

Discrimination between Atlantic Salmon (*Salmo salar*) of North American and European Origin using Restriction Analyses of Mitochondrial DNA¹

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Bermingham, E., S. H. Forbes, K. Friedland, and C. Pla. 1991. Discrimination between Atlantic salmon (*Salmo salar*) of North American and European origin using restriction analyses of mitochondrial DNA. *Can. J. Fish. Aquat. Sci.* 48: 884–893.

Twenty restriction endonucleases were used to study mitochondrial DNA (mtDNA) polymorphism in 11 hatchery strains of Atlantic salmon (*Salmo salar*) representing geographically separated populations in Europe and North America. The North American salmon mtDNAs studied were readily distinguished, by a minimum of seven restriction site differences, from fish of European origin. These results suggested that restriction analyses of mtDNA might provide a useful method for determining the proportions of European and North American Atlantic salmon caught in the West Greenland fishery. To test this proposition, we analyzed 328 salmon caught in the 1987 West Greenland fishery including 68 fish with coded wire or Carlin tags which provided the geographic source of the tagged salmon. We correctly identified the continent of origin for 67 of the 68 physically tagged salmon using two informative restriction endonucleases. This study provides a clear indication of the usefulness of mtDNA for discriminating between European and North American Atlantic salmon caught in the West Greenland fishery and for mixed-fishery analysis in general.

Vingt endonucléases de restriction ont été utilisées pour étudier le polymorphisme de l'ADN mitochondrial (ADNmt) chez 11 souches d'écloserie de saumon de l'Atlantique (*Salmo salar*) représentant des populations provenant de régions différentes, soit d'Europe et d'Amérique du Nord. Les ADNmt du saumon d'Amérique du Nord qui ont été étudiés se distinguaient facilement de ceux des espèces d'origine européenne par au moins sept différences de sites de restriction. Ces résultats indiquent que l'étude des sites de restriction de l'ADNmt pourrait constituer une méthode utile pour déterminer les proportions de saumons de l'Atlantique capturés dans la pêcherie de l'ouest du Groenland provenant d'Europe ou d'Amérique du Nord. Pour vérifier cette proposition, nous avons étudié 328 saumons capturés dans la pêcherie de l'ouest du Groenland en 1987, dont 68 pourvus de micromarques magnétisées codées ou d'étiquettes Carlin qui permettaient de déterminer leur provenance. En utilisant deux endonucléases de restriction informatives, nous avons identifié correctement le continent d'origine de 67 des 68 saumons marqués. La présente étude indique clairement que l'ADNmt est utile pour déterminer si le saumon de l'Atlantique capturé dans la pêcherie de l'ouest du Groenland provient d'Europe ou d'Amérique du Nord; cette méthode pourrait également convenir en général pour d'autres pêcheries.

Received August 30, 1990

Accepted December 6, 1990

(JA709)

Reçu le 30 août 1990

Accepté le 6 décembre 1990

Commercial marine harvests of Atlantic salmon (*Salmo salar*) represent a classic problem in fisheries biology, namely that of managing a mixed-stock fishery. There

is a major fishery for Atlantic salmon off the west coast of Greenland where the catch is composed almost entirely of North American and European origin one-sea-winter age salmon. The fish originate from both wild-run rivers and enhancement programs on both continents. For conservation and economic reasons it is important, on a year to year basis, to determine the relative contributions that different stocks of Atlantic salmon make to the West Greenland fishery. In fact, information on

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the composition and origin of the catch and estimates of the mortality rate based on these data are considered when yearly quotas are determined for the fishery. Thus the accuracy and timeliness of the data, particularly stock origin, are critical.

This paper provides information on levels of mitochondrial DNA (mtDNA) polymorphism within the Atlantic salmon and also demonstrates the utility of mtDNA as a genetic marker for distinguishing between fish of North American and European origin. Specifically, we assess restriction endonuclease analysis of mtDNA as a method for determining the proportions of European and North American Atlantic salmon caught in the West Greenland fishery. Thus our aim is identical to that of a number of other investigators who have used both genetic and nongenetic methods, with considerable success, to estimate the contribution of European and North American salmon to this fishery (see particularly Reddin 1986; Stahl 1987; Reddin et al. 1988; Verspoor 1988). Currently management advice concerning the composition and origin of the salmon catch at West Greenland is based on scale ageing and meristics (Anonymous 1990). Problems associated with both the implementation and calibration of scale analyses for this fishery motivated the present study. This research also extends the findings of additional mtDNA surveys of anadromous and nonanadromous Atlantic salmon (Birt et al. 1986, 1991; Gyllensten and Wilson 1987; Bermingham et al. 1988; Hovey et al. 1989; Palva et al. 1989).

We first investigated 11 hatchery stocks of Atlantic salmon with historical origins in 10 different rivers in North America and Europe. We used restriction analyses of mtDNAs to characterize geographic population structure within Atlantic salmon and to determine whether or not mtDNA genotypic characters could provide qualitative markers useful for differentiating between salmon from North America and Europe. We next analyzed 328 Atlantic salmon caught in the 1987 West Greenland fishery. Using restriction analyses of mtDNAs, we determined the proportion of North American and European fish in the sample. Furthermore, we were later able to obtain coded wire or Carlin tag information for 68 of these salmon. By comparing data obtained from both the physical (coded wire or Carlin tags) and genetic (mtDNA) markers, we are able to evaluate the use of mtDNA analysis for determining continent of origin of harvested Atlantic salmon.

