we used the Bayesian method of Foll and Gaggiotti (2008) implemented in BayeScan version 2.0 and a target false discovery rate (FDR) of 0.01 to identify outlier loci for which a departure from neutrality best explained observed patterns of variation (see program documentation). BayeScan was chosen because of its generally robust performance compared with other approaches (Pérez-Figueroa et al. 2010; Narum and Hess 2011) and its ability to handle variable levels of differentiation among populations (i.e., different F_{ST} values) and incorporate more ecologically realistic scenarios (Helyar et al. 2011; Nunes et al. 2011).

Results from these initial analyses uncovered possible evidence only of balancing selection, where a subset of markers demonstrated much *less* divergence than expected (see Results and Figure 3). This extreme lack of variation created a lack of fit of potential candidate markers to logistic regression models (e.g., Joost et al. 2007) and preempted our ability to relate patterns of genetic differentiation to environmental factors. We therefore focused on analyses assuming marker neutrality. We again used BayeScan to identify a valid set of loci that fell within neutral expectations and could be used for more general evaluations of population relationships (Helyar et al. 2011). Here, we chose a target FDR of 0.05 to identify the most likely neutral loci; i.e., loci that fell below this threshold were retained to comprise a neutral data set.

Using our identified "neutral" data set, we assessed each sample for Hardy–Weinberg equilibrium at each locus with FSTAT (Goudet 2001), using the program's Bonferroni adjustment of critical significance to account for simultaneous tests (a conservative approach appropriate when testing for Hardy–Weinberg equilibrium: Narum 2006). We used FSTAT to calculate Nei's (1987) unbiased measure of gene diversity (H_E) and allelic richness (R_S), a rarified estimate of the number of alleles that is independent of the sample size (El Mousadik and Petit 1996; Petit et al. 1998; Leberg 2002). We tested for differences in H_E and R_S between the hatchery samples (as a group) and the Idaho field samples (as a group) using the "comparison among groups of samples" option in FSTAT. Effective population sizes (N_e) were estimated using one-sample linkage disequilibrium method in the program LDNE (Waples and Do 2008). The LDNE program performs several corrections such as correcting for bias from sample sizes smaller than the true N_e and handling problems associated with rare alleles (see Waples 2006; Waples and Do 2008). Values for N_e reported are based on a lowest allele frequency threshold of 0.02 (P_{crit}) and using jackknifed 95% CIs, which performed better than parametric approaches in simulations (Waples and Do 2008).

Genetic differentiation among populations was evaluated based on pairwise F_{ST} values calculated in FSTAT, again using the program's conservative Bonferroni adjustment for multiple tests, which we felt appropriate for a study of invasive species genetic structure (as opposed to a species of conservation concern where a less conservative approach may be desirable (Narum 2006). Population relationships were further assessed and visualized using a neighbor-joining phenogram calculated from Nei's genetic distance (D; Nei 1972) and bootstrapped 500 times across loci using the program POPULATIONS (Langella 2002). TreeView (Page 1996) was used to visualize resulting trees and bootstrap values. Phenograms typically have limited ability to depict relationships among invasive populations and their potential sources, but may be heuristic when compared with other methods (Estoup and Guillemaud 2010).

We used two complementary Bayesian clustering approaches to determine the most likely number of genetic clusters (k) found among our field and hatchery samples. Both programs use information from individual genotypes to identify the number of clusters that maximizes the fit to theoretically expected grouping patterns (i.e., based on Hardy–Weinberg and linkage equilibrium). We first used the Bayesian clustering algorithm in STRUCTURE version 2.3.3 (Pritchard et al. 2000) based on an admixture model with correlated allele frequencies. We initially evaluated 1–40 clusters, running four separate simulations of each k and using a burn-in length of 100,000 and 100,000 Markov chain Monte Carlo (MCMC) replicates for each run. We determined the most likely number of clusters using both the mean log likelihood of the data (i.e., as recommended by the authors, see STRUCTURE documentation), as well as the Delta k method outlined by Evanno et al. (2005) based on the second-order rate of change of the likelihood function; both statistics were compiled in STRUCTURE HARVESTER version 0.6.8 (Earl and vonHoldt 2012). For the most likely number of clusters, we ran 10 additional simulations using a burn-in length of 500,000 and 500,000 MCMC replicates for each run. We used the Greedy algorithm with 10,000 random inputs in CLUMPP

version 1.1.2 (Jackobsson and Rosenberg 2007) to match clusters that might have been labeled differently across each run, and Distruct version 1.1 (Rosenberg 2004) to visualize resulting individual and population average Q values graphically.

We also implemented Bayesian Analysis of Population Structure (BAPS version 5.3), first running the program using the mixture model (Corander et al. 2006, 2008) as suggested in the program documentation. Unlike STRUCTURE, the program returns log marginal likelihood values (logmls) only for the most likely k. We wanted to visualize the changes in logmls across all k-values so we evaluated k1–30 in 30 separate analyses, as well as k20, k50, and k100 sequentially in one analysis (see program documentation) running 10 iterations for each k. Secondly, we used the results of the most likely outcomes from these mixture analyses to implement the admixture model (Corander and Marttinen 2006; Corander et al. 2008) using a minimum cluster size of five, 100 iterations for individual admixture estimates, 200 reference individuals per population, and five runs as suggested in the program documentation.

