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Conservation Genetics of Remnant Coastal Brook Trout Populations at the Southern Limit of Their Distribution: Population Structure and Effects of Stocking

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ARTICLE

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Abstract

We examined genetic variation within and among a group of remnant coastal brook trout *Salvelinus fontinalis* populations along the coast of the northeastern United States. These populations occur at the southern limits of anadromy for this species and could form the foundation of a restored anadromous metapopulation. We also tested for genetic introgression between these populations and the hatchery source that has been used to stock these sites. The overall F_{ST} for the natural populations at 12 microsatellite loci was 0.145 (95% confidence interval, 0.108–0.183), and D was 0.225 (0.208–0.243). On average, 94.6% of individuals were correctly assigned to the population where they were collected. Our results suggest that there is little gene flow even between geographically proximate populations. We found little evidence that repeated historic stocking from a known hatchery source has led to genetic introgression into these wild coastal brook trout populations. One hybrid individual appeared to be a backcross between an F_1 and a hatchery individual. Another hybrid individual could not be classified. Our results suggest that nonintrogressed and potentially locally adapted populations of brook trout persist in several small coastal New England streams. These populations should be the focus of future efforts to restore anadromous brook trout in this region.

Knowledge of the genetic composition of the populations of a particular species is a prerequisite for conservation prioritization, genetic monitoring, and population restoration (Schwartz et al. 2007; Laikre et al. 2008). Information about a species' genetic composition includes the amount of genetic variation within and the genetic divergence among populations and, if relevant, the degree of introgression with anthropogenically introduced individuals (e.g., Laikre et al. 2008). This type of information is necessary for conservation goals ranging from the prevention of further erosion of genetic diversity in the most vulnerable of a series of extant populations (Ellstrand and Elam 1993), genetic rescue of extant populations suffering from inbreeding depression through translocation (Tallmon et al. 2004), or reintroduction of

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individuals to habitats where extirpation has occurred (Hansen et al. 2001).

Genetic introgression between wild and anthropogenically introduced individuals has been extensively examined in fish (Hindar et al. 1991; Hansen et al. 2001). Introduced individuals are often the product of captive breeding and are introduced to boost population size (Fraser 2008) or result from species invasion (Allendorf et al. 2004). Captive-bred individuals can be maladapted to the natural environment following rearing in an artificial environment (Fraser 2008; Araki et al. 2009). Hybridization between captive-bred individuals and native local populations may swamp local adaptations in the native populations and cause genetically based loss of fitness (Reisenbichler and McIntyre 1977; Reisenbichler and Rubin 1999; Araki et al. 2007; Fraser 2008). Here we define hybridization as the interbreeding of individuals from distinct populations, regardless of taxonomic status (Rhymer and Simberloff 1996). Recent work has demonstrated positive stocking-pressure-dependent introgression in salmonid populations (Marie et al. 2010, 2011). Alternatively, other studies have demonstrated that hybridization between captive-bred and wild fish may not occur at all or may occur only at low levels (Hansen et al. 2002; Matala et al. 2008; Hansen and Mensberg 2009). In the latter case, genomes may remain intact and population restoration efforts can focus on nonhybridized populations or (if all populations show some level of introgression) on those that are the least affected by hybridization.

The native range of brook trout *Salvelinus fontinalis* extends from the shores of Canada's Hudson Bay south through the Great Lakes and Appalachian Mountains to inland streams in northern Georgia (Power 1980). Like other salmonids, brook trout can be anadromous (so-called sea-run brook trout) wherever there is free access to the sea and marine or freshwater habitats remain sufficiently cool throughout the summer. Adoption of a resident or anadromous life history appears to be highly environmentally sensitive, and growth rate and growth rate efficiency appear to be the most important proximate factors linked to their expression (Morinville and Rasmussen 2003; Thériault et al. 2007). Anadromous brook trout have historically occurred in coastal waters north of New York City (Power 1980). Coastal brook trout populations along the coast of southern New England and Long Island, New York, have been greatly reduced in number by habitat alteration and overfishing during the past century (MacCrimmon and Gots 1980). A survey of 74 coastal streams by the Massachusetts Division of Fisheries and Wildlife identified only 17 coastal brook trout populations that may contain anadromous individuals remaining in this state as of the 1970s (Bergin 1984). Currently, wild-reproducing coastal populations occur in a few tributaries of Nantucket Sound, Buzzards Bay, and Narragansett Bay in southern New England (Hartel et al. 2002) and at least one coastal stream on Long Island, (Ryther 1997). The degree to which the individuals in these remaining coastal populations use the ocean is unclear, but those in some of these populations reach larger sizes and have faster growth rates than resident brook trout in the same stream (Ben Letcher, U.S. Geological Survey, unpublished results). The remaining small coastal brook trout streams on Cape Cod, Massachusetts, were heavily stocked with domesticated hatchery trout between the 1940s and the 1980s. The cumulative effects of this stocking might have caused widespread hatchery introgression into the remaining coastal brook trout populations (Bigelow 1963; Ryther 1997). Alternatively, the remaining wild-reproducing populations might have resisted hatchery introgression and be an important focus of future restoration efforts.

In this paper, we genetically analyzed five remaining coastal brook trout populations on Cape Cod ($N = 4$) and Long Island ($N = 1$) with 12 microsatellite markers. First, we determined the genetic variation within and divergence among these populations. Second, because there is a history of hatchery stocking in these populations, we estimated the degree of introgression between the hatchery source and the remaining populations. This work provides a foundation for future restoration efforts of the sea-run brook trout at its southern limits.

METHODS

Study Area and Sampling

We sampled wild brook trout from four coastal streams on Cape Cod (Santuit River [SA], Mashpee River [MA], Quashnet River [QU], and Red Brook [RB]) and one coastal stream on Long Island (Connetquot River [CO]) in 2002 and 2003 (Table 1; Figure 1). All of these streams are small (average flows = 0.1–0.5 cubic meters per second), low-gradient, first-order streams fed by coldwater springs along their entire length and draining directly into their estuaries. Each stream is connected to a freshwater pond at its headwaters. The streams flow through oak and pine forests over coarse sandy soils from a glacial outwash plain from elevations not higher than 23 m above sea level.