Materials and Methods

The Atlantic salmon included in this study are divided into two groups: 47 fish, representing 10 geographical stocks of Atlantic salmon (Connecticut River stocks were founded from Penobscot River fish), were collected from hatcheries and net pen facilities in the northwestern United States (Table 1). A second group of 328 fish caught in the Greenland fishery was collected at a fish processing plant in Nuuk, Greenland, in 1987. It should be noted that in the first group, small sample sizes coupled with possible loss of genetic diversity in hatchery populations (see Allendorf and Ryman 1987) suggest that only the most common mtDNA genotypes from the original populations were detected. In the second group, salmon were collected from a single processing plant over a short period of time, and thus, this sample should not be considered to represent the full geographic or temporal range of the West Greenland fishery. The coded wire and Carlin tagged salmon in the second group represent stocks that increase the geographic range of Atlantic salmon assayed (see Table 2) and diminish, but do not alle-

viate, sampling inadequacies associated with the hatchery group.

For the hatchery group, mtDNA was isolated in closed-circular form from fresh liver and/or heart if the fish was larger than 75 mm. Smaller fish (fry) were pooled and mtDNA was purified from fresh whole bodies homogenized after removal of the skins. Following isolation, mtDNAs were purified using procedures of cesium chloride/ethidium bromide gradient centrifugation (Lansman et al. 1981). Purified mtDNAs were extracted with water-saturated butanol to remove ethidium bromide and dialyzed against TE (10 mM Tris, 1 mM EDTA, pH 7.5) to remove the cesium chloride. Aliquots of mtDNA were digested with 20 restriction enzymes per individual (or pooled sample) under the conditions recommended by the vendors (Bethesda Research Laboratories and Boehringer-Mannheim; see Table 1, footnote b, for listing of enzymes used). Resulting DNA fragments were end-labeled with appropriate [α - 32 P]dXTP(s), sized by agarose gel electrophoresis (0.7–1.5%), and visualized by autoradiography (Brown 1983; Drouin 1980). The possibility of scoring two nonhomologous fragments as identical due to chance comigration was minimized by rerunning fragments of questionable identity side by side at different gel concentrations.

For the second group of 328 Atlantic salmon, crude genomic DNA was isolated from either liver or skeletal muscle samples that had been frozen on dry ice and stored at -80°C . (All steps in the DNA isolation procedure described below were carried out in 1.5-mL snap-top Eppendorf tubes.) About 100 mg of tissue from each fish was powdered in liquid nitrogen and incubated at 37°C , overnight, in a proteinase K/SDS solution (500 μL of STE (100 mM NaCl, 10 mM Tris, 25 mM EDTA, pH 7.5), 25 μL of a 10 mg/mL stock of proteinase K in STE, and 25 μL of 20% SDS). Following incubation the samples were extracted twice with an equal volume of PCI (25:24:1 mix of phenol, chloroform, and isoamyl alcohol) and twice with an equal volume of chloroform. The DNA in each sample was ethanol precipitated, pelleted, dried, and suspended in 250 μL of TE. Typical yields were about 25 μg , and approximately 1 μg of DNA from each individual fish was digested using the restriction enzymes *Dra* I and *Bst* EII. Additional aliquots from a subset of the 328 fish were digested with *Ava* II. DNA fragments were electrophoretically separated on 0.9% agarose gels (or 1.1% agarose gels for *Ava* II fragments), denatured, and transferred overnight onto Zetabind nylon filters. Following transfer the nylon filters were hybridized, for one to two nights, with ^{32}P random-primed or nick-translated Atlantic salmon mtDNA (probe mtDNA) which had been twice purified by ultracentrifugation. After washing the filters to remove the unincorporated probe mtDNA, restriction fragments were visualized by autoradiography.

A map of all restriction sites (with the exception of *Cla* I, *Stu* I, *Hind* II, and *Ava* II) was constructed using fragment sizes produced by single and double digestions of Atlantic salmon mtDNAs (Zehetner et al. 1987) (see Fig. 1). *Bam* HI, *Bcl* I, *Bgl* II, *Bst* EII, and *Xho* I restriction endonucleases each produce one or two cuts in Atlantic salmon mtDNAs and were easily mapped. Maps were considered complete when cleavage sites for a new enzyme could be assigned to locations internally consistent with those for the above enzymes. Position 0 of the approximately 16 800 base pair (bp) mtDNA of *S. salar* is an *Eco* RI site that we believe to be homologous with a conserved *Eco* RI site located at 7.5 kbp on Thomas et al. (1986) mtDNA maps of *Oncorhynchus* species. The proposed mitochondrial

TABLE 1. Sample identification numbers, composite mtDNA genotypes, and historical source of the hatchery populations used in this study.