We performed genetic assignment tests in GeneClass2 (Piry et al. 2004) to assign individuals from our field samples to the four hatchery samples. Because there is a possibility that our hatchery samples may not include *all* hatchery sources introduced to Idaho, we did not make inferences based on direct assignments (e.g., Rannala and Mountain 1997), i.e. where an individual would be assigned to the most likely of the four possible hatchery sources even if they originated from another "ghost" source. Instead we used the Monte Carlo resampling approach of Paetkau et al. (2004) to estimate the probability that an individual originated in each reference population or, alternatively, the probability that all four sampled hatcheries were excluded as a possible origin (suggesting the influence of other unsampled sources). We followed Saenz-Agudelo et al. (2009) and Waser and Hadfield et al. (2011) in tallying probabilities, such that if an individual's probability of origin was <0.05 for *all* hatchery sources, it was considered to have originated from a source other than our four sampled hatcheries (i.e., the sampled hatcheries were "excluded" as the origin). Where an individual had a probability ≥ 0.05 of belonging only to a single hatchery, it was assigned to that hatchery. Saenz-Agudelo et al. (2009) and Waser and Hadfield et al. (2011) counted individuals with probabilities ≥ 0.05 of belonging to more than one source as "unassigned." In our case, however, we accounted for the fact that hatchery sources might have been mixed at various stages in the history of Brook Trout introductions (in Idaho hatcheries, through multiple introductions of different strains or through subsequent invasion of different sources), perhaps blurring differences among them relative to field samples; we tallied all combinations where probabilities were ≥ 0.05 for belonging to two, three, and all four hatchery sources to resolve further the various scenarios of "nonexclusion."

Finally, to explore patterns of genetic variability and individual ancestry that could have arisen from admixture of hatchery sources, we performed a series of simulations to create hybrid populations using the program HYBRIDLAB (Nielsen et al. 2006). Based on initial results from assignment and clustering analyses we used genotypes from the Paradise, Temiscamie, and Leadville hatchery samples as parental populations to generate two F_1 hybrid populations: a = Paradise $\times$ Leadville and b = Temiscamie $\times$ Leadville (Figure 2). We used these hybrid populations to perform further crosses as $c = a \times b$ (Paradise–Leadville crossed with Temiscamie–Leadville), and d = the population resulting from c crossed with itself (Figure 2). We used FSTAT as outlined above to quantify gene diversity and allelic richness for these simulated hybrid populations for comparison with field samples. Further, based on initial STRUCTURE results from analyses of our Idaho field and hatchery genetic data, we included the Paradise, Temiscamie, and Leadville hatchery samples, as well as two field samples representing several watersheds with genetic characteristics somewhat distinctive from the hatchery sources (Capehorn Creek [CPHN] in the Middle Fork Salmon River and East Dry Creek [EDRY] in the Teton Basin; Table 1), and the four simulated hybrid populations in a STRUCTURE analysis based on $k = 4$ (with five iterations, and a burn-in length of 100,000 and 100,000 MCMC replicates for each iteration). Our goal was to compare results from simulated hybrid populations with patterns observed in our field samples to add further insight into processes leading to observed genetic variation among Idaho field and hatchery-source samples.

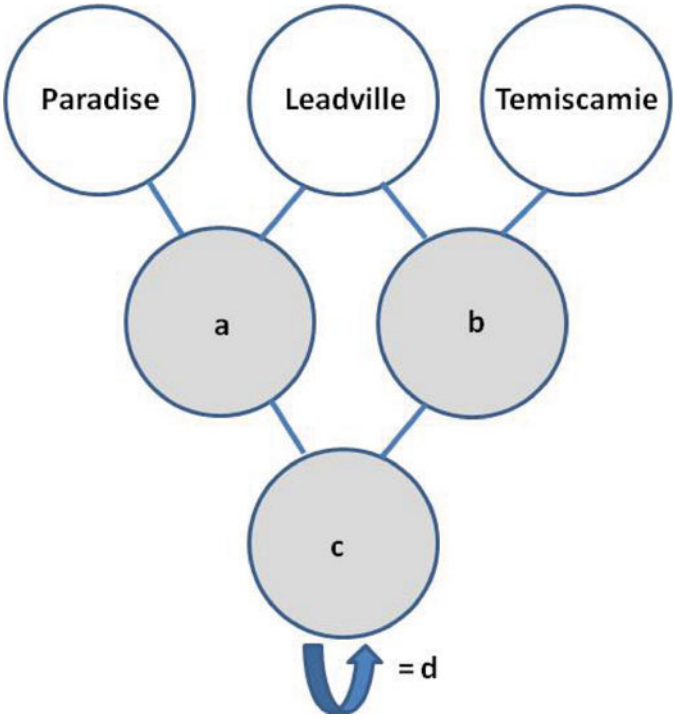

FIGURE 2. Schematic of HYBRIDLAB simulations described in the Methods, with parental Brook Trout populations in white and simulated hybrid populations in grey. [Figure available online in color.]

RESULTS

Sample Characteristics and Stocking History

A total of 1,025 Brook Trout were sampled across 34 locations in Idaho, where sample sizes ranged from 10 to 35 individuals; samples received from the four hatcheries ranged from 27 to 38 individuals each (Table 1). Of the 34 field sampling sites, 14 were not knowingly stocked with Brook Trout according to IDFG records; those that were stocked had quite different stocking histories, with some having been stocked just once to others having been stocked multiple time over decades (e.g., Lamb Creek in the Priest River was reportedly stocked 15 times over 38 years; Table 1).

SNP Marker Attributes

From an original data set of 187 SNP markers, 18 failed to amplify and nine were monomorphic and were removed from the final data set. Genetix found five pairs of loci in significant ($P < 0.0001$) linkage disequilibrium with high correlation ($r > 0.5$). Based on mapping information, four of these locus pairs were known to be physically linked (Sauvage et al. 2012); one marker from each of these five locus pairs was removed. Removal of the above markers left 155 markers for further analysis. The F_{ST} outlier test implemented in BayeScan identified 18 markers that fell above the preselected FDR of 0.01 and were considered outliers (Figure 3). However, all but one of these fit a model of balancing selection, rather than diversifying selection, with a cluster of 10 markers showing an extreme lack of diversity (circled in Figure 3). One hundred and thirty-seven markers fell below the targeted FDR of 0.05 and were retained to comprise our "neutral" data set (Figure 3).

