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Genetic analysis of fish genomes and populations: allozyme variation within and among Atlantic salmon from Downeast rivers of Maine

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Analysis of genetic variation within and among samples of naturally produced Atlantic salmon ($n = 372$) from 7 Maine (U.S.A.) and one Canadian river were conducted based on 54 allozyme loci. Eight of the 54 loci proved polymorphic, and estimated heterozygosities (H_S) based on all loci ranged from 0·012 to 0·026 (mean = 0·021, S.E. = 0·002). Only one of 56 tests revealed genotypic proportions that deviated significantly from Hardy–Weinberg expectations. Genetic distances (D) between samples ranged from 0·002 to 0·022. No obvious association existed between genetic and geographic distances. Cluster analysis of genetic distances revealed the Dennys River sample as the most differentiated when all samples were included in the analysis, though bootstrap support of the cluster analysis was generally weak. G-tests revealed significant differences in allele frequencies among samples at five of the polymorphic loci, and the G-value summed over all loci also indicated significant differences among samples. F_{ST} values indicated that 3·4% of the total genetic diversity was due to variability among samples, while 96·6% was due to variability within samples. These results indicate that the Atlantic salmon analyzed in this study had levels of genetic variability and differentiation among samples comparable to native populations from other areas collected across a similar geographic range.

Key words: allozymes; genetic variation; northeastern U.S.; population genetics; salmonidae; *Salmo salar*.

INTRODUCTION

Rivers along the eastern U.S. coast of Maine once supported large numbers of anadromous Atlantic salmon *Salmo salar* L. Presumably, these fish were substructured into multiple genetically differentiated populations both within and among river systems, as is the case for Atlantic salmon in other parts of their range (Ståhl, 1987). This genetic organization reflects the general tendency of

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salmonids to 'evolve genetically discreet, ecologically specialized populations by natural selection over thousands of generations of adaptations to local environmental conditions' (Allendorf & Ryman, 1987). Unfortunately, loss of access to spawning areas, degradation of freshwater habitat, and over-harvesting have severely reduced the number of salmon and in some cases caused the extirpation of native stocks (*e.g.*, Penobscot River; Saunders, 1981). To help restore the stocks, hatchery-reared salmon from a variety of sources have been planted in the rivers of Maine for the past several decades.

Recently, Atlantic salmon populations in six of the Maine Rivers (Sheepscot, Ducktrap, Narraguagus, Pleasant, Machias, and Dennys) sampled in this study have been added to the endangered species list in order to protect any remaining unique native stocks [United States Fisheries and Wildlife Service (USFWS), 2000]. To determine whether such action might be appropriate, information is needed to assess whether or not the original genetic diversity and integrity of the native stocks has been compromised by the extensive hatchery plantings that have occurred. The objective of this study was to genetically characterize naturally produced Atlantic salmon from six rivers in Maine, as well as artificially propagated offspring of adult returns from one river in Canada (an 'outgroup') and the Penobscot River in Maine. Fish from the Canadian river and the heavily stocked Penobscot Rivers were included to compare Canadian and Maine levels of genetic variation and assess whether genetic homogenization in stocked rivers has occurred. This characterization would serve to: 1) establish a current baseline of genetic variability for allozymes within these rivers, 2) determine the levels of genetic variability and differentiation within and among these rivers, and 3) provide an independent assessment of these levels for comparison with data derived from other molecular markers such as mitochondrial DNA (King *et al.*, 2000) and microsatellites (King *et al.*, 2001; Spidle *et al.*, 2001).

MATERIALS AND METHODS

ALLOZYME PROCEDURES

Naturally produced Atlantic salmon parr from six rivers in Maine (the Sheepscot, Ducktrap, Narraguagus, Pleasant, Machias, and Dennys), and artificially propagated offspring from adult returns to the Penobscot River (Green Lake National Fish Hatchery) and the St. John's River in New Brunswick (Fig. 1) were shipped on dry ice to the Cornell Genome Variation Analysis Facility and then stored at -80° C until analyzed. Muscle, liver, kidney, and eye tissues were subjected to horizontal starch-gel electrophoresis and histochemical staining following the procedures of May (1992). Initially eight individuals each from the Penobscot strain and four river systems (Dennys, Machias, Narraguagus, and Sheepscot) were examined for enzyme expression and allelic variation for 38 enzymes. Buffer, tissue, and enzyme combinations were chosen based on previous experience with other salmonids. The remaining individuals from each sample were examined for genetic variation at the polymorphic loci from the initial screen as well as various loci reported to be polymorphic in other Atlantic salmon populations (T.F. Cross & R.H. Payne, unpubl. data; Roberts, 1976; Cross & Payne, 1977; Cross *et al.*, 1979; Child, 1980; Ståhl, 1981; Vuorinen, 1982; Ryman, 1983; Ståhl, 1983; Verspoor & Cole, 1988; Davidson *et al.*, 1989; Koljonen, 1989; Jordan *et al.*, 1990; Verspoor *et al.*, 1991). Enzyme nomenclature followed guidelines suggested by Shaklee *et al.* (1990). At the regulatory locus *PGM-1r**, only the homozygote phenotype for the

