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Population Structure of Atlantic Salmon in Maine with Reference to Populations from Atlantic Canada

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Abstract.—Anadromous Atlantic salmon *Salmo salar* from 12 rivers in Maine, 3 rivers in New Brunswick, and 2 rivers each in Nova Scotia, Quebec, Newfoundland, and Labrador as well as 2 landlocked strains in Maine ($N = 3{,}863$) were genotyped at 11 microsatellite loci. Fish in the drainages of Maine's Kennebec and Penobscot rivers were genetically similar to those sampled from the 8 rivers recently listed as containing an endangered distinct population segment under the United States' Endangered Species Act. Genetic distance estimates confirm that Maine's Atlantic salmon, both landlocked and anadromous, represent a discrete population unit, genetically as independent from any Canadian population as the Canadian populations are from each other. Within Maine, the anadromous and landlocked populations were statistically distinct. Anadromous Atlantic salmon were more genetically similar among year-classes within rivers than among rivers, as would be expected if the river is the unit of population. The effective number of breeders estimated within each river is larger than the number of adults estimated from samples and redd counts over the 10-year period from 1991 to 2000.

The abundant Atlantic salmon *Salmo salar* in North American rivers represented an important subsistence and commercial fishery resource for both native North Americans and European settlers through the beginning of the industrial revolution. The Atlantic salmon was nearly extirpated from New England in the 19th century, when habitat loss resulting from dam construction and logging

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TABLE 1.—Adult Atlantic salmon returns to distinct population segment rivers (either 95% confidence interval from a model based on redd and adult counts or count from a trap) as estimated by the United States Atlantic Salmon Assessment Committee's technical advisory committee (USASAC 2001).

Year	Sheepscot River	Ducktrap River	Narraguagus River	Pleasant River	Machias River	East Machias River	Dennys River
1991		23–66	95	20–58	38–110		30–85
1992	19–54	11–32	73	11–31	30–87	3–8	25–73
1993	17–48	12–35	94	13–37	20–58	11–31	14–40
1994	19–55	18–51	51		22–63	12–34	10–29
1995	3–8	10–29	56	7–19	12–36		21–62
1996	9–25	20–58	64	19–55	34–99	19–55	16–45
1997	7–19	3–8	37	2–5	24–70	8–24	17–50
1998	4–12	7–21	22	7–21	28–80	28–80	16–47
1999	12–36	15–44	33		21–59	13–39	13–38
2000	10–29	3–8	23	3	13–38	8–22	2
Harmonic mean	7.6–21.4	7.7–21.4	42.7	5.8–11.0	21.3–61.9	8.7–24.2	9.5–14.5

rendered former levels of harvest unsustainable (Beland and Bielak 2002). By the 1890s, 70–90% of the United States' commercial catch of Atlantic salmon was restricted to Maine's Penobscot River (USASAC 2001). Nonetheless, anadromous fish continued to spawn in several Maine rivers, in abundances too low to support commercial harvest (Baum 1997).

Beginning in the 1870s, the Penobscot River was the primary focus of efforts to maintain and improve salmon production in Maine. Through 1947, Penobscot River production was primarily supplemented with progeny from broodstock of Penobscot origin (Baum 1997, summarized in Spidle et al. 2001). From the 1940s through the 1960s, returns to the Penobscot River were extremely low (on the order of tens of animals per year) despite the increase in stocking efforts. During the period from 1948 to 1967, hatchery releases originated primarily from broodstock taken from the Miramichi River in New Brunswick. From 1968 to 1971, most hatchery releases originated from the Machias and Narraguagus rivers in Maine (Baum 1997; Spidle et al. 2001), and adult returns to the Penobscot River rebounded. These returns resumed their former role as the primary broodstock for the Penobscot River restoration program in 1974 (Baum 1997; Spidle et al. 2001). From 1972 to 1992, fish of Penobscot River origin were used to supplement the limited wild returns in most other Maine rivers (Baum 1997). These last stocking efforts, consisting entirely of broodstock collected in Maine, would be expected to have made the greatest contribution to the current population of Atlantic salmon in Maine.

Prompted by the diminishing spawning runs and low juvenile densities indicated by adult surveys and redd counts (Table 1), U.S. resource managers have recently designated the Atlantic salmon in eight Maine rivers as an endangered distinct population segment (DPS; included are the Sheepscot, Ducktrap, Narraguagus, Pleasant, Machias, East Machias, and Dennys rivers and Cove Brook of the Penobscot River drainage) under the U.S. Endangered Species Act (ESA). At the time of listing, the Kennebec River and its tributaries were excluded because of a consensus that its native salmon had been extirpated owing to habitat loss and that the river had been repopulated by strays of unknown origin. The main-stem Penobscot River was also excluded because it was known to have received substantial supplemental stocking from Canadian rivers (see Baum 1997 and Spidle et al. 2001), even though the extent to which such stocking had contributed to the populations in the river was not clearly understood.

River-specific broodstocks were established for six of the eight populations within the Maine DPS designation as early as 1992 (the Sheepscot, Narraguagus, Pleasant, Machias, East Machias, and Dennys rivers; the Penobscot River population of Atlantic salmon has also been maintained as a river-specific strain since the 1970s but was not included in the DPS). It is hoped that the separate river-specific strains will maintain overall genetic diversity and, ideally, river-specific traits in the face of low effective population size. Rare alleles are expected to be lost in some populations and fixed in others, resulting in the overall preservation of genetic diversity. In contrast, under panmixia, alleles would be fixed or lost throughout the entire population, although the expectation of loss and fixation would be less. Because all of the river-specific strains have remained small, there has been concern that genetic drift and demographic stochasticity may overwhelm natural selection, po-

tentially resulting in lower genetic diversity and fitness. Documentation of the genetic relatedness among all the gene pools used for species recovery would aid in understanding the impacts such processes can have on the populations of salmon inhabiting Maine rivers.