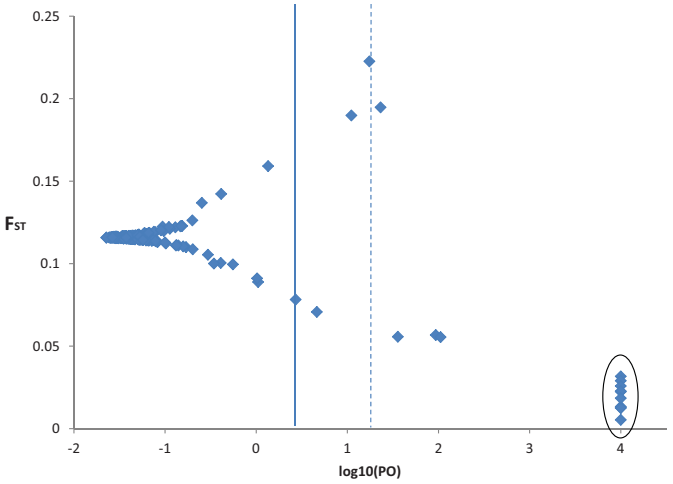

FIGURE 3. Results of F_{ST} outlier test based on differentiation in nonnative Brook Trout in Idaho and putative hatchery source populations as implemented in BayeScan. Eighteen markers fell above the targeted false discovery rate (FDR) of 0.01 (vertical dashed line) and were considered "outliers," and 10 markers (circled) fell at the extreme end of this distribution. One hundred and thirty-seven loci fell below the FDR of 0.05 (solid vertical line) and were retained for a "neutral" data set. [Figure available online in color.]

Evaluation of Genetic Diversity and Relationships Within and Among Samples

All of the loci in the remaining "neutral" data set of 137 markers met expectations of Hardy–Weinberg equilibrium based on a table-wide adjusted P-value of 0.00001. For field samples, genetic diversity (H_E) averaged across loci ranged from 0.23 (Cow Creek in the Kootenai River) to 0.34 (North Leigh Creek of the Teton River), while average allelic richness (R_S) ranged from 1.58 (Cow Creek) to 1.83 (Horseshoe Creek in the Teton River; Table 1). The diversity within hatchery samples was similar (H_E, 0.27–0.29; R_S, 1.66–1.74; Table 1), such that comparisons between the two groups showed no difference in diversity for either metric (average hatchery H_E of 0.28 versus average field H_E of 0.30, $P = 0.23$; average hatchery R_S of 1.71 versus average field R_S of 1.74, $P = 0.29$). Effective population sizes for hatchery samples alone ranged from 14.4 for the Assinica hatchery sample to 82.6 for the Paradise hatchery sample, with relatively small confidence intervals (Table 2). Field samples showed a broad range of estimated N_e values, ranging from 12.7 for Kenny Creek in the Lemhi River to 4,947.3 for Cow Creek in the Kootenai River (Table 2). Upper 95% CIs for field samples were frequently large, and in many cases were estimated to be infinity (Table 2).

Pairwise F_{ST} values indicated a relatively large degree of differentiation among samples, with values ranging from 0.005 (between Knapp and Marsh creek, neighboring tributaries of the Middle Fork Salmon River) to 0.34 (between the Temiscamie hatchery sample and Cow Creek in the Kootenai River; see Supplementary Table 1 in the online version of this article). Ninety-three percent of comparisons suggested "significant" differentiation (Table A.1). Differentiation among only field populations ranged between 0.005 (same populations as above) and 0.23 (between Cow Creek in the Kootenai River and East Dry Creek in the Henrys Fork River). In comparing relationships between hatchery and field samples, the F_{ST} values between the Assinica–Temiscamie hatchery samples versus field samples ranged from 0.11 (Temiscamie versus Garden Creek in the upper Salmon River) to 0.34 (Temiscamie versus Cow Creek); comparisons with the Paradise hatchery sample ranged from 0.05 (with Boulder Creek in the Kootenai River) to 0.20 (with East Dry Creek in the Henrys Fork River), and comparisons with the LNFH sample ranged from 0.04 (with Lake Creek in the Secesh River) to 0.14 (with Kenny Creek in the Lemhi River), and many values were on the lower end of this range (Table A.1).

The neighbor-joining phenogram relating field and hatchery samples generally had low bootstrap values and relationships often were not based on geographic location of the sample collection (i.e., samples from the same drainage were often interspersed throughout the tree; Figure 4). However, even though bootstrap values generally showed low support for relationships, there were many similarities with those identified by the clustering analysis in STRUCTURE (results presented together below).

TABLE 2. Estimated effective population sizes (N_e) and lower and upper limits of 95% CIs for Brook Trout from four hatcheries and 34 naturalized nonnative populations sampled across Idaho.