The Connetquot River, about 300 km southwest of the Cape Cod streams, is the southernmost location where anadromous brook trout occur (Ryther 1997). This 10-km stream's geohydrology is similar to that of the Cape streams. The Connetquot River has been intensively managed for trout fishing since the 1860s, first by an exclusive private club and since 1973 by the state of New York. The Connetquot River has its own specific hatchery located immediately on the stream, which does not maintain a broodstock. Returning brook trout are selected and spawned from the river each year. The hatchery then raises and releases the fish directly back to the river as adults. The hatchery has always used native returning fish for reproduction and has not introduced other brook trout stocks into the river (Gil Bergin, manager, Connetquot Hatchery, Oakdale, New York, personal communication). Natural brook trout reproduction reportedly occurs in some areas of the river.

Hatchery brook trout (hereafter HA) from the Sandwich Fish Hatchery (Sandwich, Massachusetts; Figure 1) have been released directly into the SA, MA, and QU since the 1940s

TABLE 1. Brook trout collection locations with their abbreviations, latitude–longitude coordinates, and number of fish sampled from each location (*N*).

Region and river	Abbreviation	Latitude (N)	Longitude (W)	*N*
Cape Cod				
Santuit River	SA	41°37.672	70°27.062	29
Mashpee River	MA	41°37.300	70°28.823	43
Quashnet River	QU	41°35.533	70°30.463	82
Red Brook	RB	41°45.915	70°38.035	49
Sandwich Hatchery	HA	41°45.159	70°29.381	37
Long Island				
Connetquot River	CO	40°45.783	73°09.166	40

(Table 2). The hatchery fish are from the Sandwich strain eastern brook trout broodstock, a strain registered with the National Fish Strain Registry. The registry reports the original source of the animals as the Montague, Massachusetts, state fish hatchery and various field sites (Kincaid et al. 2002). This broodstock has always been maintained using spawners from the hatchery itself, and brook trout from other locations have not been mixed with this hatchery broodstock (Craig Lodowsky, manager, Massachusetts Division of Fisheries and Wildlife, Sandwich Fish Hatchery, personal communication). The fourth Cape Cod site (RB) has been privately owned and managed as an anadromous brook trout fishing camp since the 1860s. Brook trout

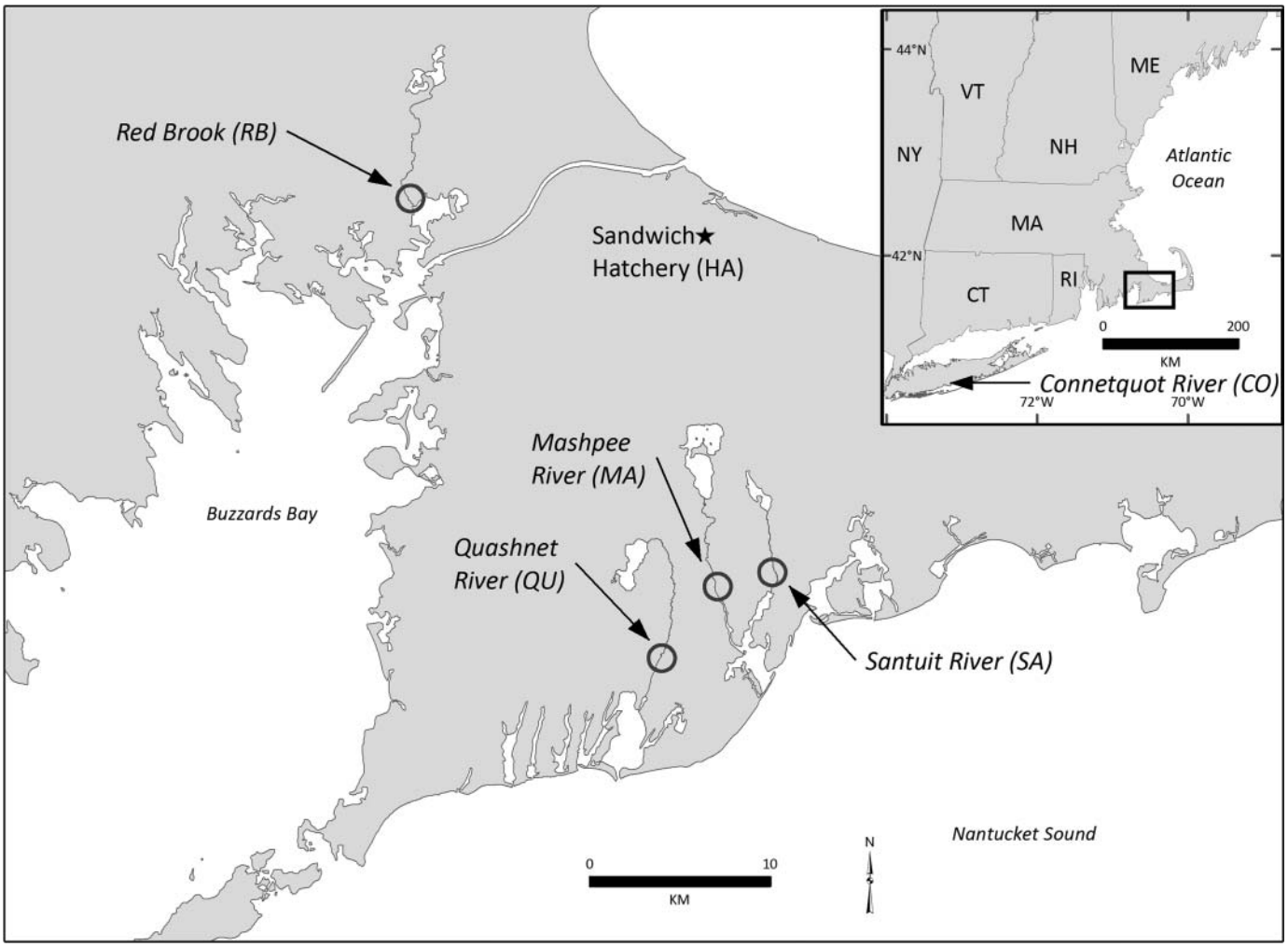

FIGURE 1. Map showing the locations of the coastal streams where brook trout were sampled. Also shown is the location of the Sandwich Fish Hatchery, from which the hatchery strain was obtained.

TABLE 2. Stocking pressure for three of the study sites examined. The entries are the numbers of Sandwich Fish Hatchery adult brook trout stocked into the Santuit River (SA), the Mashpee River (MA), and the Quashnet River (QU) during each decade from 1940 to 2000. Every decade spans the 10 years following the year listed (e.g., 1950s = 1951–1960). The numbers are based on stocking records kept by the Massachusetts Division of Fisheries and Wildlife.