Sample ID	N ^a	Composite genotype ^b	Historical source of stock
O-99		CCCCCCCCCCCCCCCCCCCC	Connecticut River ^d , CT, USA
O-100		CCCCCCCCCCCCCCCCCCCC	Connecticut River ^d , CT, USA
O-97		CCCCCCCCCCCCCCCCCCCC	Penobscot River, ME, USA
O-98		CCCCCCCCCCCCCCCCCCCC	Penobscot River, ME, USA
O-95		CCCCCCCCCCCCCCCCCCCC	Union River, ME, USA
O-96		CCCCCCCCCCCCCCCCCCCC	Union River, ME, USA
O-142	3	CCCCCCCCCCCCCCCCCCCC	Grand Lakes, ME, USA
O-143	3	CCCCCCCCCCCCCCCCCCCC	Grand Lakes, ME, USA
O-195		CCCCCCCCCCCCCCCCCCCC	Grand Lakes, ME, USA
O-103		CCCCCCCCCCCCCCCCCCCC	St. Johns River, N.B., Canada
O-104		CCCCCCCCCCCCCCCCCCCC	St. Johns River, N.B., Canada
O-101		CCCCCCCCCCCCCCCCCCCC	Grand Cascopectia River, N.B., Canada
O-102		CCCCCCCCCCCCCCCCCCCC	Grand Cascopectia River, N.B., Canada
O-364		CCCDDDCCCCCCCCCCCCCX	Vogalax River, Iceland
O-365		CCCDDDCCCCCCCCCCCCCX	Vogalax River, Iceland
O-366		CCCDDDCCCCCCCCCCCCCX	Vogalax River, Iceland
O-173R		CCCDDDCCCCCCCCCCCCCX	Wester Ross River, Scotland
O-174R		CCCDDDCCCCCCCCCCCCCX	Wester Ross River, Scotland
O-175R		CCCDDDCCCCCCCCCCCCCX	Wester Ross River, Scotland
O-369		CCCDDDCCCCCCCCCCCCCX	Wester Ross River, Scotland
O-170		CCCDDDCCCCCCCCCCCCCX	Nanske River ^e , Norway
O-134	5	CCCDDDCCCCCCCCCCCCCX/Y ^c	Morrum River, Sweden
O-137	5	CCCDDDCCCCCCCCCCCCCX/Y ^c	Morrum River, Sweden
O-138	4	CCCDDDCCCCCCCCCCCCCY	Neva River, Russia
O-139	4	CCCDDDCCCCCCCCCCCCCY	Neva River, Russia
O-140	4	CCCDDDCCCCCCCCCCCCCY	Neva River, Russia

^amtDNA was isolated from pooled samples where *N* is indicated; otherwise, mtDNA was isolated from a single individual.

^bLetters, from left to right, refer to digestion profiles produced by the following restriction endonucleases: *Bam* HI, *Bcl* I, *Bgl* I, *Bgl* II, *Bst* EII, *Cla* I, *Dra* I, *Eco* RI, *Eco* RV, *Hind* III, *Hpa* I, *Pst* I, *Pvu* II, *Sac* I, *Stu* I, *Xba* I, *Xho* I, *Ava* I, *Hind* II, *Ava* II.

^cThe two pooled samples taken from Swedish salmon displayed all fragments characteristic of both the X and the Y *Ava* II restriction fragment profiles. This is most likely the result of pooling fish with different *Ava* II mtDNA genotypes.

^dDerived from Penobscot River fish.

^eMoni A/S commercial stock.

gene organization in Atlantic salmon is the same as that in human (Davidson et al. 1989b).

We estimate that the placement of cleavage sites on the Atlantic salmon map is accurate to within plus or minus several hundred base pairs. It is possible that we have failed to map a few restriction sites because they terminate small fragments or are coincident with other sites. The presence of one such site, which bounds an *Ava* I 500-bp fragment, was inferred during mapping. However, for most endonucleases the sizes of restriction fragments revealed by each enzyme totaled very close to 16.8 kbp, suggesting that we have observed most fragments. Comparing data from different laboratories, it is clear that large fragments cannot be sized accurately, and mapping provides valuable independent information on the true positions of distant sites (see Table 3).

Unmapped restriction sites (*Cla* I, *Stu* I, *Hind* II, and *Ava* II) were inferred from the fragment data, and presence/absence of both mapped and unmapped restriction sites was used to calculate the extent of sequence divergence (*d*) differentiating mtDNA haplotypes (Nei et al. 1985).

Results

Three distinct mtDNA genotypes were observed in the 47 hatchery group Atlantic salmon mtDNAs (representing both

pooled and individual fish) surveyed with 20 restriction endonucleases (Table 1). These genotypes, or mtDNA haplotypes, are strongly patterned geographically. The North American salmon studied were readily distinguished from fish of European origin: the two mtDNA haplotypes from European fish differed at a minimum of seven restriction sites (*Bgl* II, *Bst* EII, *Cla* I, *Dra* I, and three or more *Ava* II sites) from the single mtDNA genotype observed in all North American Atlantic salmon (Fig. 1 and 2; Table 3). The minimum estimated mtDNA sequence divergence observed between the "North American" salmon mtDNA type and the "European" types is $d = 0.0072$.

The two mtDNA haplotypes obtained from European fish differ by only a single *Ava* II restriction site. The estimated sequence divergence between these two types is $d = 0.0011$. Again there is a geographic pattern to the distribution of the two mtDNA genotypes observed in European Atlantic salmon. Salmon from rivers in Iceland, Scotland, and Norway share a mtDNA halotype (referred to hereafter as the "European" type) that differs from the mtDNA haplotype from fish of Russian origin ("Baltic" type). The gain of an *Ava* II site in the mtDNAs of Baltic Sea fish relative to the mtDNAs of North Sea salmon was also observed by Gyllensten and Wilson (1987) in their study of Atlantic salmon from two Swedish rivers (Altran River,

TABLE 2. Geographic occurrence, in this and other studies, of the three mtDNA types defined in this report. Numbers in parentheses represent the coded wire and Carlin tagged Atlantic salmon collected in the West Greenland fishery.