Watershed or origin	Sample name	Code	Lower limit of 95% CI	Estimated N_e	Upper limit of 95% CI
Paradise Hatchery	Paradise	PAR	62.2	82.6	119.1
Brandon Enterprise Hatchery	Temiscamie	TEMSC	17.9	20.3	23.3
Brandon Enterprise Hatchery	Assinica	ASSIN	13	14.4	16.1
Leadville National Fish Hatchery	Leadville	LEAD	56.1	70.9	94.1
Kootenai River	Boulder Creek	BLD	136.1	595.4	Infinite
	Cow Creek	COW	123.4	4947.3	Infinite
	Myrtle Creek	MRT	56.9	103.7	438.2
Priest River	Lamb Creek	LMB	47.6	73.4	148.7
	Jackson Creek	JCKS	66.3	114.2	351.4
Secesh River	Summit Creek	SUMM	156.9	323.4	458,861.9
	Lick Creek	LICK	128.3	247.5	1,884.6
	Grouse Creek	GRSE	26.3	30.1	34.9
	Lake Creek	LAKE	204.3	547.1	Infinite
Lemhi River	Eighteenmile Creek	EIGHT	18.4	21.1	24.3
	Upper Lemhi River	UPLEM	50	63.7	85.8
	Kenney Creek	KENN	11.2	12.7	14.3
	Big Springs Creek	BGSPR	19.9	23	26.9
Middle Fork Salmon River	Knapp Creek	MFK	116.7	193.9	511.6
	Marsh Creek	MFM	108.1	171.1	377.6
	Beaver Creek	MFB	15.4	17.4	19.6
	Capehorn Creek	CPHN	30.9	36.3	43.2
Upper Salmon River	Smiley Creek	USS	267.5	379	Infinite
	Upper Mainstem Salmon River	USMS	82	112	170.7
	Valley Creek	USV	28	32.3	37.7
	Garden Creek	USG	20.3	22.8	25.8
	North Leigh Creek	NLE	204.7	518.7	Infinite
Teton River	Teton Creek	TET	25.7	29.8	34.8
	Game Creek	GAM	20.6	31.4	59.2
	Moose Creek	MOO	70.8	93.7	134.6
	Trail Creek	TRA	27.9	31.9	36.8
	Mahogany Creek	MAH	76.1	119.2	254.2
	Horseshoe Creek	HOR	67.9	168.4	Infinite
	Packsaddle Creek	PAC	32.5	37.8	44.7
	Mainstem Teton River	TETMS	134.7	382.5	Infinite
Henrys Fork River	Warm Springs Creek	WMSP	422.7	−1,950.6[a]	Infinite
	Targhee Creek	TARG	117.2	202.1	628.4
	Squirrel Creek	SQRL	61.3	83.8	127.5
	East Dry Creek	EDRY	67.4	96.4	161.1

[a]Negative N_e values are an occasional and theoretically expected outcome of this analytical approach and are typically interpreted as "infinite" estimates (see LDNE program documentation).

Clustering Analyses

STRUCTURE results showed a relatively large jump in mean log likelihood between $k = 1$ and 2 and again between $k = 3$ and 4, after which likelihoods continued to rise gradually until they began to become asymptotic and increased in variance around $k = 21$ (see Supplementary Figure 1 in the online version of this article). The Evanno Delta k method found $k = 2$ to be the most likely number of clusters, with a second jump in Delta k for $k = 4$ (Supplementary Figure 1B); The H′ statistic from CLUMPP was 0.98 and 0.99 for $k2$ and $k4$, respectively, suggesting a high degree of pairwise similarity among replicates and thus little discrepancy in calculated proportional ancestry among runs. However, $k = 2$ showed highly unresolved relationships, with all individuals being admixed between the two

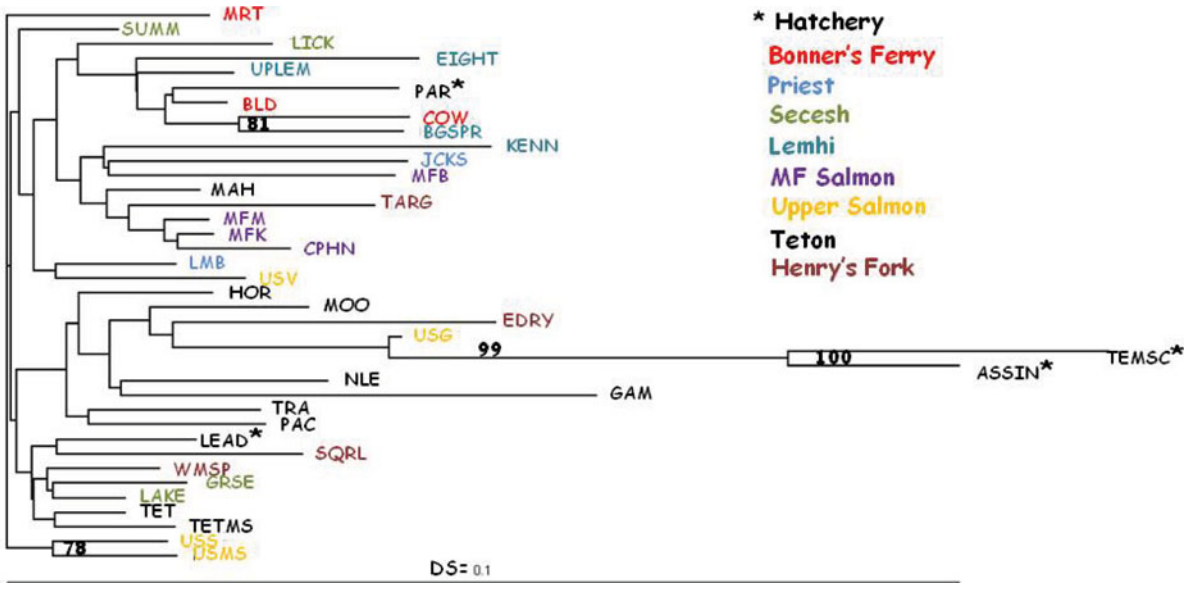

FIGURE 4. Neighbor-joining phenogram using Nei's genetic distance (D). Population abbreviations are defined in Table 1 and are colored by major drainage or hatchery origin described at the right of the figure. Bootstrap values > 50 (percent out of 500 iterations) are shown. [Figure available online in color.]

clusters (Supplementary Figure 2). Because the Delta method often underestimates biologically meaningful structure (J. Pritchard, STRUCTURE Google Group, personal communication) and a slightly higher level of structure was supported by other analyses, we focused on $k = 4$ as the most appropriate outcome and present results from an additional 10 simulations of four clusters using a burn-in length of 500,000 and 500,000 MCMC replicates.