	Decade							
Stream	1940s	1950s	1960s[a]	1970s	1980s	1990s	2000s[b]	Total
SA	0	23,400	2,400	800	2,850	0	0	29,450
MA	18,000	37,150	6,750	400	0	0	0	62,300
QU	0	30,000	4,600	800	0	0	0	36,000
Total	18,000	90,550	13,750	2,000	2,850	0	0	127,750

[a]In 1965, 5,000 1-in (2.54-cm) brook trout fry were stocked into each stream from the Montague State Fish Hatchery. The Montague hatchery is reported as one of the original sources of animals for the Sandwich broodstock. Records indicate that between the years 1959 and 1963 between 600 and 2,350 adult brook trout were stocked in each stream from sources other than the Sandwich Fish Hatchery.

[b]Through 2011.

from the Sandwich Fish Hatchery and other sources have been stocked into RB during this time period. Since the 1990s, hatchery fish are no longer stocked directly into any of the streams. However, thermally stratified headwater ponds at the headwaters of each stream are still stocked with hatchery fish. It is likely that thermal barriers (warmer surface waters) limit the movement of hatchery individuals into stream habitat (Steve Hurley, Massachusetts Division of Fisheries and Wildlife, personal communication).

Wild adult fish ($N = 247$) were collected by pulsed-DC electrofishing (400 V, 0.3–0.5 A, and 60 Hz) or seining in freshwater reaches of the coastal streams within 500 m of tidewater during the summer and fall months before spawning. Population sample sizes ranged between 29 and 82 (Table 1). To reduce the risk of collecting closely related individuals that may be schooling together, fish were sampled from multiple locations separated by approximately 100 m within each stream, except in the Connetquot River, where up-migrating fish were seined from multiple locations below a small dam. Tissue was sampled from 37 individuals from the Sandwich Fish Hatchery in the fall of 2003. Adipose fin tissue was collected from live wild fish prior to release and stored in 95% ethanol until subsequent laboratory analysis.

Genetic Data Analysis

Microsatellites.—Laboratory analysis was conducted at the U.S. Geological Survey, Biological Resources Division, Leetown Science Center, Aquatic Ecology Laboratory in Kearneysville, West Virginia. Genomic DNA was isolated from fin tissue with the Puregene DNA extraction kit (Gentra Systems, Inc., Minneapolis, Minnesota) according to the manufacturer's guidelines. Isolated DNA was resuspended in 100 μL of 10 mM tris-HCL, pH 8.0, and 1 mM EDTA before use in polymerase chain reactions (PCR). A group of 12 microsatellite loci (King et al. 2012) were selected for their demonstrated polymorphism in other brook trout population studies and examined in all fish (see the supplemental table available in the online version of this article). Each PCR consisted of 100–200 ng of genomic DNA, 0.875 × DMD multiplex PCR buffer (58 mM tris-HCl [pH 8.8], 15 mM $(NH4)_2SO_4$, 5.9 mM $MgCl_2$, 8.8 mM ß-mercapthoethanol, and 6 mM EDTA), 0.32 mM dNTPs, 0.075–0.250 μM forward and reverse primers (the forward primer labeled with TET, FAM, or HEX; Life Technologies Corporation, Carlsbad, California), and 0.1 U/μL *Taq* DNA polymerase (Promega, Madison, Wisconsin) in a total volume of 15 μL. Amplifications were carried out on a 96-well thermal cycler 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. Fragment electrophoresis and scoring were performed according to the protocols described by King et al. (2001).

Genetic diversity within populations.—Allele frequencies, deviations from Hardy–Weinberg expectations, gametic disequilibrium, observed (H_O) and expected (H_E) heterozygosity (per locus and per population), mean within-population expected heterozygosity (H_S), and the fixation index F_{IS} were calculated with GENEPOP version 4.0.10 (Rousset 2008). Mean allelic richness per population (AR; i.e., the mean number of alleles scaled to the smallest sample size; $N = 29$) was calculated with FSTAT version 2.9.3.2 (Goudet 2001). We corrected for multiple tests for Hardy–Weinberg expectations and gametic disequilibrium with the sequential Bonferroni procedure (Rice 1989). We used an initial α value of $0.05/k$, when k is the number of comparisons. We conducted tests for excess homozygosity at each locus in each population with MICROCHECKER version 2.2.3 (Van Oosterhout et al. 2004) as a test for the presence of null alleles.

Genetic divergence among populations.—Pairwise exact tests for genic differentiation were calculated with GENEPOP (Rousset 2008). *F*-statistics were calculated with FSTAT (Goudet 2001). We used θ analogues (Weir and Cockerham 1984) for overall and pairwise estimates of F_{ST}. We used the DEMEtics version 0.8-3 (Gerlach et al. 2010) package for R version 2.12 (R Development Core Team 2006) to estimate Jost's D (Jost 2008). We used 1,000 permutations to calculate 95% confidence intervals or P-values for both measures, and we applied a sequential Bonferroni correction to adjust for multiple tests (Rice 1989). We used PHYLIP version

3.5 (Felsenstein 1993) to calculate Cavalli-Sforza and Edwards' (1967) genetic distance (CSE) between each pair of populations with the GENDIST module and constructed an unrooted neighbour-joining dendrogram with the NEIGHBOR module. We used TreeViewX version 0.5.0 (Page 1996) to visualize the dendrogram. The PHYLIP module CONSENSE was used to generate a consensus tree with bootstrap values from 4,000 replicate data sets created in SEQBOOT. We performed maximum-likelihood assignment tests to further test the genetic relationships among populations. GENECLASS version 2.0 (Piry et al. 2004) was used to calculate probabilities of individuals belonging to populations following Rannala and Mountain (1997).

We tested the relationship between genetic and geographic distances between populations (isolation by distance [IBD]) to further examine the factors structuring populations. We used CSE chord distance for the genetic distances. Coastal geographic distances were measured in ArcView 3.2a (ESRI, Redlands, California) as the shortest distance from river mouth to river mouth through the estuaries and the ocean, around headlands and islands. This measure of distance is consistent with previous observations of brook trout movement patterns in the ocean (White 1942). We performed analyses with and without the Long Island population because it is geographically highly removed from the Cape Cod populations (the mean $\pm$ SD pairwise geographic distance for the Cape Cod populations was 32.0 $\pm$ 23.3 km; the mean $\pm$ SD distance including the Long Island population was 136.7 $\pm$ 136.4 km). We performed Mantel tests with Isolation By Distance Web Service version 3.21 (Jensen et al. 2005).