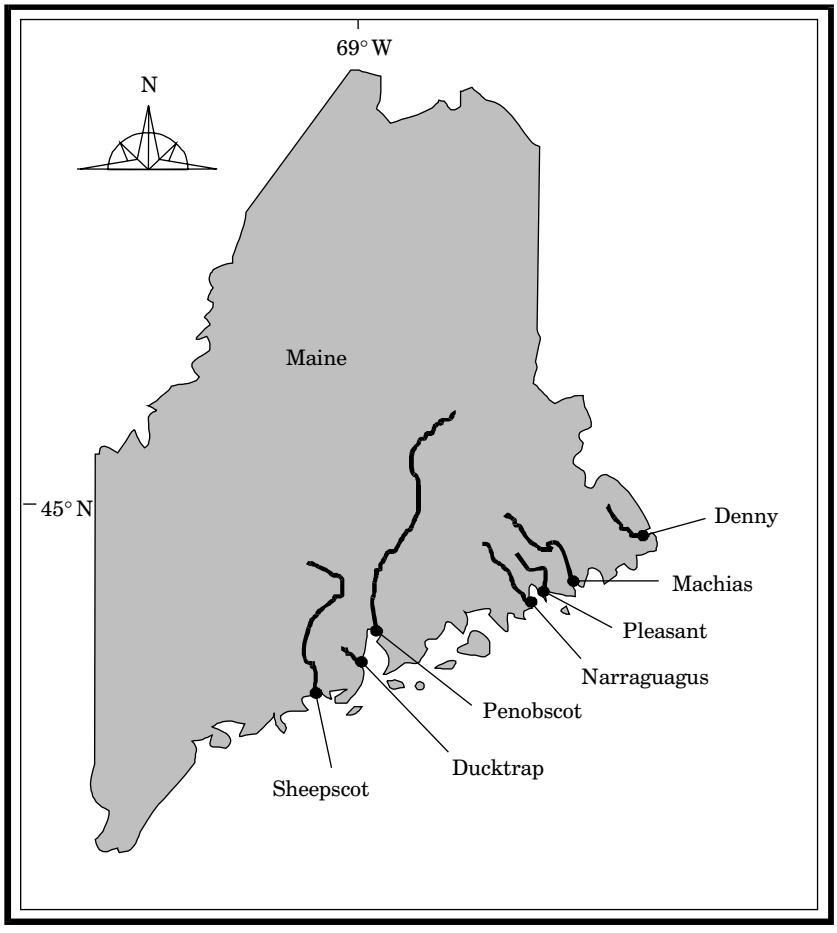

FIG. 1. Locations of rivers in Maine providing samples of naturally produced Atlantic salmon for allozyme analysis (fish representing the Penobscot strain were derived from adults returning to the Penobscot River).

lack of *PGM-1r** expression can be reliably distinguished (Johnson, 1984); therefore, allele frequencies at this locus were calculated from the square root of the frequency of the identifiable homozygote.

STATISTICAL PROCEDURES

Analyses of the data were performed using 'Genes in Populations,' a microcomputer program (May *et al.*, 1995). Conformance of allelic frequencies to Hardy–Weinberg expectations within samples was assessed by log-likelihood *G*-tests (Sokal & Rohlf, 1981). Genetic differences among samples were assessed with heterozygosities, *G*-tests, and genetic distance coefficients (*D*). Observed and expected heterozygosities and their variance estimates were calculated as described by Nei & Roychoudhury (1974) and Nei (1973). Hereafter, mean expected heterozygosity per locus will be referred to simply as heterozygosity. Allele counts by locus were compared statistically by contingency table analysis with *G*-tests (Sokal & Rohlf, 1981). The critical values used to reject the null hypothesis for the *G*-tests were increased (based on Sidak's multiplicative inequality) to

account for the increase in type-I error when multiple tests of the same hypothesis were made (Cooper, 1968). *G*-values were calculated between all possible pairs. Genetic distances (Nei, 1972) were calculated using data from seven polymorphic loci (frequency of alternate allele $>0{\cdot}05$) and subjected to unweighted pair-group method cluster analysis (UPGMA) (Sneath & Sokal, 1973). The level of differentiation among samples was measured with F_{ST} (Wright, 1965; equivalent to G_{ST} of Nei, 1977).