River	"North American"	"European"	"Baltic"	Study ^a
North America				
Connecticut (ME, USA)	2 (1)			1
Penobscot (ME, USA)	2 (37)			1
Union (ME, USA)	2			1
Grand Lakes (ME, USA)	7			1
St. John (N.B., Canada)	2			1
Miramichi (N.B., Canada)	(5)			1
Grand Cascopeia (N.B., Canada)	2			1
Nepisiquit (N.B., Canada)	(1)			1
Kedgwick (N.B., Canada)	(1)			1
Tusket (N.S., Canada)	(1)			1
Medway (N.S., Canada)	(1)			1
North (N.S., Canada)	(1)			1
Five Mile Pond ^b (Nfld., Canada)	1			2
Exploits (Nfld., Canada)	1			2
Gambo ^b (Nfld., Canada)	29	5		3
Gambo (Nfld., Canada)	28	9		3
Total	124	14		
Europe				
Vogalax (Iceland)		3		1
Corrib (Ireland)		(3)		1
Lee (Ireland)		(3)		1
Erne (Ireland)		(1)		1
Shannon (Ireland)		(1)		1
Boyne (Ireland)		(1)		1
Wester Ross (Scotland)		4		1
Lussa (Scotland)		(1)		1
Tyne (England)		(4)		1
Ware (England)	(1) ^c	(3)		1
Itchin (England)		30	10	4
Mixed stock (France)		(2)		1
Nanske (Norway)		1		1
Atran (Sweden)		4		5
Total	1	61	10	
Europe, Baltic Sea				
Lule (Sweden)			5	5
Morrum (Sweden)		10 ^d		1
Saimaa ^b (Finland)			17	6
Pielisjoki ^b (Finland)			6	6
Neva (Russia)			21	1, N=12; 6, N=9
Total			49	

^a1, This study; Bermingham et al. (1988); 2, Birt et al. (1986); 3, Birt et al. (1991); 4, Hovey et al. (1989); 5, Gyllensten and Wilson (1987); 6, Palva et al. (1989).

^bNonanadromous stocks of Atlantic salmon.

^cOnly five of the physically tagged fish in this study were assayed with *Ava* II, but included in this group is the one fish with a "North American" haplotype tagged in the River Ware, England. None of the Newfoundland fish have been assayed with *Ava* II.

^dThese 10 fish were assayed as pooled samples and presumably include individuals with both the "European" and "Baltic" genotypes; see footnote c, Table 1.

North Sea; Lule River, Baltic Sea). In our study, both the "Baltic" and the "European" mtDNA genotypes were found in salmon from Sweden's Morrum River stock (Table 1; Fig. 2)

Two of the restriction endonucleases that produce genetic patterns useful for discriminating European from North American salmon, *Dra* I and *Bst* EI1, were used to analyze genomic DNAs isolated from the 328 fish sampled from the west Greenland fishery. Seventy-four fish (23%) were identified as European and 254 (77%) as North American. Only one fish had a

mixed European/North American genotype (a *Dra* I "North American" genotype and a *Bst* EI1 "European" genotype). Tissues from this fish were resampled and analyzed a second time with *Dra* I and *Bst* EI1 and for a first time with *Ava* II. *Ava* II and *Bst* EI1 yielded typical "European" mtDNA genotypes, but again, *Dra* I revealed the "North American" pattern. This is likely to represent the secondary loss of the *Dra* I site located at 4.25 kbp on our Atlantic salmon map in the mtDNA haplotype carried by this individual.