Hatchery introgression.—We used STRUCTURE version 2.3.2 (Pritchard et al. 2000; Falush et al. 2003) to test for hybridization between the hatchery strain (HA) and each of the Massachusetts wild populations (MA, SA, QU, and RB). Each hatchery–wild population pair was examined separately without prior information for sample location. We used 500,000 replicates and 100,000 burn-in cycles under an admixture model in which we estimated a separate α parameter (i.e., the Dirichlet parameter for degree of admixture) for each population and an initial α of 1.0. We used the correlated allele frequencies model with an initial λ of one. We allowed F to assume a different value for each population, which allows for different rates of drift among populations. We performed 10 runs for $K = 1$ and 2 for each hatchery–wild population pair. The proportion of loci within each individual that were assigned to either the wild population or the hatchery strain (q) was used as an estimate of individual-level hybridization. The Sandwich strain that we examined was the sole known source of individuals introduced into MA, SA, and QU. Other unknown and unavailable sources of fish may have been introduced to RB, and therefore our analysis with the Sandwich strain as the hatchery source could represent an underestimate of introgression rates at this site.

We further examined the probability that individuals belonged to one of five distinct genetically defined categories (pure wild, pure hatchery, F_1, F_2, and backcross to either wild or hatchery fish) with the software NEWHYBRIDS version 1.1 Beta3 (Anderson and Thompson 2002). We performed a separate analysis for each of the four Massachusetts wild–Sandwich Fish Hatchery population pairs and specified the expected genotype frequency of each category. Each run of the Markov chain consisted of a burn-in period of 100,000 iterations followed by 250,000 iterations. We provided prior information on the identity of the hatchery individuals. Individuals belonging to a category with posterior probabilities >70% were considered correctly assigned (Gunnell et al. 2008).

RESULTS

Genetic Diversity within Populations

The total number of alleles observed at a locus ranged from 2 at *SfoC79* to 15 at *SfoC115* (Table 3). Mean allelic richness ranged from 4.5 to 5.3 (Table 3). Mean expected heterozygosity (H_S), ranged from 0.495 to 0.608 (Table 3). Tests of deviation from Hardy–Weinberg proportions were significant in 6% of the cases (4 of 69 tests; $P < 0.05$), where 3.5 were expected by chance at $\alpha = 0.05$ (Table 3). None of the tests for deviation from Hardy–Weinberg proportions was significant following sequential Bonferroni correction ($\alpha = 0.05$), either for the approximately 12 tests within each population sample or the approximately 6 tests per locus. However, 3 of the 4 significant tests occurred at locus *SfoD91* (in SA, MA, and CO; Table 3). Positive F_{IS} values for this locus in these three populations were consistent with the presence of null alleles (Table 3). Furthermore, tests for excess homozygosity with MICROCHECKER indicated that null alleles may occur at *SfoD91* in CO. Subsequent analyses were performed with and without *SfoD91*, but none of the inferences changed. We therefore report results from the complete 12-locus data set. Significant gametic disequilibrium was detected in 11% of the cases (41 of 374 tests; $P < 0.05$). Upon sequential Bonferroni correction for the approximately 66 locus pairs in each population, three tests remained significant ($\alpha = 0.05$), two of which occurred in QU and one in MA.

Genetic Divergence among Populations

There were 18 population-specific alleles, and qualitative differences in allele frequencies were observed at many loci (Figure 2). One hundred and sixty-nine of the 180 (94%) pairwise exact tests for genic differentiation were significant ($P < 0.05$). Six of the 11 (55%) nonsignificant pairwise exact tests involved the locus with the fewest alleles (*SfoC79*, $N = 2$ alleles). The overall F_{ST} was 0.159 (95% CI, 0.125–0.195), and Jost's D was 0.257 (0.241–0.274). Excluding the hatchery population, the overall F_{ST} was 0.145 (0.108–0.183) and Jost's D was 0.225 (0.208–0.243). Pairwise F_{ST} ranged from 0.05 between RB and CO to 0.22 between SA and QU (Table 4). Pairwise Jost's D ranged from 0.07 between RB and CO to 0.396 between HA and QU (Table 4). All pairwise F_{ST} and Jost's D values were significant following sequential Bonferroni adjustment for multiple tests.

TABLE 3. Summary of genetic variation within brook trout populations from Cape Cod (SA, MA, QU, and RB), a Massachusetts hatchery (HA), and Long Island (CO). The number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), and F_{IS} are shown for each locus. Significant results for tests of Hardy–Weinberg expectations are indicated by asterisks in the F_{IS} column ($P < 0.05$). Following the locus-specific estimates, the mean values across loci of A, allelic richness (AR; standardized to $N = 29$), H_S (mean H_E across loci), and F_{IS} are shown for each population.

	SfoB52 (12 alleles)				*SfoC24* (5 alleles)				*SfoC38* (4 alleles)				*SfoC79* (2 alleles)				*SfoC86* (5 alleles)			
Population	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}
SA	5	0.379	0.388	0.022	3	0.679	0.557	−0.219	3	0.483	0.435	−0.110	1	0.000	0.000	NA	3	0.552	0.428	−0.289
MA	6	0.548	0.628	0.128	5	0.558	0.519	−0.076	2	0.023	0.023	0.000	2	0.047	0.046	−0.012	4	0.628	0.587	−0.069
QU	7	0.476	0.511	0.068	3	0.537	0.512	−0.049	4	0.329	0.290	−0.136	1	0.000	0.000	NA	4	0.469	0.435	−0.078
RB	7	0.694	0.663	−0.046	4	0.592	0.517	−0.146	3	0.122	0.118	−0.040	2	0.041	0.040	−0.011	3	0.449	0.512	0.123
HA	5	0.460	0.453	−0.013	3	0.472	0.531	0.111	2	0.460	0.434	−0.059	2	0.297	0.328	0.094	5	0.676	0.652	−0.037
CO	6	0.650	0.748	0.131	3	0.475	0.583	0.185	3	0.300	0.268	−0.120	2	0.275	0.240	−0.147	4	0.325	0.382	0.149