Genetic distances were plotted as an unweighted pair group method with arithmetic means (UPGMA; Sneath & Sokal, 1973) dendrogram using Phylip 3.5 c (Felsenstein, 1993). The original allele frequency matrix was then resampled 1000 times by bootstrapping and the genetic distances between samples were estimated for each resulting matrix. A consensus UPGMA diagram was generated and all bootstrap values above 50% were plotted on to the dendrogram from the original sample matrix to indicate stability of the nodes.

RESULTS

GENETIC VARIATION WITHIN SAMPLES

Eight (14·8%) of the 54 loci that were adequately resolved (Table I) were classified as polymorphic (frequency of alternative allele >5%, Table II; the polymorphism at *FBALD-2** has not previously been described); however, four additional loci (*sIDHP-1**, *sIDHP-2**, *LDH-B1**, and *PEPA**) had alternative alleles with a frequency less than 5% in at least one sample. All variation at the isoloci *sMDH-B1, 2** was assigned to *sMDH-B1** for purposes of analysis. *EST-1** and *PGM-3** were initially thought to be polymorphic; however further studies revealed that phenotypes varied when muscle samples were taken from different parts of the fish; therefore, these loci were not included in the analyses. Estimated mean heterozygosity (H_S) based on a total of 54 loci was 0·021 (S.E. = 0·0016). The Pleasant River sample had the lowest H_S (0·012), as compared to 0·020 to 0·026 in all other samples (Table II). Genotypic proportions deviated significantly from Hardy–Weinberg expectations ($P < 0{\cdot}05$) for only one of 56 tests (*sMDH-B1** in the Sheepscot sample).

GENETIC VARIATION AMONG SAMPLES

Genetic distances (*D*) between samples based on seven polymorphic loci ranged from 0·002 to 0·022 (Table III). The Machias River sample generally had the lowest genetic distances, whereas the Dennys River sample generally had the highest distances. No obvious association existed between genetic distance and geographic distance between sample locations. Cluster analysis of genetic distances depicted the Dennys River sample as being the most differentiated (Fig. 2). Bootstrap values ranged from 28 to 71%, with the only value above 50% occurring at the node joining the Pleasant and Naraguagus Rivers (71%).

Based on *G*-tests, significant differences in allele frequencies existed among the samples at five of the seven polymorphic loci ($P < 0{\cdot}05$; Table II). The *G*-value summed over all loci also indicated significant differences among samples ($P < 0{\cdot}01$). Twenty of the 28 possible pair-wise *G*-tests between samples were significant ($P < 0{\cdot}05$; Table III). The Sheepscot was the only sample that had allele frequencies significantly different from all other samples. The Machias sample was the least differentiated, having allele frequencies significantly

TABLE I. Tissue sources, locus designations, and electrophoretic buffers for Atlantic salmon proteins. Enzyme numbers are as recommended by IUBNC (1984). Tissues used were white muscle (M), liver (L), and eye (E). Buffer systems used were as follows: A (Ayala *et al.*, 1973 as modified by May *et al.*, 1979); C (Clayton & Tretiak, 1972 as modified by May *et al.*, 1979); M (Markert & Faulhaber, 1965); R (Ridgway *et al.*, 1970); 4 and 9 (Selander *et al.*, 1971, 9 tray buffer adjusted to pH 8·0; 9 tray buffer diluted 1:19 for gel buffer)