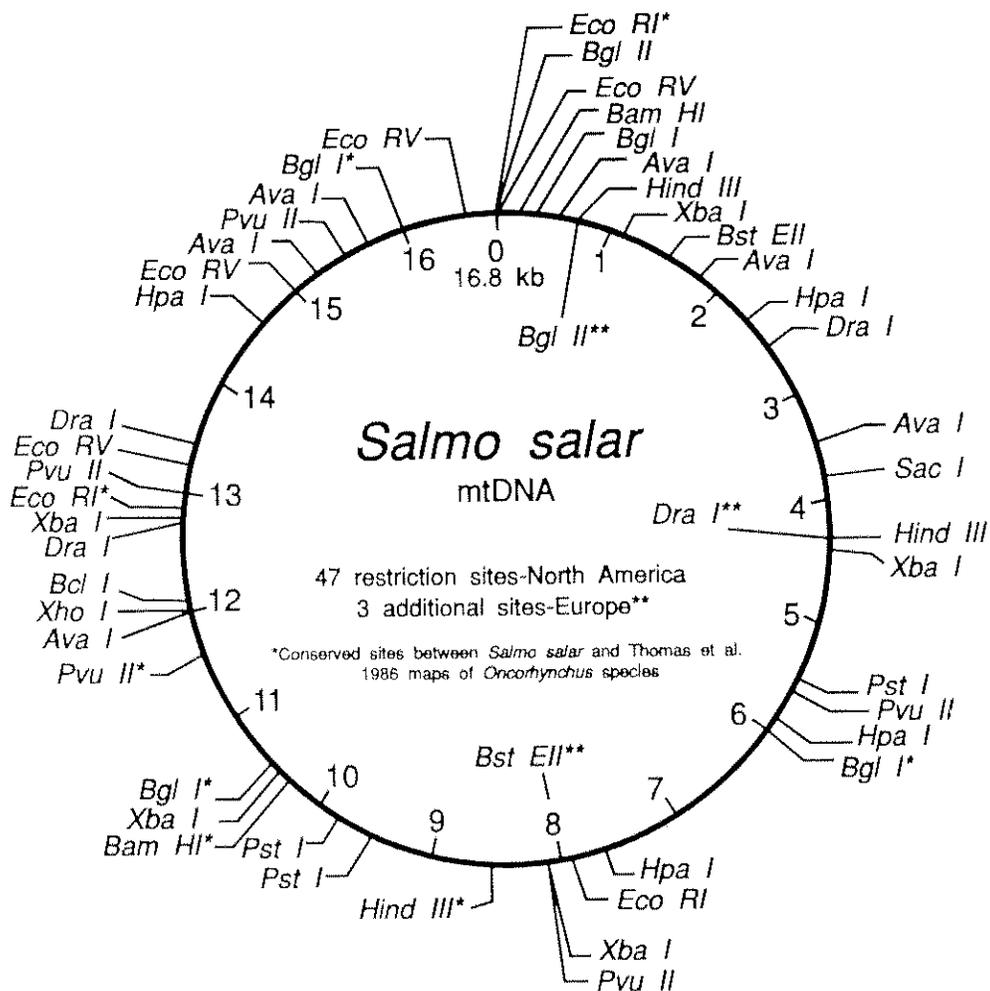


FIG. 1. Map positions of restriction endonuclease sites in the "North American" and "European" mtDNA genomes of Atlantic salmon. See text for details.

Of the 328 Atlantic salmon collected from the West Greenland fishery, physical tags indicating river, country, and continent of origin were recovered from 68 fish. Scoring the 68 marked salmon blind to the tag information revealed 47 North American salmon and 21 European salmon. Two fish had physical tag data and mtDNA genotype data that were not congruent. Resampling and digestion of the DNA with *Ava* II, *Bst* EII, and *Dra* I showed that laboratory error accounted for one of the misassignments; the new restriction patterns matched the tag identity. The other fish, which physical tag data identified as "European," was again characterized as "North American." This single true discrepancy between the genetic and physical tag data may have been due to error in tissue sampling or tag recording in the field, or the "North American" mtDNA genotype may occur in very low frequency in Europe. Nevertheless, the mtDNA data and the physical tag data agreed for 67 of the 68 Atlantic salmon tested from West Greenland.

The major feature of the mtDNA data in Atlantic salmon is the genetic divergence exhibited between North American and European forms. A secondary feature of the data is the genetic distinction between the "European" and "Baltic" mtDNA haplotypes. Figure 2, an autoradiograph showing the results of an *Ava* II digestion of both European and North American Atlantic salmon mtDNAs, depicts these genetic differences using one of the several informative enzymes. Although more detailed geographic sampling is required, the degree of con-

cordance between physical tags and mtDNA genotype markers strongly suggests that mtDNA haplotypes may be used to assign continent of origin to individual Atlantic salmon with a high degree of confidence.

Discussion

Geographic patterns of mtDNA restriction site polymorphism have previously been described for a number of economically important fish species (Wilson et al. 1985, 1987; Kornfield and Bogdanowicz 1987; Grewe and Hebert 1988; Billington and Hebert 1988; Chapman 1989; Mulligan and Chapman 1989). The present study augments earlier surveys of Atlantic salmon using restriction enzyme analyses of mtDNA (Birt et al. 1986, 1991; Gyllensten and Wilson 1987; Hovey et al. 1989; Palva et al. 1989) and additionally provides a test analysis of stock composition in a mixed fishery off the West Greenland coast. The purposes of this research have been to (1) provide a data base on intraspecific mtDNA differentiation in Atlantic salmon over a wide geographic range and (2) assess the usefulness of restriction enzyme analyses of mtDNA for discriminating between Atlantic salmon of North American and European origin.

The level of mtDNA variation observed in Atlantic salmon is considerably lower than has been reported for several species of freshwater fishes (Bermingham and Avise 1986) but is sim-

Fig. 3. mtDNA fragment sizes observed in this and other published studies of Atlantic salmon. Only restriction endonucleases used in this study and revealing polymorphic mtDNA digestion patterns are listed. Fragments on one line are presumed to be homologous.