	SfoC88 (5 alleles)				*SfoC113* (9 alleles)				*SfoC115* (15 alleles)				*SfoC129* (7 alleles)				*SfoD75* (14 alleles)			
Population	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}
SA	3	0.552	0.428	−0.289	4	0.621	0.514	−0.209	5	0.586	0.568	−0.033	3	0.586	0.511	−0.147	8	0.621	0.735	0.155
MA	4	0.628	0.587	−0.069	6	0.744	0.822	0.094	8	0.571	0.645	0.114	5	0.512	0.491	−0.041	10	0.721	0.734	0.017
QU	4	0.469	0.435	−0.078	5	0.561	0.595	0.057	6	0.622	0.655	0.050	5	0.402	0.385	−0.044	8	0.866	0.840	−0.031
RB	3	0.449	0.512	0.123	6	0.531	0.551	0.037	7	0.694	0.754	0.080	6	0.633	0.650	0.027	9	0.816	0.809	−0.009
HA	5	0.676	0.652	−0.037	5	0.676	0.689	0.020	8	0.784	0.837	0.064	4	0.806	0.702	−0.148	7	0.541	0.663	0.185
CO	4	0.325	0.382	0.149	5	0.450	0.588	0.234*	10	0.825	0.760	−0.086	3	0.550	0.519	−0.060	9	0.600	0.553	−0.085

	SfoD91 (14 alleles)				*SfoD100* (10 alleles)				Mean across loci			
Population	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	AR	H_S	F_{IS}
SA	7	0.448	0.587	0.237*	7	0.931	0.798	−0.167	4.3	4.5	0.520	−0.065
MA	8	0.581	0.660	0.119*	7	0.738	0.755	0.022	5.6	5.3	0.554	0.035
QU	7	0.817	0.766	−0.066	7	0.317	0.354	0.104	5.1	4.5	0.495	0.002
RB	7	0.735	0.807	0.089	6	0.531	0.472	−0.125	5.3	5.0	0.521	0.004
HA	7	0.784	0.828	0.053	5	0.730	0.693	−0.053	4.8	4.6	0.608	0.021
CO	9	0.575	0.826	0.304*	7	0.650	0.583	−0.116	5.4	5.1	0.539	0.073

Cavalli-Sforza and Edwards chord distances also revealed strong genetic divergence among populations (Figure 3). The results of individual assignment tests followed the general pattern observed in pairwise F_{ST}, Jost's D, and CSE comparisons. On average, 94.6% of individuals were correctly assigned to the population from which they were collected (Table 5). Pairwise genetic and geographic distances were not significantly correlated when all of the wild populations were included in the analysis ($z = 209.1$, $r = -0.242$, $P = 0.677$) or when the geographically removed Long Island population was excluded ($z = 36.5$, $r = 0.356$, $P = 0.175$).

Hatchery Introgression

The models from STRUCTURE revealed that introgression between the hatchery strain and each of the four Massachusetts wild populations was low (Figure 4). For each STRUCTURE run and each hatchery–wild population pair, the $K = 2$ model had far greater likelihood estimates. The vast majority of point estimates of q were close to 1.0, which represents a "pure" indigenous brook trout (Figure 4). The median q-values were 0.991 for SA, 0.994 for MA, 0.995 for QU, and 0.995 for RB. Only three wild-caught individuals had point estimates of individual q-values less than 90%. These included single wild-caught individuals from MA ($q = 19.7\%$), SA (61.4%), and QU (85.4%). The 90% credible intervals for q-values included 1.0 for all but the one individual from MA. This individual appeared to be a later-generation hybrid with more hatchery than wild ancestry (90% credible interval, 0.0–0.458; Figure 4).

The results from NEWHYBRIDS allowed further inferences regarding putative hybrid individuals and were generally consistent with those from STRUCTURE. The individual from MA was assigned as a backcross between an F_1 and a hatchery fish (posterior probability = 0.747). The ancestry of the hybrid individual from SA could not be resolved. For this fish, the category with the highest posterior probability (0.381) was F_1, though all cross-type categories had nonzero posterior probabilities. We did not detect any evidence of hybridization in RB (mean posterior probabilities of pure wild fish = 0.989) or QU (mean posterior probabilities of pure wild fish = 0.991) with NEWHYBRIDS. Based on these results, the analyses of genetic structure were repeated with the MA and SA hybrid individuals removed. The overall inferences did not change (data not shown).

DISCUSSION

A combination of enhanced drift in populations with small effective size and restricted gene flow likely explains the genetic differentiation that we observed. The genetic differentiation of the Cape Cod populations was similar to that observed ($F_{ST} = 0.107$) in a study of 59 anadromous brook trout populations to the north of our study region (Castric and Bernatchez 2003). Castric and Bernatchez (2003) found greater differentiation and weaker IBD among southernmost populations (Gulf of Maine, USA, and Bay of Fundy, Canada) and lower genetic differentiation and greater IBD among more northern Canadian sites. Our study sites occurred to the south of all of the sites examined by Castric and Bernatchez (2003), and therefore our work extends their results further to the south. That is, our results extend the pattern of increased genetic differentiation and weak IBD at the southern limits of anadromy for coastal populations of brook trout. This pattern is consistent with reduced rates of anadromy among more southern coastal brook trout populations. Individuals at southern sites may be more likely to remain as residents in their natal streams, and gene flow may thus be lower in southern coastal brook trout populations. Acoustic tagging research currently under way suggests that the brook trout in our study sites use the ocean environment (Andy Danylchuck, University of Massachusetts–Amherst, personal communication). Furthermore, inter-river movement of brook trout has been observed between the MA and SA sites (Mullan 1958), which drain to the same estuary and are separated by only 3 km at their mouths. However, the rates of anadromy in our study populations remain poorly understood. A nonmutually exclusive alternative is that southern populations have smaller effective population sizes than northern populations, and therefore enhanced drift without a reduction in gene flow may explain the increased genetic divergence in the south. The range of heterozygosity that we observed at 12 microsatellites (0.495–0.608) was lower than that observed at 6 microsatellites (0.600–0.780) among coastal brook trout populations to the north of our study sites (Castric and Bernatchez 2004), but not dramatically lower. Therefore, drift is not likely to be solely responsible for the genetic divergence observed. We cannot further distinguish the relative influences of drift and gene flow on genetic divergence in these populations with the data in hand.