Clear genetic differentiation between North American and European Atlantic salmon has been demonstrated (Stahl 1987; Bermingham et al. 1991; Verspoor 1994; McConnell et al. 1995a; Taggart et al. 1995; King et al. 2000, 2001), as has substantial population structure within North American Atlantic salmon (McConnell et al. 1995b, 1997; Beacham and Dempson 1998; King et al. 2001; Spidle et al. 2001). Significant genetic variation has been detected in Atlantic salmon throughout Atlantic Canada (Verspoor 1986, 1994; McConnell et al. 1997). King et al. (2001) have extended these results to show that collectively Maine's Atlantic salmon are as distinct from any Canadian population as each Canadian population is from the others. For management purposes, it is important to assay the level of genetic variation within Maine to provide effective guidance for restoring Atlantic salmon in accordance with the ESA.

In this article we report the results of a survey of microsatellite DNA variation in Atlantic salmon from throughout the six rivers designated under the ESA as well as the drainages of the Penobscot and Kennebec rivers. Several Canadian populations are included in the analysis for comparison and to help place the Maine populations in the context of Atlantic salmon population structure within all of North America. Genetic diversity was calculated within and between all the Maine samples, as was the genetically effective size of those populations. Together, the results will be useful in planning the future management of Maine's Atlantic salmon.

Methods

Tissue collection.—Fin or scale tissue samples were taken from parr, smolts, grilse, or adults from 23 anadromous populations and 2 nonanadromous strains of Atlantic salmon from Maine and Atlantic Canada. Table 2 provides a general description of the collection locations, sample sizes, and the life stage(s) year-classes sampled. The locations of all rivers sampled are shown in Figure 1. Adults from the two nonanadromous strains were sampled in hatcheries used to supplement landlocked populations in Maine. A portion of the samples from the Stewiacke and Gold rivers of Nova Scotia, Canada, were taken from parr generated in a hatchery environment from returning wild adults. All other collections were made in the river and, where possible, screened to eliminate the influence of aquaculture escapees. Due to the relative scarcity of adult fish in Maine, collection efforts were directed at parr using pulsed-DC backpack electrofishing units. Parr collected from the Sheepscot, Pleasant, Narraguagus, Machias, East Machias, and Dennys rivers were used to establish river-specific broodstocks for the restoration of each of those populations. Parr were also collected from the Ducktrap River but not used to establish a broodstock. Sample selection, handling, and DNA isolation followed protocols described in King et al. (2001). Smolt samples collected from the Pleasant River in 1999 and 2000 were found to contain a number of aquaculture escapees with evidence of European ancestry and were not included in subsequent analyses. For purposes of analysis, the age-classes sampled were assumed to be uniform (parr = age 1, smolts = age 2, and adults = age 3 and above).

Microsatellite survey.—Eleven microsatellite loci were screened in all fish (*Ssa14*, *Ssa289* [McConnell et al. 1995b], *SSOSL25*, *SSOSL85*, *SSOSL311*, *SSOSL438* [Slettan et al. 1995, 1996], *Ssa85*, *Ssa171*, *Ssa197*, *Ssa202* [O'Reilly et al. 1996], and *SSLEEN82* [GenBank accession number U86706; W. B. Schill and R. L. Walker, USGS-BRD, unpublished data]). Polymerase chain reaction conditions, electrophoresis, and scoring followed King et al. (2001).

Statistical analyses.—The genetic diversity present at each location in Maine was described with three statistics: gene diversity, the total number of alleles across loci, and the number of unique alleles. Unbiased estimates of gene diversity were obtained using the standard method (Nei 1978). The number of alleles expected in a sample varies with sample size, and the sample sizes from anadromous populations of Maine rivers varied from 64 to 644. Therefore, rarefaction (Hurlbert 1971) was used to estimate the number of alleles that might have been found if all samples had equal sizes of 50 individuals. The number of unique alleles present in each sample was estimated by randomly selecting 50 individuals from each sample without replacement. This was repeated 10,000 times and the average taken.

Observed genotype frequencies were tested for consistency with Hardy–Weinberg expectations using randomization tests implemented by GENEPOP 3.1 (Raymond and Rousset 1995). The Hardy–

TABLE 2.—Location, life stage (A = adult, F = fry, P = parr, and S = smolt), year-class, and sample size of 3,308 Atlantic salmon from Maine and 555 from Canada assayed for variation at 11 microsatellite loci. Asterisks indicate collections from distinct population segment rivers as designated under the U.S. Endangered Species Act.

Collection location	Life stage	Sample size	Year-class(es) sampled
Maine			
Bond Brook (Kennebec tributary)	P	108	1993–1995
Togus Stream (Kennebec tributary)	P	81	1993–1995
*Sheepscot River	P	271	1993–1995, 1997
*Ducktrap River	P	123	1993–1995, 1998
Penobscot River main stem	A	580	1995–1997
*Cove Brook (Penobscot tributary)	P	102	1993–1995, 1998
Kenduskeag Stream (Penobscot tributary)	P	93	1996–1997
*Narraguagus River	P	644	1993–1995, 1997
*Pleasant River	P	64	1995, 1996
*Machias River	P	573	1993–1995, 1997
*East Machias River	P	324	1993–1995, 1997
*Dennys River	P	253	1992–1994, 1997
Sebago Lake (landlocked strain)	A	50	1992–1993, 1996
Grand Lake (landlocked strain)	A	42	1992–1993, 1996
New Brunswick			
Dennis Creek (St. Croix tributary)	P	63	1995
Nashwaak River (St. John tributary)	A	66	1992–1993
Nova Scotia			
Miramichi River	A	56	1991–1992
Stewiacke River	FA	56	1984, 1993
Gold River	FP	54	1993–1994
Quebec			
St. Jean River	A	63	1996
Saguenay River	S	59	1997
Newfoundland			
Conne River	P	30	1993
Gander River	A	63	1992
Labrador			
Michaels River	P	29	1995–1996
Sand Hill River	P	16	1995–1996

Weinberg test used the Markov chain randomization test of Guo and Thompson (1992) to estimate exact two-tailed *P*-values for each locus in each sample. Global tests combined these results over loci and sampling locations using Fisher's method (Sokal and Rohlf 1994). Sequential Bonferroni adjustments (Rice 1989) were used to determine statistical significance for these and all other tests at the overall α-level of 0.05. The amount of genetic variation in each sample was summarized by gene diversity (average expected heterozygosity) and the number of unique alleles expected. The mean relatedness of each sample was estimated using the program Relatedness 5.08 (Queller and Goodnight 1989; available online at http://gsoft.smu.edu/GSoft.html).