Sample	"North American" ^a	Newfoundland ^{b,c}	"European" ^a	England, Wales ^d	Sweden ^e (N. Sea)	Sweden ^e (Baltic)	Finland, Russia ^f	"Baltic" ^g
II	16 800	16 700	—	—	ND ^h	—	—	—
	—	—	15 800	16 050	—	15 600	14 700	16 050
	—	—	860	750	—	900	860	750
EII	16 800	16 700	—	—	ND	ND	—	—
	—	—	10 200	10 300	—	—	10 430	10 300
	—	—	6 500	6 500	—	—	6 160	6 500
I	5 300	—	ND	—	ND	ND	ND	—
	—	—	—	5 100	—	—	—	5 100
	3 850	—	—	3 850	—	—	—	3 850
	3 400	—	—	3 400	—	—	—	3 400
	[2 225] ⁱ	—	—	[2 225]	—	—	—	[2 225]
	—	—	—	(200) ^j	—	—	—	(200)
I	10 150	10 200	—	—	ND	ND	ND	—
	—	—	8 500	8 450	—	—	—	8 450
	6 000	6 000	6 000	6 000	—	—	—	6 000
	—	—	1 700	1 700	—	—	—	1 700
	650	650	650	650	—	—	—	650
I	12 500	—	13 300	12 500	ND	—	13 250	12 500
	—	10 300	—	—	—	10 350	—	—
	3 900	3 800	3 800	3 900	—	[3 900]	3 730	3 900
	—	3 100	—	—	—	—	—	—
	350	—	300	350	—	—	—	350
II	—	ND	—	4 150	4 786	4 786	5 000	4 150
	2 525	—	—	2 525	2 951	2 951	2 500	2 525
	2 400	—	—	—	—	—	—	—
	2 250	—	—	2 250	[2 203]	[2 203]	2 200	2 250
	2 125	—	—	2 125	—	—	2 000	2 125
	1 825	—	—	—	—	—	—	—
	1 475	—	—	1 475	—	—	—	—
	—	—	—	1 375	1 396	1 396	1 400	1 475
	860	—	—	860	891	950	1 300	1 375
	815	—	—	—	—	—	—	—
	740	—	—	—	—	—	—	—
	550	—	—	550	582	582	[520]	550
	—	—	—	—	555	555	—	530
	415	—	—	415	414	414	380	415
	—	—	—	—	336	336	310	340
	—	—	—	[320]	322	322	300	[320]
	—	—	—	—	315	315	ND	—
	310	—	—	—	—	—	—	—
	235	—	—	235	211	211	—	235
	230	—	—	230	202	202	—	230
	—	—	—	145	143	143	—	145
	—	—	—	100	80	80	—	100

^a This study; Bermingham et al. (1988).

^b Irt et al. (1986, 1991).

^c Davidson et al. (1989a).

^d Covey et al. (1989).

^e Jyllensten and Wilson (1987).

^f Olva et al. (1989).

^g ND = no data.

^h Square brackets = presumed doublet.

ⁱ Parentheses = fragment inferred, not actually seen on gel.

to the amount of mtDNA restriction site polymorphism observed in many marine and diadromous fish species (Avisé et al. 1986, 1987; Wilson et al. 1985, 1987; E. Bermingham and M. Shulman, unpubl. data). Nonetheless, there is an obvious geographic pattern to the distribution of mtDNA haplotypes observed in Atlantic salmon. It is the readily assayable mtDNA restriction site differences between the geographically separated groups of North American and European Atlantic

salmon that make restriction analysis of mtDNA such a potentially powerful tool in the management of the West Greenland fishery. In addition, this result suggests that mtDNA may also be useful for analysis of other mixed fisheries involving anadromous species.

Both scale meristics and protein variation have been used to identify Atlantic salmon stocks (Reddin 1986; Stahl 1987; Reddin et al. 1988; Verspoor 1988). Scale samples used in the age

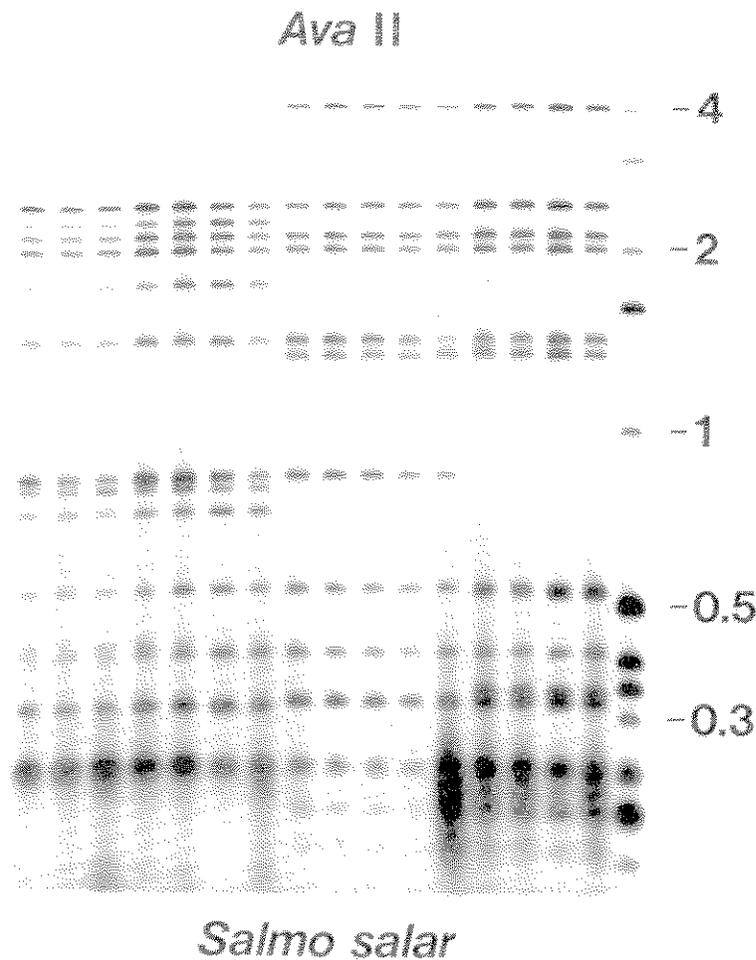


FIG. 2. Representative *Ava* II digests of mtDNAs isolated from Atlantic salmon. From left to right, the samples are O-99, O-100 (Connecticut River, USA); O-95R, O-96 (Union River, USA); O-103 (St. Johns River, Canada); O-101 (Grand Cascopectia River, Canada); O-143 (Grand Lakes, USA); O-364, O-365 (Vogalax River, Iceland); O-369, O-174R (Wester Ross River, Scotland); O-170 (Nanske River, Norway); O-134, O-137 (Morrum River, Sweden); O-138, O-140 (Neva River, USSR); molecular weight standard (BRL 1 kb ladder). mtDNAs were end-labeled and then size fractionated on a 1.5% agarose gel. (N.B. For total genomic DNA preps, however, separated on agarose gels, transferred to nylon filters, and hybridized to Atlantic salmon mtDNA, we typically are not able to resolve fragments smaller than 0.5 kb.) See Table 1 and text for comments on pooled samples.