We found little evidence that introgression between hatchery and wild individuals has occurred in the Cape Cod populations. These streams received heavy hatchery stocking for many years, up until the last 5–10 brook trout generations. More recent stocking has occurred only in headwater ponds, where introduced individuals are confined to cold, deeper waters and are unlikely to have an opportunity to reach the streams. Based on other studies of brook trout with heavy stocking pressure (Marie et al. 2010), we might have expected widespread introgression instead of the low levels observed. Captive-bred individuals can be maladapted to the natural environment following rearing in an artificial environment (Fraser 2008; Araki et al. 2009). The Sandwich Fish Hatchery breeds fish to grow quickly in the hatchery environment and these fish appear to be highly susceptible to angling upon release to natural streams (Craig Lodowsky, personal communication). Poor survival in the wild due to the effects of domestication selection along with high angling mortality could explain the low rates of introgression observed. Another factor contributing to the poor survival of Sandwich Fish Hatchery fish in coastal streams could be that the hatchery fish originated in inland streams (in western

Massachusetts) and thus had low survival rates in the coastal stream habitat. It should be noted that one of our study streams, RB, has been stocked with fish from sources other than the Sandwich broodstock and therefore that our results for this site may underestimate hatchery introgression. We also lack historical samples that would allow us to examine the change over time in the genetic makeup of these populations and would provide definitive evidence for a lack of introgression. For example, historical samples have been used to reveal introgression in brown trout *Salmo trutta* populations (Hansen and Mensberg 2009). However, the strong genetic divergence between the hatchery source and each of the wild populations and the lack of evidence for introgression suggest that mating between wild and hatchery fish has occurred infrequently in the Cape Cod populations.

The populations we considered are threatened by a variety of stressors, including estuarine eutrophication, water withdrawals, invasive species introductions, and continued habitat loss due to

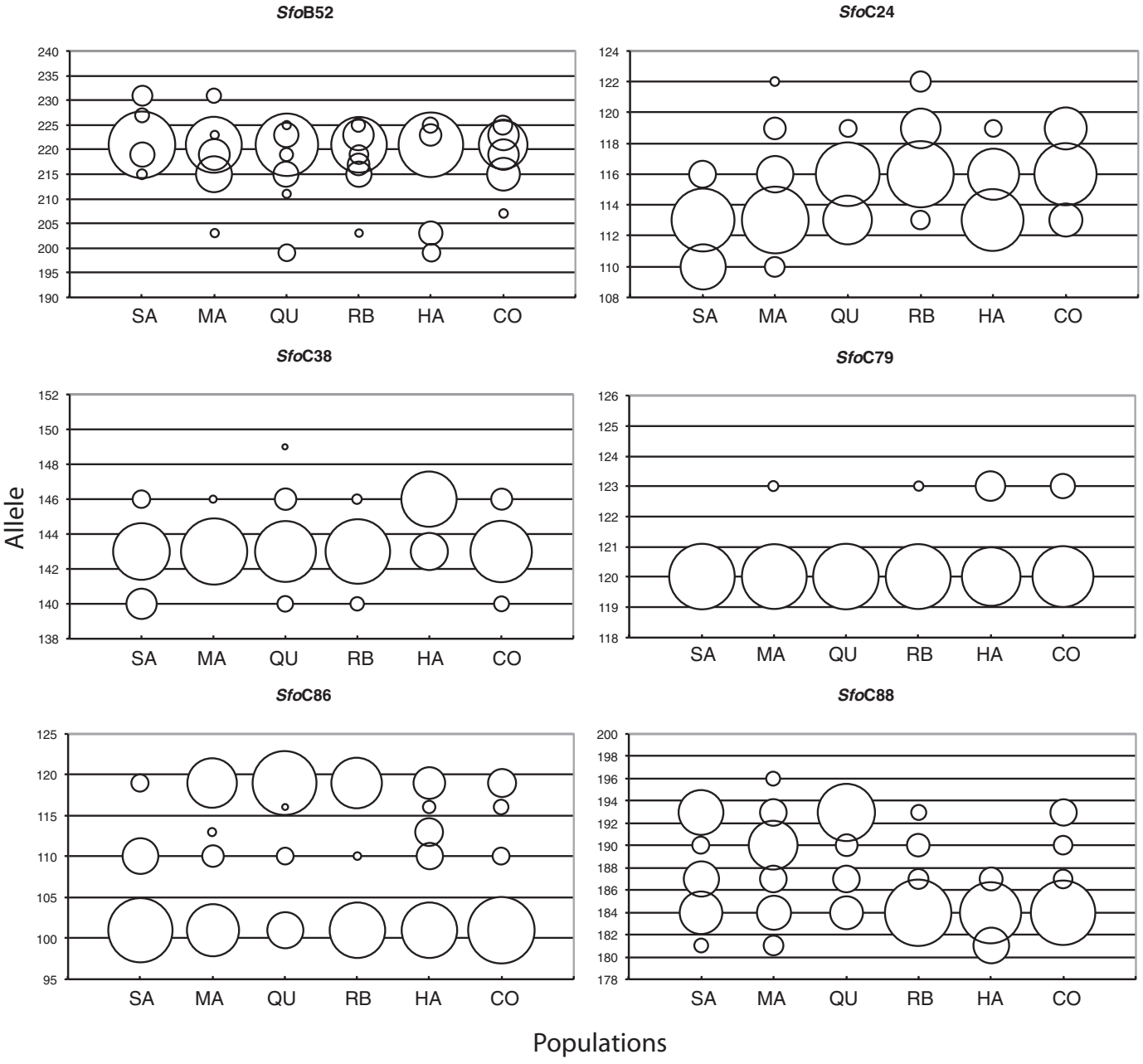

FIGURE 2. Bubble histograms illustrating the allele frequency differences among the six populations (see Table 1 and Figure 1) for each of the 12 microsatellite loci studied. The relative size of each bubble is proportional to the frequency of the corresponding allele in that population.