Evaluating structure based on four clusters showed a clear distinction of the Paradise hatchery sample, with individuals assigning strongly to one cluster (red in Figure 5); individuals from both the Temiscamie and Assinica hatchery samples also showed clear assignments to one common cluster (dark grey in Figure 5). Individuals from the LNFH were more admixed, consistent with the fact that this hatchery broodstock originated from multiple sources; proportional ancestry for LNFH individuals was partitioned among three clusters (red, white, and light grey in Figure 5), including the Paradise hatchery (red) cluster. Several field samples (Cow and Bigsprings creeks and, to a slightly lesser extent, Boulder and Eighteenmile creeks, the upper Lemhi River, and part of Teton Creek) had high assignments to the Paradise hatchery (red, Figure 5) cluster, suggesting fish from this hatchery were planted in these sites originally. These relationships were also supported by the fact that these samples clustered together with the Paradise hatchery sample in the phenogram (Figure 4). The only field sample that showed any notable relationship with the Temiscamie and Assinica cluster (dark grey, Figure 5) was Garden Creek in the upper Salmon River, for which individuals showed patterns suggesting admixture between this cluster and the clusters characterizing the LNFH sample. This influence of the Quebec Brook Trout strains in Garden Creek was also suggested in the phenogram, where there was strong support (99% bootstrap value) for grouping Garden Creek with the Temiscamie and Assinica samples (Figure 4). Many field samples had individuals with proportional ancestries similar to individuals from the LNFH sample (i.e., mixed ancestry in the red, white and light grey clusters in Figure 5), such as Myrtle Creek in the Kootenai River, all tributaries to the Priest and Secesh rivers, and three tributaries to the upper Salmon River (Smiley and Valley creeks and the main-stem river). Several field samples strongly assigned to the white cluster, including Kenney Creek in the Lemhi River, all samples from the Middle Fork Salmon River, Mahogany Creek in the Teton River, and Targhee Creek from the Henrys Fork River (Figure 5). The phenogram similarly grouped these samples (Figure 4). Finally, other samples, primarily from the Teton and Henrys Fork rivers, assigned mostly to the light grey cluster (Figure 5). When plotted geographically, assignments to the different clusters show marked variation across the state of Idaho (e.g., Middle Fork Salmon River versus those from the most northern part of the state; Figure 1); even within some watersheds populations assigned to different clusters (e.g., in the Lemhi and Henrys Fork rivers, where there were contrasting assignments of three samples mostly to the one cluster versus assignment of the fourth sample to another cluster).

Results from BAPS were generally similar to those from STRUCTURE but were slightly less resolved in terms of determining the most appropriate k, and the program's "probability of k" approach maximized a much higher level of structuring. When each sequential k was evaluated in a separate analysis, each higher level of structure was consistently maximized as the most likely, with a probability of 1. However, in evaluating