Enzyme	Enzyme number	Locus	Tissue	Preferred buffer	Polymorphism observed
Aspartate aminotransferase	2.6.1.1	*sAAT-1, 2*; 5**	M	9, R	No
		*sAAT-4**	L	4	Yes
Adenosine deaminase	3.5.4.4	*ADA**	E	4	No
Adenylate kinase	2.7.4.3	*AK-1*;2**	M	C	No
Aldolase	4.1.2.13	*FBALD-2**		A	Yes
Creatine kinase	2.7.3.2	*CK-1*; 2**	E	R	No
		CK-3; 4**	E	M, 4	No
Fructose bisphosphatase	3.1.3.11	*FBP**	E	4	No
Fumarate hydratase	4.2.1.2	*FH**	M	4	No
Glycerate-2-dehydrogenase	1.1.1.29	*GLYDH**	L	C	No
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A*; B1*; B2**	M	R	No
N-Acetyl-beta-glucosaminidase	3.2.1.30	*bGLUA**	E	R	No
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1*; 3**	M	A, C	No
β-N-Acetylgalactosaminidase	3.2.1.53	*bGALA**	L	R	No
L-Iditol dehydrogenase	1.1.1.14	*IDDH-1**	L	4*	Yes
Isocitrate dehydrogenase	1.1.1.42	*mIDHP-1*;2**	M	4	No
		sIDHP-1;2**	L	C	Yes
L-lactate dehydrogenase	1.1.1.27	*LDH-A1*; A2**	M	R	No
		*LDH-B1**	E	M	Yes
		*LDH-B2**	L	C	No
α-Mannosidase	3.2.1.24	*aMAN**	L	M	No
Malate dehydrogenase	1.1.1.37	*sMDH-A1**	E	A, 4	Yes
		*sMDH-B1,2**	M	A, 4	Yes
Malic enzyme	1.1.1.40	*mMEP-1**	M	C	No
		*mMEP-2**	M	C	Yes

Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	M	9, R	No
Methylumbelliferyl phosphatase	3.1.-.-.	*MUP-1**; *2**	M	C	No
Nucleoside phosphorylase (purine-nucleoside phosporylase; Dipeptidase)	2.4.2.1	*NP**	E	M	No
glycyl-leucine substrate	3.4.-.-	*PEPA**	E	C	Yes
leucyl-glycyl-glycine substrate	3.4.-.-	Cannot be specified	K	M	No
leucyl-alanine substrate	3.4.-.-	Cannot be specified	E	R	No
leucyl-leucyl-leucine substrate	3.4.-.-	Cannot be specified	L	C	No
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M	R	No
Phosphoglucomutase	5.4.2.2	*PGM-1r**	L	C	Yes
		*PGM-1**; *2**	M	C	No
Inorganic pyrophosphatase	3.6.1.1	*PP-3**	M	9	No
Superoxide dismutase	1.15.1.1	*sSOD**	M	R	No
Triose-phosphate isomerase	5.3.1.1	*TPI-1**; *2**; *3**	M	A, C	No

4* gel buffer adjusted to pH 8·6 with addition of 2·5 g Tris base per 1 L.
4 tray buffer adjusted to pH 7·7 with addition of 6·0 g Tris base per 1 L.

TABLE II. Allele frequencies, heterozygosities (H_S), and number of loci polymorphic for samples of Atlantic Salmon representing six rivers in Maine, one river in Canada (St John), and the Penobscot hatchery. Mean expected heterozygosities were based on a total of 54 loci. Critical values for the *G*-tests of differentiation among samples at each locus = 14·7 (alpha = 0·05) and 18·5 (alpha = 0·01)

		Sample								*G*-test
Locus	Allele	Sheepscot	Ducktrap	Penobscot	Narraguagus	Pleasant	Machias	Dennys	St John	(d.f. = 7)
*sAAT-4**	1	0·902	0·988	0·991	1·000	1·000	0·989	0·987	0·944	25·3
	2	0·098	0·013	0·009	–	–	0·011	0·013	0·056	
	H_S	0·176	0·025	0·019	–	–	0·022	0·025	0·105	
	n	41	40	53	48	40	46	39	45	
*FBALD-2**	1	1·000	0·988	0·991	0·968	1·000	0·990	0·929	0·978	16·0
	2	–	0·013	0·009	0·032	–	0·010	0·071	0·022	
	H_S	–	0·025	0·017	0·062	–	0·021	0·133	0·043	
	n	44	40	57	47	40	48	42	45	
*IDDH**	1	0·967	0·919	0·936	0·913	0·944	0·851	0·804	0·909	13·8
	2	0·033	0·081	0·064	0·087	0·056	0·149	0·196	0·091	
	H_S	0·064	0·149	0·120	0·160	0·105	0·253	0·316	0·165	
	n	30	37	47	40	36	37	28	44	
*sMDH-A1**	1	1·000	1·000	0·983	0·980	0·975	0·990	0·907	0·943	22·1
	2	–	–	0·017	0·020	0·025	0·010	0·093	0·057	
	H_S	–	–	0·033	0·040	0·049	0·020	0·169	0·107	
	n	41	40	59	49	40	50	43	44	
*sMDH-B1**	1	0·807	0·725	0·788	0·840	0·888	0·850	0·872	0·807	10·7
	2	0·193	0·275	0·212	0·160	0·113	0·150	0·128	0·193	
	H_S	0·312	0·399	0·334	0·269	0·200	0·255	0·223	0·312	
	n	44	40	59	50	40	50	43	44	
*mMEP-2**	1	0·974	0·863	0·990	1·000	1·000	0·969	0·988	0·922	35·6
	2	0·026	0·138	0·010	–	–	0·031	0·012	0·078	