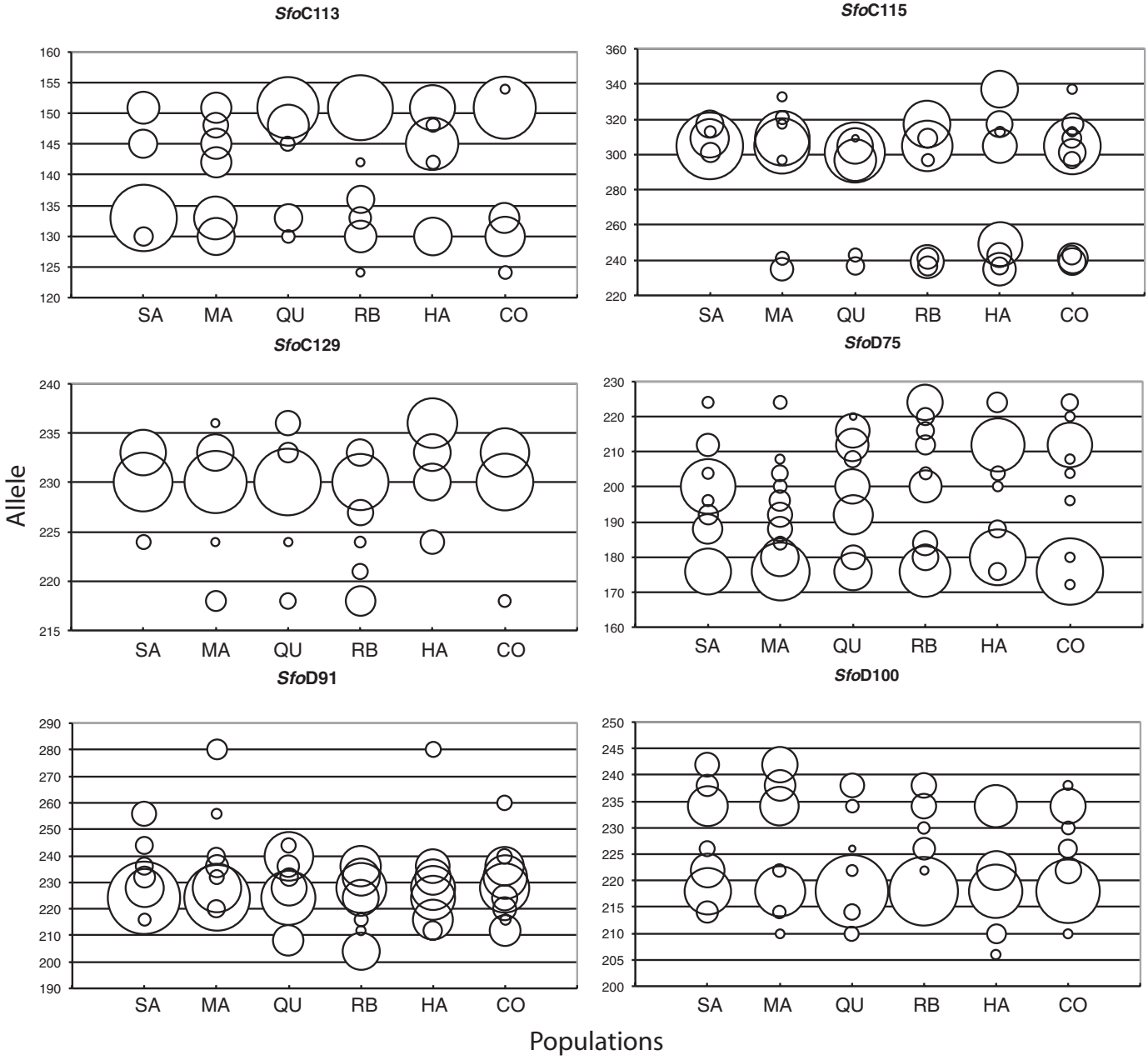

FIGURE 2. Continued.

TABLE 4. Genetic differentiation between pairs of brook trout populations from Cape Cod (SA, MA, QU, and RB), a Massachusetts hatchery (HA), and Long Island (CO). Estimates of pairwise F_{ST} are shown below the diagonal, estimates of pairwise Jost's D are shown above the diagonal. All estimates were significant following sequential Bonferroni adjustment for multiple tests ($\alpha = 0.05$).

Population	SA	MA	QU	RB	HA	CO
SA		0.140	0.287	0.290	0.345	0.254
MA	0.105		0.262	0.241	0.333	0.229
QU	0.215	0.137		0.228	0.396	0.263
RB	0.159	0.129	0.105		0.309	0.071
HA	0.183	0.183	0.228	0.160		0.274
CO	0.152	0.130	0.168	0.050	0.149	

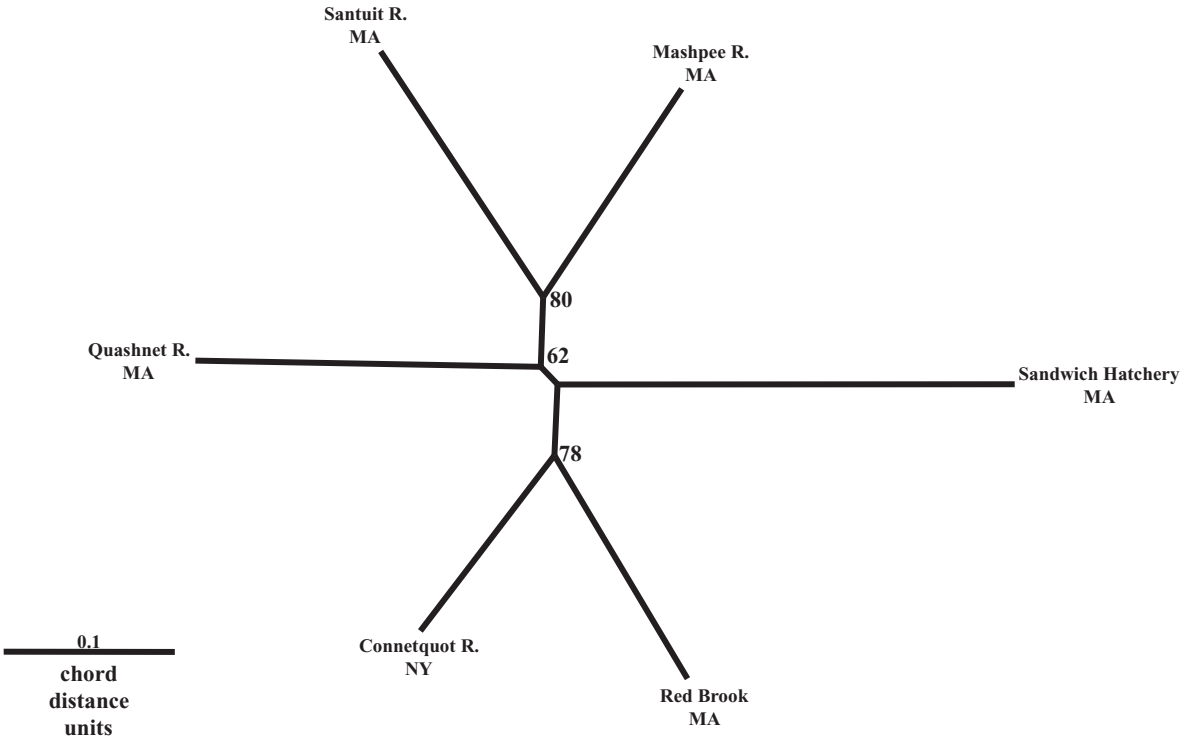

FIGURE 3. Neighbor-joining phenogram depicting the genetic distance (chord distance; Cavalli-Sforza and Edwards 1967) among six brook trout populations from Cape Cod (MA) and Long Island (NY). The numbers indicate the bootstrap support for the nearest node with 4,000 permutations.