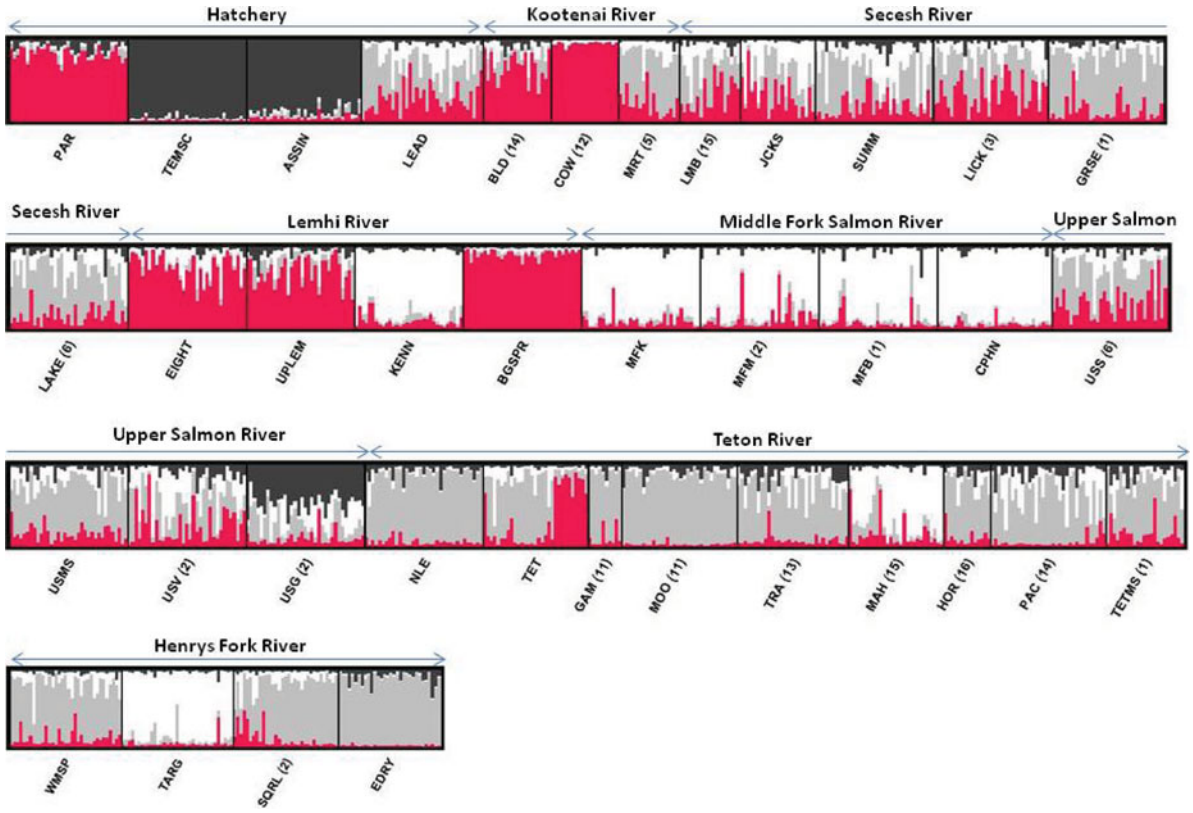

FIGURE 5. STRUCTURE results based on four clusters ($k = 4$) for samples from four hatcheries and from populations of nonnative Brook Trout across Idaho. Ten runs were implemented using 500,000 burn-in iterations and 500,000 MCMC repetitions. Individual assignments were aligned using the program CLUMPP (see text). Colors (red, light grey, dark grey, and white) represent the four clusters defined by STRUCTURE, and each vertical bar shows the proportional ancestry (Q) within an individual fish in each cluster. Dark vertical lines separate samples, names of which are abbreviated below each sample (see Table 1 for definition of abbreviations); origin (i.e., hatchery or major watershed) is given above. Where stocking was historically recorded, the number of stocking events is shown in parentheses. [Figure available online in color.]

the actual logmls values across these collective analyses (similar to the log-likelihood output of STRUCTURE), there were jumps in likelihoods between $k2$ and $k3$ and again between $k6$ and $k7$, after which values continued to increase gradually up to $k30$ (Supplementary Figure 3). The inclusive analysis of $k20$, $k50$, and $k100$ returned $k30$ as the most likely outcome in all of the 10 best visited partitions with a probability of 1. Results for the admixture model for $k3$, $k4$, $k7$, and $k30$ are presented in Supplementary Figure 4. For $k3$, the program clearly partitioned the Paradise Hatchery and the Assinica–Temiscamie hatcheries in separate clusters, while LNFH was a mixture of the Paradise and a third cluster, similar to STRUCTURE results. Also similar to STRUCTURE results, field samples were characterized primarily by either the Paradise or the two Leadville hatchery clusters, although Garden Creek (number 25 in Supplementary Figure 4) again showed an influence of the Assinica–Temiscamie cluster. One difference was that the Middle Fork Salmon River samples (numbers 18–21 in Supplementary Figure 4) assigned clearly to the Paradise cluster as opposed to the white cluster partially represented by LNFH in STRUCTURE. Results were similar for $k4$, with the fourth cluster separating many samples from the Teton and Henrys Fork rivers as with the assignment of these samples to the grey cluster in STRUCTURE (Figure 5; Supplementary Figure 4). With the further partitioning in $k7$, Garden Creek assigned to a distinctive cluster with no representation in the hatcheries. Kenny Creek (number 16 in Supplementary Figure 4 from the Lemhi River) and samples from the Middle Fork Salmon River were further separated, similar to their assignment to the white cluster in STRUCTURE; similarly, samples from the upper Salmon and Teton rivers became distinctive as in STRUCTURE, while East Dry Creek in the Henrys Fork River assigned to the seventh cluster. With $k30$, the program continued to resolve even greater structure among tributaries within the various watersheds. Overall, the BAPS admixture model showed less, but still some, evidence for individual admixture across samples.

Probability of Assignment to Hatchery Sources

GeneClass2 probability of assignment results largely corroborated other results. Of 1,025 individuals collected in Idaho, 80 were "excluded" from any assignment, having a probability of origin from any of the four sampled hatcheries < 0.05

TABLE 3. Assignments of 1,025 individuals (*N*) captured in naturalized Brook Trout populations in Idaho to four known hatchery sources. Excluded individuals had a probability <0.05 of belonging to any hatchery source, "Assignments" tally individuals with probability ≥0.05 of assignment to only one hatchery source, and dyads, triads, and "all four" tally individuals with probability ≥0.05 of assignment to two, three, and all four hatcheries, respectively. See Table 1 for definitions of hatchery codes.

Category of "assignment"	Hatchery or combination	*N*
Excluded from all hatcheries		80
Assignments	PAR	462
	TEMISC	16
	ASSIN	0
	LEAD	6
Dyads	PAR/TEMISC	68
	PAR/ASSIN	0
	PAR/LEAD	343
	TEMISC/LEAD	1
	ASSIN/LEAD	0
	TEMISC/ASSIN	1
Triads	PAR/TEMISC/ASSIN	10
	PAR/ASSIN/LEAD	0
	TEMISC/ASSIN/LEAD	0
	PAR/TEMISC/LEAD	33
All four	All four	5

(Table 3). The large majority (462 of 480) of "true" assignments, i.e., where individuals had $P \geq 0.05$ of belonging to only one hatchery, were to the Paradise Hatchery, with 16, 0, and 6 individuals assigning exclusively to Temiscamie, Assinica, and Leadville hatcheries, respectively. Of the 412 individuals with probabilities ≥ 0.05 of belonging to two hatcheries, most (343) "assigned" both to the Paradise and Leadville hatcheries, with 68 "assigning" both to the Paradise and Temiscamie hatcheries. The remaining individuals assigned to multiple hatcheries were "'assigned" to various combinations of dyads, triads, and all four hatcheries (Table 3).