	H_S	0·050	0·237	0·019	–	–	0·059	0·023	0·143	
	n	39	40	51	48	40	49	43	45	
*PGM-1r**	1	0·598	0·599	0·491	0·693	0·790	0·577	0·463	0·558	31·2
	2	0·402	0·401	0·509	0·307	0·210	0·423	0·537	0·442	
	H_S	0·482	0·467	0·500	0·428	0·320	0·489	0·497	0·494	
	n	42	39	58	50	40	48	42	45	
Mean H_S		0·020	0·024	0·019	0·018	0·012	0·021	0·026	0·025	
No. loci poly. – 5%		3	4	3	3	3	3	5	6	
*No. loci poly. -1%		5	6	7	5	4	7	7	7	

*Does not include the four loci with low-frequency alleles that were treated as monomorphic.

TABLE III. Genetic distances (Nei, 1972; top) and pair-wise *G*-test values* (Sokal & Rohlf, 1981; bottom) between Atlantic salmon samples representing six rivers in Maine, one river in Canada (St John), and the Penobscot hatchery strain. Values are based on seven polymorphic loci. The order of sample names reflects the linear order of the rivers along the Atlantic coast (see Fig. 1)

	Sheepscot	Ducktrap	Penobscot	Narraguagus	Pleasant	Machias	Dennys	St John
Sheepscot	*	0·005	0·003	0·004	0·009	0·004	0·012	0·002
Ducktrap	22·1	*	0·007	0·006	0·011	0·006	0·016	0·004
Penobscot	17·7	21·2	*	0·007	0·017	0·003	0·006	0·002
Naraguagus	29·3	28·5	17·1	*	0·002	0·003	0·012	0·004
Pleasant	21·7	34·7	28·1	9·3	*	0·010	0·022	0·011
Machias	19·2	16·1	10·2	11·4	22·6	*	0·004	0·002
Dennys	41·6	41·4	23·8	22·9	42·8	16·2	*	0·006
St John	19·5	13·5	15·5	25·2	33·9	12·5	18·6	*

*Critical values for *G*-tests = 17·5 ($\alpha = 0{\cdot}05$) and 21·9 ($\alpha = 0{\cdot}01$).

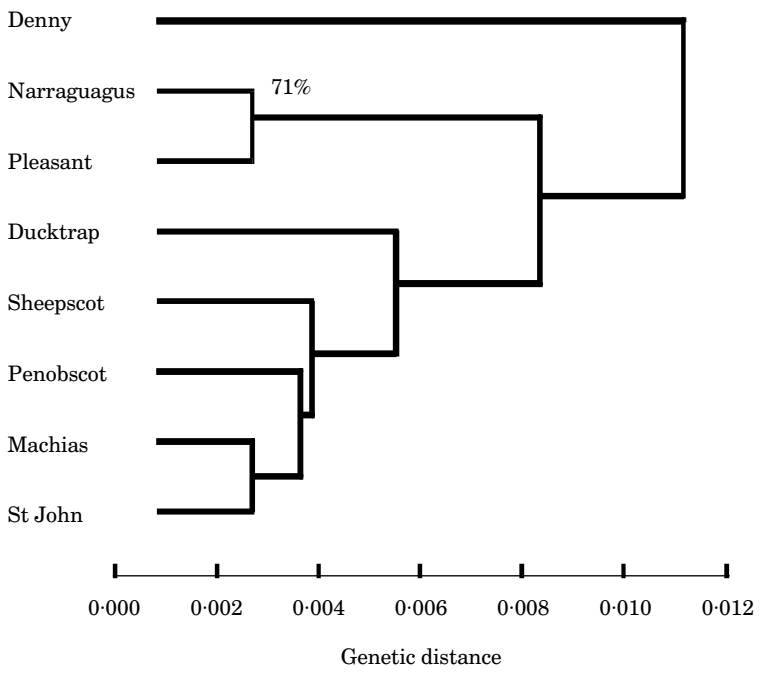

FIG. 2. Dendrogram generated by cluster analysis of Nei's (1972) genetic distance coefficients calculated among Atlantic salmon samples with data from seven polymorphic loci. Bootstrap values at the nodes indicate the percentage of times populations beyond the node grouped together based on 1000 bootstrap iterations. Only values above 50% are shown.

different from only two of the other seven samples. Of the total genetic diversity observed, the F_{ST} value of 0·034 indicated that 3·4% was due to variability among samples and that 96·6% of the variability was due to variability within samples.