analysis of salmon populations simultaneously provide a data source for continent assignments that is easy to collect and analyze. The ability to analyze numerous scale samples also ensures that the sampling design of the fishery is statistically robust. Regrettably, scale analysis has the disadvantage of being based on a character subject to annual variation. Variation in scale meristic characters is largely determined by environmental factors such as temperature, and thus, these characters are unlikely

to be stable over time. A case in point was made by Reddin (1982) who demonstrated that scale circuli counts of the first-sea-year for European salmon decreased substantially between a 1968–70 database (Lear and Sandeman 1980) and a 1980 database (Reddin 1982). Use of the 1968–70 database in the analysis of scales from Atlantic salmon caught in the West Greenland fishery in recent years underestimated the proportion of European salmon caught there.

... scale analysis is calibrated yearly by one of two... The first technique uses scale samples of two-sea-... salmon collected the following year to validate the pre-... year's scale analysis. The second method calibrates the... analysis in the same year using an independent technique. ... in the same year have recently been undertaken... electrophoretic analyses of allozymes (Verspoor 1988). ... problems in implementing the allozyme analyses of... salmon to determine continent of origin resulted in data... in the assessment of the Greenland fishery. As... by the ICES North Atlantic Salmon Working Group... (Anonymous 1990), the inability to collect the required tissue... and the inability to identify European genotypes using... has left the scale meristics continent of origin anal-... calibrated for annual variation since 1987. Clearly there... for a technique to identify continent of origin of salmon... more efficacious and has less rigorous sample collection... ments

... restriction analysis of mtDNA has advantages compared... protein electrophoresis in determining the continent of ori-... Atlantic salmon. First, the basic units of mtDNA analysis... distinct haplotypes in individual fish, in contrast with allo-... analyses which are typically based on population allele... frequencies. In other words, mtDNA restriction fragment length... polymorphisms (RFLPs) are genetic tags which can identify the... continent of origin of individual fish, even in the absence of... specific, fixed differences in allozyme alleles between pop-... In addition, mtDNA RFLPs share many of the attri-... that have made physical tags so useful in fisheries man-... They have the added benefit that all individuals carry... and thus are considerably more cost effective than... physical tags. Furthermore, we report here the continent of ori-... of 328 salmon harvested in the 1987 Greenland fishery. ... though the sample is too small and nonrandom to interpret... in the fishery, it is large enough to illustrate the potential... building up this technique beyond the sample requirements of... annual calibration for the scale analysis.

... second advantage of mtDNA analysis over allozymes... fixed fishery analysis concerns sample availability and... preservation. The statistical power of population analyses based... allele frequencies is a function of the number of polymorphic... loci that can be reliably scored. Maximizing the number... of polymorphic loci available for study in turn depends on the avail-... of several tissues (typically liver, muscle, and eye) and... adequate preservation of these tissues (samples frozen on... ice or liquid nitrogen with subsequent storage at -70°C ... (Arnold et al. 1987)). Both tissue collection and preserva-... can be problematic in remote areas. Also, the amount and... of tissues available are limited in species for which... the fish market condition is important. As noted by Ver-... (1988), "In spite of the high classification success rates... are possible, protein variation has limited potential for use... in the West Greenland fishery due to difficulties in obtaining... of liver tissue necessary for determining protein geno-

... These problems are significantly reduced when nucleic... methods are used. One hundred milligrams of tissue rou-... yields sufficient high molecular weight DNA for 10-20... restriction enzyme digestions using either liver or skeletal mus-... In addition, tissue preservation is less critical in nucleic... methods than in starch gel electrophoresis, which gener-... requires preservation of active enzymes. Although in the... study, tissues were collected on dry ice and maintained... our laboratory routinely isolates high molecular

weight DNAs and carries out mtDNA RFLP analyses from tis-
sues preserved in 70-90% ethanol.

Although restriction enzyme analysis of mtDNA appears to
be a valuable tool in the management of Atlantic salmon, cave-
ats regarding these mtDNA analyses need to be discussed. The
first is that our hatchery baseline sampling of Atlantic salmon
mtDNA genomes, even with the addition of the physically
tagged salmon collected in the West Greenland fishery, is not
fully representative of the diversity and distribution of mtDNA
haplotypes in the rivers of North America and Europe. This is
particularly true in North America where all of our samples
originated from a group of contiguous rivers in Maine and
southern Canada. In fact, Birt et al. (1991) have recently
observed what we have termed the "European" mtDNA haplo-
type in 20% of the anadromous and nonanadromous Atlantic
salmon collected from the Gambo River in Newfoundland using
restriction enzymes *Bst* EII and *Dra* I (Table 2). These are the
same enzymes that we used to survey the 328 fish collected in
the West Greenland fishery, and their data indicate that we may
have underestimated the proportion of North American fish in
that sample. Preliminary data, based on reasonable samples of
salmon from only the Gambo and Penobscot rivers (Table 2),
indicate that there may be a clinal distribution in North America
of the "European" mtDNA haplotype and that it is completely
replaced by the "North American" type in southern Canada
and the United States. At the present time it is unknown whether
or not *Ava* II will be able to distinguish between "European"
mtDNAs of fish collected in North America and Europe. Only
a single "North American" mtDNA haplotype has been
observed (this study) in four independent surveys of wild and
hatchery populations of European Atlantic salmon (this study;
Gyllensten and Wilson 1987; Hovey et al. 1989; Palva et al.
1989) (Table 2).