Several techniques were used to describe the genetic relationships among samples. Allele frequency heterogeneity was tested both globally and pairwise between collections for each locus using the genic differentiation randomization test in GENEPOP (Raymond and Rousset 1995). Nei's genetic distance (D_A) was estimated between each pair of populations (Nei et al. 1983). Multidimensional scaling plots (MDSs) were constructed from D_A genetic distances using Systat 10 (SSPS 2000). A linear Kruskal loss function was used with $1 - D_A$ as a measure of the similarity between samples. MDSs were chosen to provide a perspective on the genetic distance matrix without imposing a bifurcating evolutionary history. An unrooted phylogenetic tree was fitted to the data using the D_A distance matrix and neighbor-joining algorithm. TreeView (Page 1996) was used to visualize the tree. The strength of support for each node in the tree was tested by bootstrapping (10,000 replicates) over 11-locus genotypes using NJBPOP (J.-M. Cornuet, Institut National de la Recherche Agronomique, unpublished data). Isolation by distance (examined both across North America and

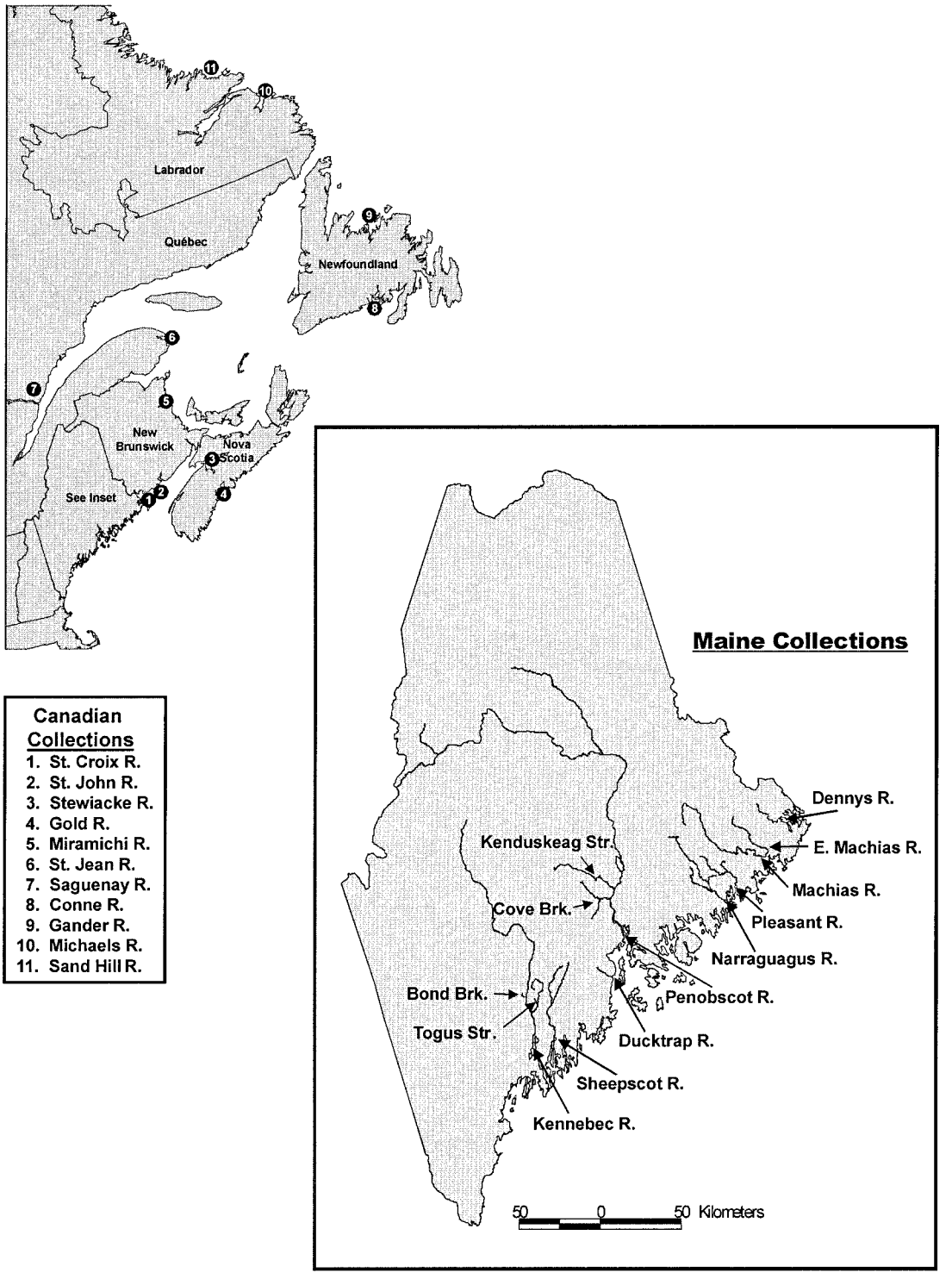

FIGURE 1.—Locations of the water bodies sampled for anadromous Atlantic salmon in Maine (excluding Cove Brook) and Canada. Abbreviations are as follows: Brk. = Brook, Str. = Stream, and R. = River.

within Maine using the Mantel test [Mantel 1967]) was implemented by the MXCOMP routine of NTSYS (Rohlf 2000) to assess correlation between matrices of D_A genetic distance and geographic distance excluding landlocked populations.