DISCUSSION

The levels of genetic variability (heterozygosity) found in this study were within the range typically found in naturally reproducing populations of Atlantic salmon. Ståhl (1987) reviewed and summarized allozyme data from 28 naturally reproducing Atlantic salmon populations representing 23 major river systems draining into the Western Atlantic Ocean, the Eastern Atlantic Ocean, and the Baltic Sea. Based on 38 loci, heterozygosities reported by Ståhl (1987) ranged from 0·011 to 0·036, whereas heterozygosities from the Maine and Canadian samples in this study ranged from 0·012 to 0·026 (based on 54 loci). Sanchez *et al.* (1996) conducted a comparison of genetic variation detected by allozyme and microsatellite loci in Atlantic salmon populations from Ireland and Spain. While both types of loci showed the same pattern of differentiation among samples, the microsatellite loci exhibited higher levels of genetic variability and greater genetic distances. King *et al.* (2000, 2001) found reduced genetic variability in North American Atlantic salmon populations relative to

their European counterparts using both mitochondrial and microsatellite markers, hypothesizing that the differences may be due to the differing glacial histories of the two continents.

Levels of genetic differentiation among samples in this study were similar to the levels of differentiation among native populations sampled by Ståhl (1987). Differentiation between the Maine and Canadian samples ($F_{ST} = 0{\cdot}034$) was higher than Ståhl (1987) reported among samples within single river drainages ($F_{ST} = 0{\cdot}015$) but less than Ståhl reported among populations from different river systems within the same region ($F_{ST} = 0{\cdot}049$). Given the larger regional scale surveyed in Ståhl's (1987) comparisons, genetic differentiation among the rivers in Maine do not appear to be significantly different compared to natural Atlantic salmon populations in other regions. This is supported by reports of multiple runs of fish within the same river system at different times of the year (*e.g.*, Penobscot River; Saunders, 1981), which are consistent with the normal diversity found among populations in other parts of the world. Whether current levels of genetic variability and differentiation accurately reflect the historical (pre-manipulated) genetic composition of these rivers is difficult to support or refute without genetic data from the unperturbed, historical populations. A more definitive way of answering questions about the historical genetic diversity would be to analyze DNA (*e.g.*, microsatellite variation) from scales sampled from fish prior to any stocking.

In November 2000 wild fish in eight Maine rivers were listed endangered as a single distinct population segment under the federal Endangered Species Act (ESA). Two questions pertaining to the current listing are: 1) were historical populations in Maine rivers sufficiently genetically distinct from Canadian rivers to warrant separate listing, and 2) has artificial stocking homogenized the existing populations to the point that they no longer represent wild populations and are no longer useful for maintaining the natural genetic variation within and among North American populations of the species. Atlantic salmon analyzed in this study exhibited levels of genetic variability and differentiation within and among samples similar to what has been reported for native populations collected over a comparable geographic area in other parts of their range. Evidence based on *G*-tests and F_{ST} values suggests significant differentiation among the Maine river samples as well as between the Maine and Canadian samples, supporting the conclusion that Atlantic salmon in Maine Rivers have not suffered genetic homogenization despite heavy stocking with various Maine and Canadian stocks. Whether the levels of genetic differentiation reported here represent genetically isolated populations or partially isolated segments of a metapopulation is open to interpretation. Analysis of a minisatellite locus (Taggart, 1995), Mitochondrial DNA (King *et al.*, 2000), and microsatellites (King *et al.*, 2001) confirmed the existence of a deep phylogenetic discontinuity between North American and European Atlantic salmon. King *et al.* (2000) also reported shallower but still significant genetic structure between Canadian and Maine rivers as well as among six of the seven Maine rivers analysed in the present study. Furthermore, Spidle *et al.* (2001) produced evidence based on microsatellite DNA that significant intra-river population structure existed in numerous Maine Rivers including the Penobscot, which remained genetically distinct from hatchery strains despite years of heavy stocking.