Simulation of Hybrid Populations and Resulting Genetic Diversity

HYBRIDLAB simulations of populations arising from hybridization of different hatchery strains produced populations with relatively similar levels of genetic diversity and internal structure as we observed in the field. In comparison with field samples, where H_E ranged from 0.23 to 0.34, gene diversity values for the hybrid populations were: 0.29 for *a*, the Paradise × Leadville population; 0.33 for *b*, the Temiscamie × Leadville population; 0.33 for *c*, the cross between *a* and *b*; and 0.33 for *d*, or *c* crossed with itself. Simulated populations had slightly higher allelic richness than did field samples, however, as R_S values were 1.92 for *a,* 1.94 for *b*, and 1.95 for *c* and *d*, compared with a range from 1.58 to 1.83 for field populations. STRUCTURE characterization of our simulated hybrid populations was similar to that of many of our field samples, such as Garden (compared with *b*), Lick and Jackson (compared with *c* and *d*), and many others (Supplementary Figure 5).

DISCUSSION

Our characterization of genetic diversity in established populations of Brook Trout across Idaho provides insight about a factor that has previously been ignored but may influence the invasive success of this fish. We found Brook Trout populations in Idaho to have variable but frequently high within-population genetic diversity, to display significant genetic structuring across and even within watersheds, and to be characterized in some cases by admixture. These characteristics reflect historical propagation (i.e., hatchery) practices as well as subsequent introduction and invasion patterns of different hatchery strains in different regions of the state. With our current data we cannot directly link observed genetic diversity to possible selective advantages in the field, but the theoretical and empirical foundation for such a scenario is clear, as genetic attributes similar to those we observed have been shown in numerous recent examples to translate to high establishment and invasion success in various taxonomic groups (Lavergne and Molofsky 2007; Roman and Darling 2007; Dlugosch and Parker 2008; Facon et al. 2008).

Propagule pressure has received increasing attention as an important factor facilitating establishment and subsequent invasions in many taxa including salmonids (Colautti 2005; Simberloff 2009; Bennett et al. 2010; Consuegra et al. 2011), emphasizing the role that high genetic diversity may play in these processes (Lockwood et al. 2005; Roman and Darling 2007). Although we were not able to evaluate the relationship between genetic diversity and propagule size directly given the poor resolution of historic records and confounding factors, we suspected our samples would have relatively high genetic diversity because multiple introductions were documented at many of our sites. Hatchery introduction events also typically involve many thousands of fish, often from different sources, and collectively these practices can create substantial propagule pressure in founding populations that may help them circumvent genetic impacts typically expected in founder or invasive populations. Indeed, diversity in many field samples was higher than that of the hatchery samples (with an upper limit of 0.34 as opposed to 0.29 for H_E and 1.83 versus 1.74 for R_S; Table 1), although this difference did not prove to be statistically significant between the two groups. Many field samples had larger estimated effective population sizes compared with the hatchery samples and to populations from native trout they frequently outcompete or replace in the west (Meyer et al. 2006; Neville et al. 2006; Narum et al. 2010; Ardren et al. 2011; Peacock and Dochtermann 2012). Furthermore, when we simulated populations arising from mixing hatchery samples, gene diversity levels were similar to those observed in our field samples (although R_S was actually higher in simulated populations).

Another factor increasingly recognized as important in explaining the "invasion paradox" is that invasive populations

often benefit from the introduction of divergent strains from across the native region (Roman 2006; Roman and Darling 2007). In eastern North America, native Brook Trout demonstrate substantial genetic differentiation even among geographically close populations as well as across major regions of their distribution, which is probably associated with the geography of glacial refugia and postglacial colonization patterns (Angers and Bernatchez 1998; Danzmann et al. 1998; Castric et al. 2001). Recent studies also showed genetically based differences in terms of growth, gene expression, and energy mobilization among Brook Trout populations from different geographic regions (Bougas et al. 2010; Crespel et al. 2013a, 2013b). In our study, the broad geographic origins of the four known hatchery sources introduced to Idaho were reflected in significant differentiation from each other with high pairwise F_{ST} values among the four hatchery samples.

Clustering analyses readily resolved this diversity. Because STRUCTURE results were mirrored more closely by other analyses (such as the phenogram and assignment tests), and to reach a balance between increased partitioning (with higher k) versus a parsimonious approach of not over-fitting models, we focus our interpretation and discussion of clustering results on the STRUCTURE analysis with $k = 4$. (Even where results from STRUCTURE and BAPS diverged, differences were slight and both supported the main conclusions that multiple hatchery sources have contributed to diversity among naturalized Brook Trout populations.) STRUCTURE grouped individuals from the Temiscamie and Assinica strains together and clearly separated these two geographically proximal Quebec-origin strains from the Paradise, New York, hatchery sample and the sample from the LNFH. Fish from the Paradise hatchery were also genetically separated from other strains. The characterization of individuals from the LNFH, however, reflected their origin from many different sources, with ancestry spread across three clusters. The fact that the Paradise (red, Figure 5) cluster was one of these, as well as the fact that many fish were dually "assigned" to both the Paradise and the LNFH samples by GeneClass2, suggests that Paradise fish may have been one of these original sources incorporated into the LNFH broodstock.