Nevertheless, it is clear that a more complete geographic
sampling of Atlantic salmon, especially in North America, is
required. It is also clear that Atlantic salmon mtDNA genotypes
need to be examined in greater detail. For example, by using
restriction enzymes that cut at four or five base recognition
sequences (see below) or directly sequencing portions of the
mtDNA genome, it may be possible to find additional nucleic
acid markers that unequivocally distinguish between fish of
European and North American origin. A first step should be
assays of Newfoundland and Quebec Atlantic salmon using the
Ava II, *Hinf* I, and *Hae* III restriction enzymes that have already
revealed informative mtDNA polymorphisms (Birmingham
et al. 1988 and this study; Hovey et al. 1989; Palva et al. 1989).

Our observation that restriction sites may blink on and off in
Atlantic salmon mtDNAs strongly suggests that salmon caught
in the West Greenland fishery need to be tested with more than
one restriction endonuclease. *Ava* II is a particularly useful
enzyme for identifying salmon to continent of origin because
it reveals multiple restriction site differences between "Euro-
pean" and "North American" mtDNA haplotypes. Thus, con-
vergent mtDNA digestion profiles are highly unlikely with this
enzyme. This is true for *Ava* II even though we typically cannot
resolve mtDNA fragments smaller than 0.5 kbp using agarose
gels and filter hybridization. To better appreciate the differ-
ences revealed by *Ava* II under conditions that would probably
be used in a routine survey of the West Greenland fishery,
imagine only the fragments larger than 0.5 kbp in Fig. 2 and
Table 3.

A primary concern of managers of the West Greenland
Atlantic salmon fishery is the determination of continent of ori-

gin of salmon harvested. However, there is also considerable interest among salmon biologists in being able to distinguish fish from different river systems. Recently published research by Palva et al. (1989) demonstrated that mtDNA markers can be used to discriminate between the geographically related Saimaa Lake and Neva River Atlantic salmon stocks used by the Finnish government in their breeding and stocking programs. Furthermore, a second group of investigators (Hovey et al. 1989) has even documented differences in the distribution of mtDNA haplotypes between Atlantic salmon spawning sites within a single river. Both of these studies are subject to the same criticism that can be leveled against our study: not enough fish have been sampled and temporal variation has not been surveyed. It remains to be seen if mtDNA analyses of Atlantic salmon will improve our knowledge of the regional genetic structure of Atlantic salmon populations beyond that which has resulted from allozyme studies (Stahl 1987; Verspoor 1988).

The northern rivers of North America and Europe have only been recolonized by Atlantic salmon following the last glaciation, within the last 8000 – 10 000 yr. Thus, not much time has elapsed for the accumulation of DNA base substitutions that are likely to distinguish between fish from geographically related drainages. Observed allozyme genotype differences in salmon populations are largely allele frequency shifts probably due to founder events and genetic drift (Stahl 1987; Verspoor 1988). It may be the case that in the absence of river stock specific mtDNA markers, allozymes representing unlinked protein coding loci or nuclear RFLPs are likely to provide better assays of local population subdivision. This is because the power of a test for population heterogeneity increases with the addition of unlinked polymorphic loci, and mtDNA is effectively a single linkage group. Nonetheless, mtDNA is a highly polymorphic locus with a large number of alleles in moderate frequency (e.g. six mtDNA haplotypes observed in fish collected from the River Lichen with the following frequencies: 0.565, 0.175, 0.135, 0.075, 0.025, and 0.025 (Hovey et al. 1989); four mtDNA haplotypes observed in nonanadromous salmon in the Gambo River (0.677, 0.177, 0.117, and 0.029); and two mtDNA haplotypes surveyed in anadromous salmon from the same river (0.757 and 0.243) (Birt et al. 1991)). If used in conjunction with allozymes or nuclear RFLPs, mtDNA may prove to be as useful for local Atlantic salmon stock differentiation as it appears to be for continent of origin determination.

This study and others cited herein provide convincing evidence that genetic stock identification of Atlantic salmon is clearly enhanced by restriction analyses of mtDNA. What is now required is a thoroughly representative geographic sampling of wild and hatchery stocks of Atlantic salmon surveyed for both informative mtDNA and allozyme characters. Only in this way will we know if the tools necessary for understanding the composition and origin of Atlantic salmon caught in the West Greenland fishery are in hand.

Acknowledgements

We owe a special debt of gratitude to Fred Utter for his commitment, overcoming inertia and adversity, to establishing a nucleic acid laboratory at NMFS/Seattle. We hope this research begins to vindicate his vision. We also thank Paul Aebersold, Laura Beal, Dave Leverage, and Owen McMillan for their help in the laboratory and Peter Downton for his help in collecting samples. We thank Dr. Willi Davidson for comments that improved the quality of this report. The work was

funded primarily by the National Marine Fisheries Service Northeast Fisheries Center. The Smithsonian Tropical Research Institute's Molecular Evolution Program supported the preparation of the manuscript.

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