Evidence from this and other studies indicate that Maine river populations of Atlantic salmon have maintained historical levels of variation and differentiation despite heavy artificial stocking, and supports their current listing as a distinct population segment. Recent evidence (King *et al*., 2001 and references therein) also points to the presence of significant inter- and intra-river genetic structuring in these rivers, which should be taken into account in any restoration efforts that might involve hatchery supplementation or the movement of fish between rivers. Further research is needed to better quantify the existing genetic structuring in the system and to compare the current state with historical patterns reconstructed via DNA analysis of archived scale samples.

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References

Allendorf, F. W. & Ryman, N. (1987). Genetic management of hatchery stocks. In *Population Genetics and Fishery Management* (Ryman, N. & Utter, F., eds), pp. 141–159. Seattle, WA: University of Washington Press.

Ayala, F. J., Hedgecock, D., Zumwalt, G. S. & Valentine, J. W. (1973). Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. *Evolution* **27,** 177–191.

Child, A. R. (1980). Identification of stocks of Atlantic salmon (*Salmo salar* L.) by electrophoretic analysis of serum proteins. *Rapports et Proces verbaux des Reunions Conseil Permanent International pour L'Exploration de la Mer* **176,** 65–67.

Clayton, J. W. & Tretiak, D. N. (1972). Amine-citrate buffers for pH control in starch gel electrophoresis. *Journal of the Fisheries Research Board of Canada* **29,** 1169–1172.

Cooper, D. W. (1968). The significance level in multiple tests made simultaneously. *Heredity* **23,** 614–617.

Cross, T. F. & Payne, R. H. (1977). NADP-isocitrate dehydrogenase polymorphism in the Atlantic salmon *Salmo salar*. *Journal of Fish Biology* **11,** 493–496.

Cross, T. F., Ward, R. D. & Abreu-Grobois, A. (1979). Duplicate loci and allelic variation for mitochondrial malic enzyme in the Atlantic salmon, *Salmo salar* L. *Comparative Biochemical Physiology* **62B,** 403–406.

Davidson, W. S., Birt, T. P. & Green, J. M. (1989). A review of genetic variation in Atlantic salmon, *Salmo salar* L., and its importance for stock identification, enhancement programmes and aquaculture. *Journal of Fish Biology* **34,** 547–560.

Felsenstein, J. (1993). *PHYLIP-Phylogenetic Inference Package, Version 3.5c*. Distributed by the author. Department of Genetics, University of Washington, Seattle.

IUBNC (International Union of Biochemistry, Nomenclature Committee) (1984). *Enzyme nomenclature, 1984*. Orlando, Florida: Academic Press.

Johnson, K. R. (1984). Protein variation in Salmonidae: genetic interpretations of electrophoretic banding patterns, linkage associations among loci, and evolutionary relationships among species. PhD Thesis, The Pennsylvania State University, University Park, PA. 197 p.

Jordan, W. C., Youngson, A. F. & Webb, J. H. (1990). Genetic variation at the malic enzyme-2 locus and age at maturity in sea-run Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **47,** 1672–1677.

King, T. L., Spidle, A. P., Eackles, M. S., Lubinski, B. A. & Schill, W. B. (2000). Mitochondrial DNA diversity in North American and European Atlantic salmon

with emphasis on the Downeast rivers of Maine. *Journal of Fish Biology* **57,** 614–630. doi: 10.1006/jfbi.2000.1338

King, T. L., Kalinowski, S. T., Schill, W. B., Spidle, A. P. & Lubisnki, B. A. (2001). Population structure of Atlantic salmon (*Salmo salar* L.): a range-wide perspective from microsatellite DNA variation. *Molecular Ecology* **10,** 807–821.

Koljonen, M. L. (1989). Electrophoretically detectable genetic variation in natural and hatchery stocks of Atlantic salmon in Finland. *Hereditas* **110,** 23–35.

May, B. (1992). Starch gel electrophoresis of allozymes. In *Molecular Genetic Analysis of Populations: A Practical Approach*, 2nd edn (Hoelzel, A. R., ed.), pp. 1–28 and 371–378. Oxford: Oxford University Press.

May, B., Wright, J. E., Jr. & Stoneking, M. (1979). Joint segregation of biochemical loci in Salmonidae: results from experiments with *Salvelinus* and review of the literature on other species. *Journal of the Fisheries Research Board of Canada* **36,** 1114–1128.