Analysis of molecular variance (AMOVA) was used to partition the genetic variation into a hierarchy of groups of populations (Excoffier et al. 1992). The software program Arlequin 2.001 was used to test the statistical significance of the differentiation in Maine both within rivers across year-classes and across rivers within year-classes, as well as to generate tests of Wright's F_{ST} value (a measure of genetic differentiation) over all populations and between each pair of populations (Schneider et al. 2000). Locus-by-locus AMOVA was used to indicate which loci contribute the most information to tests of population structure.

The effective number of breeders (N_b) present in each river was estimated from the standardized variance of allele frequency change, F, using a modification of the method of Waples (1990). The standardized variance of each allele between two samples, $\hat{F}_{a(i,j)}$, was estimated by means of the equation

$$\hat{F}_{a(i,j)} = \frac{(p_i - p_j)^2}{(p_i + p_j)/2}$$

where i and j represent years, and p indicates the frequency of the allele. The standardized variance for each locus, $\hat{F}_{l(i,j)}$, was estimated by combining the estimates for each allele at the locus, that is,

$$\hat{F}_{l(i,j)} = \frac{1}{k-1}\sum_{i=1}^{k} F_{a(i,j)}$$

where k indicates the number of alleles at the locus. The standardized variance across all loci was obtained by taking the weighted (by number of alleles) average across loci

$$\hat{F}_{i,j} = \frac{\sum_{i=1}^{L} F_{l(i,j)}(k_i - 1)}{\sum_{i=1}^{L} (k_i - 1)}$$

where L indicates the number of loci examined and k_i the number of alleles at the ith locus. An estimate of the annual effective number of breeders between year i and year j was obtained from

$$\hat{N}_b = \frac{b}{2(\hat{F}_{i,j} - 1/S)}$$

where b is a measure of the number of generations separating the two sampling years and S is the number of individuals sampled each year. The value of b was calculated from the number of years between the two samples and the distribution of spawning ages (Tajima 1992). Based on a general average of returnees across Maine rivers, we assumed that 10% of individuals spawned at age 1 (i.e., precocious parr), 10% at age 3 (i.e., grilse), 70% at age 4, and 10% at age 5 and that there were no repeat spawners,. The value of N_b was estimated between each pair of years and then averaged.

Assignment tests were used to determine the likelihood of each individual's genotype being found in the collection from which it was sampled (without replacement) using the program GeneClass (Cornuet et al. 1999). The likelihood of a particular multilocus genotype being drawn from a collection was calculated using the Bayesian method, with the prior assumption being an equal probability for allele frequencies at each locus in each population. This approach requires no other assumptions about allele frequencies or equilibrium and had the greatest assignment accuracy in simulations of loci evolving under the infinite-alleles model (Cornuet et al. 1999). In addition to the assignment to river of origin, we assessed the assignment to nation of origin, to the federally defined endangered DPS, and to the DPS plus the Penobscot River population (which has been used to restock many of the DPS rivers [Baum 1997]).

Results

Diversity

The gene diversity of Atlantic salmon collected in Maine rivers ranged from 0.54 to 0.60 (Table 3), with an average value of 0.57. (Overall allele frequencies are available from the corresponding author upon request.) Fish from Bond Brook in the Kennebec River drainage and Cove Brook in the Penobscot River drainage had the lowest diversity, followed by fish in the Dennys River. Atlantic salmon from Kenduskeag Stream, the Ducktrap River, and the Sheepscot River had the highest gene diversities. The number of alleles in samples of 50 individuals varied from a low of 60 in Cove Brook to a high of 88 in the Dennys River. There was no correlation between the gene diversity present in samples and the number of alleles (Mantel test, $P = 0.12$). The number of unique alleles present in each location varied from one or less (Bond Brook, Togus Stream, Machias River, and Penob-

TABLE 3.—The gene diversity (average expected heterozygosity, H), total number of alleles expected in a sample of 50 individuals (N_a), number of unique alleles in a sample of 50 individuals (N_u), estimated effective number of breeders (N_b), and mean relatedness of individuals (R) for each of the anadromous Atlantic salmon populations sampled in Maine.

Location	H	N_a	N_u	N_b	R
Bond Brook (Kennebec tributary)	0.54	76	0.6	61	0.0272
Togus Stream (Kennebec tributary)	0.56	72	1.0	13	0.0505
Sheepscot River	0.58	85	3.2	37	0.0114
Ducktrap River	0.60	82	1.4	33	0.0089
Penobscot River main stem	0.57	81	0.8	426	0.0193
Cove Brook (Penobscot tributary)	0.54	60	3.1	11	0.0301
Kenduskeag stream (Penobscot tributary)	0.60	81	1.4	27	0.0144
Narraguagus River	0.57	84	1.3	167	0.0096
Pleasant River	0.56	85	1.1	154	0.0101
Machias River	0.56	84	0.8	177	0.0045
East Machias River	0.57	87	1.9	94	0.0051
Dennys River	0.55	88	4.9	99	0.0022

scot River) to 4.9 (Dennys River). Cove Brook had approximately three unique alleles, even though it has a relatively low amount of genetic variation and the lowest estimated effective number of breeders (see below). There was no association between the total number of alleles present in populations and the number of unique alleles. Hardy–Weinberg disequilibrium was detected in 5 of 352 locus by population tests after correction for multiple simultaneous tests. The Sheepscot River collection was the only one with disequilibrium at two loci (*Ssa171* and *Ssa202*). Other populations had disequilibrium at only a single locus (*Ssa197* [Togus Stream] and *Ssa202* [Machias and Stewiacke rivers]). The disequilibria were all detected at loci with the greatest number of alleles (25–40 per locus) and are not regarded as compromising the data. The pairwise and global estimates of F_{ST} were significantly different from zero at every level except in the comparison of the two Labrador populations (global $F_{ST} = 0.0352$; F_{ST} within Maine $= 0.020$).