urbanization. Restoration efforts aimed at restoring anadromous coastal brook trout populations in Massachusetts are currently under way. Future efforts will involve habitat improvement and fish translocation, either among the extant coastal populations or to currently vacant habitat in an effort to establish new coastal populations with access to the ocean. Our research provides a baseline analysis of extant coastal populations to guide these efforts. Maintenance of genetic diversity in these extant populations is critical to their future potential for adaptive response to environmental changes (Jump and Peñuelas 2005). The strong genetic divergence observed among populations at this geographic scale suggests that each of these populations might be locally adapted to environmental conditions (Lenormand 2002). The overall lack of introgression from hatchery fish further suggests that native gene pools worthy of conservation have persisted. Further, if in fact the rates of anadromy in our study sites are suppressed relative to historic levels, restoration of connectivity through expression of the migratory anadromous life history is an important conservation goal. Restoration of connectivity could allow the group of Massachusetts populations to form the foundation for a metapopulation of sea-run brook trout at the southern limit of anadromy for this species.

TABLE 5. Population assignment analysis confirming strong genetic differentiation of brook trout populations from Cape Cod (SA, MA, QU, and RB), a Massachusetts hatchery (HA), and Long Island (CO). The rows designate the populations from which individual brook trout were sampled, the columns the populations to which the individuals from those populations were subsequently assigned.

	Population					
Population	SA	MA	QU	RB	HA	CO
SA	0.931	0.069	0.000	0.000	0.000	0.000
MA	0.000	0.977	0.000	0.000	0.023	0.000
QU	0.000	0.037	0.939	0.000	0.000	0.024
RB	0.000	0.000	0.000	0.959	0.000	0.041
HA	0.000	0.000	0.000	0.000	1.000	0.000
CO	0.000	0.050	0.000	0.050	0.000	0.900

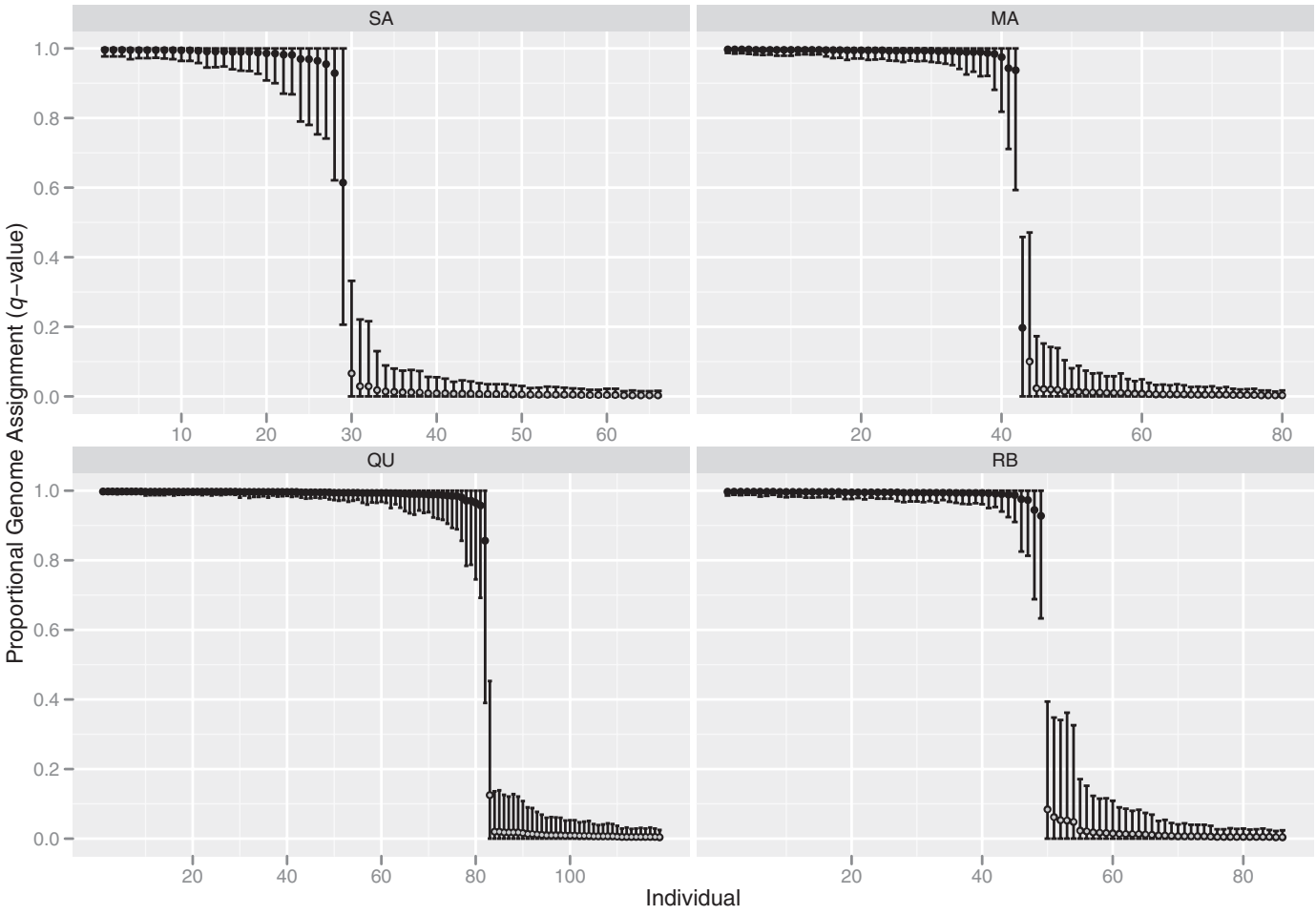

FIGURE 4. Proportions of loci within individuals (q-values; 90% credible intervals shown) assigned to either a wild population or the hatchery strain based on a STRUCTURE model for four Cape Cod brook trout populations. A q-value of 1.0 corresponds to a "pure" wild brook trout, and a q-value of 0.0 corresponds to a "pure" hatchery trout. Each point on the x-axis represents an individual. Wild-caught fish are represented by black points, hatchery fish by grey points. The same sample of hatchery fish from the Sandwich Fish Hatchery was used in each pairwise comparison with wild-caught population samples.

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