Markert, C. L. & Faulhaber, I. (1965). Lactate dehydrogenase isozyme patterns of fish. *Journal of Experimental Zoology* **159,** 319–332.

Nei, M. (1972). Genetic distance between populations. *American Naturalist* **106,** 283–292.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences U.S.A.* **70,** 3321–3323.

Nei, M. (1977). F-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics* **41,** 225–233.

Nei, M. & Roychoudhury, A. K. (1974). Sampling variances of heterozygosity and genetic distance. *Genetics* **76,** 379–390.

Ridgway, G. J., Sherburne, S. W. & Lewis, R. D. (1970). Polymorphism in esterases of Atlantic herring. *Transactions of the American Fisheries Society* **99,** 147–151.

Roberts, F. L. (1976). Biochemical genetics of the Atlantic salmon *Salmo salar*. Final Report, Research Contract No. 14-16-0008-829, U.S. Fish and Wildlife Service, Washington, D.C. 25 p.

Ryman, N. (1983). Patterns of distribution of biochemical genetic variation in salmonids: differences between species. *Aquaculture* **33,** 1–21.

Sanchez, J. A., Clabby, C., Ramos, D., Blanco, G., Flavin, F., Vazquez, E. & Powell, R. (1996). Protein and microsatellite single locus variability in *Salmo salar* L. (Atlantic salmon). *Heredity* **77,** 423–432.

Saunders, R. L. (1981). Atlantic salmon (*Salmo salar*) stocks and management implications in the Canadian Atlantic Provinces and New England, USA. *Canadian Journal of Fisheries and Aquatic Sciences* **38,** 1612–1625.

Selander, R. K., Smith, M. H., Yang, S. Y., Johnson, W. E. & Gentry, J. B. (1971). Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old field mouse (*Peromyscus polionotus*). In *Studies in Genetics IV* (Wheeler, M. R., ed.), pp. 49–90. Austin, TX: University of Texas Publication 7103.

Shaklee, J. B., Allendorf, F. W., Morizot, D. C. & Whitt, G. S. (1990). Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society* **119,** 2–15.

Spidle, A. P., Schill, W. B., Lubinski, B. A. & King, T. L. (2001). Fine-scale population structure in Atlantic salmon from Maine's Penobscot River drainage. *Conservation Genetics* **2,** 11–24.

Sneath, P. H. A. & Sokal, R. R. (1973). *Numerical Taxonomy*. San Francisco, CA: W.H. Freeman.

Sokal, R. R. & Rohlf, F. J. (1981). *Biometry: The Principles and Practice of Statistics in Biological Research*, 2nd edn. San Francisco, CA: W.H. Freeman.

Ståhl, G. (1981). Genetic differentiation among natural populations of Atlantic salmon (*Salmo salar*) in northern Sweden. *Ecological Bulletins (Stockholm)* **34,** 95–105.

Ståhl, G. (1983). Differences in the amount and distribution of genetic variation between natural populations and hatchery stocks of Atlantic salmon. *Aquaculture* **33,** 23–32.

Ståhl, G. (1987). Genetic population structure in Atlantic salmon. In *Population Genetics and Fishery Management* (Ryman, N. & Utter, F., eds), pp. 121–140. Seattle, WA: University of Washington Press.

Taggart, J. B., Verspoor, E., Galvin, P. T., Moran, P. & Ferguson, A. (1995). A minisatellite DNA marker for discriminating between European and North American salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **52,** 2305–2311.

USFWS/NOAA (2000). Endangered and threatened species; final endangered status for a distinct population segment of anadromous Atlantic salmon (*Salmo salar*) in the Gulf of Maine. *Federal Register* **65,** 69459–69483.

Verspoor, E. & Cole, L. J. (1988). Genetically distinct sympatric populations of resident and anadromous Atlantic salmon *Salmo salar*. *Canadian Journal of Zoology* **67,** 1453–1461.

Verspoor, E., Fraser, N. H. C. & Youngson, A. F. (1991). Protein polymorphism in Atlantic salmon within a Scottish river: evidence for selection and estimates of gene flow between tributaries. *Aquaculture* **98,** 217–230.

Vuorinen, J. (1982). Little genetic variation in the Finnish Lake salmon, *Salmo salar sebago* (Girard). *Hereditas* **97,** 189–192.

Wright, S. (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **19,** 395–420.

Electronic Reference

May, B., Krueger, C. C., Eng, W. & Paul, E. (1995). *Genes in Populations*. A microcomputer program. Available at: http://animalscience.ucdavis.edu/extension/software/Gene.htm