Effective Number of Breeders

Estimates of the annual number of effective breeders ranged from a low of 11 for Cove Brook salmon to a high of 426 for the main-stem Penobscot population (Table 3). Five out of 12 of the populations were estimated to have an effective number of breeders less than 50. Estimates of N_b were sensitive to assumptions regarding the age at which fish reproduced. The proportion of parentage attributable to precocious parr had a strong effect upon the estimate of N_b (see Discussion). Correlations between estimates of the effective number of breeders and indices of genetic variation were weak. Mantel tests showed that the estimates of N_b were not correlated with those of gene diversity or the total number of alleles. That the N_b estimates (Table 3) exceeded the counts of spawning adults (Table 1) indicates that there is low variance in allele frequencies among year-classes. Low levels of relatedness (Table 3) also indicate low variance in allele frequencies across the samples from each river.

Analysis of Molecular Variance within Maine

When anadromous salmon from Maine were grouped by river across year-classes, statistically significant genetic variation was found at every level of analysis (among rivers, among samples, and within samples; $P < 0.00001$). The greatest proportion of genetic variation was found within individual samples (Table 4), as might be expected from a suite of highly polymorphic loci. The AMOVA results indicated that there was significant variation among year-classes within rivers but greater variation among rivers across year-classes (Table 4). This result supported pooling samples across year-classes within rivers for subsequent analysis. Locus-by-locus AMOVA indicated significant structure for all loci at all levels with the exception of *SSOSL311*, which lacked significant structure at the among-rivers level (Table 5).

Genetic Distance

Among the anadromous Atlantic salmon that we sampled in Maine, the greatest pairwise genetic

TABLE 4.—Analysis of molecular variance in anadromous Maine Atlantic salmon grouped by river of collection across year-classes. Asterisks indicate values that are significantly greater than 0 at $\alpha = 0.00001$.

Variation	df	Sum of squares	Variance component	Percentage of variation
Among rivers	11	315.840	0.04049*	1.42
Among samples within rivers	29	205.867	0.02984*	1.04
Within samples	6,413	17,890.048	2.78965*	97.54
Total	6,453	18,411.755	2.85998	100.00

distances were between (1) the populations in the two Penobscot tributaries, Cove Brook and Kenduskeag Stream, and other populations and (2) the populations in the two Kennebec River tributaries, Bond Brook and Togus Stream, and other populations (the complete pairwise distance matrix is available from the corresponding author). The river (not tributary) with the greatest pairwise distance from other rivers was the Ducktrap, which, like the Penobscot tributaries, has little history of stocking. Among Maine anadromous populations, the main-stem Penobscot, Narraguagus, and Machias River samples are the most similar (pairwise distances < 0.015). Small populations (based on N_b estimates) are more differentiated from the Penobscot River population than large populations (Mantel test, N_b estimate versus D_A between Penobscot River and each sample; $P = 0.006$).

Among Canadian samples, the closest are from the St. John and Miramichi rivers, the most distant from the St. Jean and Conne rivers (0.0532 and 0.1794, respectively). The closest Canadian and Maine samples are from the Miramichi and Dennys rivers (pairwise distance = 0.0739). Isolation by distance was not detected using a Mantel test to compare the matrices of genetic and geographic distance ($P = 0.998$ for all North American populations, 0.1752 for all Maine populations, and 0.7738 with a post hoc subtraction of the Cove Brook genetic distance outlier from other Maine collections).

Multidimensional scaling analyses showed that Maine populations were substantially distinct from the Canadian populations (Figure 2). The landlocked samples were differentiated from all of the anadromous populations. The differentiation between the Maine and Canadian populations was approximately equal to that between the populations in different provinces of Canada.

A neighbor-joining tree of D_A distance among all samples further clarifies the general structure of North American Atlantic salmon (Figure 3). Two clusters of Canadian populations correspond to (1) Labrador and Newfoundland and (2) New Brunswick, Nova Scotia, and Quebec, respectively. The Gold River population from Nova Scotia is distinct from the other lower Atlantic province populations. The Atlantic salmon of Cove Brook, Maine, cluster nearer to those from Newfoundland and Labrador than to any other populations. Within Maine, Cove Brook and the landlocked hatchery strain are quite distinct, while populations sampled

TABLE 5.—Locus-by-locus analysis of molecular variance in anadromous Maine Atlantic salmon. The percentages of variation for each locus among rivers, among samples within rivers, and within samples are given, along with the appropriate fixation indices (F_{CT}, F_{SC}, and F_{ST}, respectively). All indices are significantly different from zero except F_{CT} for *SSOSL311*.

	Among rivers		Among samples within rivers		Within samples	
Locus	Variation	F_{CT}	Variation	F_{SC}	Variation	F_{ST}
Ssa14	4.29483	0.04295	1.26155	0.001318	94.44362	0.05556
Ssa85	1.93120	0.01931	0.57713	0.00588	97.49167	0.02508
Ssa171	1.17308	0.01173	1.04623	0.01059	97.78069	0.02219
Ssa197	1.24752	0.01248	1.33760	0.01355	97.41487	0.02585
Ssa202	1.13831	0.01138	0.99112	0.01003	97.87057	0.02129
Ssa289	0.62287	0.00623	0.72266	0.00727	98.65446	0.01346
SSLEEN82	1.22935	0.01229	0.82189	0.00832	97.94876	0.02051
SSOSL25	1.25961	0.01260	1.16080	0.01176	97.57959	0.02420
SSOSL85	1.67295	0.01673	1.37080	0.01394	96.95625	0.03044
SSOSL311	0.74165	0.00742	1.84859	0.01862	97.40976	0.02590
SSOSL438	3.20099	0.03201	1.53734	0.01588	95.26166	0.04738