This diversity among hatchery sources translated to a surprising degree of genetic structure among naturalized populations of Brook Trout in Idaho, corroborating the idea that patterns observed on the ground presently reflect the historical legacy of stocking fish from different sources across the native range followed by further genetic differentiation through genetic drift. STRUCTURE suggested a clear influence of the Paradise hatchery and LNFH fish on multiple field populations, as expected given the broad distribution of fish from both of these hatcheries across the western United States (Karas 2002; LNFH, unpublished documents; U.S. Fish Commission, unpublished documents). In other cases, assignments of individuals from field samples to certain clusters created by STRUCTURE were clear but did not directly match the characteristics of our hatchery samples. For instance, individuals in the Middle Fork Salmon River generally assigned to the white cluster (Figure 5), while those from the Teton and Henrys Fork rivers (exclusive of Mahogany and Targhee creeks and some individuals in Teton Creek) assigned to the light grey cluster; these clusters were both represented in our Leadville sample from the LNFH, but individuals from Leadville were typically admixed among the white, light grey, and the red clusters (Figure 5). Such mismatches may reflect a sampling effect due to genetic drift after introduction from the LNFH, i.e., where field populations subsequently diverged from their source (see Blum et al. 2007) or, possibly, drift between our contemporary sample from LNFH and the original broodstock used for introductions. Alternatively, they may reflect the presence of direct introductions from other sources we did not sample (but that were perhaps also used to create the admixed LNFH broodstock). For instance, records show that Wisconsin Brook Trout were one of the 13 sources used to initiate the LNFH broodstock; Wisconsin hatchery fish were also distributed widely across the United States (U.S. Fish Commission Reports, unpublished) and therefore could have been introduced directly to Idaho as well despite lack of documentation. This mismatch was corroborated by probability of assignments generated in GeneClass2, where all four of our hatchery samples were excluded as a possible origin for a subset of fish.

The lack of affinity of almost all field samples to the Temiscamie and Assinica cluster in our STRUCTURE results was somewhat surprising, particularly for collections in the Henrys Fork River watershed: these Quebec strains were purposefully introduced to Henrys Lake in relatively recent times (1980s) and subsequent introductions or invasions of these strains to other parts of this watershed might be expected. These strains were of particular interest here because of their remote northern origin and the possibility that their introduction would contribute unique genetic diversity arising from a different adaptive landscape (e.g., see Roman 2006). Though one population (Garden Creek) was characterized by introgression with these Quebec-origin fish, our results indicate these strains were not introduced broadly (at least not across the sites we sampled), or have not persisted in the wild perhaps due to low fitness of this hatchery fish in the wild.

We found evidence of admixture in many samples, which in many recent empirical studies has been associated with increased fitness in invasive populations because it can increase genetic variation (Allendorf and Lundquist 2003) and dilute inbreeding depression (Roman 2006; Sexton et al. 2011) or create novel genetic variability that subsequently proves beneficial (Lavergne and Molofsky 2007; Roman and Darling 2007; Facon et al. 2008). Our data suggest at least some of this hybridization arose from intentional mixing of fish from various sources during the hatchery propagation phase as early as the late 1890s, as was documented for the LNFH and is evident in our modern sample from this hatchery. Subsequent admixture might have also occurred in Idaho's hatcheries or when multiple introductions at a given site comprised fish from different sources. Given

our finding of populations of Brook Trout with different genetic characteristics persisting even within the same watershed (e.g., in the Lemhi and Henrys Fork drainages), it may be expected that admixture will continue to occur via movement of fish in the field, although the retention of distinct population characteristics to date is notable in many field samples.

The information our study provides about genetic diversity and population structure in naturalized Brook Trout provides an important foundation for informing effective management actions and future research on the invasive potential of the distinctive populations found in Idaho. For instance, management of nonnative Brook Trout is largely focused on efforts to eradicate them from selected habitats through either manual removal or application of piscicides, but both managers and the public are frequently frustrated by the ineffectiveness of these actions (Quist and Hubert 2004; Peterson et al. 2008). Our results suggest that even where eradications are not fully successful the imposition of bottlenecks impose an important genetic constraint on populations that could be helpful in reducing persistence or invasiveness in the future. Furthermore, even where Brook Trout already occur, the prevention of future introductions or invasion may help guard against the accumulation of high genetic variation and admixture of divergent strains in receiving populations that may increase their likelihood of becoming successful invaders themselves.

Manipulative studies could also draw on the genetic structure uncovered here and be useful for risk analysis (e.g., Stepien et al. 2005). As one example, it would be fruitful to impose bottlenecks or full extirpations of local populations across genetically distinctive regions of Idaho and compare the relative abilities of fish from different stocks to recover or reinvade (e.g., Hampton et al. 2004). Future monitoring of highly differentiated or highly variable populations may also provide insight on the evolutionary potential of this genetically diverse species in its nonnative range (Allendorf and Lundquist 2003). In this study, a lack of evidence for diversifying evolution in specific markers made us unable to achieve our initial goal of associating potentially selected markers with environmental factors, but this was not terribly surprising given the fact that local populations have not had long (~100 years) to respond to selective forces and given the observed wide broadcast and mixing of the primary hatchery strains across the state (and therefore across environmental gradients). Note also that genome scan methods frequently underestimate the number of loci under selection, especially for genes controlling polygenic phenotypic traits as well as in situations where the level of genetic differentiation between populations is relatively large, as observed here (Le Corre and Kremer 2012). Still, populations with different genetic characteristics and levels of variability may well respond differently to environmental change in the future (Allendorf and Lundquist 2003), and such variation in adaptive potential has not been incorporated, for example, in our own recent studies projecting Brook Trout responses to environmental change (Wenger et al. 2011a, 2011b).

Overall, our results indicate that nonnative Brook Trout are not as homogeneous or genetically depauperate as might have been previously assumed, and show marked genetic variation among populations, relatively high within-population genetic variability, and indications of admixture in some regions. Our findings suggest that even century-old patterns of hatchery propagation and introduction have allowed these fish to defy the "genetic paradox" early on in their nonnative history, setting the stage for successful establishment and subsequent expansion across western North America. This diverse genetic template merits consideration in future management and research related to this species' ubiquitous invasive success.

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