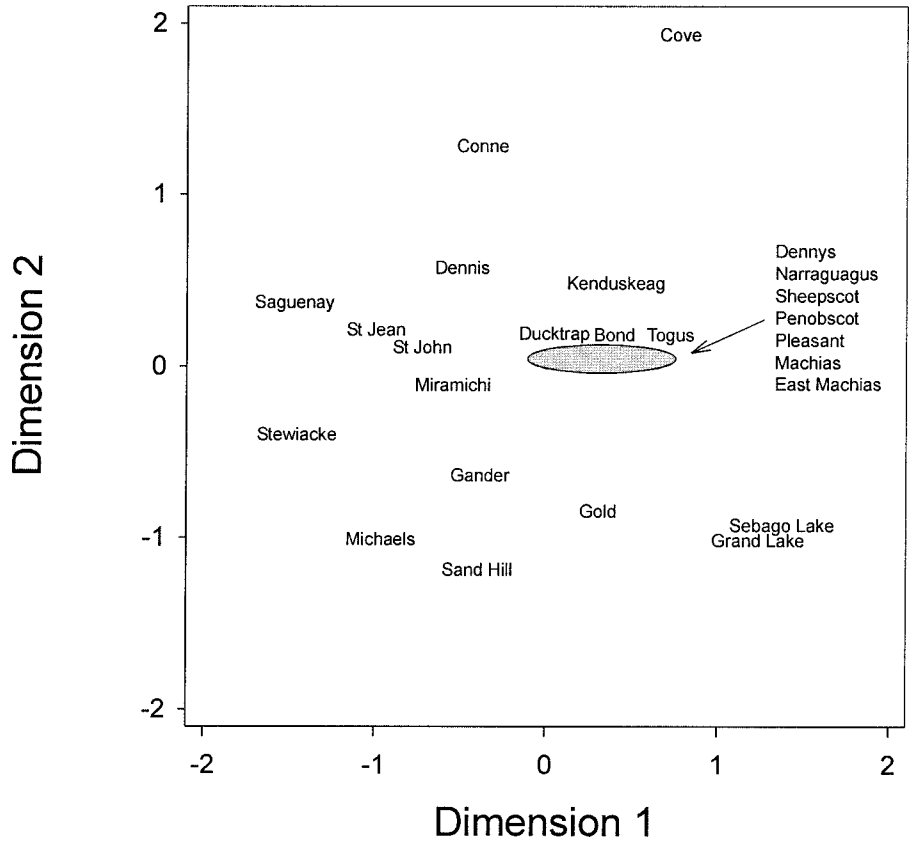

FIGURE 2.—Multidimensional scaling diagram comparing Atlantic salmon populations within North America. A linear Kruskal loss function was used with 1—Nei's genetic distance (D_A; Nei et al. 1983) as a measure of the similarity between samples.

from the remaining rivers are relatively similar in terms of D_A distance. In general, the clusters of Canadian populations were as distant from each other as each was from the cluster of Maine populations. The landlocked strains from Maine appeared to have a gene pool independent of that in Maine's anadromous populations yet closer to those fish than to any Canadian population.

Assignment Tests

Individuals were assigned to the collection (river) of origin much more frequently than would be expected by chance alone (χ^2 test, df = 24, $P < 0.05$ for each river after sequential Bonferroni adjustment [Rice 1989] for 25 simultaneous tests). Within Maine, the highest percentage of correct assignment to the collection of origin was for Cove Brook, and the lowest was for the Narraguagus River (Table 6); the latter result could reflect the contribution of Narraguagus broodstock to the present Penobscot strain as well as the release of hatchery-reared Narraguagus fish into many other Maine rivers. The mean rate of correct assignment to the collection of origin was 52.84% in Maine, somewhat lower than that in Canada (60.04%). This difference reflects the greater geographic proximity of samples taken from Maine. The rate of assignment back to country of origin was similar for each country (87.99% for Canada and 92.18% for the United States; Table 6). Within Maine, mean assignment rates to the federally designated DPS were similar to mean correct assignments to river of origin within the state (49.88% versus 52.84%). The higher rates of assignment to the DPS plus the Penobscot River (Table 6) for many collections (Sheepscot, Narraguagus, Machias, East Machias, and main-stem Penobscot rivers) indicates the similarity of the Penobscot River samples to those collected from the DPS.

Discussion

Multidimensional scaling of genetic distance shows tight clustering within the federally desig-

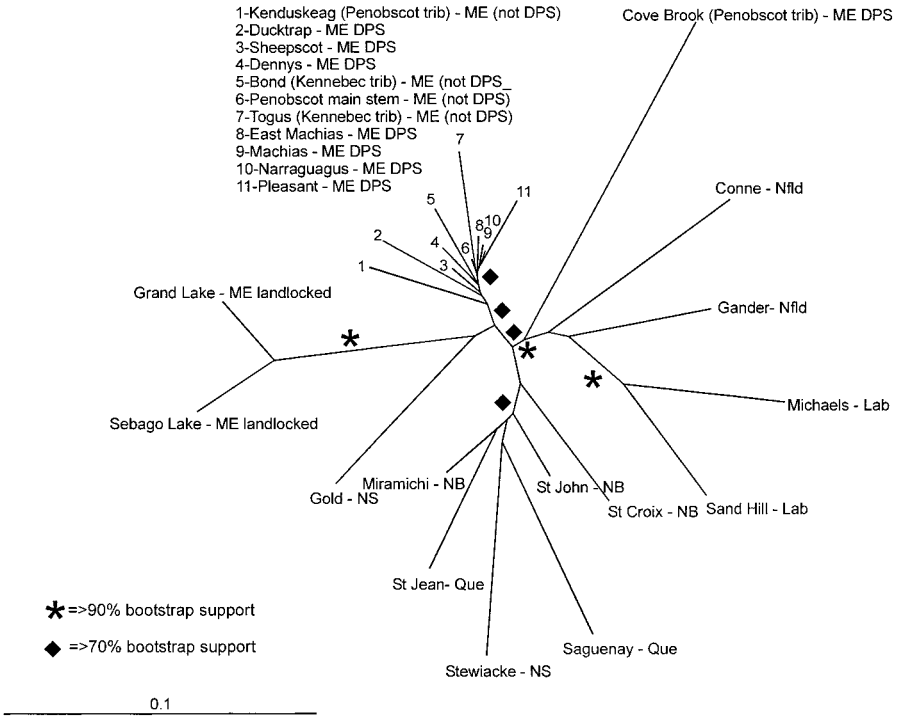

FIGURE 3.—Neighbor-joining phenogram depicting D_A among all Atlantic salmon populations when pooled across year-classes. A node immediately counterclockwise from an asterisk has at least 90% bootstrap support, a node immediately clockwise from a diamond at least 70% bootstrap support (5,000 replications in each case). Abbreviations are as follows: DPS = distinct population segment, trib = tributary, Lab = Labrador, ME = Maine, NB = New Brunswick, Nfld = Newfoundland, NS = Nova Scotia, and Que = Quebec.

nated distinct population segment of Maine (with the exception of Cove Brook) and within Maine in general, suggesting a close relationship between the fish in the Kennebec and Penobscot rivers and those in the DPS. Cove Brook and the landlocked samples were shown to be distinct from Maine's anadromous populations. The landlocked strains sampled are not known to have been supplemented (K. Warner, Maine Department of Inland Fisheries and Wildlife, personal communication). That the anadromous Maine fish cluster with landlocked Maine fish in a neighbor-joining tree of genetic distance (Figure 3) rather than with anadromous Canadian fish suggests a common ancestry within Maine due to postglacial recolonization. The effective size of the DPS and other populations within Maine, which was estimated from the temporal variation in allele frequencies, is not as small as it might have been if estimated from census numbers within the region (Table 1). All Maine populations are distinct from all Canadian populations. Finally, multilocus assignment tests both give high precision in discriminating country of origin and reflect relatively greater homogeneity among the DPS rivers (and the main-stem Penobscot) than among other sets of rivers (Table 6). The assignment tests do not show as much similarity between the Atlantic salmon in the Kennebec tributaries and other anadromous salmon from Maine as do the analyses of genetic distance.

The tree of genetic distance values indicates two primary population clusters among anadromous Maine populations. One centers around the Machias and Narraguagus rivers, which are source populations for much of the Penobscot River restoration, and the Penobscot itself, which is a source for repopulating many other rivers in Maine. The second cluster, comprising populations in Kenduskeag Stream, the Ducktrap and Sheepscot rivers to the southwest of the Penobscot River, the Dennys River to the northeast of the Penobscot, and the Bond Brook tributary of the Kennebec River, is more disjunct. Outside these two primary clusters is the Penobscot tributary of Cove Brook. Multidimensional scaling indicates a focal group of populations in the Penobscot, Machias, East

TABLE 6.—Summary of maximum likelihood tests for assigning individual fish to their collection of origin. The last four columns show the percentages of fish from each river that were correctly assigned to their river and nation of origin (United States or Canada), distinct population segment (DPS) rivers (the Sheepscot, Ducktrap, Narraguagus, Pleasant, Machias, East Machias, and Dennys rivers, plus Cove Brook) as defined under the U.S. Endangered Species Act, and the DPS plus the Penobscot River.

		Correct assignment (%)			
Location and statistic	N	To river	To nation	To DPS	To DPS and Penobscot
Sheepscot River	271	30.26	88.56	58.30	71.96
Ducktrap River	123	56.91	90.24	78.86	79.67
Cove Brook	102	96.08	98.04	97.06	97.06
Narraguagus River	644	25.16	94.10	71.74	80.28
Pleasant River	63	46.03	85.71	76.19	77.78
Machias River	573	33.33	94.42	72.08	81.15
East Machias River	324	38.89	90.43	67.28	79.32
Dennys River	263	42.97	88.21	68.44	73.38
Bond Brook	108	60.19	90.74	24.07	29.63
Togus stream	81	65.43	90.12	16.05	23.46
Penobscot River main stem	580	31.55	96.03	44.66	76.21
Kenduskeag stream	93	65.59	90.32	17.20	21.51
Sebago Lake[a]	50	76.00	96.00	4.00	4.00
Grand Lake[a]	42	71.43	97.62	2.38	2.38
St. Croix River	55	72.73	89.09	9.09	9.09
St. John River	65	36.92	78.46	16.92	16.92
Stewiacke River	56	76.79	92.86	1.79	1.79
Gold River	53	79.63	90.74	5.56	5.56
Miramichi River	56	35.71	83.93	10.71	10.71
St. Jean River	63	58.73	93.65	6.35	6.35
Saguenay River	59	79.66	100.00	0.00	0.00
Conne River	30	70.00	90.00	6.67	6.67
Gander River	63	66.67	87.30	11.11	12.70
Michaels River	29	58.62	93.10	3.45	3.45
Sand Hill River	16	25.00	68.75	25.00	25.00
Overall mean assignment (%)		56.01	90.34	31.80	35.84
Mean assignment for samples from Maine (%)		52.84	92.18	49.88	56.98
Mean assignment for samples from Canada (%)		60.04	87.99	8.79	8.93

[a] Landlocked strain.

Machias, and Narraguagus rivers, with other Maine rivers being associated more loosely (Figures 2 and 3). Considering the Penobscot's virtual extirpation and subsequent repopulation with fish from the Machias and Narraguagus rivers and the use of the reconstituted Penobscot population to supplement other Maine rivers, the structure suggested by multidimensional scaling concords with expectations.

Of the sites in the second cluster, Cove Brook has never been stocked, Kenduskeag Stream has been stocked only occasionally (Spidle et al. 2001), and the Ducktrap River received over 80,000 Penobscot strain fry over the period from 1985 to 1990 (Baum 1997; USASAC 2001). It is uniformly accepted that the Kennebec River has lost all of its native Atlantic salmon due to habitat degradation and that those now found in Kennebec River tributaries are the result of the straying of fish produced in other rivers. The Penobscot River was stocked largely with Machias and Narraguagus River fish from the late 1960s to the early 1970s (Baum 1997, summarized in Spidle et al. 2001). The Penobscot River strain has since been used to stock most Maine rivers throughout. Although the Penobscot River was heavily stocked with fish of Canadian origin (particularly from the Miramichi and Saguenay rivers) through the 1960s, the neighbor-joining tree and multidimensional scaling views of genetic distance suggest that these fish have left little to no legacy. That populations in bodies of water with little to no stocking history (Ducktrap River and Kenduskeag Stream) cluster loosely with those that have been used to repopulate each other over the previous 30 years (Penobscot, Machias, and Narraguagus) as well as with landlocked populations that have not been stocked from outside Maine (Sebago and Grand Lake strains) could indicate a common origin for all of the Maine populations examined.

The degree of population structure observed (multilocus F_{ST} = 0.020 over 11 loci within

Maine) is around the median reported from salmonid microsatellite data and is surprising in light of the hatchery supplementation that the Down East rivers have received. Olsen et al. (1998) found similar differentiation over much of the West Coast range of pink salmon *Oncorhynchus gorbuscha* (θ, an analog of F_{ST} = 0.023 over 4 loci). The population structure in Maine is intermediate to those of Newfoundland's Conne River (F_{ST} = 0.011 over four loci; Beacham and Dempson 1998) and Quebec's Ste. Marguerite River (F_{ST} = 0.034 over 5 loci; Garant et al. 2000) and less than has been observed in two putative metapopulations in Quebec (Gaspé Peninsula: Φ_{ST}, an analog of F_{ST} used in mtDNA analysis = 0.05; north shore of the St. Lawrence River: Φ_{ST} = 0.104; all values over 5 loci; Fontaine et al. 1997).

Management and Conservation Implications

The effective population size estimates of the parr used to establish the broodstock for the river-specific strains are greater, in relation to the actual number of breeders, than the estimates for much larger hatchery strains of salmonids on the West Coast (Simon et al. 1986; Waples 1990; Bartley et al. 1992; Hedrick et al. 1995). To the extent that the estimates are reliable, they do not mandate pooling the river-specific strains of Atlantic salmon maintained at Craig Brook National Fish Hatchery (East Orland, Maine). This follows from the fact that while drift may be somewhat accelerated in each individual subpopulation, global drift is not likely to result in the complete loss of alleles, as would happen if the fish were forced into panmixia. There seems to be time to analytically determine a desirable rate of gene flow among the six river-specific strains from the DPS and the Penobscot River. A program of gene flow among strains can be formulated if evidence of inbreeding depression is found in particular strains.

Our estimates of the effective number of breeders in the DPS rivers are based on three assumptions that require examination. First, the model we used assumes that the annual number of effective breeders in each population is constant. If a population is growing, the current number of effective breeders in it will probably be larger than our estimate. If the population is declining, the current population will probably be smaller than our estimate. Of these two scenarios, the latter is more serious. Fortunately, simulations have shown that estimates of effective population size quickly reflect actual population size for declining populations (Waples 1990). In contrast, estimates of effective population size respond much more slowly to increases in population size. The second assumption that we made was that the age distribution of spawners was as follows: 10% at age 1, 10% at age 3, 70% at age 4, and 10% at age 5, which one can represent as [0.1, 0.0, 0.1, 0.7, 0.1]. Different proportions produce substantially different estimates of the effective number of breeders. For example, reassigning 10% of the reproduction from age-4 reproduction to another year-class that is already reproducing (i.e., age 1, 3, or 5) would decrease the estimate by about 20%. If we had assumed that precocious parr do not mate successfully, our estimates of the effective number of breeders would have increased significantly. For example, assuming the distribution [0.0, 0.0, 0.1, 0.8, 0.1] would raise the estimate of the effective number of breeders by approximately 40%. The third assumption was that the parr we collected in rivers represented a random sample. In particular, if the assumption of a uniform age among age-classes were incorrect and the parr collected in consecutive years were full or half-siblings (which would mean less genetic variation between samples), the collections would look more similar than if the individuals were sampled completely at random, thus inflating estimates of N_b. Both the overall Hardy–Weinberg equilibrium and the low levels of relatedness estimated across samples (Table 3) support this assumption. Finally, the N_b estimate incorporates all reproduction in the system, including the hatchery, and the ratio of N_b to returning adults may not do so. Resolving this issue and reconciling the estimates of the number of breeders from census surveys is an important research priority.

Our data indicate that the Atlantic salmon listed as a distinct population segment (DPS) under the U.S. Endangered Species Act comprise a discrete, independent gene pool within North America. The similarity of Atlantic salmon from Penobscot and Kennebec tributaries to those from the DPS rivers and the similarity between Maine's landlocked and anadromous fish and collective differentiation from Canadian fish reinforce the independence of Maine's Atlantic salmon gene pool. While the Atlantic salmon in Maine rivers may constitute a limited number of metapopulations, our data support the independence of these populations from other populations in North America, an observation that is concordant with an earlier survey of genetic variation across the range of Atlantic salmon (King et al. 2001). Among Maine's anadromous salmon populations, genetic distance analysis indicates a

separation of the populations of Cove Brook, Kenduskeag Stream, and the Ducktrap, Sheepscot, and Dennys rivers from each other and those of the Penobscot, Pleasant, East Machias, Machias, and Narraguagus rivers.

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